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Poster Sessions

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POSTER SESSIONS

Poster Session 1

Sunday 5 July & Monday 6 July
08:30–19:30, Foyer Convention Center

Gen EX S1, Chromatin Structure and Epigenetic Modifications and Maintenance of the Genome

P02-005-SP
Investigation of the G4 interactome using human protein microarrays

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We report the detailed analysis of G-quadruplex (G4) interactions with human proteins. Recently, great attention has been paid toward the role of noncanonical DNA structures in genomic regulation. A large body of data supports the significance of such structures for transcription and translation control, recombination, alternative splicing and other processes. G4s have gained particular interest since their cell-cycle progression-dependant formation was quantitatively visualized *in vivo*. The present knowledge about G4-binding proteins is insufficient for elucidating all the diverse mechanisms of G4-mediated regulation of gene expression. To complement the current data on the G4 interactome, we profiled several model oligonucleotides, which represent different types of G4 architectures (parallel and antiparallel; 2- and 4-tetrad, etc.) with microarrays that contain over 9000 immobilized human proteins. The protoarray approach has been previously employed for analyzing sequence-specific DNA-protein binding. This is the first example of a protoarray-based study of conformation-specific binding. We identified several dozens of proteins affine to a particular G4 topology or all G4 topologies, including those reported in the literature and some new G4-recognising proteins. Predictably, the majority of them are related to nucleic acid processing. The new G4-recognising proteins include transcription factors, splicing factors, chromatin remodeling regulators and others. Although evaluation of their specificity for G4s in comparison with other nucleic acid structures is still underway, our preliminary data provide a more integrative view on the G4 interactome. The implications of the newly identified G4-protein interactions for some G4-associated gene expression modulation mechanisms are discussed.

Supported by RSF [14-25-00013].

P02-006-SP
Analysis of XCI mosaicism in the liver from a patient with OTC deficiency

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Ornithine transcarbamylase (OTC) deficiency is an X-linked inborn error of metabolism of the urea cycle associated with severe hyperammonemia and significant morbidity and mortality. *OTC* gene is located on Xp21.1 (subject to X-chromosome inactivation, XCI) and it is expressed mainly in the liver. Clinical symptoms of the carrier females are highly variable both in onset and severity.

Here we report a case of a female patient heterozygous for the mutation c.583G>C exon 6 (p.G195R) in the *OTC* gene. The patient underwent successful liver transplantation at the age of 14 years that was performed because of worsening of metabolic control on dietary therapy with increased frequency of hyperammonemic episodes during puberty. She has not manifested any symptoms of the disease after the transplantation.

We have examined 25 DNA and RNA samples isolated from the liver and 1 DNA sample from the blood to determine the XCI ratios and the expression ratios of the individual alleles. The standard XCI method (HUMARA) was not informative but three of our novel assays published previously were usable and we found skewing of X-inactivation in the liver ranging from 45:55 to 82:18 (mean 70:30). The XCI ratio in blood was 57:43. The results show the intra-organ variation of XCI ratios and a significant difference from the ratio in blood, however the average values were not dissimilar. X-inactivation in the liver samples obtained by biopsy in carriers may vary from the average, further research is needed to decide whether XCI ratio in blood is a useful surrogate.

P02-007-SP
DNA structural transitions upon dehydration of DNA solutions revealed by FTIR spectroscopy

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The DNA secondary structure is very important for genome functioning. B- to A-DNA transition occurs at manifold natural processes such as replication, transcription, DNA-protein interactions, etc. Aqueous environment and metal ions play the key role in DNA secondary structure maintenance. We investigated fast structural transition in DNA using attenuated total reflectance FTIR spectroscopy of drying drop of DNA solution. The main aspects we studied were the velocity of water release, the amount of water in the hydrations shells of DNA, and the manifestations of B- and A-forms in IR spectra.

We observed some indicative alterations in IR spectra of DNA confirming B to A transition during the sample dehydration. The disturbance of native DNA structure in the solution, such as partial denaturation or UVC irradiation, lead to the softening of A-form IR markers and to the increase in the amount of residual water in DNA films. In the native DNA the time of full dehydration process and the amount of the water in the hydration shells depended on the salt concentration in the sample. The effects observed in the experiment can be attributed to the ions penetration into the DNA hydration shell and dismissal of water molecules.

Part of research was performed at the Center for Optical and Laser Materials Research of Research park of St.Petersburg State University.

This work was supported by the Russian Foundation for Basic Research (RFBR 13-03-01192 and 15-08-06876) and SPbSU (11.38.644.2013).

P02-008-SP
PRE-PIK3C2B: a Human PRE with a difference?

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The transcriptional layout determined by gene specific regulators during early development is maintained by cellular memory modules consisting of Polycomb and Trithorax complexes and the polycomb/trithorax response elements (PRE/TRE). We have identified a hPRE/TRE which maps in PIK3C2B (PRE-PIK3C2B; Bengani *et al*. 2013, *PLoS One*) and HOXB3 (CE-HOXB3: Unpublished). PRE-PIK3C2B functions as a PRE in transgenic *Drosophila* and interacts with both *polycomb* and *trithorax* genes. In this background, we have delineated the interaction of PRC2 complex with PRE-PIK3C2B in human cells. Following the screening of different cell lines for the expression level of PIK3C2B and its neighbours, we analysed the probable TRE-like function of PRE-PIK3C2B. Concurrently, we carried out *de novo* search for PRE-PIK3C2B interacting proteins in human cells, using DNA based affinity columns followed by mass spectrometry, which led to the identification of MLL protein among others. Validation of the interaction of both MLL and BRM proteins with PRE-PIK3C2B strongly suggests TRE activity of PRE-PIK3C2B. We have identified the potential targets of PRE-PIK3C2B as MDM4 and PPP1R15B based on their coordinated expression and the effect of knock-down of PRC2 members on them. Furthermore, we have performed the Circular Chromosomal Conformation Capture assay to identify long range interaction of PRE-PIK3C2B.

P02-009
The toxic effect of calcium carbide on DNA damage in banana

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This study was conducted to evaluate the toxic effects of ripening accelerator (CaC_2) on DNA damage in banana. Samples were taken after 0, 4, 8, and 16 days of CaC_2 treatment and DNA was extracted from banana's leaves, peels, and fruits. We were used comet assay to detected DNA damage by measuring the head DNA (H-DNA), tail DNA (T-DNA), and olive tail moment (OTM) of the comets. The levels of DNA damage were assessed and quantified by computer image analysis. The values of H-DNA, T-DNA, and OTM exhibited significant differences from the control at 4, 8 and 16 days of CaC_2 treatment. The DNA damage was induced by CaC_2 treatment in a time-dependent manner.

P02-010
Oxidative stress induces LINE-1 hypomethylation through depletion of S-adenosylmethionine

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Increased oxidative stress and hypomethylation of long-interspersed nuclear element-1 (LINE-1) are demonstrated in patients with bladder cancer. Induction of LINE-1 hypomethylation by reactive oxygen species (ROS) is demonstrated in a bladder cancer cell line with unknown mechanism. We hypothesized that ROS-induced LINE-1 hypomethylation was mediated through the depletion of the methyl donor, S-adenosylmethionine (SAM). Bladder cancer (UM-UC-3 and TCCSUP) and normal human kidney (HK-2) cell lines were used in the experiments. No significant change of cell viability was observed in cells exposed to 20 μM H_2O_2. Intracellular ROS production and protein carbonyl content were significantly increased, but LINE-1 methylation was significantly decreased, in the H_2O_2-exposed cells. LINE-1 hypomethylation was significantly restored by α-tocopheryl acetate (TA), *N*-acetylcysteine (NAC), methionine, SAM, and folic acid. SAM level in H_2O_2-treated cells was significantly decreased while total glutathione was significantly increased. The depleted SAM in H_2O_2-treated cells was restored by NAC, methionine, SAM, and folic acid, whereas, an increased total glutathione was normalized by TA and NAC. Homocysteine (Hcy) was significantly decreased in the H_2O_2-treated cells, which was reinstated by NAC. Conclusion, SAM and Hcy were depleted, but total glutathione was raised, in bladder cancer and normal kidney cells exposed to H_2O_2. These changes were restored by antioxidants (TA and NAC), and one-carbon metabolites (SAM, methionine, and folic acid). The present findings suggest that exposure of cells to ROS activates glutathione synthesis via the transsulfuration pathway leading to deficiency of Hcy to be used for SAM synthesis. This consequently causes SAM depletion, and hence hypomethylation of LINE-1.

P02-011
Cell cycle arrest mediates Rb gene methylation patterns in APL patients

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The retinoblastoma tumor suppressor (Rb) protein binds to the E_2F transcription factors to control gene expression. Overexpression of E_2F will drive quiescent cells to reenter the cell cycle. Rb is a phosphoprotein that regulates cell cycle progression from the G1 to S phase by reversibly inhibiting E_2F-mediated transcription of genes required for S-phase entry. Epigenetic regulation is critical for mammalian development and cellular differentiation and dysregulation of this process causes human developmental diseases and cancer. DNA methylation is the most frequent epigenetic alteration seen in mammalian genomes. In cancer the genome in general is hypermethylated.

In this study, aberrant DNA methylation of target promoters in APL patients that were treated with ATO (As_2O_3) and the NB4 cell line was confirmed using Methylation Specific PCR (MSP) and was linked to changes in the expression of the Rb gene, as assessed by real time RT-PCR. We further addressed the

hypothesis that Rb gene methylation might be of value in the detection of APL.Therefore, it could be suggested that hypermethylation of the Rb promoter is one of the epigenetic factors affecting the progress of sporadic APL carcinogenesis in patients.

P02-012
Epigenetic regulation is a determinant of the cell line specific expression of the UDP glycosyltransferase 3A1 and 3A2 genes

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The UDP glycosyltransferases (UGTs) are a super-family of enzymes involved in the metabolism of xenobiotics and endogenous molecules. Variations in UGT expression have been associated with disease states and inter-individual variation in response to drug therapy. The UGT3A family was discovered and characterised by our laboratory. Upon investigating UGT3A expression in a range of cell lines, we found a large number in which UGT3A1 was not expressed. To determine if epigenetic mechanisms are responsible for this lack of expression, cell lines were treated with the demethylating agent 5-aza-2′-deoxycytidine (5-aza-dC), and the histone deacetylase inhibitor trichostatin A (TSA), and levels of UGT3A mRNA measured. In three cell lines (T47D, MCF-7 and ZR75.1), 5-aza-dC induced UGT3A1 expression from 8.9-fold (ZR75.1, $p = 0.024$) up to 12.2-fold (T47D, $p = 0.007$). TSA alone did not induce UGT3A1 expression, but in some cases, was synergistic with 5-aza-dC. Neither chemical had an effect on UGT3A2 expression in these three cell lines. In MDA-MB-453 cells, only 5-aza-dC with TSA induced UGT3A1 expression (14.5-fold, $p = 0.036$), while UGT3A2 was induced by 5-aza-dC (27.8-fold, $p = 0.002$), and 5-aza-dC with TSA (23.9-fold, $p = 0.012$). Thus our preliminary data indicates that epigenetic mechanisms, particularly DNA methylation, are a determinant of cell line specific expression of UGT3A1 and UGT3A2. Bisulfite sequence analysis will be performed to identify the methylation status of predicted promoter CpG islands. This should provide further novel insights into the epigenetic regulation of the UGT3A family.

P02-013
Epigenetic changes over long-term evolution of breast tumor

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The key feature separating cancer from genetically inherited disorders is that cancer is continuously evolving during the course of the disease. Histone modifications are well-established mediators of transcriptional programs that control the cell evolution process. Thus, an epigenetically perspective is critical for understanding the initiation and progression of cancer.

The present understanding of the relationship of dynamic epigenetic signature and cancer evolution is unclear and fragmented because comprehensive epigenetic data related to long-term evolution of cancer and clinical samples covering the whole life history of a human tumor from initiation to metastasis have been unavailable.

The mouse-based xenograft model has long been used to study the genetics underlying tumourigenesis, tumor progression and metastasis, and has been proven highly successful in human cancer biology.

In our study, we used serial passages of a human cell-derived xenograft tumor in mice to study the profiling of epigenetic dynamics in breast cancer evolution, including tumourigenesis, tumor progression and metastasis.

Our current study initially identified that the study of the dynamics of epigenetic signature is a potentially productive approach that could help us to understand the mechanisms of cancer evolution, especially tumor progression and metastasis. In our further study, we will study predicted transcription factor motifs that are enriched in a subset of dynamic H3K27ac clusters, which might link to functional pathways, and could further assist to understand the linkage between transcriptional regulatory elements and biological process of cancer evolution.

P02-014
Acetylation on the nucleoprotein of influenza A virus

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Posttranslational acetylation of lysine residues is most extensively studied in histones, and is known to play crucial roles in gene expression. This modification is also found in many other proteins and is implicated in a wide range of biological processes. Recently, acetylation was reported to occur on the non-structural protein 1 protein in H3N2 strain of influenza A virus, and this modification selectively suppressed inducible gene expression for antiviral response. Here, we report that nucleoprotein (NP), histone-like viral protein, is acetylated. First, to screen (a) novel viral acetylated protein(s), the A549 cells infected with H1N1 and H3N2 strains of influenza A viruses were homogenized and separated by SDS-PAGE. Western blotting analysis using anti-acetyl-lysine antibody indicated the positive signals around 50 kDa. The combination analysis of immunoprecipitation using anti-NP antibody and western blotting using anti-acetyl-lysine antibody showed that this acetylated protein was NP, and LC-MS/MS analysis confirmed this result. This acetylation was also detected on the NP-expressed cells, suggesting that an endogenous acetyltransferase acetylated NP. Next, we investigated which acetyltransferase acetylates NP, using the recombinant NP and various acetyltransferases. We found that recombinant GCN5, pCAF and HBO1 proteins acetylated NP upon incubation with [^{14}C]-acetyl-CoA. NP in ribonucleoprotein purified from virus particles was also acetylated by GCN5 and pCAF. This enzymatic reaction was inhibited by embelin, which was a known inhibitor of pCAF. These results indicated that pCAF and GCN5 acetylated NP of influenza A virus in infected cells. We will discuss from various aspects in the Congress.

P02-015
The impact of mm-waves on the level of DNA methylation on the plant model

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The rapid increase in the use of mobile phones (MPs), as well as the wide use of mm-waves-therapy and diagnostic equipments in medicine in recent years, has raised the problem of health risks connected with high-frequency electromagnetic fields in our environment. DNA methylation is one of the most studied epigenetic modifications in biological systems.

We explore a plant model for investigating the impact of mm-waves on the cell nuclei and DNA methylation. We have chosen wheat seeds for the model, a plant very well investigated by us. We separate DNA from control samples and samples treated by EHF (Extremely High Frequencies) EMI (Electromagnetic Irradiation) seedlings on the 4th day. After that some of the treated seedlings are grown in soil till to get a harvest (as a second generation). Investigation is carried out in the range of 45–51.8 GHz frequencies of EMI. EHF EMI can bring some changes in the DNA methylation level and aqua environment, which lead to changes in chromatin architecture. The data indicate that 5mC changes depend on EHF EMI frequencies and exposition. After that we have investigated the level of DNA methylation from 4th-day seedlings by growing seeds from the new harvest (second generation). Data obtained in our study shows that the changes in the level of DNA methylation in the first generation of seeds during the plant ontogeny are partially conserved and pass to the seedlings of second generation seeds. So we have shown in the plant model that the changes in biological systems under the influence of EHF EMI have rather epigenetic character and partially pass to the next generation.

P02-016
Investigation of DNA methylation and H4 hyperacetylation dynamics in the 5S rRNA genes family by chromatin immunoprecipitation assay

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Oogenesis is an important event in the formation of female gamete, whose role in development is to transfer genomic information to the next generation. During this process, the gene expression pattern changes concomitant with genome remodeling, while genomic information is stably maintained. Active and silent genes are distinct from one another with respect to their chromatin configurations. Two 5S RNA gene families are transcribed in oocytes, firstly the major oocyte type and secondly the somatic type. In somatic cells, only the somatic 5S RNA genes are transcribed, while the oocyte genes are repressed.

The aim of the study was to investigate the presence of H4 acetylation and DNA methylation of the oocyte and somatic 5S rRNA genes in *Triturus cristatus,* using chromatin immunoprecipitation assay (ChIP and RE-ChIP). Our findings suggest that histone acetylation and DNA methylation are critical mechanisms involved in transcriptional regulation of 5S rRNA genes family.

P02-017
Retinoic acid induced *Hoxa5* is negatively regulated by CTCF in F9 teratocarcinoma cells

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Hox genes are essential for anterior–posterior body patterning at early stage embryonic development. In mammals, 39 *Hox* genes are divided into four clusters called *HoxA*, *B*, *C* and *D* on four different chromosomes. The combinatorial expression of *Hox* plays an important role in the process of mammalian development. However, the precise mechanisms by which signal pathways are stimulated to regulate the expression of *Hox* are not clear. In the previous study, retinoic acid (RA) has been identified as a modulator of cell survival, proliferation and differentiation in the developing embryo. Interestingly, RA induces *Hox* expression in F9 cells. Furthermore, CCCTC-binding factor (CTCF) was reported as a controller of *Hox* expression. Here, we provide relationship of RA, CTCF and *Hox* expression in F9 teratocarcinoma cells. In order to investigate the expression pattern of *Hox* in response to the RA, we performed the RT-PCR in the RA treated F9 cells. The result showed that the anterior *Hoxa* mRNA levels were up-regulated in RA treated F9 cells. However, RA induced *Hoxa5* expression level was decreased in CTCF over-expressed F9 cells. This experiment demonstrated that CTCF negatively associated with up-regulation of *Hoxa5* in response to retinoic acid. In addition, to investigate whether the RA regulates the CTCF binding at *Hoxa5* promoter region, we carried out the chromatin immunoprecipitation assay. When RA was present, CTCF was dissociated from the binding site. These results altogether indicate that RA might regulate the expression of *Hoxa5* by modulating the binding of CTCF at the *Hoxa5* promoter region.

P02-019
DNA methylation contributes to consitutive telomerase gene expression by inhibition of KLF2 binding to a promoter element in huuman T cells

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Telomerase is critical for the life span of normal and tumor cells. The telomerase limiting subunit hTERT (human telomerase reverse transcriptase) is strictly regulated in its transcription and highly expressed even in normal human T lymphocytes when they grow. We previously identified a novel element in the hTERT promoter, which was unmethylated and functioned as a repressor in normal resting T cells, and have recently published that the transcription factor Krüppel-like factor 2 (KLF2) bound to the element, resulting in repression of hTERT transcription. During our studies, we noticed that KLF2 bound to the exogeously introduced unmethylated element but not to the endogenous element in a resting human T leukemic cell line (Kit 225), in which KLF2 was expressed and the endogenous element was DNA-methylated. Demethylation treatment with Zebularine restored KLF2 binding to the endogeous element in Kit 225 cells. We thus assumed that DNA methylation inhibited KLF2 binding, leading to consitutive expression hTERT that is one of the common signatures of tumor cells. Transcriptional repressive mark of histone H3 lysin nine trimethylation (H3K9me3) was associated with KLF2 binding to the promoter, indicating KLF2-mediated epigenetic silencing of the hTERT promoter. Another mark of H3K27me3 was related to cell growth but independent of KLF2 binding. Our findings demonstrate that KLF2 regulates strict transcription of the hTERT gene by direct binding to the promoter in normal T cells, while in tumor cells these important mechnisms are modulated by DNA methylation.

P02-020
CTCF regulates *HOXA10* gene expression in breast cancer cell lines

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CTCF (or CCCTC-binding factor), a ubiquitous 11-zinc finger multifunctional protein has distinct molecular functions such as transcriptional activation, transcriptional repression, or enhancer blocking activity, in a locus-specific manner. Identification of somatic mutations in CTCF in different cancers and its involvement in cellular growth, differentiation and apoptosis point towards its role in cancer progression. The *HOX* genes, a family of transcription factors, not only play a key role in body patterning during embryonic development but also regulate cell cycle, cell adhesion, migration and differentiation in adults. Many *HOX* are found to be dysregulated in different cancers. Among *HOX*, *HOXA10* is an emerging tumor suppressor for its role in activation of p53 and in countering tumorigenesis in breast cancer. *HOXA10* silencing is associated with different cancers but the underlying mechanism is still elusive. We investigated the effect of CTCF on the expression pattern of *HOX* genes and identified *HOXA10* as one of the genes directly regulated by CTCF. Our data defines the putative promoter region of *HOXA10* and CTCF binding site, using dual luciferase reporter assays and ChIP analysis, respectively. Analysis of histone modification reveals that the presence of CTCF is associated with decreased active histone marks H3K4me3 and increased repressive histone marks H3K27me3 on *HOXA10* locus. Based on the evidence in our study, we propose that enrichment of CTCF on the promoter region of *HOXA10* opposes the recruitment of transcription factors and alters the histone modification. Epigenetic silencing of *HOXA10* may contribute towards tumorigenesis by decreasing apoptosis and promoting metastasis.

P02-021
HOXB gene upregulation is associated with tamoxifen-resistance in MCF7 breast cancer cells

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Endocrine therapy, such as tamoxifen and aromatase inhibitors, has been used to treat both early and advanced estrogen receptor α (ER)-positive breast cancer. Despite improvements in treatment, resistance to the current therapeutics can occur in up to one quarter of all cases and thus presents a serious therapeutic challenge. Multiple mechanisms responsible for endocrine resistance have been proposed, however, the molecular events underlying resistance to therapeutic agents are not clearly understood. Therefore, a better understanding of gene expression alterations associated with the resistance would suggest alternative regimens that overcome endocrine resistance. HOX transcription factors have recently been implicated as strong candidates to control cancer progression and metastasis. Previously we have demonstrated *HOX* gene dysregulation in human breast cancer samples as well as breast cancer cell lines. To identify *HOX* genes involved in tamoxifen resistance, here we have generated *in vitro* model of acquired tamoxifen resistance using MCF breast cancer cells (MCF7-TamR) and analyzed expression pattern of *HOX* genes. MCF7-TamR cells were more resistant to tamoxifen in MTT assay and exhibited up-regulation of *HOXB* including *HOXB4*, *HOXB5*, and *HOXB6*. ChIP analysis of histone modification revealed that the activation of midcluster *HOXB* in MCF7-TamR cells is associated with the loss of H3K27me3. Meanwhile, Kaplan-Meier analysis of the overall survival for all patients treated with only endocrine therapy showed the correlation of high *HOXB5*, *HOXB6* expression with a poor response to endocrine therapy. These results suggest a functional role of epigenetically regulated *HOXB* in the development of aquired tamoxifen resistance in breast cancer.

P02-022
O-GlcNAc transferase impact on EZH2-dependent *FOXC1* and *FOXA1* gene expression in breast cancer cells

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EZH2 is involved in transcriptional repression by methylation of histone H3 at lysine 27. EZH2 is associated with progression of breast cancer and its expression correlates with breast cancer aggressiveness. O-GlcNAc transferase (OGT) is an enzyme which catalyzes the addition of *N*-acetylglucosamine moiety to serine/threonine residues of cellular proteins. Recent studies have suggested that OGT may be involved in transcriptional repression caused by Polycomb proteins. In this study we analyzed the effect of OGT and EZH2 on FOXC1 and FOXA1 expression as well as migration and invasion of breast cancer cells. FOXC1 and FOXA1 are expressed in a vast majority of cancers, including breast cancer, in which high expression is associated with a good prognosis. To investigate regulatory effects of EZH2 and OGT on gene expression the breast cancer cells were transfected with OGT and EZH2 siRNA. We found that OGT down-regulation caused decrease in EZH2 protein and H3K27 methylation levels as well as increased level of FOXC1 and FOXA1 expression. Similar increase of the two transcription factors expression was observed in cells with down-regulated expression of EZH2. We examined the binding of EZH2 and OGT at different regions of FOXC1 and FOXA1 promotors. Chromatin immunoprecipitation analysis was performed using EZH2, OGT and H3K27 specific antibodies. Enrichment of the FOXC1 and FOXA1 was quantified using qRT-PCR. We have found that the OGT occupancy coincides with EZH2 and the H3K27me3 at promoters. Our studies suggest the role of O-GlcNAc transferase in EZH2-dependent repression of genes activity in breast cancer cells.

P02-023
Global methylation profiles in lung tissues of silicosis patients

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A previous study has demonstrated that silica mediates the activation of the PI3K/PTEN/AKT/MAPK/AP-1 pathway in human embryo lung fibroblasts (HELFs).The purpose of this study is to identify genome-wide aberrant DNA methylation profiling in lung tissues from silicosis patients. We performed Illumina Human Methylation 450K Beadchip arrays to investigate the methylation alteration in formalin-fixed, paraffin-embedded

(FFPE) lung specimens from six early-stage silicosis patients and four advanced-stage patients. Immunohistochemistry was used to confirm the level of PI3K/PTEN/AKT/MAPK/AP-1 protein in FFPE samples. MS-PCR was used to investigate the methylation of *PTEN* and *c-Jun*. We found 86,770 CpG sites and 79,660 CpG sites significantly differed in methylation status in early-stage and advanced stage compared with normal lung methylation data from GEO, respectively. Analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG) revealed the MAPK signaling pathway was considered significant, indicating that the MAPK pathway was regulated by DNA methylation. The CpG promoter sites of *PTEN* and *c-Jun* were shown to be increased in advanced-stage cases. Early-stage cases showed the positive expression of c-Jun and PTEN protein and negative or mild expression in advanced-stage cases using immunohistochemistry. The *PTEN* promoter was no differentially methylated and the *c-Jun* promoter differed at 12 and 24 h in HELFs detected by MSP-PCR. These results suggested that abnormal DNA methylation on a genome-scale was implicated in silicosis, and *PTEN* promoter hypermethylation might be associated with the decrease of PTEN protein.

P02-024
The association between preeclampsia and K55R polymorphism and methylation levels of the soluble epoxide hydrolase gene (*EPHX2*)

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Preeclampsia is a multifactorial disease characterized by new onset of hypertension and either proteinuria or end-organ dysfunction after 20 weeks of gestation. It is a leading cause of maternal and fetal mortality and morbidity and its etiology is not yet understood. Soluble epoxide hydrolase (sEH) is involved in metabolism of epoxyeicosatrienoic acids (EETs) to their less bioactive corresponding diols. In this study the association between K55R polymorphism and methylation levels of the *EPHX2 promoter region* and preeclampsia was investigated in 520 individuals including 260 preeclamptic patients and 260 healthy pregnant women.

K55R polymorphism and methylation levels of the *EPHX2* gene promoter were determined by real time PCR using double-dye hydrolysis probes and methylation-sensitive high-resolution melting analysis, respectively.

The presence of the K55R polymorphism was significantly higher in cases than controls, and was associated with increased risk of preeclampsia (OR 1.86; 95% CI 1.09–2.63). Methylation levels of the *EPHX2* promoter region in cases were significantly lower than controls. 2.83 times increased preeclampsia risk was observed in pregnant women with *EPHX2* promoter methylation levels of <25%.

In conclusion, hypomethylation of the promoter region of the *EPHX2* gene and K55R polymorphism were associated with significant increased risk of preeclampsia. sEH enzyme may play a role in the pathogenesis of preeclampsia by contributing to reduction of the vasodilatator, anti-hypertensive and anti-inflammatory effects of EETs by rapid degradation of these molecules.

P02-025
Short telomere length and increase expression of its related proteins were associated with the level of benzo(a)pyrene exposure in human bronchial epithelial cell

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Telomeres play a key role in the maintenance of chromosome integrity and stability. Shortened telomere length (TL) is thought to been associated with genomic instability. External stressors from environmental exposures might accelerate shortening of TL and induce expression of its related proteins, e.g. telomeric repeat binding factor 2 (TRF2) and telomerase. Benzo(a)pyrene is the main form of carcinogenic polycyclic aromatic hydrocarbons which could cause lung cancer. In the present study, we investigated the association of TL and its related proteins with benzo(a) pyrene exposure *in vitro*. Human bronchial epithelial cells were treated with three concentrations of benzo(a)pyrene (1, 4 and 16 μmol/l). The TL of genomic DNA, the TRF2 and the telomerase in cells were evaluated, and were detected by real-time polymerase chain reaction, western-blotting and TRAP-ELISA, respectively. The positive control was 4 μg/ml bleomycin. Compared to the 0 μmol/l group, the TL in the 1, 4 μmol/l and positive control groups were significantly shorter ($p < 0.05$), but no significant difference was found between the 16 and 0 μmol/l groups. There was high expression of TRF2 in the 1 μmol/l and positive control groups,but there was low expression in the 16 μmol/l group, as well as the activity of telomerase. The results indicate that short TL is associated with the benzo(a)pyrene exposure and induce the expression of TRF2 and telomerase, and suggest that benzo(a)pyrene might have influence on the genomic instability by acting on telomeres in human bronchial epithelial cell.

P02-026
Functional requirement of zinc finger motif(s) in *Helicobacter pylori* Topoisomerase I function

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Helicobacter pylori, a human pathogen dominating the gastric microbial population, displays differential gene expression during various stages of stomach colonization. Topoisomerases play a crucial role in maintaining DNA superhelicity and thus gene expression. *H. pylori* has only two topoisomerases: DNA gyrase and Topoisomerase I, as opposed to four in most other prokaryotes. Biochemical characterization of Topoisomerase I from *H. pylori* (HpTopoI) revealed that HpTopoI has properties distinct from *Escherichia coli* Topoisomerase I (EcTopoI). While EcTopoI prefers ssDNA over dsDNA, HpTopoI binds both ssDNA and dsDNA with similar affinities. Although both enzymes have comparable DNA-relaxation activity, sequence comparison of HpTopoI with EcTopoI shows that there are four zinc finger motifs (ZFs) at the carboxy terminal domain (CTD) unlike three in EcTopoI. Moreover, *Thermotoga maritima* Topoisomerase I (TmTopoI) has one non-essential ZF and *Mycobacterium tuberculosis* TopoI lacks ZFs. To gain an insight on the role of ZFs in HpTopoI function, we sequentially deleted the ZFs from carboxy terminus. We observed that third and fourth ZFs are dispensable for HpTopoI function whereas deletion of distal three ZFs hampered DNA-relaxation activity with no effect on

DNA-binding. Deletion of all ZFs, however, drastically reduced DNA-binding and abolished DNA-relaxation. Thus, we hypothesize the role of first two ZFs in strand passage activity of HpTopoI. Intriguingly, atomic-spectroscopy data suggested that HpTopoI ZFs failed to co-ordinate with Zn^{2+} as documented for TmTopoI but the observation is in contrast with that of EcTopoI. Our observations would further help to understand the variability in number of ZFs in Topoisomerase I function

P02-027
Harmonious pattern of *HOXA10* gene expression with epigenetic aberration of its regulatory region in eutopic endometrium and ectopic endometriotic lesion of endometriosis patient during the menstrual cycle

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Epigenetic aberrations such as DNA methylation and histone modifications appear to be involved in various diseases such as Endometriosis. Here, we investigate the epigenetic regulation of the *HOXA10* promoter, as a crucial gene responsible for uterine organogenesis, functional endometrial differentiation and endometrial receptivity, and its correlation with mRNA expression of this gene in eutopic, ectopic and normal endometrium, during the menstrual cycle.

For this respect, chromatin immunoprecipitation using anti-MeCP2, H3K9ac, H3K9me2, H3K4me3, H3K27me3 and the real-time PCR technique were performed. Ectopic endometriotic lesions and eutopic endometrium samples were collected through laparoscopy from 36 women with endometriosis. Also, endometrial biopsies were obtained from 20 fertile women as control group. Ethical consent was gained from patients.

Epigenetic data showed a coordinate pattern with expression data. In the secretory phase, in eutopic tissues, reduction of *HOXA10* gene expression was shown along with lower H3K9ac and higher H3K9me2 incorporation on *HOXA10* gene promoter. In return in ectopic endometriotic lesions in the secretory phase, induction of *HOXA10* gene was correlated with higher H3K9ac, H3K27me3 and H3K4me3 incorporation on *HOXA10* gene promoter, all in comparison with the control group. In the proliferative phase, in the ectopic endometrium up-regulation of *HOXA10* coincides with higher incorporation of H3K4me3 on its promoter region compared with the control group.

Since *HOXA10* aberrant expression in the endometrium is involved in endometriosis pathogenesis according to previous data, epigenetics can play an important role in this gene aberrant expression.

P02-028
Epigenetic disruption of CRE transcriptional activity pathway in human spermatogenic disorders

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One of the most interesting epigenetic regulations occurs through compaction of sperm chromatin. Expression of sperm chromatin condensing genes including transition proteins (TNPs) and protamins (PRMs) are activated by a testis specific transcription factor named CREM, a DNA binding regulator necessary for spermatogenesis in mammals. CREM or cAMP response element modulator in combination with its co-factor ACT (activator of cAMP-responsive element modulator) binds to cAMP response elements (CRE) in regulatory regions of chromatin condensing genes and regulates their expression.

Regarding the critical role of compaction of sperm chromatin in male fertility, the potential role of CREM and ACT on regulation of these genes was the aim of the study.

For this reason, consent was obtained from azoospermic men referred to the Royan Institute, according to local ethical approval and then testes tissue samples were collected from three groups including complete maturation arrest, Sertoli cell only syndrome, and hypospermatogenesis (as positive control), based on pathological features. Expression levels of *TNPs*, *PRMs*, *CREM* and *ACT* were evaluated by qRT-PCR. Also, chromatin immunoprecipitation coupled with real-time PCR was performed to evaluate the incorporation of CREM and ACT into the CRE regions of *TNPs* and *PRMs*.

The results showed a significant decrease in expression of *CREM* and *ACT* as well as *TNPs* and *PRMs* genes, in two groups with spermatogenesis failure versus positive control. Also our findings revealed decreased incorporation of CREM and ACT into the CRE regions of the mentioned genes in the two mentioned groups versus control.

This study implies association of epigenetic disruption of the CRE transcriptional activity pathway and male (in)fertility.

P02-029
Evaluation of telomere length and TERRA transcription level in PCOS patients

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Telomeres are essential structures at the ends of all eukaryotic chromosomes. In human, telomeres and their transcripts, TERRA, are composed of hexa nucleotide tandem repeats which play an important role in chromosomes protection. By each cell division telomere length is shortened but this shortening is counteracted by telomerase, a ribonucleoprotein enzyme that can extend the 3′ ends of chromosomes. Some studies showed that increasing both the expression and activity of telomerase is effected by some hormones such as androgens. Based on the Rotterdam consensus, clinical or biochemical hyperandrogenism is one of the three diagnostic criteria for Polycystic Ovarian Syndrome (PCOS). Therefore, we aimed our attention on study of TERRA expression in ovary of PCOS patients parallel with evaluation of telomere length of leukocyte cells (LTL) of the same patients. For this respect, cumulus cells and blood samples were collected from PCOS patients and healthy women with male factor infertility as positive control. TERRA expression and telomere length were measured by quantitative real time PCR. Results showed significant increase of TERRA expression in cumulus cells of PCOS versus control group, but there is no difference in telomere length of blood cells between two groups.

This finding implies a considerable association between alteration of TERRA transcription and PCOS. Also, no difference of

LTL in PCOS versus control group may be interpretable by importance of TL measuring in cumulus cells.

P02-030
Histone deacetylase inhibitor, CG200745, attenuates transcriptional activity of mineralocorticoid receptor through its acetylation

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HDAC inhibitors attenuate fibrosis, hypertrophy, inflammation, and hypertension in several animal models. CG200745 {(E)-2-(Naphthalen-1-yloxymethyl)-oct-2-enedioic acid 1-[(3-dimethylamino-propyl)-amide] 8-hydroxyamide]} (CG) is a novel HDAC inhibitor that is being evaluated in phase II clinical trials for its anticancer effect. However, the antihypertension effect of CG remains unknown. We hypothesized that CG attenuates transcriptional activity of the mineralocorticoid receptor (MR) through its acetylation. Expression of MR target genes was measured by quantitative real-time polymerase chain reaction. Recruitment of MR and RNA polymerase II on target genes was analyzed by chromatin immunoprecipitation. MR acetylation was determined by western blot with an anti-acetyl-lysine antibody after immunoprecipitation with an anti-MR antibody. In f-hMR-HEK293 cells, treatment with CG increased MR acetylation and decreased expression of MR target genes. The down-regulation of target genes coincided with a decrease in the recruitment of MR and RNA polymerase II to specific hormone response element (HRE). These results demonstrate that CG can attenuate transcriptional activity of MR through its acetylation. Our data strongly suggest that CG could be used for a novel antihypertension drug.

P02-031
Aberrant methylation of *CYP19A1* gene in human endometrium throughout the menstrual cycle in endometriosis patients

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Endometriosis is defined as the presence of endometrium-like tissue outside of the uterus. It has been proved that endometriosis is an estrogen-dependent disease and one of the key enzymes in estrogen biosynthesis is CYP19A1. Regarding the important role of epigenetic mechanisms such as DNA methylation in many diseases including endometriosis, epigenetic evaluation of the *CYP19A1* gene was the aim of this study.

For this reason, ectopic endometriotic lesions and eutopic endometrium samples were collected through laparoscopy from 10 women with endometriosis in the proliferative phase. Also endometrial biopsies were obtained from 10 fertile women as a control group. Ethical approval and informed patient consent was gained for the use of tissue samples. Epigenetic analysis of the PII promoter of *CYP19A1* in collected tissues was assayed by Chromatin Immunoprecipitation (ChIP), using antibody against MeCP2, a protein which specifically binds to methylated DNA. Also, quantitative expression analysis of this gene was performed using the real-time PCR technique.

Data showed a harmonious pattern between mRNA expression of *CYP19A1* and methylation level of its promoter region (PII). Expression of *CYP19A1* was significantly higher in ectopic and eutopic endometrium of patients with endometriosis versus control group. The incorporation epigenetic mark of MeCP2 induced in both ectopic and eutpoic groups in comparison with control group.

Our findings imply that higher expression of *CYP19A1* as well as epigenetic alteration of this gene can contribute to the etiology and progression of endometriosis.

Keywords: *CYP19A1* gene, Epigenetics, Endometriosis

P02-032
Protein arginine methyltransferase five regulated encystation of *Acanthamoeba*

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Acanthamoeba is an opportunistic protozoan pathogen that can cause granulomatous encephalitis and keratitis. Under harsh conditions or chemotherapeutic drugs, *Acanthamoeba* transforms to a resistant cyst form, and it remains a significant problem in the treatment of *Acanthamoeba* infections. Recently, several encystation mediating factors were studied to inhibit the encystation of *Acanthamoeba*. However, the regulation mechanisms of encystation mediating factors are still unknown. We were interested in the inhibition of encystation in *Acanthamoeba* by the hypomethylating agent, azacitidine. We first found a protein arginine methyltransferase five from *Acanthamoeba castellanii* (AcPRMT5). AcPRMT5 showed increased expression levels during encystation of *Acanthamoeba*. EGFP fused recombinant protein of AcPRMT5 localized in the nucleus. *Acanthamoeba* transfected with siRNA against AcPRMT5 failed to form mature cysts. The information about this methyltransferase in *Acanthamoeba* may open the way to further study on understanding the encystation mechanism and improving the therapeutic efficacy of amoebicidal drugs by interrupting encystation of *Acanthamoeba*.

P02-033
G4 structures and reparation efficiency: the focus on DNA end processing

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G-quadruplexes (G4s) are noncanonical nucleic acid structures consisting of planar guanine tetrad arrangements. They have diverse functions in the human genome, which include gene expression control and the maintenance of genome stability. G4s are regarded as the hallmarks of DNA fragile sites and recombination hotspots. The role of such structures in DNA reparation is a rather complex and somewhat controversial matter. We discuss here the possible implications of G4 formation in the proximity of DNA single-strand or double-strand breaks (SSB, DSB)

for the efficiency of excision reparation and non-homologous end-joining. Regulation of both processes suggests the interplay between a number of proteins. One key DNA damage response protein is poly-(ADP-ribose) polymerase (PARP-1). In the case of low damage levels (e.g., local SSB/DSB), it promotes DNA repair by recruiting reparation proteins to the sites of damage and activating other response proteins, including the ATM kinase and p53. PARP-1 has been shown to interact with noncanonical DNA structures, in particular G4s, and induce their unwinding. We studied the impact of noncanonical structures, which are recognized and unfolded by PARP-1, on the rate of DNA end phosphorilation using model 50-mer oligonucleotides with 5′-terminal G4 or hairpin motifs and control ss-oligonucleotides. We show that G4/hairpin formation inhibits polynucleotide kinase function and may hamper ligation. We also analyzed binding of several model G4s with PARP and the extent of PARP activation. Collectively, our findings suggest that G4-unwinding by PARP-1 near SSB/DSB sites may be essential for efficient DNA end-processing during the reparation.

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P02-034
High glucose induce overall DNA hypomethylation in Human Endothelial Progenitor Cells

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Endothelial Progenitor Cells play important functions in postnatal vasculogenesis, specifically in the repair and angiogenesis of damaged tissue, however, both *in vitro* studies and studies in diabetic patients have shown that high glucose condition alters their potential for vascular repair in damaged tissue.

In this research work, mononuclear cells from healthy donors were cultured and differentiated to Endothelial Progenitor Cells, evidenced through changes in expression of cell surface markers Oct-4, CD34+ characteristics of immature cell and CD31+ and KDR which are endothelial cell markers. Cell viability assays showed that the Endothelial Progenitor Cells are able to survive in high concentrations of D-glucose (20 mM) without affecting their immunophenotype, however, overall DNA methylation was significantly reduced in stem cells cultured in high glucose media compared to control cells. This phenomenon may be involved in the loss of angiogenic functions of Endothelial Progenitors Cells in diabetic patients. Studies using changes in gene expression could help to explain the alteration in vascular repair mechanisms observed in patients with permanent hyperglycemia.

P02-035
Epigenetic regulation of endothelin-1 expression by histone acetylation/ deacetylation in diabetes

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Hyperglycemia-induced endothelin-1 (ET-1) synthesis plays a major role in the development of diabetic vasculopathies. The mechanisms of ET-1 regulation are not completely elucidated. Histone acetylation is an important epigenetic mechanism that regulates gene transcription. We have aimed at elucidating the role of histone acetylation in mediating ET-1 up-regulation in diabetes. Human umbilical vein endothelial cells EAhy926 (ECs) were exposed to normal (5.5 mM) or high levels of glucose (11–25 mM) in the absence/presence of pharmacological inhibitors of histone acetyltransferase (HAT-CPTH2) or histone deacetylase (HDAC-SAHA). Male C57BL/6J mice were rendered diabetic with streptozotocin. The animals were randomized into three groups: (i) non-diabetic, (ii) diabetic, and (iii) diabetic+SAHA, and were follow-up for 4 weeks. Real-time PCR, ELISA, and Western blot analysis were used to investigate the regulation of histone acetylation, HAT, HDAC, and ET-1. We found that high glucose induced a dose-dependent increase in H3K27ac and promoted a steady up-regulation of HAT1 and HDAC1 protein levels in ECs. Inhibition of HAT or HDAC greatly reduced the up-regulated ET-1 mRNA and protein expression levels in high glucose-exposed ECs. Treatment of diabetic animals with SAHA diminished significantly the aortic mRNA expression and the plasma levels of ET-1, and the mRNA of MCP-1, ICAM-1, and VCAM-1. Histone acetylation plays a role in the regulation of ET-1 in diabetes. HAT/HDAC may represent attractive pharmacological targets to counteract the deleterious effects of ET-1 in diabetes.

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P02-036
The investigation of genetic analysis of genes encoding sperm nuclear proteins with the effects on fertility in infertile men

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Infertility is a progressively increasing disease under control of environmental and genetic conditions. Investigation of the factors that cause infertility may play important role on patients with this disease and stimulate the developments in *in vitro* fertilization techniques.Protamines are specialized proteins which only exist in sperm DNA. These proteins act as packaging of sperm DNA and help to protect it until fertilization. Over 20 single nucleotide polymorphism (SNPs) have been reported for the protamine 1 and 2. The aim of the study is to investigate the association between the changes in three polymorphic regions in *PRM1*, *PRM2* genes and infertility. Also we analyzed the sperm DNA damage in these regions and correlated them with polymorphisms. We made genotype analyses of the *PRM1* and *PRM2* genes in 90 infertile and 70 fertile Turkish men; and three genetic markers (PRM1190C→A, PRM1G197T and PRM2C248T) were analyzed by PCR-RFLP analysis. In 90 patients, DNA damage was studied with the Comet assay. Observations were made at magnification 400× using an epifluorescent microscope. Each image was classified according to nucleus scale and tail length given a value of 0, 1, 2, 3 or 4 (from undamaged class 0 to maximally damaged class 4). Although no correlation was found between PRM1190C→A ($p = 0.40$; $p > 0.05$), PRM1G197T($p = 0.17$; $p > 0.05$) and PRM2C248T ($p = 0.23$; $p > 0.05$) polymorphisms in infertile patients compared to fertile control groups, we found statistical significant association between the 190C→A and C248T SNP regions on PRM1–PRM2 genes respectively and sperm DNA fragmentation in patients

($p < 0.05$). The results of the study suggested that, the protamine polymorphisms which were associated with sperm DNA fragmentation may be important in infertility treatment, recurrent IVF failures, recurrent pregnancy loss, healthy pregnancy and healthy development of the new born.

P02-037
High glucose-induced NADPH oxidase expression and activity is mediated by histone acetylation/deacetylation mechanisms in vascular smooth muscle cells

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High glucose-induced vascular smooth muscle cell (SMC) phenotypic alterations in diabetes are considered to be partially mediated by oxidative stress generated by activated NADPH oxidase (Nox). Still, the molecular mechanisms are not completely defined. In this study we have aimed to investigate the role of histone acetyltransferase (HAT) and histone deacetylase (HDAC) in mediating Nox regulation in diabetes.

Human aortic SMCs were exposed to glucose (5.5–25 mM) in the absence/presence of selective pharmacological inhibitors of HAT (CPTH2) or HDAC (SAHA). Streptozotocin-induced diabetic C57BL/6J mice were used: (i) non-diabetic, (ii) diabetic, (iii) diabetic + CPTH2, and (iv) diabetic + SAHA. The animals were followed up for 4 weeks. Lucigenin-enhanced chemiluminescence, dichlorofluorescein assay, real-time PCR, and Western blot analysis were employed to investigate epigenetic changes and Nox regulation. Exposure of cultured SMCs to increasing concentrations of glucose led to a dose-dependent up-regulation of H3K27ac, HAT1, HDAC1, and HDAC2 protein levels. Pharmacological inhibition of either HAT or HDAC reduced significantly the up-regulated Nox activity, as well as the mRNA and protein expression levels of Nox1, Nox4, and Nox5 subtypes in high glucose-exposed SMCs. Treatment with either CPTH2 or SAHA decreased the Nox1, Nox2, and Nox4 mRNA and protein levels in the aorta of diabetic mice. These data indicate the existence of a new epigenetic mechanism whereby a complex interplay among HAT and HDAC converges to Nox up-regulation in SMCs in diabetes.

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P02-038
Association of Contrin (YBX2) 187T>C and 1095 + 16A>G single nucleotide polymorphisms with male factor infertility

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Environmental factors or infections contribute to infertility to some extent, but genetic factors also play a pivotal role in etiology of male infertility. A number of such SNPs have been reported recently, some of these SNPs are associated with reproductive functions, such as sperm production. Y-box proteins, a highly conserved family expressed in organisms from bacteria to humans, with nucleic acid binding activity by way of the cold-shock domain, function in regulating both transcription and translation, especially in germ cells. As a member of the Y-box proteins, the *YBX2* gene is located on chromosome 17 and encodes a protein, called Contrin, with specific expression in the testis. With the essential role of *YBX2* in male infertility, it was reasonable to postulate that the *YBX2* gene might be associated with human idiopathic infertility. The aim of the study is to investigate the association between the changes in two polymorphic regions in the *YBX2* gene and infertility. We extracted genome DNA, genotyped the polymorphisms of the *YBX2* gene in 90 infertile and 70 fertile Turkish men, and two *YBX2* genetic markers (187T>C and 1095 + 16A>G) were analyzed by PCR-RFLP analysis, compared the genotype frequencies between the case and control groups. We did not find statistical correlation between the *YBX2* gene 187T>C ($p = 0.442$; $p > 0.05$) and 1095 + 16A>G ($p = 0.51$; $p > 0.05$) polymorphisms in infertile patients compared to fertile control groups. Further research in a large group of men is required to clarify the role of Contrin in male fertility and these data might be correlated with sperm motility, sperm count and sperm morphology.

P02-039
Bacterial mutagenicity, oxidative stress and DNA damage caused by airborne particulate matter PM collected from Thessaloniki

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The size segregated airborne particulate matter PM ($d < 0.49$, 0.49–0.95, 0.95–3, >3–7.2 μm) collected in Thessaloniki during winter and summer of 2013 was investigated to evaluate their possible genotoxic potencies. Their mutagenicity was evaluated by Ames test on *Salmonella typhimurium* T100 tester strain, in presence and in absence of the metabolic activation system S9. Most samples increased the number of revertant colonies, probably due to its organic components. PM can cross the membranes through ion channels and/or with the aid of transporter proteins, as well as via endocytosis. After entering into cell, they can directly interact with DNA and oxidative organelles such as mitochondria, redox active proteins, which stimulate ROS production in bacterial cells. PM charged with organic compounds induces reactive oxygen species (ROS) by various chemical reactions, resulting in DNA strand breaks. The ROS created intracellularly in the bacterial strain *E. coli* as result of organic PM was measured based on Nitroblue tetrazolium (NBT) reduction protocol and showed a dose-dependent response. DNA damage inside bacterial cells was monitored using plasmid based reporter gene assay. Blue colonies (due to the hydrolysis of X-gal by β-galactosidase enzyme) reduced significantly for the bacterial cells treated with organic PM. Finally, malondialdehyde (MDA) equivalents (nM), which is an endogenous genotoxic product of enzymatic and oxygen radical-induced lipid peroxidation and a potentially important contributor to DNA damage and mutation, were measured.

P02-040
InMethyl: the design of target-specific primer combinations for PCR amplification and bisulfite sequencing of complete CpG-islands

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The non-specificity of PCR amplification after bisulfite conversion is one of the most common issues in gene methylation studies. In fact, bisulfite treatment leads to the reduction of 4-letter alphabet (ATGC) to 3-letter (ATG, except methylated cytosines) that dramatically increases a possibility of mispriming. The second issue of the promoter region studies is coming from the features of CpG-islands sequence: low-complexity, polyN-rich, and CG-rich. We developed the InMethyl software (available at https://sourceforge.net/projects/inmethyl/), a novel Python-based application enabling the design of target-specific primers for amplification of CpG-islands and other hard-to-study genomic regions. InMethyl uses bowtie high-throughput aligner to identify potential mispriming sites and undesirable PCR products in the bisulfite treated or intact genome. A key feature of InMethyl is the balance between various characteristics that allows to pick up primers in the arduous genomic regions. This balance is based on the calculation of scoring factor including primer pair specificity, nucleotide composition (sequence complexity), thermodynamic features (melting temperature, dimers dG, etc.), presence of CpG-sites and other parameters. Users are intended to customize desired or limit ranges of these values as well as penalties for out-of-bounds values. Moreover, InMethyl software allows to optimize combination of PCR primer pairs to perform the amplification of large genomic regions, e.g. CpG-islands.

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P02-041
Treatment with clinical doses of anti-cancer drug etoposide induce specific chromosomal aberrations in leukocytes *RUNX1* gene

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Secondary leukemia is a severe side effect that affects 2–8% of cancer patients treated with etoposide, a topoisomerase II inhibitor. Genomic aberrations associated with acute myeloid leukemia, such as chromosomal translocation (8;21), are often found in those patients. This suggests that myeloid cells, including bone marrow and peripheral blood cells, are affected more than other cell types by the action of etoposide. However, the exact mechanism behind the generation of that particular type of cancer is largely unknown. Moreover, intravenous administration of etoposide implies that peripheral blood cells are the first cells that come in contact with the drug. Therefore, we hypothesize that treatment with etoposide generates specific genomic aberrations in *RUNX1* intron five in circulating blood cells. To test this hypothesis, we assessed genomic aberrations using inverse genomic polymerase chain reaction on DNA from samples of peripheral blood treated with clinically relevant concentrations of etoposide. Surprinsingly, our results show that genomic aberrations in *RUNX1* gene due to treatment with etoposide are not completely random, suggesting that this may be the first step towards cell transformation.

P02-042
RUNX1 chromosomal break point region harbors a regulatory element modulated by RUNX1

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t(8:21) is one of the most frequent chromosomal translocation found in leukemia. To date all the break points mapped for this translocation are clustered in three specifics regions (called BCRs = break clusters regions) located inside intron 5 of the *RUNX1* gene. Interestingly, two of the BCRs exhibit DNase I hypersensitive sites, which have been widely associated with *cis* regulatory elements. Therefore, we hypothesize that regulatory elements are harbored in the BCRs in intron 5 of the *RUNX1* gene. In order to test this hypothesis, we performed Chromatin Inmunoprecipitation assays to identify regions in intron 5 of the *RUNX1* gene enriched in epigenetics marks characteristic of regulatory elements. Interestingly, our results shown one region enriched in H3 and H4 acetylated and in H3K27ac. These marks have been associated with promoter modules. Indeed, when we cloned this region (PRR = Putative Regulatory Region) in the pGL3 basic reporter vector, we found that it activates expression of the luciferase reporter gene in an orientation dependent manner. Moreover, when we analyze putative transcription factors binding sites, we found one RUNX binding motif. Indeed when we transfected the promoter with RUNX1 expression vector we found that RUNX1 modulates the expression of PRR.

Taken together our results demonstrate the presence of a putative regulatory region inside a chromosomal break point cluster in intron 5 of the *RUNX1 gene,* which is controlled by RUNX1.

P02-043
DNA–caffeine interaction in the presence of Mg^{2+} and Cu^{2+} ions

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Caffeine has been regularly consumed by humans for centuries. The molecular mechanisms of the diversity of its biological effects on the organism are still not completely understood. For example, caffeine inhibits DNA synthesis and repair, affects cell cycle, modifies the impact of ionizing and UV radiation on living cells [1–3], demonstrates both antioxidant and prooxidant effects on DNA depending on the presence of Cu^{2+} ions [4]. We studied DNA interaction with caffeine and Mg^{2+} and Cu^{2+} ions in aqueous solutions and dried films using UV/FTIR absorption spectroscopy and UV circular dichroism.

We have demonstrated that the caffeine–Cu^{2+} binding is stronger compared with Mg^{2+}. The observed changes in DNA spectral parameters indicate that ternary DNA–caffeine–Mg^{2+} complexes appear which involve interactions with the nitrogen bases of DNA. Such complexation is assumed to be responsible for the prooxidant action of the caffeine. The role of water in the considered interactions is discussed.

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P02-044
KLF4 is overexpressed after treatment with 5-ITu, an inhibitor of Haspin, in mouse embryonic stem cells

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Haspin (also known as germ cell-specific gene 2 protein/GSG2 or haploid germ cell-specific nuclear protein kinase) is a serine/threonine kinase. Haspin's best characterized and conserved function to date is the phosphorylation of histone H3 on threonine 3 (PT3-H3), a phosphomark that accumulates specifically at centromeres during prometaphase and is removed after anaphase. By phosphorylating Thr3 of histone H3 Haspin promotes centromeric recruitment of the chromosome passenger complex (CPC) during mitosis. Histone H3 phosphorylation can be blocked by 5-iodotubercidin (5-ITu), a specific Haspin inhibitor. Employing a genome-wide microarray screen, we show here that elimination of the mitotic PT3-H3 mark results in modest overexpression of KLF4 in mouse embryonic stem cells. We further show that the treated cells for 26 h do not develop efficiently into embryoid bodies (EBs) and do not form teratomas in mice.

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P02-045
5-aza-deoxycytidine has differential effects on DNA methylation patterns and histone modifications

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Cancer is a multifactorial disease resulting from the accumulation of different genetic and epigenetic alterations. Epigenetic changes may affect genes that are important for cellular processes which are critical for the balance of cell homeostasis. Among these genes are MLH1 and p16. MLH1 performs important functions in DNA repair and lack of its expression results in the accumulation of errors in the cell′s DNA, while p16 is a tumor suppressor gene and its silencing triggers uncontrolled cell proliferation. A compound that prevents DNA methylation is 5-aza-deoxycytidine (5-aza-dC). Therefore, reverse aberrant DNA methylation of critically important genes, i.e. MLH1 and p16, may result in growth inhibition and even improve survival rates in cancer patients. But not all genes respond to the treatment with the compound; for example in pancreatic cancer NKX2-3 expression is not reactivated after treatment.

The aim of this research is to analyze the DNA methylation status of MLH1, p16 and NKX2-3 gene promoters (bisulfite sequencing), their relationship to histones post-translational modifications (ChIP) and transcriptional expression in HeLa and HL-60 (RT-PCR) cancer cell lines. Our results show that MLH1, p16 and NKX2-3 genes, change their expression in response to treatment in HeLa and HL-60 cells. Interestingly, DNA methylation was found in NKX2-3 gene promoter but not in MLH1 or p16 gene promoters. The effect of 5-aza-dC in different histone marks associated with MLH1, p16 and NKX2-3 gene promoters is very different in each one of them. Therefore, no unique histone modification pattern can be associated with 5-aza-dC treatment

P02-046
The study of DNA methylation based on luminometric methylation assay in *Elodea canadensis* under different salinity

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DNA methylation is one of the epigenetic mechanisms regulating the gene expression in plants' responses to environmental stressors. Salinity is a major environmental factor limiting productivity of plants including water plants in system sea–river. One of the biochemical changes possibly occurring when plants are subjected to stress conditions is the production of reactive oxygen species, which can disrupt normal metabolism through oxidative damage. The epigenetic changes in the water plant genome may be an important alternative regulatory mechanism for sensing and responding to the salt stressor also. In this study, the water plant *Elodea canadensis* were used as a model for investigation and luminometric methylation assay (LUMA) was applied to the ecological study in the first time. The changes in the status of methylation of the CCGG sequence of the nuclear genome of plants exposed to different concentrations of NaCl compared with that of untreated plants were determined by LUMA, complied with methylation sensitive restriction analysis followed by pyrosequencing. This method can be performed without a reference genomic sequence. The effect of salinity on oxidative damage of lipids, the concentrations of photosynthetic pigments and fluorescence F_v/F_m parameters in *Elodea canadensis* was studied and compared with DNA methylation status of the CCGG sequence. These results showed an alteration of DNA methylation in plants as a response to salt stress. It was assumed, the role of epigenetic changes in an adaptation process under salt stressor.

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P02-047
Evaluation of luminometric methylation assay for DNA methylation study in typical hydrobiont clonal population (*Daphnia*) under climate temperature changes

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The enzymatic methylation of nuclear DNA with creation of 5-methylcytosine (5mC) is one of the major mechanisms of epigenetic modification. Changes in the total DNA methylation pattern involving both hypomethylation and hypermethylation, are frequently observed in normal and pathological cellular processes, contributing both to development and differentiation. *Cladocera* genera (e.g. *Daphnia*) have frequently been used as model organisms for ecological genetic research. Potentially limiting factors (temperature) that influence this typical hydrobiont population were investigated on the DNA methylation level *in vivo* and *in situ*. It was the first attempt to quantitatively analyze global genomic DNA methylation in *Daphnia*, using a luminometric technology (LUMA) complicated with methylation-sensitive restriction analysis under temperature changes. Our results showed an alteration in total DNA methylation in *Daphnia* as a response to temperature changes (the effect was dose-dependent) and the role of DNA methylation in environmental sex determination. It was assumed, the role of epigenetic changes in an adaptation process in hydrobiont populations.

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P02-048
Nucleosome occupancy and epigenetic modification in the alternative splicing site of the *Kras* gene in colorectal cancer

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The chromatin structure provides essential functions in eukaryotic transcriptional regulation. Histone post-translational modifications and nucleosome remodeling are coordinate events involved in the control of gene expression. The study of the mechanisms controlling gene expression is critical in the case of human diseases, such as cancer. Colorectal cancer (CRC) is the third most common cancer in the world. Chromosomal instability (CIN) is the most common source of CRC, representing 70% of cases. Tumors originating from CIN are distinguished by the accumulation of mutations in specific oncogenes (e.g. *KRAS*), altering cellular signaling pathways. The *Kras* gene encodes two isoforms generated by alternative splicing of the fourth exon: Kras 4A and 4B, having opposite effects. Kras 4B is an anti-apoptotic isoform, while Kras 4A has a pro-apoptotic action and promotes cell migration. There are relatively few data on the chromatin regulatory mechanisms in individual nucleosomes and the *Kras* gene provides a convenient model to study the nucleosomal positioning and epigenetic modifications in the alternative splicing. The ratio of expression of both isoforms was studied in nine colorectal cancer cell lines by RT-qPCR. The HCT116 (*Kras* mutated) and SW48 (*Kras* wild-type) cell lines were selected for the study of nucleosome occupancy (by MNase protection assays) and histone modifications (by Nu-ChIP), since in these lines the change ratio between isoforms was higher. The results indicate that no significant differences between both lines exist in the position of nucleosomes.

P02-049
Accessory domains of eukaryotic abasic site endonucleases and thymine-DNA glycosylases: Their evolution and possible role in epigenetic regulation

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DNA repair has evolved to protect genetic material from ongoing damage and is present in some form in all cellular organisms. Of its several pathways, base excision repair (BER) removes small non-bulky lesions from DNA; modified bases of this kind are the most abundant source of mutations and are also involved in epigenetic regulation. Available structural data show that many eukaryotic BER enzymes consist of a well-resolved core domain (s) responsible for catalysis and/or DNA binding, and disordered terminal tails. Interestingly, bacterial counterparts of eukaryotic BER enzymes are most often compact, with little extra sequence outside the core domain. The functions of these tails in eukaryotic BER remain mostly enigmatic. We have analyzed available sequences of the terminal tails and core domains of thymine-DNA glycosylase (TDG), an enzyme involved in active removal of epigenetic 5-methylcytosine marks, and exonuclease III-like abasic site endonuclease (APEX1), which, in addition to its role in DNA repair, reactivates oxidized transcription factors. Hidden pattern search identified that the tails in both enzyme families, in contrast to core domains, mostly consist of diverged repeated elements. Disorder analysis revealed that the tails are indeed likely to possess a more flexible structure. The extension of TDG proteins and APEX1 proteins from vertebrates were evolutionarily conserved, indicating that their coupling with the core domain is functional. In contrast, in arthropodal APEX1 homologs, first described from *Drosophila* as hybrid repair/recombination proteins (Rrp), the recombination module is not conserved even within Diptera, suggesting that the repair and recombination functions are generally uncoupled.

P02-050
Understanding the role of CFP1 in regulating chromatin modification and transcription at CpG island associated genes

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The interpretation of genomic information by the transcriptional network is essential for normal gene expression and cell-type specification. To achieve this, the transcription factors that regulate gene expression recognize DNA motifs within gene promoters and other regulatory elements. CpG islands (CGIs) are central and conserved features of most vertebrate gene promoters. However, despite their initial identification more than 30 years ago, we still understand very little about how CpG islands contribute to gene regulation. Zinc-finger CxxC (ZF-CxxC) domain-containing proteins bind to non-methylated CpG dinucleotides and associate with chromatin modifying activities targeting them to CGIs. Therefore, binding of ZF-CxxC proteins at CGI promoters may dictate essential chromatin states and contribute to gene regulation.

The ZF-CxxC protein CFP1 is a developmentally essential component of the SET1 H3K4 methyltransferase complex. However, its regulatory function at CGIs still remains poorly understood. By exploiting a novel mouse embryonic stem cell line in which CFP1 can be rapidly deleted, I will investigate (i) how

acute loss of CFP1 affects H3K4me3 deposition and SET1 recruitment to CGIs. This will provide the basis to examine (ii) how loss of CFP1 affects gene expression and occupancy of the core transcriptional machinery. Finally, (iii) to understand the underlying molecular mechanisms for CFP1 function, conditional cell lines will be engineered to express versions of CFP1 that ablate CGI targeting and SET1 interaction. Together this systematic analysis of CFP1 function at CGIs will begin to elucidate the mechanisms by which CGI elements contribute to the chromatin state and regulation of gene promoters.

P02-051
Controlling the methylation writer: regulation of Dnmt3a DNA methyltransferase by oligomerisation

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Almost all our cells carry the same genetic information encoded in the DNA, yet they follow different developmental pathways and differentiate into more than 200 cell types building the human body. The cellular fate is determined by epigenetic regulatory mechanisms, the most prominent of which is DNA methylation. Methylation of cytosine residues in gene promoters is a strong repressing signal, leading to down-regulation of genes' activity. Thereby, specific methylation patterns present in different cell types regulate their transcriptional programs, leading to cell specialization. The patterns of DNA methylation are set during early embryogenesis, but are extensively altered in cancer. The Dnmt3a and Dnmt3b DNA methyltransferases play a central role in these processes, however their regulation in cells is largely unknown. We have recently discovered an unprecedented mechanism that regulates the physiological function of Dnmt3a. Using a combination of biochemical and cellular approaches, we show that complex multimerization behavior of Dnmt3a, which includes formation of protein–DNA filaments and self-oligomerisation, is relevant for all aspects of the enzyme biology, including cellular localization and catalytic activity.

P02-052
Expanding the substrate scope of the Jumonji C histone demethylases

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Lysine methylation is a ubiquitous mark, which in histones can signify both gene activation and repression, depending on the degree (mono/di/tri) and position of lysine methylation on the histone tail. Methylation is actively regulated by lysine methytransferases, which deposit the marks, and lysine histone demethylases (KDMs), which remove them. Beyond histone methylation there are a wealth of other methylated proteins for which the regulatory mechanisms are currently less clear. Understanding the regulation of methylation is of interest both with respect to its links to diseases, including inflammatory diseases and cancer, and the role of methylation in transcriptional regulation.

The Jumonji C (JmjC) demethylases constitute the largest class of KDMs. We recently demonstrated that the substrate scope of these KDMs extends beyond N^{ε}-methyl groups (e.g. to N^{ε}-ethyl- and N^{ε}-isopropyl groups). Following on from this we have profiled the substrate selectivity of representative members of each KDM subfamily, using a combination of mass spectrometry, NMR, biochemical assays and novel small molecule epigenetic "probes". Although the results largely confirmed literature assignments, our study reveals that, at least *in vitro*, the KDMs can catalyse demethylation of peptide substrates other than their currently characterised histone substrates, such as demethylation of histone 3 lysine 27 by the KDM4 subfamily. This indicates that the JmjC KDMs are likely much more promiscuous than previously thought. Given the importance of KDMs in development and disease a full understanding of the biological functions and substrate scope of these proteins will be important for the development of future therapeutics.

P02-053
Genetic evidence of the role of PCNA post-translational modifications in DNA damage tolerance

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DNA damage bypass mechanisms are required to avoid replication fork collapse. Post-translational modification of Proliferating Cell Nuclear Antigen (PCNA) plays a key role in these processes by recruiting essential proteins implicated in DNA damage bypass. PCNA can be monoubiquitylated at K164 by the Rad6-Rad18 ubiquitin ligase complex. Through this modification, PCNA can interact with low-fidelity Y-family polymerases to promote translesion synthesis. Monoubiquitylated PCNA can be polyubiquitylated by a further ubiquitin-conjugating complex to promote template switching, an error free process.

In our study we used a $PCNA^{K164R}$ mutant DT40 chicken B lymphoblastoma cell line, which is hypersensitive to DNA damaging agents such as methyl methanesulfonate (MMS) or cisplatin due to the fact that PCNA cannot be ubiquitylated. Indeed, by expressing a PCNA rescue construct we were able to restore the sensitivity similar to the wild type. PCNA-ubiquitin fusion proteins have been reported to mimic the monoubiquitylated PCNA, therefore we created and stably expressed (in the $PCNA^{K164R}$ cell line) two further constructs, $PCNA^{K164R}$-ubiquitin and $PCNA^{K164R}$-$ubiquitin^{K63R}$ fusions. Because of the ubiquitin K63R mutation, ubiquitin could not be polyubiquitylated, which facilitates the investigation of the effects of this post-translation modification. We investigated MMS and cisplatin sensitivity and also determined the influence of PCNA ubiquitylation on polymerase recruitment by measuring T–T cyclobutane pyrimidine dimer (CPD) bypass. Cell lines expressing the fusion displayed resulted similar sensitivity and rate of translesion synthesis to the wild type, suggesting that the polyubiquitylation of PCNA is not necessary to protect cells from replication-stalling DNA damage.

P02-054
Role of AhR-regulated Alu transposon in insulation and chromatin structure of pluripotency genes OCT4 and NANOG

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Local chromatin structure controls and coordinates the activation of expression domains in eukaryotic genomes. This organization

and stability can be modulated by transposable elements, likely because of their ability to bind specific transcription factors. By using enhancer blocking assays (EBAs) we report that, in the human genome, three Alu(s) retrotransposon located in the flanking region of pluripotency genes NANOG and OCT4 have potent insulation activity conferred by binding the transcription factor dioxin receptor (AhR) to consensus elements present in the transposon sequence. The insulation activity of these xs-Alu (s) elements involve direct AhR binding *in vitro* as determined by chromatin immunoprecipitation (ChIP) assays. Interestingly, these Alu(s) are present in most of the stemness-relevant genes, including OCT4 (x36s and x14s) and NANOG (x45s and x14s). Insulators can exert their regulatory activity by modifying chromatin compaction. The analyses of histone marks revealed different patterns of me3H3K4, me3H3K9 and meH3K27 upon AhR expression. Notably, these epigenetic patterns changed during differentiation. At the genomic level, insulation can also induce long-range chromatin reorganization. We have used chromosome conformation capture (3C) to address long range physical interactions between the Alu(s) flanking OCT4 and NANOG. The results obtained showed that the interaction frequency changes drastically with the RA differentiation, suggesting the formation of a new chromatin loop. Interestingly, such loop was not detected by AhR knock-down, suggesting the involvement of AhR. We propose that AhR-regulated Alu(s) elements can represent evolutionary conserved genome-wide insulators. These retrotransposon may control developmental, oncogenic or toxicological-dependent processes via physical heterochromatin modifications.

P02-055
Drosophila Opbp protein regulates divergently-paired genes with different expression levels

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Divergently-paired genes (DPGs) constitute a large fraction of *D. melanogaster* genes (32%), which are transcribed in opposite directions from TSSs located <1 kbp apart. Although genes of this class in pairs usually show similar expression profiles, there is a substantial part of DPGs that are regulated independently within a pair. Promoters of DPGs are often enriched with insulator proteins (CP190, BEAF-32, dCTCF) that potentially act as boundary elements to ensure differential expression of DPGs.

Using yeast two-hybrid assay and GST pull-down, we identified a transcription factor Opbp (Optix-binding protein), which is capable of interacting with the insulator protein CP190. Opbp can bind DNA in a sequence-specific manner via zinc-fingers domain in EMSA experiments. ChIP-seq analysis revealed that Opbp preferentially occupies promoters of DPGs, where one gene in pair is a highly expressed ribosomal gene and another one is a tissue-specific gene. Results of Opbp-knockdown demonstrate that Opbp is a likely regulator of the differential expression of these genes.

We are interested to further explore the mechanisms of DPG differential expression regulation and a role of Opbp in this process.

P02-056
In vivo structural mapping of FACT-histone interactions using genetically encoded crosslinkers

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Chromatin is a highly dynamic nucleoprotein structure that stores the genetic information in eukaryotes. Histones are the essential chromatin proteins that form the core octamers around which DNA is wound and compacted within the nucleus. Chaperones act as guards and guides of histones and are implicated in the regulation of transcription, replication and DNA repair. The essential histone chaperone complex FACT (*fa*cilitates *c*hromatin *t*ranscription), composed of three subunits in *Saccharomyces cerevisiae*, is conserved across eukaryotes and only individual domains have been structurally characterized. In order to understand how the complex achieves its various functions, it is crucial to structurally characterize the interplay between FACT and its binding partners *in vivo*. Therefore, we apply an innovative site-specific structural approach to map the interaction surface of FACT using a genetically encoded UV-inducible crosslinker, 4-Benzoyl-L-phenylalanine (pBPA) in budding yeast. Here, we present this highly reproducible crosslinking approach at nearly two hundred positions of the yFACT complex at single amino acid resolution. We found that the acidic C-terminal domain (CTD) of the Pob3 subunit interacts with the histones H2A-H2B in a defined manner *in vivo*. Further analyses revealed a nuclear localization signal at the C-terminus of the Pob3-CTD that is required for nuclear import. Deletion of acidic residues from the CTD creates a hydroxyurea sensitive phenotype in yeast, suggesting a specific role of this domain in DNA replication. A synthetic genetic array (SGA) analysis corroborates this finding by a negative genetic interaction of the deletion mutant with the DNA replication helicase complex subunit Mcm7.

Gen Ex S2, Turning Signals into Messages – the Complexity of Gene Regulation

P03-003-SP
A PARP1-ERK2 synergism is required for the induction of synaptic plasticity

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Unexpectedly, a post-translational modification of DNA-binding proteins by polyADPribosylation, which initiates the cell response to DNA damage, is also required for longterm memory acquisition in a variety of learning paradigms. Our findings disclose a mechanism underlying this phenomenon. We found that a PARP1-Erk2 synergism was required for the induction of synaptic plasticity in stimulated cerebral neurons. Electrical stimulations, inducing long-term potentiation (LTP) in cerebral synapses, caused a sequence of events initiated by PARP1 binding to phosphorylated Erk2 via docking sites of Erk in the catalytic domain of PARP1. The resulting PARP1 activation was required for the recruitment of PARP1-bound phosphorylated Erk2 to promoters of immediate early genes (IEG) that are implicated in synaptic plasticity and long-term memory. PARP1 inhibition, silencing or genetic deletion, abrogated both IEG expression and LTP induction. IEG expression was similarly abrogated by a predominate binding of PARP1 to single-strand DNA breaks, occluding its Erk docking sites. These findings dis-

close a mechanism inducing synaptic plasticity, which is impaired by an incomplete DNA repair, frequently occurring in senescence.

P03-004-SP
DNA damage response: Mechanism of transcriptional regulation by p53 leading to cell cycle arrest

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After DNA damage, a cell requires time to repair its DNA before the next cycle of cell division. One way to halt cell division is to stabilize the p53 tumor suppressor. p53 is a central transcription factor able to induce apoptosis or cell cycle arrest. Upon activation, p53 downregulates transcription of numerous genes, e.g. *PLK1, PLK4, Cyclin A, Cyclin B, CDC25C, KIF23, BUB1* and *Survivin*, thereby controlling cell cycle progression. We provide evidence that the mechanism for transcriptional repression of these genes is indirect. p53 does not contact the promoters of the repressed target genes directly. Instead, p53 directly activates transcription of $p21^{WAF1/CIP1/CDKN1A}$. The p21 protein then inhibits Cyclin/CDK complexes leading to hypophosphorylated RB-related protein p130. Hypophosphorylation of p130 results in formation of the DREAM complex consisting of E2F4/DP1/p130 and MuvB core proteins. The DREAM complex contacts CDE/CHR repressor elements in the promoters of genes repressed by p53, finally leading to downregulation of these genes. We present a genome-wide meta-analysis of several data sets on mRNA expression after p53 induction and chromatin immunoprecipitation of DREAM components. This analysis leads to the identification of more than 200 cell cycle genes repressed by p53 through this mechanism. These data suggest a general role for the p53-p21-CDK-DREAM/pRB-E2F/CDE/CHR signaling pathway in regulating transcription of cell cycle genes. In summary, we provide a mechanism for transcriptional control by p53 leading to cell cycle arrest in response to DNA damage.

P03-006-SP
Programmed translation arrest controlling antibiotic resistance genes relies on the sequence context of the nascent peptide stalling domain

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Expression of macrolide resistance genes in bacteria is controlled by drug dependent translation arrest in the regulatory leader ORF. The stalled ribosome prompts an mRNA conformational switch that allows activation of the resistance. Translation arrest requires the presence of the "stalling domain" in the leader peptide, which in many regulatory peptides conforms to the consensus Arg/Lys-X-Arg/Lys (+X+). However, in some instances the mere presence of the +X+ motif is insufficient to induce macrolide-dependent translation arrest. Which other factors are required for the +X+ sequence to serve as an efficient stalling signal?

Using the ErmDL (MTHSMRLR) regulatory peptide as a model, we found out that its N-terminal sequence preceding the arrest motif affects the efficiency of stalling. Frame-shift mutations, which changed the N-terminal sequence while leaving the RLR stalling domain intact, dramatically diminished drug-induced ribosome stalling. Furthermore, the wild type N-terminal MTHSM sequence promotes moderate stalling even when the RLR motif is partially disrupted. We concluded that both, the stalling domain and the sequence context preceding it, are necessary for efficient antibiotic-dependent translation arrest. We propose a model where the sequence of the nascent peptide in the drug-obstructed ribosomal tunnel determines the placement of the reactive amino acid residues at the peptidyl transferase center, thereby determining the efficiency of peptide bond formation and continuation or arrest of protein synthesis. The regulatory peptides likely evolved to contain not only the "stalling sequence" but also the "favorable" N-terminal context in order to efficiently cause the translation arrest necessary to activate resistance genes.

P03-007
Unraveling the members of a DNA-binding complex of a bacterial haloacid operon

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Haloacetates are structurally simple organic acids that can be found in our environment. They are generated incidentally during disinfection of water and are mutagenic. A soil bacterium, *Burkholderia caribensis* strain MBA4, produces an inducible dehalogenase that transforms these compounds to utilizable substrates. Previous studies using electrophoretic mobility shift assay has identified at least two retardation complexes that bind to the upstream non-coding region of the dehalogenase gene. These complexes were detected in extracts prepared from glycolate-grown cells and not in haloacid-grown cultures. In this presentation, oligonucleotide-conjugated metal beads were used to capture these DNA-binding proteins. Candidates were subjected to tandem mass spectrometry assays and the gene encoding for a candidate was disrupted. When extracts prepared from this mutant was used, the formation of one of the retardation complexes was disabled. This candidate protein was heterologously expressed, purified and used for bandshift assay. Two retardation complexes, different from those identified in wildtype, were detected. When this purified recombinant protein was added to the extract of the mutant and used in bandshift assay, the missing complex was re-established. This candidate, tentatively named as Peg8620 has been identified as a member of TetR family of regulators. This suggested that the retardation complex being considered is formed by the interaction of Peg8620 and another protein.

P03-009
Expression of genes, encoding enzymes of auxin biosynthesis in *Arabidopsis* plants with altered ubiquitin signaling

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AXR1 encodes an E1-like protein which interacts with E1 C-terminal related 1(ECR1) protein to activate the RELATED TO UBIQUITIN protein (RUB) for conjugation to cullins. Activity of AXR1 leads to neddylation of SCFTIR1-ligase by RUB, which is required for further poly-ubiquitination of AUX/IAAs for proteasome-dependent degradation and thus it is supposed to be a key player in auxin signal perception and transduction. Proteolysis of Aux/IAA transcriptional repressors releases auxin

response factors and initiates auxin-inducible gene transcription. We performed the complex analysis of growth, development, endogenous free IAA level and expression of genes, encoding enzymes of auxin biosynthesis (*TAA1* and *YUC* family), conjugation (*GH3* family) and transporters (*AUX1*, *PIN* and *ABCB* family) in shoots and roots of wild type and *axr1–3* mutants of *Arabidopsis thaliana* seedlings, grown on medium without or with addition of exogenous auxin (natural auxin IAA – indole-3-acetic acid). Advanced high-density and low volume microfluidic quantitative PCR platform Fluidigm and methods of multivariant statistics were implicated for gene expression analysis. Revealed alteration in free endogenous IAA concentration in shoots and roots of auxin resistant *axr1–3* mutant correlated with modulation of *TAA1* and number of *YUC*s genes expression. Exogenous IAA treatment led to free IAA accumulation only in shoots of wt plants. Changes in IAA degradation are suspected.

P03-011
The aetiology of genetic, acquired and sporadic prion diseases

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Based on the protein X or the chaperone protein hypothesis which enables the reaction of conversion from PrPC to PrPSc and the gene interaction of this protein with prion protein gene (*PRNP* gene) we propose the genotypes involved and give possible explanations of genetic, sporadic and acquired forms of prion diseases. In this context, the genetic forms of prion diseases like fCJD, fGSS and FFi have A–B genotypes. In these genotypes, the *PRNP* gene and Xchap have been subject to a dominant mutation. In the sporadic forms of prion diseases like sCJD and sFI, the Xchap gene has been subject to dominant mutation but in the acquired forms of prion diseases (kuru, iCJD and VCJD) the *PRNP* gene has been subject to dominant mutation.

Keywords: Prion diseases, PRNP, Protein X chaperone, gene interaction, gene module

P03-012
Indirect regulation of Claudin 6 gene expression by triiodothyronine in an estrogen-positive breast cancer cell line

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Claudin 6 (*CLDN6*), a protein member of the Claudin family, is involved in the formation of the GAP junction. Although some researchers reported the expression of *CLDN6* in breast cancer cell lines, its role in carcinogenesis remains controversial. Our group identified a triiodothyronine (T3) action on different genes of breast cancer cell lines through the microarray approach, including *CLDN6*. In this study we are looking for nuclear and extra-nuclear action of T3 on *CLDN6* gene expression in MCF-7, an estrogen-receptor-positive breast cancer cell line. Cells were treated with 10–8 M T3 for 10, 30, 60 and 240 min, separately. Treatments were organized with or without Cycloheximide (CHX – 50 μM), a protein synthesis inhibitor, and with or without Fulvestrant (ICI – 1 μM), an estrogen receptor antagonist. T3 decreases gene expression of *CLDN6* in MCF-7 cell lines during 10 and 30 min, but this action was converted to the control level when T3 was associated with CHX, indicating that prior protein synthesis is required in this process initially. Addition of ICI to T3 for 10 and 30 min augmented gene expression in relation to those cells treated only with T3, but after 60 min, ICI plus T3 decreased *CLDN6* gene expression when compared with T3 itself. Therefore, T3 regulation of *CLDN6* gene expression is dependent on prior protein synthesis and estrogen receptor. The alteration in *CLDN6* gene expression is dependent on the time elapsed after the treatment, indicating that metabolism changes could be altered after 30 min in T3-dependent *CLDN6* gene expression.

P03-013
Knockin' on pHeaven's Door: A fast and reliable high-throughput-compatible zero-background cloning procedure

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Cloning of DNA fragments derived from PCR reactions is a routine technique in molecular biology. We created a pair of vectors allowing simple and efficient molecular cloning of any gene of interest with minimal consumption of time, labor and material. By combining available molecular tools we developed a new method we call pHeaven's Door cloning.

The method of Golden Gate Cloning (Invitrogen) can be adapted and optimized for PCR products, thus saving time and material in the process of primer design and preparation of fragment and destination vector to be ligated. Based on type IIs restriction enzymes, which cut dsDNA outside their recognition site, our method allows the use of a single restriction enzyme for all DNA molecules to be inserted into a given vector. Additionally, this system allows simultaneous restriction and ligation in one tube.

By generating a pair of vectors with different procaryotic selection markers, possible background from a template vector in the PCR can be avoided without the need of laborious agarose gel extraction procedures to separate PCR product and template vector. Finally, through the use of a ccdb suicide gene, which is replaced by the DNA fragment of interest during the restriction/ligation reaction, background from undigested and religated vectors is eliminated. pHD cloning thus enables the production of recombinant proteins in mammalian cells starting from the cDNA within 3 days. This system is applicable for standard molecular cloning, high-throughput cloning and generation of fusion protein libraries as well as for more complex gene assembly purposes.

P03-014
Thrombin-induced IL-8/CXCL8 expression is mediated by ORMDL3, ATF6, and AP-1 signaling pathways in human lung epithelial cells

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Airway inflammation plays a major role in the pathophysiology of lung inflammatory diseases such as asthma. Thrombin is a well-known coagulation factor generated during vascular injury and plays an important role in airway inflammation. Orosomucoid 1-like 3 (ORMDL3) was strongly linked with asthma and its expression could be induced by allergen in lung epithelial cells. In

this study, we examined the role of ORMDL3, ATF6, and AP-1 in thrombin-induced IL-8/CXCL8 expression in human lung epithelial cells. Thrombin-induced IL-8/CXCL8 release was inhibited by small interfering RNA of ORMDL3 (ORMDL3 siRNA) and ATF6 siRNA. Treatment of cells with thrombin caused increase in expression of ORMDL3 and ATF6, which were inhibited by ORMDL3 siRNA. Thrombin caused an increase in ATF6 translocation in to nucleus in a time-dependent manner. Moreover, thrombin-induced IL-8/CXCL8-luciferase activity was inhibited by cells transfected with AP-1 mutation of IL-8 construct. Thrombin-induced increase in IL-8/CXCL8 release was attenuated by c-Jun siRNA and curcumin (AP-1 inhibitor). Stimulation of cells with thrombin caused an increase in c-Jun phosphorylation and JNK phosphorylation in time-dependent manners. We also found that treatment of cells with thrombin induced ATF6 and c-Jun complex formation. Taken together, these results suggest that the ORMDL3, ATF6, and AP-1 signaling pathways play important roles in thrombin-induced IL-8 expression and release in human lung epithelial cells.

P03-015
Epidermal growth factor receptor promotes prostate cancer bone metastasis through down-regulation of miR-1 and activation of TWIST1

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Dysregulation of the epidermal growth factor receptor (EGFR) signaling axis enhances bone metastases of many solid cancers. However the orderly sequence of events downstream of altered EGFR signaling that enhances metastatic prostate cancer is unclear. We have previously shown that miR-1 is a tumor suppressor in prostate cells, and its expression is correlated with reduced metastatic potential. Here we demonstrated a role for the EGFR in tumor progression through EGFR translocation that regulates primary miR-1-1 transcription, which directly targets TWIST1. Our results propose a model whereby nuclear EGFR acts as a transcriptional repressor to constrain the tumor suppressor role of miR-1 and sustain oncogenic activation of TWIST1 leading to accelerated bone metastasis. We identified a significant correlation between decreased miR-1 levels and enhanced expressions of activated EGFR and TWIST1 in a cohort of human prostate cancer specimens and in datasets. Our data support the existence of an EGFR, miR-1, and TWIST1 regulatory network where the induction of TWIST1 results in increased bone metastasis and is linked to dysregulation of the EGFR signaling pathway through inactivation of miRs.

P03-016
Wnt/β-catenin signaling pathway does not regulate *c-myc* gene expression in 42GPA9 (mouse adult Sertoli) cell line

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Sertoli cells are the nutritional and metabolic support and control of germ cell proliferation and differentiation. Wnt/β-catenin signaling is important for the development of the seminiferous epithelium during embryonic age, however, after birth this pathway is downregulated. Transgenic mice where β-catenin is constantly activated have altered spermatogenesis among other adverse effects. Cx43 is the most abundant protein within gap junctions which are essential for Sertoli cell functionality. The gene of this protein is a Wnt/β-catenin target in rat cardiomiocytes. c-Myc is a transcription factor involved in transcription of genes necessary for the maintenance of the pluripotent state of stem and tumorigenic cells. The gene of this protein is a target of Wnt/β-catenin pathway in embryonic and tumorigenic cells. In the transgenic mouse models and in studies with human prostate cancer cells, c-Myc was upregulated possibly affecting Sertoli cell functionality. In this study Sertoli cells (42GPA9) responded to LiCl or Wnt3a treatments, accumulating β-catenin within the nucleus, activating Axin2 transcription determined by RT-qPCR. We also evaluated the upregulation of these putative Wnt canonical pathway genes, *cx43* and *c-myc*, by luciferase assays in HEK293 where both promoters showed luciferase activity. Stimulated 42GPA9 showed a two-fold increase of *cx43* mRNA, while *c-myc* mRNA was not affected. By ChIP analysis we determined epigenetic marks possibly involved in this process. In both promoters we found H3K4me3 mark but not b-catenin in *c-myc* promoter. These results suggest that *c-myc* is not a direct target of Wnt/b-catenin pathway compared to *cx43* in this cell line.

P03-017
Structure and function of heme-responsive transcriptional regulator HrtR

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In a lactic acid bacterium *Lactococcus lactis*, a transcriptional regulator HrtR plays a crucial role for maintaining heme homeostasis by regulating the expression of a heme exporter system HrtBA in response to increasing intracellular heme concentrations. Heme molecule acts as the effector of HrtR to regulate its DNA binding activity. Only apo-HrtR binds to the target DNA, by which the expression of HrtBA is repressed. HrtR senses increasing intracellular heme concentrations to form heme-bound HrtR (holo-HrtR) that is not able to bind to the target DNA, which causes derepression of *hrtBA*. In this work, we have determined the crystal structures of apo-HrtR/DNA complex, DNA-free apo-HrtR, and holo-HrtR for wild type HrtR. The structures of H149A-HrtR and H72A-HrtR have also been determined. The molecular mechanisms of heme sensing and heme-responsive transcriptional regulation of HrtR are elucidated based on these structures.

HrtR forms a homo-dimer in the both of apo- and holo-forms and each monomer consists of the N-terminal DNA-binding and C-terminal heme-sensing domains. Heme is accommodated in the hydrophobic cavity with His72 and His149 as the axial ligands. A coil-to-helix transition of the first alpha helix, on which His72 is located, in the C-terminal domain is induced upon heme binding, which results in dissociation of HrtR from the target DNA. Conformational changes induced by this coil-to-helix transition cause increasing the distance between the DNA-recognition helices in HrtR dimer, by which the DNA-recognition helices are not fitted anymore to the consecutive major groove of the target DNA.

P03-018
Protein kinase CK2 mediates cross talk between auxin- and salicylic acid- signaling pathways in *Arabidopsis*

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Arabidopsis thaliana plants overexpressing an inactive catalytic subunit of protein kinase CK2 (CK2mut plants) are impaired severely in root development and auxin polar transport, and exhibit transcriptional misregulation of auxin-efflux transporters. Moreover, CK2mut roots accumulate high levels of salicylic acid (SA) and the gene that encodes isochorismate synthase (*SID2*) is overexpressed, strongly suggesting that CK2 activity is required for SA biosynthesis via the shikimate pathway. We also show that SA activates transcription of CK2-encoding genes and, thus, SA and CK2 appear to be part of an autoregulatory feed-back loop to fine-tune each other's activities. *Arabidopsis* plants incubated with exogenous SA and *Arabidopsis cpr* mutants (having high constitutive SA levels) show the same root phenotypes as CK2mut plants (decrease of root length and of number of lateral roots), suggesting that the CK2mut root phenotypes are SA-mediated effects; furthermore, inhibition of CK2 activity in SA-defective and SA-signalling mutants partially reverse these phenotypes. We also show that exogenous SA mediates transcriptional repression of most of PIN-FORMED (PIN) genes, which is the opposite effect observed in CK2mut roots. These results prompted us to propose a model in which CK2 acts as a link between SA homeostasis and transcriptional regulation of auxin–efflux transporters. However, CK2 overexpression in *Arabidopsis* has neither impact on SA biosynthesis nor on auxin transport, but it improves the *Arabidopsis* root system. Thus, unlike the outcome in mammals, an excess of CK2 in plant cells does not produce neoplasia, but it might be advantageous for plant fitness.

P03-019
A regulatory SNP modifies Cystic Fibrosis by disrupting NF-κB complexes binding on *FAS*

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We report the pathogenetic mechanism underlying how a regulatory SNP modifies Cystic Fibrosis (CF). Association studies of affected individuals identified the causal variant, a regulatory SNP rs7910656 on the non-coding region of *FAS* gene on human chromosome 10q24.1, which includes the binding site of many transcription factors especially NFκB complex, but no molecular alterations were detected by conventional approaches. Using a combination of functional *in-silico* gene expression analysis and computational transcription factor binding analysis together with electrophoretic mobility shift and supershift assays, we identified that an allele of the regulatory SNP disrupts the binding of the master transcription factor NFκB on *FAS* and which likely interferes with normal immune activation and programmed cell death.

Thus, our work demonstrates for the first time how a regulatory SNP disrupts the binding of a master transcription factor NFκB and a key signaling pathway FAS in immune regulation and cell death modifying Cystic Fibrosis.

P03-020
Investigating the pleiotropic biochemical effect of warfarin

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Warfarin is one of the world's most frequently used drugs, for the treatment and prophylaxis of thromboembolism. Few studies showed that warfarin blocks the transfer of oxidized vitamin K to the reduced form. In the past, certain warfarin concentrations have been shown to influence inflammatory signal transduction directly i.e, when it is given in low doses it acts as an anti-inflammatory but in higher doses it has pro-inflammatory effects depending upon various factors that affect the dosing requirements such as goal INR, dietary vitamin K intake, interacting medications, underlying disease states, age, gender and genetic factors. Such contrasting effects of warfarin probably arise from different expressions of COX-2 and IL-6 levels and yet have not been proven effective for clinical applications until now. So, in order to investigate the pleiotropy of warfarin, first we will identify the genetic factors responsible for warfarin resistance in human subjects i.e, genotyping and DNA sequencing of functional vitamin epoxide reductase 1 gene polymorphisms. It has been reported that warfarin in low dose might down regulate the expression of pro-inflammatory cytokines including interleukin-6 (IL-6), and studies also suggest that pro-inflammatory cytokines enhance the expression of cyclooxygenase enzyme. A human macrophage cell line will be cultured and exposed to different doses of warfarin. Therefore, the inflammatory effects will be determined, depending upon the warfarin concentrations (within 20–200 and <200 mM) by observing the IL-6 secretion. Total RNA will be extracted from the cultured cells and the expression of pro-inflammatory cytokines will be measured.

P03-021
Intestinal inflammation alters the expression of HDL genes in human and mouse cells by different mechanisms

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Chronic inflammation is a hallmark in a range of clinical disorders including inflammatory bowel diseases (IBD). Patients with IBD have low HDL levels and enhanced atherogenesis. Here we monitored changes in expression levels of intestinal genes that

could account for the above characteristics of IBD. Treatment of intestinal Caco-2 cells with TNFα resulted in a significant decrease in mRNA levels of the *APOA1*, *APOC3*, *APOA4* genes and their regulator HNF4α. Similarly, the mRNA levels of the nuclear receptor LXRα were decreased along with its major target gene *ABCA1*. We aimed to verify these findings *in vivo* by employing the DSS-induced colitis protocol in mice. Treatment of C57BL/6 mice with 3.5% DSS for 5 days caused a dramatic increase in the expression of the inflammatory genes *Il1β*, *Il6*, *Tnfα* and *Mcp1* in the large intestine. In agreement with results in Caco-2 cells, the expression of Hnf4α and its target genes *Apoa1*, *Apoc3* and *Apoa4* was significantly reduced and so was the expression of *Lxrα*. Interestingly, the expression of the Lxrα targets, *Abca1* and *Abcg1*, was not affected by the DSS treatment suggesting that different mechanisms regulate the expression of these two genes in human and mouse intestinal cells. In conclusion, intestinal inflammation is associated with altered expression of HDL genes and their transcriptional regulators in human and mouse enterocytes by different mechanisms.

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P03-022
Gene expression profile of *Thermoplasma volcanium* GSS1 under mild and severe oxidative stress

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Cells are vulnerable to the oxidative stress that is caused by the increased levels of reactive oxygen species (ROS) which severely affect all macromolecules and redox homeostasis of the cells. The living organisms have developed defense mechanisms in order to minimize adverse effects of oxidative damage. Understanding of these mechanisms can give insight into our understanding of the anti-stress mechanisms and development of new therapeutic approaches. In this study, we reported global gene expression of the thermophilic archaeon *Thermoplasma volcanium* under oxidative stress including the unique aspects of the responses to sublethal and lethal doses of the H_2O_2. We have studied gene expression profiling by using whole genome expression oligoarray and qRT-PCR techniques. Most of the differentially regulated genes under mild oxidative stress were shared by the severe stress response. The amount of differentially expressed genes were found to be five fold higher under severe stress than mild stress. About 10% of the up regulated genes under both stress conditions are related to functional categories of electron transfer, redox balance (e.g., aldo/keto reductases, Fe/S oxidoreductases, hydrogenases, thioredoxin reductase), Fe-S cluster biosynthesis and isoprenoid biosynthesis which can be important for coping with oxidative damage.

P03-023
Modulation of the host cell RNA splicing programm by the gastric pathogen *Helicobacter pylori*

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Helicobacter pylori is a Gram-negative bacterial pathogen colonizing the human stomach. Infection with *H. pylori* causes chronic inflammation of the gastric mucosa and may lead to peptic ulceration and/or gastric cancer. We performed a comprehensive analysis of changes in splicing of the host cell mRNA during infection. The transcriptome of *in vitro* infected cells was sequenced using RNA-seq and analyzed for changes in transcript abundances.

A total 15% of all regulated genes were detectable by at least two different analytic approaches. Selected candidate genes were successfully validated using quantitative RT-PCR. To identify splicing factors which govern the changes in splice site recognition and to uncover motifs enriched directly at the differentially spliced exon boundaries we used the differentially spliced junctions revealed through MATS. Emphasis was placed on motifs of the kind previously described for splicing factor binding sites. Thus, we identified motifs enriched at exons which are preferentially skipped in infected cells. These motifs were matched with already known binding motifs of alternative splicing factors. Consequently, we could show that specific, infection induced stimuli (e.g. via T4SS) induce cellular signaling, which subsequently leads to a changes in isoform abundance of specific genes. The results obtained in cultured cells were confirmed in human gastric primary cells. Alternative splicing factor binding sites were found to be enriched at regulated exons and we speculate these factors might constitute determinants of the infection induced changes in splicing preference.

P03-024
ZNF224 is a novel transcriptional repressor of the *c-myc* oncogene in Chronic Myelogenous Leukemia

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The transcription factor ZNF224 was recently identified as a Wilms Tumor protein 1 (WT1) cofactor which modulates WT1-dependent apoptotic genes transcription in chronic myelogenous leukemia (CML) cells. Furthermore, ZNF224 induction by ara-C enhances apoptotic cell death. Moreover, we demonstrated that BCR-ABL tyrosine kinase activity negatively regulates ZNF224. Indeed, BCR-ABL inhibition in CML cell lines by Imatinib and by second generation tyrosine kinase inhibitors triggers ZNF224 up-regulation via a transcriptional mechanism. It is known that Bcr/Abl induces c-myc overexpression, which is required for Bcr/Abl oncogenic transformation in CML. In fact, c-myc has a regulatory role in many cellular processes (proliferation, growth and apoptosis) and it is upregulated in many leukemia and solid tumors. We obtained preliminary data showing that ZNF224 was able to counteract WT1 mediated c-myc activation and observed a relevant anti-correlation in ZNF224 and c-myc expression levels in Imatinib treated K562 cells.

To clarify the role of ZNF224 in c-myc repression we conducted *in silico* analysis on c-myc promoter, reporting five ZNF224 putative binding sites. Chromatin Immuno-Precipitation (s) (ChIP) experiments using anti-ZNF224 antibody in Imatinib treated K562 cells and in HEK293 cells (lacking endogenous WT1), confirmed ZNF224 binding on the c-myc promoter. Furthermore, we observed a dose-dependent reduction of c-myc expression and promoter activity in HEK293 cells transfected with increasing amounts of ZNF224. Finally, we investigated ZNF224 regulation by JAK2/STAT3 pathway, which is essential for Bcr/Abl c-myc induced overexpression in CML. To this aim, we treated K562 cells with AG490 (JAK2 inhibitor) and reported a JAK2 negative effect on ZNF224 expression.

P03-025

Histone deacetylase 3 and 4 complex activates transcriptional activity of mineralocorticoid receptor

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Histone deacetylases (HDACs) act as co-repressors in gene transcription, resulting in epigenetic gene silencing by compacting chromatin. We previously demonstrated that transcriptional activity of Mineralocorticoid receptor (MR) is activated by HDAC3 which deacetylates MR. HDAC3 forms complexes with class II HDACs; however, the role of class II HDACs in the transcriptional activity of the MR is unclear. We hypothesized that HDAC3 and 4 complex activates transcriptional activity of MR. Expression of MR target genes was measured by quantitative real-time PCR. Enrichment of the MR and RNA polymerase II to promoters of target genes was analyzed by the chromatin immunoprecipitation assay. Interaction between the MR and HDACs was investigated by co-immunoprecipitation. MR acetylation was determined with an anti-acetyl-lysine antibody after immunoprecipitation with an anti-MR antibody. Among the class II HDACs, HDAC4 interacted with both MR and HDAC3 after aldosterone stimulation. Nuclear translocation of HDAC4 was mediated by protein kinase A (PKA) and protein phosphatase (PP). Transcriptional activity of MR was significantly decreased by inhibitors of class I HDAC (MS-275), PKA (H89), PP1 and PP2 (calyculin A), but not by class II HDAC (MC1568). Acetylation level of MR was increased by H89, calyculin A, and MS-275, but not by MC1568. Interaction between MR and HDAC3 was significantly decreased by H89, calyculin A, and HDAC4 siRNA. A non-genomic effect of MR mediated by PKA and PP1 and PP2 induces nuclear translocation of HDAC4 to facilitate the interaction between MR and HDAC3. Thus, HDAC3 and 4 complex activates transcriptional activity of MR.

P03-026

A case–control study of type 2 diabetes mellitus

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Introduction: Diabetes mellitus has become one of the most significant health problems worldwide. It is known that the disease is a complex phenotype, being determined by both environmental and genetic factors as well as by their interactions. The purpose of this research project was to investigate, if certain genetic factors can be considered as risk factors of diabetes mellitus. The *WFS1* gene seemed to be a promising candidate, thus three polymorphisms (two SNPs: rs4273545, rs4689388 and one VNTR: rs148797429) were selected in the promoter region of the gene for analysis.
Methods: Buccal DNA samples were collected and purified from 368 type 2 diabetic and 588 control people. Restriction endonuclease based method, allele-specific polymerase chain reaction and melting curve analysis were employed for genotyping; the obtained results were evaluated in a case–control setup. We also carried out a haplotype analysis. Putative biological function of the polymorphisms is currently examined by luciferase reporter assay.
Results: In the control population all genotype frequency values were in Hardy-Weinberg equilibrium ($p > 0.1$). The SNPs showed significant difference between the control and diabetic population. After the Bonferroni correction, the A allele of the rs4689388 ($p = 0.000225$), and the T allele of the rs4273545 ($p = 0.000341$) was significantly more common in the diabetic population. One haplotype (A-2x-T) seems to be a risk factor (OR 3.18, $p = 0.0000184$) for type 2 diabetes. *In vitro* analysis of the polymorphisms is currently in progress.

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P03-027

Casein kinase 1δ regulates Hypoxia Inducible Factor-2α by direct phosphorylation

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Cellular responses to low oxygen are mediated by Hypoxia Inducible Factors (HIFs), heterodimeric transcription factors, comprising a constitutively expressed subunit (ARNT) and an oxygen-regulated subunit, HIF-1α and HIF-2α (or EPAS1). Regulation of HIF-α subunits also involves post-translational modifications, including phosphorylation. Previous studies of our lab have shown that casein kinase 1δ (CK1δ) phosphorylates HIF-1α PAS-B domain at Ser247 and impairs its transcriptional activity. To extend this study, we investigated the effect of CK1δ on HIF-2α. Inhibition of CK1δ activity did not affect HIF-2α protein levels but enhanced its transcriptional activity in hepatoma Huh7 cells grown under hypoxia. Human recombinant full-length HIF-2α or its fragments were then used as substrates in *in vitro* phosphorylation assays with CK1δ and [γ-P^{32}] ATP. The N-terminal domain of HIF-2α, comprising the PAS domain, was directly modified by CK1δ. Mass spectroscopy analysis revealed residues Ser383 and Ser386 as possible CK1δ phosphorylation sites. Using site-directed mutagenesis, these residues were converted into alanine. These mutations increased HIF-2 transcriptional activity, without affecting its protein expression levels or subcellular localization. Our data demonstrate for the first time, that HIF-2α is a direct phosphorylation target of CK1δ and clarification of this mechanism will allow complete understanding of HIF-2 regulation.

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P03-028

Mechanism of atypical pro-death signalling mediated by the Heat Shock Factor 1

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HSF1 is the main mediator of the heat shock response typically inducing cytoprotective Heat Shock Proteins (HSPs) that enables cells to survive. However, in spermatocytes, which are the most heat-sensitive spermatogenic cells, HSPs are not produced, instead apoptotic cell death is induced by active HSF1. Results of our genome-wide transcriptional analysis combined with glo-

bal mapping of HSF1 binding sites by ChIP-Seq revealed, that HSF1 binds to sequences located in the intron(s) of the proapoptotic *Pmaip1*/*PMAIP1* gene and this binding was correlated with up-regulation of the gene transcription. We found similar up-regulation of *Pmaip1*/*PMAIP1* by HSF1 also in certain somatic mouse and human cells. Notably, these cell lines were more sensitive to hyperthermia-induced cell death. Hence, HSF1 not only can activate the expression of *HSPs* genes, which prevent apoptosis, but also likely positively regulates the expression of the proapoptotic *Pmaip1*/*PMAIP1* gene, which facilitates cell death.

HSF1-mediated activation of PMAIP1 in combination with differential expression of cytoprotective HSPs, could be the main molecular mechanism involved in switching from pro-survival to pro-death signaling in cells subjected to elevated temperatures.

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P03-029
Cadmium, cobalt and nickel inhibit sequence-specific DNA binding of p63 and p73 proteins *in vitro* and in cells

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Tumor suppressors of p53 protein family (p53, p63 and p73) have important functions in many cellular processes. The p53 family members are known mainly to be important for the prevention of cancer, but p63 and p73 are crucial also in controlling of development and differentiation. For correct function as tumor suppressors, sequence-specific binding to responsive DNA sequences of target genes is required. This type of DNA binding is performed by a central DNA-binding domain (DBD), which is highly homologous among p53 family proteins. It has been shown that oxidation of cysteines in DBDs of all members abolishes sequence-specific DNA binding by affecting zinc coordination at the protein-DNA interface. It has been also found that excess of zinc ions inhibits sequence-specific DNA binding of p53 protein similarly as ions of heavy metals.

In our work we dealt with an effect of divalent transition metal ions – cadmium, cobalt and nickel – on sequence-specific DNA binding of p53 family members *in vitro* and in cells. Based on results of electrophoretic mobility shift assay and luciferase reporter assay, we conclude that cadmium inhibits sequence-specific DNA binding of all three core domains to p53 consensus sequences and abolishes transactivation of several promoters (e.g. BAX and MDM2) by micromolar concentrations. Similar inhibition effect for cobalt and nickel ions was observed by millimolar concentrations. Moreover DBD domains bound to DNA are protected against the inhibition effect of heavy metal ions. We also found that excess of EDTA restores binding activity of p63 and p73 proteins.

P03-030
Expression of CacyBP/SIP gene in colon cancer HCT116 and neuroblastoma NB2a cells

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CacyBP/SIP was discovered as a calcyclin (S100A6) binding protein (Filipek and Wojda, 1996) and later as a Siah-1 interacting protein (Matsuzawa and Reed, 2001). At present, it is known that CacyBP/SIP binds several targets and through the influence on Elk-1 activity might be involved in the regulation of cell proliferation and differentiation (Topolska-Woś *et al.*, submitted).

In this work we have examined the regulation of gene expression of CacyBP/SIP. We have found the binding sites for transcription factors such as NFAT (a key regulator of T-cell development and function), CREB (which may link CacyBP/SIP expression with the activity of other kinases and phosphatases), E2F (promoting proliferation or cell death), USF (involved in immune response and cell cycle regulation) and DREAM (which may transmit the effect of intracellular Ca^{2+} concentration on CacyBP/SIP gene expression). Among those transcription factors, NFAT, CREB and USF seem to modulate the activity of CacyBP/SIP gene promoter and to evoke changes in CacyBP/SIP mRNA and protein level. The potential regulation by NFAT was confirmed using different methods such as ChIP. Moreover, we have found that the effect of NFAT on the CacyBP/SIP protein level might depend on intracellular Ca^{2+} concentration. Comparison of CacyBP/SIP expression in colon cancer HCT116 and mouse neuroblastoma NB2a cells shows that, depending on the cell line, different factors can be engaged in its regulation.

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P03-031
Epidermal growth factor induced intestinal sodium-glucose cotransporter 1 gene expression through activation of cAMP response element binding protein

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The intestinal sodium-glucose cotransporter 1 (SGLT1) is the main apical transporter for glucose uptake. Its expression is decreased in the intestine of individuals with intestinal inflammatory disorders and pathogenesis. Thus, a better understanding of the regulatory mechanism of the SGLT1 gene may provide important insights for the development of therapeutic strategies. Using the trinitrobenzene sulfonic acid (TNBS)-induced model of IBD, we observed decreased SGLT1 expression and glucose uptake in the IBD intestine, and this decrease was positively correlated with the mucosal level of epidermal growth factor (EGF). Western blot and Q-PCR analysis demonstrated that the effect of EGF on glucose uptake was due primarily to the increased level of SGLT1 in mice and in Caco-2 cells. Transient transfection assays using SGLT1 promoter-luciferase reporters revealed an essential cAMP response element (CRE), which is required for EGF-mediated induction of SGLT1 gene expression. ChIP assay demonstrated the increased binding of CRE-binding protein (CREB) and CREB-binding protein (CBP) to the SGLT-1 gene promoter in EGF-treated cells. Further studies using specific kinase inhibitors indicated that PI3K, ERK, and p38 signaling pathways are involved in EGF-mediated effect on SGLT-1 expression. Importantly, in the intestine of mice with IBD, the level of activated CREB was found to be positively correlated with the SGLT1 protein level. These findings provide novel insights into the pathogenesis of IBD and suggest that the decreased intestinal mucosal EGF level results in decreased CREB activation, which consequently impairs glucose uptake and leads to manifestation of the disease.

P03-032

Combined interactions of plant homeodomain and chromodomain regulate NuA4 activity at DNA double-strand breaks

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DNA double strand breaks (DSBs) represent one of the most threatening lesions to the integrity of genomes. In yeast *Saccharomyces cerevisiae*, NuA4, a histone acetylation complex, is recruited to DSBs, wherein it acetylates histones H2A and H4, presumably relaxing the chromatin and allowing the access of repair proteins. Two subunits of NuA4, Yng2 and Eaf3, can interact *in vitro* with methylated K4 and K36 in histone H3 via their plant homeodomain (PHD) and chromodomain, respectively. However, the roles of the two domains and how they interact in a combinatorial fashion are still poorly characterized. In this study, we demonstrate that the combined mutations in both the PHD and chromodomain lead to dramatic defects in cell growth and DSB repair, while mutations in the Yng2 PHD or Eaf3 chromodomain alone have no significant effects. In addition, chromatin immunoprecipitation experiments reveal that high levels of H4K12ac, H3K4me2, H3K4me3, and H3K36me2 are enriched at the DSB site, thus providing the binding sites for the PHD and chromodomain. Our results suggest that multivalent interactions between PHD, chromodomain and methylated H3 act in a combinatorial manner to regulate the NuA4 activity at the DSBs.

P03-033

AIBp regulates mitotic entry and mitotic spindle assembly by controlling activation of both Aurora-A and Polo-like Kinase 1

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We previously reported that Aurora-A and hNinein binding protein, AIBp, facilitates maintaining centrosomal structure and contributes to spindle formation. Here, we identify AIBp also interacts with Plk1, raising the possibility of functional similarity to Bora, which subsequently promotes Aurora-A-mediated Plk1 activation at Thr210 as well as Aurora A activation at Thr288. In kinase assays, AIBp acts as not only a substrate but a positive regulator towards both Aurora-A and Plk1. However, AIBp functions as a negative regulator to block the phosphorylation of hNinein mediated by Aurora A and Plk1. These findings suggest a novel AIBp-dependent regulating machinery to control mitotic entry. Additionally, knockdown of hNinein caused AIBp fail to target to the centrosome, whereas depletion of AIBp does not affect the localization of hNinein and microtubule nucleation. On the other end, unlike hNinein target to r-tubulin, overexpressed AIBp appears to affect Aurora-A and Plk1 on hNinein phosphorylation which may indirectly prevent cells to enter mitotic phase. Notably, knockdown AIBp in HeLa cells impairs both Aurora-A and Plk1 kinase, which results in phenotypes of multiple spindle pole formation and chromosome misalignment. Moreover, our data showed that depletion of AIBp reveals the mislocalization of TACC3 and ch-TOG, but not CEP192 and CEP215, suggesting loss of AIBp more dominantly affects Aurora-A target proteins causing mitotic aberration. Collectively, these data demonstrate that AIBp contributes to mitotic entry and spindle assembly, may partially via hNinein, to control localization, phosphorylation and activating both Aurora-A and Plk1 during mitotic progression.

P03-034

Search for new genes involved in the integrated stress response

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The Integrated Stress Response (ISR) is a program of gene expression regulated by Activating transcription factor 4 (ATF4) and aimed to increase cell survival under the different stress conditions. We have shown previously that the switch from upregulation to downregulation of ATF4-dependent transcription after sustained inhibition of mitochondrial electron transport chain complex III can be prevented by supplementation of cell culture medium with uridine that abolishes the activation of tumor suppressor p53. We identified the set of human genes with a similar regulation mode based on the mRNA-seq data. The functional clustering among the gene list has revealed the enrichment with transcripts of previously identified ATF4 target genes confirming the workability of our approach. The results provided the list of genes for a search of potential new ATF4 transcription targets, including Angpt4, Adm2, MDGA1, VGF, ZFP36, HKDC1, FAM129A, Krt6A and Krt16. The dependence of the candidate gene expression on the levels of ATF4 was studied by RT-qPCR in two lines of cultured human cancer cells using increase in ATF4 levels by ectopic expression and suppression of ATF4 expression by RNA interference.

Since ATF4 expression is elevated in various diseases, as well as in many tumors, ISR signaling pathways are the attractive targets for anti-cancer and other types of therapy. The identification and study of still unknown molecular targets of ATF4 may contribute to the development of new strategies for disease control.

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P03-035
Functional investigations of the monogenic diabetes gene *HNF1A* identify rare variants as risk factors for type 2 diabetes in a general population

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The role of rare genetic variants in common disease is currently not well known. In a previous study, we found that a significant fraction of individuals from the Framingham and Jackson Heart Studies carry rare protein damaging variants in one of seven monogenic diabetes genes although most individuals were euglycemic. To investigate whether functional characterization of such variants can improve disease risk prediction, we studied all non-synonymous coding variants in the most common of the seven monogenic diabetes genes, *HNF1A*, identified among 4003 individuals in the Framingham and Jackson Heart Studies. Of 27 rare *HNF1A* variants, *in silico* prediction tools reported four variants as putative pathogenic but no borderline association with diabetes was detected. All variants were functionally investigated focusing on effect on HNF-1A transcriptional activity (luciferase reporter assay in HeLa cells), DNA binding (Electrophoretic Mobility Assay) and subcellular localization (immunofluoresence). By functional investigation, eleven variants were characterized as functionally damaging based on the mutant proteins demonstrating impaired HNF-1A function (Transcriptional activity and/or reduced nuclear translocation <60%). Moreover, the clinical phenotype of carriers with functionally affected variants was significantly associated with diabetes ($p = 8.1 \times 10^{-5}$, OR = 6.33; CI 2.22–18.1). Thus, functional verification of variant effect in individuals carrying rare *HNF1A* variants is needed to estimate risk of developing type 2 diabetes.

P03-036
Angiotensin converting enzyme II deficiency accelerates the progression of COPD via irregular signaling STAT3 phosphorylation

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Inflammation-mediated abnormal renin-angiotensin system (RAS) and matrix metalloproteinases (MMPs) expression are implicated in chronic obstructive pulmonary disease (COPD) development. However, interaction between angiotensin converting enzyme II (ACE2), an ACE homologue having antagonist effects on ACE/angiotensin II (Ang II) axis, and MMPs expression related to COPD development is still unclear. Therefore, we attempted to investigate the molecular mechanism of ACE2 in regulating MMPs activity and related signaling pathways in the early-stage COPD by cigarette-smoke (CS) exposure. C57BL/6 (WT) and ACE2 KO mice ($ACE2^{-/-}$) were applied in CS-induced COPD. COPD hallmark in the mice was detected, and biochemical and pathological changes in lungs of CS-exposed mice were also determined. Increases of resting respiratory rate, pulmonary immunokines, leukocyte infiltration and bronchial hyperplasia were observed in CS-exposed mice. Compared to WT mice, more serious in these physiological, biochemical and pathological changes were found in $ACE2^{-/-}$ mice. CS exposure could increase pulmonary ACE and ACE2 activity in WT mice and a significantly higher ACE in $ACE2^{-/-}$ mice compared with that in WT mice. Furthermore, pulmonary MMPs activity was decreased in smoke-exposed WT mice; whereas, MMPs activity was increased in $ACE2^{-/-}$ mice. In $ACE2^{-/-}$ mice, a significant increase of cellular signaling p-STAT3 was determined; however, no effect on p-STAT3 level was observed in WT mice. ACE2 deficiency is more sensitive to exposing CS-induced COPD pathogenesis because of excessing ACE/Ang II axis activation. Our results support the hypothesis that ACE2 deficiency influences signaling STAT3 phosphorylation to promote CS-induced pulmonary inflammation and MMPs activity concerning with lung tissue remodeling.

P03-037
Histone deacetylase inhibition (HDACi), but not an mineralocorticoid receptor (MR) antagonist spironolactone, attenuates transcriptional activity of activating mutant MR_{S810L}

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Rationale: Spironolactone (Spiro), an antagonist of mineralocorticoid receptor (MR), activates mutant MR (MR_{L810}) and causes hypertension in mutant MR carriers. Thus, it is hard to attenuate transcriptional activity of mutant MR with Spiro.
Objective: We hypothesized that histone deacetylase inhibition (HDACi), but not an MR antagonist Spiro, attenuates transcriptional activity of activating mutant MR.
Methods and Results: We established wild type MR (MR_{WT}) or MR_{L810} overexpressing HEK293T cells. Transcriptional activity of MR_{WT} and MR_{L810} was determined by luciferase assay. Expression of MR target genes was measured by quantitative real-time PCR. MR_{WT} and MR_{L810} acetylation was determined by western blot with anti-acetyl-lysine antibody after immunoprecipitation (IP) with anti-MR antibody. Overexpression of MR_{L810}, but not MR_{WT}, increased expression of MR target genes by treatment with Spiro in accordance with increased transcriptional activity of MR_{L810}. Treatment with HDAC inhibitor attenuated transcriptional activity of MR_{L810} and expressions of MR target genes induced by Spiro. HDACi promoted MR_{L810} acetylation, leading to attenuated transcriptional activity of MR_{L810}.
Conclusion: These results indicate that HDACi, but not an MR antagonist Spiro, attenuates transcriptional activity of activating mutant MR.

P03-038
Signal integration by the *CYP1A1* promoter – a quantitative study

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Genes involved in the metabolism of foreign compounds exhibit complex spatiotemporal expression patterns in liver. The expression of cytochrome P450 1A isoforms (CYP1A1, CYP1A2), for example, is restricted to the pericentral region of liver lobules in response to the interplay between two major signaling pathways/transcription factors: the aryl hydrocarbon receptor (AhR), activated by exogenous compounds such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and β-catenin, a downstream effector in the canonical Wnt signaling pathway. The mechanisms by which the two pathways orchestrate gene expression are still poorly understood. With the help of 29 mutant constructs of the human *CYP1A1* promoter and a mathematical model that combines Wnt/β-catenin and AhR signaling with the statistical mechanics of the promoter, we systematically quantified the regulatory influence of different transcription factor binding sites on gene induction within the promoter. The model unveils how different binding sites cooperate, establishes the promoter logic and is able to quantitatively predict two-dimensional stimulus-response curves. Furthermore, it shows that crosstalk between Wnt/β-catenin and AhR signaling is crucial to understand the complex zonated expression patterns found in liver lobules. This study exemplifies how statistical mechanical modeling together with combinatorial reporter assays has the capacity to disentangle the interplay of xenobiotic-activated signaling and endogenous pathways to regulate physiological gene expression patterns.

P03-039
In response to alien RNA polymerase: bacteriophage T7 evolves promoters by their electrostatic properties

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(JJ Bull *et al.* 2007) replaced the RNA polymerase (RNAP) gene in bacteriophage T7 with that of phage T3. T3 RNAP was supplied in trans by the bacterial host to T7 genome lacking the RNAP gene and a phage population was propagated on native bacteria throughout adaptation. Evolution of the T3 RNAP gene was thereby prevented, and selection was for evolution of phage genome regulatory signals. T3 RNAP transcribes from T7 promoters at low levels, but a single mutation in the promoter confers high expression, providing mechanism for reevolution of gene expression.

In 30 mutations observed, changes were in 9 of 16 promoters. Only 7 of 13 mutations were to T3 consensus, one even reversed. At conservative −11 position, considered important to T7–T3 promoters distinguishing, only one of six mutations leaded to consensus, prejudicing sequence text-encoding promoters distinguishing principles.

We found main differences in the electrostatic profiles between promoters of T7 phage and that of T3 and mutated T7 lie at starting point region (−2 ... −5 bp). Electrostatic potential of T7 promoters is considerably less than that of T3 and mutant. Mutated promoters demonstrated largest potential shift, though non-mutated also showed some potential gain due to mutations in flanking regions.

Though fitness is integral indicator of promoter strength, we can still make an assumption that differential recognition of promoters by T7 and T3 RNAPs can by driven by their electrostatic properties. DEPPDB was used to make the analysis.

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P03-040
Evolutionary dynamics of DNA-binding sites and direct target genes of a floral master regulatory transcription factor

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Flower development is controlled by the action of key regulatory transcription factors of the MADS-domain family. The function of these factors appears to be highly conserved among species based on mutant phenotypes. However, the conservation of their downstream processes is much less well understood, mostly because the evolutionary turnover and variation of their DNA-binding sites among plant species has not yet been experimentally determined.

Here, we performed comparative RNA-seq and ChIP-seq analysis of the MADS-domain transcription factor SEPALLATA3 (SEP3) in the two closely related Arabidopsis species, *A. thaliana* and *A. lyrata*. To our surprise, we found a low level of conservation of SEP3 binding sites (BSs) between both species, which have very similar floral morphology. Binding site conservation is associated with DNA sequence conservation, the presence of the CArG-box BS motif and on the conservation of relative position of the BS to its potential target gene. Differences in genome size and structure can explain that SEP3 BSs in *A. lyrata* can be located more distantly to their potential target genes than their counterparts in *A. thaliana*. In *A. lyrata*, we identified transposition as a mechanism to generate novel SEP3 binding locations in the genome. In summary, this study investigates the evolutionary dynamics of DNA BSs of a floral key-regulatory transcription factor, and explores factors affecting this phenomenon.

P03-041
The nuclear dioxygenase Jmjd6 regulates macrophage host responses

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The jumonji domain containing protein 6 (Jmjd6) belongs to the extended family of Jumonji C (JmjC) domain containing proteins that catalyse hydroxylation by coordinating di-iron Fe(II) and the co-substrate 2-oxoglutarate in their enzymatic active centres (1,2). In the nucleus, Jmjd6 associates with nascent RNA and modulates splicing through oxidative hydroxylation of the splicing proteins U2AF65 and LUC7L2 (3,4). Studies of Jmjd6 *in vivo* functions have been hampered by the early lethality associated with *Jmjd6* deficiency in mice (5). We have now generated conditional *Jmjd6* knockout mice to investigate *Jmjd6* loss-of-function effects in macrophages.

We report here that Jmjd6 expression is up-regulated in bone marrow derived macrophages (BMDM) in the late phase of lipopolysaccharide (LPS) activation. When analysed for anti-viral host responses, *Jmjd6*-deficient BMDM showed lower levels of vaccinia virus replication at 8 and 24 h post infection when com-

pared to wild type BMDM. Furthermore, confocal microscopy imaging revealed well-delineated, focal viral factories with few free virions within the cytoplasm. This suggests that Jmjd6 is needed for VACV replication in macrophages and has in addition a function in the modulation of pro-inflammatory innate immune responses.

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P03-042
A quantitative study of dual signal integration by the *CYP1A1* promoter

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Cytochrome P450 1A1 (CYP1A1), a gene involved in detoxification of foreign compounds in the liver, is restricted to the pericentral region of liver lobules in response to the interplay between aryl hydrocarbon receptor (AhR) and Wnt/β-catenin signaling pathways. The mechanisms by which the two pathways orchestrate the spatiotemporal gene expression patterns are still poorly understood. With the help of 29 mutant constructs of the human CYP1A1 promoter and a mathematical model that combines Wnt/β-catenin and AhR signaling with the statistical mechanics of the promoter, we systematically quantified the regulatory influence of different transcription factor binding sites on gene induction within the promoter. The cooperation of binding sites and the logic of the promoter could be unveiled with the help of a thermodynamic model that could also quantitatively predict two-dimensional stimulus-response curves. Furthermore, it shows that crosstalk between Wnt/β-catenin and AhR signaling is crucial to understand the complex zonated expression patterns found in liver lobules. This study exemplifies how statistical mechanical modeling together with combinatorial reporter assays has the capacity to disentangle the promoter logic that establishes physiological gene expression patterns.

P03-043
Disturbance of gene expression in primary human hepatocytes by hepatotoxic pyrrolizidine alkaloids: a whole genome transcriptome analysis

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1,2-unsaturated pyrrolizidine alkaloids (PA) are widely distributed plant metabolites that predominantly occur in the plant families Asteraceae, Boraginaceae and Fabaceae. Acute and chronic PA poisoning due to contaminated food or feed causes severe hepatotoxicity. So far, the molecular mechanisms of PA toxicity are not well understood. For the analysis of the mode of action of PA, primary human hepatocytes were exposed to 100 μM of four structurally different PA: echimidine, heliotrine, senecionine and senkirkine. Changes in mRNA expression were analyzed by a whole genome microarray. Employing cut-off values with a fold change of −2/2 and a q-value of 0.01, data analysis revealed numerous changes in gene expression patterns after PA exposure. 4556, 1806, 3406 and 8623 genes were regulated by echimidine, heliotrine, senecione and senkirkine, respectively. A total of 1304 genes were identified as commonly regulated by all four PA. Data were analyzed using a pathway analysis tool. PA affected pathways related to cell cycle regulation, cell death and development of cancer. The transcription factors p53, MYC, NFκB and NUPR1 were activated upon PA treatment. Gene expression data showed a considerable interference with lipid metabolism and bile acid flow. The associated transcription factors FXR, LXR, SREBF1/2, as well as PPARα, γ and δ were predicted to be inhibited by PA.

In conclusion, although structurally different, all four PA significantly regulated a great number of genes in common. This proposes a similar mode of action for all PA, albeit to different extents, reflected by potential hepatotoxicity and individual PA structure.

P03-044
Bilirubin neurotoxicity involves inflammatory response via ER stress

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Recent data suggest an important role of ER-stress in unconjugated bilirubin (UCB) induced neurotoxicity. ER stress signaling involves three main proteins (PERK, IRE-1, and ATF6) bound by the chaperone BiP. Misfolded proteins accumulation results in the recruitment of BiP away from ER stress sensors and leads to transcription factors up-regulation (ATF4 and CHOP by PERK, XBP1 by IRE-1 and ATF6). Connections exist between ER stress and inflammation, with NFκB activation and IL-8 and TNFα induction. Since no evidence exists on the contribution of ER stress in mediating the inflammatory response induced by UCB, we performed an *in vitro* comparative study using SH-SY5Y neuroblastoma and U87 astrocytoma cell lines. Cells were treated with toxic concentrations of free bilirubin (Bf) for 24 h. SH-SY5Y showed a mortality of 30% (Bf 140 nM) and 70% (Bf 300 nM), while no effects were seen on U87 cells. Bilirubin treatment (140 nm Bf treatment for 8 h) induced m-RNA up-regulation of ATF4 (3-fold), CHOP (46-fold), XBP1 (12-fold), ATF6 (2-fold), BiP (4-fold), NFκB (3-fold), TNFα (23-fold), and IL-8 (60-fold) in SH-SY5Y. Cells pre-treated either with an NFκB inhibitor or transfected with PERK siRNA and then exposed to 140 nM Bf for 8 h showed a decreased induction of IL-8 (90% and 70% respectively). TNFα expression was unchanged. Pre-treatment with PKC inhibitor showed a decrease in the induction of IL-8 (80%) and an increase of TNFα (80%). We conclude that UCB causes ER-stress and inflammatory response. IL-8 induction is NFκB dependent and regulated by PERK and PKC upstream signaling.

P03-045
Transactivation of the human *ADAMTS-2* gene promoter through proinflammatory cytokine TNF-α in osteoblast-like cells

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TNF-α is a proinflammatory cytokine produced by numerous cell types, including macrophages, lymphocytes, fibroblasts and keratinocytes in response to inflammation, infection and other environmental stresses. TNF-α has critical functions in inflammatory disorders such as rheumatoid arthritis and osteoarthritis. Extracellular matrix (ECM) degradation is the main concern of these conditions and an important component in morphogenesis, organogenesis, and tissue remodeling, as well as in wound healing and tissue repair. ADAMTS family has been implicated in osteoblast and cartilage. As well as MMPs, ADAMTS protease family has the capacity to degrade the ECM. A member of this family, ADAMTS-2, processes the amino terminus of the type I, II, III and V procollagens, main component of the ECM

In the present study we explored effects of TNF-α to transcriptional activity of the human ADAMTS-2 gene promoter in osteoblast like cells, Saos-2. Cells were transiently transfected with previously constructed ADAMTS-2 promoter fragments and the reporter gene activity in these cells that were either left untreated or exposed to TNF-α was determined. TNF-α dependent induction in reporter gene activity was obtained with all ADAMTS-2 promoter constructs. The main signalling pathways that were involved in ADAMTS-2 induction were also determined.

Keywords: ADAMTS-2, IL-α, Saos-2, Collagen.
Acknowledgement: This work was supported mainly by the Scientific and Technological Research Council of Turkey (TUBITAK) (212T200) and partially by the Balikesir University Scientific Research Projects Unit (BAP) (2010/39).

P03-046
Transcription of the *PSMD4* gene is upregulated in hypoxia in prostate cancer cells

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The *PSMD4* (Proteosome 26S non-ATPase subunit 4) gene is located human chromosome 1. This gene encodes three proteins, namely S5A, antisecretory factor (ASF) and Angiocidin. S5a is the polyubiquitin binding subunit of the 26S proteasome. ASF is a plasma-bound protein known to inhibit cholera toxin-induced intestinal fluid secretion in rats. Most importantly, Angiocidin is a protein over-expressed in many different solid tumors.

Hypoxia is a critical stimulus in many physiological and disease states like cancer. In addition, regulation studies of the *PSMD4* gene are rather limited. The aim of the study is to identify the hypoxic regulation of the *PSMD4* gene. For transcriptional regulation of the *PSMD4* gene, the truncated promoter constructs were cloned into a luciferase-based expression vector. Basal transcriptional activities of the promoter constructs were determined in normoxic and hypoxic conditions in PC3 (prostate cell line). We found that the 262 bp promoter construct (−191/+65) was affected by hypoxia. Bioinformatics analysis also reveals that the promoter of the *PSMD4* gene contains several putative HREs (Hypoxia Response Element) that is a target of HIF1α transcription factor. The expression of the *PSMD4* gene was also determined at mRNA and protein level.

Keywords: PSMD4, hypoxia, HIF1α, PC-3.
Acknowledgement: This work was supported mainly by the Balikesir University Scientific Research Projects Unit (BAP) (2014/109).

P03-047
High fructose diet up-regulates insulin and its down-stream signaling components in abdominal ommentum tissues: Effects of resveratrol

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Recent studies demonstrated persuasive evidence that diets rich in high fructose cause hyperinsulinemia together with vascular and hepatic insulin resistance in experimental rat models. However, there has been much less direct experimental data that sustained the changes in insulin signaling pathway in the adipose tissue of rats treated with high fructose diets. With the aim of investigating the molecular mechanism of fructose induced metabolic syndrome and the normalizing effect of resveratrol, expression levels of genes that have a role in the insulin signaling pathway – *IR, IRS-1, IRS-2, PI3K, Akt1, mTOR, eNOS, iNOS, Nrf2, NfkB, PPARg* and *SIRT1* – were evaluated in abdominal ommentum tissues by real-time polymerase chain reaction.

The results of this study revealed that consumption of a high fructose diet causes an absolute increase in gene expression levels of *IR, IRS-1, IRS-2, PI3K, Akt1* and *mTOR* which are the genes of the proteins that insulin hormone exerts its effects in abdominal ommentum tissues. Findings from this study also demonstrated the potential efficacy of resveratrol in ameliorating the changes induced by high fructose. Resveratrol treatment significantly decreased the expression levels of the insulin signaling pathway components and normalized the effects of the high fructose diet.

Induction of gene expressions in insulin signaling pathway components might be associated with the increase in fat and body weights of the rats which demonstrates the acceleration of lipid biosynthetic metabolism. Reversal of fructose-induced alterations indicated the therapeutic roles of resveratrol for the defense against high fructose-induced obesity.

Keywords: High fructose, Abdominal ommentum, Insulin signaling, Resveratrol

P03-048
Strong *E. coli* rrnB P1 promoter mutants possess pronounced electrostatic up-elements

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It is known that not only the consensus sequence text is essential for RNA polymerase–promoter recognition and regulation, but physical properties, especially electrostatics, play important role particularly at the early stages of this process. One of the elements that plays a crucial role in the promoter strength regulation is a so-called "up-element", which interacts with the alpha subunit of RNAP and thus facilitates its binding to the promoter.

High AT content is often associated with the up-element, however, no text consensus is discovered and functionality of this region is defined by its physical properties. We have shown earlier that strong T4, early T7-like, phage Lambda and *E. coli* ribo-

somal promoters with a pronounced up-element have high levels of the electrostatic potential within it, and electrostatics are responsible for its functioning during the global transcription switch under the T4 bacteriophage infection accompanying reversion of the polymerase alpha subunit local charge.

(ST Estrem *et al*. 1998, 1999) made a series of mutations in the up-element of strong *E. coli* ribosomal *rrnB* P1 promoter up to complete elimination. We found that these promoters' strength depends upon the size of the electrostatic up-element so that the bigger the element, the stronger the promoter is, and the mutant with no electrostatic up-element has zero strength. However, if the element is too big, the strength decreases slightly due to possible over-sticking (trapping) of the polymerase. DEPPDB was used to make the analysis.

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P03-049
TGF-β upregulates URG-4/URGCP gene expression in Hepatoma cells

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URG-4/URGCP has been found to contribute importantly to multistep carcinogenesis. Over-expression of URG4/URGCP accelerated tumor development in nude mice and also induced in some cancer cell types such as hepatocellular carcinoma, osteosarcoma, gastric and leukemia. However, the mechanism of URG-4 regulation by TGF-β1 and its significance in cancer progression remains largely unknown. To our knowledge, this is the first study analyzing mRNA and protein level changes of URG-4/URGCP gene in the presence or absence TGF-β1 in Hepatoma Cell line. Hep3B (Human Hepatoma Cells) cells were cultured in DMEM supplemented with 10% heat-inactivated Fetal Calf Serum and 2 mM L-Glutamine. For cytokine treatment, serum-starved cells were treated with 10, 100, 200 and 500 U/ml TGF-β1 cytokine at certain time points (1, 3, 6, 24, 48 and 72 h). We obtained the effect of TGF-β1 on URG-4/URGCP expression at mRNA level with Real-Time PCR analysis and protein level with Western blotting. Our results showed an increased mRNA level of URG-4 gene during early (1–3 h) and late (48 h) time with 200 U/ml TGF-β1 treatment. In addition, we investigated dose-response of TGF-β1 on URG-4/URGCP expression. Basically, 100 and 200 U/ml of TGF-β upregulated URG-4 gene with 2- and 4-fold, respectively. Consistent with the mRNA results, the level of the URG-4 protein was significantly increased. Quantitative analysis shows a two-fold increase of the URG-4 protein level 48 h after TGF-β1 induction.

P03-050
Response of novel *Bacillus marmarensis* GMBE 72^T to extreme conditions: Poly (3-hydroxybutyrate)

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Petroleum-based plastics are used in various operations in different sectors including food packaging, communication, transportation, textile, shelter and health care etc. Approximately, 100 million tons of plastics are produced worldwide annually (according to 2011 data). On the other hand, chemically synthesized non-degradable conventional plastics derived from petroleum are a serious threat to the environment and organisms in the ecosystem. Production of bioplastics (polyhydroxyalkanoates) as alternatives to petroleum based non-biodegradable plastics are possible solutions for an eco-friendly technology. Polyhydroxyalkanoates (PHAs) are polyesters synthesized by numerous bacteria including *Bacillus* species as intracellular carbon and energy reserve, and are accumulated as granules in the cytoplasm.

Due to their good biodegradability, biocompatibility and also physical similarity with synthetic plastics, poly (3-hydroxybutyrate; PHB), is the most commonly used PHA with current applications including medicine, packaging industry, printing materials, agriculture, food industry, pure chemicals production, etc. Despite many advantages of PHB, there are some limitations in industrial applications one of which is few number of PHB producers.

The genome of *B. marmarensis* GMBE 72^T features alkaliphilic adaptations and pathway for PHB synthesis. In this study, it's aimed to investigate the response of *B. marmarensis* GMBE 72^T to extreme conditions for PHB production.

Keywords: Poly (3-hydroxybutyrate), Bioplastics, *B. marmarensis* GMBE 72^T, Extreme Conditions.

P03-051
Electrostatic properties of *Mycobacterium leprae* genes starts reflect massive pseudogenization and strictly intracellular parasitic life

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It is known that physical properties, especially electrostatics, play an important role for transcription regulation. We revealed earlier some common properties of the electrostatic potential distribution around transcription factors binding sites, promoters, especially their up-elements, and transcription start sites overall. All of them possess an increase in the electrostatic potential value that helps to locate and connect to binding sites for transcription regulation proteins – transcription factors and RNA polymerase. The resulting profile around transcription start sites has a common architecture among all prokaryotic taxa with variations in specific proportions between its elements.

Mycobacterium leprae is an intracellular parasitic bacterium that causes leprosy in human in tropical countries. It has undergone massive genome reduction over time as a result of its parasitic nature, discarding more than half its genes with many important metabolic activities and their regulatory circuits. Twenty seven percent of the *M. leprae* genome consists of pseudogenes that have functional counterparts in *M. tuberculosis*.

We have found that the electrostatic properties of *Mycobacterium leprae* gene starts reflect massive pseudogenization and strictly intracellular parasitic life with reduced transcription regulation. It has a smoothed electrostatic profile compared to its close relatives and far less pronounced increase of electrostatics over the upstream region where extended electrostatic deep is commonly found. Moreover, the genes remaining functional overall possess even less electrostatic typical features, reflecting the diminishing need for extensive transcriptional regulation. Obviously the lost genes needed more diverse regulation with more prominent role of electrostatics. DEPPDB was used to make the analysis.

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P03-052

A functional distinction between SAFB1 and SAFB2 via their distal Gly/Arg rich C-terminal domain

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The nuclear SAFB (Scaffold Attachment Factor B) proteins constitute a three-member family, with SAFB1 and SAFB2 displaying the closest evolutionary relationship (75% identity). The higher similarity between them is at their N-terminal region where are found the DNA (S/MAR) and RNA binding domains and it decreases towards the C-terminus, where a Glu/Arg- and a Gly/Arg-rich regions reside. SAFB1 and SAFB2 have been shown, among other major functions, to be transcriptional co-repressors of the ERa (Estrogen Receptor a).

The aim of this work was to distinguish between the functional properties of the SAFB1 and SAFB2 homologs. We have examined the interaction of SAFB2 with molecules sorted out in a two hybrid system with the SAFB1 C-terminal part of the molecule. Among the clones tested, one displayed strong interaction with SAFB1, but none with SAFB2. The clone encodes for ERH, a small protein (104 aa) of strikingly high inter-species conservation. ERH was bacterially expressed and shown to interact with mammalian and bacterially expressed human SAFB1, it interacts however also with the bacterially expressed SAFB2 (but not with the mammalian expressed). ERH abolishes SAFB1 but not SAFB2 co-repressor activity, indicating a functional interaction only with SAFB1. The SAFB1/2 region interacting with ERH was limited to the 207 most distal C-terminal amino acids.

These results indicate that the distal C-terminal part of the protein contains structural elements able to distinguish functionally between the two paralogs in an eukaryotic context.

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P03-053

Bending and electrostatics in transcription regulation are evident in genes starts in the context of organisms' temperature preferences

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Genome DNA's physical properties, such as curvature and electrostatics, play an important role in transcription regulation. The distribution of curved DNA in promoter regions is evolutionarily preserved, and is mainly determined by temperature of habitat. Mesophilic genomes may have different intensity in curvature, while thermophiles and hyperthermophiles lack it overall because of the life under temperature above the curvature-relaxing point that renders this property useless in transcription regulation. Magnitude of DNA curvature is related to AT content; it was shown that strongly curved DNA fragments must possess high A + T content (reverse is not true).

Our studies of electrostatic potential distributed along genome DNA found its local value also correlates with the AT content, though does not correspond exactly, and strongly depends on sequence arrangement and context.

We revealed some common properties of the electrostatic potential distribution around transcription factors binding sites, promoters, especially their up-elements, and transcription start sites. All of them possess an increase in the electrostatic potential value that helps to locate and connect to binding sites for transcription regulation proteins – transcription factors and RNA polymerase. The resulting profile around transcription start sites has a common architecture among all prokaryotic taxa with variations in specific proportions between its elements. There is no decrease in size and prominence of electrostatic deep in extremophyles, proving the importance of electrostatics and its differential role versus curvature. Intracellular parasite has smoothed electrostatic profile and no increase over the upstream region reflecting diminishing need for extensive transcriptional regulation. DEPPDB was used to make the analysis.

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P03-054

The determination of effect of some antibiotics on paraoxonase 2 (PON2) enzyme activities in human macrophages cell

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The human paraoxonase (hPON) gene family includes three members: PON1, PON2, and PON3, located adjacent to each other on the long arm of chromosome 7. The three hPONs share approximately 65% identity at the amino acid level and 70% identity at the nucleotide level. In humans, hPON1 and hPON3 are primarily expressed in liver while expression of hPON3 is also found in kidney. In contrast, hPON2 is more widely expressed and is found in a variety of tissues including the heart, kidney, liver, lung, placenta, small intestine, spleen, stomach, and testis. The effect of PON2 enzyme that is strictly expressed in macrophages. The aim of the study is to determine the *in vitro* effect of Cefazolin sodium, Gentamycin sulphate, ampicillin sodium and Chloramphenicol sodium succinate on PON2 enzyme in human macrophages cell, namely U937 cells. Two different substrates were used for the determination enzyme activity of PON2, lactose and phenylacetate Different concentrations of drug were used in order to see any effect on PON2 enzyme. These antibiotics reveal the differential effects on PON2 enzyme in U937 macrophages at different time points and concentrations of drugs.

Keywords: Paraoxonase, paraoxonase 2, antibiotics, inhibition, activation, U937 cells

P03-055

Suppression of *RUNX1-RUNX1T1* gene expression leads to activation of both survival and proliferation signaling in acute myeloid leukemia cells

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The t(8;21)(q22;q22) rearrangement represents the most common chromosomal translocation in acute myeloid leukemia (AML) and results in appearance of a transcript encoding for the fusion protein RUNX1-RUNX1T1 which is considered to be an attractive target for treating t(8;21) leukemia. However, expression of

this gene alone is insufficient to cause transformation, and thus the potential of such therapy remains unclear whether the RUNX1-RUNX1T1-driven activation of key genes, participating in tumor progression, may be sufficiently reversed by downregulation of RUNX1-RUNX1T1. We used the RNA-interference (RNAi) approach for elucidation of the functional consequences of *RUNX1-RUNX1T1* suppression in the t(8;21)-positive AML Kasumi-1 cell line. We observed significant decrease of *KIT* expression, inhibited growth and enhanced apoptosis of the leukemic cells. We obtained the model t(8;21) cell line with suppressed *RUNX1-RUNX1T1* able to proliferate for many passages, and performed a genome-wide microarray-based screening of gene expression. We showed that the supression of *RUNX1-RUNX1T1* can lead to up- and downregulation of signaling pathways enhancing cell survival and proliferation. We provide evidence that the inhibition of this oncoprotein or gene alone may be insufficient to treat the leukemic cells. We identified that the protein ERK2, one of the key regulators responsible for cell proliferation, can mediate activation of 23/29 (79%) of these pathways and, thus, may be regarded as the key player in establishing the anti-RUNX1-RUNX1T1 therapy resistant phenotype. We speculate that supplementing therapy with targeting ERK2 and/or other members of RUNX1-RUNX1T1 regulatory network may be advantageous in developing treatments of t(8;21)-positive leukemia.

P03-056
Rho-independent terminators may rely on electrostatics in their function

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Electrostatic and other physical properties of genome DNA, such as curvature, are important to its interactions with RNA polymerase.

Distribution of curved DNA in terminators is known to be mainly determined by temperature of habitat and is belived to play role in transcription pausing, facilitating termination. Mesophilic genomes may have different intensity in curvature, while thermophiles and hyperthermophiles lack it because of life under temperature above curvature-relaxing point, rendering it useless in regulation.

We found that Rho independent lambda and *E. coli* terminators have the same characteristic symmetrical M-like electrostatic potential profile in the core terminators area, reflecting their palindrome nature. Terminators sites have a pronounced rise in the negative potential value around them, that spans for nearly a hundred b.p. and clearly exceeds the terminator hairpin zone. Overall they have the same electrostatic potential profile scale, compared to RNA polymerase size, despite three-fold different annotated palindrome length.

The same overall properties are typical in a diverged range of bacterial taxa. There is no decrease in size and prominence of electrostatic deep in extremophyles, proving importance of electrostatics and its differential role versus curvature.

Quite different underlying sequences form the same electrostatic profile that carry out their biological functions. The rise of the potential in the surrounding area, long enough to be comparable with RNA polymerase size, may lead to transcription pausing and facilitate the termination by hairpin formation.

DEPPDB (deppdb.psn.ru) and its tools were used to make the analysis.

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P03-057
Sex description of cells supplied by commercial vendors

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Recently, sex/gender of the subjects began to be perceived as an important variable in researches, and guidelines have been established requiring the inclusion of both sexes in clinical and preclinical studies. However, recognition that sex of the cells can also influence the experimental results remains significantly low. To prove or nullify sex of the cells as an experimental variable, knowing and reporting the sex of the cells should become a prerequisite in basic research. Many researchers use commercially available cells without further characterizing them. Thus, researchers often do not know the sex of the cells they obtained unless the vendors provide the information. To comprehend how the major cell banks which supply cells around the world describe the sex of the cells, we analyzed the homepages of three representative cell banks, American Type Culture Collection (ATCC, http://www.atcc.org), European Collection of Cell Cultures (ECACC, http://www.phe-culturecollections.org.uk/collections/ecacc.aspx), and Japanese Collection of Research Bioresources (JCRB, https://cellbank.nibio.go.jp/english/). We found that the sexes of the cells provided by the aforementioned vendors are not clearly indicated in general. The perception of sex/gender as an important variable and the efforts to integrate this perception into research can directly result in the precise understanding of the etiology of a disease, increasing the effectiveness of treatment, and minimizing any side effects. By marking the sexes of the cells they provide, the commercial vendors would help researchers to evaluate sex as a possible variable in basic biomedical research.

P03-058
Paraoxonase1 gene polymorphisms and serum paraoxonase activity in Turkish non-Hodgkin's lymphoma patients

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Objective: The incidence of non-Hodgkin's lymphoma (NHL) has been significantly increasing since the 1950s. One possible mechanism may be oxidative stress as a result of environmental exposure to carcinogens. Paraoxonase (PON1) plays a preventive role against oxidative stress. The aim of this study was to investigate the possible association of PON1 192/55 polymorphisms in NHL Turkish patients.
Materials and methods: In our study, blood samples were optained from 93 patients with NHL and 93 healthy subjects as controls. PON1 192/55 gene polymorphisms were determined by real time PCR. PON1 activity was measured by spectrophotometric assay of p-nitrophenol production following addition of paraoxon.
Results: Allele and genotype analysis showed no significant differences between the risk of NHL and PON1 polymorphisms. The PON1 192 and 55 genotypes distributions were assesed in NHL and control groups: NHL: 48.4% (RR) and 38.7% (LL),

38.7% (QR) and 45.2% (LM), 12.9% (QQ) and 16.1% (MM) and control: 44.0% (RR) and 40.9% (LL), 45.2% (QR) and 50.5% (LM), 10.8% (QQ) and 8.6% (MM). No significant differences were found between controls and the PON1 genotypes (192 and 55) in NHL. Serum PON1 activity was lower in NHL patients (137.7 ± 96.2 U/ml) than the healthy controls (166.6 ± 110.4 U/ml), the difference was not statistically significant ($p > 0.05$). The highest PON activities were detected in QQ and LL genotypes ($p < 0.01$, $p < 0.05$), and the lowest activities in RR and MM genotypes ($p > 0.05$).

Conclusion: Our results suggest that the PON 1 activities are affected by PON1 genetic variability in Turkish NHL patients. Further studies are necessary to clarify the PON1 gene polymorphisms in larger series.

P03-059
Genes starts electrostatic profiles of phages resemble that of their hosts in a wide variety of prokaryotic taxa

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Physical properties, especially electrostatics, play an important role in transcription regulation.

We have shown earlier several common properties of electrostatic profiles around transcription factors binding sites, promoters, especially their up-elements, and transcription start sites overall. All of them possess an increase in the electrostatic potential value that help to locate and connect to binding sites for transcription regulation proteins – transcription factors and RNA polymerase. The resulting profile around transcription start sites has a common architecture among all prokaryotic taxa with variations in specific proportions between its elements. Most intriguing is the perfect alignment in the physical space of the major inflection points of electrostatic profiles of all the examined procariotic genomes in bacteria, archaea, viruses, mitochondria, and plastids.

We have found that the properties of electrostatic profiles of phages' gene starts frequently resemble that of their hosts in a wide variety of prokaryotic taxa, both Bacteria and Archaea, mimicking their main architecture details. However, they have a less pronounced increase of electrostatics over the upstream, especially far upstream, region where extended electrostatic deep is commonly found in cellular genomes. This may reflect an overall diminishing need for extensive transcriptional regulation compared to free-living host cells, in particular – the lack of transcription factors and their binding sites with prominent role of electrostatics. Also many phages have their own RNA polymerase that is usually a relatively small single-subunit protein which does not have an alpha subunit and thus does not rely on an electrostatic up-element in its functioning. DEPPDB was used to make the analysis.

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P03-061
Functional transcription factor binding sites from the *IL2Ra* locus that contain SNPs associated with autoimmune pathologies

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The Interleukin 2 receptor alpha (IL2RA) chain is required for the development of regulatory lymphocytes which play a central role in the control of autoimmunity. Not surprisingly, numerous single nucleotide polymorphisms (SNPs) that are associated with various autoimmune diseases have been located in the human *IL2RA* locus. One of these SNPs, rs61839660, overlaps with a number of characteristic epigenetic signatures of regulatory elements. In particular, this SNP maps to a putative binding site of Mef2c (myocyte-specific enhancer factor 2C) that is highly expressed in bone marrow and in peripheral B-cells. Using pull-down assay, we demonstrated rs61839660-dependent Mef2c binding to the corresponding DNA probe from *IL2RA* locus, with stronger binding to the common variant of the polymorphism. Currently, we are testing the ability of the sequences containing various SNPs from the first intron of *IL2RA* gene to regulate the activity of the *IL2RA* promoter, depending on the variant of the polymorphism. We aim to determine which transcription factors bind to SNPs that can directly influence IL2RA expression.

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P03-062
Characterization of genomic island responsible for the increased thermotolerance in *Cronobacter* strains

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Cronobacter spp. are opportunistic foodborne pathogens associated with serious infections in neonates and immunocompromised adults. *Cronobacter* is particularly tolerant to desiccation and some strains are also tolerant to elevated temperatures. Up to 10% of *Cronobacter* strains contain a genomic island, which is responsible for increased thermotolerance.

The aim of our study was to compare the thermotolerance genomic island from several *Cronobacter* strains. The locus from the *C. sakazakii* ATTC 29544 type strain is 18 kbp long and contains *thr*B-Q genes. In addition the whole thermotolerance island was found in two *C. malonaticus* strains and a shortened version containing only the *thr*BCD and *thr*OP genes was present in two *C. sakazakii* strains. Survival of strains containing the thermotolerance island at 58°C was significantly better compared with the deletion mutant lacking the whole locus. Strains with the longer island were 2–3 times more tolerant then the strains with the shortened version. These results were further confirmed by complementation of the deletion mutant with the thermotolerance island cloned in a low copy vector. We also observed that different thermotolerance between strains containing identical thermotolerance island was caused by different level of *rpo*S expression encoding for general stress-response sigma factor.

P03-063

Guanine-rich sequence-binding factor 1 binds to G-quadruplex structures in RNA

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Guanine-rich sequence binding factor 1 (Grsf1) is an RNA-binding protein (RBP) that functions as a regulator of post-transcriptional gene expression in the nucleus, cytoplasm as well as in mitochondria. The Grsf1 protein contains three quasi-RNA recognition motifs (qRRM) that bind to RNAs containing a cognate G-rich $AGGG^{A}/_{G}$ motif.

Our work is aimed at characterizing the properties of Grsf1 substrates. G-rich stretches of nucleotides have the tendency to form G-quadruplex structures. And these structures play roles in various post-transcriptional processes. By using CD-spectroscopy we are showing for the first time that the Grsf1 substrate RNAs fold into G-quadruplex structures. These structures are stabilized by K^+ and Na^+ ions. To investigate whether the G-quadruplex is necessary for recognition by Grsf1, we created mutated RNA substrates, in which either the cognate $AGGG^{A}/_{G}$ motif was deleted or the surrounding GG-repeats supporting the G-quadruplex were replaced by GA-repeats. CD spectroscopy revealed that $AGGG^{A}/_{G}$ deletion does not impair G-quadruplex formation. Instead GG- to GA-mutation of G-quadruplex stabilizing nucleotides abolished G-quadruplex formation. We then measured the effect of G-quadruplex formation on Grsf1 binding by employing quantitative RNA gel shift assays. Deletion of the cognate $AGGG^{A}/_{G}$ binding site impaired Grsf1 binding about 30-fold. Mutation of the neighboring GG-repeats however completely abolished Grsf1 binding. Taken together, these data indicate that the presence of the G-quadruplex is more important for Grsf1 binding than the so-called cognate binding motif $AGGG^{A}/_{G}$. The next step will be to investigate which domain interacts with $AGGG^{A}/_{G}$ and to study the functional relevance of these interactions.

P03-064

Dynamic transitions in gene expression states during neuronal differentiation

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In mouse Embryonic Stem Cells (ESCs), important developmental regulator genes are characterized by Polycomb repression in the presence of an unusual form of RNA polymerase II (RNAPII), which keeps these genes poised for activation. Polycomb is also present in an additional group of genes, important for signaling and energy metabolism, which are active in ESCs but fluctuate between active and repressed states in different cells or alleles. Many of these Polycomb-active genes may have roles in maintaining ESCs in a specific metabolic state (Brookes *et al.*, 2012).

To study how the complex interplay between RNAPII and Polycomb evolves during differentiation, we have mapped the occupancy of phosphorylated forms of RNAPII and Polycomb genome-wide along neuronal differentiation. Our time series starts from ESCs, capturing the early exit from pluripotency and extends to immature and mature post-mitotic neuronal states.

To gain understanding about the fluctuations between Polycomb-repressed and Active states, we have applied two new techniques to identify single-cell transcriptional profiles. We have used STAMM (State Transitions using Aggregated Markov Models, Armond *et al.*, 2014) to model single-cell states from mixed-cell population transcriptome data. Moreover, we have produced single-cell mRNA-seq transcriptional profiles at different time-points.

We characterized genome-wide the dynamics of the RNAPII-Polycomb interplay during neuronal differentiation. Our work shows that the Polycomb-active state is an important and dynamic promoter state, and shows a potential link between Polycomb and metabolic regulation.

P03-065

Sec16 alternative splicing controls the adaptation of the COPII machinery to higher secretory cargo load upon T-cell activation

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T-cell activation is a complex and dynamic process which forces the cell to dramatic changes like migration, proliferation and secretion of effector molecules. For the latter an increase in the T-cell's protein secretion capacity is required. Protein secretion begins at ER-exit sites, where newly synthesized proteins are packed in CopII coated vesicles for further transport. One major player in this process is Sec16, a scaffold protein with putative regulating properties.

We recently found that the C-terminal region (CTR) of Sec16, which is required for the interaction with other CopII members, is amongst others encoded by two regulated exons (E29 and E30). We detect four splice isoforms of which the isoform containing only E29 is substantially upregulated upon T-cell stimulation. We could show that the increase in the E29 isoform is required for an increase of CopII vesicles upon T-cell activation and thereby regulates efficient protein export. Furthermore, we found that the four isoforms exhibit different abilities to interact with CopII proteins thus providing a molecular basis for their different ability to control CopII transport.

In recent studies we generated genome engineered cell lines lacking Sec16 E29 via a CRISPR/Cas9 approach. These cells show obvious defects as they exhibit a reduced number in CopII vesicles and a reduced ER-export efficiency confirming a crucial role of Sec16 alternative splicing in controlling the CopII pathway.

Altogether our results describe how a signal induced splicing switch is of functional relevance for cells to adapt their secretory pathway to new environmental circumstances.

P03-066
Inhibition of ERN1 signaling of endoplasmic reticulum stress affects the expression of TNF receptor genes in U87 glioma cells

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Endoplasmic reticulum (ER) stress as well as hypoxia is an important factor of the tumor growth. Endoplasmic reticulum to nuclei-1 (ERN1) is a central mediator of the unfolded protein response. Blockade of the ERN1 in glioma U87 cells leads to a suppression of tumor growth in these cells. Tumor necrosis factor receptor superfamily (TNFRSF) plays an important role in the regulation of cell death and proliferation. The main goal of this work is to examine the association between the expression of genes encoded TNF receptors and function of ER stress signaling, mediated by ERN1, in normoxic and hypoxic conditions in U87 glioma cells. The expression of investigated genes in U87 glioma cells and its subline with ERN1 knockdown was measured by qPCR. Cells were treated by hypoxia (3% O2) for 16 h. We have shown that the blockade of ERN1 in glioma cells leads to significant up-regulation of the TNFRSF10D, TNFRSF21 and TNFRSF11B gene expressions and to down-regulation of the expression of TNFRSF10B and TNFRSF1A genes as well as to reduction of glioma cell proliferation. Hypoxia usually affects the expression levels of TNFRSF genes and effect of hypoxia on the expression of these genes mostly depends upon the ERN1 signaling enzyme function. Results of this study demonstrate the dependence of TNFRSF gene expressions in U87 glioma cells upon the ERN1 function indicating its participation in the regulation of metabolic and proliferative processes via ER stress. Moreover, hypoxia controls the expression of TNF receptor genes mostly through ERN1 signaling.

P03-067
FGF21 is down regulated by fasting in mice under leucine deficient diet

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Fibroblast growth factor 21 (FGF21) is a member of the FGF family with endocrine properties that functions as a regulator of energy, lipid and glucose metabolism. FGF21 is produced primarily in liver, but also in other tissues such as white and brown adipose tissue, skeletal muscle and pancreas.

During fasting, FGF21 plays an important role in eliciting and coordinating the adaptive response. Other conditions in which FGF21 expression and secretion are induced are leucine deficiency, carbohydrate restriction and fatty liver disease. Thus FGF21 is a regulator of physiological responses to metabolic stress.

FGF21 regulation in liver is complex and involves several transcription factors. During fasting it is strictly regulated by PPARa and during leucine deprivation ATF4 is the major regulator. In addition, PGC-1alpha negatively regulates hepatic FGF21 expression by modulating the heme/Rev-Erba axis.

In this work we show that FGF21 levels are increased – as expected – in mice under a leucine deficient diet. Surprisingly, when mice under leucine deficient diet were fasted, FGF21 levels decreased.

Our results suggest that Heme/Rev-Erba axis is regulating FGF21 levels in these situations; decreasing the repression under leucine deprivation and increasing repression when fasted.

P03-068
Sumoylation of histone deacetylase 2 regulates tumor relevant gene expression patterns

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Histone deacetylase 2 regulates biological processes by deacetylation of histones and non-histone proteins. It is subject of intensive research how posttranslational modifications (PTMs) govern histone deacetylase (HDAC) dependent control of cell proliferation and apoptosis. HDAC2 is modified by small ubiquitin-related modifier 1 (SUMO1) at K462 and this modification regulates the tumor suppressor p53 and NF-κB-dependent gene expression antagonistically. HDAC2 sumoylation enables HDAC2 to counteract PCAF-mediated p53 acetylation at lysine K320. Cells with sumoylated HDAC2 have less K320-acetylated p53, which is less efficient in binding to DNA both, *in vitro* and *in vivo*, and fails to regulate p53 target gene expression. In contrast, NF-κB-dependent gene expression is upregulated by HDAC2 and sumoylation is needed for this augmentation of NF-κB activity. At the molecular level, several factors were identified for being necessary for HDAC2-mediated NF-κB gene induction. The inhibiting effect of HDAC2 sumoylation on p53 signaling and the increased NF-κB-dependent transcription ultimately result in diminished apoptosis rates when challenged with genotoxic stress. An enhanced sumoylation of HDAC2 might provide an explanation for selection of high HDAC2 levels in tumors and contribute to giving these cells increased robustness against chemotherapy. The sum of these data shows that HDAC2 governs the survival of cancer cells and could be used as an important diagnostic marker and a useful therapeutic target in chemotherapy.

P03-069
CK1δ inhibits HIF-1-dependent induction of lipin-1 and reduces both lipid accumulation and cell proliferation under hypoxia

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Cell proliferation under hypoxic conditions requires metabolic adaptation, which is mainly mediated by the transcriptional activator Hypoxia Inducible Factor-1 (HIF-1). Expression and activity of its regulatable subunit (HIF-1α) is controlled by oxygen as well as by oxygen-independent mechanisms involving phosphorylation. Our previous *in vitro* studies have shown that CK1δ phosphorylates HIF-1α in its N-terminus and reduces the affinity for its heterodimerization partner ARNT. Furthermore, our *in situ* studies in intact or living cells have confirmed that CK1δ impairs the intranuclear formation of the HIF-1α/ARNT complex. To evaluate the physiological significance of these findings, we inves-

tigated the involvement of CK1δ in the induction of the HIF-1 target gene lipin-1, which plays a critical role in triglyceride synthesis and lipid droplet formation under hypoxia. We now show that CK1δ inhibition using D4476, a specific chemical inhibitor, causes increased HIF-1 binding to the *Lpin1* promoter and upregulates lipin-1 protein expression under hypoxia. Treatment with D4476 also significantly increases lipid droplet formation under hypoxia while overexpression of CK1δ causes the opposite effect. Importantly, CK1δ inhibition also stimulates cellular proliferation under hypoxia in both cancer and primary non-transformed human cells. This stimulation is HIF-1- and lipin-1-dependent as it is abolished by siRNA-mediated repression of HIF-1α or lipin-1 expression, respectively. Collectively, our results suggest a novel role of CK1δ in HIF-1-mediated adaptation of cells to low oxygen conditions and can lead to the development of new molecular strategies for the treatment of hypoxia-related pathological conditions.

P03-070
RNA half lives and transcriptional delays determine transcript dynamics in response to MAP kinase signalling

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The classical MAP kinase signalling pathway controls fundamental cellular processes such as growth and differentiation and is deregulated in at least one-third of all cancers. Whereas the quantitative details and dynamics of signal transduction have been studied extensively the gene regulatory response to MAP kinase signalling and its network organisation lacks detailed understanding. To investigate how MAP kinase signalling regulates transcription and transcript stability, we obtained gene expression time course data from a human cell culture system which synthetically activates the oncogene RAF. With help of mathematical modelling and computational data analysis, we estimated RNA half lives and transcriptional delays. Thereby, we identified a new cluster of immediate long-lived genes (ILG) that could have a poised promoter state like immediate early genes (IEG) although their response times are in the range of delayed early genes (DEG). Taking temporal organisation of primary and secondary response genes into account, we furthermore inferred gene regulatory networks from the given expression data to develop characteristic transcriptome-wide response profiles to both normal and aberrant MAP kinase signalling. Here, we describe different transcriptional waves and how they relate to each other. Moreover, we reveal different transcript clusters sensitive or insensitive to subsequent targeted inhibition of MAP kinase signalling in our highly controllable model system. By illuminating the gene regulatory dynamics in response to both normal and aberrant MAP kinase signalling we help to better understand crucial regulatory layers controlled by this highly relevant pathway and how they are affected by targeted therapy.

P03-071
Investigating a model of combinatorial gene regulation by FOX and E-box-binding factors in FLT3-ITD Acute Myeloid Leukaemia

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Internal tandem duplication (ITD) of the FLT3 locus occurs in up to 34% of Acute Myeloid Leukaemia (AML) cases, and correlates with a poor clinical outcome. Genome-wide DNase I data generated in this laboratory has identified a signature of 500–1000 *cis*-regulatory elements which are specifically activated in FLT3-ITD AML patients. *De novo* motif discovery performed on these *cis*-regulatory elements revealed enrichment of several DNA motifs predicted to be bound by transcription factors. These included a novel FOX/E-box (FOX/E) composite binding motif. The aim of this study is to investigate how these FOX/E composite sites are regulated in FLT3-ITD AML.

Analysis of genome-wide mRNA expression data found a broad range of both FOX and E-box binding transcription factors to be abundantly expressed in AML patient samples and cell lines bearing FLT3-ITD. Furthermore, Electrophoretic Mobility Shift Assays (EMSAs) performed with FOX/E sites confirmed specific binding to the E-box motif within the element. However, evidence of a FOX-binding pattern was observed in just one of three FLT3-ITD AML cell lines investigated, suggesting that either these are poor models of AML or that the EMSA conditions used were sub-optimal.

To further optimise EMSA conditions, we have achieved specific binding activity in EMSAs using recombinant FOX proteins and are performing assays with material from a FLT3-ITD patient sample. We are also investigating the biological activity of FOX/E elements using luciferase reporter constructs. Future work will dissect further the function of these composite motifs to gain insight into their role in FLT3-ITD AML.

Gen Ex S3, Translational Control and Protein Turnover

P04-005-SP
Mechanistic dissection of the early phase of UsnRNP biogenesis uncovers a role of ribosomes in assembly and RNP homeostasis

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Formation of macromolecular complexes within crowded cellular environment requires aid from assembly chaperones. An interplay between PRMT5- and SMN-complexes has been shown to mediate formation of the common core structure comprising of seven proteins of the Sm/Lsm protein family bound to snRNA of the spliceosomal UsnRNPs. After spatial arrangement of Sm/Lsm proteins by assembly chaperone pICln, SMN-complex catalyzes snRNP formation by transferring these Sm/Lsm proteins onto snRNA. How newly synthesized proteins engage with the cellular assembly machinery to evade aggregation and/or mis-assembly has remained elusive. Here we report that newly synthesized Sm/Lsm proteins remain bound to the ribosome near the polypeptide

exit tunnel upon translation termination. Release from ribosome is dependent on pICln, analogous to the role of folding chaperones. pICln ensures formation of cognate Sm/Lsm heterooligomers and their chaperoned guidance into late assembly stage mediated by SMN-complex. Inactivation of SMN-complex leads to block of Sm protein flow through the assembly line and accumulation of Sm proteins on pICln. However, removal of pICln leads to the retention of Sm protein on the ribosome and eventually their translational down-regulation. Our results identify an elaborate assembly line for UsnRNPs in which the ribosome plays a crucial part as a quality control hub and starting point for the chaperone-mediated assembly. Furthermore the coordinated hand-off of newly synthesized UsnRNP subunits from ribosome to specialized assembly chaperones likely safeguards individual subunits from mis-assembly and aggregation and determines cellular snRNP homeostasis. These snRNP assembly principles may also find use in the assembly of other macromolecular complexes.

P04-006-SP
The Ubiquitin-Proteasome System as a central regulator of cellular antioxidant responses, mitostasis and proteostasis

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Proteome quality control is critical for cellular functionality and it is assured by the curating activity of the proteostasis network. Central to the proteostasis network functionality is the Ubiquitin-Proteasome System. We recently reported that proteasome is differentially regulated in the gonads and somatic tissues of *Drosophila* flies during ageing. We also found that transient proteasome dysfunction triggers the activation of an Nrf2-dependent tissue- and age-specific regulatory circuit aiming to adjust the cellular proteasome activity according to temporal and/or spatial proteolytic demands; prolonged deregulation of this proteostasis circuit accelerates ageing. We report herein that RNAi of various proteasomal subunits in *Drosophila* flies exerts differential effects on proteasome functionality and organism viability. Also, proteasome dysfunction in flies' somatic tissues resulted (apart of Nrf2 activation) in upregulation of molecular chaperones; the induction of the selective autophagy adaptor protein p62/SQSTM1 and in the activation of cellular cathepsins. It also disrupted the mitochondrial respiration and membrane potential; the assembly of the mitochondrial respiratory complexes and the structure of the mitochondrial cristae. On the other hand, overexpression of the β5 or the β2 20S proteasome peptidases in transgenic flies induced higher proteasome activities and increased resistance to stress. The increased proteasome activity in the transgenic flies related to a FOXO-dependent upregulation of the Rpn6 subunit that enhanced the 26S proteasome assembly. These findings indicate that proteasome activity maintenance is regulated by both the Nrf2 and FOXO transcription factors; they also suggest that proteasome functionality is critical for mitostasis and cellular proteostasis.

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P04-007-SP
Cardiac sympathetic neuron distribution controls myocardial cell size by local modulation of cardiomyocyte proteostasis

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Purpose: The myocardium is highly innervated by Sympathetic Neurons (SNs), that distribute within the tissue with a well-define pattern, and we have previously shown that neuronal input to cardiomyocytes represses proteolysis and activates protein synthesis, through the β2-AR dependent signaling (*Zaglia et al. Cardiovasc Res, 2013*). We here tested the hypothesis that regional differences in SN distribution reflect on heterogeneity in cardiomyocyte proteostasis and cell size.
Results: In the mouse heart, SNs are predominantly found in the outer myocardial cell layers, and, consistently, CMs in the outer layers (EPI) are significantly larger than the similarly oriented ones in the innermost (ENDO) layers (SNs/CM: EPI 0.45 ± 0.06 versus ENDO 0.15 ± 0.02; CM volume: EPI 8590 ± 2121 versus ENDO 4697 ± 1433, in μm^3). Such differences disappear upon SN ablation in the adult mouse, and never establish when SNs are ablated before the postnatal period in which sympathetic innervation of myocardium takes place (P3–P21). Moreover, genetic ($\beta 2\text{-}AR^{-/-}$ mice) or pharmacologic (ICI118,551) interference with β2-AR signaling results in the ablation of the CM size heterogeneity. Furthermore, we demonstrated by ISH that CM size heterogeneity is the result of local control on the UPS operated by SN. This concept holds true regardless of the innervation pattern, as in the heart of other rodents and mammals (e.g. rat, rabbit, pig), characterized by different cardiac neuron distribution pattern, cardiomyocyte size follows neuronal density.
Conclusions: This is the first evidence, so far, of an otherwise homogeneous tissue shaped by a superimposed innervation pattern through the modulation of intracellular protein turnover.

P04-008-SP
Stat1 stimulates cap-independent mRNA translation to inhibit proliferation and promote survival in response to anti-tumor drugs

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The signal transducer and activator of transcription 1 (Stat1) functions as tumor suppressor via immune regulatory and cell-autonomous pathways. Herein, we report a novel cell-autonomous Stat1 function, which is its ability to exhibit both anti-proliferative and pro-survival properties by facilitating translation of mRNAs encoding for the cyclin-dependent kinase (cdk) inhibitor $p27^{Kip1}$ and anti-apoptotic proteins X-linked inhibitor of apoptosis (XIAP) and B-cell lymphoma xl (Bcl-xl). Translation of the select mRNAs requires the transcriptional function of Stat1 resulting in the up regulation of the p110γ subunit of phosphoinositide 3-kinase (PI3K) class IB and increased expression of the translational repressor translation initiation factor 4E (eIF4E)-binding protein 1 (4EBP1). Increased PI3Kγ signaling promotes the degradation of the eIF4A inhibitor programmed cell death protein 4 (PDCD4), which favors the cap-independent translation

of the select mRNAs under conditions of general inhibition of protein synthesis by upregulated 4EBP1. As such, Stat1 inhibits cell proliferation but also renders cells increasingly resistant to anti-proliferative effects of pharmacological inhibitors of PI3K and/or mammalian target of rapamycin (mTOR). Stat1 also protects Ras-transformed cells from the genotoxic effects of doxorubicin in culture and immune deficient mice. Our findings demonstrate an important role of mRNA translation in the cell-autonomous Stat1 functions with implications in tumor growth and treatment with chemotherapeutic drugs.

P04-009
Low expression of Stem-Loop Binding Protein (SLBP) in G1 is ensured by coordinate action of different postranscriptional mechanisms

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Expression of replication-dependent histone mRNAs is tightly regulated during cell cycle and much of regulation is at posttranscriptional level. SLBP binds to 3′ end of histone mRNAs and is required for histone pre-mRNA processing, export, and translation. SLBP mRNA expression is not significantly changing throughout the cell cycle but protein expression is cell cycle regulated. SLBP expression is low outside the S phase and this is a major mechanism that limits histone biosynthesis to S phase. Previously, it was proposed that inefficient translation was responsible for low SLBP expression during G1. Here, using synchronized cells, by pulse labeling newly synthesized proteins with S^{35} methionine, we showed that low production rate of SLBP is limited to early G1 and rate of translation reaches to S phase level somewhere between early and mid-G1. Interestingly, despite the enhanced rate of synthesis, expression of SLBP is still kept low for couple of more hours until G1/S transition, which suggests presence of another mechanism with contrary effect. Therefore, we checked the stability of SLBP and revealed that SLBP is unstable during this period of G1 due to rapid degradation by proteosome. When we treated those G1 cells with proteosome inhibitor, we found pronounced accumulation of SLBP showing that SLBP is produced and degraded simultaneously in this period of the cell cycle. In conclusion, we found that in G1, SLBP is kept low by coordinate action of translation regulation and proteosome mediated degradation in order to ensure the shutdown of histone production before S phase.

P04-010
The tumor suppressor gene TIP30 impedes pressure overload induced cardiac hypertrophy by inhibiting protein synthesis

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Cardiac hypertrophy describes a state of heart muscle overgrowth that is characterized by enhanced synthesis of contractile proteins, which often triggers the development of heart failure. Regulatory mechanisms of increased protein synthesis during hypertrophy remain poorly defined. Here, we examined the cardiac function of *Tip30*, which was previously reported as tumour suppressor gene.

We identified myocardial *Tip30* mRNA as downregulated in mouse models of cardiac hypertrophy and in human failing hearts. In order to assess the functional consequences of diminished myocardial TIP30 in disease, we subjected heterozygous TIP30-mice (HET) to experimental cardiac pressure overload (TAC) to induce hypertrophy. Compared to wild-type mice, HET mice developed aggravated cardiac growth and dysfunction. In turn, TIP30 overexpression reduced cardiomyocyte hypertrophy in isolated rat cardiomyocytes as well as in mice.

Mechanistically, proteomic analysis after pull-down screens revealed the eukaryotic translation elongation factor 1A1 (eEF1A1) as binding partner of TIP30. Mapping of the binding domains showed that TIP30 bound eEF1A1 at the same region as its co-factor 1β2 (eEF1B2). When analysing the eEF1A1-eEF1B2 interaction *in vitro*, we found that addition of increasing TIP30 concentrations interfered with the association of both molecules. This was confirmed by proximity ligation assays in TIP30 overexpressing rat cardiomyocytes. Likely as a result, we found a significantly reduced protein-synthesis rate in isolated cardiomyocytes after TIP30 overexpression as well as an enhanced synthesis rate of contractile proteins in TIP30 HET mice after TAC.

Myocardial TIP30 inhibits hypertrophy and cardiac dysfunction during pressure overload in mice, most likely by interfering with protein synthesis.

P04-011
Sumoylation of thymidylate synthase enhances its stability

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We showed previously that depletion of SUMO ligase hMMS21 decreased thymidylate synthase (TYMS) levels in MCF-7 breast cancer cells. In the present study, we further investigated the underlying mechanism. Analyses of mRNA levels by real-time PCR and time-dependent changes in protein abundance following cycloheximide treatment showed that the stability of TYMS was decreased by hMMS21 depletion. To further assess whether sumoylation was involved, we constructed a His-tagged TYMS

expression vector (pcDNA3.1-TYMS-Myc/His), and various deletion and site-directed mutants which did not include the predictable sumoylation sites. Co-transfection of cells with pcDNA3.1-TYMS-Myc/His (or its mutants) and HA-tagged SUMO expression vectors, followed by pull-down experiments with Ni-resin, and immunoblot analyses with appropriate antibodies, demonstrated that only His-tagged TYMS or its mutant containing the sumoylation site (K308) could be sumoylated in the presence of hMMS21. In addition, the expression of His-tagged TYMS rather than its mutants lacking the SUMO sites was down-regulated by depletion of hMMS21. Taken together, the results suggest that the stability of TYMS is increased by hMMS21-mediated sumoylation.

P04-012
Human LACE1 mediates degradation of nuclear-encoded complex IV subunits, acts pro-apoptotic and functionally interacts with p53 tumor suppressor

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LACE1 (Lactation elevated 1) protein is human homolog of yeast Afg1 (ATPase family gene 1) ATPase. Afg1 was shown to be required for degradation of mitochondrially encoded complex IV subunits. LACE1 protein consists of ATP/GTP binding P-loop motif and common five domain structure and predicted ATPase activity.

The aim of our work was to perform detailed cell biological characterization of human LACE1 using human embryonic kidney cell line (HEK293).

Human LACE1 is mitochondrially localized integral protein with apparent molecular weight of 50 kDa and resides in the membrane as complex of 130–140 kDa. Prepared stable shRNA LACE1 KD HEK293 showed reduced LACE1 protein levels and elevated protein levels of complex IV nuclear-encoded subunits COX4, COX5a, COX6a and COX7a. YME1L and PARL proteases and p53 were found upregulated and LON and HTRA2 proteases and MRPS31 protein were found attenuated. Under constitutive condition and staurosporine-induced LACE1 KD cells exhibited attenuated levels of cleaved PARP. Increased activity of complex I and complex II were found. Using affinity purification of LACE1-FLAG expressed in LACE1 KD background we show that protein physically interacts with COX5a subunit. Fluorescent microscopy showed that LACE1 KD cells have fragmented mitochondrial network and plasmid-based reintroduction of wild-type LACE1 into KD cells fully rescued phenotype. Transmission electron microscopy of LACE1 KD cells showed altered mitochondrial shape and cristae morphology.

Our results demonstrate crucial and complex involvement of LACE1 in maintenance of mitochondrial proteostasis.

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P04-013
Expression of bovine cytochrome P450scc enzyme system in *Saccharomyces cerevisiae* cells as a self-processing polyprotein

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A tool for co-expression of proteins to study properties of multicomponent protein systems has been provided by the so called 2A peptide technology, based on picornavirus-derived 2A peptide self-cleavage trait. The 2A peptide is used for the synthesis of multiple protein moieties from single transcript. During translation dissociation of the polyprotein occurs at the C-terminus of the 2A sequence, resulting in formation of individual proteins.

Mammalian cytochrome P450scc system includes the cytochrome P450scc (CYP11A1) and electron transfer proteins, adrenodoxin reductase (AdR) and adrenodoxin (Adx). Cytochrome P450scc in adrenal cortex catalyzes cholesterol side-chain cleavage, the initial step of steroidogenesis.

A coexpression system was established in *S. cerevisiae* yeast using plasmid with genes of P450scc system proteins linked by the FMDV 2A region in a single ORF. A polycistronic plasmid pYeDp/HGL_2A was constructed with cDNAs for P450scc-system proteins located in the transcription unit in oder: P450scc-2A-Adx-2A-AdR. pYeDp/HGL_2A was shown to direct the synthesis of heterologous proteins in yeast to a high yield. Western blot analysis showed that the each component derived from polyprotein (P450scc-2A, Adx-2A, and AdR) was expressed as individual protein. Additionally, significant portion of AdR and Adx are presented in cells in a form of hybrid Ax-2A-AdR. Therefore, the first 2A linker provides efficient "cleavage" of the polypeptide, the efficiency of the second 2A linker is lower.

As evidenced by ELISA, cell-free homogenate of the recombinant demonstrated hydroxylase/lyase activity towards 22(R)-hydroxycholesterol, which testified to the presence of catalytically active P450scc enzyme system.

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P04-014
Role of ZFAND family members in proteasomal protein degradation

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Proteasomal protein degradation plays a major role in eukaryotic protein quality control. The chaperone-related, ubiquitin-selective AAA protein Cdc48 (a.k.a p97/VCP) segregates ubiquitylated proteins from their cellular environments for subsequent proteasomal degradation. Cdc48 is involved in various cellular pathways including ER-associated protein degradation (ERAD), ribosome-bound quality control, DNA damage repair pathways and many others. Control of these distinct Cdc48 activities is performed by a large number of regulatory cofactors that control identity and fate of Cdc48 substrates [1].

Recently, the human AIRAP (arsenic-inducible proteasomal 19S regulatory particle-associated protein; a.k.a Zfand2a) and AIRAPL (a.k.a. Zfand2b) proteins were found to be induced by exposure to arsenite and to activate the clearance of damaged proteins by the 26S proteasome [2,3]. AIRAP and AIRAPL belong to a subfamily of ZFAND (zinc finger AN1-type domain) proteins characterized by two aminoterminal AN1-type zinc fin-

gers mediating proteasome binding. This subfamily also comprises their worm homolog AIP-1, the uncharacterized human Zfand1 protein and its putative yeast homolog Cuz1. Here, we show that Cuz1 interacts directly with Cdc48, consistent with recent reports [4,5]. We mapped the binding sites of Cuz1 and Cdc48/p97 and demonstrate an increased interaction upon arsenite stress. In ongoing work, we characterize the role of Cdc48 and its novel cofactor Cuz1 in response to arsenite proteotoxicity and in other cellular stress responses.

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P04-015
Evidence for translational regulation of the ZAC1 transcription factor in prostate cancer cells

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The ZAC1 transcription factor regulates several members of an imprinted gene network (IGN) coordinating growth and metabolic activity especially in the embryo. In prostate cancer (PCa), expression of the network genes including ZAC1 is commonly diminished. We have therefore investigated the effect of ZAC1 overexpression on PCa cell lines.

In transient transfection experiments, both major ZAC1 isoforms induced the imprinted genes H19, IGF2 and CDKN1C/p57KIP2 in 22Rv1 cells. In stable transfection experiments, both isoforms suppressed clonogenicity of the PCa cell lines 22Rv1, LNCaP and PC-3. In all these experiment, the ZAC1delta isoform exerted the strongest effects. Intriguingly, cell clones derived from each cell line with inducible mRNA expression of the major form ZAC1 displayed no discernable increase in protein expression. Likewise, expression of the major ZAC1 isoform protein was weak compared to the ZAC1delta isoform in transient transfection experiments, but was strongly enhanced by proteasome inhibitors or more moderately by PI3K pathway inhibitors. However, neither ZAC1 mRNA nor protein half-life were particularly short. Removal of 5′-sequences from the ZAC1 cDNA bolstered ZAC1 protein expression.

Our data confirm that ZAC1 is a nodal regulator of the IGN in prostatic cells. Its downregulation in prostate cancer may therefore contribute to the overall decreased expression of the IGN genes. Moreover, ZAC1 reexpression is growth-inhibitory in prostate cancer. In addition, our findings uncover an hitherto unreported posttranscriptional regulation of ZAC1, which most likely occurs at the level of translation.

P04-016
Kallikrein 11 as a novel biomarker in meningioma and glioblastoma tumors

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Kallikreins are a subgroup of serine protease family and are widely expressed in different tissues. The human tissue kallikrein family consists of 15 genes which are located on the 19th (19q13.4) chromosome. Several studies have shown that the human kallikrein family may be related with cancer. Their expression profiles vary in several malignancies. Kallikrein 11 (*KLK11*) is expressed in many tissues such as brain, skin, stomach, prostate and intestine. KLK11 mRNA and protein expression was detected previously in various human cancer tissues including breast, gastric, colon and ovarian carcinoma. In this study it was aimed to determine KLK11 mRNA and protein expression in meningioma and glioblastoma brain tumors.

mRNA and protein expressions of KLK11 were examined by using reverse transcriptase polymerase chain reaction (RT-PCR) and Western blot techniques respectively in meningioma (n = 15) and glioblastoma (n = 15) samples. Pearson Chi-Square and Yate's correction tests were used for the statistical analyses and $p < 0.05$ was considered statistically significant.

KLK11 mRNA and protein expression was determined in 86.7% of meningioma group and in 73.3% of glioblastoma group. The results suggested that both mRNA and protein expression of KLK11 were relatively higher in meningioma group than those were in glioblastoma group. Since KLK11 mRNA and proteins were found to be expressed more frequently in meningioma group, further studies need to be carried out in order to consider KLK11 as a novel biomarker in brain tumors.

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P04-017
Regulation of p53-dependent metabolic functions by zinc finger protein ZPR9

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The p53 tumor suppressor protein plays a key role in the regulation of metabolic homeostasis. The zinc finger protein ZPR9 is a physiological substrate of murine protein serine/threonine kinase 38 (MPK38), a member of the AMPK-related kinase family. Here, ZPR9 physically interacts with p53 through a disulfide linkage involving Cys^{238} and Cys^{242} of p53 and Cys^{221} and Cys^{254} of ZPR9. Wild-type ZPR9, but not C221S/C254S and T252A (MPK38-mediated phosphorylation-defective) mutants, positively regulated p53 signaling by destabilizing complex formation between p53 and its negative regulator Mdm2 or by increasing complex formation between p53 and its positive regulators, serine-threonine kinase receptor-associated protein (STRAP) and 14-3-3. In addition, ZPR9 promotes the p53 nuclear translocation via a direct interaction. ZPR9 functionally potentiates the p53-mediated inhibition of SREBP1, PPAR-γ, NF-kB, pyruvate dehydrogenase kinase 2 (PDK2), glucose transporter 1/4 (GLUT1/4), or glucose-6-phosphate dehydrogenase (G6PD) transactivation and stimulates the p53-induced glutaminase 2 (GLS2) transactivation, leading to beneficial metabolic profiles. MPK38-mediated phosphorylation of ZPR9 at Thr^{252} is also required for these metabolic processes. These findings suggest that ZPR9 functions as a potential regulator responsible for controlling the metabolic functions of p53.

P04-018
An inter-organ crosstalk exists to regulate the bioavailable copper levels in circulation

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In mammals copper participates in signaling and catalytic activity of vital cuproenzymes. Imbalance of copper homeostasis results in severe disorders. Copper homeostasis in tissues is strongly dependent on the extracellular copper level, which is defined by the level of oxidase activity of ceruloplasmin (Cp, multicopper ferroxidase and major copper-transporting protein including 95% of serum copper). The existence of whole body mechanisms, supporting Cp-required bioavailable copper level was examined in present work. Three animal models were used: mouse, growing tumor, adrenalectomized rats, and Ag-rats characterized by Cp-responsible copper deficit (rats were fed with fodder containing Ag(I) from birth to sexual maturity). The last model is based on the electronic similarity between Cu(I) and Ag(I) that allows Ag(I) to use copper-transporting proteins. As a result Cp loses oxidase activity and Cp-copper deficit is developed. In the models copper status indexes (oxidase activity, both Cp and metallothionein proteins concentration, Cu(Ag) pools associated with them) were measured. Cu(Ag) distribution into body was determined. In organs the activity of genes coding cuproenzymes, Cu(I)-chaperons, copper-reserving proteins were valued by RT-PCR and Western-blot. It was shown that growing tumors of various origins increase copper metabolism in the liver. The adrenals control copper excretion in the liver. In Ag-rats as was shown in pulse-chase experiments, non-hepatic Cp isoform appeared in the bloodstream; moreover the induction of Cp gene activity was found in the fat tissues. The inter-organs crosstalk controlling copper status in whole body is discussed.

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P04-019
The mechanism of the crocin-induced apoptosis in primary epithelial breast cancer cells

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Natural products including saffron have been used to prevent and treat various diseases and might be good candidates for the development of anticancer drugs. Carotenoids (crocin and crocetin) and monoterpene aldehydes (safranal and picrocrocin) are potent ingredients of the saffron stigma. Breast cancer is the second leading cause of cancer death and the most common malignancy among women. Mechanism of action of many cancer therapeutic agents involves the induction of apoptosis. Apoptosis occurs through two main pathways; extrinsic (cytoplasmic) and intrinsic (mitochondrial) pathway. The intrinsic pathway arises when stimulated leads to the release of cytochrome C from the mitochondria, activation of caspase-9 and induction of the death signal. PI3K is up-regulated in many cancers. Akt kinase activation leads to phosphorylation of caspase-9 that blocks the induction of apoptosis.

In this study, the freshly isolated normal and cancerous human epithelial cells from the mammoplasty and human breast tumors, respectively; were exposed to different concentrations of crocin at different time intervals. Flow cytometric analysis and caspase-9 expression were shown that crocin treatment resulted in the apoptosis induction and increasing in the expression of caspase-9 in cancerous epithelial cells (determined by western blot), while there was no effect on normal cells. These results are compatible with our previous data obtained on both preventive and therapeutic effect of crocin on NMU-induces breast cancer in rat. In conclusion, crocin could be used as a chemopreventive as well as a chemotherapeutic agent for breast cancer in human.

P04-020
Interactions of DnaA protein with DnaA boxes in Origin of replication in acetic acid cells

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DnaA protein is encoded on the chromosome of the bacteria. It is replication-initiating protein and it belongs to the family of AAA + proteins. Homologous proteins to DnaA are found in all domains of life. It is composed of four domains, each with a specific function [1]. This protein has the ability to interact with specific binding sites at the origin of DNA replication- so called DNAA boxes. Moreover it interacts with other proteins, for example, DnaB helicase, Hu protein, IHFα and IHFβ proteins. In addition to the initiation of replication DnaA protein works as a transcription factor [2]. This protein has been extensively studied in the model organism *E. coli*. In this work we focused on the study of the mechanism of replication initiation in acetic acid bacteria cells. We cloned *dnaA* gene into expression vectors pET28a+ and pET30a+, to ensure fusion of target protein with His-taq for the needs of the DnaA purification. We overexpressed and purified recombinant proteins on nickel column. In previous work we have identified the origin of replication in *Acetobacter pasteurianus*. We have prepared mutated forms of *Acetobacter pasteurianus* origins of replication. We are using theese in EMSA assays to study interactions of DnaA protein with specific DNAA boxes. Our goal is to identify the minimal necessary structure in origin of replication which is able bind DnaA protein and initiate the DNA replication process.

P04-021
Human translational elongation factor eEF1γ subunit utilizes its GST-like domain to form an eEF1B complex and interact to aminoacyl-tRNA synthetases

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Two elongation factors, eEF1A and eEF1B are necessary for the elongation step in eukaryotic protein biosynthesis. eEF1A requires GTP for the transportation of an aminoacyl-tRNA to the ribosome and eEF1B promotes the exchange of GDP to GTP. Human eEF1B consists of α, β and γ subunits and assembles into a heavy complex, eEF1H with eEF1A. Both α

and γ subunits have a GST-like domain at their N-terminus. Using the GST-like domain, one γ subunit can bind another γ and α subunits simultaneously to form a heterotetrameric complex. The GST-like domain of γ subunit can also bind ValRS and CysRS, aminoacyl tRNA synthetases containing a GST-like domain. These results suggest that eEF1γ employs a GST-like domain to play a role for channeling to tRNA in human translational system.

P04-022
A complex structure of human EPRS and AIMP2 GST-like domains

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Aminoacyl-tRNA synthetases are essential enzymes catalyzing the ligation of their cognate amino acids and tRNAs. In the mammalian, nine different aminoacyl-tRNA synthetases (ARS) are bound together in the multi-synthetase complex, with three ARS interacting multi-functional proteins (AIMPs). In this complex, glutamyl-prolyl tRNA synthetase (EPRS) forms a specific association with the AIMP2. Both EPRS and AIMP2 contain a GST-like domain at the N-terminus and C-terminus, respectively. The two proteins can form a complex using their GST-like domains, and the complex structure of their GST domains was determined at the resolution of 2.6 Å. Both GST domains consist of GST-N and GST-C subdomain. The GST-N of EPRS has four-stranded b-sheet while that of AIMP2 has five-stranded b-sheet. In the binding interface, two arginine residues from each GST-N domains are stacked and stabilized by aspartate residues from GST-C domains. The overall interaction of the heterodimer is that of a canonical GST dimer.

P04-023
Investigation of factors influencing the heterologous production of polyomavirus-like particles in yeast

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Efficient production of heterologous virus proteins in yeast is dependent on multistep processes involving regulation at the level of transcription, mRNA turnover, protein translation, post-translational modifications or assembly of virus like-particles (VLPs). On the other hand, heterologous gene expression is dependent on cellular protein factors that may be involved in supporting or inhibiting these processes in the yeast cells. In this study we explored yeast expression system to demonstrate that human polyomavirus 12 (HPyV12) VP1 protein is most likely synthesized starting from the second of two potential translational initiation sites within the VP1 gene. This resulted in an efficient translation of the 364 aa-long VP1 protein and its self-assembly into VLPs. We also evaluated the influence of codon bias on the production of HPyV12 VP1 protein in yeast by expressing two codon-modified open reading frame variants: one encoding HPyV12 VP1 protein with native virus codons and the second with codons optimized for *S. cerevisiae* expression. Our study showed that there was a significant positive correlation between the gene expression level and the degree of its codon bias towards the favorable yeast codon usage. This effect most probably was dependent on the improved translational elongation.

P04-024
TCTP is induced early in colorectal cancer, it is translationally regulated via the Akt/mTORC1 pathway, and it contributes to the resistance of HCT116 colon cancer cells to 5-FU and oxaliplatin

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Translationally controlled tumour protein TCTP is a highly conserved, cytoprotective protein that is frequently overexpressed in cancers. To understand how TCTP is regulated in human colon cancer, we investigated TCTP levels in colorectal tumours using immuno-histochemistry. We show that TCTP levels are low in normal colon tissue, but increase in adenomas and further to the adenocarcinoma stage. Growth induction of TCTP levels in cancer cells is translationally regulated through the PI3K/Akt/mTORC1 pathway.

We hypothesised that, as an anti-apoptotic protein, TCTP might be involved in the development of anti-cancer drug resistance in colon cancer. First, we asked how TCTP levels are regulated in HCT116 colon cancer cells in the response to two commonly used anti-cancer drugs, 5-fluorouracil (5-FU) and oxaliplatin. We find that TCTP levels are up to 5-fold upregulated under such conditions. This effect is not paralleled by an increase in TCTP mRNA levels, but it is largely inhibited by the TOR kinase inhibitor AZD8055, indicating that TCTP is translationally regulated through mTOR complex 1 under these conditions.

To investigate whether alterations in TCTP levels affect the anti-cancer drug resistance of colon cancer cells, we performed TCTP-knockdown on HCT116 colon cancer cells and monitored the cytotoxicity exerted by 5-FU and oxaliplatin in real time using the X-Celligence Cell Analysis System and also using the MTS endpoint assay. TCTP-knockdown sensitised HCT116 cells to the effects of these drugs. Conversely, a TCTP overexpressing cell line displayed increased resistance against these drugs, compared to control cells.

P04-025
The catalytic core of HIV-1 integrase is essential for the binding of integrase to its cellular co-factor Ku70

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Ku70/Ku80 is a heterodimer complex displaying essential functions for eukaryotic cell survival. Recently Ku has been identified as a co-factor for HIV-1 integrase (IN), which protects it from proteasomal degradation. In/Ku70 complex appears to be a promising drug target. The drug design would be facilitated if a detailed structure of Ku70/IN complex were available. The exact structure of HIV-1 integrase is not yet known, and only single

domains of IN can be effectively crystallized. To learn the structure of Ku70/IN complex, we performed a systematic analysis of subdomains within IN that are required for the complex formation. Earlier by pull-down assay, we have shown that IN and Ku70 form a stable complex with a Kd about 70 nM. Here the use of the same technique and SPR-analysis allowed us to elucidate interactions of full-length Ku70 with various IN fragments. N-His6-tagged HIV-1 IN individual domains [N-terminal (1–50 aa), catalytic (51–220 aa) and C-terminal (220–270 aa)] and several truncated variants containing amino acids 1–160, 1–220, 51–160, 51–220 and 51–280 were expressed in *E. coli*. A full-size Ku70 with an N-terminal GST-tag was also expressed in *E. coli*. Our data shows that neither N-terminal nor C-terminal domains of IN are essential for its binding to Ku70. The affinity to Ku70 of the catalytic core (51–220 aa) was comparable to the affinity of the full-size IN, whereas its truncated variant (51–160 aa) bound to Ku70 only weakly. Thus, the region of IN from 160 to 220 aa is extremely important for Ku70/IN complex formation.

P04-026
Melanogenesis: different molecular mechanisms are involved in cutaneous and uveal melanocytes

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Melanocytes are specialized cells that produce the melanin-based pigment, responsible for coloration of the eye, skin and hair.

It is well known that tyrosinase is the rate-limiting enzyme of melanogenesis and that MITF is its major transcriptional regulator. Several studies regarding cutaneous melanocytes showed that the activation of ERK and AKT lead to ubiquitination and degradation of MITF resulting in reduction of tyrosinase expression and activity.

The embryologic origins of uveal and cutaneous melanocytes are identical; however, the two types of cells show different features. For instance, cutaneous melanocytes respond to ultraviolet irradiation or α-MSH stimulation while uveal melanocytes do not.

Taking into account these differences and the lack of information about melanogenesis in uveal melanocytes, the aim of our study has been to investigate whether the relationship between the ERK and AKT pathways and the regulation of tyrosinase activity also exists in uveal melanocytes.

To this purpose, we used B16 cutaneous mouse melanoma and 92.1 human uveal melanoma cell lines, treated or not with 25 μM of MEK inhibitor PD98059 (that prevent ERK phosphorylation) or with PI3K inhibitor mix (that prevent Akt phosphorylation), made by wortmannin (50 nM) and LY294002 (20 μM). The expression of tyrosinase evaluated by Western Blot analysis was strongly increased by either inhibitor in cutaneous B16 cells whereas a drastic reduction of tyrosinase expression in presence of either inhibitor was observed in uveal 92.1 cells.

These data, although preliminary, suggest that the regulation of tyrosinase expression and melanogenesis are differently regulated in cutaneous and uveal melanocytes.

P04-027
Transforming growth factor-b3 regulates cell junction restructuring via MAPK-mediated mRNA destabilization and Smad-dependent protein degradation of Junctional adhesion molecule B (JAM-B)

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Junctional adhesion molecule-B (JAM-B) is found between Sertoli cells at the blood-testis barrier (BTB) as well as between Sertoli and germ cells at the apical ectoplasmic specializations (ES) in the testis. The expression of JAM-B is tightly regulated to modulate the passage of spermatocytes across the BTB as well as the release of mature spermatozoa from the seminiferous epithelium. Transforming growth factor beta (TGF-b) family is implicated in the regulation of testicular cell junction dynamics during spermatogenesis. This study aims to investigate the effects of TGF-b3 on the expression of JAM-B as well as the underlying mechanisms on how TGF-b3 regulates JAM-B expression to facilitate the disassembly of the BTB and apical ES. Our results revealed that TGF-b3 suppresses JAM-B at post-transcriptional and post-translational levels. Inhibitor, siRNA knockdown and co-immunoprecipitation have shown that TGF-b3 induces JAM-B protein degradation via ubiquitin-proteasome pathway. Immunofluorescence staining further confirmed that blockage of ubiquitin-proteasome pathway could abrogate TGF-b3-induced loss of JAM-B at the cell-cell interface. siRNA knockdown and immunofluorescence staining also demonstrated that activation of Smad signaling is required for TGF-b3-induced JAM-B protein degradation. In addition, TGF-b3 reduces JAM-B mRNA levels, at least in part, via post-transcriptional regulation. mRNA stability assay has confirmed that TGF-b3 promotes the degradation of JAM-B transcript and TGF-b3-*mediated mRNA destabilization requires the activation of ERK1/2 and p54 JNK signal cascades*. Taken together, TGF-b3 significantly downregulates JAM-B expression via post-transcriptional and post-translational modulation and results in the disruption of BTB and apical ES.

P04-028
A transcriptional and translational approach of silica nanoparticles exposure to human lung fibroblasts

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Silica nanoparticles (SiO_2) are increasingly used in different biomedical applications. However, the cellular and proteomic effects of SiO_2 exposure are not completely understood. The aim of this study was to assess expression of heat shock proteins (Hsps) at transcriptional and translational level, induced by exposure to a 6.25 μg/ml dose of amorphous SiO_2 nanoparticles (7 nm) in cultured human lung fibroblast cells (MRC-5) for 24, 48 and 72 h of exposure.

The Hsp (27, 60, 70, 90) gene expression were analyzed through quantitative real time PCR (qRT-PCR) and their expression at protein level were quantified by Western blot analysis as well as by proteomic technology – 2DGE.

Our results showed that mRNA of Hsp27 increased after 24 h of exposure, followed by a slight decrease later on. The mRNA expression of Hsp60 significantly increased after 48 respectively 72 h, whereas that of Hsp90 and Hsp70 increased in a time dependent manner. At protein level, a strong inhibition of expression of Hsp27 and Hsp60 was noticed. The Hsp90 protein expression had the same trend as the mRNA one. The Hsp70 protein level did not change. The results of proteomic analysis were in accordance with the Western blot ones.

In conclusion, our data indicated that the exposure of human lung fibroblasts to SiO_2 nanoparticles can induce proteomics alterations.

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P04-029
Regulation of E3 ligases: Conservation of the auto-inhibitory mechanism inside the Nedd4-family

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Nedd4-family ubiquitin ligases (E3s) play a key role in several signal transduction pathways. Hence, addressing which mechanisms regulate their activity under normal conditions is of high relevance, considering they are promising targets for drug discovery.

Although Nedd4 ligases exhibit high levels of conservation, diverse regulatory mechanisms have been proposed within the family. The activity of various Nedd4 E3s, such as Smurf2 and Nedd4, is controlled through an auto-inhibitory interaction of the N-terminal C2 domain with the catalytic HECT domain.

Using our recently develop NMR approach "methionine scanning" we have characterized the C2 domain-binding surface of the Smurf2 HECT domain, which partially overlaps with a non-covalent ubiquitin binding surface (UBS).

Our *in vitro* ubiquitination assays and pull-downs show that the overlap of the C2 and the UBS interferes directly with the enzyme's activity. Point mutations in the C2-HECT binding surface were able to release the C2-mediated auto-inhibition.

In addition, structural models show that the conformation adopted by the full length protein does not allow for the transfer of Ub form the E2 to the HECT domain. These results also supported by our transthiolation assays. Lastly, analogous results were obtained for Nedd4, validating the same auto-inhibitory mechanism.

Although sequence conservation between Smurf1–Smurf2 is higher than 80%, previous studies do not support auto-inhibition as the mechanism responsible for Smurf1 regulation. Using the aforementioned NMR approach we were able to detect an interaction between the C2 and HECT domain of Smurf1, what lead us to investigate whether auto-inhibition is indeed not conserved in Smurf1.

P04-030
Production of the recombinant tetrameric butyrylcholinesterase with improved pharmacokinetic properties for the protection against organophosphate poisoning

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Butyrylcholinesterase is a natural human bioscavenger against poisoning by pesticides or nerve agents. Design of the recombinant antidotes capable to neutralize organophosphorus compounds (OP) is a problem of high social importance. The main factors limit of intensive utilization of the recombinant human butyrylcholinesterase (rhBChE) is (i) an inefficient methods of the rhBChE production and (ii) extremely high clearance of monomeric rhBChE from bloodstream ($t_{½} \approx 3$ min). Up to the present day, there was no effective expression system for tetrameric rhBChE (4rhBChE) production. Here, we develop the high productive CHO-based expression system for production of 4rhBChE exclusively in tetrameric form. It was achieved as the result of co-expression of genes of hBChE and proline rich peptide (PRAD), responsible for tetramerization of hBChE, linked by "self-processing" F2A peptide. We used strong hybrid hEF1-HTLV promoter. Insertion of Matrix Attachment Regions upstream of the promoter provided three-fold increase in 4rhBChE production. Subsequent transfection of the best-producing clone with additional hBChE gene copies allowed us to obtain clone with the production level up to 70 mg/l of recombinant protein. 4rhBChE have improved pharmacokinetic properties ($t_{½} = 32.4 \pm 1.2$ h, MRT = 43 ± 2 h). We showed that 4rhBChE major accumulation and degradation compartment is liver and products of degradation are removed by kidneys. The bioscavenging efficiency of the 4rhBChE was demonstrated in mice, preliminary treated by plasma carboxylesterase specific inhibitor CBDP. We found that administration of 50 mg/kg of 4rhBChE protects against 100% lethal dose of paraoxon.

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P04-031
Amino acid specificity of the *Escherichia coli* leucyl-tRNA synthetase editing domain

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Leucyl-tRNA synthetase (LeuRS) covalently attaches leucine to cognate tRNA in an ATP-dependent manner. This enzyme also catalyses reactions with norvaline; a noncanonical amino acid that accumulates in *Escherichia coli* under microaerobic conditions and presents major threat for accuracy of Leu-$tRNA^{Leu}$ synthesis. Norvaline is eliminated from protein synthesis by deacylation of Nva-$tRNA^{Leu}$ in the LeuRS editing domain. To prevent futile ATP consumption, the editing domain has evolved mechanisms to efficiently discriminate against cognate aminoacyl-tRNA. Both, binding and catalysis may contribute to specificity. It was shown that LeuRS discriminates against Leu-$tRNA^{Leu}$

with 10^3-fold specificity that arises from decreased rate of deacylation in the editing domain. To determine contribution of ground-state binding in specificity, we examined interactions between the LeuRS editing site and the non-hydrolysable analogues of the 3′ end of aminoacylated tRNA (Nva2AA and Leu2AA) using isothermal titration calorimetry. The dissociation constant for Leu2AA was only 10-fold higher than for Nva2AA, challenging a general perception that editing domain operates as a fine sieve that prevents binding of the cognate substrate. To explore specificity determinants of the editing site, we introduced T252A substitution that enables Leu-tRNA^{Leu} hydrolysis. Interestingly, this substitution did not increase the affinity for Leu2AA, implying that distinct determinants govern discrimination against Leu-tRNA^{Leu} at the binding and chemical steps. The full length Nva-tRNA^{Leu} and Leu-tRNA^{Leu} bind to deacylation-defective LeuRS with similar affinity, as measured by microscale thermophoresis. This further supports the notion that the substrate specificity in deacylation is preferentially established at the transition rather than at the ground-state.

P04-032
Liver iron regulates hepcidin expression. Studies in a rat model of chronic renal failure under recombinant human erythropoietin therapy

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Iron has essential roles in several physiological processes, including red blood cell formation. The body iron concentration is tightly controlled as there is no physiological excretion mechanism. Hepcidin is the main regulatory peptide of iron metabolism. However, regulation of hepcidin is not completely understood. We aimed to study the effect of recombinant human erythropoietin (rHuEPO) effect on iron metabolism in a rat model of chronic renal failure (CRF).

Male Wistar rats, 12 weeks old, were divided in 6 groups: CRF (induced by a two-stage 5/6 nephrectomy), rHuEPO-CRF treated (100, 200, 400 and 600 IU/kg/week) during 3 weeks and Sham-operated (surgery without kidney mass reduction). Hematological and iron data were evaluated, as well as, the expression of several genes involving iron metabolism and the quantification of some proteins by western blot. Mann-Whitney test was used to evaluate differences between groups.

At the end of protocol, CRF group presented anemia ($p < 0.05$), whereas rHuEPO-treated groups showed an improvement in hematological parameters ($p < 0.05$) compared to Sham group. Iron levels were similar in all groups, but in the 600 IU rHuEPO group increased serum ferritin levels and hepatic iron deposits were found, together with up-regulation of hepcidin expression and the activation of the BMP6/SMAD pathway, despite the high erythrocyte concentration.

Our results suggest that in conditions of excessive and effective erythropoiesis, liver iron regulates hepcidin expression, mainly, through the BMP6/SMAD pathway and that erythropoiesis seems not regulate directly hepcidin expression.

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P04-033
Development of highthroughput screening systems based on FACS or microfluidic devices for the directed evolution of chitinases

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The use of tailor-made enzymes offers many advantages for industrial applications such as reducing processing costs and avoiding of harmful chemical reagents. Chitin is the second most abundant polysaccharide in nature and it largely exists in wastes from the processing of marine food products. Therefore chitinases are very important for the biofuel industry but they find numerous applications in pharmaceuticals and biomedical fields as well. The method of choice to get improved enzyme properties is directed evolution. The most challenging step of the evolution experiment is the development and validation of the screening or selection method used for the property of interest. The commonly used screening methods are based on microtiter plates or the solid-phase screening on agar plates are expensive, laborious, time-consuming and only a small number of variants can be screened (10^3–10^6 in moths).

This work focuses on the establishment of an ultra-high-throughput screening assay that can be used for screening chitinase mutant libraries using either fluorescence activated cell sorting or microfluidic devices where more than 10^7 mutants can be evaluated in a shorter timeframe. The HTS method that is still in testing phase is suitable for screening chitinase variants with higher activity, thermostability and different pH optima. The gene of interest will be mutated by error-prone PCR and gene shuffling. The fluorescence assay setup was compared with other chitinase benchmark assays like *Dinitrosalicylic Acid* method. Further the assay will be tested using yeast cells expressing active chitinase and FACS detection systems.

Mem Biol S1, Organelle Dynamics and Communication

P08-005-SP
Fatty acid beta-oxidation promotes normal peroxisome distribution, morphology and function in *Arabidopsis* seedlings

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In *Arabidopsis*, fatty acid beta-oxidation occurs exclusively in peroxisomes and provides the energy needed for seedling development following germination. To identify genes important for peroxisome function, we screened mutagenized *Arabidopsis* seedlings expressing a peroxisomally-targeted GFP reporter (GFP-PTS1) for abnormal fluorescence distribution. Using recombination mapping and whole-genome sequencing, we identified 26 causal lesions in 14 genes. Most identified mutants have impeded fatty acid beta-oxidation, including several mutants defective in core beta-oxidation enzymes; additional mutants disrupt peroxisomal valine catabolism, the glyoxylate cycle, a lipase, and hydrogen peroxide detoxification. Some of these mutants display clustered

GFP-PTS1 puncta even while displaying apparently normal seedling growth in the absence of an external fixed carbon source, perhaps because slight defects in fatty acid catabolism cause clustering of peroxisomes around inefficiently mobilized lipid bodies. Interestingly, some mutants with disrupted beta-oxidation genes showed unexpected defects in peroxisomal matrix protein import and/or processing. Mutations in the *PXN* gene encoding a peroxisomal CoA transporter lead to enlarged GFP-PTS1 puncta. We observed that puncta size is restored when fatty acid transport into peroxisomes is blocked. Given that CoA is required for beta-oxidation, this result suggests that the peroxisomal enlargement is due to the accumulation of a beta oxidation intermediate. In agreement with this hypothesis, we found that peroxisomes become larger during early seedling development when fatty acid beta-oxidation is maximal and then shrink to the size they display as adults after lipid body depletion.

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P08-006-SP
Nup50, a novel key factor required for postmitotic assembly of nuclear pore complexes

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Nuclear pore complexes (NPCs) are large protein assemblies located within the nuclear envelope. They function as barriers and regulators of molecule transit between the cytoplasm and the nucleoplasm. NPCs assemble in two distinct stages of the cell cycle: after mitosis and throughout the whole of interphase. The nucleoporin Nup50 is localized on the nucleoplasmic side of the pore during interphase and is well known as an auxiliary factor in nuclear transport: in fact, it binds transport receptors as well as the small GTPase Ran, key components of the NLS-containing cargo import machinery. In addition to this well-studied function, we have determined that successful assembly of NPCs at the end of mitosis requires Nup50: depletion of the protein in *Xenopus* egg extract blocks NPC assembly i*n vitro*, whereas formation of nuclear envelope is unaffected. A similar phenotype has previously been described for Mel28/ELYS depletion, which is required for the same process and which tethers components of the core NPC onto the decondensing chromatin in the early stages of the nuclear assembly. However, our experiments show that Nup50 and Mel28 function independently in NPC assembly. Thus, this work establishes that Nup50 is critically involved in NPC assembly at the end of mitosis in a separate event that occurs in parallel and probably simultaneously to Mel28/ELYS mediated chromatin seeding of NPC core components.

P08-007-SP
The role of Septin 1 in maintaining the Golgi architecture

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Septins are evolutionary conserved GTPases with essential roles in cytokinesis and cytoskeletal organization. In mammalian cells septins comprise a group of 13 different isoforms that form homo- and heterooliogomers and thereby polymerize into non-polar filaments. Golgi biogenesis, positioning and architecture are highly dependent on cytoskeletal elements, but so far no role for septins has been reported. We have used small interfering RNA (siRNA)-mediated depletion of septins to explore their function in organellar compartmentalization and protein trafficking. Depletion of SEPT1 leads to severe morphological changes of the Golgi complex, including extensive tubulation and fragmentation. Following the ligand-induced secretion of a mutant FKBP reporter protein from the endoplasmic reticulum, we could show that exit from the Golgi in SEPT1-depleted cells is inhibited. In line with a role for septins in the maintenance of Golgi morphology we found that the septin specific inhibitor Forchlorfenuron (FCF) partially phenocopies the effects observed upon SEPT1 knockdown. Furthermore FCF affects Golgi reassembly after nocodazole-induced Golgi fragmentation. Little is known about physiologically relevant interaction partners of SEPT1. We thus followed a mass spectrometry-based approach to identify SEPT1-binding proteins. These analyses reveal a putative involvement of proteins implicated in cytoskeletal organization and centrosomal microtubule assembly. Collectively, our data suggest that SEPT1 forms part of a regulatory scaffold that supports Golgi integrity.

P08-008-SP
miRNAs targeting MPRs and AP1 subunits regulate lysosomal function

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miRNA are involved in the post-transcriptional regulation of many cellular processes, such as cell differentiation and tissue development, implicating the modulation of lysosomal function. Therefore we investigated the post-transcriptional regulation of lysosomal genes by miRNAs. A database on lysosomal genes was realized (http://lysosome.unipg.it). It integrates miRNA binding predictions from different softwares and its interrogation revealed that genes coding for proteins involved in lysosome biogenesis, vesicle trafficking and autophagy are targeted by a higher number of miRNAs with respect to lysosomal hydrolases. In particular genes relevant for hydrolases sorting to lysosome, i.e. receptors for mannose 6-phosphate (M6PR, IGF2R), other receptors (SORT1) or subunits of the Adaptor Protein complex 1 (AP1), were predicted to be regulated by miR-9 and miR-211. Predictions were confirmed by reporter assay. miR-9 has been studied in neurogenesis and is involved in epithelial-mesenchymal transition. We observed that miR-9 over-expression impaired the processing of cathepsin D, affecting its transport to lysosomes. miR-211 is transcribed within the Transient Receptor Potential 1 (TRPM1) gene. TRPM1 is a non-selective cation conductance channel, whose expression inversely correlates with melanoma. TRPM1/miR211 is regulated by MITF transcription factor, that belongs to the MIT subfamily also including TFEB, a pivotal lysosomal biogenesis and autophagy regulator. These findings suggest a relevant role of the MITF/miR211/TRPM1 cascade in the regulation of melanocytes function and indicates that this approach can shed light on gene regulatory networks relevant for specialized cell types.

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P08-009
Use of fungi as bilogical decontamination of organophosphorouse compounds and heavy metals pollutants in water

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The effect of *Aspergillus* sp. on decreasing the concentration of (P&S) presence in water by increasing time where it reaches the minimum level after 14 days. Weight of growing fungus represented which indicates that activity of fungus increased by increasing time till reach the maximum growth and activity after 14 days then the activity decreased. Concentration of (P&S) uptake from water inside fungus represented which shown increasing the concentration of (P&S) inside fungus by increasing time, indicating the ability of *Aspergillus* sp. on removal of organphosphorous compounds from water, this ability is more than of *Fusarium* sp. But, the removal rate is highly increased after using mixture of Aspergillus sp. And *Fusarium* sp. on removal of organphosphorous compounds from water is more than *Fusarium* sp. by increasing time. The results obtained indicate the removal rate of organphosphorous compounds from water increased by increasing cultural mass of fungi, and this meaning that the increasing of the cultural mass leads to activity the of fungus and its ability on removal of organphosphorous pollutants from water.

P08-010
The effects of bile acids on the liver mitochondria in the presence and absence of Ca^{2+}

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In this paper we studied the effect of bile acids (BAs): lithocholic (LA), chenodeoxycholic (CDA) and ursodeoxycholic (UDA) on the cyclosporine A (CsA)-insensitive Ca^{2+} (Sr^{2+})-dependent nonspecific permeability of the inner membrane of rat liver mitochondria. Our studies have shown that the addition of BAs to the suspension of mitochondria, loaded with Ca^{2+} or Sr^{2+}, leads to a swelling of organelles, indicating the pore opening in the inner membrane. Furthermore, these BAs promoted Ca^{2+}-release from the mitochondrial matrix and drop of membrane potential ($\Delta\psi$). CsA significantly inhibited the swelling of organelles, but not prevent it. The data obtained indicate that these BAs are able to induce nonspecific permeability of the inner membrane (pore opening) of liver mitochondria. In case of replacement of Ca^{2+} by Sr^{2+}, the effectiveness of these BAs as inducers of pore opening is significantly reduced. Under our conditions, UDA, which has the highest hydrophilic-hydrophobic index (HHI) among used BAs, is the most effective.

In addition, it was found that under Ca^{2+}-free conditions BAs are able to reduce $\Delta\psi$, showing protonophore uncoupling activity. However, the decrease in $\Delta\psi$ induced by these BAs was reversible and then replaced by the almost complete restoration of $\Delta\psi$. It is assumed that the effect of BAs on the mitochondrial energetics depends on the HHI of these acids. The most hydrophobic BAs (with highest HHI) were the most effective as protonophore uncouplers under Ca^{2+}-free conditions.

P08-011
Uncouplers inhibit clonal expansion of mutant mitochondrial DNAs

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The presence of multiple variants of mitochondrial DNA (mtDNA) in one cell induces a number of pathologies and age-related disorders. Such condition, heteroplasmy, occurs as a result of mtDNA mutation and subsequent clonal expansion of these molecules. Here we searched for the ways to prevent the clonal expansion of mtDNA with large deletion in *Saccharomyces cerevisiae*. As diploid yeast cells inherit mitochondria from both gametes, to produce heteroplasmic cells we mated strains carrying normal and mutant mtDNA. The ability of mutant mtDNA to displace the wild type one (supressivity) was determined by percentage of respiratory incompetent diploid cells in the progeny. We found that anionic uncouplers significantly suppress such displacement, whereas neither respiratory chain inhibitors, nor antioxidants demonstrated such an effect. Importantly, the impact of uncouplers was due to improved selection towards functional mtDNA within individual cells and not due to intercellular selection. Furthermore, we showed that this effect was much lower in the strains with suppressed Dnm1 (a key protein in mitochondrial fission process) or Atg32 which is indispensable receptor of mitophagy-selective degradation of mitochondria. Moreover, it was shown that the uncoupler FCCP stimulates the degradation of mtGFP in zygotes implying the activation of mitochondrial turnover. Together, these results point that competition of wild type and mutant mtDNAs is significantly affected by the mitochondria quality control system. We suggest that anionic uncouplers can be used to prevent the clonal expansion of mutant mtDNAs.

P08-012
Retrograde signaling pathway controls survival during cell cycle arrest in yeast cells

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While the role of mitochondria in the cell cycle progression was extensively studied, the involvement of retrograde mitochondria-to-nucleus signaling (Rtg cascade) in this process is still unclear. To address this question we studied Rtg cascade during cell cycle arrest in yeast *Saccharomyces cerevisiae*. First, we tested the role of mitochiondria in pseudohyphal growth. Pseudohyphae are specialized elongated cells arrested in G2. We found that pseudohyphae formation is promoted by activation of Rtg cascade induced by energy deprivation. At the same time, the energetic function of mitochondria *per se* was not crucial for filamentation: the inhibition of pseudohyphae formation induced by uncouplers was suppressed by the downstream mutation. Next, we studied the role of Rtg signaling in cell survival under prolonged cell cycle arrest. It appeared that the long-term arrest in mitosis or S phase, but not in G1, kills the cells. At the same time, addition of the uncouplers or depletion of mtDNA significantly increased the survival of arrested cells, while neither antioxidants nor mitochondrial protein synthesis inhibitors showed any effect. However the inactivation of retrograde signaling was found to positively affect cell survival under a prolonged arrest in the

S-phase induced by temperature-sensitive mutation in *CDC13* gene, encoding telomere-binding protein. Deletion of Rtg genes decreased the arrest-induced death in *cdc13-1,* whereas a deletion of a negative regulator of retrograde signaling, *MKS1,* decreased the survival. These results suggest that dysregulation of mitochondria-to-nucleus signaling stimulates cell death under prolonged cell cycle arrest.

P08-013
The J protein Djp1 is involved in the targeting of mitochondrial precursor proteins

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In yeast there exist several HSP70s. They are involved in different processes like protein folding, assembly of complexes and protein targeting. To fulfill its function the HSP70 machinery needs co-chaperones like NEFs (nucleotide exchange factors) and the J proteins. In yeast the specificity of the different HSP70s is given by the 20 J proteins. The binding of client proteins to HSP70s is mediated by the J proteins. Djp1 is a member of the J protein family and is required for the peroxisomal protein import. We show here that Djp1, beside its function in peroxisomal biogenesis, is also involved in the targeting of mitochondrial precursor proteins like Oxa1.

P08-014
Induction of mitochondrial permeability transition by dequalinium

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Dequalinium, a typical antimicrobial drug, was reported to be selectively accumulated into mitochondria, and to show inhibitory effects on electron transport chains between the sites of NADH and ubiquinone. These activities are often observed with hydrophobic cationic compounds, and hydrophobic cationic compounds also often induce mitochondrial permeability transition. In the present study, we examined whether dequalinium causes mitochondrial permeability transition.

When dequalinium was added to the mitochondrial suspension, the turbidity of the mitochondrial suspension decreased, in a manner dependent on its concentration. The decrease of the turbidity of the mitochondrial suspension induced at low concentration of dequalinium was suppressed by addition of cyclosporin A, but that at high concentration was not. These results strongly suggested the induction of mitochondrial permeability transition by dequalinium. This conclusion was further supported by the morphological analysis of mitochondria by electron microscopy. When mitochondrial permeability transition was induced by dequalinium, release of cytochrome c from mitochondria was also observed, as often observed with typical inducer of mitochondrial permeability transition.

P08-015
CRISPR/Cas9-mediated endogenous protein tagging for super-resolution microscopy and its application for studying mitochondrial dynamics

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The majority of live-cell microscopy studies in human cells rely on cells transiently or constitutively overexpressing a host protein fused to a fluorescent protein (FP). Overexpression of fusion proteins may cause a multitude of artefacts including mislocalization, protein aggregation, imbalanced gene dosage, and others. Such artefacts are presumably even more articulate in studies using diffraction-unlimited super-resolution microscopy, which facilitates the visualization of protein localizations and dynamics on a scale inaccessible by conventional light microscopy. Still, so far all live-cell super-resolution microscopy studies of mammalian cells using FPs relied on overexpressed fusion proteins.

Recent advances in genome engineering technologies, such as the CRISPR/Cas9 system, permit site-specific endogenous tagging of proteins from their chromosomal loci. Genomic labelling might eliminate overexpression concerns and would allow direct quantification of the abundance, localization and dynamics of proteins under native expression conditions. Therefore we generated CRISPR/Cas9 endogenously FP-tagged cells and show (i) elimination of overexpression-induced artefacts and (ii) RESOLFT super-resolution microscopy for imaging protein dynamics of cellular model structures on different timescales in the genome edited cells. We further applied CRISPR/Cas9-mediated endogenous labelling to study mitochondrial inner membrane protein complexes where (i) overexpression leads to mitochondrial fragmentation and (ii) suitable antibodies for fluorescence and electron microscopy are lacking. Using an approach that combines biochemical assays, electron microscopy and live-cell as well as super-resolution imaging we obtained novel insights into the localization, interaction and trafficking of mitochondrial inner membrane proteins unattainable or obscured in overexpression-based methods.

P08-016
Long-Chain inorganic polyphosphate is highly enriched in osteoblastic matrix vesicles

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Evidence is emerging that long-chain inorganic polyphosphates play a variety of critical roles in mammalian cells that remain little understood. In particular it has been posited that polyphosphate is found at a relatively high concentration in osteoblasts but many unanswered questions remain regarding polyphosphate function and synthesis. One significant challenge is the lack of tools to observe and analyse polyphosphate function. Here, we report evidence that polyphosphate is highly enriched in SaOS-2 osteosarcoma osteoblastic matrix vesicles. In one line of evidence, we use various chromatographic approaches coupled to use of DAPI as a wavelength-dependent polyphosphate specific dye. In a second line of evidence, we use a heavy metal labelled polyphosphate binding domain (PPBD) of *Escherichia coli* exopolyphosphatase (PPX) as a tool for the observation of polyphosphate by transmission electron microscopy. Results correlated in observing a particularly strong enrichment of poly-

phosphate in osteoblastic matrix vesicles. Polyphosphates likely play important roles in mineralization in matrix vesicles, suggesting polyphosphate could be an overlooked key molecule in extracellular matrix biology.

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P08-017
Endothelial mitochondrial homeostasis is enhanced by shear stress

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The hemodynamic flow-induced shear stress trigger adaptive responses in vascular endothelial cells (ECs) are crucial for normal vascular functions. Maintenance of mitochondrial homeostasis is essential for cellular function and cell survival. The effects of shear stress on mitochondrial homeostasis and functions in ECs remain unclear. We examined the dynamic changes of mitochondrial phenotype and regulatory proteins involved in the control of mitochondrial homeostasis and functions in ECs exposed to shear stress. Cultured human umbilical vein ECs were subjected to a laminar flow with shear stress (12 dynes/cm^2) generated by a parallel-plate flow chamber system. Shear-treated ECs demonstrated a time-dependent increment of mitochondrial interconnected tubular formation. This phenomenon was associated with an increase of fusion proteins (MFN2, OPA1) and a decrease of fission protein (FIS1). Consistently, an increase of phosphorylation at S637 but decrease at S616 on DRP1 after shear treatment was observed, indicating shear stress promoted mitochondrial fusion process. Moreover, shear stress increased the expression of mitophagy proteins (PINK1, PARKIN) suggesting a quality control of mitochondria was enhanced. Shear stress also promoted mitochondrial biogenesis with the elevated expression of peroxisome proliferator-activated receptor γcoactivator 1α (PGC-1α), mitochondrial transcription factor A (TFAM) and mitochondrial proteins. Furthermore, shear stress increased the expression of mitochondrial antioxidant enzymes MnSOD2, TRX2, PRX3 and PRX5. As a consequence, sheared ECs improve mitochondrial functions revealed by the increased mitochondrial membrane potential, ATP production and a decrease of mitochondrial reactive oxygen species (ROS) levels. Our results clearly indicate that shear stress promotes mitochondrial homeostasis in ECs.

P08-018
Nonthermal effect and safety of DBD-bioplasma on fibroblasts: a new molecular validation of bioplasma using the levels of HSP70 in cells

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Plasma is an ionized gas mixture, consisting of neutral particles, positive ions, negative electrons, electronically excited atoms and molecules, radicals, UV photons, and various reactive species. Also, plasma has unique physical properties distinct from gases, liquids, and solids. Until now, non-thermal plasmas (called bioplasma) have been widely utilized in bio-medical applications and developed for the plasma-related devices that are used in the medical field. Although numerous bioplasma studies have been performed in biomedicine, there is no confirmation of the non-thermal effect induced by bioplasma. Standardization of the biological application of bioplasma has not been evaluated at the molecular level in living cells. In this context, we investigated the heat effect of bioplasma on living cells. Heat shock protein 70 (HSP70) up-regulates its own protein level in response to physical treatments, such as heat. Hence, we exposed mouse embryonic fibroblasts (MEFs) on dielectric barrier discharge (DBD), and assessed the molecular level of heat HSP70, one of essential cellular responses. Interestingly, DBD-plasma induced cell death, but there was no difference in the level of HSP70, indicating that the DBD-plasma is non-thermal and applicable to biomedical sciences. Our data provide the basic information on the interaction between MEFs and DBD, and can help to design a molecular approach in this field.

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P08-019
Intersectin-1s: a novel nucleo-cytoplasmic endocytic protein

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A growing number of proteins involved in endocytosis are reported to undergo nucleocytoplasmic trafficking and interact with different nuclear factors. This mechanism, known as protein moonlighting, is emerging as a key factor in many biological processes, though little is known about the correlation between endocytosis and nuclear functions.

Here we investigated the nuclear location of the multi-domain scaffold endocytic protein intersectin-1s, a protein involved in clathrin mediated endocytosis, cell signalling and actin cytoskeleton rearrangements.

A bioinformatic analysis of the protein sequence allowed the identification both of putative nuclear localization sequences (NLS) and nuclear export sequences (NES). Immunofluorescence studies and biochemical nuclei isolation experiments revealed the presence of small amounts of intersectin-1s in the nuclear compartment, distributing between the nucleoplasm and the nuclear membrane, where it partially colocalizes with nuclear lamins. Despite the protein is mainly located in the cytoplasm at the steady state, cell treatment with Leptomycin B, a specific inhibitor of the chromosomal region maintenance 1 (CRM-1)-dependent nuclear export, resulted in its nuclear accumulation, suggesting that intersectin-1s is a moonlightining protein. Finally, by analyzing the subcellular localization of several Intersectin 1-s domains fused with YFP, or point mutants derivatives thereof impairing the putative NLS/NES, we could demonstrate that indeed intersectin-1s possesses several redundant signals mediating its nucleocytoplasmic shuttling.

In conclusion, our results show for the first time the existence of a nuclear pool of intersectin-1s, which shuttles between the cytosol and the nucleus and is localized in different intranuclear compartments.

P08-020
Protein profile of erythrocyte membranes in acute pancreatitis: potential targets for therapeutic intervention

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Acute pancreatitis (AP) is a clinical condition with complications and a mortality rate up to 20%. The changes in the erythrocyte membrane can be expected to reflect the metabolic changes occurring within the acinar cell of pancreas and help reveal the molecular basis for the basolateral cell membrane changes in AP.

In a prospective study 42 consecutive patients with acute non-biliary pancreatitis (ANBP) were included. In all patients the erythrocyte membranes were examined within the first 24 h of admission and 10 days thereafter. Twenty-one age matched volunteers were used as a control group. The various red blood cell membrane proteins were separated by electrophoresis on SDS gels.

On admission, patients with ANBP have abnormal protein spectrum, in which most membrane proteins are damaged. There is a strong correlation between the clinical symptoms and intracellular malonyl dialdehyde concentration that underlines the crucial role of oxidative stress in pathogenesis of the disease. A structural network of proteins is located on the inner surface of the lipid bilayer, therefore defects in membrane associations result in loss of unsupported phospholipids. Standard treatment only improves up to 28% of protein and lipid profile of the erythrocyte membrane. This suggests that there is much to be gained by introducing into the traditional therapy drugs with immunomodulating, antioxidant and membrane protective properties. 22 patients were administered S-adenosylmethionine, ethylmethylhydroxypyridine succinate, and hydroxyethyl starch. This new therapeutic strategy normalized levels of α-, β- spectrin, pallidin, actin and lead to a significant decrease of malonyl dialdehyde concentration.

P08-021
What is the role of FAB1C in PSY1 mediated cell growth?

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In plant cells elongation depends on the increase of the size of the vacuole. The peptide hormone PSY1 stimulates cell elongation in root and hypocotyls. Based on a microarray study performed in our lab studying the effect of PSY1 treatment, the nine fold down regulated phosphatidylinositol-3P 5-kinase FAB1C is suggested to play a role in vacuole growth and acidification. In yeast it is shown that FAB1 affects membrane homeostasis and size as well as structure of the vacuole. Recent studies of FAB1C homologues Fab1A/B in *Arabidopsis thaliana* gave similarly results. Loss-of-function and gain-of-function of FAB1A/B affects endomembrane homeostasis and results in developmental abnormalities. Additionally, these mutants have shown an abnormal vacuolar phenotype. FAB1C belongs together with FAB1D to a unique group, only existing in the plant kingdom. All these plant FAB1 proteins share some features, like the C-terminal kinase domain and the central Grol-domain. Only the N-terminal FYVE domain is missing in the plant FAB1.

The Aim of this study is to investigate the unknown function of FAB1C. We hypothesize that FAB1C play an important role in the signal transduction from plasma membrane to vacuole. Therefore, localization and complementation studies are carried out in *Arabidopsis thaliana* as well as in *Saccharomyces cerevisiae*. These studies will cast light on the signal transduction between the membrane compartments. Investigations of vacuole development in different plant mutants will take place and we will perform the analysis of the PIP_2 composition. Further more, we will analyze the pH in *fab1c* plant lines.

P08-022
Lys-plasminogen affects platelet secretion and cytoskeleton rearrangement

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Plasminogen/plasmin system takes part not only in fibrinolysis but also in regulation of functional state of different cells. Platelet membrane can provide a surface for assembly of plasminogen and its activators. On the surface of certain blood cells native Glu-plasminogen is transformed into Lys-form, which possesses open conformation and can be more easily activated with the plasmin formation. We previously showed that Lys-plasminogen but not Glu-form inhibits human platelet aggregation. This study was aimed to evaluate effects of Lys-plasminogen on actin cytoskeleton reorganisation and α-granule secretion of human platelets. Exposition of platelet secretion markers, P-selectin and vitronectin, was measured by flow cytometry. Cytoskeletal reorganization was assessed by Western blot of fibrillar, globular and membrane-associated actin pools. It was shown that Lys-plasminogen, but not Glu-plasminogen, decreased thrombin-induced P-selectin expression (that indicates supression of α-granule release) and increased vitronectin exposition on the surface of activated platelets. Lys-plasminogen prevented association of membrane cortex actin into filamentous network, thus interferring thrombin-induced cytoskeleton reconstruction. It is likely that alterations of platelet secretion and aggregation occur due to impaired reorganization of actin cytoskeleton caused by Lys-plasminogen. Vitronectin, secreted from α-granule during platelet activation and stays bound to platelet surface also may interact with Lys-plasminogen sorbed on the platelets .The lack of Glu-plasminogen effect on vitronectin exposition can be explained by the fact that Lys-plasminogen expressed higher affinity for the vitronectin as compared with Glu-form. In summary, Lys-plasminogen which is formed on the cell surface can be involved into regulation of functional state of blood cells.

P08-023
Proteins of plasma membranes of villous syncytiotrophoblast and their posttranslational modification in case of placental insufficiency

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Objective: The study of the spectrum of villous syncytiotrophoblast membrane proteins and their posttranslational modification in case of placental insufficiency (PI).
Methods: The study was made in women with physiological pregnancy (n = 32) and with pregnancy complicated with PI (n = 27). Membranes of villous syncytiotrophoblast were released by means of the differential ultracentrifugation. Solubilized mem-

brane proteins were fractionated using two-dimensional electrophoresis (1st: IPG strip pH 3–10, 17 cm; 2nd: 8–16% polyacrylamide gel). The quantitative evaluation of proteins stained with *Coomassie R250* was made densitometrically. The intensity of posttranslational modifications of proteins was determinated using kits.

Results: In case of PI, the solubilization of proteins by soft detergents is intensified, the quantity of slowly migrating proteins with a relative molecular mass from 150,000 to 200,000 decreases (by 27%) and the quantity of proteins with a molecular mass of 25,000–30,000 increases (by 35%). This indicates a disaggregation of protein associates of membranes. The posttranslational modification of membrane proteins in case of PI consists in the intensification of carbonylation (by 25%) that indicates an increase of their oxidative destruction. Opposite dynamics is revealed for glycation, phosphorylation and amidation: their intensity is reduced by 28%, 37% and 29%, respectively, resulting in the change of the charge, contact interaction of proteins and communication properties of plasma membranes.

Conclusion: Disturbing the functions of these structures, the modification of placental membrane proteins is evidently a molecular basis for the development of pregnancy complications, the integral manifestation of which is PI.

Keywords: Membrane proteins, posttranslational modifications, placenta.

P08-024
siRNA mediated downregulation of LETM1 results in decreased expression of OPA1

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First identified in Wolf-Hirschhorn syndrome, LETM1 is a conserved protein of the mitochondrial inner membrane. It has been suggested that LETM1 serves as a mitochondrial shaping protein while the underlying mechanism of this function is not clear. Some studies investigated its relationship with OPA1 but the results are controversial. Here, we demonstrated that LETM1 silencing in mouse embryonic fibroblasts results in 1-fold decrease in OPA1 expression as determined by quantitative real time-PCR. A decrease in OPA1 protein level was also confirmed by immunoblotting in cell lysates and in isolated mitochondria without a significant change in the ratio of long and short isoforms of OPA1. In addition, we investigated cyt-c release. For this purpose, isolated mitochondria were incubated with recombinant tBID for 0, 3 and 6 min and released cyt-c levels were quantified by ELISA. According to our data, there was no difference in cyt-c release patterns between LETM1 silenced and control cells, whereas initial release of cyt-c (time 0, e.g. no tBID stimulation) was higher in LETM1 silenced mitochondria. Beside this, nigericin, a K^+/H^+ ionophore, restored OPA1 expression levels as well as initial cyt-c release. These data suggest that LETM1 indirectly contributes mitochondrial shape by decreasing OPA1 expression.

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P08-025
Isolation and characterization of different local *Salmonella* strains from Northern Iraq

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The different pathogenic bacterial strains have an important impact on the health of both of human and animals with consequence of both life conditions and economic situation. Isolation and characterisation of the different strains as well as the new emerging species and sub-species of these bacteria helps us to develop new antibiotic agents and the modification of the ones in use in order to cure both the human and the livestock animals from the infection with these microorganisms. During our study 80 different strains of *Salmonella* bacterium were isolated with later characterisation from a group 80 patients consisted of 44 male and 36 female individuals from Sulaimaniya, Erbil and Duhok governorates. Characterisation of the strains were accomplished be applying the E-test, disc diffusion test and the multiplex PCR with five different primer pairs for the identification of genes responsible of the O-antigene or the H-antigene respectively. 94.6% of the individuals were bearing the O-antigene, 5.4% were bearing the H-antigen. All the isolated strains were resistant against different antibiotics but all of them were resistant against chloramphenicol and gentamicin and seven different local strains were characterized. We also can conclude that these new local strains can be a good source for further studies on the molecular determination of the antibiotic resistance in gram negative bacteria and provide essential information for the developmental status of these bacteria with further development of new antibiotics in order to contain the outbreak of disease caused by bacterial microorganisms.

P08-026
The role of ATF6 in endoplasmic reticulum stress response in pancreatic β cell

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Protein folding homeostasis in the endoplasmic reticulum is defended by signal transduction pathways that are activated by an imbalance between unfolded proteins and chaperones so called ER stress. This homeostatic response is initiated by three known ER stress transducers: IRE1, PERK and ATF6. The high insulin production causes heavy load in the ER of pancreatic cells. So it can be speculated that as a cellular response autophagy may be induced by ATF6 in pancreatic β cells.

The INS-1E cells of rat pancreatic beta-cells were incubated with palmitic acid, palmitic acid+rapamycine and rapamycin. At 3th, 6th, 9th, 12th, 24th hours ER stress genes ATF6, PERK, GRP78, XBP1, CHOP, autophagy genes Beclin-1, Atg5,Atg7, LC3-1 apoptosis genes Bcl-2, Bcl.-XL, Bax expression rates were analysis in all three experimental conditions. The most effective response to palmitic acid induced stress was in the 6th hour. Palmitic acid, palmitic acid+rapamycine conditions autophagy starts at 6th hour and apoptosis starts at 12th hour. An increase ATF 6 expression has been observed as a result of the autophagy induced by ER stress thus may contribute to the cell survival. ATF6 level, can be sufficient for the GRP78 expression. Under ER stress condition PERK and ATF6 contribute to CHOP expression.

P08-027

Super-hub mechanism of calcium signaling in atria

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In typical heart muscle cells (ventricular cardiomyocytes; VM), a transverse-oriented membrane tubule network (TN) mediates electrical coupling of intracellular Ca^{2+} release signals through TN nanodomain membrane contacts with the sarcoendoplasmic reticulum (SER). However, current models of atrial myocytes (AM) predict no or only few short tubules at the cell surface. Here, we identify for the first time an AM-specific TN mainly comprised of axial tubules (AT). We hypothesized that the previously not recognized AT structures function as Ca^{2+} signaling hubs in AM.

Using membrane-optimized AM isolation and live superresolution STED microscopy, we show continuous AT structures with diameters of 293 ± 8 nm (n = 30), and significantly larger compared to VM (201 ± 9 nm; n = 27; $p < 0.001$). AT-located intracellular Ca^{2+} imaging (fluo4-AM) showed rapid high-amplitude Ca^{2+} transients confined to ATs. Moreover, Ca^{2+} release at AT hubs occurred significantly faster relative to surface membrane (Ca^{2+} upstroke latency: surface 3.0 ± 0.5 ms, AT 1.3 ± 0.5 ms; n = 19, $p < 0.05$). Biochemical analysis and *in situ* phosphorylation mapping revealed AT-associated ryanodine receptor (RyR2) channel clusters with locally increased protein kinase A phosphorylation in close proximity to junctophilin two clusters, a tail-anchored SER protein and TN membrane tether.

Our data suggest a fundamental signaling role of membrane tubules and a new AM model of excitation-contraction coupling, where ATs function as signaling super-hubs that control ultrarapid Ca^{2+} signals at the cell center. This may enable centrifugal recruitment of myofilament bundles for rapid atrial contraction, graded recruitment of non AT-associated RyR2 clusters, and specific atrial functions and disease mechanisms significantly different from ventricles.

P08-028

The language of telocytes: understand their involvement in tissue morphogenesis/ regenerative medicine

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Recently, a new type of interstitial cells was described – the telocytes. Among some of the hypothesized roles we can enumerate: tissue morphogenesis, homoeostasis and remodeling/renewal. It was shown that during tissue regeneration a low intensity current electrical field (EF) is generated. This EF induces a favorable gradient for the cells involved in regeneration processes, facilitating their orientation and migration to the targeted area. Telocytes are considered to be promoters of regeneration processes both through cytokine secretion and their capacity to form a tandem with stem cells. Telocytes were cultured in a special chamber, with parallel electrodes, placed in a cell culture incubator, attached to an inverted microscope. We examined telocytes from pregnant myometrium in primary cell culture and subsequent passages under influence of DC EF by phase contrast microscopy and time lapse-video microscopy for 24–72 h. Telocytes present migration and orientation behavior directed by DC EF. Telocytes orientation was obtained after 30–100 min. This study might be an *in vitro* model of a tissue injured state with the involvement of telocytes and contributes to the understanding of the processes and phenomena that occur during the tissue recovery, in our case myometrial regeneration.

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P08-029

Defining the role of SEPT9 in ciliogenesis

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Septins are a family of filamentous GTPases conserved from yeast to man. They play a number of important roles in biological processes as diverse as cell division and neurotransmission. Due to their direct association with negatively charged lipids, they have been implicated as diffusion barriers for integral membrane proteins. However, they also function as multimolecular scaffolds by serving as a platform for important signaling molecules. Depletion of septins results in significant impairment of ciliogenesis and initial studies suggested that this was due to loss of the diffusion barrier, although their precise role in ciliogenesis remains unclear.

We have localized septins to the axoneme and basal body of primary cilia and have also shown that the N-terminus of SEPT9 binds directly to a GTP exchange factor (SA-RhoGEF) for Rho. More importantly, SEPT9 binding increases its exchange activity for RhoA. Both SA-RhoGEF and RhoA are seen to localize to the basal body at the base of primary cilia. Depletion of SA-RhoGEF or inhibition of RhoA function, like septin depletion, significantly inhibits ciliogenesis and results in similar shortening of cilia. Using knockdown-rescue approaches where we target components of the pathway directly to the basal body, we have determined that septins and the other components of this pathway are only required at the basal body to achieve ciliogenesis and normal cilium length. Together these studies indicate that a major role for septins in ciliogenesis is the targeted activation of a signaling axis through the GTPase RhoA and that this pathway regulates microtubule stability.

P08-032
Novel insights into the molecular mechanisms that mediated incorporation of the amyloid precursor protein into multivesicular bodies

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Introduction: It has been proposed that amyloid precursor protein (APP) levels contribute to Aβ production and amyloid plaque formation. Recent evidence has shown that APP can be localized in multivesicular bodies (MVBs), however it is unclear whether this localization is connected to lysosomal degradation or for secretion through exosomes. Moreover is still unknown the contribution of ubiquitination in this process. In this work we investigated the contribution of TSG101, the product of the tumor susceptibility gene 101 that participate in the biogenesis of MVBs intraluminal vesicles (ILVs), and of Rab27a, an essential GTPase in the formation of exosomes.
Material and methods: We developed stable neuroglioma cell lines (H4) expressing different versions of APP or C99 fused to EGFP including wild-type and mutated versions in all putative ubiquitination sites. These cells were depleted of either TSG101 or Rab27a, by RNAi transfection or shRNA stable expression with lentiviral particles, respectively. The levels of APP and C99 were analyzed by immunoblot, flow cytometry, and fluorescence microscopy.
Results: Depletion of TSG101 and Rab27a caused a strong accumulation of GFP-signal in intracellular compartments, positive to markers of recycling endosomes. Biochemical analysis confirmed these results, observing a significant increase in C99 levels and in an ubiquitin-dependent manner.
Discussion: We propose that affecting incorporation of C99 into ILVs can facilitate its intracellular accumulation and cleavage by gamma-secretase.

P08-033
The combined role of long chain acyl-CoA synthetase 2, long chain acyl-CoA synthetase 4 and long chain acyl-CoA synthetase 9 in lipid metabolism of *Arabidopsis*

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Lipids are necessary components of plant cells to maintain the integrity of the whole plant. Integral parts of most lipid molecules are fatty acids. To become metabolically available fatty acids need to be activated by coenzyme A or by acyl carrier protein. In *Arabidopsis* a gene family was described which encodes nine different long-chain acyl-CoA synthetase (LACS) enzymes which are generating acyl-CoAs. The analysis of specific biological roles of individual LACS enzymes is hindered by overlapping functions masking the effects of gene inactivation. To get a better understanding of the role of each LACS gene, different LACS mutant combinations were generated in our laboratory. This study proved the importance of LACS for proper development of *Arabidopsis thaliana*. The morphological and biochemical analyses revealed that LACS2 and LACS4, which are localized at the ER and have roles in cuticle synthesis, together with LACS9, which performs almost 90% of plastidial LACS activity, impact the oil content and fatty acid composition of seeds dramatically. It was detected that the seed oil content was decreased by up to 30% compared to wild type. Besides, the inactivation of all three genes prevented the elongation of roots after germination if the seedling establishment was not rescued by provision of an exogenous carbon source. Overall, different stages of plant development as well as seed oil biosynthesis were affected by the combined action of the three LACS enzymes showing that even activities in different subcellular locations can at least partially overlap in their functions.

P08-034
New insights in telocytes role: intercellular signaling from novel *in vitro* approaches

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Telocytes, a new type of interstitial cells, are mainly involved in intercellular signaling. Intercellular communication is accomplished by protein signaling cascades as well by signaling vesicles that eventually cause the reprogramming of the transcriptome. System biology studies in mammalian cells have typically focused on individual cell types far away from reality of cellular heterogeneous structure of the tissues. We examined telocytes from pregnant myometrium in primary cell culture and subsequent passages by phase contrast microscopy and time lapse-video microscopy for 24–72 h. Telocytes release microvesicles into the extracellular environment targeting the surrounding cells. These vesicles are shed by direct release or transported via cellular processes called telopodes to the target cell. This behavior suggests that telocytes play major role as supporting cell. This study provides clues to better understanding of how telocytes and different types of cells communicate with each other in *in vitro* systems, thus, paving the way for advances in intercellular and organ-level system biology.

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P08-035
SEPT9 negatively regulates ubiquitin-dependent downregulation of EGFR

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Septins constitute a family of GTP-binding proteins that are involved in a variety of biological processes. Several isoforms have been implicated in disease such as cancer and neurodegeneration, but the molecular mechanisms underlying pathogenesis are only poorly understood. Here, we investigate the role of SEPT9 in epidermal growth factor receptors (EGFRs) stability. Our study shows that depletion of SEPT9 decreases surface levels of

epidermal growth factor receptors (EGFRs) by enhancing receptor degradation. We identify a consensus motif within the SEPT9 N-terminal domain that supports its association with the adaptor protein CIN85. CIN85 and SEPT9 co-localize exclusively at the plasma membrane, where SEPT9 is recruited to EGF-engaged receptors in a CIN85-dependent manner. We demonstrate that SEPT9 negatively regulates EGFR degradation by competing binding of the ubiquitin ligase Cbl to CIN85, resulting in reduced EGFR ubiquitylation. Taken together, these data provide a mechanistic explanation of how SEPT9, though acting exclusively at the plasma membrane, impairs endosomal sorting of EGFRs into the degradative pathway. On-going proteomic analyses indicate that SEPT9 also modulates the recruitment of Vav3 and PI3KC2b to active EGFRs, and thus suggest additional functions of SEPT9 during growth factor-induced rearrangements of the actin cytoskeleton.

P08-036
Improvement of endothelial function in cardiac syndrome X by metoprolol

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The important factor in the treatment of cardiac syndrome X (CSX) is to improve endothelial function. We aimed to investigate endothelial function in CSX patients by measuring important endothelial marker namely vascular cell adhesion molecule-1 (VCAM-1) and assess the effects of metoprolol therapy on VCAM-1 levels.

We enrolled 25 consecutive CSX patients (5 male/20 female, mean age: 55.36 ± 10.31 years). Age- and gender-matched (5 male/20 female, mean age: 54.32 ± 9.27 years) healthy volunteers were completely served as controls. Sandwich ELISA method used for VCAM-1 evaluation.

The levels of VCAM-1in CSX (109.08 ± 48.21 ng/ml) are significantly higher than controls (57.12 ± 17.42 ng/ml), respectively; $p = 0.002$. Moreover, levels of VCAM-1 in CSX patients after treatment (84.63 ± 30.88 ng/ml) decreased significantly in comparison with baseline, $p < 0.001$.

Metoprolol can improve CSX symptoms and may be is a good choice for CSX treatment. Probably effort for treatment and improvement of endothelial function is one of the best treatment lines for CSX.

P08-037
Mitochondrial and lysosomal permeabilization and reactive oxygen species mediate Patulin and Stergmatocystin cytotoxicity on CHO-K1

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Ingestion of contaminated food is a main route of exposure to different industrial and environmental contaminants. Mycotoxins constitute an example of naturally occurring contaminants; they are hazardous secondary fungal metabolites which are found in feed and other food materials that occur simultaneously in food or raw materials. The objectives of this study were to evaluate the inhibition of cell viability, induction of the reactive oxygen species (ROS) production and damage to sub-cellular organelles (mitochondria and lysosome) in cultured ovarian cells exposed to low concentrations of Patulin (Pat; 0.025; 0.05 and 0.1 μM) and Sterigmatocystin (Ste; 0.078; 0.78 and 3.12 μM) similar to those detected in food. Both, Pat and Ste reduce cell viability with an IC50 equal to 2.82 and 25 μM respectively after 24 h of exposure. ROS measurement every 5 min during 2 h showed that ROS increased for the two mycotoxins compared to to control. Pat reduced mitochondrial membrane potential (MMP) at all concentration tested with a reduction of 20% respect to control while the range of reduction for STE is 25–40%. Besides, the two mycotoxins increased the lysosomal membrane stability (LMS). Our results suggest that ROS, MMP and LMS may be incriminated in cell viability reduction.

Mem Biol S2, Autophagy and Degradation

P09-005-SP
Glutathione depletion in spermatogonia-type germ cells: Autophagy and Ago2 function

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Argonaute (Ago) proteins interact with miRNAs to mediate translational repression and enhance degradation of mRNA. Ago2 is degraded as miRNA-free entities by autophagy. This process is the major intracellular degradation system and acts as a pro-survival response during several conditions. Glutathione (GSH) plays an important role in the antioxidant defenses of the spermatogenic epithelium. In this work, we evaluated whether autophagy is involved in spermatogonia-type germ cell line (GC-1) survival during GSH depletion and if Ago2 is affected during this condition. We showed that disruption of GSH metabolism with L-buthionine-(S,R)-sulfoximine (BSO) decreased GSH content in GC-1 cells, without altering ROS production and cell viability. Autophagy was assessed for levels of LC3BI processing to LC3BII and for its sub-cellular distribution. Immunoblot and immunofluorescence analyses showed a consistent increase in LC3BII levels and accumulation of autophagosome under GSH-depletion conditions. This process did not affect the activity of AMP-activated protein kinase (AMPK) or the ATP content. However, inhibition of autophagy resulted in decreased ATP content and increased caspase-3/7 activity in GSH-depleted GC-1 cells. Ago2 protein level decreased during GSH depletion in GC-1 and HeLa cells and when these cells were treated with BSO and chloroquine to inhibit autophagy, accumulation of Ago2 was observed. Finally, GSH depletion and autophagy inhibition affect the interaction of Ago2 with Let7a miRNA. These findings suggest that GSH deficiency triggers an AMPK-independent autophagy and that GSH and autophagy machinery are important for the miRNAs function.

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P09-006-SP
ERK- and AMPK-mediated autophagy protects Burkitt lymphoma cells from oxidative stress by increasing the activity of the ROS transforming enzymes SOD1, SOD2 and catalase

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Cellular ROS-control by autophagy is important to protect from oxidative damage in highly metabolic active cells. In this study, we investigated the role of autophagy in Burkitt lymphoma (BL) cells under oxidative stress.

We performed Western Blot analysis of key autophagy proteins and quantified autophagosomes in BL cell lines that were treated with Pyrogallol, Antimycin A or hydrogen peroxide to induce ROS.

We found LC3II increased after 12 h incubation with Antimycin A/Pyrogallol and 6 h with hydrogen peroxide. The number of autophagosomes elevated after 24 h Antimycin/Pyrogallol treatment, after 12 h hydrogen peroxide treatment. Treatment with the autophagy inhibitor chlroquine led to increased apoptosis after 24 h under Antimycin A/Pyrogallol and after 12 h under hydrogen peroxide. In addition, cell proliferation was significantly reduced. Activities of SOD1/2/Catalase increased significantly when autophagy is activated under Antimycin A/Pyrogallol. After hydrogen peroxide treatment the activity of Catalase elevated. We next analyzed the expression levels of phospho-ERK, and -AMPK in ROS induced BL cells. Phospho-ERK increased significantly after 8 h of Antimycin A/Pyrogallol treatment in both BL cell lines tested, while phospho-AMPK remained unchanged over 12 h. Hydrogen peroxide treatment led to elevated levels of phospho-AMPK reaching the peak after 6 h. Over a time period of 36 h, autophagy is not initiated after treatment with the ERK-inhibitor FR180204 under Antimycin A/Pyrogallol. Under hydrogen peroxide autophagy initiation failed in AMPK-inhibited cells.

We conclude that ERK/AMPK-mediated autophagy helps BL cells to survive and proliferate under ROS by increasing the activity of ROS transforming enzymes.

P09-007-SP
The dual role of proteases in regulation of autophagic cell death

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Intracellular proteolytic degradation plays a major role in both cell death and cell survival. *N*-α-tosyl-L-phenylalanine chloromethyl ketone (TPCK), an inhibitor of chymotrypsin-like proteases (CLP), was shown to induce apoptosis. We recently reported that TPCK provokes autophagic cell death in several cell lines. Using a fluorescent-labeled TPCK analog, the protease inhibitor α1-Antitrypsin (AAT) was "fished out" as a TPCK intracellular protein target. We further showed that AAT levels were downregulated during TPCK or tamoxifen-induced autophagy, suggesting an inhibitory role for intracellular AAT against autophagic cell death. In view of these findings, the present study investigates changes in activity of proteolytic enzymes that are involved in the autophagic process. We found an increase in trypsin-like activity, which could be attributed to AAT downregulation. Furthermore, TPCK or tamoxifen-induced autophagy appears to be associated with early stage inhibition of the activity of several proteases. These proteases however, are not inhibited directly by TPCK but rather by chymostatin and conditions of acidic pH. Mixing of control and treated lysates did not result in inhibition of these proteases. These results imply that the observed inhibition requires signaling through intact cells, and that an inhibitor is lacking in the lysate. The results also show that TPCK-induced autophagy is associated with inhibition of chymostatin-sensitive proteases, which may play a role in protecting the cells against autophagic cell death.

P09-008
The effect of long-chain α,ω-dioic acids on mitochondria

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In mammals and humans, α,ω-dioic acids (DAs) are formed predominantly in the liver and kidney cells as a result of ω-oxidation (formation of the second carboxyl group at the opposite end of the acyl chain) of the corresponding monocarboxylic fatty acids (MFA). It is noted that in liver cells under normal physiological conditions, the ω-oxidation of MFA does not exceed 10% of the total metabolism. ω-Oxidation rate is significantly enhanced under conditions that are characterized by increased levels of MFAs, various disorders of their metabolism, and under the effect of xenobiotics or ethanol. In the absence of Ca^{2+} one of the DAs-α,ω-tetradecanedioic acid (TDA) stimulates respiration of isolated liver mitochondria without affecting the $\Delta\psi$. It is assumed that the effect of TDA on mitochondria is similar to the action of agents that are supposed to switch the respiratory chain complexes to the idle mode (perform the inner uncoupling). In Ca^{2+}- or Sr^{2+}-loaded liver, heart and kidney mitochondria DAs [among them α,ω-hexadecanedioic acid (HDA) is the most effective] are able to induce the pore opening by the mechanism insensitive to CsA. Under these conditions, the release of cytochrome *c* from mitochondria is observed. Therefore, the accumulation of long-chain DAs in the liver cells is considered as one of the possible factors of their death under various pathological conditions associated with disturbance of lipid metabolism.

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P09-009
EGCG antagonizes Bortezomib cytotoxicity in prostate cancer cells by an autophagic mechanism

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The proteasome inhibitors Bortezomib (BZM) and MG132 are known to trigger cancer cell death via induction of endoplasmic reticulum (ER) stress and unfolded protein response, as a result of the accumulation of obsolete and damaged proteins.

Epigallocatechin gallate (EGCG), the most active green tea polyphenol, has been proved to have strong anticancer properties, to inhibit proteasome activity and to induce ER stress.

We investigated whether the combination of BZM or MG132 (IC_{50} doses) with 5 or 50 μM EGCG would enhance prostate cancer cell (PC3) death through increased ER stress induction. We focused our attention on the molecular mechanisms involved in the control of protein homeostasis, induction of stress response and commitment to cell death.

Paradoxically, EGCG antagonized BZM cytotoxicity already when used at the lowest concentration. At difference, MG132 dose-response curve was not affected by co-administration of EGCG. Consistently, apoptosis (PARP-1 and caspases), proteasome inhibition (accumulation of polyubiquitinated proteins) and ER stress (CHOP and p-eIF2α) were inhibited in PC3 cells treated with BZM+EGCG but not with MG132 + EGCG. We also observed that EGCG enhanced autophagy induction only in BZM-treated cells. Autophagy inhibition by chloroquine, a lysosomotropic agent, restored cytotoxicity in BZM + EGCG-treated cells concomitantly with CHOP and p-eIF2α up-regulation. Taken together, our results show that EGCG is able to antagonize BZM but not MG132 toxicity by exacerbating the activation of a compensatory autophagic degradation of proteotoxic aggregates, which in turn mitigates ER stress and reduces CHOP up-regulation, finally protecting PC3 cells from apoptotic cell death.

P09-011
SIRT1/autophagy: a cardioprotective response to Zearalenone-induced endoplasmic reticulum stress

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Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin produced by several species of *Fusarium* in cereals and agricultural products. In this study, we demonstrated that ZEN induces toxicity in cardiomyoblasts (H9C2). In fact, ZEN-treatment induced endoplasmic reticulum (ER) stress as evidenced by over-expression of GRP78 and Gadd34. In cardiomyocytes, ER stress is known to trigger autophagy, a dynamic process responsible for the degradation of cell components by the lysosomal pathway. Here, we demonstrated that cell exposure to ZEN showed an increase of autophagy markers as evidenced by western blot (LC3-II and Beclin) and flow cytometry using Cyto-ID autophagy detection probe. Besides, cell-pretreatment with Chloroquine, a well-known inhibitor of autophagy, significantly increased ZEN-induced toxicity in H9C2 cells.

The NAD-dependent deacetylase SIRT1, the founding member of the sirtuins family, has been shown to be activated in response to different heart stresses to promote cell adaptation and survival. We thus hypothesised that SIRT1 might be involved in the regulation of ER stress-induced autophagy in heart to provide a cardioprotective adaptation. To evaluate the role of SIRT1 in H9C2 following ZEN-treatment, we used a specific pharmacological inhibitor of this deacetylase, EX527. We showed that cell pretreatment with EX527 significantly increased ZEN-induced cell death. However, pretreatment with Resveratrol, a SIRT1 activator, decreased ZEN cytotoxicity which better confirm the implication of this deacetylase in the protection against ZEN-induced cell damages.

In conclusion, the SIRT1/autophagy pathway may play a critical role as a protective response to help prevent ZEN-induced ER stress in H9C2 cells.

P09-012
Modulation of SUMOylation by cholesterol-dependent cytolysins

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SUMOylation is a reversible protein post-translational modification indispensable for viability in all eukaryotes. The conjugation process is a sequential catalytic cascade involves E1, E2 and E3 enzymes. Modulation of SUMOylation has emerged as a strategy exploited by many pathogens during infection. Previous report showed that cholesterol-dependent cytolysins (CDCs), which is a group of pore-forming toxins, suppressed global SUMOylation of host cells by degrading SUMO E2 Ubc9. Here we demonstrated that recombinant CDCs, LLO, PLY, SLO and SLY, all possessed inhibitory effect on both Ubc9 stability and SUMOylation, when applied to both HeLa cells and THP-1 cells. We found that structural element of Ubc9 is related to the CDC-induced degradation, while neither SUMO-conjugation nor phosphorylation of Ubc9 is related. Surprisingly the reduced level of Ubc9 induced by CDCs was due to Ubc9 translocation, instead degradation. Our data further suggested that this process involves Ca^{2+} influx, K^{+} efflux and ATPase activities. The rapid response of Ubc9 translocation is thought to be associated with membrane repairing during the exposure to CDCs.

P09-013
Diazinon induces endoplasmic reticulum stress and autophagy in cardiomyoblasts (H9C2)

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Organophosphorous (OP) pesticides are widely used in the agriculture and home. Diazinon (DZ) is a worldwide used insecticide for pest control. Exposure to this pesticide has been reported to induce cardiotoxicity. This study investigated the mechanism of DZ-induced toxicity in cardiomyoblasts (H9C2). We demonstrated that DZ enhanced ROS generation and activated endoplasmic reticulum (ER) and Unfolded Protein Response as evidenced by up-regulation of GRP78 and GADD34 and expression of the pro-apoptotic factor CHOP. In cardiomyocytes, ER stress is known to trigger autophagy, a dynamic process responsible for the degradation of cell components by the lysosomal pathway. This ER stress response was accompanied by the induction of autophagy markers as evidenced by western blot (LC3-II) and flow cytometry using Cyto-ID autophagy detection probe. Cell-pretreatment with Chloroquine, a well-known inhibitor of autophagy, significantly increased DZ-induced toxicity in H9C2 cells indicating that DZ-induced autophagy has a cytoprotective role. In other hand, we demonstrated that DZ-induced cell death with an apoptotic process. Our results demonstrated that DZ induced the drop in mitochondrial membrane potential and caspase activation.

P09-014

The investigation of possible protective effect of taurine on extracellular matrix and related signaling pathway in renal ischemia/reperfusion model

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Renal ischemia/reperfusion injury (I/R) is a serious medical condition that might lead to acute kidney failure (AKF), and there is no specific treatment for AKF. Taurine is a naturally occurring sulfonic acid with known antioxidant properties. In addition, taurine has been shown to alleviate the formation of reactive oxygen species (ROS), the side products of elevated oxidative stress during I/R. Hence, we aimed to investigate the potential protective effect of taurine against renal I/R injury.

Nineteen Wistar rats were randomly allocated to I/R, sham, and treatment (I/R + taurine) groups. Animals in the I/R group were exposed to 1 h of ischemia, followed by 6 h of reperfusion. Treatment group received intraperitoneal taurine (200 mg/kg) 45 min before I/R. Light microscopy and biochemical analyses were used to evaluate structural and biochemical changes.

I/R group had characteristic morphological changes compared to the sham group. Moreover, I/R group had elevated creatinine, BUN (Blood urea nitrogen) and MDA (malondialdehyde) levels, which decreased after taurine treatment. SOD (superokside distumase) levels were lower in the I/R group, but increased after taurine treatment. I/R group had higher matrix metalloproteinase-2 (MMP-2) and MMP-9 mRNA levels, compared to the sham group, while taurine treatment decreased expression of both mRNAs. I/R group had higher MMP-9 activity, which decreased after taurine treatment. In addition, phospho-p38 protein levels were significantly higher in the I/R group, whereas taurine treatment caused a significant decrease.

Taken together, our results suggest that taurine exerts its protective effects through the modulation of MMP-2, MMP-9, and p-p38 levels and/or activities.

P09-015

EBR induced autophagy in colon carcinoma cell lines

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Epibrassinolide (EBR), a member of brassinostreoids with a structural similarity to mammalian steroids, play an important role to induce cell proliferation in plants. Our previous data suggested that EBR induces programmed cell death in cancer cell lines. Autophagy is a process of self-digestion in which sequestration of bulk cytoplasm and organelles occurs in autophagosomes formed by the fusion of autophagic vesicles and lysosomes. During this process ATGs, LC3 and Beclin-1 proteins are involved in the formation of autophagic vacuoles and autophagosome formation via ULK-1 signalling. Autophagy has multiple physiological functions in multicellular organisms, including protein degradation and organelle turnover. Malignant transformation is frequently associated with suppression of autophagy. We found that EBR treatment (30 mM) caused 50% cell viability loss in time dependent manner and induced autophagy by activating ULK-1 and upregulating Beclin-1 expression. The upregulation of cleaved-LC3 and Atg family proteins following EBR treatment also showed the formation of autophagic vacuoles. Lysosomes and autophagosome formation were also determined by acridine orange and monodansyl-cadaverine staining, respectively by fluorescence microscopy and FACS flow analysis after 48 h of EBR treatment in SW480 and DLD-1 cells. Surprisingly, we observed that exposure of $ATG5^{-/-}$ MEF cells, which are known to be autophagy deficient, to EBR induced autophagy. Therefore while EBR induces autophagy in SW 480 and DLD-1 colon cancer cells, the lack of ATG5 is not a limiting factor for MEF $ATG5^{-/-}$ cells after EBR treatment. Therefore ATG5-independent signaling mechanism should be discussed in future work.

Mem Biol S3, Redox-Regulation of Biological Activities

P10-003-SP

The specificity of thioredoxins and glutaredoxins is determined by electrostatic and geometric complementarity and not by redox potential

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The thiol-disulfide oxidoreductases from the thioredoxin (Trx) family of proteins have a broad range of well documented functions and possess distinct substrate specificities. The mechanisms and characteristics that control these specificities are key to the understanding of both the reduction of catalytic disulfides as well as allosteric disulfides (thiol switches). Here, we have used the catalytic disulfide of *E. coli* 3′-phosphoadenosine 5′-phosphosulfate (PAPS) reductase (PR), that forms between the single active site thiols of two monomers during the reaction cycle, as a model system to investigate the mechanisms of Trx and Grx protein specificity. Enzyme kinetics, $\Delta E^{0\prime}$ determination, and structural analysis of various Trx and Grx family members suggested: The redox potential does not determine specificity nor efficiency of the redoxins as reductant for PR. Instead, the efficiency of PR with various redoxins correlated strongly to the strength and extent of a negative electric field of the redoxins reaching into the solvent outside the active site, and electrostatic and geometric complementary contact surfaces. These data suggest that, in contrast to common assumption, the composition of the active site motif is less important for substrate specificity than other amino acids in or even outside the immediate contact area.

P10-004-SP
G6PC3 deficient human white blood cells exhibit distinct endoplasmic reticulum stress response

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Lack of glucose-6-phosphatase-beta (G6PC3) causes Severe Congenital Neutropenia type 4 (SCN4). G6PC3 is a metabolic enzyme in the endoplasmic reticulum (ER) suggested hydrolysing glucose-6-phopshate (G6P). In the lumen of ER G6P can be metabolized by hexose-6-phosphate dehydrogenase (H6PD) as well, which is responsible among others for ER redox homeostasis. SCN4 phenotype comprises congenital heart defects, urogenital anomalies, facial dysmorphism, growth and developmental delay.

How can lack of a metabolic enzyme lead to developmental malignancies? Total white blood cell (WBC) fractions from control and SCN4 patients were used for enzymatic and Western-blot measurements.

Neutrophils from G6PC3-deficient WBC-s proved the lack of G6PC3. We could not identify any metabolic aberrations in this WBC-s. G6PC3-deficient cells showed increased levels of Grp78 and phosphorylated eIF2-α compared to the control ones. Other ER stress enzymes are still under investigation. H6PD was also decreased in the mutant cells.

Lack of ER G6P metabolism in these cells suggests that endoplasmic reticulum stress can be responsible for increased apoptosis and functional anomalies in G6PC3-deficient neutrophils. However involvement of the PERK-eIF2α-ATF4 signalling pathway in SCN4 is still unclear, these alterations may at least in part be responsible for the phenotype of G6PC3 deficiency. Chemical breakdown of G6P in the ER lumen is directed mainly towards H6PD as it is suggested by the different K_M values. Maintaining NADPH in the lumen of ER is almost a sensible, important and underestimated parameter in development and differentiation.

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P10-005-SP
Redox regulation of Na,K-ATPase activity at pathological conditions

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Na,K-ATPase produces a transmembrane gradient of sodium and potassium ions required for vital functions of all animal cells. Disruption of the Na,K-ATPase activity underlies a whole range of pathological conditions involving a change of redox-status of cells, such as ischemic tissue damage, cancer and Alzheimer's disease. We have studied the redox-regulation of Na,K-ATPase in the cells associated with redox-modifications of cysteine residues of the enzyme, such as glutathionylation, nitrosylation and oxidation.

It has been shown that ischemia induces an increase of glutathione level and decrease of NO level in cells. This is accompanied by an increase in the level of glutathionylation of Na,K-ATPase catalytic-subunit leading to inhibition of the enzyme.

Treatment with beta-amyloid causes dose-dependent reduction of intracellular glutathione and a rise in NO levels. Changes of the redox-status of cells result in decreased levels of glutathionylation and increased nitrosylation of the Na,K-ATPase catalytic-subunit.

It has been shown on the myelogenous leukemia Kasumi-1 cells that changes of AML1-ETO and KIT oncogenes expression affect the Na,K-ATPase activity. Transfection of model cells by both oncogenes leads to a typical for the cancer cells increase of the Na,K-ATPase activity. Expression of oncogenes results in decrease of glutathionylation levels of catalytic-subunit, and increase its nitrosylation level, which correlates with a decrease of glutathione level, and increase of the activity of Na,K-ATPase. Thus, changes in the Na,K-ATPase activity in cells at ischemia or oncotransformation may be due to changes in the glutathionylation and nitrosylation level of the enzyme.

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P10-006-SP
Unfolded protein response to the hypercholesterolemia induced endoplasmic reticulum stress in atherosclerosis

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Hypercholesterolemia is the major risk factor for the development of atherosclerosis which is a leading cause of the death worldwide. The ubiquitin-proteasome system (UPS) may influence atherosclerosis by affecting disease-relevant cellular processes or by affecting cellular stress responses. NF-E2-related factor 2 (Nrf2) is a transcription factor that controls the expression of antioxidant genes and as well as the expressions of molecular chaperones and proteasome subunits. In the present study, we investigated unfolded protein response (UPR) and its relation with Nrf2 signaling pathway in stress conditions induced by high cholesterol diet and the effects of vitamin E in an *in vivo* model of atherosclerosis.

In order to elucidate the *in vivo* role of oxidative stress and ER stress in hypercholesterolemic model, we investigated serum levels of cholesterol, MDA and vitamin E, protein levels of Nrf2, GSTα, GRP78, GRP94, PERK, IRE1 and the proteasomal activity in aortic tissues. We found that p-PERK and p-IRE1 as ER stress markers and GRP78 and GRP94 as ER chaperons were significantly increased in the aortea. In addition, Nrf2 pathway was activated whereas the proteasomal activity was impaired by high cholesterol diet. Regarding to our results, vitamin E showed its protective effect by reducing ER stress and increasing GSTα as an antioxidant defence. We also showed that there is a reverse correlation of Nrf2 and the proteasomal system and vitamin E also affords protection via activation of the proteasomal system during this process.

P10-007
Cardiac hypertrophy induced in mitochondrial NADP+-dependent isocitrate dehydrogenase knockout mice

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Cardiac hypertrophy, a risk factor for heart failure, is associated with enhanced oxidative stress in the mitochondria resulting from high levels of reactive oxygen species (ROS). The balance between ROS generation and ROS detoxification dictates ROS levels. As such, disruption of these processes results in either increased or decreased levels of ROS. In previous publications, we have demonstrated that one of the primary functions of mitochondrial $NADP^+$-dependent isocitrate dehydrogenase (IDH2) is to control the mitochondrial redox balance, and thereby mediate the cellular defense against oxidative damage, via the production of NADPH. To explore the association between IDH2 expression and cardiac function, we measured myocardial hypertrophy, apoptosis, and contractile dysfunction in IDH2 knockout ($idh2^{-/-}$) and wild-type ($idh2^{+/+}$) mice. As expected, mitochondria from the hearts of knockout mice lacked IDH2 activity and the hearts of IDH2-deficient mice developed accelerated heart failure, increased levels of apoptosis and hypertrophy, and exhibited mitochondrial dysfunction, which was associated with a loss of redox homeostasis. Our results suggest that IDH2 plays an important role in maintaining both baseline mitochondrial function and cardiac contractile function following pressure overload hypertrophy, by preventing oxidative stress.

P10-008
c-Src-dependent EGFR transactivation mediates CORM-2-induced HO-1 expression in human tracheal smooth muscle cells

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Carbon monoxide (CO), a reaction product of the cytoprotective heme oxygenase (HO)-1, displays an anti-inflammatory effect in various cellular injuries, but the precise mechanisms of HO-1 expression remain unknown. We used the transition metal carbonyl compound carbon monoxide-releasing molecule-2 (CORM-2) that acts as carbon monoxide donor. The effects of CORM-2 on expression of HO-1 in human tracheal smooth muscle cells (HTSMCs) were determined by Western blot, real-time PCR, and promoter activity assay. In HTSMCs, CORM-2 activated Nrf2 through the activation of a c-Src/EGFR/PI3K/Akt-dependent pathway, resulting in HO-1 expression. We showed that CORM-2-induced HO-1 protein and mRNA levels were inhibited by the inhibitor of c-Src (PP1 or SU6656), EGFR (AG1478), PI3K (LY294002), Akt (SH-5), JNK1/2 (SP600125), or p38 MAPK (SB202190) and transfection with siRNA of c-Src, EGFR, Akt, p38, JNK2, or Nrf2 in HTSMCs. We also showed that CORM-2 stimulated c-Src, EGFR, Akt, p38 MAPK, and JNK1/2 phosphorylation. CORM-2 also enhanced Nrf2 translocation from the cytosol to the nucleus and antioxidant response element (ARE) promoter activity. Moreover, CORM-2 mediated p38 MAPK and JNK1/2 activation via a c-Src/EGFR/PI3K/Akt pathway, which further enhanced Nrf2 activation and translocation. Finally, we observed that CORM-2 induced *in vivo* binding of Nrf2 to the HO-1 promoter. CORM-2 activates the c-Src/EGFR/PI3K/Akt/JNK1/2 and p38 MAPK pathways, which in turn trigger Nrf2 activation and ultimately induces HO-1 expression in HTSMCs. Thus, the HO-1/CO system might be potential therapeutics in airway diseases.

P10-009
The Yin and Yang of hydrogen turnover. [FeFe]-Hydrogenases analysed by ATR FT-IR

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[FeFe]-hydrogenases catalyse hydrogen turnover in bacteria and algae. The enzymes harbour a unique active site, the so-called H-cluster, which is buried deep inside the protein. Here, two protons are reduced to molecular hydrogen at the expanse of two electrons, and *vice versa*: $2H^+ + 2e^- \Leftrightarrow H_2$

Evolutionary [FeFe]-hydrogenases have been optimized to supply and remove reactants in a well-controlled manner. Protons are guided towards the H-cluster by a proton pathway of charged residues and, potentially, water. Molecular hydrogen reaches the active site via hydrophobic gas channels, and electrons tunnel between H-cluster and protein surface across accessory iron-sulphur clusters. Whether [FeFe]-hydrogenases catalyse hydrogen evolution or uptake is controlled by the ratio $[H^+]/[H_2]$ as well as the availability of redox partners.

We analysed the "photosynthetic" [FeFe]-hydrogenase of *Chlamydomonas reinhardtii* by ATR FT-IR spectroscopy. Under H_2 supply the enzyme adopts a mixture of reduced states while with N_2 the oxidized state is formed. Interestingly, when probed at $[H^+] > 10^{-5}$ under 1 bar of H_2 the hydrogenase adopts a strongly oxidized state. Formation of HDO under 1 bar of D_2 indicates uptake activity, albeit restraint in comparison to pH $\geq$6. ^{13}CO exchange identifies the distal iron ion (D) as reductant. Site-directed mutagenesis of the proton pathway produces enzyme that adopts the "superoxidized" state with H_2, irrespective of $[H^+]$. The same effect is observed upon prolonged dehydration in presence of H_2.

P10-010
Mitochondrial Ca^{2+} uptake is regulated by the Ca^{2+}-dependent interaction of a disulfide-linked MICU1-MICU2 dimer which is formed by Mia40

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The essential oxidoreductase Mia40 mediates disulfide bond formation and protein folding in the mitochondrial intermembrane space. Here, we investigated the interactome of Mia40 thereby revealing links between thiol-oxidation and apoptosis, energy metabolism and Ca^{2+} signaling. Among the interaction partners of Mia40 is MICU1 – the regulator of the mitochondrial Ca^{2+} uniporter (MCU) which transfers Ca^{2+} across the inner membrane. We show that Mia40 introduces an intermolecular disulfide bond which links MICU1 and its inhibitory paralog MICU2 in a heterodimer. MICU2 binding to MCU depends on MICU1. In line with this we demonstrate that absence of the disulfide results in increased ATP-induced mitochondrial Ca^{2+} uptake. In the presence of the disulfide bond MICU2 levels are controlled by dissociation of the heterodimer from MCU upon high Ca^{2+} concentrations. Our findings support a model of Ca^{2+}-dependent remodeling of the Ca^{2+} uniporter that depends on the presence of a disulfide bond in the MICU1-MICU2 heterodimer.

P10-011
Inhibition of human peroxiredoxin 5 by catechol derivaties: an enzymatic kinetic approach

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Strokes are a common cause of death and disabilities effecting millions worldwide. Ischemic strokes are the most common type. They are characterized by clotted brain vessels that restrict cerebral blood flow, ultimately resulting in the death of brain cells. Since stroke patients suffer from a multitude of pathological effects as a consequence of brain inflammation, research is currently focused on preventative measures to delay post-ischemic inflammatory response. Recently, human peroxiredoxin proteins (hPrx1, 2, 5 and 6) have been found to regulate the brain inflammation cascade. It is therefore of interest to characterize inhibitors against Prx's. Through NMR-screening and modeling studies, small molecules catechol derivatives have been identified to bind and interact with hPrx5. In this study, hPrx5 peroxidase activity is assessed through an *in vitro* enzymatic assay, to understand how these catechol derivatives can bind and potentially inhibit hPrx5. It has been determined these catechol derivatives can bind and reversibly inhibit hPrx5. The catechol inhibitors, have been ranked according to their half maximal inhibition concentration value (IC_{50}) and correlate to the binding dissociation constants (K_d) previously reported by NMR. Currently, the inhibition mechanism of these inhibitors is being evaluated through enzymatic kinetics. Overall, this research can provide greater insight for designing higher affinity inhibitors to bind and inhibit Prx's and therefore contribute to the delay and prevention of post-ischemic inflammation.

P10-012
Functional state of rat heart muscle cells and blood antioxidant system under psycho-emotional stress

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It is widely accepted that due to any kind of stress, response reactions are launched in a cell of the living organism, namely: free radical oxidation, diminution of the energetic metabolism, etc. eventually ending up with forming a whole list of pathologies. During last year's special attention was drawn to study of the influence of these factors on the process to development of various types of disease of the cardiovascular system. We have studied Functionality of the antioxidant system in laboratory rat heart muscle cells and blood under psycho-emotional stress. It has been found that 30-day isolation and violation of the diurnal cycle among the animals is accompanied by intensification of lipid per oxidation process and marked with a reduced activity of antioxidant system enzymes, such as catalase and superoxid-dismutase activity. It has been suggested that psycho-emotional stress is accompanied by oxidative stress, which is reflected by the reduction in the intensity of energy metabolism in heart muscle cells. Such suggestion is strengthened by the fact that the activity of the enzymes involved in the metabolic process in progress in mitochondria is reduced, as well as by reduction in the activity of enzymes participating in the process of glycolysis. Based on the results we suggest that psychological stress is one of the factors contributing to development of cardiological diseases.

P10-013
The effect of various antioxidants in cell death-related oxidative stress

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The majority of reactive oxygen species (ROS) is produced by mitochondria. ROS could have a double role – low concentrations could be involved as cellular signals in various molecular pathways, while high concentrations could lead to oxidative stress and cause molecular damage. Effective cellular antioxidant systems are complemented by exogenous antioxidants in therapy.

The aim of our study is to test the capability of antioxidants to affect induced oxidative stress in different cells and to resolve the exact effect of antioxidants that might be beneficial in some therapeutic approaches.

In our study cells' media was enriched by the selected antioxidants: NAC, α-tocopherol, MnTBAP and tempol. Various inducers of cell death were used as triggers of oxidative stress: menadione (MD), staurosporine (STS), and tumour necrosis factor-α (TNF-α), where necessary in combination with cyclohexamide and caspase inhibitor.

Based on our results we can conclude that MD, STS and TNF-α treatment caused ROS, followed by cell death. All used antioxidants showed reducing effect on ROS production by either MD or STS, while they were less efficient in scavenging TNF-α induced ROS. Furthermore, NAC seemed to be more efficient antioxidant in comparison to α-tocopherol and MnTBAP. Notably, cell death was not always reduced by antioxidant usage. While, usage of tempol as an antioxidant is problematic due to its low scavenging capacity and contrary action. With this study we alert that tempol is often used incautiously and its effect should be re-evaluated, although tempol seems to have a great potential for co-treatment with other chemotherapeutics.

P10-014
Molecular determinants for cytosolic Fe/S cluster insertion

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Up to 50 cytosolic and nuclear Fe/S proteins act in a wide range of cellular processes in eukaryotes. These proteins are of key importance for ribosomal maturation, tRNA modification, DNA replication and repair, and are thus found in all eukaryotes. By the socalled cytosolic iron sulfur protein assembly (CIA) machinery composed of nine proteins biosynthesis and insertion of the Fe/S clusters is carried out. The determinants for the recognition of the 50 proteins among the 6000–25,000 of cytosolic proteins, which a typical eukaryotic organism contains, remain completely obscure. A path driven by cluster transfer to a thermodynamically favoured binding site at the target is tacitly assumed, similar to the belief in a spontaneous path before discovery of the mitochondrial and cytosolic Fe/S biosynthetic machineries. This "thermodynamic model" has been challenged by various observations, suggesting a highly selective protein-guided process. First, several factors of the mitochondrial Fe/S biosynthetic machinery

have specific target apo-Fe/S proteins. Second, a Lys-Tyr-Arg (LYR) primary sequence motif of Fe/S proteins has been identified as determinant for maturation in mitochondria. Third, most relevant for the CIA machinery, different affinities for target apo-Fe/S proteins were detected by mass spectrometric analysis of late CIA machinery components.

Here, we report on phenotypic analysis of mutants and activity measurements of intrinsic and ectopic enzymes to identify amino acid sequence motifs, which are critically required for maturation.

P10-015
Biochemical characterization of a novel azoreductase from *Rhodococcus opacus* 1cp

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Azo dyes are characterized by one or more R_1–N = N–R_2 bonds and can be enzymatically metabolized. The gene from the strain *Rhodococcus opacus* 1CP encoding a novel flavin-containing azoreductase (25 kDa), which is able to catalyse the degradation of azo dyes, was identified and overexpressed in *Escherichia coli* BL21 (DE3). The recombinant azoreductase was purified through nickel affinity columns by fast protein liquid chromatography (FPLC). NADH served as an electron donor and 2-(4-dimethylaminophenylazo)benzoic acid (Methyl Red) served as the azo-substrate for activity assay. Biochemical characterization demonstrated this azoreductase performed higher degradation velocity between pH 3.8–5 in acetate buffer. But, through stability analysis, it lost activity when pH was 4.0. From thermal assay the highest activity was reached at 53°C from a range 10–65°C. This azoreducatase was stable from 20–35°C and the unfolding temperature was 60°C. Metal ions like Mg^{2+}, Ca^{2+}, Zn^{2+} and Fe^{3+} inhibited the enzyme activity while Cu^{2+} and Mn^{2+} accelerated. Enzyme kinetics suggested that the association between the enzyme and the substrate methyl red was strong. HPLC analysis verified this azoreductase cleaved methyl red into *N,N*-dimethyl-*p*-phenylenediamine and 2-aminobenzoic acid.

P10-016
Identification and characterization of novel bacterial [FeFe]-hydrogenases for exploitation as highly efficient H_2-producing catalysts

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The identification and characterization of novel [FeFe]-hydrogenases offers the opportunity to cover the lack of information on these enzymes and to address the ever increasing demand of catalysts able to produce H_2 at high rates and low costs. Bacterial [FeFe]-hydrogenases represent a promising source of inspiration – mimicking nature – for the design of artificial catalysts to be used in green chemistry, for H_2 high efficiency production and in fuel cells.

In this study, culturable microbiological flora was isolated from green waste biomasses during dark fermentation and grouped in 11 classes on the basis of restriction fragment length polymorphism (RFLP) profile analysis of 16S rDNA and of morphological features. H_2 production efficiency of each member belonging to the classified groups was assayed by gas chromatography.

Clostridium beijerinckii and *Clostridium tyrobutyricum* were found to be two high hydrogen producers (299.4 ± 2.5 and 246 ± 6.7 H_2 ml/g glucose respectively).

Six uncharacterised *C. beijerinckii* [FeFe]-hydrogenases were identified and the most promising enzymes were produced in active form after gene cloning and recombinant heterologous expression in *E. coli*.

Green waste biomass was enriched with these two hydrogen-producing strains isolated from the same matrix; studies were performed on the effect of microbial consortia on H_2 production and on the modulation of seven different [FeFe]-hydrogenase genes in fermentative process by qPCR.

The resulting data suggest a complex cellular regulation and possible interplay between different metabolic pathways involving hydrogenases with different roles, time and mode of expression. These results lead to a better knowledge of the mechanisms of catalysis for improved biotechnological applications.

P10-017
Sulfation of quercetin reduces its biological activity

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The flavonoid quercetin is one of the most extensively studied plant secondary metabolites. It is well known for its antioxidant, anti-inflammatory and anticancer effects. One of the main metabolites of quercetin in mammals is quercetin-3′-*O*-sulfate (Q3′S). In this study we prepared Q3′S enzymatically and compared its biological activity with that of quercetin. We found that the sulfation of quercetin significantly decreased its ability to scavenge DPPH radicals. Quercetin reduced DPPH radicals with the EC_{50} of 3.8 μM while the EC_{50} value found for Q3′S was 23.5 μM. We also found that Q3′S, in contrast to quercetin, did not induce the expression of heme oxygenase-1 in murine macrophage RAW264.7 cells and the expression of cytochrome P450 1A1 in human hepatoma HepG2 cells. We further tested the uptake of both compounds in HepG2 cells by using HPLC/MS. We found that Q3′S was absorbed to a much less extent than quercetin. For instance, our analyses of HepG2 cells exposed for 6 h to 50 μM flavonoids showed that the yield of quercetin was 1.14 nmol per 10^6 cells whereas the yield of Q3′S reached only 38 pmol per 10^6 cells. We conclude that the sulfation of quercetin at 3′-OH attenuates the antiradical and biological effects of the parent molecule.

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P10-018
Redox control of cytoskeletal dynamics: toggling the thiol switch in CRMP2

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This project addresses the redox regulation of collapsin response mediator protein 2 (CRMP2/DPYSL2), a mediator of the semaphorin-plexin signaling pathway. A thiol disulfide redox switch in CRMP2 controls actin and maybe tubulin dynamics by affecting its direct interactions with other proteins [1]. Cytosolic glutaredoxin 2 (Grx2c), that is essential for embryonic brain development [2] and is specifically induced in many cancer cells [3], specifically reduces the allosteric disulfide in CRMP2 [4,5]. This regulation is required for normal axonal outgrowth and brain development [2]. Cancer cells, like HeLa cells, expressing Grx2c show dramatic alterations in morphology and gain the ability to actively migrate and invade a collagen matrix, a hallmark in cancer progression (submitted). We identified a specific and reversible intermolecular thioldisulfide switch in homotetrameric CRMP2 that determines 2 conformations of the complex and is efficiently reduced by Grx2c *in vivo* and *in vitro* [5]. Here, we analysed the potential function of the FAD-dependent monooxygenases "molecule interacting with CasL" (MICAL) 1 and 2 as specific oxidases of the thiol switch in CRMP2 *in vivo* and *in vitro*.

P10-019
Oxidizer and reducer different effects on proton-translocating F_oF_1-ATPase activity of *Rhodobacter sphaeroides* membrane vesicles

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Anaerobic growth of purple bacterium *Rhodobacter sphaeroides* from Armenian mineral springs upon light is coupled with drop of redox potential (E_h) from positive to low negative values [1]. In these conditions the F_oF_1-ATPase of *R. sphaeroides* operates as ATP-driven H^+- pump with generation of proton motive force (Δp) [1]. E_h can affect Δp by changing proton gradient across the bacterial membrane and activity of H^+-translocating ATPase. For understanding the H^+-translocating F_oF_1-ATPase role in bacterial redox sensing the effects of oxidizer ferricyanide and reducer dithiothreitol (DTT) on DCCD-inhibited ATPase activity of *R. sphaeroides* MDC6521 membrane vesicles were studied. Oxidizer and reducer affect the ATPase activity in different manner. DCCD-inhibited ATPase activity of bacterium, grown in medium with 1 mM DTT, was increased ~1.5-fold in compare to control. *R. sphaeroides* membrane vesicles, grown with 1 mM ferricyanide, demonstrated ~1.6-fold DCCD-inhibited enzyme activity. Many membrane proteins contain thiol-groups as cysteine residues, which redox states might affect the enzymes activity. The increase of ATPase activity by reducer might be connected with the changes of enzyme dithiol/disulfide status. The effects of redox reagents on accessible thiol-groups number in bacterial membrane vesicles were also determined. The thiol-groups number was enhanced by addition of ATP. An additional increase in thiol-groups number was observed in presence of reducer, but not oxidizer. The interaction DTT with ATPase can lead to the break of disulfides in membrane proteins and inhibition of a dithiol-disulfide interchange.

Reference

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P10-020
Recombinant human HSP60 produced in ClearColiTM BL21 (DE3) lacks cytokine activity mediated by the NFκB pathway

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HSP60, an intracellular molecular chaperone has been largely described as an alarmin or Damage-Associated Molecular Pattern when released outside the cell. HSP60 has been reported as a possible ligand of TLR2 or TLR4 inducing NFκB-dependant signalling pathway leading to cytokine secretion. However, recent publications suggested that HSP60 could not act as an activator of TLR4 by itself. The observed effect could be due to the presence of endotoxin in HSP60 preparation especially LPS. In order to clarify the controversy, we produced recombinant human HSP60 in two different strains of *Escherichia coli*, standard strains for protein overproduction, BL21 (DE3), and the new ClearColi BL21 (DE3) strain which lacks LPS-activity through TLR4. Undoubtedly, we have shown that recombinant HSP60 by itself was not able to induce NFκB-dependant signalling pathway in a model of THP1 monocyte cell line. Our data suggest that HSP60 needs either pathogen-associated molecules, specific post-translational modification and/or other host factors to activate immune cells via NFκB activation.

P10-021
Inhibition of glycerol-3-phosphate oxidase activity of liver mitochondria by palmitic acid in the presence of ATP and *tert*-butylhydroperoxide

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The effect of palmitic acid (Pal) on glycerol-3-phosphate oxidase (GP-oxidase) activity of liver mitochondria was investigated in the presence and absence of ATP, and under *tert*-butylhydroperoxide (TBH)-induced oxidative stress. We found that Pal inhibits GP-oxidase activity of de-energized mitochondria formally in the competitive manner. It is noted that the competitive inhibition of mitochondrial GP-oxidase activity by Pal may be related to its ability to increase the negative charge density on the outer surface of the inner membrane. ATP eliminates the ability of Pal to inhibit mitochondrial GP-oxidase activity. This effect of ATP is not observed in the presence of F_OF_1-ATP-synthase inhibitor oligomycin. Apparently, in the case of vector transport of H^+ from the matrix to the intermembrane space of mitochondria induced by ATP-hydrolysis there is occurs protonation of Pal^- on the outer surface of the inner membrane and subsequent movement of its neutral molecules to the inner monolayer of the inner membrane. TBH in the presence of ATP and Pal inhibits their GP-oxidase activity formally in the competitive manner without effect on mitochondrial ATP-ase activity. We assumed that

TBH-induced oxidative stress in the case of ATP-energized mitochondria results in an increase in transport rate of Pal anions from the inner monolayer of the inner membrane to its outer monolayer. This, in turn, is accompanied by an increase in the density of negative charges on the outer surface of the inner membrane and inhibition of mitochondrial GP-oxidase activity formally in the competitive manner.

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P10-022
The evaluation of certain biochemical antioxidant markers in the blood of patients with schizophrenia

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The activity of the body's antioxidant defenses in schizophrenia was the object of study for many researches in the biomedical field of activity. For our particular researches we chose both some well known components of the antioxidant defensive mechanisms (namely the SOD and GSH-Px enzymes) and also some other biochemical compounds known mainly for other roles and functions but which present also antioxidant properties (albumin, direct bilirubin, total bilirubin, indirect bilirubin and uric acid).-For the first research presented here we have chosen a group of eight patients diagnosed with schizophrenia. All the patients received, prior to the research, antipsychotic medication. We determined for this group the levels of albumin, direct bilirubin, total bilirubin, indirect bilirubin and uric acid. The blood serum samples were biochemically analyzed and the results were later statistically compared with the normal average values using the One-Sample T-test. The results for albumin and direct bilirubin were found to be statistically significant ($p < 0.01$ and Cohen's $d = 2.02$, $p < 0.05$ and Cohen's $d = 1.13$, respectively), indicating decreases as compared with the normal values.For the second research we chose to investigate the levels of SOD and GSH-Px. For GSH-Px we chose a group of 11patients, and for the SOD a group of 9 patients. All these patients received also antipsychotic medication prior to the research. The technical material and the statistical methods used were the same. The results showed statistically significant decreases both for GSH-Px ($p < 0.001$, Cohen's $d = 1.91$) and for SOD ($p = 0.002$, Cohen's $d = 1.57$).

P10-023
Vesicular transport and small G proteins are involved in glutoxim and molixan effect on intracellular Ca^{2+} concentration in macrophages

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Synthetic analogues of oxidized glutathione (GSSG) disulfide-containing drugs glutoxim® (GSSG disodium salt with d-metal nanoaddition, "PHARMA-VAM", St. Petersburg) and molixan® (complex of glutoxim with inosine nucleoside) are used as a wide range immunomodulators. However, cellular and molecular mechanisms underlying these drugs action are still unclear.

Recently, we showed for the first time that glutoxim and molixan cause intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, increase due to Ca^{2+} mobilization from thapsigargin-sensitive Ca^{2+} stores and subsequent store-dependent Ca^{2+} entry in rat peritoneal macrophages. Also, it was found that actin filaments and microtubules are involved in signalling cascade induced by glutoxim and molixan and leading to $[Ca^{2+}]_i$ increase in macrophages. It invites the assumption that macrophage activation induced by these agents is mediated by vesiclular traffic. Thus, the aim of the present work was to elucidate the possible involvement of vesicular transport and small G proteins, important components of the exocytosis pathway, in glutoxim and molixan effects on $[Ca^{2+}]_i$ in macrophages.

Using Fura-2AM microfluorimetry we have shown for the first time that macrophage preincubation with small G proteins of the Ras superfamily inhibitor *N*-acetyl-S-farnesyl-L-cysteine and vesicle transport inhibitor brefeldin A, which inactivates small G proteins of Arf subfamily, that are central to the regulation of vesicular transport, considerably suppress both phases of Ca^{2+}-responses induced by glutoxim or molixan.

Results suggest that Ca^{2+} responses induced by glutoxim and molixan in macrophages depend critically on small G proteins of the Ras superfamily, as well as on vesicular traffic.

P10-024
The involvement of actin-binding proteins in glutoxim and molixan effect on intracellular Ca^{2+} concentration in macrophages

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The redox system glutathione/glutathione disulfide (GSH/GSSG) plays an important role in redox regulation of cellular functions. The disulfide-containing drugs glutoxim® (GSSG disodium salt with d-metal nanoaddition, "PHARMA-VAM", St. Petersburg) and molixan® (complex of glutoxim with inosine nucleoside) belong to the special group of immunomodulators and hemostimulators (thiopoetins) widely used in therapy of oncological and infectious diseases. However, cellular mechanisms of glutoxim and molixan action are poorly understood.

Previously we showed that glutoxim and molixan increase intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, due to Ca^{2+} mobilization from thapsigargin-sensitive Ca^{2+} stores and subsequent store-dependent Ca^{2+} entry in rat peritoneal macrophages. Using actin depolymerizers and stabilizers we found that actin reorganization is crucial for glutoxim- and molixan-induced Ca^{2+} responses. Thus, investigation of actin-binding proteins involvement in glutoxim and molixan effect is also of particular interest.

$[Ca^{2+}]_i$ measurements were performed with Fura-2AM microfluorimetry. To examine actin-regulating proteins participation in the effect of GSSG-based drugs on $[Ca^{2+}]_i$ we used compound CK-0499666, which disrupts Arp2/3 (actin-related protein) complex involved in actin branching and new microfilaments formation, and wiskostatin, inhibitor of WASPs (Wiskotte–Aldrich syndrome proteins) that are necessary for Arp2/3 complex activation. It was demonstrated for the first time that both wiskostatin and CK-0499666 significantly decrease Ca^{2+} mobilization as well as Ca^{2+} entry induced by glutoxim or molixan in rat peritoneal macrophages. Thus, the results suggest that dynamic actin reorganization triggered by Arp2/3 complex and WASPs is important to initiate complex signaling cascade activated by glutoxim and molixan and leading to $[Ca^{2+}]_i$ increase in macrophages.

P10-025
Correlations between some enzymatic antioxidants and some cations in patients with affective depressive disorder

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The depression has a complex fundamental cause of genetic vulnerability accompanied by a number of biochemical changes and a sensitivity towards certain life events, known as events that induce a high degree of stress on individuals.

The aim of this study was the determination of some enzymic antioxidants (SOD, GPx, GR) and of serum iron in order to establish some statistical correlations that could help to confirm the influence of the oxidative stress in depression diagnosis.

The studied group included 30 patients diagnosed with affective disorder according to clinical criteria from DSMIV and to the scores obtained to Hamilton Depression Scale. The Study Group was made up of 30 patients (56.67% females and 43.33% males) and the Control Group was composed of 30 patients without hydroelectrolyte imbalances and affective disorders in antecedents.

For the biochemical parameters analyzed in this study, between their mean values in male and female groups, separately for each plot (study and control), there are no statistically significant differences at $p > 0.05$. Also, there are no statistical correlations between these enzymatic antioxidants and between them and serum iron in study group.

Survey results have shown that there are significant statistical differences between SOD values between the two groups, which reflects the fact that oxidative stress lead to quantitative changes in case of this enzymatic antioxidant. These results support the idea that free radicals are implicated in the etiopathogenesis of depressive disorder and they can produce some neurodegenerative changes.

P10-026
Modification of the mechanism regulating the iron metabolism and correlation with oxidative stress in associated pathology of chronic hepatitis C and rheumatoid arthritis

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Studies on serum iron in different pathologies show that there is an alteration of the mechanisms regulating iron homeostasis. Hepcidin, a polypeptide synthesized in the liver, is involved in these mechanisms, blocking the export of iron deposits from the liver and decreasing Fe absorption in the intestine. Hepcidin activity is influenced by diverse factors, for example the HVC infection or inflammation in rheumatoid arthritis. In these two pathologies, opposing mechanisms on the iron homeostasis are activated. Fe is also considered a prooxidant factor.

Our study assessed serum iron and oxidative stress markers, superoxide-dismutase (SOD) and glutathion peroxidase (Gpx), in an associated pathology, chronic hepatitis with HVC and rheumatoid arthritis (HCVC + RA). We compared a group of patients HCVC + RA with three groups: control, HCVC and RA.

It was found that serum iron level increases in HCVC group, and decreases in the RA group, confirming the opposite mechanisms occurring in Fe metabolism regulation.

Serum iron level in HCVC + RA group is lower than the reference groups; hyposideremia may be induced by altering modulation of hepcidin expression. There are statistically significant differences ($p < 0.05$) between the pathological groups.

Evaluating the oxidative stress versus serum iron the presence of a strong negative correlation in the HCVC group was revealed ($r = -0.312$, $p = 0.001$; $p < 0.05$).

In the associated pathology, regulatory mechanisms of Fe metabolism are profoundly altered. The presence of oxidative stress may be seen in particular in viral pathology where the level of serum iron is higher.

P10-027
Silver Nanoparticles as an antihemolytic agent

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In this study, the effect of silver nanoparticles on the hemolysis index of Wistar rats was studied. Fourteen days was the duration of treatment of the animals. Female rats were randomly divided into five groups including: reference, control, experimental 1, experimental 2, and experiment 3. Group 1 (control) received enough food and water per each day during this period. Group 2 (reference) received physiologic serum in each day of the treatment period. The empirical group 1 in the fourteenth treatment day received 50 mg Isoniazid drug for each kilogram of the body weight of the animal by injection into the peritoneum. To the experimental groups 2 and 3, nanoparticles of silver with doses of 0.25 and 0.5 kg/mg/bw were injected into the peritoneum during 13 consecutive days. After passing 24 h (fourteenth day), nanoparticles in combination with the isoniazid drug, with the

dose of 50 mg/kg animal body weight, were injected again. The obtained results showed that the isoniazid drug makes no change to the red blood cell count and hematocrit, also it causes the decrease of hemoglobin; while, silver nanoparticles caused increase of the hemoglobin. Also fragility of the red blood cells increased because of isoniazid; but in the presence of silver nanoparticles this effect was reduced. The results obtained in the experiments show the anti-oxidant and anti-hemolytic role of silver nanoparticles.

P10-028
Assessing the level of medium-weight molecules in the semen of men of reproductive age in the area of environmental crisis of Aral Sea region

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Reproductive health is an important part of overall health and is central to human development. One of the crisis regions of Kazakhstan is recognized as the Aral Sea region. The sanitary and ecological situation in the Aral Sea region currently continues to deteriorate. Thus, all of the above identifies the urgency of the problem, there is a need for studying the causal link between the incidences in the population and leading toxic environmental factors that will identify the criteria for early diagnosis and prenosological forms of diseases caused by exposure to the environment in the Aral Sea region. The aim of the study was to assess the level of medium-weight molecules in men of reproductive age in the area of environmental crisis in the Aral Sea region at the molecular–cellular level. Clinical and laboratory studies were conducted in men of reproductive age in the zone crisis areas Zhusaly, Zhalagash and Shieli of Kyzyl-Orda region. The age of persons surveyed was 18–49 years. The material of the study was the sperm. The total number of men surveyed was 524 people. For the determination of middle molecules in sperm in the investigated persons we used the technique of Kovalevsky A.N and Nifanteva O.E.

The results of this study suggest the action of the negative factors of the ecological crisis in the germ cells of men of reproductive age, the accumulation of middle molecules has negative consequences for the organism, because they are well adsorbed on the membranes and lead to disruption of membrane transport induced by the endogenous intoxication in the cells.

P10-029
Oxidative status of neutrophils from patients on chronic hemodialysis

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Susceptibility to bacterial infections is considered as one of the major causes of morbidity and mortality in patients with end stage chronic renal failure (CRF). There is no detailed information about metabolic status of neutrophils from CKF patients. The purpose of our research was to study the effect of chronic hemodialysis on oxidative status of neutrophils from end stage CRF patients. Thirty seven end stage CRF patients on chronic hemodialysis treatment participated in these studies. Twenty-five of these patients had chronic pyelonephritis as the initial form of disease (1st group), and 12 had chronic glomerulonephritis as the initial form of disease (2nd group). As control, neutrophils from 32 healthy ones were used. In neutrophils, advanced oxidation protein products (AOPP), protein reactive carbonyl derivatives were detected. Our results have demonstrated the decrease of AOPP in neutrophils of all patients with end stage CRF on chronic hemodialysis (by 1.5 times, $p < 0.05$) compared with control ones. We have fixed the decline of intracellular protein reactive carbonyl derivatives in neutrophils from end stage CRF patients of the 1st group (by 1.3 times) and of the 2nd group (by 1.57 times, $p < 0.05$) compared with control ones.

We have demonstrated the presence the neutrophils with altered oxidative status from end stage CRF patients on chronic hemodialysis. We have surmised that impaired protective function of neutrophils from end stage CRF patients on chronic hemodialysis may be connected with the suppression of oxidized proteins formation.

P10-030
The relation between the erythrocytes' glutathione-dependent antioxidant enzymes and the consumption of a nutritional supplement in post-acute stroke patients

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Objective: This study was designed to investigate the relation between the activity of erythrocytes' glutathione-dependent enzymes and the consumption of a nutritional supplement (ALAnerv®) in post-acute stroke patients.
Material and methods: The study population comprised 28 patients which were randomly divided into a control group [(−) ALA] and a study group [(+) ALA]. All the subjects were hospitalized for a period of 2 weeks. Beside the standard medication, patients from the (+) ALA group received ALAnerv® (2 pills/day/2 weeks). Blood samples were taken at the admission and at the discharge moment, respectively. Erythrocytes were isolated and on the corresponding lysates the activities of glutation peroxidase (GPx), glutatione reductase (GRed) and glutatione transferase (GT) were assessed. The statistical analysis was performed usingGraphPad InStat 3 and a p value < 0.05 was considered to be statistically significant.
Results: In (−) ALA group GT and GPx activities increased, while GRed activity decreased. In (+) ALA group GT and GPx activities decreased, while GRed activity increased.
Conclusions: These results suggest that a longer treatment period could lead to a correction of the erythrocytes' redox status, including the activities of the glutathione-dependent enzymes.
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P10-031
Hypoxia/reperfusion injury evaluated in a cardiac cell model: protection by antioxidant plant extracts

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Ischemic heart disease is one of the main causes of death worldwide and its relationship with ROS has been well established.

We have implemented an i*n vitro* model of cardiac ischemia, using H9c2 cells, based on GOX/Cat activities that are described as rapidly generate a stable oxygen atmosphere of about 2%. With this enzymatic system we intend to clarify the role of ROS in hypoxia-induced cardiomyocyte cell death and evaluate the effects of promising novel antioxidant plant extracts in the prevention of ischemia/reperfusion cardiac damage.

Incubation with the enzymatic system results in cell death, depending on the ratio of GOX to Cat and on the time incubation period. For 24 h incubation we achieved more than 50% of cell death evaluated by the SRB assay. This is a much more effective result than that obtained with $CoCl_2$ incubation, as oxygen depleting agent. This hypoxia-induced cell death was characterized by Hoechst staining, which confirmed more than 40% of apoptotic cells, after 16 h incubation. Mitochondrial membrane depolarization was also affected by the enzymatic system, as accessed by TMRM fluorescence. Trolox was shown to protect H9c2 cells from GOX/Cat system-induced hypoxia, reverting the decrease in cell proliferation and the number of apoptotic nuclei. We also present evidence that these hypoxic cells are able to recovery when incubated for 24 h more, with fresh media. The effect of antioxidant plant extracts, are being evaluated as potential new agents useful in the overcoming of cardiac infarction.

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P10-033
Expression of cellobiose dehydrogenase from *Phanerochaete chrysosporium* in yeast *Saccharomyces cerevisiae* for directed evolution

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Cellobiose dehydrogenase (CDH) gene from *Phanerochaete chrysosporium* has been cloned in yeast *Saccharomyces cerevisiae* for extracellular expression. Recombinant CDH produced in yeast had lower specific activity of 0.6 U/mg of pure protein than native CDH produced in *P. chrysosporium*. Recombinant enzyme showed similar substrate specificity for cellobiose and lactose. Optimal temperature and pH stability was slightly different compared to native CDH. The molecular weight of recombinant CDH was higher than molecular weight of native CDH (90 kDa) with a broad band on SDS electrophoresis gel at 120 kDa that was result of hyperglycosylation. Results showed that CDH can be expressed in yeast *S. cerevisiae* which can be used in directed evolution experiments. CDH gene library was generated using error-prone PCR to create random mutations and obtained mutants were tested in microtiter plates for improved activity using adapted DCIP assay. Several mutants with increased activity were detected in microtiter plates.

P10-034
Molecular dissection of Mia40 functions in *Saccharomyces cerevisiae*

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The majority of mitochondrial proteins are encoded in the nucleus, synthesized in cytosol and imported into the mitochondria. Proteins targeted to the mitochondrial matrix carry an N-terminal targeting sequence which allows the translocation across the outer and the inner mitochondrial membranes. Many proteins of the mitochondrial intermembrane space (IMS) lack the presequence but contain conserved cysteine residues that are organized in so-called Cx_3C or Cx_9C motifs. In addition, IMS proteins contain special hydrophobic binding sequences named MISS regions. The import of these proteins into the IMS is mediated by the mitochondrial oxidoreductase Mia40. Mia40 possesses two characteristics which are combined within a single protein – the ability to oxidize incoming substrates via a redox-active CPC motif and a chaperone-like activity via a hydrophobic binding cleft. In this study, we addressed the possibility to separate both Mia40 functions on molecular level. Our preliminary results show that both functions have to be present within a single protein. However, different Mia40 substrates differ considerably in their dependence on the chaperone and oxidase activities of Mia40.

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P10-035
Molecular dissection of the mitochondrial protein import machinery

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Nuclear encoded mitochondrial proteins are synthesized in the cytosol and are translocated to their respective destinations within the mitochondria. The TIM23 complex mediates the translocation of precursor proteins that are targeted to the mitochondrial matrix or to the mitochondrial inner membrane. Tim17 is an integral component of the TIM23 translocase containing four transmembrane domains. The highly conserved Tim17 protein contains two conserved cysteines that are located directly adjacent to the first and second transmembrane domains facing the intermembrane space (IMS). These cysteines are oxidized at steady state under *in vivo* and *in vitro* conditions, whereas the other two cysteines are found to be in a reduced state within the transmembrane domain. The cysteines at position 10 and 77 are involved in a disulfide bond formation. The disulfide bond in Tim17 presumably has a stability rather than a regulatory function and can only be released upon treatment with unphysiologically high concentration of reductants. Severely affected import kinetics of radiolabelled Tim17 into Mia40 down regulated mitochondria indicates a role of Mia40 for the import. However, it is not clear whether the disulfide bond of Tim17 is formed directly by Mia40 or by another IMS protein that accumulates in the IMS in a Mia40-dependent manner. The implications for the role of Tim17 and its cysteine motif in regulatory and mechanistic function remain yet to be examined.

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P10-036
RAW 264.7 response to quantum dots generated ROS decides cellular fate: activation versus necrosis

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Quantum dots (QDs) are useful tools in biomedicine, nonetheless they are often composed of cytotoxic heavy metals. Therefore silicone QDs are considered promising alternatives. Macrophages have an important role in generating the tissue-level lesions associated with exposure to silicone nanoparticles, and we reveal the link between oxidative stress and the activation of RAW 264.7 cells upon exposure to Si/SiO_2 QDs.

MTT assay revealed that LD_{50} was 15.3 μg QDs/ml after 24 h of exposure. The LIVE/DEAD assay and LDH test indicated a loss of cell membrane integrity as soon as 6 h of exposure to 15 μg QDs/ml. Microscopic analysis revealed at this time interval extensive vacuolisation, while after 24 h, cellular fusion resulted in multinucleated giant cells formation. RAW 264.7 cells can form osteoclasts under Nf-kB activation, a scenario supported by our data that showed COX activity increased after 6 h of exposure supporting prostaglandin E2 gradual accumulation in the culture supernatant.

The expression of the 89 kDa transcriptionally active Nrf-2 increased with time exposure and was positively correlated with ROS levels and the activation of protective antioxidant glutathione S-transferase. These results suggest that QDs exposure generated a crosstalk between pro-inflammatory NF-kB and antioxidant Nrf2 transcription factors.

In conclusion, early NF-kB activation might induce Nrf2, in order to promote cellular survival and activation during oxidative challenge.

P10-038
Regulation of redox status in placenta in case of physiological pregnancy and complicated pregnancy

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Objective: Study the production of a number of proteins involved in maintaining the redox status of placenta as well as the their influence on condition of plasma membranes of syncytiotrophoblast in case of physiological pregnancy and pregnancy complicated with placental insufficiency (PI).
Methods: Placentas were received after delivery (39–40 weeks of gestation) in women with physiological pregnancy (n = 27) and with PI (n = 25). The protein production was evaluated by means of proteomic analysis, which includes two-dimensional electrophoresis with the subsequent time-of-flight mass spectrometry. Syncytiotrophoblast membranes were released using the method of differential ultracentrifugation. Microviscosity of the lipid bilayer of membranes was determined using a pyrene fluorescent probe. The content of Schiff base and activity of enzymes in membranes were determined using kits.
Results: In case of PI, the production of peroxiredoxin-4, aconitase, SOD, carbonyl reductase that take part in the regulation of accumulation of reactive oxygen intermediates is disturbed, the production of prohibitin that influences the reception of important antioxidants (estrogens) is also reduced. In syncytiotrophoblast membranes the microviscosity increases, the content of Schiff base and activity of phospholipase A_2, Na^+-K^+-ATPase, and neuraminidase that intensifies the degradation of glycoproteins grow. A correlation analysis revealed the correlation between the disturbance of production of the studied proteins and modification of the condition of syncytiotrophoblast membranes.
Conclusion: The imbalance of pro- and antioxidant processes in placenta results in deviations in the structure and enzyme activity of syncytiotrophoblast plasma membranes that impedes a transplacental exchange and fetal life-support processes.
Keywords: Redox regulation, plasma membranes, placenta

P10-039
Full-length adiponectin protects platelet from activation and apoptosis

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Platelets are activated by different stimulants in the circulation. Previous studies revealed that adiponectin deficiency in circulation leads to enhanced thrombus formation and platelet aggregation. Although platelets are anucleated cells, they express at least part of apoptotic markers. The aim of the study is to investigate recombinant full-length adiponectin (fAd) on platelet function and platelet apoptosis.

Venous blood was obtained from healthy volunteers who had not taken any antiplatelet and anti-inflammatory drugs for the prior 10 days in acid citrate dextrose containing tubes.

Platelets were isolated and suspended with HEPES buffer and incubated with/without fAd (10 μg/ml) in the presence of ADP. For the effects of fAd on platelets activation, platelet aggregation and P selectin levels were determined. Platelet aggregation in platelet-rich plasma (PRP) was measured using light absorbance. For the effects of fAd on apoptotic response, protein expressions of caspase-3, gelsolin and mitochondrial membrane depolarization (MMP) were investigated. Caspase-3, gelsolin levels were analyzed by immunoblotting.

Platelet aggregation and P selectin expression decreased in fAd treated platelets ($p < 0.05$). Results showed, protein expression of caspase-3, cleavage of gelsolin and mitochondrial membrane depolarization in the fAd group was significantly lower than in the ADP group ($p < 0.05$).

In conclusion, fAd protects platelets from aggregation and apoptosis. Enhancing adiponectin levels in circulation may be a therapeutic *strategy* to *prevent* thrombus formation.

P10-040
Changes in hepatic insulin signaling and inflammatory responses in streptozotocin induced diabetes: Effects of resveratrol

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Diabetes mellitus is a heterogeneous metabolic disorder essentially characterized by deficiency of insulin secretion and insulin receptor or postreceptor events. This study aims to investigate the effects of resveratrol administration on the metabolic charac-

teristics, hepatic functions, histopathological features and insulin signaling pathway components in streptozotocin induced diabetes.

Male Wistar rats were randomly divided into four groups; (1) control/vehicle; (2) control/20 mg/kg resveratrol; (3) diabetic/vehicle; (4) diabetic/20 mg/kg resveratrol. Histopatological examinations were carried out to reveal hepatic tissue damage and inflammation. In addition to hepatic glucose, lipids, insulin, ALT, AST, resistin and XOD contents, gene and protein expressions of insulin signaling pathway components such as insulin Rβ, IRS-1, IRS-2, eNOS, PI3K, Akt, and FOXO3a were analyzed by qRT-PCR and Western blot. The rats in the diabetes group had significantly lower terminal body weight and hepatic insulin level, but significantly higher hepatic glucose, total cholesterol, triglyceride and resistin concentrations. Diabetes triggered the inflammatory process in the liver tissues that was evidenced by histopathological deformations and increase in the hepatic ALT and AST levels. Hepatic inflammation was considerably associated with insulin signaling pathway ever since a significant down-regulation of insulin signaling components; IRS-1, IRS-2, PI3K, Akt and mTOR have been identified in diabetic group. To some extent; resveratrol treatment reversed the diabetes-induced changes in liver tissues.

Resveratrol substantially improved hepatic dysfunction induced by diabetes. This may be due to the healing activity of resveratrol on insulin signaling pathway and resistin levels and decreased liver glucose-lipids contents.

P10-041
Effect of apple polyphenol extracts on glycoxidation of intestinal cells

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Oxidative stress is recognized as one of the primary processes underlying the initiation and progression of inflammation and tissue injury in inflammatory bowel disease. Therefore under physiological conditions, the balance between reactive oxygen species (ROS) generation and ROS scavenging is tightly controlled in intestinal cells. Elevated plasma glucose levels and advanced glycation end products (AGEs), formed during hyperglycemia, generate free radicals resulting in decline of antioxidant defense mechanisms and can cause inflammation and cell damage.

Apple fruit has been reported to have high antioxidant effectiveness that is potentially linked to its richness in polyphenolic molecules. The aim of the study was to investigate the role of apple extracts on glyoxidation using intestinal Caco-2 cells. Apple extracts were obtained from freeze dried apples (*Golden* and *Del Papa*) and cell glycoxidation was induced by incubating Caco-2 cells with 25 mM glucose.

The results showed that the incubation of Caco2 cells with apple extract reduced the formation of intracellular ROS, lipid peroxidation markers and AGEs formation induced by high glucose treatment. The protective effect was dependent on the concentration of polyphenolic compounds in apple extracts.

The intestine is highly vulnerable to glycoxidative damage due to its constant exposure to aerobic metabolism, high glucose concentration and/or oxidants and advanced glycation end products from ingested nutrients. A diet rich in apple antioxidants might prevent or delay the progression of intestinal diseases characterized by oxidative stress and inflammation, especially because they reach higher concentrations in the gut than in other tissues.

P10-042
Mitochondrial complex I and II activities of lymphocytes in children with traumatic brain injury

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Respiratory chain enzymes activities (complex I (NADH dehydrogenase, NADH-D) and complex II (succinate dehydrogenase, SDH) were evaluated in 67 patients with severe head injury (GCS <8 on admission) in five time periods after the injury (1–3 days; 4–7 days; 7–14 days; 14–21 days; 1 month) and age-matched control subjects (n = 27) by quantitative cytochemical method, based on ability of p-nitrotetrazolium violet to form insoluble formazan granules during enzymatic reduction in lymphocytes. All patients were divided using the Glasgow Outcome Scale (GOS) into favorable (n = 51; GOS: 4–5) and unfavorable (n = 16; GOS: 1–3) prognosis groups. There was no significant difference of NADH-D activity between the favorable prognosis group and the control group during 1 week after injury, beginning with the 7th day activity of the enzyme was increased. The high activity NADH-D is stored in the first month of TBI in children with a favorable outcome. Group with poor outcome is characterized by decreased activity NADH-D throughout the post-traumatic period. Changes in the activity of SDH had a similar phase pattern in both groups of patients. In the first 24 h we detected increased activity of the enzyme in children with TBI compared with the control group. Starting from 2 day there is a decline of activity SDH during 3–5 days, which increased and returned to near-normal level by the second week after injury. We found the potential association between mitochondrial dehydrogenase activities of peripheral blood lymphocytes and clinical outcomes after traumatic brain injury in children.

P10-043
Modeling experimental atherosclerosis in rabbits for investigation of antioxidant proteins expression

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Ischemic heart disease (IHD) is the main cause of human mortality, lifetime and its quality worldwide. In most cases, anatomical "ground" of its development are fatty deposits in the coronary arteries walls (atherosclerosis), appearing during pathophysiological unbalance of lipid and carbohydrates metabolism. It is well established that oxidative stress and reactive oxygen species (ROS) play an important role in coronary atherosclerosis initiation and subsequent development with inflammation system involvement. Naturally occurring defense antioxidant system was noticed to protect against ROS aggressive and disruptive capabilities. However, involvement of many antioxidant proteins remains contradictory and uncompletedin coronary atherosclerotic processes.

In understanding of the basic processes of atherosclerotic pathogenesis, model objects as rabbits are the most suitable system, as biochemical parameters of lipid-carbohydrate metabolism

are the closest to human with the possibility of spontaneous atherogenesis. At this point, the purpose of our study was to create an experimental model of atherosclerosis in rabbits in order to further investigation the expression of the most important antioxidant proteins (ecSOD, GPX1,3,4) in plaques by immunohistochemistry.

For this purpose, we selected ten normal (without physical pathology) white purebred rabbits with average weight 500 g and observed them after 16 weeks until the animals reached weight of about 1 kg and 12 months age. Then rabbits were exposed to high 2% cholesterol and trans fat diet for 12 weeks to develop hyperlipidemia and atherosclerosis. After slaughter, pieces (0.5 × 0.7 mm) of the thoracic aorta were cut, prefixed in formalin solution and embedded in paraffin blocks by classical method for further immunohistochemistry.

P10-044
How does mitochondrial Grx2 protect from doxorubicin toxicity, cardiolipin peroxidation and apoptosis?

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The cytostatic antracyclinic antibiotic doxorubicin/adriamycin redox cycles with complex I of the respiratory chain generating a semi-quinone radical and initiating the production of superoxide and, subsequently, oxidation of cellular components. High levels of mitochondrial Grx2 prevent cytochrome C release, attenuate caspase activation and apoptosis. Silencing of Grx2 sensitizes cells towards doxorubicin-induced apoptosis. The aim of this project is to identify and characterize Grx2's protective functions using cells with silenced an increased Grx2 levels. Experiments will be performed using galactose supplemented medium to induce oxidative phosphorlation and increase the amount of mitochondrial proteins [1]. Changes in the mitochondrial redox-state after doxorubicin treatment of Hela cells are examined with the oxICAT technique to identify potential substrates of Grx2. In addition, protein interaction partners for Grx2 will be identified by intermediate trapping and mass spectrometrical analysis. Special emphasis will be put on membrane proteins such as subunits of complex I, that have been suggested to contain susceptible thiol groups before [2]. Changes in mitochondrial respiratory complex composition after doxorubicin treatment are analyzed by complexome profiling [3]. We investigate how cardiolipin oxidation changes the physico-chemical properties of the membrane and the attachment of cytochrome C to it. Therefore, we cloned, expressed and purified human cytochrome C to measure interactions with different cardiolipin species using (FT-) Infrared Reflexion-Absorption Spectroscopy (IRRAS). Furthermore, we will investigate how doxorubicin treatment changes the extent of cardiolipin oxidation in Hela cells.

P10-045
Activity of succinate dehydrogenase in T-lymphocytes subsets in children with genetically diagnosed glycogen storage disease type I

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Introduction: Glycogen storage disease (GSD) is a group of rare genetic diseases, characterized with glycogen accumulation in tissues (muscle or liver) with development of the secondary mitochondrial disfunction. GSD is frequently accompanied by intercurrent diseases and increased risk of autoimmune disorders.
Aim: To evaluate functional activity of T-lymphocytes subsets in children with genetically diagnosed GSD of Ia and Ib type.
Materials and methods: Forty-nine patients with GSD were examined, 11 of them have Ia type and 38 have Ib type. 34 practically healthy children composed the control group. SDH activity in T-lymphocytes was measured by quantitative cytochemical method with a help flow cytometry [Beckman Coulter FC500 (USA)].
Results: In all children with I type GSD, a decrease of SDH activity in the general lymphocytes population in comparison with the norm was revealed ($p < 0.02$, Kolmogorov-Smirnov test). It was detected that patients with Ib type, characterized by a more severe disease course, have greater decrease of mitochondria functional activity than children with Ia type ($p < 0.05$).

T-cells' absolute number was decreased in most patients. Absolute number CD4+-cells, CD8+-cells, activated T-helpers demonstrate Fig. 1.

T-cells' SDH activity was decreased in Ia by 17.2%, in Ib by 18.4%. SDH activity in CD4+ lymphocytes was decreased in Ia by 20.4%, Ib by 18.1%. SDH activity in CD8+ lymphocytes was decreased by 16% in all types. We found a significant increase of activated T-helpers and regulatory T-cells (T-reg) numbers in children with GSD. SDH-activity in activated T-helpers was not different to the control group. SDH-activity in T-reg cells was significantly decreased.
Conclusions: It is necessary to carry out regular investigation of immunity status and lymphocytes functional activity assessment in patients with GSD for timely prevention of intercurrent diseases.

P10-046
The effects of quercetin on mildly oxidized LDL-induced oxidative modifications and reduced NO bioavailability in platelets

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Previous studies indicate that midly oxidized LDL (mox-LDL) activates platelets and increases free radical production. Platelet hyperactivation plays an important role in the development of cardiovascular diseases. Therefore, antioxidants might be important in preventing oxidative-stress-induced platelet activation or aggregation. In the current study, we evaluated the effects of quercetin on mox-LDL-induced oxidative modifications and

nitric oxide (NO) and endothelial nitric oxide synthase (eNOS) levels in platelets. For *in vitro* effects of quercetin on platelets, ADP activated platelets were incubated with mox-LDL for 1 h at 37°C with or without preexposure to quercetin. Then, platelet mitochondrial reactive oxygen species (mROS), malondialdehyde (MDA), NO, 3-nitrotyrosine (NT) levels and protein content of eNOS were measured. P selectin level of platelets was also investigated. Mitochondrial ROS, MDA, NT and P selectin levels in platelets treated with mox-LDL significantly increased compared to the control group. Expression of eNOS protein significantly decreased with ox-LDL. Quercetin pre-treatment caused reduction in mROS, MDA, NT and P selectin levels of mox-LDL-treated platelets. The effect of mox-LDL on the platelet eNOS levels was inhibited by quercetin. Quercetin pre-treatment of ox-LDL-treated platelets markedly increased NO levels. These data suggest that quercetin may protect platelets from ox-LDL-induced oxidative modifications and nitrosative stress.

P10-047
Nucleoredoxin – a potential cytosolic dithiol oxidase

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Redox modifications play an important role in the regulation of cellular processes. Many members of the thioredoxin (Trx) family share a common structural fold and active site (Cys-X-X-Cys) and are involved in specific protein reduction. In the cytosol, the formation of protein disulfides is less well understood. H_2O_2 can act as a signal to activate redox signaling cascades to specifically oxidize target proteins but is not sufficient to oxidize proteins itself because it is scavenged by highly abundant peroxidases. We hypothesize that nucleoredoxin (Nrx), a member of the Trx-family acts as the transducer and thus as a dithiol oxidase in this potential cascade. Nrx contains a dithiol active site-motif Cys-Pro-Pro-Cys and is active in the insulin reduction assay.

We identified potential Nrx substrates performing intermediate trapping approaches with distinct samples using a Nrx mutant lacking the resolving active site cysteine residue (Cys-Pro-Pro-Ser). Among others, we identified cytoskeletal proteins and redoxins. Using quantitative redox proteomics of the neuroblastoma SH-SY5Y cells with silenced Nrx expression compared to controls, we could identify proteins with altered thiol redox state – also identified by the intermediate trapping. Interestingly, most proteins identified in cells lacking Nrx were more reduced, leading us to hypothesize that Nrx might act as a specific cytosolic dithiol oxidase instead of a reductase. This hypothesis was further supported by determining the redox potential of recombinant Nrx and by the analysis of the redox state of potential Nrx substrates in Nrx knock-down and control cells using Western-Blot analysis.

P10-048
Inhibition of Monoamineoxidase A (Mao-A) by some herbal medicines

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In this study, the inhibition effects of *Hypericum perforatum* L., *Ginkgo biloba* L., *Lavandula angustifolia* Miller, *Zingiber officinale* Roscoe, *Tilia tomentosa* Moench., *Cinnamomum aromaticum* J. Graham, *Menthae piperita* L., *Thymus cherlerioides* Vis., *Portulaca oleracea* L., *Trachystemon orientalis* (L.) G. Don, *Allium sativum* L., *Petroselinum crispum* A.W. Hill and *Spinacia oleracea* L. were investigated and were used as inhibitors of the activity of rat liver monoamine oxidase. It was determined that the best inhibitor plant extract for MAO-A (I_{50} = 0.071 mg/ml) was ethanol extract of *Hypericum perforatum* L. with 70.15% inhibition. Following ethanol extract of *Hypericum perforatum* L., the best inhibition effects were determined in ethanol extract of *Spinacia oleracea* L. with 0.074 mg/ml of I_{50} value, ethanol extract of *Ginkgo biloba with* 0.075 mg/ml of I_{50} value, ethanol extract of *Allium sativum* L. with 0.080 mg/ml of I_{50} value and distilled water extract of *Hypericum perforatum* L. with 0.081 mg/ml of I_{50} values, respectively. In addition, the protein amount of the rat liver homogenate is determined. The protein amount of the rat liver homogenate was calculated as 0.21 mg/ml.

P10-049
Evolution of b-type cytochromes in prokaryotes

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Prokaryotes use a wide variety of bioenergetic pathways but the order of emergence of these pathways and their evolutionary relationships are still unresolved issues. Further, the patchy distribution of bioenergetic pathways in different taxonomic groups, suggests rampant horizontal gene transfer. In this study we focus on the evolutionary relationships of different familes of b-type cytochromes, which form part of a variety of bioenergetic enzymes (the cytochrome b6f complex, ubiquinol and menaquinol reductases, formate dehydrogenases, Ni/Fe-hydrigenases, and succinate dehydrogenase). We use data from 272 species of fully sequenced bacteria and archaea, which represent the full diversity of prokaryotic lineages and multiple bioenergetic modes, to examine the distribution of these cytochromes across lineages, and ask the question of whether sequences from different species group by cytochrome b family, by phylogenetic mode or by taxonomic group. We find that species do not group based on bioenergetic mode. Different cytochrome-b types are found in many lineages of the bacteria and archaea, and form distinct groups in phylogenetic analysis, which indicates an ancient origin and diversification of different cytochrome b types before the diversification of lineages. We also re-examine data from previous studies using this expanded sample of organisms spanning the full diversity of prokaryotic lineages. Concerning the b6f complex, our expanded phylogenies do not show significant bootstrap support for a "green clade" of cytochrome b6, and the split form of b6 is not monophyletic. We also present data on the similarities between prokaryotic and eukaryotic cytochrome b561 sequences.

P10-050
Hypochlorous acid influence on neutrophils functional activity

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Hypochlorous acid is formed in the reaction of chloride ions oxidation by hydrogen peroxide catalyzed by myeloperoxidase (MPO) of blood neutrophils and monocytes. HOCl is the main microbicidal agent during phagocytosis. The high level of HOCl in organism can provoke a halogenating stress resulting in degradation of biomolecules, cells, and tissues.

The aim of this work was to establish the mechanisms of HOCl influence at low concentration on neutrophils functioning.

It has been established that HOCl at non-toxic for cell concentrations (15–50 μmol/l) rendered the priming effect on neutrophils. This effect is appeared as the increase of reactive oxygen and chlorous species (ROCS) generation rate by neutrophils stimulated by adhesion, latex particles, LPS and chemotactic peptide fMLP. It was shown that MPO and phosphatidylinositol-3-kinase are the targets for HOCl in cells stimulated by fMLP. The contribution of these enzymes to ROCS production by neutrophils was decreased at HOCl addition at micromole concentrations. Treatment of neutrophils with HOCl led to ROCS yield increase owing to NADPH-oxidase and phospholipase A2 activation and redistribution of lipoxygenase and cyclooxygenase pathways of arachidonic acid metabolism in the formation of cells oxygen-activating response.

In cells exposed to HOCl in non-toxic concentrations, the reorganization of cytoskeleton has been revealed. This was evidenced by the change in cell shape, the number of filopodia, the redistribution of F-actin and increased secretion of MPO from azurophilic granules into the extracellular medium.

P10-051
Neutrophil to lymphocyte ratio as a measure of systemic inflammation in psoriasis

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Introduction: Psoriasis, a common immune-mediated disease, affects approximately 2% of the population worldwide. The relationship between psoriasis and systemic inflammation has been researched in several studies. Many different markers of inflammation have been used to assess inflammatory status in psoriasis. Recently, the neutrophil to lymphocyte ratio (N/L ratio) has been used as a sign for systemic inflammatory status. The N/L ratio, which is the total count of neutrophils divided by those of lymphocytes, is seen as an indicator of inflammation and poor prognosis in different diseases, such as myocardial infarction, diabetes mellitus, hypertension and cancer. The aim of this study was to evaluate the N/L ratio in patients with psoriasis.

Methods: A total of 166 patients with psoriasis and 192 control subjects admitted to Selcuk University Favulty of Medicine Dermatology Clinic were enrolled to this study. Whole blood counts were performed with Abbott Cell DYN3700 instrument.

Results: The median N/L ratios were 1.72 (0.72–5.74) and 1.94 (0.79–1.79) for patients and control respectively ($p = 0.774$).

Discussion: Our data demonstrate the N/L ratio can be used for evaluating the inflammatory status in psoriasis. These results should be supported with further studies which evaluate the N/L ratio and before and after treatment and use of other markers for systemic inflammation at the same time.

P10-052
Relations between concentrations of asymmetric dimethylarginine and homocysteine in chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is one of the most common chronic diseases and represents an important cause of morbidity and mortality. The inflammation has a direct or indirect potential for disturbing the equilibrium between oxidant and antioxidant systems. The relationship between conventional risk factors, together with malnutrition and chronic inflammation appear to be a major source of oxidative stress, playing an important role in the pathogenesis of dependent-NO endothelial dysfunction. Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NO syntheses that can modulate NO production. Homocysteine is an inflammatory amino acid that is produced as a by product of protein metabolism. It is a sulfur-containing amino acid, is an emerging risk factor for vascular disease, including coronary artery disease, stroke, peripheral vascular disease, and venous thrombosis. The oxidative stress plays an important role in the pathogenesis of COPD. The aim of this study is to assess ADMA and homocysteine levels in COPD. In this study, 35 patients with COPD and 25 healthy non-smokers control group included. Serum ADMA and h*omocysteine* levels were measured using high performance liquid chromatography (HPLC). Serum ADMA and homocysteine levels were observed statistically to have increased when compared with those of the control group in patients groups. Our study shows that ADMA and homocysteine levels are increased in the COPD patients. Also, ADMA and homocysteine plays an important role in the pathogenesis of COPD.

P10-053
Oxidative stress is involved in the antimalarial activity of dehidroepiandrosterone

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P. falciparum is highly dependent of the redox state for its survival; therefore, the antioxidant system in this parasite is a therapeutic target. The mechanism of action of many antimalarial drugs is the induction of death through oxidative. Dehydroepiandrosterone (DHEA) is a pre-hormone with activity against *P. falciparum in vitro*. In this work, we tested the hypothesis that the antiplasmodial activity of DHEA is mediated though the induction of oxidative stress.

Female and male mice were treated with DHEA and infected with *P. berghei* ANKA. Parasitaemia was measured in Giemsa stained blood smears and oxidative stress was evaluated by measuring the activity of superoxide dismutase (SOD), catalase and malonildialdehyde (MDA) levels. Male and female mice treated with DHEA decreased significantly the level of parasitaemia compared to control mice treated with vehicle. SOD activity in blood, spleen, liver and brain significantly increased in male mice treated with DHEA. Catalase activity increased in male, but significantly decreased in spleen of female mice both groups treated with DHEA. MDA levels were significantly increased in blood, spleen and brain of female mice treated with DHEA compared to control mice. In contrast, male only showed a significant increment in brain compared to control group.

Conclusion: DHEA induces strong oxidative stress which is higher in female compared to male mice infected with *P. berghei* ANKA. Our results suggest that at least in part the antiplasmodial activity of DHEA is mediated by the induction of oxidative stress.

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P10-054
Oxidative stress and antioxidant status in patients with asthma

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Asthma is a common chronic inflammatory disease of the airways and has common symptoms include wheezing, coughing, chest tightness, and shortness of breath. Airways are exposed to high level of environmental oxidants and several inflammatory mediators including a range of toxic reactive oxygen species (ROS). And also, the imbalance between oxidants and antioxidants that is called oxidant stress is critical to asthma pathogenesis. Malondialdehyde (MDA) is a stable end product of the peroxidation of membrane lipids by ROS, and thus, it is used as an indicator of increased lipid peroxidation. Glutathione (GSH) is an antioxidant whose role is to prevent damage caused by ROS. The objective of this study was to evaluate free radical and antioxidant metabolism of 44 patients with asthma disease compared to 25 healthy controls. The levels of serum malondialdehyde (MDA) and glutathione (GSH) were determined by high-performance liquid chromatography (HPLC). The levels of MDA were higher than control group. Besides, GSH levels were lower than in controls. Statistical analysis showed that MDA and GSH levels differed significantly in patients with asthma compared to controls. Immune defects can disturb the oxidant/antioxidant balance of the organism and can accelerate the formation of free radicals. The cytotoxic effects of free radicals lead to cell damage and increase oxidative stress. These parameters have important roles in oxidant/antioxidant defense in asthma.

P10-055
Determination of transforming growth factor beta-1 levels in hemodialysis-treated non-diabetic patients

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Chronic renal failure (CRF), characterized by irreversible loss of renal function, is a major public health problem in the world. Patients with chronic kidney failure suffer from some immune disorders due to the changes on cell adhesion molecules and cytokine levels for various causes. Transforming growth factor beta (TGF-β) is a multifunctional cytokine involved in the cellular growth, differentiation and migration, formation and degradation of extracellular matrix components, chemotactic processes, and apoptosis, as well as immune regulation. Among the three TGF-β isoforms, TGF-β1 plays a key role in the pathogenesis of many forms of renal diseases. Our study focuses on the determination and evaluation of TGF-β1 levels of patients receiving hemodialysis treatment because of chronic renal failure. We studied 24 patients who were on regular hemodialysis, with non-diabetic nephropathy. Twenty healthy people who proved to be in a good state of health and free from any signs of chronic diseases or disorders were enrolled as a control group. Blood samples were collected both before and after hemodialysis treatment from each patient. TGF-β1 serum levels were determined by Enzyme Immunoassay method. TGF-β1 levels were found significantly higher in the hemodialysis patients than those of the control groups. Also, the value of TGF-β1 in serum was significantly reduced after hemodialysis treatment but it was still higher than control groups.

P10-056
The influence of physical exercise program with whole-body cryostimulation treatment on activity of antioxidant enzymes in obese human

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The aim of this study was to determine the effect of 6-month-long physical exercise program with a two-time exposure to whole-body cryostimulation (WBC) in 20 sessions on the activity of superoxide dismutase 1 (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GSSG-R) in erythrocytes. Forty five overweight and obese men participated in the experiment. The exercise program consisted of moderate-intensity cardiovascular exercise, three times a week during 6 month. Additionally, in the second and last month of the exercise program the cryostimulation procedure was implemented, with a scheme of 20 daily treatments. During the experimental period, venous blood samples were collected before and 6 months following the exercise program, after the second WBC treatment. The enzymes activities were measured in the hemolysate samples with a BIOXYTECH® kit (Oxis Research, Portland, OR, USA) using a UV/VIS Lambda 40 spectrophotometer (Perkin-Elmer, Wellesley, MA, USA). The nature of changes in the activity of respective antioxidant enzymes during successive months of the exercise program was not similar. SOD activity significantly increased in response to 6 month of exercise with cryostymulation procedure from 354.75 to 406.8 U/g Hb. As regards CAT and GST a significant decrease was observed in its activity from 167.95 to 121.28 U/g Hb, and 0.176 to 0.048 U/g Hb properly. No statistically significant changes for GPx and GSSG-R occurred. The present results are consistent with our earlier reports on WBC as a stress-inducing factor that activates favorable adaptation mechanisms.

P10-057
Alterations of the antioxidant status induced by the exposure to phospholipidic micelles magnetic nanoparticles in mouse lung tissue

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The aim of our research was to analyze the possible toxic effects of a new type of contrast agent composed of an iron oxide core encapsulated in phospholipidic micelles on mouse lung tissue.

CD-1 mice were randomly divided into three groups (seven individuals each) per interval of experiment: a control group injected intravenously with 0.7% sodium chloride, and the other ones with solutions of nanoparticles corresponding to 5 and respectively 15 mg Fe/kg body weight. After 2, 3, 7 and 14 days, pulmonary tissues were collected. Oxidative stress status was evaluated by kinetic measurement of specific enzymatic activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione

reductase (GR), as well as by determination of reduced glutathione (GSH) concentration and malondialdehyde levels.

After 2 days of nanoparticles exposure, the results showed a significant increase in lung catalase activity for both doses related to control, whereas the other ones were slightly increased (SOD, GPx) or significantly decreased (GR, GST). After 3 and 7 days a decrease in antioxidant activities of all enzymes occurred which was maintained up to 14 days when the activities have slightly increased. GSH concentration, as non-enzimatic antioxidant, decreased significantly compared to control values during the entire analyzed period. Regarding the level of lipid peroxidation, an ascending trend was observed between 3 and 14 days. No major differences of response were observed between doses.

Given these results, we concluded that lung antioxidant defense system suffered modification in the capacity to counteract nanoparticles induced-oxidative stress.

P10-058
Redox-active metal complexes with functionalized 1,2-dihydroxybenzene ligands as antimicrobial agents: evaluation of cytochromes c as probable molecular targets

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Novel redox-active metallotherapeutic agents were used to develop effective antimicrobial remedies differing from standard antibiotics in their mechanism of action. For this purpose redox-active compounds carrying sterically hindered 1,2-dihydroxybenzene moieties along with various metal ions into a single scaffold have been synthesized and characterized by means of chemical and physicochemical methods as well as screened for their antimicrobial activity against bacteria and fungi. This screening revealed metal complexes that may be considered as potential metallotherapeutic agents with activity higher than or comparable to that of some standard drugs. Using the method of cyclic voltammetry, we have shown some 1,2-dihydroxybenzene derivatives as well as their metal complexes to be also of a pronounced reducing ability. Bacterial cytochrome *c*-like enzymes are among the first targets for redox-active antimicrobials on their way into the cell. Thus, spectrophotometric investigation was carried out in order to estimate the rate of reduction of a common cytochrome *c* from bovine heart with the compounds. The oxidation of 1,2-dihydroxybenzene derivatives and their metal complexes by cytochrome *c in vitro* under anaerobic conditions can include two successive one-electron steps of oxidation of their ionic forms to yield reactive *o*-benzoquinones on interaction *via* intermediate *o*-benzosemiquinone formation. The results obtained are discussed in view of presumed correlation between the capability of the compounds for reducing cytochrome *c*, their antimicrobial activity, and physico-chemical characteristics (redox properties determined electrochemically, lipophilicity, ionization constants of the ligands and stability constants of the metal complexes).

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P10-059
Antioxidant capacity of daily consumed peach fruit juice and its antimicrobial effect on *Proteus mirabilis*

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Fruit juices used in our regular diet may have importance in the protective treatment of some infectious diseases. In this study, the selected dietary beverage, peach (*Prunus persica* L.) was investigated for its antioxidant capacity and antimicrobial activity against *Proteus mirabilis*, a well known bacteria in urinary tract infections.

Fresh peach juice was lyophilised to dryness and then was used throughout this study. Antioxidant capacities of the extract were carried out by using 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging (ABTS) and 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH) methods along with the determination of total phenolic compounds in the extract. Antimicrobial activities of the extract were determined by disc diffusion test, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods.

Peach has revealed DPPH EC_{50} and TEAC values of 1.148 ± 0.034 mg/ml and 0.492 ± 0.041 mmol/g respectively. It may be an effective antimicrobial for *P. mirabilis* infections with 1.5 mg/ml MIC and 12 mg/ml MBC value and 8 mm inhibition zone.

The antimicrobial effects of active natural chemicals obtained by chemical methods might be important for producing new antibiotics in the future. Getting bacterial infections under control before their progress by consuming fresh fruit juices is very important for human health. Therefore, peach juice may be used for urinary tract infections that *P. mirabilis* causes.

P10-060
Anti-oxidant properties of the seeds of *Gundelia tournefortii* L.

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Gundelia tournefortii L., which grows in the Mediterranean area and Middle East, is a spiny, thistle-like flowering plant. It is a member of the *Asteraceae* Family. The flowers, leaves, seeds and stems of plant are consumed as food sources and it is used as folk medicine against diabetes, epilepsy, diarrhoea.

In this study the antioxidant properties of *Gundelia tournefortii* (*G. Tournefortii*) seeds were determined. Seed extract was prepared in methanol and investigated for its anti-oxidant capacity by using the methods of ABTS and DPPH. The seeds of *G. Tournefortii* presented an anti-oxidant potential, with EC_{50} value of 0.601 ± 0.008 mg/ml for DPPH scavenging capacity.

Similarly, the radical scavenging capacity of *G. Tournefortii* seeds were examined for ABTS cationic radical and found the value of 0.975 ± 0.001 μM/mg as the trolox equivalent anti-oxidant capacity (TEAC). At the same time, total phenol and flavonoid content of the seed extract were determined as 21.905 ± 3.13 μg/mg of gallic acid equivalents and 57.185 ± 8.774 μg/mg of catechin equivalent respectively.

Consequently, leaves of *Gundelia tournefortii* revealed high amounts of phenolics and flavonoids as well as strong radical scavenging properties.

P10-061
Post-translational redox modifications of proteins in mitochondria

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Post-translational modifications (PTMs) of proteins, for example phosphorylation, are an important mechanism for regulating protein activity and function. Recently oxidative modification of cysteine residues including disulfide bonds, sulfenic acids or S-glutathionylation has been recognized to play a role in regulating protein function, but the transient nature of these modifications makes identification and characterization difficult.

To identify proteins with cysteine modifications *in vivo* we developed a probe based on the redox-regulated transcription factor Yap1, which harbors a nucleophilic cysteine can attack sulfenic acid and disulfide modifications in proteins, forming stable intermolecular disulfides. As mitochondria are believed to be a major source of reactive oxygen species (ROS), we reasoned that they represent a promising compartment to look for proteins with oxidative modifications. We targeted our Yap1-probe to the mitochondrial matrix and used stable isotope labeling of amino acids in cell culture (SILAC) to screen for interaction partners. Cells expressing the probe were grown in either light or heavy isotope containing amino acids, and subsequently either left untreated or were treated with H2O2. Probe-substrate protein complexes were immunoprecipitated from cells and proteins enriched upon oxidative challenge were identified by mass-spec.

The analysis revealed several candidate proteins with oxidative modifications involved in a range of metabolic pathways. We focused on one of these proteins, Ilv5, that is involved in the synthesis of branched-chain amino acids and a major consumer of mitochondrial NADPH. Hence we plan to investigate the interplay between mitochondria, redox changes and Ilv5 activity.

P10-062
Degradation of extracellular NAD and its metabolites in cultures of human cells

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NAD is an essential redox carrier, whereas its degradation is a key element of important signaling pathways. Human cells replenish their NAD contents through NAD biosynthesis from extracellular precursors. These precursors encompass bases (nicotinamide, Nam, and nicotinic acid, NA) and nucleosides (nicotinamide riboside, NR, and nicotinic acid riboside, NAR), now collectively referred to as vitamin B3. Even extracellular NAD and related mononucleotides serve as precursors of intracellular NAD. However, it is still debated whether nucleotides enter cells directly or whether they are degraded to nucleosides and bases prior to uptake into cells. Therefore, an important issue is the possibility of extracellular degradation of nucleotide precursors.

Here, we demonstrate the degradation of extracellular NAD metabolites in cultures of human cells using NMR-based metabolite measurements. As expected, in cultures of HEK293 cells, in medium containing 10% fetal bovine serum (FBS), nucleotides (NMN, NAD, NAMN and NAAD) were degraded to the corresponding nucleosides. Moreover, the nucleosides were further degraded to their corresponding bases. Surprisingly, degradation was also observed in culture medium alone, in the absence of cells. These results indicate that FBS contains enzymatic activities which degrade NAD metabolites. When cultivating HEK293 cells in serum-free medium, we found that NAD and NAAD are still efficiently cleaved to NMN and NAMN; NMN, but not NAMN, is dephosphorylated to NR, while NR, but not NAR, is hydrolyzed to Nam. These observations suggest that NAD metabolizing enzymes on the outer surface of HEK293 cells can contribute to the utilization of extracellular NAD and its metabolites.

P10-063
Presence of chronic varicose veins related to increased serum ceruloplasmin levels in adults: a cross sectional study

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Background: One-fifth of the population suffers from varicose veins (VV). Varicose veins are not only a serious cosmetic and emotional problem, but can also result in loss of limbs. Our aim was to evaluate the relationship between serum Ceruloplasmin (Cp) levels and VV.
Materials and methods: Thirty-five patients with superficial and symptomatic varicose veins of the lower extremities, and 35 age and sex matched control subjects were included in this study. Ceruloplasmin levels were measured by Erel's method, an automated and calorimetric method.
Results: In the VV group, Cp was significantly higher than in the control group ($p = 0.012$). No other factor was associated with serum Cp levels. Before and after surgical treatment, serum Cp levels were similar in the patient group.
Discussion: This study demonstrated higher serum Cp levels in patients with VV, which decreased slightly, though not statistically significant, after surgical treatment.

P10-064
High serum total free thiol levels in patients with high serum HDL-C: a cross-sectional study in adult healthy volunteers

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Introduction: We aimed to investigate the levels of serum total free thiol (STFT) in healthy volunteers grouped according to serum HDL-C levels.
Materials and methods: Twenty-five patients admitted for check-up to our hospital were included in this study. Using measurements of Cholesterol in Adults (Adult Treatment Panel III), patients were divided into three groups based on HDL-C values. Group 1 = HDL-C 0–39 mg/dl, Group 2 = HDL-C 40–59 mg/dl and Group 3 = HDL-C $\geq$ 61 mg/dl. Free sulfhydryl groups in serum samples were assayed according to Hu's modified method.
Results: Glucose, urea, creatinine, uric acid, alanine and aspartate transaminase, total cholesterol, sodium and potassium similarity ($p > 0.05$), triglycerides, low density lipoprotein cholesterol, white blood cells, hemoglobin and STFT levels were different ($p < 0.05$) among the groups. Subgroup analyses showed that differences of STFT were due to differences between Group 3

and the other groups. Pearson and Spearman correlation analysis showed that hemoglobin, urea and TG were positively correlated and HDL-C negatively correlated with STFT levels ($p < 0.05$, Table 2). Linear regression analysis showed that only the level of urea independently affected STFT levels ($p < 0.05$, Table 2).

Discussion: We have found that STFT levels in the high HDL-C group were higher compared to the groups with moderate and low HDL-C levels. The reason for this may be a reduction of HDL-C degradation due to thiol. Based on this information, increasing HDL may be more successful by raising serum levels of thiol.

P10-065
Hydrogen peroxide-induced oxidative damage in human mononuclear leukocyte: the anti-genotoxic effects of *H. Perforatum* extract on DNA damage

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Background/Aim: Oxidative stress can leads to many pathophysiological conditions in the body. The genotoxic and anti-genotoxic effects of *Hypericum perforatum* seed flower and fruit extracts were investigated. Our aim of this study, *Hypericum perforatum* methanol extracts against to H_2O_2 induced DNA damage with a single cell alkaline gel electrophoresis were analyzed.

Materials and methods: The amount of total phenolics, measured by Folin–Ciocalteu method. DNA damage was evaluated by alkalin comet assay.

Results: The highest anti-genotoxic effects were seen 50 μg/ml concentrations. In the positive controls that has only H_2O_2 in the environment at the same concentrations of DNA damage as an indicator of genotoxic effects were 297 Arbitrary Unit (AU), whereas in negative controls the damage was 22 AU. The highest level of anti-genotoxic effects were seen at 50 μg/ml concentration in that the damage was 47 AU with the seed extract and 258 AU with the fruit extract and 83 AU with the seed extract.

Conclusion: The present results suggested that *H. perforatum* could be a good source of natural antioxidants. The optimum doses of *H. perforatum* flower, fruit and seed extracts have a strong anti-genotoxic effect and this effect is more powerful in the seed extract than in the fruit and flower. We recommend that further investigations are necessary to evaluate the *in vitro* and *in vivo* benefits of *H. perforatum* methanol extracts.

P10-066
Research of indicators of secondary catabolites of lipid peroxidation (LPO) and the endogenous intoxication of men living in ecologically unfavorable Kyzylorda region (Kazakhstan)

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The aim of our study is to assess the health status of men of reproductive age from 18 to 35 years living in ecologically unfavorable Kyzylorda region on the molecular–cellular level, for the accumulation content of malondialdehyde (MDA) and the average molecular masses as the endogenous intoxication.

Materials and methods: The study involved 900 men of reproductive age. The object of the study was blood. To evaluate the activity of free radical processes in men of reproductive age to determine the content of secondary products (malondialdehyde) the method of E.N. Korobeinikova (1989) was used and the determination of medium-molecular peptides studied individuals used the technique of A.N. Kovalevsky.

Results: In the blood of men living in the settlement of Zhusaly there was an increase in the content of malondialdehyde level and medium-weight molecules compared to settlement Shieli.

Conclusions: Thus, comparing the data obtained, it should be noted the accumulation of catabolites secondary lipid peroxidation and average mass molecules in the blood of men of reproductive age in the ecologically unfavorable Kyzylorda region, indicating a shift of lipid peroxidation, suggested a role of free radical reactions in the development of endogenous intoxication.The obtained results indicate the negative impact of toxicants and dust-salt aerosols on the body of men of reproductive age, leading to the accumulation of malondialdehyde as catabolites reflects the degree of activity of the process of peroxidation and causing damage to the structures of cell membranes and violation of their functions.

P10-067
Glutathione reductase is essential for growth and its overexpression leads to defective hyphal growth and attenuated virulence of *Candida albicans*

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Glutathione plays a pivotal role in biological events and glutathione reductase (CGR1) is necessary to maintain the high glutathione/glutathione disulfide ratio. Herein, *Candida albicans CGR1* was disrupted and overexpressed. The *CGR1*-deficient strain was non-viable when *CGR1* expression under the control of CaMAL2 promoter was repressed conditionally and its growth could be partially overcome by exogenous thiols. The *CGR1* expression was increased by several types of oxidants. The virulence- or hyphae-specific genes were down-regulated by *CGR1* overexpression, confirming attenuated virulence and morphological transition mediated by *CPH1*-dependent pathway. Our results demonstrate that the *CGR1* is indispensable for cell growth, differentiation and virulence.

P10-068
Catalase from larvae of the camel tick *Hyalomma dromedarii*

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Catalase plays a major role in protecting cells against toxic reactive oxygen species. Here, Catalase was purified from larvae of the camel tick *Hyalomma dromedarii* and designated TLCAT. It was purified by ammonium sulfate precipitation and chromatography on DEAE-cellulose, Sephacryl S-300 and CM-cellulose columns. Gel filtration and SDS-PAGE of the purified TLCAT indicated that the protein has a native molecular weight of 120 kDa and is most likely a homodimer with a subunit of approximately 60 kDa. The Km value of TLCAT is 12 mM H_2O_2 and displayed its optimum activity at pH 7.2. $CaCl_2$, $MgCl_2$, $MnCl_2$ and $NiCl_2$ increased the activity of TLCAT, while $FeCl_2$, $CoCl_2$, $CuCl_2$ and $ZnCl_2$ inhibited the activity of TLCAT. Sodium azide inhibited TLCAT competitively with K*i* value of 0.28 mM. The presence of TLCAT in cells may play a role in

protecting *H. dromedarii* ticks against oxidative damage. This finding will contribute to our understanding of the physiology of these ectoparasites and the development of untraditional methods to control them.

P10-069
Novel NAD(H)-linked methylglyoxal dehydrogenase is induced in glutathione-depleted *Candida albicans*

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NAD^+-linked methylglyoxal dehydrogenase (MGD1) activity that catalyzes the oxidation of methylglyoxal to pyruvate was found in a glutathione-depleted g-glutamylcysteine synthetase disruptant in *Candida albicans*. MGD1 also had a NADH-linked methylglyoxal- and pyruvate-reducing activity. The K_m values corresponding to the methylglyoxal oxidation, reduction and pyruvate reduction were 1.21, 4.03×10^{-2} and 5.51×10^{-2} mM, respectively. Interestingly, the *MGD1* gene was expressed only within glutathione-depleted cells as intracellular methylglyoxal accumulated. Furthermore, alcohol dehydrogenase 1 (*ADH1*) gene expression was significantly induced in glutathione-depleted cells and in the *MGD1* disruptant, as confirmed by northern blot analysis. In *MGD1* disruptants of both wild-type and glutathione-depleted cells, the intracellular concentration of methylglyoxal increased significantly leading to defects in cell growth, viability, virulence and a G1-phase arrest of the cell cycle.

Chem Biol S1, Probing Cellular Function with Small Molecules

P14-005-SP
Tetraphosphate cap analogues modified in polyphosphate bridge are inhibitors of Dcp1/2 decapping complex

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Dcp1/2 is the major eukaryotic RNA decapping complex composed of regulatory (Dcp1) and catalytic (Dcp2) subunits, which release m^7GDP molecule from m^7G capped transcripts. Dcp1/2 activity is crucial for RNA quality control and turnover hence deregulation of those processes may be detrimental. Since m^7GDP is bound by Dcp2 with only milimolar affinity we screened a small library of synthetic m^7G nucleotides (cap analogues) bearing modifications in the oligophosphate chain in order to find better Dcp1/2 binders. Using a radioactivity-based decapping assay we identified compounds binding Dcp2 much tigher than m^7GDP. All these nucleotides are "two headed" 5′ mRNA cap analogues based on m^7Gppppm^{7}G structure. These compounds contain either boranophosphate or phosphorothioate moiety in the phosphate chain and some of them possess an additional methylene group between β and γ phosphorus. The most potent inhibitor, $m^7Gp_Sppp_Sm^7G$ is about 20-times more potent than m^7GDP, thus it was subjected to kinetic and structural studies. NMR binding experiments revealed that both regulatory and catalytic domains of Dcp2 recognise that compound with submicromolar affinities. Single-turnover kinetics inhibition assay showed that mentioned compound is a mixed inhibitor with higher affinity for *apo* enzyme than for enzyme-substrate complex. Finally, DLS measurements showed higher hydrodynamic radius for inhibitor-Dcp2 complex if compared to *apo* form. We propose that $m^7Gp_Sppp_Sm^7G$ blocks a conformational transition to a catalytically active conformation. It is the first report of a small molecule inhibitor of Dcp2 and it may be beneficial for further studies on RNA decapping and development of new therapeutical.

P14-006-SP
A genome-wide RNAi screen to dissect retrograde membrane traffic to the Golgi complex

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Despite our considerable knowledge of the molecular machinery associated with forward trafficking pathways, much less is known about retrograde transport pathways linking the cell surface with early secretory pathway organelles such as the Golgi complex and endoplasmic reticulum. Nevertheless, these routes are important, as they are known to be exploited by a number of viruses and protein toxins as part of their mechanism of action. This retrograde route also holds promise for the delivery of therapeutic agents, and as such it is essential that we understand its regulatory machinery.

In order to identify the proteins that regulate retrograde transport to the Golgi complex a genome-wide RNA interference screen coupled to high-content image analysis was performed in HeLa cells treated with Shiga-like toxin, a probe for this transport pathway. These studies revealed roles for members of the Rab family of GTPases and components of the cytoskeleton not previously described to be involved in this process. In addition, previously uncharacterised proteins and other classes of proteins were also identified as key regulators of this pathway.

The results from this screen not only contribute to our general knowledge of the mechanism of retrograde traffic, but are also valuable in the development of more efficient and targeted drug delivery strategies to cells.

P14-007-SP
How oncogenic mutations affect qualitative and quantitative wiring of signalling

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Most tumors arise by genomic mutations, leading e.g. to uncontrolled growth and resistance to apoptosis. Many mutations act by changing the signalling network, which produces vulnerabilities that can be exploited in targeted therapy. Therefore it is crucial to understand the effect of an oncogene on the level of signalling.

We apply a combined experimental/theoretical approach to characterize isogenic cancer cell lines that genomically differ by a single oncogenic mutation within the Ras/PI3K signaling network. More specifically, we systematically perturb the network at the receptor level by growth factors and within the signalling cascade by small molecule inhibitors, and measure multiple relevant phosphorylation signals by using a multiplex bead-based Elisa

platform. From the resulting perturbation data we reconstructed the signalling networks around the introduced oncogene in a semiquantitative manner using an enhanced version of Modular Response Analysis. With the knowledge obtained by comparing the models for different genetic backgrounds, we further attempt to incorporate the oncogenic mutations as additional perturbations within a single model.

P14-008-SP
A survey of the inhibition of Arf GTPases and their GEFs by small molecules

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Small GTPases of the Arf family are major regulators of membrane traffic in eukaryotic cells. They are activated by Guanine nucleotide Exchange Factors (ArfGEFs), which stimulate GDP/GTP exchange. Five subfamilies of eukaryotic ArfGEFs, carrying distinct regulatory and membrane binding domains, activate Arfs on different membranes in the cell. The identification of the natural compound Brefeldin A as the first known GEF inhibitor, which inhibits Arf functions at the Golgi, established the Arf machinery as model systems to investigate the druggability of small GTPases and their GEFs in diseases. Six chemical compounds of unrelated structure have been reported by us and others to inhibit ArfGEF subfamilies. Some of them, recently discovered, remain poorly characterized *in vitro*, which hampers their use as relevant biological tools. Here, we compared the efficiency and specificity of these inhibitors towards representative members of the major ArfGEF subfamilies. The kinetics of Arf GTPase activation by their GEFs in the presence of the inhibitors was monitored by fluorescence spectroscopy, using purified recombinant proteins reconstituted in artificial membranes. This study reveals that most inhibitors are active towards more than one ArfGEF, with the notable exception of Brefeldin A, which is restricted to Golgi ArfGEFs. However, the patterns of inhibition vary between the different inhibitors. This survey also shows that membranes modulate the efficiency of some, although not all inhibitors compared to their effects in solution. These findings provide novel insight into the inhibition of small GTPases and their GEFs by small molecules and their use to interrogate cellular pathways.

P14-009
Characterizing the role of CPSF6 in HIV-1 infection by using small-molecule inhibitors

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The HIV-1 Core Capsid is built by 1500 subunits. After the viral entry the Core is uncoated in the cytoplasm. The stability of the core in the cytoplasm is crucial for HIV infections, mutations that stabilize or destabilize the core show lower infection. The stability provided by the capsid protein assembled into the viral core is important for the occurrence of reverse transcription and productive infection.

In the recent years several small molecule Drugs have been developed which target the HIV-core and affect HIV-1 core stability. This work explored the mechanism of the small inhibitors PF74 and BI-2. We found by Biochemical and structural analysis that the binding side of this drugs to the HIV-1 Capsid overlaps with the binding site of cleavage and polyadenylation specificity factor subunit 6 (CPSF6). (1,2)

A short cytosolic Form of CPSF6 has been found previously to inhibit HIV-1 Infection. (3) We could demonstrate that overexpression of a cytosolic full-length CPSF6 blocks HIV-1 infection at the nuclear import step and enhances stability of the HIV-1 core contradictory to PF74 and Bi-2. (4,5).

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P14-010
Protective effects of ginseng extracts on age-related phenotypes of *Sod1*$^{-/-}$ mice

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Aging is characterized by increased oxidative stress, chronic inflammation, and organ dysfunction, which occur in a progressive and irreversible manner. Superoxide dismutase (SOD) serves as a major antioxidant and neutralizes superoxide radicals

throughout the body. *In vivo* studies have demonstrated that copper/zinc superoxide dismutase-deficient ($Sod1^{-/-}$) mice show various aging-like pathologies in organs. Here, we found that keorean ginseng (*Panax ginseng* C.A. Mey) extracts, including in ginsenoids and syringaresinol (also known as Lirioresinol B), treatment attenuated the age-related changes in $Sod1^{-/-}$ liver, bone, and skin. Interestingly, syringaresinol normalised skin atrophy of $Sod1^{-/-}$ mice, and promoted outgrowth of fibroblasts from $Sod1^{-/-}$ skin *in vitro*. These results strongly suggested that ginseng extracts exhibit beneficial effects on age-related organ changes in mice.

P14-011
The functioning of parameters haemostasis system under influence IgG from patients with ischemic stroke

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One of the most dangerous and deadly diseases in 21 century is stroke. Therefore the aim of our study was to determine the influence of IgG from blood plasma of patients who had suffered from different types of ischemic stroke on chosen parameters of hemostasis system: thrombin/prothrombin, factor Xa, and protein C.

In the project three groups of patients took part (40 atherothrombotic ischemic stroke patients, 40 cardioembolic ischemic stroke patients, and 40 individuals without stroke) and 20 healthy donors. IgG separated by affinity chromatography on protein A sepharose was used in the experiment as well as commercial preparations: thrombin, factor X, activators of thrombin and protein C and their specific chromogenic substrates.

The results of the experiment did not show any influence of IgG all types (in the concentrations 100 and 300 μg/ml) on the process of substrate degradation by FX. At the same time, the addition of IgG all factions (300 μg/ml) accelerated splitting substrate by thrombin for 45–57% depending on the patient group. The research also demonstrated deceleration splitting of substrate by the transformation proenzyme to active enzyme: thrombin for 20% and protein C for 33%.

To conclude: Cardioembolic ischemic stroke was accompanied by significant elevation of the IgG level, whereas atherothrombotic ischemic stroke led to a minor decrease in IgG. IgG of ischemic stroke patients reduced blood clotting on the level of prothrombin and protein C on the one hand and activated blood clotting on the level of thrombin on the other hand.

P14-012
Lactoferrin and its complex forms as bioregulators of cell process

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Lactoferrin (LF) is one of the most important proteins of the acute phase and nonspecific defense against different types of microbial and viral infections. It is a Fe-binding glycoprotein contained in milk, other epithelial secretions and blood. LF possesses anticancer activity, demonstrates antibacterial, antiviral and antifungous properties, and greatly increases activity of antibacterial and antifungus drugs. LF is an acute phase protein: its level significantly increases during almost all inflammatory processes where it works as the inflammation regulator. LF activates transcription; regulates immunomodulation and cell growth and proliferation; possesses DNase, RNase activities.

We have proposed that since LF is a relatively small protein (80 kDa) its polyfunctional properties may result from its existence in several oligomeric forms and in complex with other cell components. In this study we investigated complex forms of lactoferrin. It was found that in human milk LF forms a high molecular mass (~1000 kDa) protein complex which is stable in the presence of high salt concentrations. LF is the main protein of the complex, and α-lactalbumin, albumin and β-casein, and sIgA were present in moderate or minor amounts. As nonprotein components of the complex were detected RNA (near 10 kb) and mono-, di- and triglyceride. It was shown that the complex possesses protein kinase and DNase activity. Research of cytotoxic effect on cancerous human and mouse cells showed that this complex of milk proteins with RNA demonstrates higher cytotoxicity in compare to single proteins (in particular lactoferrin).

P14-013
Design, synthesis and biological evaluation of specific rhomboid inhibitors

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Rhomboid proteases comprise a large family of intramembrane serine proteases which are present in various prokaryotic, archaeal and eukaryotic organisms. They regulate a wide array of biologically significant cellular processes, include growth factor signaling, host cell invasion, and mitochondria integrity etc., making them potent drug targets. Functional genetics has played a major role in understanding rhomboids. However, this approach is complicated by the redundancy of rhomboid genes in many organisms. Hence, specific chemical inhibitors would be extremely valuable tools to probe rhomboid functions. Although rhomboids have been reported to exhibit weak inhibition by some soluble serine protease inhibitors, e.g. isocoumarin derivatives, none of them were recognized as highly potent and specific for these membrane proteases. Consequently, a number of promising leads have been generated through a candidate based approach, followed by molecular modeling using Molecular Operating Environment software (MOE) and these candidates have been ranked on the basis of their ligand efficiency values derived from the consensus scoring approach. Henceforth, considering molecular docking results, a series of most favorable chemical leads was synthesized and tested against Rhomboid protease in cell-free and cell-based activity assays. Moreover, we have also made a progressive step for its mechanism of inhibition. This led us to successfully identify a new class of mechanism-based inhibitors for Rhomboids, with inhibitory potency in the low micromolar range. Our results represent significant achievement in the development of new inhibitor class for rhomboid study which will also provide us new insight into its biological roles and catalytic mechanism.

P14-014
Antibodies from sera of HIV-infected patients hydrolyzing histones

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Catalytic antibodies (abzymes) are distinctive feature of autoimmune diseases. It was found that abzymes isolated from sera of HIV-infected patients hydrolyze DNA, integrase, reverse transcriptase and casein. According to the literature, blood of HIV-infected persons contains histones in high concentrations. Histones are involved in the regulation of gene activity, replication, repair and recombination of DNA, so controlling the amount of histone by antibodies may be important to the functioning of cells in viral diseases. In our study electrophoretically and immunologically homogeneous IgGs were isolated from sera of HIV-patients by affinity chromatography. After separation of IgG preparations on histone-Sepharose, the same fractions of immunoglobulins hydrolyzed histones and the myelin basic protein (MBP). Several rigid criteria showed that hydrolysis of histones and MBP are an intrinsic property of immunoglobulins from blood of HIV infected patients, but not from healthy donors. Similar to other proteolytic abzymes, histones- and MBP-hydrolyzing IgGs from some HIV-infected patients were inhibited by specific inhibitors of serine and metal-dependent proteases. However a significant activity inhibition by specific inhibitors of acidic- and thiol-like proteases was observed for the first time. Although HIV-infection leads to formation of abzymes against many viral and human antigens, a possible biological role for most of them is not known. A negative role in counteracting the infection of histones- and MBP-hydrolyzing of IgGs cannot be excluded. We propose that detection of these hydrolyzing activities can be used for diagnostic purposes and for estimation of the immunological status of HIV-infected patients.

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P14-015
Effects of herbicides and fungicides on the soil chitinolytic activity. A molecular docking approach

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It is well known that an important amount of pesticides reach a destination other than their specific targets affecting the aquatic and soil environments, as well as human settlements. A molecular docking approach has been used to assess the effects of two herbicides (nicosulfuron and chlorsulfuron) and two fungicides (difenoconazole and drazoxolone) upon chitinases from *Bacillus, Serratia*, and *Streptomyces* sp. These species are commonly found in soil and reveal chitinolytic activity by secreting chitinases hydrolysing the chitin and its chitooligomers. All the considered pesticides show favorable binding to investigates chitinases, the herbicides exposing a higher potential to bind to the active site of chitinases. These data illustrate the inhibitory potential of both herbicides and fungicides on the soil chitinolytic activity with direct consequences on soil biodiversity and health.

P14-016
Semicarbazide-containing drug attenuates lung extracellular matrix deposition under the chronic ovalbumin-induced asthma, but does not affect histaminase activity in the bronchoalveolar lavage fluid

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Previously we demonstrated that semicarbazide intake led to essential structural changes in the rat extracellular matrix evoked by the collagen cross-links reduction and affected on aldehydes level through amine oxidase inhibition. The current investigation aims to examine the influence of semicarbazide-containing drug on lung fibrosis formation and some allergic hypersensitivity markers under chronic ovalbumin-induced asthma.

Guinea pigs were randomly assigned to: intact group (*normal*); ovalbumin (OVA) sensitized animals (*OVA*/*0.9% NaCl*); OVA challenged group (*0.9% NaCl*/*OVA*); OVA-induced asthma group (*OVA*/*OVA*); OVA-induced asthma group under *per os* treatment (*OVA*/*OVA*/*D*) of w/v: 2% L-lysine, 1% L-glycine, 0.5% *N*-acetyl-L-cysteine, 0.05% semicarbazide.

Sensitization to ovalbumin (*OVA*/*0.9% NaCl*) was associated with 4.5-fold growth of plasma IL-4 content compared with intact group ($p < 0.05$). Plasma IL-13 level attained 1.5-fold increase in *OVA*/*OVA* and *0.9% NaCl*/*OVA* versus normal ($p < 0.05$), whereas drug intake group (*OVA*/*OVA*/*D*) had non-reliable changes versus normal ($p > 0.05$). These findings were confirmed with histology results, that detected mucus hypersecretion by goblet cells and mucous glands, basement membrane thickening in *0.9% NaCl*/*OVA*, and acute obstructive emphysema, thickening and sclerosis of interalveolar walls, lung tissue fibrosis in *OVA*/*OVA*. Indeed, IL-13 activates TGFβ1-dependent pulmonary fibrosis pathway. We found that *OVA*/*OVA*/*D* histology indicated a fibrosis formation block. In this case, collagen deposition was prevented with lysyl oxidase (LOX) activity impairment through drug administration, which led to 1.7-fold LOX activity decrease (*OVA*/*OVA*/*D* versus *OVA*/*OVA*; $p = 0.05$) and as expected to 2.9-fold plasma histaminase (DAO) activity reduction (*OVA*/*OVA*/*D* versus *OVA*/*OVA*; $p < 0.05$). However treatment did not influence DAO activity in bronchoalveolar lavage fluid (*OVA*/*OVA*/*D* versus *OVA*/*OVA*; $p = 0.82$).

P14-017
Inhibition of NorA pump of *Staphylococcus epidermidis* by essential oils from *Salvia* spp.

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Bacterial multidrug efflux pumps are the major contributors of microbial resistance to several classes of antibiotics. The multidrug efflux transporter NorA of *Staphylococcus aureus* and *S. epidermidis* confers multidrug resistance (MDR) to a broad spectrum of compounds, including fluoroquinolones, quaternary ammonium compounds, dyes and others. As the inhibition of efflux pumps can potentially improve the clinical efficacy of antibiotics and decrease the selection of resistant mutants, there is urgent need for identification of novel efflux pump inhibitors, which may be potentially useful in combination therapy. The benefits of these inhibitors will be the ability to reuse the tradi-

tional antibiotics that became no longer effective due to the development of bacterial resistance through the efflux pumps.

From the collection of clinical isolates of *Staphylococcus epidermidis* we chose ciprofloxacin resistant strains with higher expression of *nor*A gene (estimated by q-RT-PCR) and wihout mutations in gyrase and topoisomerase IV genes (estimated by sequencing of *gyr*A and *par*C subunits). Essential oils (EOs) from *Salvia fruticosa*, *S. officinalis* and *S. sclarea* lowered the MIC of ciprofloxacin and ethidium bromide 2- to 4-fold. Using checkerboard assay we estimated synergistic interactions of EOs and ciprofloxacin. In order to investigate, if the synergistic and killing effect of EOs are the result of efflux pump inhibition, we performed functional analysis using real-time fluorometry and we found, that EOs increased accumulation and reduced efflux of model substrate ethidium bromide.

P14-018
Profiling and inhibition of multivalent WW domain interactions

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The recruitment of proline-rich sequences (PRS) by WW domains in the spliceosome is characterized by low affinities which can be enhanced by multivalent binding. However, the underlying mechanism of multivalent recognition is still poorly understood. We focus on the spliceosomal protein FBP21 which contains two WW domains separated by a short and flexible linker. Analyzing the interaction between the tandem WW domains (tWW) and ligands containing one or more PRS we could show that the tandem arrangement of WW domains and the valency of the proline-rich ligand both contribute to an apparent affinity enhancement.

FBP21-tWW has been shown to activate pre-mRNA splicing. In addition, FBP21-tWW has been put in to context with splicing of clinically relevant in to targets such as vascular endothelial growth factors (VEGF a/b isoforms). In order to understand and alter FBP21s cellular function, we aim at creating an inhibitor against FBP21-tWW.

We are following several ideas for inhibitor design. We are trying to increase binding affinity of the monovalent WW ligand. To achieve this, we optimized a peptide ligand by screening a nonapeptide phage display. The best binding candidate was WPPPPRVPR which we could improve further in a collaborative effort with AG Kühne.

We are also presenting the binding peptide on polymeric scaffolds, to gain avidity through multivalent ligand presentation. This is done in collaboration with AG Haag, using their dendrimeric polymer, and in collaboration with AG Rademann, in which a multivalent dextran is employed to display the peptide ligand.

P14-019
The investigation of endogenous intoxication and lipid peroxidation in patients with giardiasis before and after treatment

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The level of middle molecules of peptides (MMP) allows to evaluate the severity and prognosis of the disease and is a criterion for the effectiveness of the treatment.

Purpose of the study was to evaluate the state of endogenous intoxication and indicators of lipid peroxidationin patients with giardiasis before and after treatment.

Materials and methods: The amount of MMP and products of lipid peroxidation weredetermined in the blood of 198 patients with giardiasis, 129 of them were women (65%), 69 were men (35%).

The MMP level was detected for comparison in the blood of 84 healthy volunteers. Data were processed by conventional methods of variation statistics, we calculated the arithmetic mean (M) and standard dispersion (m). t-test (t) was used to assess differences.

Results: MMP concentration in the blood of women with Giardia was 2.5 times greater than that of the comparison groups of women. The level of MMP exceeds more than 6 times in men with giardiasis. The decrease in the intensity of endogenous intoxication was 2 weeks after antigiardia therapy.

A statistically significant increase in the level of all the studied parameters lipid peroxidation cascade was observed in the blood of men with giardiasis. The treatment of giardiasis helped to stabilize the level of almost all metabolites of lipid peroxidation cascade.

Conclusion: The MMP level was significantly higher in blood of patients with giardiasis than in comparison group. The accumulation of primary and secondary products of lipid peroxidation was observed in the blood of men and women.

P14-020
Effect of nanoparticles in the utilization of fatty acids by human microbiota

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The production of rosmarinic acid (RA), Sage (S) and Savoury (V) extracts solid lipid nanoparticles (SLN), using Witepsol (w) and Carnauba waxes (c) was already studied (Campos *et al.,* 2014). These systems are to be further incorporated in oral formulations but it is unknown if gut microbiota will metabolize these systems. With this aim, the evaluation of total fatty acids (FA) according to Castro-Gómez *et al.* 2014 in samples obtained from human faeces fermentation processes (8 and 24 h) was performed.

Samples w0 (empty SLN) were mainly composed of saturated (SFA; 48.84%), namely C12:0 (21.56%), and monounsaturated FA (MUFA, 49.48%; mainly C18:1c9, 37.24%). The addition of RA, S and V ingredients decreased ($p < 0.05$) the total SFA and

C18:1t9 concentration while increasing C18:1c9 and total polyunsaturated FA (PUFA), this latter by increments in the level of CLA isomers.

In Carnauba samples (s0) C18:1c9 was the main FA (66.26%) together with C18:1t9 (7.24%), C16:0 (5.65%) and C18:2CLAtt (3%). Preparations cRA, cS and cV resulted in higher amounts of C18:1c9 and CLA isomers.

After the assayed time, C4:0 increased in all samples (from 1.15% to 10.04–7.76% in Witepsol samples; from 2.37% to 18.42–16.98% in Carnauba) as result of the fermentative process. In all preparations C18:1c9, C18:2c9t11 and C18:2t10c12 contents decreased.

The assayed preparations did not alter the fermentative process of human microbiota and some healthy FA seems to be used by the bacteria. However, further research is needed to know if these effects may help to improve the human microbiota function.

P14-021
Adjuvant properties of Alternanthera mosaic virus virions and virus-like particles.

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Developing new adjuvants and vaccination strategies has paramount importance for successfully fighting against infectious diseases. Recently we have identified the new strain of Alternanthera mosaic virus (AltMV-MU, FJ822136 in GenBank). Flexible filamentous virions of AltMV-MU are typical for the family *Alfaflexiviridae* (genus *Potexvirus*) and closely related to Papaya mosaic virus (PapMV) (Ivanov *et al*., 2011). We have shown previously that the AltMV-MU coat protein (CP) can be efficiently reassembled *in vitro* under different conditions into helical RNA-free virus-like particles (VLPs) antigenically related to native virus (Mukhamedzhanova *et al*., 2011). AltMV-MU CP is able to form VLPs *in vitro* at pH 4.0 and at low ionic strength as PapMV. However, in contrast to PapMV (Erickson *et al*., 1976) AltMV-MU CP formed filamentous VLPs at pH 8.0. Here we demonstrated that in physiological conditions AltMV-MU CP formed stable VLPs with the morphology identical to the native virions but varying the length with the average of 400 nm. We examined adjuvant properties of AltMV virions and VLP. We showed that immunogenicity of a model antigen in the presence of AltMV virions and VLP increased significantly. Plant viruses and VLP are biologically safe for humans and may be considered as promising novel adjuvants.

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P14-022
Accelerating small compounds discovery targeting protein–protein interaction

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Protein–Protein Interactions (PPIs) are now considered as major targets to develop new drugs. The main issue to discover PPI modulators for complexes of interest is to find molecules able to inhibit (or stabilize) this type of interaction in chemical libraries compatible for experimental screening.

To overcome this issue a PPI-focused chemical library ($2P2I_{3D}$) has been designed, based on successful (published) stories that validated chemical molecules with an orthosteric mode of action. Selected targets, based on their structurally diverse interfaces of interaction (p53/MDM2, TCF/beta catenin complexes, two proteins containing a PDZ motif, bromodomain and viral proteins), have been screened using Homogeneous Time Resolved Fluorescence (HTRF) assays. All primary screenings, performed at 20 μM final of compounds, on these targets have revealed a higher hit rate than the one obtained with non focused libraries (0.6–2.4% versus 0.1–0.4%), which represent an enrichment factor between 5 and 15 times.

After screening steps, compounds have been characterized using orthogonal and cellular experiments showing their interesting affinity, without any chemical improvement of the molecules, and their activity on a more complex cellular environment revealing, at least, their potential as tools to study PPI complexes of interest.

All together these results suggest a new avenue to select compounds in order to improve enrichment factor during screening campaigns directed against PPI targets and to accelerate the discovery of biologically active compounds.

P14-023
Pepsin digestion of C-phycocyanin releases chromopeptides with potent anticancer and antioxidant activities

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C-phycocyanin is the most abundant protein of cyanobacteria Spirulina (genus *Arthrospira*), which is *commonly* used dietary supplement due to high nutritional value. Numerous *in vitro* and *in vivo* studies have showed that C-phycocyanin possesses significant antioxidant, anticancer, anti-inflammatory and immunomodulatory effects, ascribed to phycocyanobilin, blue linear tetrapyrrole chromophore. Phycocyanobilin is covalently bound (*via* tioether bond) to multiple cystein residues of apoprotein.

There are no literature data about C-phycocyanin bioavailability and digestibility, and whether released peptides with bound chromophore (chromopeptides) have biological activity. In this study we tried to give answers to aforementioned questions.

We found that C-phycocyanin (previously purified from commercial Hawaiian Spirulina Pacifica powder) is easily and rapidly digested by pepsin in simulated gastric fluid. Five dominant chromopeptide fractions, obtained from digest by preparative HPLC, were analyzed by mass spectrometry. Six chromopeptides were characterized, varying in size from 2 to 13 amino acid residues.

Reducing power and oxygen radical absorbance capacity (ORAC) assays showed that chromopeptides have significantly higher antioxidant capacity than vitamin E *analogue* Trolox: from 5.9 to 9.1 and from 7.8 to 12.8 times greater activity for reducing power and ORAC test, respectively. Chromopeptides, at concentration in the range of 10^{-5}–10^{-4} M showed cytotoxic effect on human cervical adenocarcinoma HeLa and human epithelial colonic carcinoma Caco 2 cell lines, with Caco 2 being more sensitive in comparison to Hela cells.

Our results indicates that orally administrated C-phycocyanin is quite digestible, and released chromopeptides obtained after pepsin digestion could have significant benefits on human health.

P14-024
Biochemical characterization of the alternative heme *b* biosynthesis pathway of *Desulfovibrio vulgaris* Hildenborough

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Desulfovibrio genus belongs to the sulfate reducing bacteria (SRB), a group of anaerobic prokaryotic organisms that perform reduction of sulfate coupled with oxidation of hydrogen or organic substrates. Since these organisms are metabolically versatile, they can also use other electron donors and acceptors. SRB are found in soils, marine and fresh waters and sediments. Although *Desulfovibrio* are responsible for biocorrosion of oil and gas pipelines, they have potential in bioremediation because of their ability to reduce several toxic metals. Additionally, they are present in the human and animal gut microflora and recently, it was proposed a correlation between *Desulfovibrio* and diseases, such as autism and inflammatory bowel diseases.

Desulfovibrio's metabolic processes rely on a wide range of modified tetrapyrrole-containing proteins. Modified tetrapyrroles include hemes, chlorophylls, siroheme and vitamin B_{12}, which are key metalloprosthetic groups for virtually all organisms and are formed in a complex and branched biosynthetic pathway. Despite the importance of these molecules in several metabolic pathways, their biosynthesis in *Desulfovibrio* was poorly understood. Our work has contributed to elucidate the synthesis of modified tetrapyrroles in *Desulfovibrio*, from the first universal precursor, aminolevulinic acid. More importantly, it demonstrated that in *D. vulgaris*, heme *b* is formed via an unprecedented pathway designated alternative heme biosynthesis (Ahb), which is likely also active in other SRB and archaea. In Ahb, the siroheme cofactor is hijacked as an intermediate and transformed into heme *b* in three enzymatic steps involving unparalleled chemistry, providing a biosynthetic and functional evolutionary correlation between heme *b* and siroheme.

P14-025
Interaction of NBD-labeled fluorescent steroids and a fatty acid with *Escherichia coli*

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Escherichia coli is known to lack its own steroids, but genetically engineered strains of the bacteria have been successfully used for heterologous expression of steroid-converting enzymes and creation of artificial steroid-converting whole cell biocatalysts [e.g., see Makeeva *et al*., *AJMB* (2013), 3(4), 173–182]. Interaction of four 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD)-labeled cholesterol-like compounds with *E. coli* DH5α cells have been evaluated; non-steroidal dyes Nile Red (NR) and 6-(NBD-amino)-hexanoic acid (6NHA) have also been used for comparison. The NBD-labeled steroids included 22-NBD-cholesterol (22NC), 25-NBD-cholesterol (25NC) as well as 5α-H,3α-H-isomer of 3-(NBD-amino)-cholestane (3NCII) and 20α-H-isomer of 20-(NBD-amino)-pregn-5-en-3β-ol (20NPα). It was shown that all the steroids stain *E. coli* cells. Methyl-β-cyclodextrin was shown to cause reduction of the cellular uptake of the compounds, whereas EDTA and toluene slightly enhance the cell-to-medium fluorescence ratios. Fluorescence microscopy and flow cytometry data demonstrate the ability of the steroids, NR and 6NHA to stain both membrane(s) and intracellular compartments of the bacterium. 6NHA has been found to be strongly metabolized into non-fluorescent products according to thin layer chromatography-fluorescence imaging and flow cytometry data whereas 25NC, 22NC, 20NP and 3NC seem to be stable. The difference is in accordance with our docking simulations of the NBD-labeled compounds interaction with a nitroreductase of *E. coli*. The revealed properties of NR, 25NC and 22NC as substrates for steroid-converting oxidoreductases together with the findings presented create perspectives for using the compounds with *E. coli* strains, expressing steroid-converting enzymes.

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P14-026
Preliminary studies on structure–function relationship of bitter-taste dipeptides derived from food proteins – *in silico* approach

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The taste is a chemoreceptive sensation generated by the interaction between chemical substance and taste receptors. Human organism perceives the taste in the *cavum oris,* where the taste buds containing the taste receptors are located. The taste receptors are distributed in the mucosal membrane of palate, epiglottis, and throat. The chemical information generated due to the interaction between the taste medium and a protein receptor migrates through neural system to brain.

Human organism distinguishes the following taste sensations: bitter, sweet, sour, astringent, salty and umami. These tastes can be generated by e.g. peptides obtained from food proteins after the enzymatic hydrolysis. The biological activity of a peptide is often a sum of physicochemical properties resulting from its chemical structure. These properties are expressed in numbers

and they are called descriptors. Currently, the structure descriptors characterizing the small biomolecules can be acquired from the chemical and biological databases.

The aim of the study was to apply bioinformatic/chemoinformatic techniques to elucidate the relationships between the structure of bitter-taste dipeptides derived from food proteins and their activity (bitterness). Molecular dipeptide structure descriptors were calculated by computer programs freely accessible in internet. The structure–function relationship of dipeptides possessing bitterness was estimated by multivariate linear regression analysis.

For some of the peptides analyzed, their experimental versus predicted activities were concurrent. Seventeen dipeptides were similar in terms of their chemical nature – they consisted of Pro, Leu, and Val. Our findings were consistent with the literature data concerning the analysis of bitterness of peptides.

P14-027
In cerebellar neurons the enzyme glutamate dehydrogenase is crucial for glutamate oxidation

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Glutamate is the most abundant excitatory amino acid in the brain. The enzyme glutamate dehydrogenase (GDH) catalyses the oxidative deamination of glutamate to α-ketoglutarate or the reverse reaction. This reaction connects the major cellular pathways the tricarboxylic acid cycle, amino acid metabolism and neurotransmitter metabolism. To investigate the role of GDH in neuronal metabolism, glutamatergic neurons were cultured from mice with a central nervous system specific deletion of glutamate dehydrogenase 1 (GDH1-deficient neurons) or from control mice (control neurons). The neurons were incubated with 0.1 mM [U-^{13}C]glutamate and the occurrence of ^{13}C-labelled metabolites was investigated by gas chromatography mass spectrometry. GDH1-deficient neurons had a significantly higher cellular percent labelling of [U-^{13}C]glutamate compared to control neurons. The metabolites generated directly from [U-^{13}C]glutamate, the subsequent tricarboxylic acid cycle intermediates [U-^{13}C]succinate and [U-^{13}C]fumarate were significantly less labelled in GDH-deficient neurons suggesting a slower metabolism of [U-^{13}C]glutamate. Furthermore, in GDH-deficient neurons a significantly reduced labelling in the isotopomers generated via the first turn of the tricarboxylic acid cycle, i.e. triple labelled α-ketoglutarate and triple labelled glutamate, and double labelled succinate, double labelled fumarate and double labelled aspartate, were observed compared to control neurons. These data suggest that GDH plays an important role in oxidative catabolism of glutamate in glutamatergic neurons.

P14-028
Teratogenic and biochemical effects of a selective pesticide on rabbits

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Many chemical pesticides have been becoming a serious health hazard exerting effects on environment and on human health. Many commercial formulations in market have high concentration of pesticides that they have labelled. Dichlorvos is one of these pesticides. In our study we have studied effects of this by animal model to elaborate the effects of dichlorvos. We studied concerning effects of organophosphorus on acetyl cholinestrases, butyrlcholinestrase, biochemical, hematological and teratogincal parameters.

The aim of this study was to evaluate the effects of this insecticide on the cholinesterases activity in serum as well as in other tissues like brain, heart, liver and kidney during complete pregnancy of rabbits. The 30 pregnant rabbit were categorized into six groups, one control and other five groups treated with 36, 18, 8, 1.48 and 0.986 mg/kg of body weight DDVP on gestational days 6, 13, 18 and 24 respectively. Rabbit serum samples were collected at 1, 7, 13 and 25 gestational day to measure the acetyl- and butyryl cholinestrases profiles. Results showed a significant decrease in acetyl- and butyryl cholinestrases activity in serum as well as in each organ during complete pregnancy exposure to high dosage of dichlorvos.

P14-029
Levels of MMPs and TIMP-1 in esophageal tissue after burn injury

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According to the World Health Organization there has been a steady increase in the number of chemical burns of the esophagus. The main result of such injury is the formation of scarring. It is known that violation of the healing process of post burn wounds is the cause of excessive collagen synthesis, which is caused by an imbalance in the correlation of MMPs and TIMPs.

The purpose of the work was to determine the levels of MMP-1, MMP-2 and TIMP-1 in esophageal tissue after burn injury

In our experiments we used nonlinear immature white rats (1 month) weighing 90–110 g, which were kept on a standard vivarium diet. The animals were experimentally simulated with the alkali esophageal burn (AEB) with NaOH 20% (2nd degree burn). Materials for research were collected at 1st, 7th, 15th and 21st days. The levels of TIMP-1 and MMP-1, MMP-2 were measured by ELISA. Data analysis was conducted using Microsoft Excel 2010.

We have shown the increase of MMP-1 and MMP-2 levels in esophageal homogenate after burn injury. Thus, the levels of MMP-1 and MMP-2 were higher than the reference values in during all days of the experiment. The highest levels of tissue inhibitor TIMP-1 were observed on 15th and 21st days of the experiment and exceeded the control in 55% and 60% respectively. Future studies addressing changes of MMPs and TIMPs levels after burn injury may delineate more specifically roles for these and other MMPs in the processes of post burn tissue remodeling and scar formation.

P14-030
The Kv-channel blockers as potent modulators of platelet reactivity

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Membrane potential and its transient changes play an important role in regulation of cellular physiology and response to the different types of stimuli. The resting potential of platelets that equals approximately –60 mV is defined by the equilibrium

potential of K^+ and undergoes changes at the early stages of platelet activation prior to thrombus formation. In this research several blockers of voltage-dependent K^+ channels were tested for the ability to modify the response to platelet agonists such as ADP and thrombin. The activation process was assessed by the release of pH-sensitive dye acridine orange (spectrofluorimetric registration), changes in the dot plots of side scatter versus forward scatter (flow cytometry) and by secretion of dense granule constituent L-glutamate (glutamate dehydrogenase assay). It has been shown that stable depolarization of plasma membrane of platelets by tetraethylammonium, tetramethylammonium and 4-aminopyridine enhanced subsequent activation and aggregation of rabbit and human platelets stimulated by ADP. The significant differences have also been exhibited in the action of tested Kv-channel blockers on the release of endogenous glutamate after platelet stimulation. This study provides the basis for understanding the influence of changes in E_m on platelet reactivity. It was also demonstrated significant differences in the action 4-aminopyridine and tetraethylammonium on platelet functional state *in vitro*.

P14-031
Efficient *in vitro* inhibition of topoisomerase I and DNA binding by novel acridine derivatives

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Topoisomerases are enzymes performing the relaxation of supercoiled DNA essential for DNA replication and reparation. They are considered as important targets for antibacterial and anticancer therapy. So the search for new efficient topoisomerase inhibitors is of significant interest.

A series of novel topoisomerase I (Topo I) inhibitors were synthesized. Their structures were based on acridine core with strong intercalating properties containing one or two basic substituents (*N*,*N*-dialkylamino, pyridyl, *N*-methylpiperazinyl, morpholyl fragments) attached at various positions of acridine ring via linker groups. Being protonated under physiological conditions, these fragments are able to interact with DNA phosphates and acidic groups of the enzyme to enhance the binding of ligands to topoisomerase complex and their inhibiting activity.

Over 20 derivatives were tested in the *in vitro* system of supercoiled pBR322 DNA relaxation by eukaryotic Topo I. Six compounds inhibited the enzyme with IC_{50} below 15 μM. The most efficient inhibitors containing two basic fragments demonstrated IC_{50} as low as 2.5–3.1 μM. Structure-activity relationship was analyzed to identify the structural features determining high biological activity of drugs.

Since inhibitors were designed to interact with DNA, their binding affinity was determined by FID (Fluorescent Intercalator Displacement) assay. Binding constants for active inhibitors were in the range $(1.0–2.6) \times 10^6$ M^{-1}, i.e. they are highly efficient DNA binders. At the same time, no direct correlation between DNA binding efficiencies and IC_{50} values was observed. This may indicate that ligand interaction with topoisomerase also contributes to the inhibiting effect.

Keywords: Topoisomerase, inhibitors, acridines

P14-032
Biosensor on based of immobilized Lipoxygenase for determination of leukotrienes and lipoxins

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Lipoxygenase (LOX) is an enzyme that is found in many plants and animals, which catalyse the oxygenation of polyunsaturated fatty acids (PUFA) to form fatty acid hydroperoxides. They are present in a wide range of biological organs and tissues, but they are particularly abundant in grain legume seeds (beans and peas) and potato tubers [1].

Linoleic and linolenic acid are the major polyunsaturated fatty acids in plant tissues, and insertion of the oxygen takes place at either the 9 or 13 position to generate the corresponding 9- or 13-hydroperoxides [2].

In this study, we describe the development of enzyme sensors for the determination of products by ω-3 and ω-6 fatty acids. Since linoleic and α-linolenic acids show differences in first and second oxygenation activities, it is possible to analyses each of them products [3].

While most LOXs so far characterized are soluble cytosolic enzymes, some are chloroplastic, mitochondrial, or located in the vacuoles.

Leukotrienes use lipid signaling to convey information to either the cell producing them (autocrine signaling) or neighboring cells (paracrine signaling) in order to regulate immune responses. Leukotriene production is usually accompanied by the production of histamine and prostaglandins, which also act as inflammatory mediators [4].

In mammals, dioxygenation products of LOX are precursors to tissue hormones such as leukotrienes and lipoxins that regulate a variety of cellular inflammation and immune responses. Such as, they play a critical role in the arachidonic acid cascade [5].

P14-033
Molecular composition, function and physiology of Kainate Receptors (KARs) in pancreatic endocrine cells

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KARs are one of the three classes of ionotropic glutamate receptors (iGluRs). KARs are located throughout the central nervous system (CNS) at both presynaptic and postsynaptic sites where they modulate neurotransmitter release or mediate excitatory neurotransmission, respectively. While KARs and other iGluRs have been identified outside the CNS, the molecular organisation and function of these receptors in non-neuronal cells remain unclear. The aim of this study was to establish the molecular composition and functional role of KARs in pancreatic endocrine cells.

The presence of KAR subunit mRNAs and proteins were investigated in cultured pancreatic clonal beta-cells, alpha-cells and primary rodent islets of Langerhans. Reverse-transcriptase PCR identified mRNAs for GluK2–5 and their auxiliary subunits Neto 1 and Neto 2 in all cell types. This was confirmed using immunoblotting with GluK2/3 and GluK5 selective antibodies.

FURA-2AM epifluorescence imaging of cultured MIN6 beta-cells showed that activation of KARs with kainate induced an increase in intracellular calcium concentration at stimulatory glu-

cose concentrations. A similar effect was observed on glucose-induced insulin secretion. These effects of kainate were blocked by KAR antagonist but not by antagonists of other iGluRs.

MTT cell viability assay showed that chronic exposure to kainate, glutamate and dihydrokainic acid significantly reduced the viability of both cultured pancreatic endocrine and neuronal cells.

These results indicate that a range of functional KAR subunits are expressed in the endocrine pancreas. Activation of these receptors is likely to have an impact on pancreatic hormone secretion and viability of endocrine cells in the islets of Langerhans.

P14-034
Cytotoxic activity of proteins and small molecules isolated from whole plant extracts and latex of *Chelidonium majus* L. towards HeLa cells

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Greater Celandine (*Chelidonium majus* L.) is a member of the Papaveraceae and is broadly distributed in Europe and Western Asia. Extracts and latex (milky sap) from this plant have been widely used in traditional folk medicine and contemporary pharmacology in the light of their antimicrobial, antitumor, anti-inflammatory, antifungal, and antiviral activities. Our previous research showed that proteins which are contained in fractions with nucleolytic activity are responsible for biological activity and belong mainly to PR (pathogenesis-related) proteins (Nawrot *et al.* 2007, *Phytochemistry* 68:1612–22; Nawrot *et al.* 2008, *Folia Histochem Cytobiol* 46:79–83).

The goal of the project was to determine the cytotoxic activity of purified nucleolytic proteins from *C. majus* extracts against HeLa cells and to identify using LC-MS/MS which non-protein substances (small molecules) are associated with them.

Extracts of *C. majus* whole plants and latex were purified using fast performance liquid chromatography (FPLC) on preparative heparin sepharose columns. Fractions after purification were monitored for DN-ase activity using gel-based zymography, SDS-PAGE and analyzed with LC-ESI-MS/MS. Proteins were identified using Mascot.

Mass spectrometry results for the proteins of applied fractions showed that they contained plant defense- and pathogenesis-related (PR) proteins. Further analysis showed that the main non-protein constituents of the samples were alkaloids, like coptisine, protopine, chelidonine and berberine. Studied samples were applied to HeLa cells and showed cytotoxic potential. Cytotoxic effect was dependent on the time which elapsed after addition of protein to the incubation medium.

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P14-035
Aflatoxin B1 induces macrophage activation and inflammation through TLR-MyD88 pathway

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Mycotoxins are structurally diverse metabolites of fungus which cause serious health problems. Aflatoxin is the most harmful mycotoxin produced by *Aspergillus flavus* and *A. parasiticus*, known to cause hepatocellular damage. Hepatocytes got much attention to study aflatoxin mediated toxicosis, compared to macrophages. Toxicity of mycotoxins on cellular system depends on dose and time of exposure. Moreover, consumption of lower dose of mycotoxins sometimes does not produce any apparent symptoms, although suppress the immune functions and activates macrophages. In this study, we have elucidated the molecular mechanism of aflatoxin induced inflammation in murine macrophage Raw 264.7 cells. Aflatoxin B1 (AFB1) treatment elevated the expressions of TLRs (TLR 2, 4) and its downstream signals, MyD88 and IRAK4 as detected by western blot. AFB1 enhances the nuclear translocation of p50 and p65 subunits of NF-κB, thereby, increased the secretions of cytokines, like TNF α and IL-1β, as determined by ELISA. To confirm the macrophage activation and nuclear translocation of NF-κB after AFB1 treatment, we analysed nuclear localization of p50 by using immunofluorescence method. We detected an increased in expression of p50 in nucleus in macrophage after AFB1 treatment. AFB1 treatment also increased the DNA binding efficiency of p50 subunit, as confirmed by electrophoretic mobility shift assay. These results suggest that AFB1 activates macrophages and induces inflammation via TLR-MyD pathway. Although there are many reports documented about inflammatory nature of AFB1, but the mechanism of action was remain unclear. This finding could be helpful to understand the molecular mecnism of aflatoxin induced inflammatory diseases.

P14-036
Ubiquitin-independent degradation of myelin basic protein by immunoproteasome contributes to cytotoxic T-cell-mediated demyelination in experimental autoimmune encephalomyelitis

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Proteasome plays an essential role not only in continual turnover of intracellular proteins but also in antigen processing, generating peptides which can be presented on MHC I molecules and recognized by cytotoxic T-lymphocytes. Recently we have shown that myelin basic protein (MBP), one of major autoantigenes in multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis (EAE), is degraded by 26S proteasome ubiquitin-independently. As proteasomal degradation of MBP is not

controlled by ubiquitylation system, rate of MBP degradation and composition of the resulting peptide pool is dependent mainly on the protesome substrate specificity. $\beta 1i^{high}$ immunoproteasome, which is upregulated in the brain of SJL mice with EAE, generates increased amount of $MBP_{83–90}$ peptide epitope (ENPVVHFF). This peptide can be presented on mouse MHC I molecules of H2-K^k and H2-K^s haplotypes and induces cytotoxic CD8+ T cells to target oligodendrocytes *ex vivo*. Inhibition of immunoproteasome activity, inhibition of MBP deimination, or increase of MBP expression level can affect the oligodendrocytes' susceptibility to cytotoxic T-cells-mediated damage.

P14-037
In vitro immunosuppressive activity and immunological alterations induced by H1 receptor antagonist, Astemizole on mouse immune system

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Astemizole (AST) is a H1 receptor antagonist, and by signaling through these receptors AST modulates histamine mediated inflammatory and hypersensitivity responses. In this study, we have elucidated the molecular mechanism underlying the immunosuppressive effects of increasing concentration of AST *in vitro*. Splenocyte cell were isolated from mouse and viability was analyzed with comitogenic assay. Phagocytic potential of macrophages were determined using neutral red uptake, NBT reduction assay and lysosomal enzyme release. The expression of p38-MAPK pathway was detected with western blotting. AST inhibited the growth of mouse T cells and also suppressed the phagocytic activity of macrophages in dose dependent manner, there by caused the activation of p38-MAPK signaling which caused cytokine secretion. These results suggest that supplementation of immune cells with AST could suppress cellular immune responses. The mechanism might shows potential of AST as a countermeasure to the immune dysfunction and suggesting an interesting use in inflammation related diseases.

Keywords: Immunosuppressant; H1 receptor antagonist; T cell; Phagocytosis; p38-MAPK signaling.

P14-039
Bisubstrate-analogue inhibitors targeting mitotic protein kinases Aurora A and Haspin

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Mitotic PKs ensure the progression of a cell through the division process, whereas the abnormal signalling of these PKs contributes frequently to cancer. We report development of inhibitors and fluorescent probes targeting important players in mitosis, Aurora A and Haspin.

For the design of compounds, we used the bisubstrate-analogue approach where a fragment targeting the ATP-site was conjugated with a fragment targeting the protein substrate site of the PK. In case of Aurora A probes, attachment of the oligoarginine to VX-689 contributed to increased affinity of the conjugates in the presence of Aurora A activator, TPX2. Interestingly, the probes possessed slow association and dissociation kinetics. The conjugates were further converted into photoactivatable probes binding irreversibly to PK upon UV-irradiation.

In case of Haspin-targeting compounds, we achieved 6000-fold gain in affinity of the conjugates as compared to their fragments. The selectivity profiling against a panel of 43 protein kinases showed that the conjugate incorporating a peptidic moiety derived from the Histone H3 was remarkably selective towards its target, whereas inclusion of a positively charged oligoarginine sequence into the structure of the compound abolished selectivity. The co-crystals of Haspin with two novel conjugates confirmed the bisubstrate character of the inhibitors.

The wealth of data was gained using homogeneous binding/displacement and inhibition assays with detection of fluorescence anisotropy and/or intensity. In addition, we have examined novel compounds in live cells using fluorescence microscopy (co-localization with antibody staining and interference with mitosis during prolonged incubation) and western blot (reduction of phospho-substrate levels).

P14-040
Intracellular distribution and DNA binding activity of glyco-tacrine conjugates

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This study examines the binding properties of a series of newly synthetized tacrine derivatives **1–2** and their anticancer effects. Spectroscopic techniques were used to study DNA binding properties and to determine the types of DNA interaction with the studied derivatives. Large values of binding constants K in the range from 5.4×10^4 and 4.8×10^5 M^{-1} prove a high affinity of ligands to DNA-base pairs. Both of glycol-tacrine derivatives possessed strong DNA binding activity and the low cytotoxicity of **1** can be associated with its intracellular distribution. To explain differences in cytotoxicity of **1** and **2** their intracellular distribution has been determined. An intracellular distribution of **1** and **2** was investigated by a method of co-localization/overlay of the fluorescence of our substances and organelle-specific fluorescent dyes using a confocal microscopy. It was found that only fluorescence **2** and SytoRed overlay, indicating that **2**, but not **1**, substances was localized inside of the nucleus.The study of colocalizations of glyco-tacrine derivatives with a mitochondrial dye MitoRed and an endoplasmic reticulum dye ER-Tracker, indicated accumulation of the derivatives in mitochondria and ER, despite only a partial overlay of both conjugates with organelle-specific fluorescent dyes was recorded.

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P14-041
Characterization of Potato virus X spherical virus-like particles

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Spherical particles (SPs) generated by thermal transition of rigid rod-like Tobacco mosaic virus (TMV, genus *Tobamovirus*, family *Virgaviridae*) were described by our group previously [1,2]. Recently we obtained virus-like particles (VLPs) with morphology similar to the spherical shape by thermal denaturation and structural remodeling of filamentous Potato virus X (PVX, genus *Potexvirus,* family *Alphaflexiviridae)* [3]. The formation of PVX

VLPs starts at 70°C and fully completes by 90°C. Thus, in the case of PVX, VLPs thermal transition requires a lower treatment temperature (90°C) in comparison with TMV (94°C). The size of spherical PVX VLPs varies from 50 to 150 nm and unlike TMV SPs size does not depend on the initial virus concentration. The evidences that PVX VLPs are RNA-free and consist only of PVX coat protein were obtained. In this work we demonstrated that PVX VLPs have adsorption properties and could bind with different model antigens (recombinant Rubella virus antigen, recombinant Plum pox virus antigen, TMV coat protein). The adjuvant properties of spherical PVX VLPs were examined. Immunostimulating properties of native PVX virions were demonstrated and compared with spherical PVX VLPs. PVX spherical VLPs generated by thermal treatment of helical filamentous virions could become a promising platform for the development of functional active complexes. The work was funded by the Russian Science Foundation (grant no. 14-24-00007).

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P14-042
Functioning change of serotonin metabolism in blood of patients with type 2 diabetes mellitus and ischemic stroke

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It is known that cerebral atherosclerosis is one of the leading factors of ischemic stroke, and type 2 diabetes is an independent factor in their development. View of the relationship pathogenetic mechanisms of atherosclerosis and type 2 diabetes was expressed by several authors (Gallacher, 2013; Snell-Bergeon, 2014). Current studies indicate the involvement of serotonin in energy metabolism and the effect of serotonin on the concentration of glucose in the blood (Lam, 2007). These data give reason to assume that the serotonergic system may be involved in the pathogenesis of type 2 diabetes.

We have analyzed the content of serotonin, tryptophan, MAO activity and aggregation of platelets in the blood of patients with ischemic stroke, which suffer from type 2 diabetes. Studies have shown an increase of serotonin content of 46% and tryptophan to 5.6 times in patients with ischemic stroke and type 2 diabetes compared with the values of the control group. We found that activity of monoaminooxidase in patients with type 2 diabetes was reduced by 40% against to the activity in a group of healthy donors. Analysis of platelets aggregation showed an increase on 22% relative to this value of the donors.

These data may indicate a significant imbalance in serotonin metabolism in two groups of patients and the necessary for further research of metabolic enzymes of serotonin in the bloodstream and functioning platelet hemostatic links to additional information that could explain the differences in the content of these metabolites.

P14-043
Bioconversions of lipophilic dyes Nile Red and 25-NBD-cholesterol into mycobacteria

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Previously we have found that fluorescent *N*-alkylarylamine Nile Red (NR) is converted by mammalian cytochrome P450 steroid 17α-hydroxylase CYP17A1. Mycobacteria possess various P450s, so we tested of the dye with *M. smegmatis* and *M. tuberculosis*. It has been found that fluorescent oxidized derivatives of NR form during long incubations of the dye with both of the bacteria. Docking simulations have demonstrated that CYP130 & CYP125 can bound NR close to their heme cofactors affinely, pointing out on a possible involvement of the P450s in the oxidation of NR. Analogously, fluorescent steroid 25-NBD-cholesterol (25NC) has been confirmed to be converted *via* formation of its 4-en-3-one derivative, indicating that 25NC is a substrate for cholesterol oxidases and/or 3β-hydroxysteroid dehydrogenases from *M. smegmatis*. The bioconversions of the dyes by mycobacteria have reported for the first time. Due to uptake and metabolism of mammalian lipids by *M. tuberculosis* are essential for persistence of the pathogen, the established bioconversions should be taken into consideration if both NR and 25NC will be used for staining of lipids cells during a study of living *M. tuberculosis* or the host-pathogen interactions.

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P14-044
New antihistamine Kunitz-type polypeptides of the sea anemones, *Heteractis crispa* and *Stichodactyla mertensii*

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The Kunitz-type protease inhibitors participate in regulation of vital processes, such as inflammation, blood coagulation, digestion and others. Poisonous animals have Kunitz-type polypeptides which, in addition to inhibiting serine proteases, can modulate voltage-gated channels (K_v, Ca_v, Na_v) and the TRPV1 receptor. Beside this, two Kunitz-type polypeptides from the sea anemone *Heteractis crispa* have shown antihistamine activity *in vivo*.

In this work we investigated cDNA transcripts of the sea anemones *H. crispa* and *Stichodactyla mertensii* and gene sequences encoding Kunitz-type polypeptides, which share a high sequence similarity with K_v channel blocker SHTXIII from *Stichodactyla haddoni*, were identified. The sequences of all mature polypeptides are characterized by point substitutions. Three chosen polypeptides of *H. crispa* and *S. mertensii* were produced in

the *Escherichia coli* expression system. They inhibited trypsin activity and did not have toxic effects in mice up to a dose of 5 mg/kg. Electrophysiological assays on eight potassium channel isoforms (K_v1.1–1.6, *Shaker* IR, h*ERG*) revealed the absence of modulatory activity. *In vitro* antihistamine activity study was conducted on bone marrow-derived macrophages from mice Balb/c and a statistically reliable inhibitory effect induced by the two polypeptides was observed. Noteworthy is the fact that the polypeptides are 10-fold more potent than fexofenadine (a selective antagonist of the H1 receptor). Electrophysiological analyses on the cloned H1 receptor in the *Xenopus laevis* expression system are planned to unravel the molecular interaction between the polypeptides and the H1 receptor.

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P14-045
A new multigene family of Kunitz-type IQ-polypeptides from sea anemones

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Protease dysfunction underlies various disorders including cancer, neurodegenerative and cardiovascular diseases. One of the biggest treatment challenges is to inhibit unwanted protease-related activity. A great number of protease inhibitors have been found in terrestrial and marine venomous animals including sea anemones that have been a promising source of new protease inhibitors. Previously we discovered the multigene superfamily of Kunitz-type GS-polypeptides from the tropical sea anemone *Heteractis crispa*. These polypeptides can inhibit different protease classes demonstrating analgetic, anti-inflammatory, antihistaminic and hypothermic activities.

Here we report on the discovery of a novel sea anemone Kunitz-type inhibitors family named IQ-polypeptides. In *H. crispa* cDNA library, we revealed transcripts encoding for IQ-polypeptides characterized by a large positive charge (8.5–9.3). The mature IQ-polypeptides consist of 58 residues with conserved spacing of six cysteine residues. Unlike GS-polypeptides, IQ-polypeptides have a propart peptide following the signal sequence. The IQ-polypeptide genes belong to a multigene superfamily of Kunitz-type GS-polypeptides based on a very high similarity of its signal peptide-coding sequence. Using cDNA libraries from different sea anemones, such as *H. magnifica* and *Stichodactyla mertensii,* we found that IQ-polypeptide genes are widely presented in different species of Stichodactylidae. Comparing the mature IQ-polypeptides of sea anemones, we revealed that they were high conserved and differed from each other by point amino acid substitutions probably indicating similar biological activities. Further obtaining and characterizing of recombinant IQ-polypeptides as potential therapeutic agents are of great interest.

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P14-046
Inhibition of DNA-topoisomerase I/II activity with selected bistacrine-thiourea/urea derivatives and their biological effect

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Topoisomerases influence the topological state of DNA by relaxing torsion tension in supercoiled DNA and their activity is essential for cell viability. Both forms of the enzyme, Topo I/II, may also have the capability to damage the genome leading to cell death in both healthy and cancerous cells. Well-known anticancer agents such as acridine or camptothecine derivatives act by interfering with DNA synthesis and by inhibiting Topo I/II activity(1). Previous studies have shown that some tacrine derivatives can act as dual-effect Topo I/II inhibitors, thereby suggesting that these agents may show potential for development as novel anticancer agents(2).

Bistacrine-thiourea/urea derivatives (**1**–**4**) are a novel class of cytotoxic agents which combine both 9-amino-1,2,3,4-tetrahydroacridines linked with thiourea/urea and various lengths of alkyl chains. In this study, Topo I/II inhibition mode assays were performed and verified that the novel compounds are topoisomerase suppressors rather than poisons. The cytotoxic action of **1**–**4** on human leukemic cancer cell line HL-60 were assessed using different techniques, such as MTT assay, the detection of mitochondrial membrane potential, cell viability measurements and cell cycle distribution analysis after 24, 48 and 72 h incubation. The studied derivatives were found to be more cytotoxic than the positive control, tacrine. Binding studies of **1**–**4** with ctDNA were also performed in order to characterize the effect mechanism using a variety of techniques (UV-Vis and fluorescence spectroscopy, thermal denaturation, linear dichroism and viscometry).

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P14-047
Two novel antioxidants with diverse biological effects on curcumin-induced apoptosis in C2 skeletal myoblasts; signaling mechanisms involved

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Excessive levels of reactive oxygen species (ROS) contribute to a number of pathological conditions including muscle disorders. Since redox equilibrium imbalances may seriously affect skeletal muscle performance of daily activities, there has been a substan-

tial rise of the scientific interest in the possible salutary impact of antioxidants. In this context, in the present study, we aimed at evaluating the mechanism of action of two newly synthesized antioxidant compounds (named AK1 and AK2), in C2 skeletal myoblasts, a routinely used skeletal muscle experimental setting. At first, both compounds were found to exhibit a very efficient interaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH), indicative of their reducing potential and radical scavenging properties. Using H_2O_2 and curcumin as pro-oxidants (MTT assay), we next observed that while AK1 as well as AK2 were found to inhibit the deleterious effect of H_2O_2 on C2 myoblasts viability, AK1 failed to enhance survival after treatment with curcumin. AK1 equally failed to block activation of c-Jun NH2-terminal kinases (JNKs), in skeletal myoblasts exposed to curcumin, a signaling pathway identified to mediate curcumin-induced apoptosis, evidenced by cleavage of poly(ADP-ribose) polymerase (a routinely used marker of apoptosis). Collectively, despite their minimal differences in structure, AK1 and AK2 show a different antioxidant mode of action, with AK1 exhibiting its antioxidant activity more rapidly versus the more delayed and sustained activity of AK2. Future studies are required so as to assess the exact mechanism of action of these compounds and potentially support their application in therapeutic protocols against ROS-related muscle disorders.

P14-048
Unexpected anti-platelet and promising proangiogenic effects of calix[4]arene C-145 *in vivo*

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Calix[4]arenes are macrocyclic compounds with intramolecular lipophilic cavity formed by aromatic fragments that can carry out different biological effects depending on modification of their carbon skeleton. *In vivo* studies of calix[4]arene C-145 showed that after its injection the TT and APTT were prolonged in 2 and 1.5 times respectively, but the total fibrinogen and prothrombin level, parameters of fibrinolytic and anticoagulant systems remained constant.

The aim of a present work was to study calix[4]arene C-145 action on activation and aggregation of platelets *in vivo*, as well as on proliferation and apoptosis of endothelial cells (EC) in cell culture.

Aggregometry and flow-cytometry showed that calix[4]arene C-145 did not activate platelets nor affect their aggregation *in vitro*. However intravenous injection of calix[4]arene C-145 in the dose of 7.5 mg/kg into the bloodstream of healthy rabbits leads to strong inhibition of platelet aggregation and changes of shape and granularity of most of the platelets. But we did not observe any additional appearance of EC activation marker tPA *in vivo* and any inhibition of proliferation of EC in cell culture. Moreover, calix[4]arene C-145 decreased the level of apoptotic cells in EC culture. The population of proliferative cells (G_2/M + S) in the presence of calix[4]arene C-145 was 2,5 times bigger when compare to control probes.

In conclusion calix[4]arene C-145 selectively inhibits fibrin polymerization, possesses strong anti-platelet effect *in vivo* and proangiogenic effect on EC culture. These characteristics allowed us to assume the possibility of calix[4]arene C-145 use as an effective antithrombotic agent.

P14-049
Coumarin-tacrine hybrid molecules as potential anticancer agents

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Naturally occurring coumarins are frequently used by researchers as a base for the development of novel synthetic and semisynthetic coumarin based therapeutic agents. This is primarily due to their wide spectrum of activities including, among others, antioxidant, anti-inflammatory and anticancer effects etc. Many of these agents are hybrid molecules and have shown evidence of multiple pharmacological activities. It is therefore conceivable that these hybrid compounds could also be used as potential drug candidates for multifactorial diseases such as cancer or Alzheimer's disease(1).

Recent years have seen the increasing pharmacological interest in DNA topoisomerase I, an important enzyme which is found in all living organisms and which participates in many metabolic cellular processes, such as replication, transcription, recombination and repair(2). In this study, a series of novel coumarin-tacrine hybrids were designed, synthetized and biologically evaluated for their potential inhibitory effect of this enzyme. Our research investigated the nature of the interactions of N1-[n-1,2,3,4-tetrahydroacridin-9-ylamino)alkyl]-2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetamides (***1a–d***) with DNA and the ability of these hybrid molecules to inhibit topoisomerase I was also studied using electrophoretic techniques. The electrophoretic results proved that ligand 1c inhibited topoisomerase I at a concentration of 30 μM. Drug-DNA interactions were studied using spectroscopic techniques (UV-Vis, fluorescence spectroscopy and circular dichroism), and the binding constants for the coumarin-tacrine hybrids with DNA were determined from UV-Vis spectroscopic titration.

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P14-050
Investigation of biocompatibility and antifungal activity of silver doped hybrid materials based on silica and cellulose derivates

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In the present study two types of silver-doped organic-inorganic hybrid materials were prepared by sol gel method. As precursor of silica was used tetraethylorthosilicate (TEOS), precursor of silver nanoparticles (AgNPs) – silver nitrate and as cellulose derivates were chosen hydroxypropyl cellulose (HPC) and hydroxy-

propyl methyl cellulose (HPMC). The structure of obtained hybrids was analyzed and characterized using AFM analysis and static contact angles measurements. The antifungal behavior of the matrices against *Penicillium chrysogenum* was tested by measuring the size of the inhibition zone formed around the materials. Other important aspect of the work was the investigation of biocompatibility of hybrids. For that purpose a cytocompatibility test (MTT test) and cell adhesive behavior (actin cytoskeleton organization) of 3T3 cells as a function of surface functional groups were studied. AFM analysis showed that with the increasing of silver concentration the roughness of the materials became higher. Static contact angles measurements revealed hydrophilicity or hydrophobicity of these hybrid coatings. It has been experimentally demonstrated that these silver loaded organic-inorganic hybrids have a very good antimicrobial behavior. The experiments revealed that the biocompatibility of the obtained hybrids depends on the amount of AgNps. By increasing the content of the silver nanoparticles in the hybrid materials their surface became rougher resulting in low cell adhesion and spreading.

One possible way for stabilization of AgNps is to introduce them into an appropriate organic-inorganic matrix, the choice of which is very important for their use in the biomedical field as tissue engineering and regenerative medicine.

P14-051
Stereospecific synthesis of molecules with physiological effect on the cell functions by means of lipase isolated from *Pseudozyma antarctica*

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The adverse effect of certain external factors such as viruses or bacteria, existing in the environment, leading to infection and disturbance of cellular functions in the body. Small molecules with physiological activity, which by its operation can affect the organism and to restore the normal function of cells can be synthesized or modified using enzymes. This can be done under catalysis of lipolytic enzymes, as they possess unique characteristics: substrate specificity, regio-specificity and stereo-selectivity. Lipases are serine hydrolases defined as triacyl glycerol acylhydrolases (EC 3.1.1.3). The production of lipolytic enzymes has been performed by plant, animal, bacteria, fungi and yeasts. *Pseudozyma antarctica (Candida antarctica)* strain, isolated in the cold conditions of the Antarctica, can produce a thermostable extracellular lipases. Many methods and techniques for optimization and obtaining of isolation and purification of lipase are reported. Generally, in a practice fractional precipitation, dialysis, ultrafiltration, gel filtration, various types of chromatography or IEF are used. A lot of research groups attempt to innovate and optimize this process. In our work we report the found optimal conditions for isolation and purification of extracellular lipases obtained from *Pseudozyma antarctica* by fractional precipitation with ammonium sulfate and subsequent ultrafiltration. Further, thus obtained lipase was subjected to electrophoretic separation by SDS PAGE with silver staining. In perspective, the obtained lipase will be used for stereoselective reaction of protection/deprotection of primary, secondary, and phenolic hydroxyl groups of the amino acids Ser Tyr, Thr as a model substrates, as well as carbohydrate-containing molecules with physiological activity.

P14-052
"Humanised" biotin protein ligase provides clues about inhibitor selectivity

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Biotin protein ligase (BPL) is a ubiquitous enzyme involved in the attachment of biotin onto biotin-dependent enzymes. Due to the pivotal role of biotin-dependent enzymes in important metabolic pathways in bacteria, BPL has been proposed as a novel antibiotic target. Bacterial drug targets that have a closely related human homologue such as BPL represent a new frontier in antibiotic discovery. However, to avoid potential toxicity to the host these inhibitors must have very high selectivity for the bacterial enzyme. A triazole-based class of compounds has recently been shown to inhibit *Staphylococcus aureus* BPL (*Sa*BPL) but not the human enzyme. A number of BPL crystal structures have been published but the structure of human BPL, which would be useful in understanding the molecular explanation for selectivity, is yet to be reported. Although the amino acid residues in the biotin-binding site are highly conserved, the nucleotide pocket shows a high degree of variability that can be exploited to create selective compounds towards BPLs from pathogens. Here, we converted the seven variable amino acids in the nucleotide binding site of *Sa*BPL to the corresponding equivalents in human BPL. The resulting "humanized" enzyme has similar kinetic and inhibitory properties to human BPL but importantly can be purified at >20-fold greater yield. Crystal trials of this chimeric protein have commenced as this structure may help us design species selective compounds in future drug discovery efforts.

P14-053
The development of inflammation and its impact on brain indoleamine 2,3-dioxygenase activity under conditions of obesity

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Obesity is presently viewed not only as a metabolic disorder but also as an inflammatory disease. However, the relationship between inflammation and metabolic changes in the mechanisms of obesity development remains unknown. The possible link, that connects these two processes might be the activation of brain tryptophan catabolism by kynurenine pathway, which is regulated by cytokine-sensitive, speed-limiting enzyme indoleamine 2,3-dioxygenase (IDO). Therefore the aim of this work was to determine the development of inflammation and its possible impact on IDO activity under conditions of obesity.

The study was performed on white nonlinear rats, which were randomly divided into two groups. Animals of the first group ("NC") have been fed with a standard chow for 10 weeks. Obesity in the second group of animals ("HCD") was caused by the consumption of high-carbohydrate diet. Serum content of proinflammatory (IFN-γ, IL-1β, IL-12) and anti-inflammatory (IL-4, IL-10) cytokines was determined by ELISA using standard kits. Brain IDO activity was measured was measured spectrophotometrically using standard protocol.

Studies have shown an increase in proinflammatory cytokines production by 1.3 times (IFN-γ) and 1.4 times (IL-1β, IL-12), and a decrease of anti-inflammatory cytokines production by 1.3 times in HCD group compared to the NC group. Also studies

have shown an increased brain IDO activity in HCD group by two times compared to the NC group.

Thus, we have shown that the development of obesity is accompanied by inflammation and activation of brain kynurenine pathway, which can lead to alteration of tryptophan metabolism and impairment in monoamine production.

P14-054
The early effect of coronary surgery on serum Nt-Pro Bnp levels

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Introduction: The estimated population prevalence of heart failure in the developed world is 1–2%. In the United States, an estimated 5.1 million adults are living with heart failure and at least one-half have heart failure with reduced ejection fraction (HFREF). NT-proBNP can be seen as quantitative markers of HF that summarise the extent of systolic and diastolic left ventricular dysfunction. In general, levels of NT-proBNP are directly related to the severity of HF symptoms and to the severity of the cardiac abnormality. Among all investigated neurohormones and natriuretic peptides, BNP and NT-proBNP are the best markers for ruling out left ventricular dysfunction. The aim of this study was to determine the effect of cardiac surgery on serum NT-proBNP levels.
Methods: Twenty-eight male and 11 female coronary artery patients from cardiac surgery clinic of Selcuk University Faculty of Medicine were enrolled to this study. NT-proBNP was analyzed by Simens IMMULITE 2000 XPI Immunoassay System (US)
Results: The mean Nt-pro BNP values were 759 (20–5594) and 1796 (216–9945) pg/ml for pre-op and post-op patients, respectively ($p < 0.001$).
Discussion: After early onset of cardiac surgery, there may not be a improvement in cardiac contraction and shear stress. It may be useful to establish Nt-proBNP values in late periods to establish the real effects of surgery.

P14-055
The regulatory mechanisms of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, fluvastatin and lovastatin, for the induction of p21 expression in HeLa cells

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p21 is a cyclin-dependent kinase inhibitor that acts a tumor suppressor or oncogene in response to a variety of intracellular and extracellular stress signals. Various signaling molecules regulate p21 expression at the transcriptional and post-translational levels. p21 gene is known to be regulated via the p53-dependnet or/and the p53-independnt manner. However, the detailed regulatory mechanisms of p21 gene expression via potential anti-tumor agents, such as statins, still remain to be investigated. Up to now, their possibility of statins as an anticancer reagent is still controversial. Here, we demonstrated that statins, fluvastatin and lovastatin, worked as general histone deacetylase inhibitor to induce p21 expression via the p53-independent manner and both combinations further selectively cooperated pathways, such as apoptosis, DNA damage, cell cycle progression and autophagy, involved into the p21 functions in HeLa cells. Another drug repositioning of cardiovascular medication, digoxin, was combined with fluvastatin and lovastatin and further suggested that the p21 induction mediated through the p53-dependent manner, as well as p53-independent manner. Digoxin modulated the effects of statins on p53, p21, cyclin D1 and ATF3 expressions, whereas fluvastatin enhanced its DNA damage effect and lovastatin interfered its DNA damage effect. Our subcellular localization data of fluvastatin and lovastatin combined with digoxin further supported the localization specificity of their cross-talk interactions. In summary, this work will not only provide the regulatory mechanisms of p21 induction and the rethinking of widely used cardiovascular medications for clinical applications and drug repositioning.

P14-056
Different graphene oxide flakes dimensions impact on whole gene expression and molecular interactions in immune cells

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Graphene oxide (GO) is gaining the interest of the scientific community for its revolutionary future biomedical applications. [Geim AK *et al. Nature Materials* 2007]. In this context, the possible immune cells impact of GO is a fundamental area of study for a translational application in medicine [Orecchioni M. *et al. JTM* 2014, Pescatori M *et al. Biomaterials* 2013]. We focused on the molecular effects, on human lymphomonocytes (PBMCs), of two types of GOs, deeply characterized, which differed in lateral size dimension (GO-Small and GO-Large). To clarify the molecular impact on immune cells of GOs we provided a wide range of assays looking at cells viability, cell activation, cytokines release and genome expression. We let in lights also the action of GOs on immune response-related 84 genes. A whole genome analysis was conducted on T cells and monocytes to deeply evaluate the GO-cell molecular interactions. GOs didn't impact the cell viability. We identified 37 upregulated genes in the GO-Small samples compared to 8 genes for GO-Large, evidencing a clear lateral dimension-dependent impact on cell activation. The size-related effect at the protein level was confirmed by multiplex ELISA. Results were supported also by microarray analysis. Data evidenced the GO-Small-induced downregulation of oxidative phosphorylation followed by a glycolitic switch-on in both cell types giving future perspectives for anticancer nano-graphene systems. This work represents a comprehensive characterization of different-sized GOs' impact on immune cells giving crucial information of the molecular effect of graphene to improove its chemical and physical design for biomedical applications.

P14-057
5-Aminouracil derivatives downregulate human adenovirus replication

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Human adenoviruses (HAdVs) are non-enveloped DNA viruses causing various infections; their pathogenicity varies dependent on virus species and type. Currently there is no causal therapy, which is effective to counteract diseases caused by HAdVs. Several 5-substituted pyrimidine nucleosides (1-benzyl-5-(arylamino) uracil derivatives) have potent antiviral activities against HIV-1 and EBV. The purpose of our study was to synthesize new 5-aminouracil derivatives and screen them for anti-adenoviral activity. The synthesized compounds (Z380, Z383, Z384, and Z446) were dissolved in dimethyl sulfoxide (DMSO) in 100 mM stock solutions. All compounds were non-toxic to H1299 cells at concentrations of 5, 25, and 100 μM after 24 h of incubation, according to MTT test. Less than 10% of apoptotic cells were detected by apoptosis assessment using double staining with Annexin V-FITC and propidium iodide, followed by flow cytometry analysis. H1299 cells were infected with HAdV-5 at an MOI of 1 FFU/cell. The 5-aminouracil derivatives at concentrations of 0.5, 2.5, 5, 10, 15 and 25 μM were added to H1299 cells 3 h post infection. 24 h later, newly synthesized viral genomes were detected via qPCR. Notably, we observed a strong reduction of HAdV-5 genome replication in adenovirus-infected H1299 cells treated with Z380 (IC50 0.3 μM) and Z383 (IC50 2.8 μM) as compared to H1299 cells treated with DMSO alone. As positive control we used H1299 cells treated with 2-{[2-(benzoylamino)benzoyl] amino}-benzoic acid (IC50 6.9 μM). We have shown that 5-aminouracil derivatives are potent non-nucleoside inhibitors of adenoviral replication and hold promise to be developed into efficient anti-adenoviral therapy.

P14-058
Kinetic investigations on small molecules, inhibitors of soybean lipoxygenase with potential activity on cellular functions in different diseases

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Lipoxygenase (LOX) dioxygenates lipids with cis,cis-pentadiene parts. LOX takes part of metabolic pathway of cells and their activity disturbance leads to many human diseases. Recently, there are many scientific efforts for modeling different pharmacological agents, which specifically influence LOX pathways in order to restore their function and to successfully treat different illnesses. It is also essential to obtain small molecular weight agents, which does not affect other normal physiological functions. The highest level of sequence identity between plant and mammalian LOX is in the area of the catalytic domain, containing the non-heme iron atom. Taking into account this fact, soybean LOX is a good alternative for model design of such kind of small molecules able to influence cellular function, interacting as inhibitors or activators of LOX metabolic pathways. According to Steele E. *et al*., the presence of primary amino or hydroxyl function is a sign for potential inhibition activity against LOX. Herein, we report our investigation on the kinetic parameters (Ki, Vmax) and type of inhibition of soybean LOX in presence of two natural inhibitors (ribavirin, galantamine) and one synthetic analogue of ribavirin (2,3,5-triacetyl-1-β-ribofuranosyl-thiourea) in presence of linolenic acid as a substrate. We defined that the type of inhibition for natural inhibitors is noncompetitive with prerogatives of mixed type of inhibition. The synthetic analogue of ribavirin demonstrates mixed type of inhibition.

Key words: soybean lipoxygenase, disease, inhibition, ribavirin, galantamine.

P14-059
A new affinity method for purification of bovine testicular hyaluronidase enzyme and an investigation of the effects of some compounds on this enzyme

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In this study, a new affinity gel for the purification of bovine testicular hyaluronidase (BTH) was synthesized. L-Tyrosine was added as the extension arm to the Sepharose-4B activated with cyanogen bromide. m-Anisidine is a specific inhibitor of BTH enzyme. m-Anisidine was clamped to the newly formed Sepharose-4B-L-tyrosine as a ligand. As a result, an affinity gel having the chemical structure of Sepharose-4B-L-tyrosine-m-anisidine was obtained. BTH purified by ammonium sulfate precipitation and affinity chromatography was obtained with a 16.95% yield and 881.78 degree of purity. The kinetic constants K_M and V_{Max} for BTH were determined by using hyaluronic acid as a substrate. K_M and V_{Max} values obtained from the Lineweaver-Burk graph were found to be 2.23 mM and 19.85 U/ml, respectively. *In vitro* effects of some chemicals were determined on purified BTH enzyme. Some chemically active ingredients were 1,1-dimethyl piperidinium chloride, β-naphthoxyacetic acid and gibberellic acid. Gibberellic acid showed the best inhibition effect on BTH.

P14-060
The effects of acute malathion exposure on renal oxidant & antioxidant balance in rats

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Malathion, which is a member of organophosphate chemical family, is used to control pests and therefore it is helpful in agriculture and public health practices. On the negative side, many evidences suggest that malathion has hazardous effects to human health such as disrupting the antioxidant defence systems. In this study, 3 groups were designed to evaluate the acute effects of two different doses of malathion in rat kidney tissues. Group 1, the control group, was given plain corn oil. Malathion was given to Group 2 (100 mg/kg) and Group 3 (200 mg/kg) in corn oil via oral gavage. The rats were sacrificed 24 h after administration of the chemical. In an attempt to evaluate the oxidative injury, malondialdehyde (MDA), advanced oxidation protein products (AOPP), superoxide dismutase (SOD) and catalase activity levels

were determined in renal tissues of rats. All of the parameters were measured spectrophotometrically. A statistically significant increase of MDA levels and SOD enzyme activity was observed in Group 3, compared to Group 1. There was also a statistically insignificant increase in MDA and SOD activity in Group 2 with respect to the control. There was no significant change in either AOPP levels or Catalase activities among any of the groups.

P14-061
Hydrolytic enzymes marine organisms as an instrument for investigating protein–protein interaction

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Protein molecules with hydrolytic activity have one of a major role in the signaling mechanisms in the body. This property of these molecules is realized through the formation active protein molecules and the advent of the body fragments this protein molecules. The fragments can be a variety of mechanisms launchers molecules functioning as normal and in various pathology of the organism. The search for alternative protein with similar activity is essential for a better understanding of the processes degradation and to create novel pharmaceuticals. The purpose of this study was to analyze the presence of protein molecules in the tissues of marine organisms of Antarctic region with hydrolytic activity. The objects of study were typical representatives of this region. We have developed a 2-step extraction method allowing keeping the hydrolytic activity of the test samples. Analysis of the extracts revealed in them amidolytic, esterase, amylolytic and caseinolytic activity. The study of these samples by zymography method showed presence hydrolytic enzymes with different molecular weights. Using inhibitors revealed the presence of enzymes with different structure of the active center. These results suggest that hydrolytic enzymes in the analyzing samples have different taxonomy. A deeper study of these protein molecules must necessarily include cleaning them until smooth. These protein molecules may be useful for the further study of various cascade processes, in which they have an effect, and to provide new pharmacological agents.

P14-062
The effect of low concentrations of some biologically active agents on the aerobic respiration of lymphocytes *in vitro*

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The aim of our work was the study of the exposure of ultralow concentrations of selected biologically active agents (SBAA) on the activity of enzymes of energy metabolism of succinate dehydrogenase (SDH), glycerol-3-phosphate dehydrogenase (GPDH). To the lymphocytes culture was added the physiological solution (untreated control). To the treated control was added the solution of aerobic respiration inhibitor – NaN_3. The experimental culture of lymphocytes (ECL) contained of SBAA and the NaN_3 solution.The studies have revealed the increased activity of SDH in ECL relative to the treated control, 2.6–1.3 times after 4, 24, 48, 72 h of cultivation. The SDH activity in ECL after 4 and 24 h after starting of the experiment did not differ significantly from the SDH activity in the untreated control cells. The latter shows that the action of NaN_3 in ECL on the initial stages of the experiment (4, 24 h) was completely neutralized by ultralow influence of SBAA. However, after 48, 72 h of incubation, we have found a certain reduction of the SDH activity in ECL in reference to untreated control, which is evidence of partial neutralization of inhibitory NaN_3 action. Activity of the core enzyme of glycerophosphate shuttle – GPDH in ECL was certain lower relative to the treated control of the whole experiment. In these conditions the GPDH activity in ECL was at the untreated control level. Thus, we have found that ultralow concentrations of SBAA enable neutralization of NaN_3 inhibitory effect on the process of lymphocytes respiration.

P14-063
Some indicators of oxidative metabolism of neutrophils of patients with community-acquired pneumonia

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It is considered, that oxidative stress plays a leading role in the pathogenesis of pneumonia. However, the results of the study of oxidative metabolism of neutrophils in pneumonia are not enough, although neutrophils affect almost all the basic mechanisms of the inflammatory response and induce oxidative stress. The aim of our study: to determine the content of advanced oxidation protein products (AOPP), carbonyl derivatives and the activity of adenosine deaminase (ADA) of neutrophils of patients with community-acquired pneumonia. Object of study: blood neutrophils of 25 patients with community-acquired pneumonia, who were accepted to hospital for treatment, and 14 healthy donors. The content of AOPP in the lysate of neutrophils was determined by the method Witko-Sarsat *et al*. (1996). The content of carbonyl derivatives of proteins was determined by the method R.L Levine *et al*. (1990) and was registered spectrophotometrically. Activity of ADA was determined by the method I.B Nemecek *et al*. (1993). Mann-Whitney test was used for statistical analysis. The level of carbonyl derivatives and the activity of ADA in blood neutrophils of patients with community-acquired pneumonia were significantly lower than control ($p < 0.02$). No significant differences were found between research groups in content AOPP; however, this indicator in patients with pneumonia was 25% lower than in control group. Such character of metabolic disorders of neutrophils, in our opinion, can determine their lack of effectiveness in the progression of pneumonia.

P14-064
Flavonostilbens from *Vexibia alopecuroides* (L.) Jakovl with antimicrobial and proliferative properties

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Diabetic foot infection is one of the most frequent and complex problems among patients suffering from diabetes. This study was designed to evaluate the antibacterial and proliferative activities of the flavonostilbens obtained from *Vexibia alopecuroides* (L.).

The methylene chloride crude extract showed good antimicrobial activity, and high fibroblast proliferation. Therefore, the methylene chloride crude extract was subjected to bioassay-guided fractionation. Column eluate was collected on the basis of TLC similarities and recombined into 10 fractions. Antimicrobial

activity of crude extract from *Vexibia alopecuroides* (L.) and extract fractions were evaluated using NCCLS modified version of broth micro-dilution assays. Granulation formation was made using fibroblast proliferation assay. The antibacterial activity of the samples was more effective inhibiting the growth of Gram-positive bacteria than Gram-negative bacteria. One fraction (H) showed the highest antibacterial activity against both methicillin-resistant *S. aureus* and *S. aureus* with IC50 value < 0.8 μg/ml compared to the IC_{50} values of 0.1 μg/ml of standard drug ciprofloxacin. Fractions showing antibacterial activity were purified; the structures of compounds were elucidated by high-resolution LC/MS analysis and ^{1}H and ^{13}C NMR spectra, which were in agreement with the literature data of alopecurone A, B, C, D, F. Also these compounds significantly stimulate the proliferation of fibroblast cells. The present study demonstrated promising results which represents an important step in searching and developing new antibacterial and regenerative agents against diabetic foot infections.

P14-065
Uptake of polymeric nanoparticles by different cell types from oral epithelium

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Over the last years nanoparticle interaction with various organs and tissues has developed into an interdisciplinary research field of great interest. Polymeric nanoparticles offer several distinct advantages such as biocompatibility, biodegradability and reduced side effects. While the oral route represents an important route of delivery, to date, very limited data is available on the interaction between polymeric nanoparticles and oral mucosa. The specific aim of the present work is to focus on the uptake of poly(lactic-co-glycolic acid) nanoparticles by normal, transit amplifying and oral keratinocyte stem cells. We show that depending on cell type and chemical nanoparticle structure the cellular uptake, proliferation and oxidative stress induction have significant differences. Thus a maximum cell uptake ($30.2 \pm 4.3\%$ vs $15.4\% \pm 1.3\%$ versus $10.4 \pm 3.2\%$) could be observed in stem versus transit amplifying versus normal keratinocyte population after 24 h of incubation with 5 μg/ml of chitosan nanoparticles. Higher concentrations or prolonged incubation times revealed an increase in oxidative stress markers in all cell types. The present work shows that chitosan based nanoparticles are more suitable for delivery through the oral mucosa and more specifically through the oral stem compartment.

P14-066
Probing biocatalytic phosphorylations with small molecules

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The analysis of the biocatalytic functions of phosphorylating enzymes like phosphotransferases/kinases with small molecule acceptors using suitable phosphoryl donor like ATP, pyrophosphates or polyphosphates has been a key theme in biochemistry from its beginnings. Although the stereochemistry of many important biochemical phosphorylations has been investigated in detail and phosphorylating enzymes have been shown to catalyze the phosphorylation of many small molecules with high enantioselectivity, many phosphorylating enzymes can phosphorylate both enantiomers. New methods for directly analysing the enantiomers of phosphorylated small molecules have been an important prerequisite for finding highly enantioselective kinases. Enzymes hydrolyzing phosphate esters lack enantioselectivity if used in the reverse reaction. The results of probing cellular phosphorylations are not only of interest for cellular functions and signalling pathways, but also for transitioning to biocatalytic asymmetric phosphorylation reactions in synthesis. Therefore the future of probing biocatalytic asymmetric phosphorylations of small molecules in cellular functions and synthesis looks bright.

P14-067
Conventional inflamation and oxidative stress markers of the nonalcoholic fatty liver disease diagnosed patients

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Nonalcoholic Fatty Liver Disease (NAFLD) is a condition which causes increased insulin resistance at peripheral tissues, and secondly causes fatty liver with oxidative stress. In this study, NAFLD patients are grouped according to their ALT levels. The aim of this study is to compare the levels of serum MDA and AOPP -which are oxidative stress markers that have a role at the pathogenesis and the progression of the disease- with serum TNF-α, IL-6, and HA levels -which are inflamation markers. A total of 133 non-obese and non-diabetic individuals are included in this study who applied clinics with dyspeptic symptom or for routine control. Groups are categorized according to their serum ALT levels and ultrasonography findings. The groups are formed as follows: Nonalcoholic fatty liver disease diagnosed patients with normal ALT levels as Group 1 (n = 53), nonalcoholic fatty liver disease diagnosed patients with high ALT levels as Group 2 (n = 35) and the control group which includes individuals without any known systematic disease (n = 45). HA, TNF-α, and IL-6 serum levels of all patients are studied by using ELISA method; AOPP and MDA levels are studied by using spectrophotometric methods. Results indicate that serum AOPP levels are not statistically significantly different between groups ($p > 0.05$). On the other hand MDA and HA levels increased significantly depending on high ALT levels while TNF-α, IL-6 serum levels increased significantly independent of ALT levels ($p < 0.05$).

Keywords: Nonalcoholic Fatty Liver Disease, ALT, Oxidative Stress, MDA, AOPP, Inflamation, IL-6, TNF- α, HA.

P14-069
Reduced melanogenesis by si-RNA of P-protein in Melan-A cells

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Hyperpigmentation diseases, especially melasma and lentigines, are major psychological diseases in most of the societies. The obsession for the clean, fair and healthy skin is one of the basic instincts of every individual. For such diseases, a number of melanogenesis inhibitors have been screened. They were effective but their side effects cause many complications. Pink eye dilution

protein (P-protein) is a structural protein in the melanosome that plays a critical role in cellular melanogene sis. The aim of the present study was to investigate the effect of P-Protein inhibition, by using P-protein small interfering RNA (siRNA), on melanogenesis in Melan-a melanocyte. For this purpose, si-RNA for p Protein was introduced into Melan-A cells. Melanin content, cell viability, PCR and western blot for tyrosinase were performed. In this research, it has been observed the both Pprotein and mRNA level were significantly lowered by the siRNA treatment. siRNA of P-Protein also suppressed melanin synthesis without any cytotoxicity in the melan-a melanocyte cells. These results suggest that molecular approaches using siRNA targeting P-protein may provide a novel approach for the control of the melanogenesis.

P14-070
The photoactivated fluorescent dye for probing cellular organelles and lipid monolayers

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One of the most promising objects on the "edges" of modern chemical biology, biochemistry and biotechnology fields is a novel type of the photoactivated fluorescent dyes (PFD). These dyes can penetrate in cells and provide ultra-high optical resolution in biological microscopy and fluorescent nanoscopy. The aim of this work was to study the spectral and interfacial properties of PFD (synthesized by Dr. V.N.Belov and coworkers in MPI-BPC, Göttingen, FRG) and to investigate cell staining by PFD using laser confocal microscopy. The interactions of PFD with the lipid biomembrane component – dimyristoylphosphatidylethanolamine (DMPE) in monolayers at the air-water interface and after transfer to glass plates were studied before and after PFD photoactivation. The PFD forms stable monolayers with collapse pressure of about 30 mN/m. The PDF absorption band with maximum at 560 nm is clearly observed by photoactivation (5–15 min. of exposure). These spectroscopic investigations of PDF813 demonstrate that the precursors of fluorescent dyes can indeed be photoactivated to the fluorescent dye in an environment similar to biomembranes. Microphotographs and fluorescence spectrum were obtained using PFD for staining A431 cells. The dyes are selective for subcellular organelle staining and show that the dye mostly distributes to mitochondria. The differences in brightness of different subcellular organelles in living cells, as well as the unique spectral and interfacial properties of PFD813, are important for various applications in biomedicine and nanobiophotonics. This work was supported by the Russian Scientific Foundation (grant 14-16-00046).

P14-071
The curative effects of exendin-4 on renal oxidative damage, inflammation and fibrosis in diabetic mice

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Diabetic nephropathy is the consequence of a process including formation of reactive oxygen species (ROS), inflammation and fibrosis. Although the effects of exendin-4, is an analog of glucagon like peptide-1 (GLP-1), on diabetic nephrophaty is known, there is limited data about its molecular mechanisms of action. Hence, we aimed to show the effects of exendin-4 on oxidative damage, inflammation and fibrosis. We set an experiment with four different animal groups: solvent injected, exendin-4 injected, diabetic, and exendin-4 injected diabetic BALB/c mice. Exendin-4 (3 μg/kg, daily) was injected for 30 days after the mice were rendered diabetic by a single dose streptozotocin (200 mg/kg). Renal malondialdehyde (MDA) level was measured by colorimetrically; the level of tumor necrosis factor- α (TNF-α) was assessed by ELISA; CD68, intercellular adhesion molecule-1 (ICAM-1), monocyte chemotactic protein-1 (MCP-1), tumor growth factor-β1 (TGF-β1) and fibronectin levels were evaluated by western blotting, and CD68, ICAM-1, MCP-1 localizations were also shown by immunofluorescent technique. As the results exendin-4 attenuated the level of MDA, proinflammatory cytokine TNF-α, chemokine MCP-1, ICAM-1 and fibrosis related molecules TGF-β1 and fibronectin. In addition, we demonstrated the tubular cells as the main source of MCP-1 and ICAM-1. All of these data show us that exendin-4 has a curative effect on renal tubule centered injury including oxidative damage, inflammation and fibrosis in diabetic nephropathy.

P14-072
A novel streptococci-conserved β-lactamase involved in ampicillin resistance of *Streptococcus pneumoniae*

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Streptococcus pneumoniae, an ampicillin-resistant bacterium, is recognized as a major cause of pneumonia, which globally affects about 450 million people and results in 4 million deaths every year. At present, the mechanism of ampicillin resistance of this pathogenic microbe is controversial. To unveil the mechanism of this resistance of *S. pneumoniae* is an important issue to treat streptococcal disease that might consequently save millions of lives around the world. In this work, we isolated a streptococci-conserved hypothetical protein, SMU290. *In vitro*, SMU290 reveals a β-lactamase activity, which is able to deactivate a penicillin-based antibiotic by hydrolyzing the amide bond of the β-lactam ring. The Michaelis parameter (Km) = 2.5 μM and turnover number (kcat) = 2/s were obtained when nitrocefin was utilized as an optically measurable substrate. SMU290-overexpressed E.coli exhibits the ampicillin-resistant ability. Confocal images of the SMU290-GFP distribution exhibit that SMU290 is a protein associated with the cellular membrane. With western blot analyses and a SMU290-specific antibody, we confirm that the gene SMU290 is active in S. pneumoniae but silent in S. mutans. We propose that SMU290 is a membrane-associated β-lactamase involved in the antibiotic-resistant property of *S. pneumoniae*.

P14-073
Modern bioanalytical techniques for determination of pesticides as multienzyme system inhibitors

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Pesticides are group of compounds with low molecular weight, that can affect the cellular functions. In recent years, they are

widely used and consequently, their residues may get into animal tissues, milk, honey, eggs, etc. They inhibit cholinesterase enzymes, allowing accumulation of acetylcholine. Therefore, food safety is an integral part of the EU policy for protection of consumer's health and maximum residue levels for pesticides are defined in specific Regulations. Pesticides can be grouped into chemical families. Prominent insecticide families include organochlorines, organophosphates, and carbamates. Herein, we report the parameters of two different methods for extraction of N-methyl carbamates and organochlorine pesticides, in various foods. The pesticides were analyzed by chromatographic methods developed in accordance with the regulations in this area. Modern gas-chromatography and liquid chromatography with post column derivatization and fluorescence detector were used during this study. Analytical parameters of methods and tools will be discussed and they were compared with data from experiments conducted on the potential of biosensors for pesticides analysis. Optical biosensor with multi-enzyme system was used as comparative technique. It has many advantages as short response time, low cost, easy maintenance, and in addition providing some absolutely acceptable parameters as sensitivity, limit of detection, linear range, and reproducibility.

P14-074
The effect of malathion on oxidant & antioxidant status in rat brain tissue

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Malathion, which is an organophosphorus compound, is a widely used pesticide all over the world. However it is also known to be highly toxic to the organism. We aimed to analyse the harmful effects of acute malathion exposure on brain tissues of rats in terms of oxidative stress. To test this we set 3 groups to administer a single dose of malathion dissolved in corn oil via oral gavage at the doses of 100 mg/kg (Group 2), 200 mg/kg (Group 3). Only plain corn oil was given to Control group (Group 1). The rats were sacrificed after 24 h following administration. In order to assess the dose dependent oxidative damage we studied lipid peroxidation product malondialdehyde (MDA), superoxide dismutase (SOD), catalase and advanced oxidation protein products (AOPP). All of the parameters were measured spectrophotometrically. We found a significant increase of MDA in Group 3, compared to control group. We have also found a significant difference in SOD levels of Group 2 compared to both Control group and Group 3. There was no difference between catalase activities. AOPP levels of Group 2 were diminished compared to control group. There was also an increase in AOPP levels of Group 3 with respect to Group 2; however this did not reach to a statistical significance.

P14-076
Malathion-induced oxidative stress in rat liver

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Organophosphates are great of importance in agriculture as food production enhancers as they are well known insecticide. Malathion is one of the most popular organophosphate compounds. Despite its benefits, malathion has detrimental effects like oxidative stress on many tissues including liver. Our study was designed to evaluate the acute effects of malathion on oxidant and antioxidant status of rat liver tissues. For this purpose 3 groups were formed. Rats in Group 1 served as control group animals which were only given corn oil. Group 2 and Group 3 were administered 100 mg/kg and 200 mg/kg malathion, respectively, dissolved in corn oil by oral gavage. The rats were sacrificed after 24 h following administration. We measured malondialdehyde (MDA), superoxide dismutase (SOD), catalase and advanced oxidation protein products (AOPP) in rat liver tissue homogenates. We observed a statistically significant increase in catalase activity in Group 3 compared to Group 1. AOPP levels of Group 3 were significantly higher than Group 2. While we could not find any significant change in MDA and SOD activity levels between groups.

P14-077
The destruction of amido-containing biomolecules exposed to UV radiation

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UV radiation is an established human carcinogenic, pro-aging and pro-cataract factor. So studies of the cellular and molecular effects of UV exposure to biological and also model systems are of great importance. DNA is the most critical target for damage caused by UV radiation, but it also applies to other significant biomolecules [1, 2]. In our work we've concentrated on such amido-containing biomolecules as peptides containing serine (Ser) and threonine (Thr) residues as well as N-acetylglucosamine (GlcNAc). The first ones are building blocks of proteins, being also essential parts of active sites of many important enzymes and receptors, the second is the monomeric unit of mucopolysaccharides. We have performed the photolysis of aqueous solutions of investigated compounds. We've shown earlier that amino sugars and peptides with ethanolamine moiety in aqueous solutions undergo •OH-induced carbon skeleton destruction *via* nitrogen-centered radicals formation and their further fragmentation [3]. It has been established in the present study that in the case of compounds with N-acetylethanolamine moiety (GlcNAc, Val-Thr, Thr-Thr, Ala-Ser) UV irradiation of their aqueous solutions leads to starting molecules C-C bond destruction products formation. It is possible because nitrogen-centered radicals can be generated due to Norrish Type I decomposition [4] of excited starting molecules.

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P14-078
Cell toxicity of water-soluble [C60] fullerene derivatives

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[C60] fullerenes have antioxidant properties, furthermore, they are under studies as potential anticancer drugs and nanovectors

for the drug delivery across biological barriers. In micromolar concentrations, the fullerene derivatives have anti-viral activity. But the toxicity of [C60] is still controversially discussed. In order to assess the toxicity of 3 fullerene derivatives, a standard MTT-assay on HELFs was performed. The derivative with N-metylpiperazine substituents turned out to be the most toxic of all the three: in a 50 nM concentration it induced cell death. For derivatives with 3-phenylpropionil and phosphonate substituents those concentrations were 50 mcM and 1 mM, respectively. The investigated 3-phenylpropionyl derivative is cytotoxic in a concentration exceeding 25 mcM: it decreases PCNA and γH2AX expression as well as the activity of NF-kB and p53, whereas the level of ROS increases resulting in necrotic cell death. 0.1 to 20 mcM concentrations caused a significant increase in the amount of both SSB and DSB, which was shown by means of flow cytometry and comet assay. NOX4, VCAM and RHOA expression rises and SOD1 expression decreases. A 4 nM concentration stimulated proliferative activity of HELFs. As a result, the level of phosphorylated form of p53 also increases and the Ki-67 level deflates. The amount of NF-kB (mRNA and protein) increases, moreover, NF-kB translocates to the nucleus. Thus, the investigated derivative causes oxidative stress and double-strand breaks resulting in an arrest of the cell cycle in a concentration range of 0.1 to 20 mcM. Higher concentrations cause necrotic cell death.

P14-079
Identification of the binding pocket of different hY_2R selective antagonists

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The human Y_2-receptor (hY_2R) is one out of four human neuropeptide Y (NPY) receptor subtypes named hY_1R, hY_2R, hY_4R, hY_5R. The hY_2R consists of 381 amino acids and binds three different ligands with different affinities: neuropeptide Y, peptide YY (PYY) and pancreatic polypeptide (PP). It is involved in angiogenesis, appetite regulation, bone formation, and the regulation of the circadian rhythm. In various tumor tissues the hY_2R is overexpressed and promotes tumor growth and vascularization. Therefore, the hY_2R has great therapeutic potential and antagonists could represent promising drugs for the treatment of neuroblastoma or glioblastoma. So far, several different non-peptidic antagonist of the hY_2R have been reported but very little is known about their binding sites at the receptor. In this study the interaction of BIIE0246, compound 40 and compound 46 at the hY_2R are characterized in more detail. Therefore receptor mutants were generated and the proteins were tested for signal transduction by inositol phosphate accumulation assay in presence of pNPY and pNPY/antagonist. All receptor variants were localized in the cell membrane comparable to the wild type hY_2R as demonstrated by fluorescence microscopy. Preliminary results showed that the replacement of residues in transmembrane helix 2 and 7 reduces the activity of pNPY and all antagonists. Variation of $Q^{6.55}$ however, reduced antagonistic activity for BIIE0246, compound 40 and compound 46. Our data suggest specific binding modes for hY_2R agonists and antagonists, which opens up the possibility for the development of selective drugs.

P14-080
Study of the expression of catalytic antibodies influenced by murine B cell repertoire: implication in autoimmune disease

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There is increasing evidence of the involvement of catalytic autoactive B cells in autoimmune disease, yet the origin and the role of these self-produced catalytic antibodies are not well understood [1]. With today′s phage display technology, we are able to recreate and study model immune repertoires, closely approximating their natural states. Here, we report the construction of four scFv libraries from healthy or experimental autoimmune encephalomyelitis murine models, each being either naive or immunized. Exploiting an optimized cloning method [2] and a novel "bar-coding" procedure to provide four distinct but easily identifiable host vectors, we are able to pool the libraries into a single repertoire of size 2.4×10^9, among the largest reported in the literature. A comparative study of the distribution of immunoglobulin gene subgroups between the four libraries has revealed potential shifts in the B cell repertoire originating from differences in genetic background and immunological status of mice [3]. We use this pool of libraries to select for catalytic antibodies against a reactive target. We then study the influence of the B cell repertoire nature in the expression of these abzymes, their genetic characteristics and physicochemical properties. Our results will further elucidate the origin of catalytic antibodies and the unique characteristics of B cell repertoires that provide a framework for the expression of these abzymes.

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P14-081
Synthesis, DNA binding study and biological activity of novel first row transition metals complexes

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The search for new pharmacologically active drugs has led to the discovery of many small molecules that bind to DNA [1]. Toward this goal, in this work a new Mn(II), Fe(III), Co(II), Ni (II), Cu(II) and Zn(II) complexes based on chloro-quinoline ligand were synthesized and characterized by UV, IR and X-ray analysis. Moreover, the DNA-binding properties of metal complexes were investigated by electronic absorption, fluorescence, and CD spectra. The observed trend in hypochromism of absorption bands, reflects strong DNA-binding properties of drugs. Additionally, competitive binding studies with EB have revealed through the quenching of DNA-EB fluorescence the ability of the complexes to displace EB from the EB-DNA system suggesting intercalation as a possible mode of their interaction with DNA. All tested compounds exhibit good binding propensity to CT-DNA, the maximum value of binding constant was established for Ni(II) complex ($K_{SV} = 5.69 \times 10^4$ per M). Although all of the

complexes targeted topoisomerase I and can be qualified as mildly effective topoisomerase inhibitors. The cytostatic effect of all complexes against A549, HCT-116 and MDA-MB cells was investigated. It was found, that Fe(III) complex was significantly more cytotoxic against MDA-MB cells then cisplatin at all tested concentrations.

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P14-082
Alkoxyresorufin O-dealkylase activities in rats treated with 7,12-dimethylbenz[a]anthracene and endosulfan

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Living organisms are exposed to a variety of toxic chemicals together in daily life. 7,12-Dimethylbenz[a]anthracene (DMBA) is a lipid soluble toxic molecule generated during combustion of organic materials. Endosulfan is an organochlorine pesticide that is used in the agricultural areas to control insects. Humans are exposed to both of these chemicals with smoking cigarette. The aim of this study is to determine the effects of co-administration of DMBA and endosulfan on alkoxyresorufin O-dealkylase activities in rat liver. For this purpose, 28 male Wistar rats (weighing 170-255 g) were randomly selected and divided into four groups. The rats in DMBA treated groups were gavaged with 30.0 mg/kg body weight (b.wt). DMBA three times during the administration period. The rats in endosulfan treated groups were gavaged with 5.0 mg/kg b.wt. three times in a week. Rats were killed by cervical dislocation on the 54th day of the administration period. Microsomes were prepared for each liver tissue and histopatological studies were also performed. 7-ethoxyresorufin O-deethylase (EROD) activities of control, endosulfan, DMBA and DMBA+endosulfan were 71 ± 7, 121 ± 5, 126 ± 6 and 151 ± 15 pmol/min/mg protein, respectively. 7-methoxyresorufin O-demethylase (MROD) activities of control, endosulfan, DMBA and DMBA+endosulfan groups were 34.0 ± 0.7, 36.0 ± 4.3, 52.1 ± 1.9 and 68.6 ± 6.3 pmol/min/mg protein, respectively. 7-pentoxyresorufin O-depentylase (PROD) activities of control, endosulfan, DMBA and DMBA+endosulfan groups were 18.0 ± 1.0, 15.3 ± 0.9, 16.9 ± 0.7 and 16.4 ± 1.4 pmol/min/mg protein, respectively. Co-administration of DMBA and endosulfan caused 2 fold increase in EROD and MROD activities. The histopathological studies indicated that co-administration of DMBA and endosulfan increased liver damage in rats.

P14-083
Untangling mitogenic signalling in living cells by information theory

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Mammalian cells harbor complex and highly interlinked signal transduction networks that relay information from the outside of the cell to the inside. Here, we address the question of which mechanisms allow cells to maximize the amount of information gained from external and internal sources to ensure a proper physiological response. As a prototypic example for a mammalian signaling pathway, we analyze the information theoretical properties of the MAP Kinase signaling cascade using a combination of single-cell experiments, computational models and information theoretical analysis. The terminal kinase of the pathway, Erk, has a multitude of targets, of which roughly 100 are nuclear targets. Thus, a valuable readout of pathway activity is the nuclear translocation of Erk, which we monitor on a single cell level using quantitative live cell imaging. To quantify the information flow from ligand concentration to temporal changes in Erk translocation we use measures from the field of information theory, namely mutual information and channel capacity. We observe that Erk translocation time-courses of single cells upon ligand stimulation are heterogeneous and noisy. To define different types of Erk translocation time-courses, we cluster the time-courses of the single cells and relate the distribution of clusters to the distribution of different ligand concentrations. We show that the MAPK cascade behaves like a stochastic binary channel with a capacity around 1 bit. The capacity of this pathway can be enhanced up to 2 fold by inhibiting other pathways and taking into contextual information.

P14-084
Inhibition of amylin fibrilogenesis and protection of Islet β-cells by extracts and fractions of medicinal plants

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Misfolding of some proteins, their self-assembly into insoluble amyloid structures underlies many diseases, constituting a group of amyloid-related pathology. Fibrillation of peptide hormone of pancreas amylin is considered as one of the causes of death of β-cells in type 2 diabetes. In this work, the protection of islet β-cells by ethanol extracts from melilot, rose petals, leaves of grape, blackberry and sorrel, and several isolated constituents, from killing by preaggregated amylin was studied. The inhibition of amylin aggregation by plant preparations was studied using the fluorescence of thioflavin-containing samples (λ_{ex} = 430 nm, λ_{em} = 485 nm). IC_{50} values for several preparations in reducing the amylin aggregation and killing of cells were calculated. The obtained results allowed recommending: a) frequent use by persons at risk group of plants, effectively decreasing the impact of amylin on the islet cells; b) considering these plants, their extracts and constituents as sources for developing antidiabetic agents.

P14-085
Screening for anti-diabetic adjuvants in *balanites aegyptiaca*

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Diabetes mellitus, represents one of the most incident disease in the world, with only symptomatic treatments, for either type I or type II. From their on-set, the disease progresses, leading severe

complications, such as neuropathy, nephropathy, retinopathy, vasculopathy, all of which lead to organ failures. Oral antidiabetics or insulin are usually associated with adjuvant therapies, such as plant extracts and/or synthetic drugs to alleviate the complications. *B. aegyptiaca* extracts are used in Egypt and surrounding countries as a medicinal plant. Extracts and fractions of *B. aegyptica* were exposed to *in vitro* studies in order to identify the antidiabetic fractions, to further assess cytotoxicity and to estimate the action. The assessment evaluated the modulation of secretory cytokines, feature that is often present in various polyphenolic extracts, since inflammatory cytokines are often elevated in diabetic disease. A more disease specific mechanism, involved in the onset of diabetic retinopathy and nephrophathy, involving the enzymes aldose-reductase and sorbitol dehydrogenase were investigated. An *in vitro* cytotoxicity screen was performed using Jurkat cells and total blood (MTS as end-point). Secretory cytokines were determined in total blood cultures by xMAP assay. Further testing was based on inhibition of aldose reductase. The most active primary extract, upon further fractionation, gave the most active fraction, expressing maximal inhibitory activity of 60% at 0.01 microM/ml. Thus, a clear inhibiton of aldose-reductase was proved, that may provide protection against neuropathic disorder and retinopathy.

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P14-086
Membrane-bound carbonyl reducing enzymes as targets of an oracin immobilised affinity carrier

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Approximately 30% of all human proteins are supposed to be associated with biological membranes. Although they heavily interact with currently used drugs (up to 70% of substances), there is still little known about their physiological function and structure. The best described membrane-bound enzymatic system involved in phase I of biotransformation is cytochrome P450 superfamily (CYP). It is believed that 9 of 10 clinically used drugs are substrates for CYPs. Besides CYPs, membrane of endoplasmic reticulum also contains carbonyl reducing enzymes which are playing an important role in the metabolism of the xenobiotic compounds. However, until today there is still only one well characterized membrane-bound carbonyl reducing enzyme participating in the metabolism of xenobiotics; 11-beta-hydroxysteroid dehydrogenase 1 (HSD11B1). Based on the previous research of the reduction stereospecificity of anticancer drug oracin for HSD11B1 and human liver microsomes, additional participating microsomal carbonyl reducing enzymes were targeted for the isolation and identification. The methods based on the molecular recognition are currently the most powerful tool for the separation and isolation due to their prominent selectivity and recovery. Such affinity method was developed in our laboratory. After the initial ion-exchange chromatography separation, the immobilized oracin affinity carrier was implemented into the purification scheme of the carbonyl reducing enzymes from the human liver microsomes. Three proteins, DHRS1, RDH16 and HSD11B1 were successfully isolated and identified. Further characterization of these enzymes could significantly extend our knowledge about the membrane-bound carbonyl reducing enzymes that metabolise the xenobiotic substrates. This project was supported by GAUK no.926213/C/2013.

P14-087
Apitherapy with the Venom of *Apis sp.* (Insecta: Hymenoptera:Apidae)

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Apitherapy application of bee venom to treat various diseases has been used since ancient times in traditional medicine. *Apis sp.* (honey bee) venom is a well-known pharmacologically active product of the hive. It is synthesized by the venom glands associated with the sting apparatus of worker and queens, stored in the venom reservoir, and injected through the sting apparatus during the stinging process. A mature defender or forager contains about 100–150 μg of venom, and it injects 0.15–0.30 mg of venom via its stinger, a honeybee can inject 0.1 mg of venom via its stinger. *Apis* venom contains a number of very volatile compounds which are easily lost during collection, it is considered a rich source of enzymes, peptides and biogenic amines, it is specific weight. At least 18 pharmacologically active components have been described, including various enzymes, peptides and amines. Due to its anticoagulant and anti-inflammatory properties of bee venom was mainly used to treat many inflammatory disorders such as arthritis, bursitis, tendinitis, dissolving scar tissue, joint disease, rheumatoid arthritis, Lyme disease Multiple Sclerosis, osteoarthritis and ect. As a result, the main aim and the composition of this study is to analysis the Apitherapy applications with the Venom of *Apis sp.* (Insecta: Hymenoptera: Apidae)

Keywords:
Bee, Venom, Apitherapy, Toxicology, Insecta, Hymenoptera, Apidae:

P14-088
Exendin-4 impairs intestinal tissue damage through its proliferative and anti-fibrotic effects in diabetic rats

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Diabetes has many effects on the organs including small intestine. Exendin- 4 is an receptor agonist of the Glucagon like peptide-1 (GLP-1) which regulates glucose homeotasis. The aim of this study was to determine the effects of exendin-4 on the small intestine of chronic diabetic rats. We generated four experiment groups with *Wistar albino* rats. The first group was given saline and citrate buffer, the second group was injected exendin-4, the third group recieved STZ and the fourth group was given both STZ and exendin-4. Exendin-4 (3 μg/kg) was administered by a subcutaneous injection for 30 days after the animals were rendered diabetic by administration of STZ (200 mg/kg). As the results, Exendin-4 treatment was restored intestinal morphological alterations observed in diabetic rats. We observed especially a remarkable decrease in the deterioration of the villi integrity, increase in hyperplasia of the epithelial cells and mitotic crypt cells by Exendin-4 treatment to diabetic rats. Furthermore, we evaluated the mitotic cell number by immunohistochemical staining of proliferative cell nuclear antigen (PCNA). Exendin-4 treatment was also increased $PCNA^+$ crypt cell number in diabetic and control rats. Moreover, we observed that exendin-4 treat-

ment was decreased Transforming Growth Factor-β1 (TGF-β1) level in the small intestine of diabetic rats. Collectively, these results suggested that Exendin-4 treatment to diabetic rats, damaged villi cells could replaced by the new epithelial cells generated from crypt cells and this process results in epithelial hyperplasia of villi. Moreover, Exendin-4 contributes to tissue recovery with its anti-fibrotic effects.

P14-089
4-methylcatechol prevents hyperglycemia-induced inflammation and acute renal failure

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The increase in blood glucose level impair immune function by modifying cytokine production in macrophages. We assessed the regulation of glucose-induced inflammation by NGF in acute hyperglycemic rats. We preferred moderate hyperglycemia instead of severe hyperglycemia, because of the molecular mechanisms that regulate inflammation is triggered response to damage. The aim of this study was to investigate the effects of 4-Methylcatechol (4-MC), is a catechol compund and is known as a enhancer NGF synthesis in nerve cells, in the kidney cortex of streptozotocin (STZ)-induced acute hyperglycemic rats. STZ, is a naturally occuring chemical that is particularly toxic to insulin-producing beta cells of the pancreas in mammals. We generated four experiment groups with *Wistar albino* rats. The first group was given saline and citrate buffer, the second group was injected 4-methylcatechol (4-MC), the third group received STZ, the fourth group was given both 4-MC and STZ. 4-MC (10 lg/kg) was administered by daily intraperitoneal injection for 10 days before the animals were rendered hyperglycemic by administration of single dose STZ (75 mg/kg). With 4-MC pretreatment to hyperglycemic rats the following results were noted in the kidney tissues: i. A marked reduction in desquamated nuclei and cytoplasmic debris in the distal tubules; necrosis in glomerules; hemorrhage and hyperemia, ii. A significant increase in NGF synthesis; iii. Remarkable decreased in the level of TNF-α. Consequently, these results demonstrated for the first time that 4-MC can be a candidate molecule for using to prevent hyperglycemia-induced acute kidney damage due to its NGF-mediated anti-inflammatory effects.

P14-090
Sodium butyrate reduces *Staphylococcus aureus* internalization via TLR2 in bovine mammary epithelial cells

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Staphylococcus aureus is an etiological agent of human and animal diseases. This bacterium is able to internalize into non-professional phagocytic cells (e.g. bovine mammary epithelial cells, bMEC), event related to chronic and recurrent infections in bovines. Epithelial cells contribute to host innate immune response (IIR), which is mediated by receptors such as TLRs. TLR2 is the most relevant receptor for *S. aureus* recognition cooperating with CD36. In a previous report, we demonstrated that sodium butyrate (NaB, 0.5 mM), a short chain fatty acid able to modulate the IIR, reduced the *S. aureus* internalization into bMEC modulating the IIR. However, the molecular mechanism of this process has not been described, which is the aim of this study. The results obtained show that NaB (0.5 mM, 24 h) induces ~1.6 fold membrane abundance (MA) of TLR2 in bMEC, but CD36 MA was not modified. NaB activates 8 transcriptional factors (TF) related to IIR (CBF, AP-1, E2F-1, ER, FAST-1, MEF-1, PPAR, and HSE). Nevertheless, all the TFs were down regulated when bMEC were challenged with *S. aureus*. Interestingly, NaB pre-treatment augmented the mRNA expression of antimicrobial peptides (BNBD4, BNBD5, BNBD10 and TAP), effect that was not modified by bacterial internalization, except for BNBD5, which showed an induction of 3-6 fold. In conclusion, our results suggest that NaB activates bMEC via TLR2 and up-regulates IIR, which leads to a better response against invading pathogens.

P14-091
Prolactin-stimulated internalization of *Staphylococcus aureus* by mammary cells: role of TLR2 and α5β1 integrin

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Staphylococcus aureus has the ability to invade mammary epithelial cells (bMECs) causing mastitis. This event depends primarily on the α5β1 integrin in the host cell. In addition, bMECs are a target for the hormone prolactin (PRL), which can regulate β1 integrin-dependent actions related to differentiation and lactation. Previously, we demonstrated that bovine PRL (bPRL, 5 ng/ml) stimulates *S. aureus* internalization into bMECs. TLR2 is important during *S. aureus* infections, but its activation by PRL has not yet been established. The objective of this study was to determine the role of α5β1 integrin and TLR2 during *S. aureus* internalization into bMECs stimulated with bPRL. We demonstrated that the prolactin-stimulated internalization of *S. aureus* decreases in response to the blockage of α5β1 integrin (~65%) and TLR2 (~55%). bPRL does not modify the membrane abundance (MA) of a5b1 integrin but induces TLR2 MA (~2-fold). *S. aureus* reduces the α5β1 integrin MA in bMECs treated with bPRL (~75%) but induces TLR2 MA in bMECs (~3-fold). Bacteria and bPRL did not modify TLR2 MA compared with the hormone alone. bPRL induces the activation of the transcription factor AP-1, which was inhibited by *S. aureus*. In general, bPRL induces both pro- and anti-inflammatory responses in bMECs, which are abated in response to bacterial challenge. Interestingly, the canonical Stat-5 transcription factor was not activated in the challenged bMECs and/or treated with bPRL. Taken together, these results support novel functions of prolactin as a modulator of the innate immune response that do not involve the classical prolactin pathway.

P14-092
Hepatoprotective effect of *Cotinus Coggygria* Scop. Extract on ethanol-induced liver injury of rats

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Cotinus coggygria Scop. belongs to the family Anacardiaceae. *Cotinus coggygria* is found South and Central Europe, South Russia, Caucasia and Turkey. In folk medicine, *Cotinus coggygria* Scop. is used as an antiseptic, antiinflammatory, antimicrobial, antihaemorragic, antiulcer, antifungal and wound healing.

In this study, the effects of *Cotinus coggygria* Scop. against ethanol-induced liver damage in rats was investigated morphologically and biochemically. Sprague Dawley rats were randomly divided into four groups; Group I, control animals; group II, control animals receiving *Cotinus coggygria* Scop. water extract (50 mg/kg); group III animals receiving 1 ml absolute ethanol; group IV, animals receiving *Cotinus coggygria* Scop. extract (50 mg/kg) 1 h prior to the administration of absolute ethanol (in same dose). *Cotinus coggygria* Scop. extract and absolute ethanol were given only one dose to rats by gavage. Liver tissue was taken from animals for biochemical and morphologic analysis. Biochemical analysis were made in serum and liver tissue. Serum aspartate aminotransferase (AST) alanine aminotransferase (ALT) and alkaline phosphatase activities (ALP), and liver glutathione (GSH), lipid peroxidation (LPO) levels and catalase (CAT) activity were determined. Serum AST, ALT and ALP activities, liver LPO level increased, liver GSH level and CAT activity decreased in ethanol group. In light microscope, moderately hyperemia and slightly swollen hepatocytes that has light cytoplasm were observed in experiment group given ethanol. Treatment with *Cotinus coggygria* Scop. extract reversed these effects. As a result, we can say that *Cotinus coggygria* Scop. extract has a protective effect on ethanol induced liver injury.

P14-093
Laser processing of novel collagen-hydroxyapatite thin coatings with potential uses in bone regeneration

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The aim of this work was to prepare a bioactive, thin coating based on hydroxyapatite (HAP), collagen (Coll) and the Zinforo antibiotic, deposited by Matrix Assisted Pulsed Laser Evaporation Tehnique (MAPLE). The prepared thin coatings were characterized by TEM, AFM, SEM, XRD, SAED and InfraRed Microscopy. Biological characterization consisted in the *in vitro* evaluation (using qualitative and quantitative assays) of the biocompatibility of the prepared thin coatings on human osteoblasts and stem cells and of the antimicrobial efficiency against the methicillin-resistant *Staphylococcus aureus* (MRSA). The qualitative and quantitative results (MTT, AFM, SEM) demonstrated that the prepared thin coatings have good cytocompatibility, as revealed by the normal morphology, as well as by the slight stimulation of osteoblasts and stem cells attachment and growth, suggesting that these surfaces have the potential to be used in the regenerative medicine by stimulating the fixation at the bone-implant interface. Qualitative and quantitative analyses performed on MRSA clinical strains showed that the obtained surfaces have an inhibitory activity against microbial colonization and biofilm development maintained for up to 3 days. Furthermore, both microscopy (SEM, AFM) and viable cells count analyses proved the ability of the thin coating to interfere the MRSA biofilm development. All these data recommend this type of surface for the prevention of microbial contamination, colonization and biofilm development and for the utilization in bone tissue regeneration and bone prostheses fabrication.

P14-094
Biocompatible magnetite nanoparticles functionalized with the plant-derived compounds eugenol and limonene interfere with biofilm formation and persistence of *Pseudomonas aeruginosa*

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The purpose of this study was to develop a biocompatible nanomaterial based on magnetite and plant-derived compounds, such as eugenol and limonene in order to alter biofilm development and decrease resistance and persistence of the opportunistic pathogen *Pseudomonas aeruginosa*. This study used five clinical and one reference *P. aeruginosa* strains. Functionalized magnetite nanoparticles (Fe_3O_4@) have been synthesized by precipitation method, characterized by IR microscopy, SEM and TEM and functionalized with eugenol and limonene. The minimum inhibitory concentrations and biofilm formation were assessed by microdilution method followed by viable count. The impact of the obtained nanosystems on *P. aeruginosa* resistance and persisters formation was assessed for both planktonic cultures and biofilm embedded bacteria after treatment with the antibiotics gentamicin and norfloxacin. The biocompatibility of these nanostructures was assessed *in vitro* by fluorescence microscopy and MTT assay, using human cultured endothelial cells. Our data demonstrated that attachment and biofilm formation is significantly altered in all tested *P. aeruginosa* strains in a dose and strain dependent manner. Results revealed that functionalized magnetite nanostructures alter the percentage of persisters development after the antibiotics treatment with at least two fold for Fe_3O_4@ limonene and three fold for of Fe_3O_4@ eugenol, as compared with the situation in which bacteria are grown in the absence of the prepared nanosystems. These nanomaterials proved a good cytocompatibility *in vitro*. These results contribute to the development of modern therapies based on bioactive nanoparticles to fight resistant infections through modulating biofilm formation and persistence of the highly resistant pathogen *P. aeruginosa*.

P14-095
Serum prolidase activity in different clinical forms of Scleroderma

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Scleroderma is a chronic systemic autoimmune disease characterised by hardening of the skin and by increased synthesis of collagen.

Collagen is a powerful protein that is observed in the bones, tissues, and the skin. Prolidase is an enzyme responsible for the breaking down of iminodipeptides. It splits dipeptides that contain C-terminal proline or hydroxyproline.

The aim of this study is to search the Serum Prolidase Activity (SPA) in the clinical forms of Scleroderma Limited Scleroderma (LS) and Diffuse Scleroderma (DS).

For this purpose 35 Scleroderma patiens (24 DS and 11 LS) and 41 healthy control subjects were included to this study. SPA were determined by Myara method which is modification of Chinard's method.

SPA was not different between the Scleroderma patiens and controls ($p = 0.467$). However, SPA was significantly lower in DS both than LS patients and than control subjects ($p = 0.021$ and $p = 0.024$ respectively). It was not different between the LS patients and control subjects ($p = 0.145$).

Different clinical forms of Scleroderma have shown different SPA levels. This difference may be sourced from the difference in collagen metabolism. Collagen synthesis may be faster and degradation may be slower in DS when compared with healthy subjects. However, collagen metabolism is similar in LS and in healthy subjects. These findings should been confirmed with studies include other collagen metabolism markers such as collagen I propeptide, collagen III propeptide and the cross-linked telopeptide of type I collagen and gen expression studies.

P14-096
Biocatalytic asymmetric synthesis of glycolytic pathway metabolites

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Synthesizing glycolytic pathway metabolites provides important substrates for the functional analysis of enzymes in glycolytic and connected pathways. While the chemical synthesis of chiral 2-keto-deoxy-sugar acids from highly reduced raw materials involve numerous reaction steps, only a few reaction steps are required when the metabolic pathways of carbohydrate utilization are taken as a guiding route to the same 2-keto-3-deoxysugar acids from monsosaccharides using biocatalytic water elimination as key step. Enantiomerically pure (R)- and (S)-2-hydroxy-aldehydes have been prepared in nearly quantitative yield by chemoenzymatic synthesis for probing glycolytic pathways. Over-coming metabolite stability issues has been found as a key success factor, not only for developing a viable synthetic route, but also for understanding cellular degradation paths of these glycolytic pathway metabolites.

P14-097
Studies on the reversion of Kidney damage generated by diabetes

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Introduction: Chronic hyperglycemia in patients suffering from diabetes is the main generator of micro and macro-vascular damage in peripheral tissues, resulting in the increased amount of associated-pathologies. One of these conditions is diabetic nephropathy, where processes such as fibrosis, inflammation and oxidative stress, cause cellular damage and the following decline of renal function. Previous studies from our laboratory have shown that AY1966 causes normalization of blood glucose levels in a model of streptozotocin-induced diabetic rat. Our current goal is to elucidate the reno-protective effect of AY1966 and its molecular mechanism

Material and methods: Streptozotocin-induced diabetic rats were treated orally with AY1966. Biochemical and histopathological analyzes were performed to corroborate kidney damage. Protein levels of TGF-b, fibronectin, collagen and b-catenin were analyzed by western blot and immunohistochemistry.

Results: AY1966 treatment was able to normalize blood glucose, proteinuria and other biochemical parameters. Histopathological analysis showed a significantly decrease of kidney damage caused by diabetes. AY1966 restored the expression of fibrotic markers to normal levels.

Discussion: The effect of AY1966 indicates that this drug is able to reverse these processes at the cellular level by inhibiting oxidative stress, and decreasing inflammation and fibrosis, which are closely linked to the generation of renal damage during development of diabetic nephropathy. These results reveal some of the mechanisms induced by AY1966 in the reversion of diabetic nephropathy, and propose its potential application in the treatment of this disease. Supported by FONDECYT 1131033.

P14-098
Enzyme substrates for probing epoxidehydrolase functions

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Epoxide hydrolysing enzymes have been discovered in microbial, plant, animal and human cells and are not only of key importance for toxic epoxides but also for epoxides with high biological activities to living cells in a variety of metabolic pathways. New routes for the preparation of epoxides have been developed for analyzing epoxide hydrolase activities leading to selective biocatalytic ring-opening of epoxides by water leading to vicinal diols or other reaction products. This strategy is also used by nature to prepare a range of important metabolites and natural products by the epoxidehydrolase-catalyzed ring-opening reactions.

P14-099
In cardiac fibroblasts and myofibroblasts Toll like receptor 4 (TLR4) activation releases proinflammatory and profibrotic cytokine

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The Toll like receptor 4 (TLR4) plays a key role in the initiation and resolution of inflammatory responses in the cardiac tissue. In immune cells, TLR4 activation increased the releases of proinflammatory and profibrotic cytokines; however, in resident structural heart cells, such as cardiac fibroblasts (CF), and myofibroblasts (CMF: cell responsible for wound healing), the contribution of this receptor is unknown. CF were isolated from adult rats and differentiated to CMF by incubation with TGF-β1 (10 ng/ml) for 72 h. The identity of CMF was determined by the expression levels of α-SMA by western blotting and immunocytochemistry. The Th1 (IFN-γ, TNF-α, IL-2, IL-12) and Th2 (IL-4, IL-5, IL-10, IL-13) cytokines; as well as the main monocyte attractant chemokine (MCP-1) were measured by Luminex kit MILLIPLEX (Merck Millipore). CF and CMF were stimulated with ultrapure LPS (1 mg/ml) in presence/absence of TAK-242 (4 uM an TLR4 signaling pathway specific inhibitor). In CF and

CMF LPS increases TNF-α, IL-10 and MCP-1 secretion levels, and large differences were observed between CF and CMF. In the presence of TAK-242 these cytokine secretion levels decreased to near control values. Cytokines IL-2, IL-4, IL-5 and IL-12, did not exhibit significant differences in their secretion after incubation with LPS; whereas IL-13 and IFN-γ were undetectable. After TLR4 activation CF secretes higher levels of TNF-α than CMF; while IL-10, IL-5 and MCP-1 levels were secreted in higher levels in the MFC.

P14-101
EU-OPENSCREEN: Novel chemical tool compounds for molecular biologists

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Small molecules that can be applied as chemical 'tool' compounds (or 'probes') have become indispensable in basic research for the elucidation of fundamental biological mechanisms. They act directly with the protein-of-interest and often allow for the interrogation of biological processes that cannot be properly studied with traditional genetic or RNA interference approaches. EU-OPENSCREEN (www.eu-openscreen.eu) is the largest emerging academic chemical biology research infrastructure initiative in Europe with the aim to collaboratively develop novel research tool compounds with independent cell biologists. As a joint effort of national networks in 16 European countries, EU-OPENSCREEN offers access to high-throughput screening platforms, chemistry services and a large compound collection. It welcomes biologists who have a robust and suitable biological assay and are interested in collaboratively developing chemical tool compounds to validate their targets-of-interest. Selected assays are screened against a collection of more than 100,000 compounds, incl. confirmatory and counter screening, IC/EC50 determination, SAR (structure-activity relationships) and QC of confirmed hit compounds. EU-OPENSCREEN will start operations in 2016, but it can already look back on a growing number of transnational activities: joint screening projects, exchange of local compound libraries, development of new design principles for its compound collection; exchange of experimental data through its pilot database etc.

P14-102
Induction of L-, D-amino acids oxidases and urea cycle enzymes of *Aspergillus niger* R-3 by hydrogen peroxide

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L-amino acid oxidase (LAAO) and D-amino acid oxidase (DAAO) catalyze the stereo-specific oxidative deamination of L-D-amino acids oxidases into α-keto acid along with the production of ammonia and hydrogen peroxide via an amino acid intermediate. According to research the low concentration of H_2O_2 induces many cellular functions. Our studies shown, that LAAO and DAAO of *Aspergillus niger* R-3 induces by adding 0.002M H_2O_2 in growth media for enzymes activity determination. LAAO activity increased to 9.5 μmol NH_3 on 1 g mycelium and DAAO activity increased to 7.3 μmol NH_3 on 1 g mycelium. We can conclude that adding H_2O_2 to growth media of *Aspergillus niger* R-3 induces deamination of L-,D-amino acids. Reaction of hydrogen peroxide on the activity of urea cycle enzymes of *Aspergillus niger* R-3, which does not produce hydrogen peroxide, was studied within our research. It was found out, that adding of H_2O_2 in growth media of *Aspergillus niger* R-3 stimulated enzymes carbamoyl phosphate synthetase I, ornithine transcarbamylase, argininosuccinate synthetase and arginase. The hydrogen peroxide plays a significant role in protective and oxidational biological reactions. The results of our research show, that addition of H_2O_2 in growth media *Aspergillus niger* R-3 stimulates the NH_3 production through LAAO, DAAO and its neutralization through urea cycle.

Keywords: L-amino acid oxidase (LAAO), D-amino acid oxidase (DAAO), urea cycle, hydrogen peroxide, enzyme induction, Aspergillus niger R-3

P14-103
Effects of Hsp90 inhibition on galectin-3 expression in human monocytic cell line THP-1

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Inhibition of Hsp90 is a promising therapeutic approach with clinical relevance for treatment of specific tumour types since both Hsp90 homologous (Hsp90α and Hsp90β) account for maturation and functional stability of a plethora of polypeptides (Hsp90 client proteins), including many proteins involved in tumorigenesis (*e.g.* anti-apoptotic and signal-transduction proteins, transcription factors, Tyr-kinase receptors, *etc.*). Galectin-3, a β-galactoside lectin is a multifunctional protein, ubiquitously expressed in both intracellular and extracellular environments as well as on the surface of different cell types. Intracellular galectin-3, recognised as a strong anti-apoptotic molecule, is well known for its roles being correlated with the development and malignancy of cancers and cancer drug resistance. We studied the effects of antibiotic geldanamycin, 17-DMAG [17-(dimethylaminoethylamino)-17-demethoxygeldanamycin], an orally bio-available derivative of ansamycin and siRNA directed to Hsp90α mRNA (Hsp90α-siRNA) on the expression of galectin-3, different heat shock protein family members and Hsp90 client proteins involved in cell cycle regulation in human monocytic cell line THP-1. Although slight transient increase of galectin-3 expression was observed after 24 h of both treatments, with 17-DMAG, and with Hsp90α-siRNA, inhibition of Hsp90 had no significant effect on galectin-3 expression. 17-DMAG slightly induced the expression of both Hsp90α and Hsp90β, while Hsp90α-siRNA treatment resulted in inhibition of Hsp90α expression, but did not affect Hsp90β expression. While 17-DMAG tremendously up-regulated expression of HSP70, cdk1, p(Tyr)-cdk1, and cdk2, and did not affect Hsp27 expression, Hsp90α-siRNA did not affected any of aforementioned proteins. These results encourage for further studies focused on elucidation of galectin-3 in apoptosis and tumorigenesis.

P14-105
Alteration in xenobiotic metabolizing enzyme activities with morin and 7,12-dimethylbenz(a) anthracene in diabetic male rats

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Diabetes mellitus is a metabolic disorder causing damage in organs. The effects of toxic chemicals increase within the body in the presence of metabolic disease. 7,12-Dimethylbenz(a)anthracene (DMBA) is one of the carcinogens. Morin is a flavonoid

found in plants. This study is aimed to determine the effects of morin on 7-ethoxyresorufin O-deethylase (EROD), 7-methoxyresorufin O-demethylase (MROD), total glutathione S-transferase (GST) and glutathione reductase (GR) activities in DMBA-treated diabetic rats. Diabetes was induced in all groups by intraperitoneal injection of streptozotocin at doses of 60.0 mg/kg body weight (b.wt.). Morin was given to morin-treated groups at a dose of 25.0 mg/kg b.wt. three times in a week. The rats in DMBA-treated groups were gavaged with 30.0 mg/kg b.wt. DMBA three times during the administration period. Rats were killed on the 43rd day of the administration period. Microsomes and cytosols were prepared for each liver tissue. EROD activities of diabetes, diabetes+morin, diabetes+DMBA and diabetes+DMBA+morin were 245 ± 22 (n = 5), 272 ± 12 (n = 3), 346 ± 20 (n = 3) and 202 ± 26 (n = 3) pmol/min/mg protein, respectively. MROD activities of diabetes, diabetes+morin, diabetes+DMBA and diabetes+DMBA+morin were 59.4 ± 4.5, 77.5 ± 10.6, 85.6 ± 3.7 and 42.8 ± 4.1 pmol/min/mg protein, respectively. GST activities of diabetes, diabetes+morin, diabetes+DMBA and diabetes+DMBA+morin were 820 ± 43, 499 ± 31, 591 ± 41 and 529 ± 30 nmol/min/mg protein, respectively. GR activities of diabetes, diabetes+morin, diabetes+DMBA and diabetes+DMBA+morin were 56.0 ± 1.6, 54.9 ± 4.1, 58.8 ± 1.0 and 52.9 ± 4.1 nmol/min/mg protein, respectively. In conclusion, CYP1A-related EROD and MROD activities in DMBA+morin group were less than DMBA group. Co-administration of morin with DMBA significantly decreased the toxic effect of DMBA in diabetic rats.

P14-106
Recognition of linear B-cell epitope of betanodavirus coat protein by RG-M18 neutralizing mAb inhibits giant grouper nervous necrosis virus infection

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Betanodavirus is a causative agent of viral nervous necrosis syndrome in many important aquaculture marine fish larvae, resulting in high-mortality globally. The coat protein of Betanodavirus is the sole structural protein which can assemble the virion particle by itself. In this study, we used a high-titer neutralizing mAB, RG-M18, to identify the linear B-cell epitope on the viral coat protein. By mapping a series of recombinant proteins generated using the *E. coli* PET expression system, we demonstrated that the linear epitope recognized by RG-M18 is located at the C-terminus of the coat protein, between amino acid residues 195 and 338. To define the minimal epitope region, a set of overlapping peptides were synthesized and evaluated for RG-M18 binding. Such analysis identified the $_{195}$VNVSVLCR$_{202}$ motif as the minimal epitope. Comparative analysis of Alanine scanning mutagenesis with dot-blotting and ELISA revealed that Valine$_{197}$, Valine$_{199}$, and Cysteine$_{201}$ are critical for antibody binding. Substitution of Leucine$_{200}$ in the RGNNV, BFNNV, and TPNNV genotypes with Methionine$_{200}$ (thereby simulating the SJNNV genotype) did not affect binding affinity, implying that RG-M18 can recognize all genotypes of Betanodaviruses. In competition experiments, synthetic multiple antigen peptides of this epitope dramatically suppressed giant grouper nervous necrosis virus (GGNNV) propagation in grouper brain cells. The data provide new insights into the protective mechanism of this neutralizing mAB, with broader implications for Betanodavirus vaccinology and antiviral peptide drug development.

P14-107
DHRS7, newly identified enzyme with overlapping function in metabolism of steroids and retinoids?

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The group dehydrogenases/reductases (SDR family) called DHRS consists of 17 members. So far, only 6 have been described to various extents, the rest of the members are considered to be orphan. However, there are first signs indicating possible significance some of these members in humans. Recently, our research group identified DHRS protein, dehydrogenase/reductase (SDR family) member 7 (DHRS7) as an integral membrane protein which faces the lumen of endoplasmic reticulum that shows, at least *in vitro*, NADPH-dependent reductive enzymatic activity towards some eobiotics with carbonyl moiety (androsten-3,17-dione, cortisone) as well as xenobiotics (e.g. the potent carcinogen NNK, 9,10-phenanthrenequinone). Moreover, some studies mention its changed expression in several pathological states as e.g. liver diseases, prostate cancer or during early development. The aim of the study is to provide a further biochemical description of the previously, partially characterized human DHRS7. Its recombinant form was purified and reconstituted to the liposomal system that enables its further study, including a structure analysis or detailed catalytic activity with substrates (e.g. all-trans-retinal, androsten-3,17-dione, cortisone). For preliminary estimation of potential role of the human DHRS7 in organism, the expression of mRNA and/or protein in various human tissues and cell lines was investigated. The highest levels of mRNA DHRS7 were found in the prostate, thyroid, kidney and adrenal gland, conversely, the protein was detected primarily in the adrenal gland, prostate and liver. Based on current findings, it seems that DHRS7 might be a significant human enzyme e.g. implicated in metabolism of important intracellular messengers: steroids and retinoids.

P14-108
Nontraumatic osteoarthritis is associated with increased the levels of serum cystatin-C: A cross-sectional study

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Introduction: In previous studies, it is shown that cysteine cathepsins associated with pathogenesis of osteoarthritis (OA). Literature data of Cystatin-C (Cys-C) natural inhibitor of cathepsins are limited in humans. The purpose of this study, in patients with OA, Cys-C and serum total free thiol (STFT) levels of serum and synovial liquid is to investigate
Material and methods: This study is included 40 consecutive patients with OA and 40 healthy individuals. In serum samples from patients and controls, STFT levels were measured with Elman method's and Cys-C levels were measured with commercial kitt in autoanalyser.
Result: Serum FT levels were similar between the groups ($p > 0.05$) and Cys-C levels were significantly higher in the

patient group compared to the control group ($p < 0.05$). Pearson correlation analysis, serum Cys-C levels were associated with serum STFT levels (correlation coefficient = 0.80, $p = 0.0002$). Linier regression analysis revealed that the levels of STFT independently affect levels of Cys-C in serum (reg. Coefficient = 0.80, $p = 0.003$).

Discussion: In OA, serum Cys-C levels had been identified in high compared to controls. It may be associated with this disease pathogenesis, increased oxidative stress or chronic inflammation in osteoarthritis.

P14-109
Protein knockout mice: a novel *in vivo* approach for functional genomics

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We demonstrate for the first time that endoplasmatic reticulum retained small antibody fragments can induce a knock down phenotype in transgenic mice on the protein level. The intrabodies bind to Vascular Cell Adhesion Molecule (VCAM1), a cell surface mediator of immune functions. VCAM1 is brought to the cell surface via vesicles of the endoplasmatic reticulum. When intrabodies were produced in the same vesicles, they inhibited the transport of their antigen (here: VCAM1) to the cell surface. Consequently, surface VCAM1 expression was suppressed in the bone marrow of the anti-VCAM1 intrabody expressing mouse line. Significantly, these mice did not have the lethal phenotype of the genetic VCAM1 knockouts. The phenotype was already achieved in mice heterozygous for the intrabody, and stronger in homozygotes. Physiological effects found in adult mice were aberrant distribution of immature B-cells in blood and bone marrow. The availability of this new and broadly applicable method combined with the power of today′s *in vitro* antibody fragment generation pipelines which deliver specific antibody genes within a few weeks will spark a new level for the functional study of membrane and plasma proteins *in vivo*. It will be further of particular value for generating mouse models that more closely resemble disease states than nucleic acid based knockout methods can do.

P14-110
Gaucher disease: Phenotypic and genotypic diagnosis in Algeria

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Gaucher disease is the most common lysosomal storage in our population, it is due to a deficiency of β-glucosidase acid. The enzyme deficiency causes a pathological accumulation of undegraded substrate in lysosomes. This metabolic overload is responsible for a multisystemic disease with hepatosplenomegaly, anemia, thrombocytopenia, and bone involvement. Neurological involvement is rare. The laboratory diagnosis of Gaucher disease consists of phenotypic diagnosis by determining the enzymatic activity of β-glucosidase by fluorimetric method, a study by genotypic diagnosis in the GBA gene, limiting the search recurrent mutations (N370S, L444P, 84 GG); PCR followed by an enzymatic digestion. Abnormal profiles were verified by sequencing. Monitoring of treated patients is provided by the determination of chitotriosidase. Our experience spans a period of 6 years (2007-2014) has enabled us to diagnose 78 patients out of a total of 328 requests from the various departments of pediatrics, internal medicine, neurology. Genotypic diagnosis focused on the entire family of 9 childrens treated at pediatric CHU Mustapha, which help define the clinical form; 5 of them had type III disease, carrying the L444P mutation in the homozygous state. Three others were composite (N370/L444P), (N370S/other unintended mutation in our study), and only one family no recurrent mutation has been found. This molecular study permits screening of heterozygous essential for genetic counseling.

P14-111
Regulation of HIF1α expression by a natural compound; a new regulatory factor

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Hypoxia induces HIF1α expression, leading to the malignant cell transformation. In screening of inhibitors against HIF1α expression using a reporter gene assay system, a moracin derivative, MO, was found to strongly reduce the level of HIF1α in HeLa cells treated with a hypoxia-mimetic, $CoCl_2$. Identification of binding proteins using agarose-bead conjugated MO (AC-685) combined with subsequent MS data revealed several proteins affected by MO. AC-685 co-localized with a nuclear protein (Np-K) in the nucleus of $CoCl_2$ treated HeLa cells. Amongst several cytoplasmic or nuclear proteins, Np-K was only found to be responsible for $CoCl_2$-induced HIF1a expression as supported by Np-K siRNA. In this study, detailed regulatory mechanism of HIF1α expression by MO in association with some nuclear and cytoplasmic proteins will be presented, with a novel finding of a factor which might be a clue to cancer treatment in hypoxic environment.

Chem Biol S2, Targeted Cancer Therapy

P15-003-SP
Antibody Directed Enzyme Prodrug Therapy: Discovery of novel genes, isolation of novel gene variants and production of long acting drugs for efficient cancer treatment

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Background: Cancer accounts for 13% of the mortality rate worldwide. Antibody-Directed Enzyme Prodrug Therapy (ADEPT) is a novel strategy to improve the selectivity of cancer treatment. The ADEPT uses the bacterial enzyme, glucarpidase to produce the antibody-enzyme complex. Also the glucarpidase is a very effective enzyme for detoxification of methotrexate, (MTX) which serves as an important component of various chemotherapeutic regimens for the treatment of cancer patients. Repeated cycles of ADEPT and the use of wild type glucarpidase in detoxification are essential but are hampered by the human antibody response to the enzyme. Additionally, glucarpidase has a relatively slow action in detoxification.

Objectives: 1. Screening for novel glucarpidase producers and cloning and overexpression and purification of the novel glucarpidase(s)

2. Production by DNA shuffling of novel glucarpidase variants with ultra-activity and variants with lower immunogenicity

3. Production of long acting glucarpidase(s)
4. Synthesis of Tubulysin derivatives, as a Prodrug, on a gram scale for conjugation experiments

Results: We successfully produced, by DNA shuffling an ultra-active glucarpidase that degrades MTX with a high efficiency. We also isolated three novel glucarpidase producing bacteria from Qatari soil. Two novel glucarpidases were successful cloned, overexpressed and purified. We managed to introduce a new mutation into one of the newly isolated glucarpidase gene which led to a 500% increase in the glucarpidase activity. Novel long acting gulcarpidases was produced which can avoid or minimize the patient immune system response. Novel tubulysin based prodrug was synthesised via Multicomponent reaction. The talk will cover other issues.

P15-004-SP
Breast cancer cell line MCF7 escapes from G1/S arrest induced by proteasome inhibition through a GSK-3β dependent mechanism

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Targeting the ubiquitin proteasome pathway has emerged as a rational approach in the treatment of human cancers. Autophagy has been described as a cytoprotective mechanism to increase tumor cell survival under stress conditions. Here, we have focused on the role of proteasome inhibition in cell cycle progression and the role of autophagy in the proliferation recovery. The study was performed in the breast cancer cell line MCF7 compared to the normal mammary cell line MCF10A. We found that the proteasome inhibitor MG132 induced G1/S arrest in MCF10A, but G2/M arrest in MCF7 cells. The effect of MG132 on MCF7 was reproduced on MCF10A cells in the presence of the glycogen synthase kinase 3β (GSK-3β) inhibitor VII. Similarly, MCF7 cells overexpressing constitutively active GSK-3β behaved like MCF10A cells. On the other hand, MCF10A cells remained arrested after MG132 removal while MCF7 recovered the proliferative capacity. Importantly, this recovery was abolished in the presence of the autophagy inhibitor 3-methyladenine (3-MA). Thus, our results support the relevance of GSK-3β and autophagy as two targets for controlling cell cycle progression and proliferative capacity in MCF7, highlighting the co-treatment of breast cancer cells with 3-MA to synergize the effect of the proteasome inhibition.

P15-005-SP
Intracellular lysogens to augment the anti-tumoral efficacy of targeted toxins

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Targeted toxins are anti-tumor drugs that consist of a targeting and a toxin domain. Commonly used targeting domains are growth factors, such as the epidermal growth factor (EGF) or monoclonal antibodies, such as trastuzumab. The toxin domain originates from plants or prokaryotes. Prominent examples of toxins are saporin from *Saponaria officinalis* L. or diphtheria toxin (DT) from *Corynebacterium diphtheriae*. Ontak®, which is a fusion between a truncated version of DT and an interleukin-2 sequence, is approved for the treatment of cutaneous T-cell lymphoma. After endocytosis into the target cell, targeted toxins need to escape from endosomes or lysosomes into the cytosol. Failing to do this, they are degraded within the lysosomes. Overcoming and escaping the endo- or lysosomal membrane is critical for the efficacy of targeted toxins. High dosages are in part necessary to compel the endosomal escape process of targeted toxins. This dose increase is juxtaposed with severe side effects such as the vascular leak syndrome. Here we show that the anti-tumoral efficacy of EGF-based and trastuzumab/cetuximab-based targeted toxins is drastically augmented by plant derived amphiphilic molecules (triterpene saponins). When applying a targeted toxin in combination with a triterpene saponin in a tumor model, 8 out of 10 mice showed complete remissions. By confocal live cell imaging, we demonstrate that triterpene saponins augment the escape of internalized toxin molecules out of endosomes and lysosomes into the cytosol. Triterpene saponins do not influence the plasma membrane permeability thus having great potential as a new class of intracellularly acting lysogens.

P15-006-SP
Molecular engineering of L-asparaginases used in antileukemic therapy

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Our work aims to ameliorate the enzyme L-asparaginase (L-ASNase) which is a protein drug of high value used in antileukemic therapy. L-ASNases catalyze the deamidation of the free amino acid L-Asn to L-Asp and ammonia. Bacterial L-ASNases are FDA-approved therapeutic enzymes for use in the treatment of various blood cancers to deplete serum L-Asn levels. Their efficacy as protein drugs is based on the fact that several hematological malignancies, in particular Acute Lymphoblastic Leukemia (ALL), depend for growth on the extracellular supply of L-Asn. To avoid various side reactions inherent to the bacterial enzymes, it would be beneficial to substitute them with human L-ASNases. One human isoform, hASNase-3, belongs to the N-terminal nucleophile (Ntn) hydrolase superfamily where the protein is synthesized as a single polypeptide chain that is devoid of activity. Autoproteolytic cleavage of this protein generates two tightly associated subunits that constitute the catalytically active enzyme. The free amino acid glycine was found to selectively accelerate intramolecular processing of hASNase-3 both in isolated form and in human cells. We evolve the enzyme *in vitro* aiming to select for variants of enhanced activity. To increase serum half-life of the enzyme, we package L-ASNases into microcapsules, thus improving protein stability and potentially preventing exposure of the enzyme to the immune system. The Layer-by-Layer (LbL) strategy of biocompatible microcapsule formation has been applied under mild conditions to preserve catalytic activity, using calcium carbonate particles as core templates for protein adsorption, followed by coating with poly-L-arginine and dextran sulfate polymers.

Chem Biol S4, RNA-Based Disease Mechanism and Therapy

P17-005-SP
The role of microRNA cluster MIR23A~27A~24-2 in the development of aggressive B-cell lymphoma

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MicroRNAs (miRNAs) are small non coding RNAs, which negatively regulate protein biosynthesis. It was shown that high expression of miR-23a correlates with poor overall survival in diffuse large B-cell lymphoma (DLBCL) patients, suggesting that miR-23a might act as an oncomiR in B-cell Non-Hodgkin lymphoma (B-NHL). Our aim is to investigate the cause and consequences of MIR23A~27A~24-2 cluster deregulation in B-NHL. We observed that specific factors present in the microenvironment of germinal centers of the lymph nodes can induce MIR23A~27A~24-2 cluster expression in B-NHL cell lines. In contrast, healthy germinal center B (GCB) cells did not respond to these factors, indicating an aberrant regulatory process of MIR23A~27A~24-2 cluster expression in transformed GCB cells. This hypothesis was supported by our observation and that of others that miR-23a shows higher expression levels in primary DLBCL compared to healthy controls. In order to study the biological function of MIR23A~27A~24-2 cluster in aggressive B-NHL, we investigate the targetome of miR-23a by immunoprecipitation of Ago2-bound target-mRNA (Ago2-RIP) followed by RNA sequencing. Therefore, DLBCL cell lines overexpressing miR-23a or a non-silencing control were generated. Comparison of the Ago2-enriched mRNAs from miR-23a overexpressing cells with controls identifies potential miR-23a targets, which are subject of our future studies. Notably, the metabolic and proliferative effects described for miR-23a in other types of cancer could not be verified in our miR-23a overexpressing DLBCL cell line, strengthening the need to identify the specific targetome of miR-23a in B-NHL to understand its cellular function.

P17-006-SP
miR-155 modulates IFNγ signaling pathway by targeting SOCS1 expression in biliary atresia

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MicroRNA (miRNA) are ~22-nucleotide RNAs that negatively regulate gene expression and inflammatory responses in eukaryotes. The aim of this work was to evaluate the contribution of miR-155 on the interferon-γ (IFN-γ) induced response in biliary atresia (BA). A strong up-regulation of miR-155 expression was observed in BA samples treated with IFN-γ. miR-155 down-regulation the protein expression levels of SOCS-1 by targeting its mRNA. Up-regulation of miR-155 expression by IFN-γ in BA cells lead to activation of STAT1 and inflammatory cytokines through the JAK/STAT pathway. Specifically inhibiting miR-155 expression by anti-miRNA oligonucleotides significantly decreased the mRNA or protein production of these inflammatory cytokines and STAT1. Overall, our results suggest that the miR-155 regulate IFN-γ signaling pathway by targeting SOCS-1 expression and miR-155 may be a potential target in BA therapy.

P17-007-SP
miRNA target enrichment network analysis in Hepatocellular carcinoma

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Hepatocellular Carcinoma (HCC) is one of the most common worldwide causes of cancer death. Molecular mechanisms involved in the tumor are not completely known. microRNAs (miRNAs) exhibit aberrant expression in HCC; however, the regulatory networks are not fully understood. A miRNA target enrichment analysis was performed in order to better understand the complex regulatory networks in HCC. MiRNA expression meta-analysis was conducted to identify differentially expressed miRNAs ($P < 0.05$). miRNA targets were downloaded from mirTarBase. A Gene onthology and KEGG pathways enrichment analysis was performed using genecodis 3.0. P-values were computed using hipergeometric distribution with the FDR correction. Clusterization analysis was performed using the genesis software. 911 articles on miRNA in HCC were retrieved from literature and 94 eligible studies were assessed for miRNA expression data. miRNA expression data were collected from 3,064 HCC patients. 34 up-regulated and 19 down-regulated miRNAs were identified in tumor compared to adjacent noncancerous tissue. Target analysis identified 402 validated targets, 256 shared among different up-regulated miRNAs. Down-reguated miRNA shared 88 target among 250 validated targets. GO and KEGG pathway enrichment analysis of the meta-miR targets identified a statistically relevant ($P < 0.001$) pathways characteristics of HCC such as apoptosis, drug resistance, virus response, that link miRNAs to the cancer hallmarks. Clusterization analysis identified miRNA that share common targets and common functions, most of them belonging to the same cluster. Revealing miRNA regulatory network can be useful in identifying clinically relevant miRNAs and targets that could be candidates for improved diagnostics, prognostics and therapeutics in HCC.

P17-008-SP
Anti-miRNA zymes as a potential tool for therapy of brain tumors

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The most frequent brain tumors in adults are malignant gliomas, arising from glial cells or their precursors. Glioblastoma multiforme (GBM) represents the most common and aggressive type. It is still perceived as a daunting challenge to reveal miRNA-target networks, miRNA functions and expression profile of miRNAs specific for tumor/cancer type. While malignant gliomas represent highly heterogeneous group of tumors, miRNA profiling could be advantageous not only for further research but also for diagnostic and therapeutic purposes. We established miRNA

profile in GBM and found an effective tool for the down-regulation of selected, oncogenic miRNAs. In the first step, we have focused on the identification of miRNA expression signatures in malignant gliomas using miRNA microarrays and deep sequencing approach. We have profiled global miRNA expression in adult malignant gliomas and non-tumor brain tissues. Next, we preformed meta-analysis of differentially expressed microRNAs. Further, we designed anti-miRNA catalytic nucleic acids specific for several of miRNA defectively over-expressed in GBM. They are capable of specifically cleaving RNA molecules, what enables them to act as potential anti-miRNA and anti-cancer agents. Designed ribozymes bind and cleave miRNA molecules specific for GBM and their activity has been confirmed *in vitro* and in glioblastoma cell lines. The most meaningful ribozymes that will affect gliomagenesis process will be investigated towards therapeutic applications.

P17-009
Transcription-coupled RNA surveillance in human genetic diseases caused by splice site mutations

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Current estimates indicate that approximately one third of all disease-causing mutations are expected to disrupt splicing. Abnormal splicing often leads to disruption of the reading frame with introduction of a premature termination codon that targets the mRNA for degradation in the cytoplasm by nonsense mediated decay (NMD). In addition to NMD there are RNA surveillance mechanisms that act in the nucleus while transcripts are still associated with the chromatin template. However, the significance of nuclear RNA quality control in the context of human genetic diseases is unknown. Here we used patient-derived lymphoblastoid cell lines as disease models to address how biogenesis of mRNAs is affected by splice site mutations. We observed that most of the mutations analyzed introduce premature termination codons and trigger mRNA degradation in the cytoplasm. However, for some mutant transcripts, RNA levels associated with chromatin were found down-regulated. Quantification of nascent transcripts further revealed that a subset of genes containing splicing mutations have reduced transcriptional activity. Following treatment with the translation inhibitor cycloheximide the cytoplasmic levels of mutant RNAs increased, while the levels of chromatin-associated transcripts remained unaltered. These results suggest that transcription-coupled surveillance mechanisms operate independently from NMD to reduce cellular levels of abnormal RNAs caused by splicing mutations.

P17-010
Associations of Polymorphisms in the Vitamin D receptor gene (FOK I and BSMI) with COL1A1-sp1 Polymorphism in Relation to low bone mineral density in young osteoporotic Turkish women

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Background: Osteoporosis is a complex metabolic bone disease characterised by a low bone mass and higher risk of fragility. Bone mineral density (BMD) is determined by several gene poymorphisms.The vitamin D receptor gene is the first gene that was described.Type 1 collagen is the major protein in bone matrix encoded by the by the Col 1A1 and Col 1A2 gene. A genetic variation (Sp1) in intron 1, of Col 1A1 gene is considered a candidate gene for osteoporosis.The aim of our study was to determine the distribution of gene polymorphisms and association of them with low bone density.

Method: We studied 50 women ranging in age from 35-57 years. All patients had low BMD with Tscore ≤2,5 SD in the lumbar spine. Blood samples were obtained.with EDTA.The FOK1 (VDRF-FOK1) and BSMI(VDRB-BSMI)of VDR gene and CoL1 A1 gene was analysed with Genomica clinical array system.

Results: Genotype frequencies 62% FF, 32% Ff, %6 ff allele for FOK1, 24 % BB, 58% Bb, 18 % bb allele for BSMI, 36% SS, 62% Ss,, 2% ss for Col1A1 gene identified. Allele frequencies were 78% for F, 22% for f allele, %53 for B and %47 for b allele. And they were in Hardy-Weinberg equlibrium.($p = 0{,}632$), ($p = 0{,}245$) respectively. Allele frequencies were 67% for S and 33% for s allele.İt was not consisted with Hardy-Weinberg equlibrium($p = 0.044$).No linkage disequlibrium between BSMI, FOK1 and Col1 A1, showed that they are independent each other. Among the combined haplotypes Ss-Bb (20%) was most frequent type related to low BMD in early osteoporotic women.

P17-011
L-Dopa decarboxylase (DDC) mRNA expression: implication in insulin-signaling in human β-pancreatic cells

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DDC, the enzyme catalyzing the biosynthesis of the neurotransmitters dopamine and probably serotonin, has been implicated in glucose metabolism and function of pancreas. These data in combination with the widely supported association between dopaminergic system and glucose metabolism triggered us to investigate DDC expression under insulin-pathway modulation conditions, in human β-pancreatic cells. Our previous results revealed that treatment of 1.2B4 immortalized human β-pancreatic cells with either glucose, insulin or metformin leads to alterations in *DDC* mRNA levels. We are now focused on the investigation of DDC expression pattern, upon treatment with various inhibitors of the insulin-signaling pathway. 1.2B4 β-cells were subjected to treatment with either the LY294002 PI3K inhibitor (50μM), PD98059 MAPK inhibitor (25μM), or rapamycin (mTOR inhibitor) (10 nM) for 1 hr, followed by glucose treatment (16,7 mM) for 15 min. *DDC* mRNA levels were estimated by qRT-PCR. Cell viability was tested by flow-cytometry analysis upon annexin-V/propidium iodide staining.Treatment with any of the abovementioned inhibitors resulted in the down-regulation of *DDC* mRNA levels [RQ values (mean of 3 independent experiments±SD): for control=1; for LY294002 = 0.54 ± 0.06; for PD98059 = 0.57 ± 0.11; for rapamycin=0.43 ± 0.04]. Cell viability was unchanged in any of these conditions (>95%). Our up to now data suggest the association of *DDC* expression with insulin signaling, supporting the dopaminergic system-glucose metabolism link, that is for the first time studied on cells of human origin. Further investigation is required to elucidate its governing

molecular mechanisms that may serve as potential therapeutic targets for diabetes.

P17-012
Study of the expression of 28 diabetes-related genes in peripheral blood: indications for clinical significance in type 2 diabetes mellitus (T2DM)

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T2DM, a chronic metabolic disorder with increased cardiovascular morbidity and mortality, is regulated by both environmental and genetic components. It currently accounts for one of the global epidemics with ever growing prevalence. T2DM diagnosis is often delayed primarily due to the absence of efficient screening tests. We aimed to investigate the expression pattern of 28 diabetes-related genes in peripheral blood (PB) of T2DM patients and explore their potential as possible biomarkers. For that purpose, total RNA was isolated from PB samples of 38 patients and 27 healthy individuals, and reverse transcribed to cDNA. mRNA levels were studied by qPCR. Possible correlations with clinical features and laboratory data were investigated with appropriate statistical tests. Expression levels of *CDK5* and *CDC123* were found significantly ($p < 0.05$) higher in patient compared to control group (logistic regression OR=2.998 and 1.679, respectively). Moreover, *TCF7L2*, *JAZF1*, *CDC123*, *KCNJ11*, *THADA*, *LPL*, $p14^{ARF}$, *SLC30A8*, *FTO*, *CDKAL1*, *CDKN2A*, *WFS1* and *TSPNA8* mRNA levels significantly correlated with certain disease features (BMI, hyperlipidemia, metabolic syndrome, HbA1c, fasting glucose or fasting insulin levels; $p < 0.05$ for all). Herein, specific diabetes-related genes, either associated with the functionality of the insulin-signaling pathway or involved in cell-cycle regulation, are found to be differentially expressed in PB of T2DM patients. Further sequencing analysis of these genes are now in progress for the elucidation of their potential to serve as possible biomarkers for the diagnosis and monitoring of genetically predisposed individuals.

P17-013
The impact of human HAX-1 protein expression and localization on granulopoiesis

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HAX-1 is an ubiquitously expressed protein which has been implicated in multiple cellular processes including cell migration and adhesion, apoptosis and RNA binding. Homozygous *HAX1* mutations have been found in patients with autosomal recessive severe congenital neutropenia (SCN, Kostmann disease). Kostmann patients are characterized by a complete lack of HAX-1 protein expression and a pronounced maturation arrest in granulopoiesis at the promyelocytic/myelocytic stage. So far the exact mechanism by which *HAX1* mutations cause autosomal recessive SCN has not been fully elucidated. To study HAX-1 function, expression and protein localization in granulopoiesis we used promyelocytic HL-60 cell line which can be induced to differentiate into mature granulocytes by ATRA (all-trans-retinoic acid). In the early stages of differentiation *HAX1* mRNA and protein expression increased slightly to drop in the later stages. In undifferentiated cells HAX-1 was localized in a membrane fraction (mainly, but not exclusively in mitochondria) and, to a much lesser extent, in cytoplasm and nuclear matrix. No changes in localization were observed during differentiation. To create a model of Kostmann disease we established stable HL-60 cell lines with *HAX1* silencing and corresponding control cell lines. Cells with silenced *HAX1* showed impaired differentiation and proliferation, but, quite surprisingly, no evident changes in apoptosis levels. The levels of LEF-1 mRNA, a decisive trascription factor in granulopoiesis, dropped dramatically. In summary, we characterized *HAX1* mRNA and protein expression as well as protein localization during granulopoiesis and established a working model of autosomal recessive SCN caused by *HAX1* mutations.

P17-014
Biotechnological synthesis of new nucleosides based on 2-aminopurine with a bulky 7,8-difluoro-3,4-dihydro-3-methyl-2 *h*-[1,4] benzoxazine residue at C6 position

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For the first time, novel modified nucleosides based on 2-aminopurine, which contains bulky moieties of enantio pure derivatives of 7,8-difluoro-3,4-dihydro-3-methyl-2*H*-[1,4]benzoxazine at C6 position, were synthesized using the transglycosylation reaction. The corresponding nucleobases were shown to be good substrates for recombinant *E. coli* purine nucleoside phosphorylase (PNP). The best substrates for transglycosylation reaction proved to be bases, in which 2-aminopurine moiety was separated from benzoxazine fragment by an aminohexanoyl spacer (conversion up to 98%). In case of conjugates without a spacer the arabinosylation proceeded slowly. The efficiency of the reaction depended on configuration (*S* or *R*) of the 7,8-difluoro-3,4-dihydro-3-methyl-2*H*-[1,4]benzoxazine fragment. According to our data, *S*-configuration of the methyl group in benzoxazine residue prevents nucleobase binding with the active site of *E. coli* PNP during arabinosylation. However, those nucleobases were good substrates in transribo- and transdeoxyribosylation irrespectively of the 7,8-difluoro-3,4-dihydro-3-methyl-2*H*-[1,4]benzoxazine fragment configuration (*S* or *R*). The nucleosides synthesized are considered as potential inhibitors of intracellular adenosine deaminase. The increasing activity of this enzyme is observed in hepatitis, cirrhosis, hemochromatosis, obstructive jaundice, prostate and bladder cancer, hemolytic anemia, rheumatic and typhoid fever, gout, and Cooley's anemia. The work was financially supported by the Russian Scientific Foundation (grant 14-13-01077).

P17-015
Coordinated expression down-regulation of three small phosphatase genes *CTDSP1/2/L* in lung but not in renal cancer

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SCP family is comprised of three highly homologous small C-terminal serine phosphatases – CTDSP1, CTDSP2 and CTDSPL/RBSP3. We evaluated mRNA level alterations of *CTDSP1/2/L* genes in 24 non-small cell lung cancer (NSCLC) and 44 clear cell renal cell cancer (ccRCC) samples using qPCR. In the majority of squamous cell lung carcinomas (79%, 11/14) including stage I all three genes were down-regulated simultaneously (5-fold decrease in average). In case of lung adenocarcinoma (ADC) *CTDSP1/2/L* genes showed statistically significant increase in the down-regulation frequency during metastases development (up to 100% in metastatic ADC, $P < 0.05$). In ccRCC samples *CTDSPL* showed expression profile distinct from *CTSDP1/2* genes. *CTDSPL* gene revealed down-regulation in 48% of cases (21/44, 4-fold in average), while *CTDSP1/2* were stable in the majority of samples and up-regulated in 20-25% (4-fold in average). Moreover, *CTDSPL* mRNA level decrease was more pronounced in stage III samples compared to stages I+II ($P = 0.03$). Correlation analysis revealed strong positive correlation between *CTDSP1* and *CTDSP2* relative mRNA levels (r_s=0.67-0.86, $P < 0.01$) in both NSCLC and ccRCC. Significant correlation between expression levels of *CTDSPL* and other phosphatases was observed only in ADC (r_s=0.65-0.79, $P < 0.05$). Thus, we showed distinct expression profiles of SCP family phosphatase genes in lung and renal cancers: strong coordinated down-regulation in NSCLC and opposite directional alterations in ccRCC. High correlation coefficients of *CTDSP1* and *CTDSP2* expression levels could suggest common mechanism of their inactivation, likely miRNA. This work was financially supported by grant 14-50-00060 from the Russian Science Foundation.

P17-016
MEFV gene mutation spectrum in Familial Mediterranean fever (FMF) in the South- east region of Turkey

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Background: FMF is an autosomal recessive inflammatory disorder predominantly affects Jews, Armenians, Turks, and Arabs. The FMF gene studies revealed that this 3,505 nucleotide long gene containing 10 exons and 9 introns called the MEFV (Mediterranean fever) gene was coding a protein transcript of 781 amino acids termed as "pyrin" expressed predominantly in granulocytes. Most serious complication is development of amyloidosis. It is caused by several mutations within pyrin.

Methods: In this study, 5198 patients who attended to Dicle University Medical Faculty between 2010–2015 with fever, joint, and abdominal pain complaints were studied with FMF suspicion. DNA was isolated from blood with EDTA. Six mutations were determined with Real-time PCR based TaqMan-Fluoresence methodology.

Results: Mutations have been detected in 1839 (35.4%) patients. Most frequent mutations among the patients were**:** E148Q, M694V, V726A, R761H, M680I, M694I respectively. In 780 patients E148Q mutation (56 homozygous), in 305 patients M694V mutation (86 homozygous), in 292 patients V726A mutation (7 homozygous) in 130 patients R761H mutation (9 homozygous), in 77 patients M680I mutation (19 homozygous) in 21 patients M694I (5 homozygous) were observed. 233 were compound heterozygous for different combinations of mutations. 1 patient had different (triple heterozygous) complex mutation.

Conclusion: This study showed the high frequency and spectrum of FMF mutations in the South- east of Turkey. Turkish, Kurdish, Middle Eastern Arabs, and other ethnic groups have settled in this area. As a result, genetic analysis must be performed in all suspected patients for early treatment in order to prevent possible complications.

P17-017
RNA-based epigenetic mechanism of the malignant transformation: a novel theory of carcinogenesis

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According to our recent studies, non-coding RNAs, hyperexpression (or reactivation) of which is essential for cancer cells, silence:

1) genes encoding DNA repair enzymes and other key elements of DNA repair networks;

2) genes of histone deacetylases, histone methyltransferases and *de novo* DNA methyltransferases;

3) genes encoding nuclear lamina proteins and elements responsible for nuclear lamina-cytoskeleton connections;

4) proapoptotic genes, tumor suppressor genes and genes of cell cycle inhibitors;

5) genes encoding cell adhesion molecules, cytoskeleton components and elements of contact inhibition pathways;

6) genes counteracting expression of the stem cell reprogramming factors.

This leads to genome instability as well as to overall derepression of chromatin. As a result, reactivation of silent mobile genetic elements becomes possible, causing a positive feedback loop between DNA damage level and following derepression of mobile genetic elements. Genes of other non-coding RNAs, which counteract the tumor transformation, undergo silencing. Moreover, in view of the genome instability, we speculate, that some of these genes can undergo the target damage (endogenous gene knockout) as a result of mutations in R-loop during transcription or RNA-dependent DNA methylation. This allows reactivation (or overexpression) of genes responsible for: 1) chromatin remodeling; 2) nuclear transport; 3) proliferation and survival; 4) expression of heteroorganic antigens; 5) cell motility and cell anchoring in other tissues; 6) cell stemness. Therefore, shifts in non-coding RNA profile can themselves cause cell transformation and facilitate the cancer cell stemness. Following mutations of oncogenes and other coding genes, important for transformation, only consolidate their role in carcinogenesis.

P17-018
Expression profiles of 20 miRNAs – predicted regulators of chromosome 3p genes in breast and renal carcinomas

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MiRNAs play a great role in epigenetic regulation of gene expression in tumors. Expression alterations of 20 miRNAs (miR-124a, -125b, -127, -129, -132, -137, -148a, -17, -191, -193-5p, -203, -212, -219, -24 -2, -339-3p, -34a, -34b, -34c-3p, -375, -9) predicted as regulators of some chromosome 3p genes were evaluated in paired samples of breast cancer (BC) and clear cell renal cell cancer (ccRCC) by quantitative real-time PCR with TaqMan MicroRNA Assays Kit (Applied Biosystems, United States). RNU6 mRNA was used as a reference gene. Significant down-regulation of these miRNAs was shown in both BC and ccRCC. Only two miRNAs, miR-203 and miR-9, showed enhanced expression in single cases along with the reduction in the majority. Strong (27-90-fold) down-regulation was shown for miR-193-5p, -129, -34b, -148a, -34c-3p, -375 in 50-75% of BC samples. Equally strong (24-350-fold) down-regulation was found for miR-129, -375, -34b, -124a, -127, -125b, -34c-3p in 50-80% of ccRCC samples. These miRNAs could be suggested as BC and ccRCC diagnostics markers. Comparative analysis of obtained results and the data on expression alterations of chromosome 3p genes, which were predicted as targets according to miRWalk (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/index.html), revealed negative correlation between expression levels in some miRNA-target gene pairs. For example, between expression of RASSF1A mRNA and miR-129, miR-9, and miR-148a, that is important for regulatory networks analysis and searching for new target genes for cancer therapy. This work was supported by grant 14-15-00654 from the Russian Science Foundation and grant 13-04-00828a from the Russian Foundation for Basic Research.

P17-019
Effects of platelet derived serotonin on renal injury

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Platelet activation is associated with tissue damage occured in acute and chronic renal diseases. Serotonin released from activated platelets is suggested to be participate in inflammation and in fibrosis observed after renal injury. Renal proximal tubular cells play a role in response to renal damage by changing their phenotypes. Present study was conducted for investigating whether platelets and platelet-released serotonin are involved in the functional regulation of proximal tubular epithelial cells. Tubular cells were obtained with primary cell culture; confirmed by immunocytochemistry. The phenotypic transition of these cells into myofibroblasts after stimulated with platelet lysate or serotonin were evaluated. Tubulointerstitial fibrosis is characterized by indicating the infiltration of inflammatory cells. TGF-β1 and IL-6 levels determined as regulator of transdifferentiation and indicator for chronic renal disease were found to be increased in mRNA and protein levels following the stimulation with serotonin and platelet lysate suggesting that platelet activation and platelet-released serotonin play a key role in renal tissue damage. Furthermore, relative expressions of MMP-2 and TIMP-1 were found to be also upregulated in mRNA levels after the stimulation. This study was supported by Hacettepe University Scientific Research Project Coordination Unit (Project number: 012 07 301 001).

P17-020
Coordinated down-regulation of the genes encoding neuronal cell adhesion molecules in lung and renal cancers

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Cell adhesion molecules (CAM) play a dual role in cancer. At the early stages these proteins may act as tumor suppressors, but at the later stages CAMs may significantly contribute to the invasive tumor growth and metastasis. We revealed coordinated expression down-regulation of four members of neuronal CAM family L1 – *L1CAM*, *CHL1*, *NFASC*, and *NRCAM* in both metastatic and non-metastatic clear cell renal cell cancer (3–19-fold in 56–85% of samples) and non-small cell lung cancer (2–9-fold in 50–75% of adenocarcinomas and 60–90% of squamous cell carcinomas). These genes demonstrated highly consistent expression alterations (Spearman correlation coefficients r_s=0.42–0.67, $P < 0.001$) which suggests common mechanisms of expression regulation. Using miRStat, a Python-based tool enabling combined analysis of miRNA target prediction resources (TargetScan, mirSVR, DIANA microT, PicTar), we identified miR-182/183 as a high-confidence common regulator of the neuronal CAMs. MiR-182/183 are known to play a crucial role in carcinogenesis via stimulation of epithelial-mesenchymal transition and cell proliferation. Thus, obtained results allow to suggest these genes as important cancer-associated genes with dual role in carcinogenesis. Moreover, the coordinated CAMs down-regulation indicates a presence of common mechanism of their inactivation, e.g. miR-182/183; this needs further experimental validation. This work was supported by grants 13-04-02072-a, 14-04-32084-mol_a from the Russian Foundation for Basic Research; the grant from RAS Presidium Program "Molecular and Cellular Biology"; contract 14.621.21.0001 (project's unique identifier RFMEFI62114X0001) from the Ministry of Education and Science of the Russian Federation."This work was performed using the equipment of EIMB RAS "Genome" center (http://www.eimb.ru/RUSSIAN_NEW/INSTITUTE/ccu_genome_c.php).

P17-021
In utero pesticides exposure and generation of acute myloid leukemia associated translocation (8;21)

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Background: Although the etiology of childhood acute myeloid leukemia (AML) is not known, environmental and genetic contribution were reported. The aim of this study was to detect the

relationship between *in utero*-exposure to pesticides and development of acute myeloid leukemia (AML) associated translocation (8;21).

Subject and methods: Cord blood and fetal meconium were collected from 190 subjects. Four Pesticides (DDT, Lindane, Diazinon, and Malathion) were detected in meconium by gas chromatography and mass-spectrometry (GC-MS). AML translocation (8;21) was detected by RT-PCR on RNA extracted from cord blood.

Results: Thirty eight out of 190 (20%) of the cord blood samples were positive for the AML1-ETO translocation. The mean levels of the 4 tested pesticides were higher in meconium of the AML-ETO translocation carriers; P value is < 0.001 for DDT, and Malathion, 0.004 for Diazinone, and 0.042 for Lindane. Rural residents showed higher frequency of translocation detection than urban residents (P value = 0.007), they also expressed higher values of pesticides; P values are 0.04, 0.02, 0.04, and 0.01 for DDT, Lindane, Malathion, and Diazinon respectively. Maternal age, gestational age, birth weight and working status of the mothers showed no impact on the rate of translocation detection or pesticides levels.

Conclusion: Pesticides exposure is potentially related to the occurrence of AML (8;21)translocation in cord blood of the apparently healthy newborn. Being rural resident seems to increase the possibility of exposure to pesticides; it subsequently imparts a higher risk for carrying such leukemia translocation. Strict regulation for pesticides uses is indicated.

P17-022
Therapeutic plasma exchange restore expression profile of monocytes in antiphospholipid syndrome

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Antiphospholipid antibody syndrome (APS) is an autoimmune disease that is characterized by vascular thrombosis and recurrent miscarriages. Persistence presence of antiphospholipid antibodies (aPL) is well linked to the disease pathogenesis, however the precise mechanism of aPL-mediated thrombus formation remains unknown. Recent studies have implicated critical role of monocytes activation in hypercoagulable state in APS. Our study was aimed to determine the impact of plasma exchange therapy (PE) on transcriptional state of monocytes in APS patients. This treatment modality is accepted method for treatment of pregnant women with thrombotic complications or autoimmunity, but rarely used in APS. mRNA levels of eleven selected genes were assessed in monocytes from nine healthy subjects and eleven APS patients with recurrent miscarriages before/after PE course, using qRT-PCR method. Baseline expression of IL-1β, IL-6, IL-23, CCL2, TLR2, and STAT3 was significantly down-regulated and CXCL10 up-regulated in APS monocytes as compared with healthy cells. PE therapy resulted in increased IL-1β, IL-6, IL-23, CCL2, P2X7, TNFα and decreased STAT3 mRNA levels in APS monocytes. Comparison of gene expression profiles in APS patients after PE and healthy subjects showed that up-regulated mRNA levels of APS monocytes tended to return to normal ranges. Furthermore, PE therapy counterbalanced the expression levels of CCL2 and CXCL10 which levels are indicative of Th1/Th2 balance. Thus, our results showed that peripheral blood monocytes from APS patients characterized by distinct profile of gene expression. PE therapy exerts its effect by normalizing transcriptional activity of APS monocytes.

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P17-023
Long noncoding RNA-ABHD11-AS1 in gastric juice using as a new biomarker for screening gastric cancer

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Aim: Long noncoding RNAs (lncRNAs) play vital roles in tumorigenesis and tumor progression. However, the clinical diagnostic values of most lncRNAs in the screening of gastric cancer are largely unknown. The aim of this study is to investigate whether gastric juice ABHD11-AS1, a lncRNA, can be a potential biomarker for screening patients with gastric cancer.

Methods: Total of 173 tissue samples and 130 gastric juice samples from four stages of gastric tumorigenesis were first collected and its ABHD11-AS1 levels were detected by real-time reverse transcription-polymerase chain reaction. Then the relationships between ABHD11-AS1 levels and clinicopathological factors were further investigated. Finally, receiver operating characteristics (ROC) curves were constructed and ABHD11-AS1's diagnostic value was determined.

Results: ABHD11-AS1 levels in gastric cancer tissues were significantly higher than those in other tissues. And its levels in gastric juice from patients with gastric cancer were significantly higher than those from cases of normal mucosa or minimal gastritis, atrophic gastritis and gastric ulcers. Its levels in gastric juice from patients with gastric cancer were associated with gender, tumor size, tumor stage, Lauren type and blood CEA levels. The area under the ROC curve was up to 0.653.

Conclusion: Gastric juice lncRNA-ABHD11-AS1 may be a potential biomarker for screening gastric cancer.

P17-024
Tobacco mosaic virus-based vectors displaying conserved Influenza antigens: host range, tissue localization and peculiarities of joint infections

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Recombinant TMV-based viruses containing different versions of conserved Influenza M2e epitope on the surface of chimeric particles were created by our group previously (Petukhova *et al.*, 2013, 2014). They provide appropriate model system for studying some poorly known aspects of long-distance movement and host-virus interactions. Accumulation of coat proteins synthesized in systemic and inoculated leaves during infections of recombinant TMV-M2e-cys, TMV-M2e-ser and TMV-M2e-ala viruses was determined. For *Nicotiana tabacum* and *Chenopodium quinoa* the long-distance movement via vasculature was quite efficient. None of the viruses was capable of infecting *Nicotiana rustica* systemically. Cuts of inoculated and upper leaves with clear symptoms of TMV-M2e infections (14 days post inoculation) were stained with Toluidine blue and examined using transmitted light microscopy. We did not observe significant morphological differences between these mutants in conducting tissues. Histochemical analysis of TMV-M2e viruses' localization with M2e-specific antiserum in major and minor veins as well as mesophyll was per-

formed. Additional virus carrying conserved fusion peptide (fp) from N-terminus of Influenza hemagglutinin HA2 subunit (14 predominantly hydrophobic amino acid residues) was constructed. The sequence coding for this antigen was cloned into the TMV-U1 coat protein gene instead of M2e epitope between 155th and 156th residues. Symptoms and development of mixed infections of *Nicotiana benthamiana* (TMV-M2e and TMV-fp) were significantly distinguishable from separate inoculations. For example, TMV-fp led to systemic necrosis and flexion of the stem within 3 weeks comparing with white mild chlorosis of upper leaves. Expression of both antigens in upper leaves was confirmed by Western blotting.

P17-026
RNA effectors in combination with small molecule drugs for cancer treatment

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MiRNAs are often deregulated in cancer. We have found that the oncogenic Pim-1 kinase is a target for miRNA regulation [1-3] and we have applied several RNA-based strategies to explore Pim-1 as a tumor target in mouse xenografts of colon carcinoma and glioblastoma. Delivery of miRNA mimics or Pim-1-specific siRNAs into tumors was achieved by using polyethylenimine (PEI) as a delivery agent. This approach was also used to establish a new antisense strategy *in vivo* which is called U1-interference (U1i) [4]. Combinatorial approaches using RNA effectors and small molecule drugs for cancer treatment might be an interesting option to reduce the risk of chemoresistance and to lower unwanted side effects. We are testing RNA-based inhibition of Pim-1 in combibation with statins, natural compounds and 5-FU to lower the effective doses of these molecules. Statins have well-known pleiotropic antitumor effects at low micromolar concentrations and we found that Pim-1 levels can be reduced by statins. Importantly, strategies combining statins with siRNA or other Pim-1 inhibitors improve statin-dependent effects, thus lowering the statin concentrations for Pim-1 inhibition towards the nanomolar range. We will now evaluate Pim-1 specific RNA effectors with therapeutic relevant statin concentrations in mouse xenografts.

P17-027
Influence of microRNA expression profiles on the efficacy of radiochemotherapy in locally advanced head and neck squamous cell carcinoma

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The treatment of locally advanced head and neck squamous cell carcinoma (HNSCC) is still challenging with 5-year survival rates less than 60% resulting from a high frequency of local and/or regional tumor recurrence. Hence,the identification of molecular markers, which predict for therapy efficacy and improve patient selection for optimized treatment are therefore of high clinical relevance. MicroRNAs (miRNA) as modulators of cancer progression can function as such biomarkers. In this study we evaluated the impact of miRNA expression on the efficacy of radiochemotherapy on HNSCC. Formalin-fixed, paraffin-embedded tumor material was collected from patients with locally advanced HNSCC, who had been treated with hyperfractionated accelerated radiotherapy in combination with either 5-fluorouracil/cisplatin (5-FU/CDDP) or 5-fluorouracil/mitomycin C (5-FU/MMC) within the ARO0401 phase III trial. microRNA profiles of 50 tumors tissues were established by Affymetrix miRNA microarrays. Results were validated in samples from 149 HNSCC patients by quantitative real-time PCR (qRT-PCR). The expression levels of 15 miRNAs were identified to correlate with overall survival of patients treated with MMC-based chemoradiation, whereas the expression levels of 10 other microRNAs were correlated with overall survival of patients treated with CDDP-based chemoradiation. The expression pattern of miR-200b and miR-146a were validated by qRT-PCR, each of them being significantly correlated to overall survival in the respective study arm. Our results revealed that the correlation of the miRNAs expression was dependent on the tumor subsite and correlate with local recurrence or metastases. Therfore miRNA expression levels can be used as predictive markers for the efficacy of chemoradiation.

Mol Neu S1, Neuronal Ion Channels and their Role in Disease

P20-005-SP
Scorpion toxin fused with fluorescent protein is a novel probe to study potassium channels

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Scorpion venoms are a rich source of active polypeptides that interact with ion channels modifying their properties. Such molecules, called toxins, were successfully used in pioneer works where the structure and functions of various channels were studied. In recent years, polypeptide ligands acting on ion channels have demonstrated an immense potential in the field of drug discovery and development of diagnostic systems. The major target of these investigations is potassium channels, one of the most widespread superfamily of membrane proteins involved in many pathological processes. Here we present a fluorescent protein-scorpion toxin chimera that can be used in research of potassium channels for imaging purposes. We designed and constructed a fusion molecule based on eGFP and OSK1 (toxin purified from the venom of *Orthochirus scrobiculosus* in our laboratory). The chimera showed expected selectivity with nanomolar affinity to several potassium channel isoforms. We rationalize that our tool is easy to use and can be produced just by the recombinant technique, avoiding any chemical modifications. As an outlook, we suggest that molecules with similar design will find successful applications throughout neurobiology. This work was supported by Russian Science Foundation (Grant no. 14-14-00239).

P20-006-SP
KcsA-Kv1.2 hybrid channel embedded in *E.coli* cell membrane: design, properties, applications

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KcsA-Kv1.2 hybrid channel was designed by forming the ligand-binding site of a voltage-gated potassium Kv1.2 channel within the scaffold of a bacterial KscA channel. Enhanced KcsA-Kv1.2 expression and embedding into membrane of *E.coli* cells were achieved. After transformation of cells into spheroplasts, KcsA-Kv1.2 binds specifically Kv1.2 pore blockers including gene-coded variants of fluorescent hongotoxin and maurotoxin created by us. Following a general approach proposed by us previously [1,2] an advanced analytical system for search and study of Kv1.2-channel blockers was developed. It provides recognition of high-affinity pore blockers (Kd of 1pM to 1 μM) even in complex mixtures, measurement of their dissociation constants and investigation of molecular determinants of Kv1.2 binding. Kv1.2 is widely presented in the brain, involved in the regulation of neuronal action potential and muscle contractionin the cardiovascular system, participating in the perception of neuropathic pain. Specific blockers of Kv1.2 may become promising therapeutic agents. Our analytical "mix and read" system is shown to be convenient alternative to radioligand assay in seeking and construction of selective Kv1.2-channel blockers of high scientific and medical importance. This work was supported by the grant 14-14-00239 from Russian Science Foundation.

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P20-007
Toluene, hippocampus structure and recognition memory: adult and adolescent rats

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Toluene and toluene-containing volatile substances are the most widely abused solvents with demonstrative addictive potential in humans. Clinical and experimental studies have demonstrated that the exposure to toluene vapor leads to diverse consequences at the level ranging from the cell to the whole organism. The present study has been undertaken to determine whether toluene chronic exposure provokes immediate and/or persistent effect on the structure of hippocampus, learning and memory in adolescent and adult rats. We exposed male Wistar rats at ages P 28–32 (adolescents) and P 150–160 (adults) to 2000 ppm inhaled toluene for 40 days. The immediate and persisting effects of toluene misuse (immediately after the end of toluene chronic inhalation and 90-day after the end of toluene chronic inhalation, correspondingly) on pyramidal cell loss in the CA1 and CA3 of the hippocampus and exploratory behavior and recognition memory in the open field were evaluated. The results reveal that toluene chronic exposure affects the structure of the hippocampus, exploratory activity and recognition memory in the open field in adolescent and adult rats. In all cases the effect is age-dependent. In particular: in adolescent rats the more significant structural and behavioral alterations were observed immediately after toluene chronic exposure, while in adult rats the most considerable was persisting effect (90 days after withdrawal). Such data indicate that character of alterations depends upon the postnatal age of testing of the animals.

P20-008
The elevated level of full-length presenilin-1 associated with Alzheimer's disease enhances store-operated calcium currents in neuronal cells

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Around 43% of associated with Familiar Alzheimer's disease mutations are located in presenilin-1 (PS1) gene. The PS1 protein undergoes endoproteolysis, whereupon acts as a catalytic subunit of γ-secretase participating in production β amyloid. A decreased endoproteolysis level of PS1 was shown in the brain tissues of Alzheimer's disease patients. We examined the role of PS1 endoproteolysis in disturbances of SOC entry in Alzheimer's neuronal cell models. Calcium imaging experiments with Neuro2a mouse neuroblastoma cells incubated with γ-secretase inhibitor L-658,458 demonstrated significantly elevated SOC entry compared to control cells. Whole-cell electrophysiological recordings proved an increase in SOC currents in Neuro2a cells with attenuated endoproteolysis of exogenous human PS1, as well as of native mouse PS1. The integral SOC currents increase was observed in Neuro2A cells expressing PS1 D257A, which does not undergo endoproteolysis, but not in wild-type PS1 expressing cells. The elevated SOC currents were observed in mouse hippocampus neurons expressing PS1 D257A compared with control. The expression neither PS1 D257A nor wild-type PS1 did not affect the expression levels of main SOC entry players. We suppose that the elevation of SOC currents is due to the increased ratio of full-length PS1 to its terminal fragments. These fragments may compete with full-length PS1 for the interaction with common targets, thereby attenuating the effect of the full-length PS1. This work was supported by the Russian Scientific Foundation, projects №14-14-00720, the program of "Molecular and Cellular Biology" RAS, the Russian Basic Research Foundation №14-04-31280, the President of Russia Scholarship and SS-1721.2014.4.

P20-009
Deregulation of store-operated calcium channels in Huntington-specific human neurons

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Huntington's disease (HD) is an autosomaldominant hereditary neurodegenerative disorder which manifests in neural loss predominantly of GABA-ergic medium spiny neurons (GABA-MSNs) in striatum. HD is caused by polyglutamine expansion within Huntingtin protein. Previously we noted that neuronal store-operated calcium (SOC) channels could play a significant role in HD pathogenesis. Here we examined the changes in SOC current in human GABA-MSNs differentiated from induced pluripotent stem cells (iPSCs), obtained by somatic reprogramming

of patient-specific fibroblasts. We studied 3 different GABA-MSN lines from patients, suffering from HD. As a control, we used 2 GABA-MSN lines from healthy subjects and 1 line of GABA-MSNs differentiated from healthy embryonic stem cells (ESCs). All used HD-GABA-MSNs had an endogenous expression of mutant Huntingtin with only 40-45 glutamine residues that is close to be norm. Nevertheless we recorded the significant (2-fold) increase of the SOC currents in these cell lines compared to control GABA-MSNs.Also we indicated no differences in SOC currents in control neurons differentiated from iPSCs or ESCs. It should be noted that all lines of HD-GABA-MSNs reveal similar characteristics of the SOC currents that indicates a validity and well-reproducibility of this iPSCs-based HD model. Further we showed that EVP4593 (quinazoline-derived compound) could decrease abnormal SOC entry in HD-GABA-MSNs. The described abnormalities in Ca homeostasis in HD-GABA-MSNs give the opportunity to regard this model as a most adequate for both fundamental and applied studies of neurodegeneration. The study was supported by the RSF, RFBR (14-04-31137) and the fellowship of the President of RF.

P20-010
Structure-function study of human secreted Ly-6/uPAR related proteins SLURP-1 and SLURP-2 suggests multiple molecular targets

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SLURP-1 and SLURP-2 are produced in various human tissues, including epithelium and immune system. SLURPs play a role of autocrine/paracrine hormones regulating growth and differentiation of epithelial cells and take part in the control of inflammation and oncogenic transformation. As supposed, SLURP effects are mediated by interaction with nicotinic acetylcholine receptors (nAChRs). Using affinity purification from human cortical extracts, we demonstrated that recombinant SLURP-1 binds only with α7 nAChR subunits, while recombinant SLURP-2 binds with α3, α4, α5, α6, α7, β2, and β4 subunits. Study of SLURP-1 and SLURP-2 effects on human colorectal adenocarcinoma cells HT-29 revealed marked antiproliferative effect. Incubation of cells with 1 mM SLURP-1 and SLURP-2 during 48 h, led to reduction of a cell number down to ~ 54 and 63 % relative to a control, respectively. Dose-response curves revealed the concentration-dependent mode of SLURP-1 and SLURP-2 action with EC_{50} ~ 0.1 and 0.2 nM, respectively. Spatial structure of SLURP-1 was determined by NMR-spectroscopy. Unusual conformational plasticity of 'three-finger' SLURP-1 structure was revealed. Computational modeling points to the central loop of SLURP-1 as the major determinant of interaction with α7-nAChR. In contrast, mapping of mutations involved in development of Mal de Meleda autosomal skin disease revealed other functional epitope located on the C-terminal loop of SLURP-1. These findings imply the presence of alternative molecular targets of SLURP-1 action. The work was supported by the Russian Scientific Foundation (project № 14-14-00255) and Russian Academy of Sciences (the Program of Molecular and Cell Biology).

P20-011
Structure-function study of human SLURP-1 and SLURP-2 suggests multiple molecular targets

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Secreted Ly-6/uPAR Related Proteins SLURP-1 and SLURP-2 are produced in various human tissues, including epithelium and immune system. These proteins play the role of autocrine/paracrine hormones which regulate growth and differentiation of epithelial cells and take part in the control of inflammation and oncogenic transformation. As supposed SLURP effects are mediated by interaction with nicotinic acetylcholine receptors (nAChRs). Here we describe bacterial expression systems and the refolding protocols for SLURP-1 and SLURP-2. Comparative study of SLURP-1 and SLURP-2 effects on human colorectal adenocarcinoma cells HT-29 revealed the marked antiproliferative effect. Incubation of cells with 1 mM SLURP-1 and SLURP-2 during 48 h, led to reduction of a cell number down to ~ 54 and 63 % relative to a control, respectively. Fluorescent microscopy did not reveal nor apoptotic nor necrotic cell death. Dose-response curves revealed the concentration-dependent mode of SLURP-1 and SLURP-2 action with EC_{50} ~ 0.1 and 0.2 nM, respectively. Spatial structure of SLURP-1 was determined by NMR spectroscopy. Unusual conformational plasticity of the 'three-finger' SLURP-1 structure was revealed. Computational modeling points to the central loop of SLURP-1 as the major determinant of the interaction with α7-nAChR. On the other hand, mapping of mutations involved in development of Mal de Meleda autosomal skin disease revealed other functional epitope located on the C-terminal loop of SLURP-1. These findings imply the presence of alternative molecular targets of SLURP-1 action. The work was supported by the Russian Scientific Foundation (project № 14-14-00255) and Russian Academy of Sciences (Program "Molecular and Cellular Biology").

Mol Neu S2, Mechanisms of Nervous System Development and Regeneration

P21-003-SP
The small GTPase RAB6 regulates localization of the Cohen syndrome-associated protein COH1 to the Golgi complex

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Postnatal microcephaly, intellectual disability, and progressive retinal dystrophy are major clinical features of autosomal recessive Cohen syndrome, which is caused by mutations in the gene *COH1*. *COH1* encodes a protein of 3997 residues, which harbors two short regions homologous to yeast Vps13p. Previously, we identified COH1 as a peripheral scaffold protein that contributes to the structural maintenance and function of the Golgi complex. Another study showed that disturbed Golgi complex homeostasis

affects glycan maturation and that COH1-deficient cells display a reduced amount of early endosomes and abnormally enlarged lysosomes, pointing to a role of COH1 in endosomal-lysosomal trafficking. Here, we show that association of COH1 with the Golgi complex depends on RAB6. RNAi-mediated knockdown of RAB6A/A' prevents the localization of COH1 to the Golgi complex. In line, expression of the constitutively inactive RAB6_T27N mutant led to an increased solubilization of COH1 from lipid membrane preparations. Co-immunoprecipitation experiments confirmed the physical interaction of COH1 with RAB6, which is in line with studies on yeast Vps13p. Our ongoing work focusses on *Coh1* expression analyses, cortical development studies using RNAi and identification of other COH1 interactors similar to the known yeast Vps13p network. Initial experiments demonstrate that depletion of COH1 in primary neurons negatively interferes with neurite outgrowth, indicating a causal link between the integrity of the Golgi complex and axonal outgrowth. We conclude that COH1 is a RAB6 effector protein and that reduced brain size in Cohen syndrome patients likely results from impaired COH1 function at the Golgi complex, causing decreased neuritogenesis.

P21-004-SP
Neuronal NOS is involved in the neuronal differentiation of hippocampal neural progenitor cells

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In this study, we investigated the possible role of nNOS in the neuronal differentiation. Employing neural progenitor cells from the brain hippocampus of E16 rat embryos, we showed the expression level of nNOS increased during neuronal differentiation. In addition, expression levels of neurotrophin-3 (NT3), neurotrophin-4/5 (NT 4/5), Synapsin I and Tuj1 were increased, but they were decreased by nNOS inhibitor, 7-nitroindazole (7-NI), resulting in suppressed neurite outgrowth. To figure out the effect of nNOS in neuronal differentiation, we transfected nNOS siRNA into hippocampal neural progenitor cells. Knockdown of nNOS decreased expressions of NT3, NT4/5, Synapsin I and Tuj1 as well as neurite outgrowth, while the increased expression of an astrocyte marker, GFAP, was not changed by nNOS knockdown or 7-NI. These results suggest that nNOS plays a critical role in neurite outgrowth during differentiation of hippocampal neural progenitor cells. To elucidate the mechanism by which nNOS regulated neuronal differentiation, we studied neuronal NO signaling such as phosphorylation of PLCγ, PKCα, Akt, Src and ERK1/2. We showed that nNOS knock-down or 7-NI decreased phosphorylation of PLCγ and PKCα, but had no effect on the phosphorylation of Akt, Src and ERK1/2. In conclusion, this is the first evidence to show that nNOS acts as an important regulator of neurite outgrowth in hippocampal neural progenitor cells by promoting neuronal differentiation through PLCγ/PKCα signaling.

P21-005-SP
Role of hippocalcin in early developmental stage of hippocampal neurogenesis

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The purpose of this study is to examine the role of hippocalcin in early developmental stage of hippocampus. Mechanically dissociated cells from rat brain hippocampus of embryonic (E) day 16 or 17 were used to isolate hippocampal neural progenitor cells (HNPCs). When hippocalcin was overexpressed in E16 HNPCs, neurotropnin-3 (NT-3), neurotropnin-4/5 (NT4/5), Brain-derived neurotrophic factor (BDNF), Neurogenin 1 (Ngn1), and NeuroD were dramatically increased during proliferation but not in E17 HNPCs. In addition, Neuron-specific class III beta-tubulin (Tuj1)-positive cells were increased in E16 hippocalcin-transfected cells compared to control vector-transfected cells. On the other hand, Tuj1-positive cells were decreased in E17 hippocalcin-transfected cells compared to control vector-transfected cells. These results indicate that hippocalcin has an opposite role in hippocampal neurogenesis between E16 and E17 HNPCs. Next, we transfected hippocalcin siRNA into E16 or E17 HNPCs. Interestingly, expression levels of NT3, NT4/5, BDNF, Ngn1 and NeuroD were decreased by knockdown of hippocalcin in E16, but they were increased in E17. Taken together, we suggest that hippocalcin might be an important regulator of hippocampal neurogenesis in early developmental stage.

P21-006-SP
SNX482 inhibits semaphorin 3A induced sensory axon growth cone collapse

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During axon navigation and regeneration after injuries a guidance molecule semaphorin 3A (sema3A) is one of the principal proteins in repulsing axons and inhibiting their growth. It is now known that calcium (Ca^{2+}) is important in axon response to attractive guidance cues. On the other hand it still remains elusive if this is also true for repulsive guidance cue sema3A. In this study intracellular calcium ([Ca2 +]i) imaging was used to evaluate if sema3A-induced growth cone collapse is Ca^{2+} dependent. $[Ca^{2+}]_i$ imaging with ratiometric dye Fura-2 AM showed Ca^{2+} increase in E15 mice dorsal root ganglia neuron growth cones in response to sema3A. Therefore Sema3A effects on growth cones after modifying $[Ca^{2+}]_i$ and $[Ca^{2+}]_e$ channels that we have shown are expressed in E15 mouse embryos were evaluated. Results of our study showed that sema3A indeed increased growth cone collapse rate that was blocked by the non-selective R- and T- type Ca^{2+} channel inhibitor $NiCl_2$ and by the selective R-type Ca^{2+} channel inhibitor SNX482. Ca^{2+} channel inhibitors used have decreased the sema3A-induced $[Ca^{2+}]_i$ concentration elevation. Results of this study demonstrated that sema3A-induced growth cone collapses are related to the increase in $[Ca^{2+}]_i$ concentration in sensory axon growth cones and this is mediated through the R-type calcium channels.

P21-007
Deciphering the genetic program of neuronal axon remodeling during development

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Developmental neuronal remodeling is essential for sculpting the mature nervous systems of vertebrates and invertebrates during development. Neuronal remodeling often involves pruning of

exuberant neuronal connections and regrowth to new targets as a mechanism to refine neural circuits during development. The stereotypical remodeling of the *Drosophila* mushroom body γ neurons offers a unique opportunity to study both axon pruning and axon regrowth. Mounting evidence from our lab and others suggest that the γ neurons developmental axon remodeling is regulated, at least partially, by distinct transcription factors. My goal is to uncover the genetic program underlying neuronal remodeling of γ neurons during development. First, I established a system for obtaining high quality gene expression profiles from 1000 γ neurons isolated from intact brains at different developmental stages. Each step in this system – labeling the cells, dissociate the brains, purifying the labeled fraction and extract the mRNA was well optimized. Then, I utilized state of the art techniques in next generation sequencing optimized to uncover the transcription profile of small quantities of RNA. Preliminary sequencing data indeed highlighted many genes known to participate in remodeling. I am currently improving my developmental data by obtaining more developmentally relevant time points as well as isolating mutant neurons to identify targets of specific transcription factors. In a few months, we will know the genetic landscape of the mushroom body neurons during development. Following up this genomic data with genetic screens should increase our mechanistic understanding of neuronal remodeling.

P21-008
Biosensor approach to the detection of neuroactive steroids

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The detection of neurosteroids in complex environment is urgent aim requested by neurophysiology. Usually such methods as radioimmunoassay, mass spectrometry, gas chromatography are using for the neuroactive steroids detection. However these methods are quite costly, time consuming and does not give information in real time. Biosensor systems based on optoelectronic transducers make it possible to design devices that generate an informative signal in real time without any additional labelling of target molecules. Analytical approaches based on surface plasmon resonance (SPR) phenomena provide biosensor methods suitable for a wide range of both fundamental and practical applications. Here we present one of such approaches based on the inhibition-type competitive analysis. As a model system we used Estradiol. We performed our investigations using an SPR spectrometer "BioHelper" (with a GaAs laser as source of excitation at the wavelength $\lambda = 650$ nm) that was developed at the V. Lashkaryov Institute of Semiconductor Physics of the National Academy of Sciences of Ukraine. For the performance of the competitive analysis we used the Estradiol-BSA conjugate immobilization at the NCS-modified surface. The importance of the solution pH for the binding of the specific antibodies with the immobilized conjugate was demonstrated. Preliminary results for model solution were: standard curve limits: 0.1–1000 ng/ml; limit of detection: 0,1 ng/ml; limit of quantification : 1 ng/ml, which is suitable for the measurements in human blood serum.

P21-009
Study of the homophilic binding of the neural cell adhesion molecule (NCAM)

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The neural cell adhesion molecule (NCAM) is a glycoprotein of the immunoglobulin (Ig) superfamily. It is a cell adhesion molecule, mediates cell-cell and cell-matrix interaction via homophilic NCAM-NCAM binding and also via heterophilic binding. The exact mechanism of homophilic binding is still unknown and causes controversy. There are different models and theories, which most focuses on the first three immunoglobulin domains. It is assumed, that the cis-interaction is mediated by the Ig1 and Ig2, whereas the trans-interaction is mediated by Ig3, which binds to Ig1 and Ig2. We wanted to investigate the mechanism of the homophilic NCAM-NCAM binding in cell culture. For that reason we used NCAM-GFP-constructs and expressed NCAM-GFP in Hela cells. The NCAM-GFP-fusion proteins are localized along the cell-cell contacts and can be visualized via fluorescence microscopy. We designed different NCAM Ig-deletion mutants, to determine the influence of the different domains on homophilic NCAM binding. In contrast to the classical *in vitro* studies we could show in cell culture that after the deletion of each of the first three immunoglobulin domains, the signals along the cell-cell contacts and therefore the homophilic binding decreases in amount and intensity.

P21-010
A novel normalization based approach for somatic Alu insertions identification in human brain cells

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Retroelements (RE) comprise substantial part of mammalian genomes including humans. However, only a minor part of genomic elements are still active giving rise to new germline and somatic insertions in the genomes of normal and malignant cells. Somatic mosaicism resulted from new RE insertions is speculated to play a significant role in adult neurogenesis in particular contributing to individual neurons plasticity. Essential part of REs known to be active belong to the AluYa5, a nonautonomous primate specific SINE subfamily. Identification of somatic Alu insertions is complicated by the presence of an enormous amount of very similar though inactive elements in each human cell. Although implication of high throughput sequencing technologies resulted in great progress in somatic RE studies it still remains expensive and labor consuming. Here we describe a novel normalization based approach for somatic Alu insertions identification in the human genome. The method includes selective amplification of sequences flanking Alu insertions, genomic normalization using the Kamchatka crab duplex-specific nuclease and high throughput sequencing by Illumina. The approach was used to find somatic Alu insertions in the genomes of human brain cells. The use of this method leads to a more than 20 fold

increase in the efficiency of somatic Alu identification. We were able to identify 399 somatic insertions in approximately 50 000 nuclei from the human frontal cortex. This work was supported by the state contract 14.604.21.0118 and Russian Foundation for Basic Research (RFBR-12-04-33065).

P21-011
Proglyprol conjugates with docosahexaenoic acid and dopamine induce neuromorphogenesis in C6 glioma and PC12 pheochromocytoma cell lines

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Tripeptide Pro-Gly-Pro (PGP, or proglyprol) is a member of the group of regulatory peptides known as glyprolines. Proglyprol is involved in the formation of the inflammatory reaction in some respiratory diseases and has protective effects in experimental models of cerebral ischemia, diabetes, septic shock, and gastric ulcer. The aim of this work was to study the long- and short-term effects of PGP derivatives with dopamine (DA) and docosahexaenoic acid (DHA) on cancer cells.

C6 and PC12 cells were grown according to the ATCC recommendations. For the experiments, cells were seeded at the density of $1.5x10^5$ or 500 cells/cm^2 and incubated with various concentrations of the test compounds for 20 h (short-term) or 10 days (long-term). Cell viability was assessed using the MTT test. Cell morphology was evaluated using phase contrast light microscopy. Gene expression was analyzed with RT-qPCR using commercially available kits.

Both short- and long-term incubation revealed that PGP, PGP-DA and DHA-PGP were neither cytostatic, nor cytotoxic. DHA-PGP-DA and DHA-DA were cytotoxic for both cell lines with LD_{50} values in the range 4-60 μM after a short-term incubation. After the long-term incubation with the LD_{50} of these compounds cell bodies of both cell lines enlarged and long processes appeared (2-3 per cell for C6 and 3 or more for PC12). Differentiation marker (NSE, beta-3 tubulin, GFAP, MBP) expression analysis revealed astrocytic differentiation in C6 cells and neuronal differentiation in PC12 cells. The differentiation was reversible. The work was partially supported by the MK-3842.2015.4 and RFBR 13-04-40085-N, 13-04-40083-N grants.

P21-013
Catalytic soman scavenging by non-aging acetylcholinesterase mutant assisted with novel site-directed aldoximes

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Poisoning caused by the nerve agent soman calls for immediate treatment, which usually consists of a combined administration of an anticholinergic drug and an oxime as the reactivator of the enzyme acetylcholinesterase (AChE). However, due to the rapid dealkylation of the soman-AChE conjugate known as aging, there are no effective reactivators or satisfactory antidotal therapies for soman exposure. The efficacy of the recommended nerve agent bioscavenger, butyrylcholinesterase, administered intravenously, is limited by strictly stoichiometric scavenging. To overcome this gap, we tested *ex vivo* in human blood and *in vivo* in soman-exposed mice, the capacity of the aging-resistant human AChE mutant Y337A/F338A in combination with oxime HI-6 to act as a pseudo-catalytic bioscavenger of soman. The pyridinium oxime, HI-6, was previously shown *in vitro* to be the most efficient reactivator of this mutant following soman, as well as VX, cyclosarin, sarin and paraoxon inhibition. Here, we demonstrated that *ex vivo* 1 μM of soman was hydrolyzed within 30 minutes when supplemented with 0.5 μM Y337A/F338A and 100 μM HI-6. This combination was further tested *in vivo*. Catalytic scavenging of soman in mice improved the therapeutic outcome and resulted in a delayed onset of poisoning symptoms. Furthermore, to identify a more efficient oxime than HI-6, we screened novel imidazole-pyridinium 2-aldoximes, for reactivation of soman-inhibited Y337A/F338A. Oxime RS2-170B [4-carbamoyl-1-(3-(2-((hydroxyimino)methyl)-1H-imidazol-1-yl)propyl)pyridinium showed the reactivation superiority over HI-6. This could be due to the smaller imidazole ring, as indicated by computational molecular models, which may allow a more productive angle of nucleophilic attack.

P21-014
Targetting PTEN and associated signalling networks in axonogenesis

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The protein PTEN is a tumour suppressor that functions by antagonizing the activity of PI3K and its downstream signalling pathways. PTEN is highly expressed in neurons and its de-regulation affects important neuronal functions in the nervous system. Loss of PTEN expression has been shown to promote axonal elongation and increase the survival of cell bodies and axon terminals of degenerating motor neurons, as well as promotes regenerative growth of axonal processes following injury to the CNS. Currently it is believed that PTEN-deficiency induced neuronal growth responses involve an up-regulation of the PTEN effector mTor. Therefore, we believe that other PTEN-dependent signalling pathways act in concert with mTor in axonogenesis. Firstly, PTEN absence is likely to inhibit GSK3 activation, which has been shown *in vitro* studies to improve microtubules stability in neurons. Secondly, by regulating membranous phosphoinositides, PTEN is likely to act directly at the level of cell membrane by recruiting protein complexes known to regulate actin dynamics. One of these proteins, Lamellipodin (Lpd), is a scaffold protein linked to the actin dynamics. Lpd is able to recruit actin binding proteins participating in the lamellipodia formation, the morphogenesis of the axon and the development of the dendrites. Lpd recruitment to the leading edge is tightly connected with the phosphoinositides regulation and PI3K/PTEN pathway. Indeed, our work indicates that Lpd is recruited to the membrane after insulin stimulation in neuronal cell lines and in peripheral neurons, whilst PI3K inhibition inhibits this phenotype, indicating a correlation between PTEN/PI3K regulation and Lpd function.

P21-015
Identification of transmembrane pseudo-phosphatase Plasticity related gene 2 as an interacting partner of PTEN

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We identified the transmembrane protein Plasticity Related Gene 2 (PRG2) as a novel binding partner of PTEN in mouse brain. PTEN is an important tumor suppressor and negative regulator of the PI3K pathway with established roles in neuronal circuit formation. PRGs belong to the family of lipid phosphatases/phosphotransferases and share high homology with bioactive lipid (e.g. LPA, S-1-P) inactivating phosphatases, influencing cell migration and neurite retraction. We hypothesize that the C-domain of PRG2, containing a unique and highly acidic polyglutamate stretch, may control PTEN membrane localization and/or activity. For example, PRG2 may sequester PTEN away from the membrane and provide an efficient 'off'-switch for PTEN-mediated inhibition of the PI3K/Akt pathway. Our work supports this idea: Overexpression of PRG2 in HEK cells antagonizes PTEN function towards decreasing PI3K/Akt signaling. Further analyses demonstrate that PTEN interacts with PRG2, whilst different regions within the C-terminal PRG2 domain, participate in PRG2-PTEN interaction. Importantly, a mutant PRG2 lacking the acidic stretch, still binds PTEN but is ineffective in relieving PTEN-dependent downregulation of pS473 Akt phosphorylation in cells. To study the role of this interaction, we established inducible ES cell clones expressing tagged versions of PRG2 and deletion variants. Following neuronal differentiation into ES cell derived motor neurons and induction, PRG2 localizes prominently to plasma membrane domains and active growth cones, increases in early axonal outgrowth and filopodia length. Our results suggest that PRG2 may regulate neuronal cell morphology and growth by fine-tuning the efficacy of the PTEN/PI3K/Akt pathway.

P21-016
Optimizing CNS-delivery by lactyl stearate-coupled liposomes

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Meningitis is the inflammation of tissues which covers brain & spinal cord. Thus lactyl stearate coupled liposomes bearing rifampicin (highly lipophilic) is used for effective management of meningitis. Brain drug targeting brings a healthy skepticism to the study of the BBB, which is the most frustrating obstacle for pharmacologists wishing to find treatments for brain disorders. Synthesized Lactyl stearate was used to prepare liposomes bearing rifampicin by Lipid cast film method. Formulations were characterized for vesicle shape by Transmission Electron Microscopy (TEM), vesicle size, drug entrapment efficiency, *in-vitro* drug release. The *in-vivo* studies the drug distribution in various organs and blood of albino rats was assessed after I.V. administration. The quantitative uptake of the formulations by the brain in albino rats was assessed by fluorescent microscopy. The % encapsulation efficiency was 41% & 34% in uncoupled & coupled liposomes. Brain uptake was increased about 2-3 times in case of uncoupled liposomes and plain drug. Accumulation was increased about 6-8 times with coupled liposomes in comparison to uncoupled and about 10–12 times higher compared to drug solution. Fluorescence study indicates that the preparation is crossing basal carotid system & accessing the nervous system. This delivery system not only increased the brain uptake of the drug but it also reduces the administered dose and toxic effect of the drug. Thus, Lactyl stearate coupled liposomes effectively delivers the drug to the brain and has great potential for brain targeting.

Mol Neu S3, Degeneration and Ageing of the Nervous System

P22-005-SP
Defective cross-talk between the ubiquitin proteasome system and the autophagy lysosomal pathway under proteasome stress in aged rat hippocampus

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Autophagy plays a key role in the maintenance of cellular homeostasis participating in essential cell-fate decisions concerning cell death and survival. Autophagy deregulation gives rise to severe disorders, such as cancer and neurodegeneration. Despite autophagy machinery is well known, many of the signaling pathways regulating autophagy under stress situations are still poorly understood. Using a model of proteasome stress in rat hippocampus, we have analyzed the age-related modifications in the cross-talk between the two major cellular proteolytic systems: the ubiquitin proteasome system (UPS) and the autophagy-lysosome pathway (ALP). We demonstrated that under proteasome stress both autophagy activation and resolution were efficiently induced in young but not in aged rats. Protein homeostasis was rapidly restored in young animals, whereas aged animals accumulated aggregates of ubiquitinated proteins, as well as non-digested autophagic vacuoles, in pyramidal neurons. Importantly, proteasome inhibition inhibited GSK-3β in young but not in aged rats, which could have consequences on both the b-catenin stabilization and the transcription factor EB (TFEB) signaling. Moreover, the age-related difference in the GSK-3β signaling could be due to a dysfunction in the signaling pathway of the insulin grow factor-1 (IGF-1). Considering that alteration of proteostasis represents a hallmark of neurodegenerative diseases, present data highlight the role of GSK-3β as a master regulator in restoring proteostasis, representing a key molecular target in order to sort out this deleterious effect.

P22-006-SP
Molecular links between aberrant protein oligomers and neurodegeneration in Alzheimer's disease

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Aberrant protein oligomers have been identified as the primary pathogenic agents in many protein deposition disorders including

Alzheimer's disease. The same polypeptide sequence can assemble into different types of oligomer displaying similar morphologies yet with different abilities to cause cellular dysfunction. The pathogenic nature of oligomeric species results from their ability to diffuse through biological fluids and to interact with cell membranes. Thus, the role of lipid rafts and their ganglioside (notably GM1) content have attracted increasing attention. Here, we quantify the contribution of GM1 content to the cytotoxic effect of two different types of oligomers, grown from the $A\beta_{42}$ peptide associated with Alzheimer's disease or the model protein HypF-N. We found a quantitative relationship between membrane GM1 content in neuroblastoma cells and oligomer binding. In particular, it appears that toxic $A\beta_{42}$ oligomer binding to the cell membrane occurs with high affinity and apparent saturation kinetics whereas the GM1-dependence of non-toxic $A\beta_{42}$ oligomer binding follows linear kinetics and displays low affinity. Similar trends for membrane permeabilization, Ca^{2+} influx and cell viability were also found in cells with different GM1 content exposed to the oligomers, confirming that the observed cytotoxicity is closely related to oligomer affinity to the membrane. Overall, we provided a robust molecular basis of the role performed by membrane GM1 not only as aggregation promoter but also as key aggregate binding site and hence as initiator of different responses eventually resulting in neurodegeneration. This study was supported by the Fondazione Cassa di Risparmio di Pistoia e Pescia (2014.0251).

P22-007-SP
The dysfunction of retrograde transport is sufficient to disrupt Aβ clearance in astrocytes via disturbed endosome trafficking

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We previously showed that aging attenuates the interaction between dynein-dynactin complex, which mediates intracellular retrograde transport system, in cynomolgus monkey brain and that dynein dysfunction reproduces age-dependent endocytic pathology such as intracellular accumulation of abnormally enlarged endosomes. Accumulating evidences suggest that endocytic disturbances is involved in Alzheimer's disease (AD) pathogenesis, and we also demonstrated that dynein dysfunction-mediated endocytic disturbance causes the accumulation of intracellular β-amyloid protein (Aβ), the key factor for AD pathogenesis. Thus dynein dysfunction would be one of the causative factor for age-related endocytic disturbance leading to AD pathogenesis. On the other hand, it remains unclear whether such age-dependent endocytic disturbance also occurs in glial cells. Here, we show that intracellular accumulation of enlarged endosomes occurs even in astrocytes of aged monkey brains. Moreover, we found that Aβ accumulates in these enlarged endosomes. RNA interference studies demonstrated that dynein dysfunction reproduces astroglial endocytic pathology and disrupts Aβ clearance in astrocytes via disturbed endosome trafficking. Interestingly, dynein dysfunction did not affect Aβ uptake itself. These findings suggest that endocytic disturbance in astroglial cells may also be involved in age-dependent Aβ pathology.

P22-008-SP
Label free quantitative proteomic analysis of astrocytes directly converted to neurons

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Mesodiencephalic dopaminergic (mdDA) neurons play a key role in motor control, cognition and arousal. Their dysfunction or loss is known to cause Parkinson's disease (PD). To date, only pharmacological treatment and deep brain stimulation (DBS) is able to retard the progression of PD. Here, we investigate future options of cell replacement therapies for the treatment of PD. In order to avoid immunological rejection application of autologous transplants is the preferable method. Since efficient reprogramming of patient-derived fibroblasts to neurons is still under debate, we here propose to use astrocytes as a predominant cell type in the CNS that is more prone to generate neurons. By applying cDNA transfections with the transcription factors Sox2, Mash1, Lmx1a and Nurr1 we describe a method to convert astrocytes directly into mdDA neurons. For characterization of the conversion we used label free quantitative proteomic analysis. A number of neural and pro-neuronal specific proteins were newly expressed such as Calm1, Gpm6b, Pacsin2 and Tubb6. Expression was verified at the level of downstream target mRNAs using quantitative real time PCR and key candidate genes were identified by immunocytochemistry. Unfortunately, above methods cannot exclude unwanted side effects caused by insertional mutagenesis of foreign nucleic acids. Here, we depict the functional transduction of membrane permeable HTN-Mash1 and HTN-Lmx1a proteins, which was validated by Rhodamine-labeling and analysis of downstream target mRNA. Taken together these results provide first insights into proteome profiles during the direct conversion of astrocytes to neurons.

P22-009
Rosmarinic acid redirect lysozyme from its normal amyloid formation pathway into nontoxic amorphous aggregates and reduces cellular toxicity

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Misfolding and aggregation of various proteins and peptides is associated with a growing list of diseases, including neurodegenerative disorders such as Alzheimer′s, Parkinson′s, and Huntington′s diseases and peripheral disorders such as systemic amyloidosis and type II diabetes. Consequently, inhibition of protein misfolding and amyloid fibril formation might provide a feasible therapeutic approach for preventing amyloid-related diseases. A promising strategy is to identify compounds that inhibit amyloid fibril formation. In this study, using a range of techniques including Thioflavin T (ThT) and ANS fluorescence assays, electron microscopy and circular dichroism, we describe the efficacy of rosmarinic acid (RA), on the inhibition of fibrillogenesis and hindering cytotoxicity induced by amyloid fibrils of hen egg white lysozyme (HEWL). Our data demonstrated that

RA effectively inhibit the fibrillogenesis and destabilize preformed fibrils of HEWL in a concentration-dependent manner. Moreover, result obtained by cell viability MTT assay indicates that the compound effectively protects cultured PC12 cells against HEWL fibril-induced cytotoxicity. We conclude that RA directly influences HEWL aggregation by interacting with amyloidogenic prefibrillar structures and diverting protein from its normal amyloid formation pathway into nontoxic amorphous aggregates with low solvent-exposed hydrophobic patches. The presented result may be useful for gaining a deeper insight into possible mechanisms of inhibition of amyloid fibril formation and toxicity exerted by polyphenolic compounds and may provide useful guidelines in relation to screening for novel inhibitors against protein misfolding and aggregation associated with neurodegenerative diseases.

P22-010
Synthetic fragment of receptor for advanced glycation end products prevents memory loss in mice with experimentally induced Alzheimer's disease

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It is known that oligomeric beta-amyloid binds receptors on neuronal cell surface and this interaction can mediate cell death and amyloid plaque formation during Alzheimer's disease. We proposed that short receptor fragments representing the potential binding sites of beta-amyloid are able to bind beta-amyloid and to prevent its interaction with the receptors. Thus, administration of these receptor fragments will decrease brain level of beta-amyloid and improve the memory state. We have selected and synthesized 12 peptide fragments from three potential neuronal receptors targeted by beta-amyloid: acetylcholine receptor alpha7-type, prion protein and receptor for advanced glycation end products. Synthetic peptides were intranasally administrated into animals with experimentally induced form of Alzheimer's disease – bulbectomized mice. Then memory of mice was examined in the water Morris test. We have found that administration of only one fragment of receptor for advanced glycation end products effectively prevented the murine memory from impairment and decreased beta-amyloid level in the brain of experimental mice. We investigated the level of autoantibodies against the revealed fragment in blood sera of patients with a clinical diagnosis of Alzheimer's disease and discovered that the level of antibodies against this peptide is higher in a group of patients with Alzheimer's disease than in healthy donors. Thus, the revealed synthetic fragment plays an important role in pathogenesis of Alzheimr's disease and, therefore, seems perspective for development of new medicine for Alzheimer's disease therapy. Supported by RFBR Grants No. 14-04-31232 and 15-04-01360.

P22-011
Induction of Nanog displays protective effects against amyloid β (Aβ)-induced cytotoxicity

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Alzheimer's disease (AD) is the most common neurodegenerative disorder characterized by amyloid β (Aβ) deposition in the brain. Currently, AD therapies can only alleviate symptoms rather than Aβ-induced neurodegeneration, thus providing no pathway to overcome Aβ toxicity. Previous studies have demonstrated that Nanog, a homeodomain-bearing protein required for maintenance of pluripotency, may be linked to the pathogenesis of AD. However, the exact mechanisms underlying Nanog's contribution to AD are still unclear. In the present study, we evaluated the protective pathways by which Nanog protects against Aβ-induced cytotoxicity. Our results indicate that Nanog overexpression can counteract oxidative damage by neutralizing excessive ROS, thus contributing to the alleviation of Aβ-induced neurotoxicity. In addition, Aβ induced the loss of mitochondrial membrane potential and activation of caspase 3 and PARP, whereas overexpressed Nanog significantly attenuated these forms of deterioration. This protective effect may be due to the activation of AMP-activated protein kinase (AMPK) in a sirtuin 1 (Sirt1)-dependent pathway by shifting endogenous reactive oxygen species (ROS) detoxification responses away from cell death and toward survival. We expect our results can provide the basis for molecular mechanisms involved in the pathogenesis of brain Nanog signaling and AD. Accordingly, stimulation of Nanog signaling by targeting Nanog may lead to novel therapeutic strategies by slowing or halting AD progression in future.

P22-012
RNA aptamers against autoantibodies related to multiple sclerosis as a basis for detection probes

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Multiple sclerosis (MS) is an autoimmune CNS disease characterized by presence of proteolytic autoantibodies against myelin basic protein (MBP) contributing to the destruction of myelin sheath. There is no specific laboratory test to diagnose MS nowadays. Aptamers are widely used now to develop novel diagnostic systems. The present work is devoted to the generation of RNA aptamers against MS-related autoantibodies as well as an investigation of a possibility of their use as a diagnostic platform. To produce RNA aptamers, we used *in vitro* selection against polyclonal anti-MBP IgG autoantibodies isolated from the blood of MS patients. Incubation with IgG from healthy donors was added as a counterselection step. All pyrimidine ribonucleotides in RNA libraries were replaced by their 2'-fluoro analogs. After sequencing of enriched RNA libraries and data analysis, a series of 71-nt 2'-fluoro modified RNA aptamers were obtained. Screening of affinity and sequence minimization resulted in several aptamers with high affinity and specificity towards pathogenic anti-MBP antibodies as compared to antibodies of healthy donors. The obtained aptamers can be employed as recognizing elements for the development of heterogeneous assays for the detection of MS-related autoantibodies. Optical aptasensors for bioluminescent and fluorescent detection were designed and their affinity, specificity and sensitivity are now studied in model assays using anti-MBP antibodies and summary IgG pools from blood of MS patients to choose the best candidate for the future development of lab tests. This work was supported by and RFBR grant No14-04-01611 and FASIE grant 2014-2015.

P22-013
Ly6C^{high} monocytes control experimental autoimmune encephalomyelitis progression

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Monocytes arise from progenitors in the bone marrow and transmigrate from the circulatory system to peripheral tissues, where they differentiate into recruited macrophages. Macrophage infiltration is essential to generate the proinflammatory and chemoattractant microenvironment that permits the subsequent repair and healing processes. However, the mechanism how these cells are able to replenish the tissues and to trigger a response is poorly understood. We developed a novel method for the *in-vitro* generation of bone marrow-derived Ly6C^{high} monocytes. Flow cytometry analysis confirmed the expression of extracellular markers characteristic of circulating monocytes. Data derived from gene expression determined their ability to polarize following proinflammatory or anti-inflammatory stimuli. *In-vivo* imaging (IVIS) and laser scanning confocal microscopy (LSCM) confirmed the migratory capacity of these cells in experimental autoimmune encephalomyelitis (EAE) mice model. Homing of Ly6C^{high} monocytes follows restricted kinetics. Intravenously injected Ly6C^{high} cells migrate into the central nervous system to lumbar and cervical spinal cord only during scores two and three of disease. This migration correlates with the entry of leucocytes and macrophages and reduces the progression of the EAE pathology. This data demonstrates the essential role of Ly6C^{high} monocytes in a model of autoimmune disease. Further studies will elucidate the interaction of these cells with the other cellular elements involved in this autoimmune process, as well as the genes expressed in macrophage leading to the clinical modifications.

P22-014
Prion protein mislocalized in the cytosol causes loss of dendritic spines

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Dendritic spines are protrusions on dendritic shaft where excitatory synapses are located. Dendritic spine pathology has been observed in neurodegenerative diseases. Actin cytoskeleton is the major structure that controls spine formation and dynamics. It has also been shown that growing microtubules (MTs) can enter dendritic spines and influence their morphology and stability. Prion protein (PrP) mislocalized in the cytosol has been presumed to be the toxic entity responsible for neurodegenerative process in transmissible spongiform encephalopathies (TSE). Previously we have demonstrated that PrP interacts with tubulin and disrupts microtubular cytoskeleton by inducing tubulin aggregation. Here we show that exposition of primary neuronal cells to membrane-penetrating peptide encompassing first 30 amino acid residues of PrP results in loss of dendritic spines. Taxol was able to prevent this effect, confirming important role of MTs formation in dendritic spines stability. Microtubule associated proteins (MAPs), such as Tau and MAP2 proteins are known to enhance MTs stability. Phosphorylation of MAPs diminishes their microtubule-stabilizing function. Interestingly, hyperphosphorylated Tau has been detected in TSE. In order to inhibit activity of GSK3, main kinase responsible for modification of Tau and MAP2, we employed LiCl. This study demonstrates that both taxol and LiCl can prevent loss of dendritic spines implying substantial role of altered MTs dynamics in the neurodegenerative processes in TSE. Presented observations are in accordance with the recent discoveries of involvement of MTs in dendritic spines structure and contribute to our understanding of the molecular mechanism of neurotoxicity of cytosolic PrP in TSE.

P22-015
The yeast model of Huntingtin disease in studies concerning the role of human VDAC isoforms in the disease pathomechanism

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Huntington disease (HD) is an autosomal-dominant and fatal neurodegenerative disorder caused by CAG trinucleotide repeat expansion in exon 1 of *IT15* gene encoding huntingtin (Htt). As the trinucleotide codes for glutamine, its repeat number higher than 35 results in an abnormally long polyglutamine tract in N terminus of Htt that gives rise to its mutated form (mHtt). It is now obvious that mitochondria play a vital role in HD pathogenesis but the underlying mechanism is still not clear. Moreover, the functional relationship of Htt to mitochondria is still uncertain. On the other hand, it is becoming increasingly apparent that mHtt can impair mitochondrial function directly by affecting mitochondrial bioenergetics and dynamics. Interestingly the proposed "mitochondrial targets" of mHtt include processes that are known to be affected by voltage-dependent anion-selective channel (VDAC). Importantly, three different VDAC isoforms are present in vertebrate mitochondria including human ones but their specific role is still elusive. To investigate the role of human VDAC isoforms in HD pathogenesis we applied the yeast *Saccharomyces cerevisiae* model of the disease. The model enables to determine the effect of Htt and mHtt on status of mitochondrial coupling in intact cells expressing a given isoform of human VDAC. The obtained results indicate that Htt and mHtt may influence the status differently, their effects changed in time and depend on the presence of a given human VDAC isoform that may be an important element of HD pathogenesis mechanism. The studies were supported by the grant: NCN 2011/01/B/NZ3/00359.

P22-016
Characterization of Smn-dependent gene expression changes underlying motor neuron degeneration and synaptic mysfunction in SMA

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Spinal Muscular Atrophy (SMA), a lethal inherited neurodegenerative disorder, is characterized by low levels of the Survival of Motor Neuron (Smn) protein, which is essential for the assembly of spliceosomal small nuclear ribonucleoproteins (snRNPs). Strikingly, low levels of this ubiquitous protein mainly affect motor neurons (MNs), disrupting neuromuscular junctions

(NMJs) and leading to MN degeneration. Despite robust knowledge of SMA's genetics, the exact molecular mechanisms underlying the disease's phenotype remain largely elusive, preventing the development of rational therapeutics. One possibility is low levels of Smn have a higher impact in the expression and splicing of genes critical for MN function and survival, or that these cells are intrinsically more sensitive to global changes in RNA processing. Alternatively, Smn may be involved in MN specific functions. Possibly both hypothesis are applicable. To address the relevance of Smn-dependent changes in neuronal gene expression, we performed RNA-seq to obtain an unbiased profile of the central nervous system transcriptome of a *Drosophila melanogaster* SMA disease model. Upon SMN down-regulation we observe changes in exon usage in a particular subset of genes crucial for neuronal development, viability and NMJ function. This suggests that SMN-dependent changes in the splicing machinery do not have widespread effects, affecting specific genes possibly due to the existence of certain features in their sequence/structure. Interestingly a large proportion of identified genes with altered splicing are known genetic modifiers of the NMJ phenotype in SMA fly models, thereby supporting the biological relevance of our data.

P22-017
GDNF-family growth factors in the treatment of neurodegenerative diseases

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Neurodegenerative diseases, including Parkinson's disease, affect millions of people and constitute a very serious medical and social problem. All available treatments only alleviate symptoms and disease modifying therapies that could slow down or stop disease progression in neurodegenerative diseases are still lacking. Glial Cell Line-Derived Neurotrophic Factor (GDNF) family consists of four closely related proteins: GDNF, NRTN, PSPN, and ARTN. They stimulate differentiation, migration, neuritogenesis and promote the survival of neurons.

Unfortunately, we know very little about the biology and cell biology of these factors in particular in the aging brain. GDNF family factors specifically bind to GFRα co-receptors and mediate their signals to the cells via receptor tyrosine kinase RET. In order to better understand GDNF cell biology we have studied its secretion, internalization and intracellular trafficking; the processing and section of GDNF having at least two major splice isoforms that differ in their pro-region. We found that (alpha) pro-GDNF is mainly secreted by constitutive pathway and the (beta)pro-GDNF is using the activity-dependent pathway. Sortilin receptor family member SorLA acts as sorting receptor for the GDNF/GFRα1 complex, directing it from the cell surface to endosomes. Through this mechanism, GDNF is targeted to lysosomes and degraded while GFRα1 recycles, creating an efficient GDNF clearance pathway. The SorLA/GFRα1 complex further targets RET for endocytosis but not for degradation, affecting GDNF-induced neurotrophic activities. We are currently investigating secretion and trafficking of other GDNF family members and searching for new proteins (receptors) that recycle GDNF and direct RET and GFRa1 to degradation.

P22-018
HSP70 protects neuronal cells from toxic effect of amyloid beta and its isoforms

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Accumulation of beta-amyloid (Aβ) in the form of amyloid plaques in the brain is a major neuromorphological feature of Alzheimer's disease (AD). Pathological properties of Aβ are caused by neurotoxic effect of its soluble oligomers. Molecular factors that promote the formation of such oligomers are the complexes of Aβ isoforms and metal ions. Previously, we found that the metal-binding domain 1-16 of Aβ plays an important role in the pathological oligomerization of Aβ. In this study we have shown that the AD-associated species of Aβ, containing modifications in the 1-16 fragment, have a stronger toxic effect on human neuronal cells NSC-hTERT and neuroblastoma cells SK-N-SH compared to the intact Aβ. Treatment by peptides incorporating the "Taiwan" mutation D7H (D7H-Aβ), "English" mutation H6R (H6R-Aβ), isomerized aspartic acid residue at position 7 (isoD7-Aβ), phosphorylated serine residue in position 8 (pS8-Aβ), and by the combined peptide isoD7-pS8-Aβ resulted in a decrease of mitochondrial potential in cells and induction of apoptosis. Recombinant human heat shock protein HSP70 fully protects NSC-hTERT and SK-N-SH cells from the toxic effect of these isoforms of Aβ. At the same time, the production of tumor necrosis factor by THP-1 cells exposed to Aβ isoforms in the presence of HSP70 is reduced, which should lead to a decrease in neuronal cell death at the organism level. This indicates that HSP70 may have both direct and indirect protective effect on neuronal cells exposed to the Aβ peptides. Supported by the Russian Scientific Foundation (grant #14-24-00100).

P22-019
The effect of Glycation on the permeability of an *in vitro* blood–brain barrier model

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The blood–brain barrier (BBB) provides a physiological barrier between the blood system and the central nervous system. It protects the brain and is necessary for cerebral function. Endothelial cells are the main components of the blood–brain barrier. These cells are connected through tight junction proteins with each other. Thus, they form a physiological barrier with low permeability. The disruption of the BBB is associated with chronic-inflammatory diseases and viral infections. It is also associated with a so-called delirium after cardiac surgery in elderly patients. In this study we outline that low permeability of the BBB could be induced by posttranslational modifications through advanced glycation endproducts (AGEs). Therefore, we established an *in vitro* blood–brain barrier model. Transfected human brain microvascular endothelial cells (THBMECs) were seeded into cell culture inserts until the permeability coefficient validated an optimal

tightness of the BBB. THBMECs were glycated using methylglyoxal (MGO). The appropriate amount of MGO, which leads to AGE-formation but is not toxic to the cells, was determined by performing cell viability test. The tight junction proteins occludin, claudin and ZO-1 seal the BBB and provide a functional barrier. Tight junction proteins are analysed after glycation. Therefore, permeability measurements after glycation of the THMBECs are performed and validated by western blot and immunofluorescence analysis. Studies on glycation of the extracellular matrix proteins (ECM), in this case collagen IV and fibronectin will be performed as well. Our study indicates that posttranslational modifications, particularly advanced glycation endproducts influence the permeability of the *in vitro* blood–brain barrier.

P22-020
The effect of toluidine blue O on amyloid-β peptide levels in human neuroblastoma cells

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Alzheimer's disease (AD) is characterized by neurofibrillary tangles and neuritic plaques caused by aggregates of Aβ peptides generated from amyloid precursor protein (APP) via the amyloidogenic pathway. Recent drug strategies for AD focus on the cholinergic-based therapies with neuroprotective effects. In our earlier studies, toluidine blue O (TBO), a phenothiazine dye was found to be a highly effective inhibitor of human cholinesterases. The aim of the present study was to investigate whether TBO may effectively lower the level of $A\beta_{1\text{-}42}$, the major Aβ peptide that aggregate. Human neuroblastoma (SK-N-SH) cells were treated with 0-10 μM TBO or vehicle control for 24 hours. Methylene blue (MB), which inhibits the formation of amyloid plaques and neurofibrillary tangles, was also included in the study for comparative purposes. $A\beta_{1\text{-}42}$ levels were assayed by sandwich-based ELISA and normalized to total protein levels, determined by BCA protein assay. Treatment of SK-N-SH cells with TBO or MB resulted in a decrease in intracellular $A\beta_{1\text{-}42}$ levels at 24 h, compared to vehicle-treated cells. While intracellular $A\beta_{1\text{-}42}$ level was reduced by 52 % in 10 μM TBO-treated cells, it was decreased by 40 % in 10 μM MB-treated cells at 24 h. These preliminary results suggest that TBO may be a potential drug candidate for the treatment of AD.

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P22-021
Plasma levels of matrix metalloproteinases-2,-9 and tissue inhibitors of metalloproteinases-1,-2 in Alzheimer's disease

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Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder. It is characterized by its progressive clinical course, irreversable memory loss and cognitive disorders. Deposition of amyloid-β in senile plaques and in cerebral blood vessels is one hallmark of the pathogenesis of AD. Matrix metalloproteinases (MMPs) can degrade components of the extracellular matrix in a variety of physiological and pathophysiological conditions such as stroke and intracerebral hemorrhage. There is growing evidence that matrix metalloproteinases play an important role in the pathogenesis of AD, and, in particular, may be involved in the processing pathway of amyloid-β. In this study, we investigated MMP-2 and MMP-9 and tissue inhibitor of metalloproteinases (TIMPs), TIMP1 and TIMP2, levels in plasma of AD patients and age matched healthy controls. MMP-2, MMP-9, TIMP-1 and TIMP-2 levels of 30 AD and 30 control subjects were measured by ELISA. MMP-2 and TIMP-1 levels were significantly lower in the AD group than the control group ($p < 0.05$). On the other hand, there were no statistically significant difference between AD patients and control group in terms of TIMP2 and MMP9 levels. This study suggests that matrix metalloproteinases and their inhibitors can play a role in amyloid-β peptids catabolism which is responsible for the Alzheimer's disease pathogenesis.

Key Words: Alzheimer's disease, matrix metalloproteinases, tissue inhibitor of metalloproteinases, ELISA

P22-022
Risk effect of polymorphisms of serotonin transporter gene and the dopamine d4 receptor gene in undergraduate students for negative life events

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Functional polymorphisms in the promoter region of the serotonin transporter gene (5-HTTLPR) and in the dopamine D4 receptor gene (DRD4) encodes a receptor for dopamine have been a highly suspect genetic marker for personality. In this study, a functional polymorphism in the promoter region of the serotonin transporter gene and in the dopamine D4 receptor gene (DRD4) encodes a receptor for dopamine was used to characterize genetic vulnerability to negative life events in representative nonclinical undergraduate students. We observed 9-repeat allele, 10 repeat allele and 12 repeat allele for VNTR polymorphism of this gene. A number of genetic variants moderate the effects of environmental risk. Frequencies of short (s/s) and long alleles (l/l and l/s/) of 5-HTTLPR were found as mean: 20% and mean: 39%. Short allele ("s") in the 5-HTTLPR gene was significantly associated with the environmental experiences of these students. The 5-HTTLPR gene can interact with the environmental conditions. We also demonstrated that the DRD4 polymorphism significantly affected the risk effect conferred by an increasing level of exposure to TLE.

P22-023
Metabolic peculiarities of the mechanism for neuron protection against heat shock during human aging and initial stage of Alzheimer's disease

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Prolonged stress and information pressure on organisms cause intensive protein synthesis in neurons. Secondary products of synthesis accumulate in the region of protein synthesis. Upon hydrolysis, pyrophosphates release large amounts of energy which dissipates into thermal energy, negatively affects neurons and can lead to development of heat shock. Formation of a high concentration of pyrophosphates and phosphates in the region of protein synthesis is restricted to phosphorylation of transport proteins, including APP and tau-protein (TP) which perform an important function – evacuation of high-energy molecules from the region of protein synthesis to the periphery on the neuron membrane. Maximal phosphorylation of APP molecules proceeds with the involvement of ATP. Phosphorylated APP molecules activate α-secretase, and further processing of APP occurs in non-amyloid pathway with evacuation of phosphates to neuron membranes. Final result of non-amyloid processing of APP is the formation of fragments of membrane proteins which perform their functions: stabilize neuron membranes and stimulate the work of synapses. As a result of intensive protein synthesis, ATP in the region of synthesis becomes deficient and the level of APP phosphorylation decreases. These processes activate β-secretase and the following processing of APP switches to the amyloid pathway. Thus, APP performs a principal function in the trigger mechanism of AD pathology as well as important protective function – evacuation of power-consuming molecules from region of protein synthesis to the periphery of neuron membrane. Tau proteins have similar protective functions carrying AMP, phosphates and pyrophosphates. This study was supported by Russian Science Foundation 14-14-00536.

P22-024
Multiplex genome engineering of Amyotrophic lateral sclerosis mutant SOD1 gene using CRISPR/Cas9 systems

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by motor neuron death. Superoxide dismutase 1 (SOD1) mutations account for 20% of familial ALS. More than 160 mutations in *SOD1*, including G93A, have been identified in ALS patients and most of the mutants are thought to form protein aggregates in cytosol. Recently, RNA-guided nucleases (RGNs), which are derived from CRISPR/Cas9 systems, are used as efficient and rapid tools for genome engineering. By using CRISPR/Cas9 systems with multiple guide RNAs (gRNAs), RGNs cleave specific sites of chromosomal DNA to create double-stranded breaks (DBSs). Here, we show that the *SOD1* gene can be engineered by multiplex CRISPR/Cas9 systems using multiple gRNAs, and that the G93A mutation of the *SOD1* gene can be corrected by replacing the mutated sequences with normal sequences. Furthermore, correction of the G93A mutation in human cells normalizes pathogenic ALS signaling pathways. Our results will provide a possibility of editing other genes causing neurodegenerative diseases for their therapeutic purpose.

Keywords: ALS, SOD1, G93A mutation, CRISPR/Cas9 systems, gRNAs.

P22-025
SOD1 regulates intracellular aggregates and neuronal toxicity of the amyloid beta (Aβ) : Treating strategies for Alzheimer's disease (AD) from Amyotrophic lateral sclerosis (ALS)

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Several lines of evidence suggest that intracellular amyloid beta (Ab) is associated with the pathogenesis of Alzheimer's disease (AD). Altered proteolytic processing of the amyloid precursor protein (APP) results in the production and aggregation of neurotoxic forms of Ab, which is the central causing mechanism of AD. Therefore, a promising therapeutic approach could be to reduce levels of aggregate formation of Aβ in neurons. We previously published that intracellular Ab specifically interacts with mutant SOD1. This leads us to study the acceleration of neuron impairment and Ab aggregation by the SOD1-Ab interaction in AD. To assess this idea, we investigated the effect of the mutant SOD1 on the Ab aggregation by using immunofluoresent techniques. Mutant SOD1 induced the aggregation of Aβ in neuronal cells. Consistent with this result, Ab aggregation was three-fold higher in the brains of mutant SOD1 Tg mice than in those of wild-type mice. Furthermore, we found that the N-terminal region of SOD1 is required for the interaction with Ab. Therefore, these results indicate that this N-terminal region may be associated with intracellular Ab aggregation and Ab aggregation-linked neuronal toxicity in AD. Our study provides new insights into the development of therapeutic approaches for AD from ALS.

P22-026
Time-resolved thioflavin-T fluorescence-expanding the amyloid characterisation toolbox

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The benzothiazole dye thioflavin-T (ThT) is widely used as a fluorescent stain for detection and characterisation of amyloid fibrils whose formation underlie the pathology of neurodegenerative diseases, including Alzheimer's-related amyloid-β (Aβ) and Parkinson's-related α-synuclein fibrils. Despite extensive use it is still unclear exactly how amyloid fibrils enhance ThT fluorescence and as a consequence there is no straight-forward correlation between its steady-state emission intensity and the amount of amyloid fibrils in a sample, not even in cases where highly similar amyloid proteins are compared. We use time-resolved fluores-

cence spectroscopy to explore how the ThT fluorescence lifetime responds to amyloid fibril formation, in order to identify new read-outs that can expand the present amyloid characterisation toolbox. We demonstrate how fluorescence lifetime and emission intensity recordings can be used together with methods that determine absolute fibril content in order to reveal structural and morphological differences in fibrils formed by the AD-relevant peptides Aβ40 and Aβ42 [ref]. Further, we find that ThT fluorescence lifetimes are strongly dependent on the dye:monomer ration in the fibril, suggesting that ThT undergoes self-quenching interactions at higher loading ratios. Here, we discuss how this signal, which has a larger dynamic range than mere emission intensity, can be used to extract information on the formation of amyloid species in the lag phase of amyloid forming reactions, at time-points where conventional steady-state ThT emission shows no signal . This is of integral importance for characterisation of toxic amyloid oligomers that have been reported to be particularly abundant in the lag phase.

P22-027
The human Tp53 *Arg72Pro* polymorphism increases neuronal vulnerability to apoptosis after experimental intracerebral hemorrhage

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Intracerebral Hemorrhage (ICH) is responsible for 9-27% of all strokes worldwide. Differences in genetic susceptibility to apoptosis, can account for the different functional recovery on people suffering a stroke. Recently, we described that Tp53 *Arg72Pro* single nucleotide polymorphism (SNP) is associated with functional outcome in patients after ICH. To study mechanisms underlying this phenomenon, we used the collagenase ICH model in *knock-in* mice each one carrying a humanized allele of the *Arg72Pro* SNP. We performed immunohistochemical techniques at 6 h, 1, 3, 7 and 14 days to analyse cellular survival. Endothelial Progenitor Cell (EPC, $CD34^+$/ $VEGFR2^+$ cells) mobilization was measured by flow cytometry. Serum levels of VEGF and SDF-1a were determined by ELISA. Here we describe that residual lesion volume was higher in Arg^{72}-p53 mice. Moreover, neuronal apoptosis was increased in Arg^{72}-p53 mice at 1, 3, 7 and 10 days after ICH, shown in NeuN-TUNEL co-staining. EPC mobilization and neovascularization through growth factor signalling has been associated with a better functional recovery in patients suffering stroke. We found that serum VEGF and SDF-1a levels and the increase in the number of CD34 + /VEGFR2 + cells were higher in Pro^{72}-p53 mice than in Arg^{72}-p53 ones. Our results indicate that the Arg^{72}-p53 variant is accountable for a higher level of apoptosis in rodents under ICH. *Arg72Pro* SNP also controls VEGF and SDF-1a release and EPC mobilization after ICH, which may account for the different brain damage recovery. In conclusion, the *Arg72Pro* SNP controls functional recovery after ICH. Funded by ISCIII (PI12/0685; RD12/0014/0007; RD12/0014/0001; CD012/00685), FEDER.

P22-028
Uni-molecular investigation of copper-induced misfolding mechanism over an amyloidic fragment with D, L-amino acids

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Alzheimer's Disease (AD) stands out as one of the most common form of dementia, characterized by deposition of insoluble amyloid-beta (Aβ) fibrils in between nerve cells, and accumulation of neurofibrillary tangles in cell bodies of neurons. Although the etiologic role of Aβ in AD is accepted, the molecular mechanism of neurotoxicity remains unclear and is currently under debate. The interaction of d-block metal ions (Cu, Zn, Fe) with intrinsically disordered proteins (IDPs) gained interest due to their proposed roles in neurodegenerative diseases. A member of the IDPs group is the Aβ peptide that upon bonding with Cu or Zn it results into a misfolded peptide, that aggregates into metal-enriched amyloid plaques, a hallmark of AD. Considering the fact that the coordination sphere of Cu^{2+} in the Ab1-16 peptide involves mainly the amino acids His-6, His-13, but also Asp-1 or Ala-2, we designed peptide mutants of the Ab1-16 that were engineered to contain L/D enantiomers of those sites. We investigated the distinct Cu^{2+} binding geometries and affinities provided by the change in the local coordination environment by analyzing stochastically the interactions between the mutant peptides and a single protein nanopore immobilized in a planar lipid membrane, while incubated in various concentrations of Cu^{2+}. The obtained data showed a micromolar rage of the Cu^{2+}-binding affinity, and a decrease in its value as L-amino acids were replaced with its D-enantiomer, with the effect being most noticeable when His-6 residue was changed.

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P22-029
Identification of apomyoglobin regions responsible for amyloid formation

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Amyloid fibrils formation in organs and tissues causes serious human diseases. Therefore identification of protein regions responsible for amyloid formation is one of important tasks of theoretical and experimental investigations. Most known algorithms (for example TANGO or FoldAmyloid) correctly predict amyloidogenic regions of peptides or proteins under destabilizing conditions, were polypeptide chain is accessible to the solvent. The aim of our work was to identify the apomyoglobin regions responsible for amyloid aggregation under physiological conditions and to compare them with amyloidogenic regions predicted theoretically. The structural properties of aggregates, formed by apomyoglobin mutant form V10F, have been studied with use of infrared spectroscopy, electron microscopy and X-ray diffraction. To determine the amyloidogenic regions we used limited proteolysis of fibrils with further mass-spectrometry analysis of obtained peptides. Received data evidence that A-, part of B-, E- and G-helices of apomyoglobin are responsible for amyloid aggregation. Then we compared these data with amyloidogenic regions pre-

dicted theoretically by TANGO and FoldAmyloid methods and obtained a good agreement between experimental and theoretical results. Thus, we can conclude that localization of apomyoglobin amyloidogenic regions under physiological conditions is determined by properties of amino acids residues and can be predicted from amino acids sequence alone. This work has been supported by the Russian Science Foundation Grant № 14-24-00157.

P22-030
Investigation of polymorphism of Aβ-peptide amyloids from different firms

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The aim of this research was to study the process of amyloidogenesis of Aβ-peptide. Fluorescence spectroscopy, electron microscopy, mass spectrometry, and X-ray diffraction were chosen as methods for studying amyloidogenesis. In many publications it is stated that the process of fibril formation by Aβ-peptide depends strongly not only on conditions of fibril formation (ionic conditions, pH, temperature, mixing etc.) and the way of production (synthetic or recombinant), but also on the method of synthesis or isolation. In the literature there are no exact recommendations for preparing samples for studying; moreover researchers use Aβ-peptide preparations produced by different firms. We have used Aβ-peptide preparations of a number of firms and found that the final result of the studies may depend on what preparation is used. We are the first to have checked preparations of Aβ-peptide produced by five companies (Anaspec, Invitrogene, Enzo, Sigma-Aldrich and SynthAssist) and have received evidence that even the lot of the preparation plays its role. All these preparation form amyloid-like fibrils at pH 3-6 and the fibrils contain no cross-beta structure. Preparations of Anaspec, Invitrogene, and Enzo form one type of amyloid-like fibrils, and preparations of Sigma Aldrich and SynthAssist form another type of fibrils. The obtained structural polymorphism of Ab-peptide just emphasizes the capacity of the peptide that can act as a prion agent with different structural characteristics. The obtained data have allowed us to propose a possible scheme of formation of amyloid-like fibrils. This study was supported by Russian Science Foundation (14-14-00536).

P22-031
Modulation of metabotropic glutamate mGlu5 receptor and its signaling pathway in human brain of Alzheimer's disease and Schizophrenia

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Alzheimer's disease (AD) is the major cause of dementia in the elderly and Schizophrenia (SZ) is a mental disorder of unknown origin. Both diseases are characterized by deterioration in cognitive functions and selective neuronal loss in several brain regions. The aim of the present work was to study the levels of metabotropic glutamate mGlu5 receptor, Phospholipase C (PLC) and Protein kinase C (PKC) activity in Parietal (PC) and Temporal (TC) Cortex from AD, SZ and SZ+AD cases as compared with non-demented samples used as controls. mGlu5 receptors were analyzed by radioligand binding assays and Real Time-PCR. PLC activity was determined by accumulation of IP_3 and PKC activity by ELISA. The mGlu5 receptor level was decreased in both areas analyzed with lowest levels at highest stages in AD samples. SZ samples did not show significant changes. However, mGlu5 level in SZ+AD cases decreased in PC, but was preserved in TC. Furthermore, PLC activity was decreased in both areas from AD samples and only in Parietal Cortex from SZ and SZ+AD cases as compared with controls. Finally, PKC activity was significantly decreased in AD, SZ and SZ+AD cases in both areas analyzed. Results presented herein show that protein levels and gene expression of mGlu5 receptor, PLC and PKC activity are modulated in Parietal and Temporal Cortex of AD, SZ and SZ+AD patients, being this modulation dependent on the progression of disease and suggesting this receptor and its signaling pathway as promising targets for diagnostic and therapy of Alzheimer's disease and Schizophrenia symptoms.

P22-032
Human Tp53 *Arg72Pro* polymorphism dictates neuronal susceptibility to amyloid ß-neurotoxicity

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Alzheimer's disease (AD) is the most common form of dementia in the elderly. It is described that p53 plays an essential role in AD. This protein naturally occurs in humans in two functional variants with single nucleotide polymorphism (SNP) resulting in Arg or Pro at residue 72. Recently, we have demonstrated that the Arg^{72}-p53 genotype increases neuronal susceptibility to ischemia-induced apoptosis. The objective of this project is to unravel the mechanism that places p53 as a key point in amyloid ß (Ab)-induced damage and the relevance of *Arg72Pro* SNP in Aß neurotoxicity. For this purpose, we cultured cortical primary neurons from both knockout p53 ($p53^{-/-}$) and Knock-in p53 ($p53^{Pro/Pro}$ and $p53^{Arg/Arg}$) mice. Neurons were treated with 10 mM Ab(25-35) oligomers for 24 h. In some experiments, neurons were lipotransfected with siCdk5 or plasmids containing apoe2, e3 and e4 variants. Then we analysed protein expression levels, mitochondrial function and apoptosis. Our results demonstrate that Aß induced Cdk5-mediated p53 stabilization, which triggers mitochondrial dysfunction, leading to neuron apoptosis. Furthermore, the Arg^{72}-p53 polymorphic variant increases neuronal susceptibility to Aß-caused mitochondrial depolarization and neurotoxicity, in comparison with the Pro^{72}-p53 variant. The expression of the well-known major risk factor for AD, apoe4 in neurons abrogated this effect and both Arg^{72}-p53 and Pro^{72}-p53 neurons presented high susceptibility to Aß neurotoxicity. Thus, Tp53 *Arg72Pro* polymorphism modulates neuronal susceptibility to Aß toxicity and determines damage extent. These results make this SNP a possible biomarker of genetic risk for AD. Funded by ISCIII (PI12/0685; RD12/0014/0007), FEDER fonds, Ministerio de Educación (FPU).

P22-033
Behavioral and neurochemical effects of monosodium glutamate in neonatal rats

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Monosodium glutamate (MSG) is one of the quite widely used artificial sweetener in food products over the last decade. Previous studies showed that MSG has behavioral, neurochemical and histological effects on different organisms. To determine behavioral and neurochemical effects of MSG, we used neonatal male Wistar rats (after lactation), and repeated and applied at different doses. We used eight arms radial maze and open field tests to detect behavioral effects of MSG. Training of eight arms radial maze test was carried out for all rats before starting MSG dose injections then rats were divided into four groups as; control (n:6), MSG1 rats (n:6, 50 mg/g/day), MSG2 rats (n:6, 100 mg/g/day), MSG3 rats (n:6, 200 mg/g/day). Injections were performed a total eight applications with one day intervals as intraperitoneal treated. We found statistically significant differences in number of mistakes between MSG 200 mg/g/day group and control group (Mann-whitney U test: $P < 0.001$). Furthermore, when we compare the pre-dose and post-dose statistically significant differences were observed in line crossing, rearing, grooming and defecation for all MSG doses. Dopamine, glutamate, GABA and catecholamine levels were measured in brain tissue for all rats using by colorimetric ELISA assay methods (450 nm). We found that, catecholamine ($p < 0.05$) and glutamate ($P < 0.05$) levels were statistically different from control group in the MSG 200 mg/g/day group. Although, we observed a reduction in the level of dopamine and an increase in the levels of GABA based on MSG dose, there were not statistically significant differences between groups.

P22-034
Fluorescent carbon dots: Neuromodulatory effects on exocytotic release, uptake and ambient level of glutamate and GABA in brain nerve terminals

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Carbon dots (C-dots), a recently discovered class of pure carbon nano-sized particles with fluorescent, emission-color-tuning and non-blinking features, have great bioanalytical potential. We analyzed neuromodulatory and neurotoxic properties of fluorescent C-dots obtained from β-alanine by microwave heating. They were assessed based on the analysis of key characteristics of GABA- and glutamatergic neurotransmission in isolated rat brain nerve terminals. It was found that C-dots (40-800 mg/ml) in dose-dependent manner: (1) decreased exocytotic release of [^{3}H]GABA and L-[^{14}C]glutamate; (2) reduced acidification of synaptic vesicles; (3) attenuated the initial velocity of Na^+-dependent transporter-mediated uptake of [^{3}H]GABA and L-[^{14}C]glutamate; (4) increased the ambient level of the neurotransmitters, but (5) did not change significantly the potential of the plasma membrane of nerve terminals. Almost complete suppression of exocytotic release of the neurotransmitters was caused by C-dots at a concentration of 800 mg/ml. Fluorescent and neuroactive features combined in C-dots create base for their potential usage for labeling and visualization of key processes in nerve terminals, and also in theranostics. From toxicological point of view, it may be suggested that air pollution with similar carbon-containing nanoparticles may provoke the development of neurologic consequences.

P22-035
Diesel Particles (DEP) effects on an endothelial cell linw (hCMEC/D3) and hippocampal neurons (HT22)

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Alzheimer's disease (AD) is a neurodegenerative illness affecting the elderly population, characterized by plaques of Aβ42 aggregates, neurofibrillary tangles and neuronal loss (Allsop, 2000). In AD vascular factors could precede the neurodegenerative process (de la Torre, 2002, 2008); Aβ42 accumulation in the cerebral capillary may be a consequence of a local production in the vascular domain (Natté *et al.*, 1999). Air pollution has been associated with CNS diseases. Inhaled UFPs (< 100 nm) could easily translocate cross the air-blood barrier, reach the bloodstream and be distributed to the cardiovascular system or the CNS (Oberdorster *et al.*, 2002), thus affecting systemic microvasculature (Nurkiewicz *et al.*, 2011). hCMEC/D3 and HT22 cells have been exposed to different DEP concentrations for different times. The following parameters have been measured: cell viability, oxidative stress and inflammation markers (HO-1, iNOS, Cyp1b1, COX-2, TNFa, IL1b, IL8, VEGF), tight junction proteins (claudin-5, occludin), an amyloidogenic processing marker (BACE-1), besides an AD marker (Tau). In both cell lines, none of the concentrations induced cytotoxicity. In hCMEC/D3, DEP caused increases in HO-1, COX-2 and BACE-1 levels; moreover, the lower dose elicit a significant VEGF release. In HT22, after 3 h all the concentrations caused an increase in HO-1, iNOS, HSP70 and Cyp1b1, whereas after 24 h iNOS and Cyp1B1return almost to control levels. Finally, after 24 h a decrease in Tau levels has been found. In conclusion, all the parameters, except cytotoxicity, were differently affected in hCMEC/D3 and HT22, confirming the major susceptibility of neurons to toxic insults. Supported by Cariplo Fondation.

P22-036
β-amyloid compromises Reelin signaling in Alzheimer's disease

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Reelin is a large glycoprotein which influences synaptic neurotransmission, plasticity and memory in the adult brain through the apolipoprotein E receptor 2 (ApoER2). A growing number of studies demonstrate the interaction between Reelin and its signaling pathway with β-amyloid. However, there is no consensus on whether Reelin levels are increased or decreased in brain regions affected by Alzheimer's disease (AD) and if the Aβ peptide influences Reelin expression and compromises its biological activity. In this study we show that Reelin interacts with β-amyloid in brain extracts by co-immunoprecipitation. We demon-

strate that Reelin increases at transcriptional level in AD brain extracts, and that Reelin accumulates in association with insoluble (guanidine-extractable) β-amyloid deposits. However, characterization in cerebrospinal fluid (CSF) of ApoER2 fragments, generated after Reelin binding, indicate that the Reelin-receptor interaction may result compromise by the presence of β-amyloid. The soluble ApoER2 fragment containing the binding domain is found at lower levels in AD CSF respect to non-dementia samples. Together our result indicate that Reelin levels trend to increase in brain from AD subjects, but Aβ compromises its binding to the receptor and its biological function probably resulting in impaired Reelin signaling and contributing to pathological progression.

P22-037
Anticonvulsant activity of some new Nafimidone derivatives: Effects on GABA metabolism

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Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures. There has been a considerable interest in the development of many antiepileptic and anticonvulsant agents for controlling epilepsy with fewer side effects and improvement of quality of life. The newer agents include amino acids, amides, heterocyclic and enaminones, which can prove useful for the design of future targets and development of new drugs. Among these structures, (arylalkyl)azoles are distinct class of antiepileptic drugs which include imidazole and triazole analogs. Nafimidone is the example of imidazole analogs is a representative of novel triazole anticonvulsants with broad-spectrum activity. Although nafimidone oxime does not show anticonvulsant activity, aryl/arylalkyl substituted oxime ether and aryl substituted oxime esters which have been synthesized by modifications on nafimidone showed anticonvulsant activity. Therefore, we aimed to develop some new oxime ester derivatives of anticonvulsant nafimidone and evaluate the effects on GABA metabolism. To this end, the anticonvulsant activity of the compounds was tested against maximal electroshock (MES) and GABA metabolism parameters were analyzed in mice. We found that oxime ester derivatives of nafimidone showed anticonvulsant activity against MES test, but significant differences in glutamic acid decarboxylase and GABA transaminase activity and GABA levels were found in the group of valeric acid derivatives among nafimidone oxime esters, when compared to epilepsy group.

P22-038
Synthesis of the PCL nanoparticles containing neuroprotectants as efficient (brain) drug delivery systems

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Prevention and treatment of stroke and neurodegenerative diseases such as Alzheimer's and Parkinson's are major and unresolved problems of contemporary medicine. Despite of the progress in understanding of molecular mechanisms of neuronal injury and preventing them, only few neuroprotective substances are used in the clinic. However their efficiency in the treatment of stroke and neurodegeneration is not satisfactory. One of the major limitations is an inefficient delivery of neuroprotective drugs by the blood-brain barrier to the affected part of the brain. Therefore, The main aim of the research is to develop a new strategy of delivery of neuroprotectants by the nanocarriers, which are able to cross the blood-brain barrier without imposing side effect on its normal function. In this work we were focused on preparation of a neuroprotectant loaded PCL (polycaprolactone) nanocarriers. PCL nanoparticles containing active neuroprotectants (Polydatin and/or Resveratrol) as well as model drugs (Cumarin-6, Clozapine and/or Vitamin D_3) were prepared using emulsification by a solvent evaporation technique. All nanocarriers were characterized by size, size distribution, zeta potential, imaged by SEM and their stability in the simulated body fluid (SBF) was determined. Biocompatibility and neuroprotective action of the loaded PCL nanocarriers were evaluated in the SH-SY5Y human neuroblastoma cell line using cell viability/toxicity assays (MTT reduction, LDH release).

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P22-039
Tauroursodeoxycholic acid activates Nrf2 antioxidant system in the MPTP mouse model of Parkinson′s disease

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disease. Although several hypotheses have been proposed to explain the pathogenesis of PD, impaired mitochondrial function and oxidative stress seem to be the most prevalent mechanisms. Nuclear factor erythroid 2-related factor 2 (Nrf2) plays an important role in the defense of oxidative stress by regulating the expression of several Phase II antioxidant enzymes. Tauroursodeoxycholic acid (TUDCA) is an endogenous bile acid, neuroprotective in different models of neuropathological conditions. Importantly, we showed that TUDCA protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurodegeneration, but the mechanisms involved are still incompletely identified. In the current study, we aimed to elucidate part of the possible protective effects of TUDCA against dopaminergic neuron injury in a mouse model of PD induced by MPTP. Twelve-week-old male C57BL/6 mice were treated with MPTP and/or TUDCA before or after the neurotoxin. Our results show that in mice striatum TUDCA is able to up-regulate the expression of Nrf2, heme oxygenase-1 and glutathione peroxidase, as well as superoxide dismutase 2 and DJ-1. Importantly, TUDCA significantly increased glutathione peroxidase activity in the striatum. Interestingly, the effects of TUDCA are also significant when the bile acid is administered after MPTP. Together these results indicate that TUDCA positively regulates Nrf2 maintaining the redox balance, leading to the attenuation of dopaminergic neuron damage. The effectiveness of TUDCA in the modulation of Nrf2 in this experimental model of PD potentially leads to interesting therapeutic perspectives. Supported by FCT grant

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P22-041
The function of the *wrap53* gene in neuronal survival after ischemia

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The *WRAP53* gene encodes an antisense transcript (*WRAP53α*) that stabilizes the endogenous p53 mRNA levels and a protein (*WRAP53*β) involved in Cajal body maintenance, telomere elongation and DNA repair. SNPs in *WRAP53* have been correlated with an increased risk for various sporadic tumors. However, the underlying molecular mechanisms remain unknown. Since neuronal death caused by cerebral ischemia has been linked to accumulation of DNA damage, we investigated the role of WRAP53β in DNA repair and, hence, neuronal survival after ischemia. Moreover, we analyzed the possible association between two SNPs in the *WRAP53* gene (rs2287498;rs2287499) and the functional outcome after ischemic stroke. Primary cultured neurons were exposed to oxygen and glucose deprivation (OGD) for 3 h and were further incubated in culture medium. By RT-q-PCR we verified that OGD/reoxygenation protocol induces *WRAP53* expression. Accordingly, a significant increase in WRAP53 protein levels, determined by Western blotting, was observed during the first 4 h after OGD. This response was maintained during 24 h, after which levels of mRNA and protein decrease to basal levels leading to neuronal death. Polymorphism study in a cohort of ischemic stroke patients revealed that those harboring the *T* allele of rs2287498 poor functional outcome three months after the ischemic insult. In conclusion, WRAP53 could modulate neuronal susceptibility to ischemia. Furthermore, poor prognosis in stroke patients is associated with rs2287498 SNP in *WRAP53* and then this SNP could be considered as a genetic marker for functional prognosis after stroke. Funded by ISCIII (PI12/0685; RD12/0014/0007), Junta de Castilla y León and ESF (ISM).

P22-042
Unequivocal nanomechanics of tau

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Neurodegenerative diseases are incurable disorders with high social impact usually associated with the deposition of insoluble protein deposits in the brain, called amyloid. In tauopathies like Alzheimer disease, the aggregation of a microtubule-associated protein (tau) into amyloid is a key process in the development of the disease. However, the underlying molecular mechanism remains elusive. To understand how natively unfolded tau explores and form aggregation-prone conformations that trigger the pathological amyloidogenic pathway, we applied Single-Molecule Force Spectroscopy based on Atomic Force Microscopy (AFM-SMFS) using a unequivocal single-molecule identification strategy (carrier-guest strategy) to analyze the behavior of tau microtubule-binding domain (*pseudo*-repeats, MBD), a key region for microtubule binding aggregation. Prior to the SMFS analysis, bulk experiments show that fusion protein used in AFM-SMFS experiments is amyloidogenic (CD, TEM, NMR, Congo Red and turbidity) and cytotoxic (protein microinjection) as the isolated MBD structure . This single-molecule analysis shows a landscape characterized by a rich conformational polymorphism that includes highly mechanostable conformers, which resemble previous observations of other representative neurotoxic proteins. Furthermore, this conformational polymorphism is not inhibited by the anti-amyloidogenic peptide QBP1, similar to the findings with amyloid-β, the other key polypeptide associated with Alzheimer's disease. Taken together, our preliminary results further reinforce the hypothesis of the existence of a common mechanism at the early stages of the amyloidogenic cascade in all neurotoxic proteins analyzed this far.

P22-043
Dysfunction of glucose utilization in the brain as a trigger mechanism for neuropathology

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Brain hypometabolism is a characteristic feature of many neurodegenerative diseases (Alzheimer′s, Parkinson′s, and Huntington′s diseases, epilepsy, age-related memory disorders, Friedreich′s ataxia, disseminated sclerosis etc.) used in clinical diagnostics. Until recently it was believed that dysfunction of glucose utilization is a consequence of progressing pathology. However, recent data evidence the primary role of hypometabolism in neuropathology. The purpose of this study is to determine whether a chronic decrease in glucose utilization causes pathological changes in neural network functioning in experimental animals. Wistar rats (male, 240–300 g weight, n = 17) were used for the study. Glucose hypometabolism in the rat brain was created by chronic i.c.v. administration of 2-deoxy-D-glucose (2-DG, 2.5 mkL, 20 mM). 2-DG were injected through the guide cannula daily for 6 weeks. Animals with 0.9% NaCl i.c.v. administration were used as control. Hippocampal EEG analysis revealed that chronic glucose hypometabolism in the brain leads to pathological activity manifested as a significant increase in frequency and duration of high-frequency/high amplitude oscillations (480% and 200%, respectively), as well as in the number of high amplitude spikes. Administration of 2-DG also significantly changed the parameters of hippocampal theta rhythm. Biochemical analysis confirmed deficiency in energy metabolism (the rate of ADP/ATPase phosphorylation in mitochondria was reduced as well as the levels of adenosine and glycogen). These results are important for understanding the general mechanisms of formation of pathological activity in the brain and indicate that the deficiency in glucose utilization may be a triggering mechanism for neuropathology.
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P22-044
Carrier mediated delivery system bearing dopamine for effective management of Parkinsonism

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Delivery of drug and sustaining it in effective concentration in brain is challenging due to blood brain barrier (BBB). In the present investigation, amino acid coupled liposomes bearing dopamine-HCl were prepared to deliver drug to the brain utiliz-

ing receptor-mediated transcytosis for effective management of parkinsonism. L-lysine stearylamine conjugate (LSC) was synthesized & LSC coupled liposomes bearing dopamine HCl was prepared by lipid cast film method. Formulations were analyzed for average vesicle size, drug entrapment, *in-vitro* drug release and *in-vivo* efficacy of the formulations was assessed by measuring the reduction in the degree of drug induced catatonia in albino rats. Average particle size was found in the range of 1.92–0.80 mm. There was increase in the size for coupled liposomes due to the inclusion of LSC in liposomal bilayers. The percent encapsulation efficiency decreased from $46.82 \pm 2.17\%$ in uncoupled to $38.13 \pm 1.18\%$ in coupled liposomes. The *in-vitro* drug release after 24 hrs was $58.9 \pm 2.94\%$ with uncoupled while the coupled liposomes showed $43.7 \pm 2.18\%$ drug release. The lower value for coupled formulation could be due to the retardation of drug release caused due to the incorporation of LSC in the liposomal bilayers, which enhanced the structural integrity of the bilayer. *In-vivo* study reveals that the animals receiving uncoupled liposomes showed partial reduction and animals that received coupled liposomes showed almost complete reduction in catatonia. Fluoresence study clearly indicates the uptake of 6-CF in blood vessels and accumulated in brain. This could be due to enhanced uptake of Lysine coupled liposomes through amino acid transporters present at BBB surface.

Sys Biol S2, Molecular Clocks

P27-005-SP
Analysis and identification of circadian-regulated metabolic pathways in tumourigenesis

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Most organisms evolved an internal timing system which generates circa 24 hour endogenous rhythms allowing the entrainment of physiological and behavioural processes to geophysical time. This time-generating system is termed circadian clock. Virtually every cell possesses a molecular clock, which consists in mammals of a defined set of 14 core-clock genes interconnected in regulatory feedback loops and able to produce oscillations in the expression of target genes. There is increasing evidence that malfunctions of the circadian clock are tightly associated with cancer. Various studies link the disruption of the clock to an enhanced susceptibility to develop cancer and attempts have been made to apply chronotherapy in cancer treatment. In this project, we focus on the connection between the circadian clock and cancer related pathways, particularly metabolic and detoxification pathways. We established a new bioinformatics approach to generate a novel network of circadian regulated genes, which we screened for metabolism and detoxification related genes. For these studies, we use human colon cancer cell lines representing different stages of tumour progression. We found clear phenotypic differences, among the cell lines, with respect to their oscillatory behaviour. We are currently focusing on a set of candidate genes involved in the above mentioned pathways and their contribution to cancer development and progression, as they might be involved in novel ways by which a deregulated clock contributes to tumourigenesis. Results of this work will contribute to a deeper understanding of the role of the circadian clock in cancer with possible consequences in chronotherapeutic treatment strategies.

P27-006-SP
Transcriptomics-based approach to determine subchronic repeated-dose toxicity of GM food in the small intestine of rats and associated *in vitro* models

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Safety assessment of genetically modified (GM) plants is currently performed in animal studies following OECD test guidelines. In order to improve the mechanistic basis of GM food risk assessment and to reduce required animal numbers, the development of alternative *in vitro* testing approaches is needed. In this study, we focused on the intestine as a significant target organ of toxicity. The intestine is a site of extra-hepatic metabolic modification and massive exposure to xenobiotics. We evaluated transcriptomic profiles of rat ileal tissues from two independent 90 day feeding trials with GM-maize (MON810) vs. control maize variants. Mechanistic data sets were generated reflecting potential perturbations of global intestinal signaling pathways by GM-food/feed. Analysis of RNA sequencing (RNA-seq) data revealed differential expression profiles in rat ileum. Notably, significant differences in expression of genes associated with circadian rhythm and metabolism were observed. Circadian clock genes dbp, per3 (males) and nr1d1 were significantly and at least 6-fold up-regulated in the GM group, while bmal1 was 5-fold (males) or 3-fold (females) down-regulated. In addition, we studied the impact of GM food/feed on intestinal cell cultures. Rat small intestinal epithelial cells (IEC-6) were exposed to aqueous plant extracts and phenotype data was acquired by fluorescence-based imaging and real-time impedance measurements. Analysis of *in vitro* assays did not point to any impact of GM-plant materials on cellular phenotypes. In conclusion, circadian effects on transcript pattern have to be considered when applying RNA-seq based analyses on feeding experiments.

P27-007-SP
Circadian regulation of the immune system: a role in tumourigenesis

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Almost all organisms evolved an endogenous circadian clock which regulates the timing of central biological processes and provides a way to adapt physiology and behavior to daily dark/light rhythms. Perturbations of the circadian system were found to be associated to pathological phenotypes including obesity, sleep disorders and increasing incidence of cancer. The mammalian circadian system is hierarchically organized. A main pacemaker is located in the suprachiasmatic nucleus and peripheral oscillators exist in virtually in every body cell. An interconnected set of 14 genes forms the cellular circadian core-clock network (CCN). The CCN regulates the expression of so called clock-controlled genes via transcriptional/ translational loops. To extend the network of clock related genes, we developed a bioinformatics pipeline by combined text-mined literature data with high-

throughput gene co-expression data to obtain new elements and interactions with the direct neighboring targets of CCN. Interestingly, among our top candidates is a group of genes directly or indirectly associated with the regulation of both cancer and immune system: Elavl1, APOH (Apolipoprotein H), IFNAR1 (Interferon Alpha, Beta, Omega Receptor), SP1 (Sp1 Transcription Factor) and NCL (Nuclein). We are now analyzing the circadian phenotype of these genes in cancer cell lines and our preliminary data indicates differences in the oscillatory profiles pointing to their circadian regulation. Taken together, our findings bring us one step forward in the identification of new potential circadian regulated genes highlighting the influence of circadian deregulation in cancer and the emerging evidences indicating the "circadian" immune functions in cancer development and progression.

P27-008-SP
SJL mice immunized with epstein-barr virus antigen LMP1 develop autoantibodies towards myelin basic protein

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Multiple Sclerosis (MS) is an autoimmune chronic inflammatory disease of central nervous system (CNS). At the present time it is evident that activation of B cells is necessary for pathology development. Despite numerous studies on autoreactive B cells and particularly characterization of autoantibodies still there is no actual description of pathologic autoimmune antibodies. One of the possible mechanisms of MS triggering is crossreactivity. It was shown earlier in our laboratory that myelin basic protein (MBP)-specific IgGs are cross-reactive with epstein-barr virus (EBV) protein LMP-1. Here using deep sequencing technique we characterized the common features of cross-reactive antibodies from human MS scFv phage-display library. Utilizing *in vivo* SJL mice model we showed that antibodies initially derived against viral protein LMP1 are able to recognize autoantigen MBP and thus might be the potential MS triggers or enhance its development. We further state that discovered cross-reactivity is rising mainly due to the production of autoantibodies recognizing both antigens simultaneously rather than consequent bystander activation.

P27-009
Taxonomic profile of type II NADH:quinone oxidoreductases and evolutionary implications

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Type-II NADH:quinone oxidoreductases (NDH-II) are membrane proteins involved in respiratory chains and recognized as suitable targets for novel antimicrobial therapies as well as potential therapeutic agents for human neurodegenerative diseases, including Parkinson's disease and aging, caused by complex I failures.This is because 1)NDH-II are the only enzymes with NADH:quinone oxidoreductase activity in many pathogenic organisms, both bacteria and protozoa and 2)its expression restores the mitochondrial activity in animals with complex I deficiency.Thus, in addition to understand their role in the bioenergetic metabolism, the investigation of NDH-II may have social repercussions, namely in health and quality of life. Using a thorough sequence analyses we aimed at recognizing strictly or highly conserved structural elements (amino acid residues or motifs). We consider if a structural element is conserved and thus retained through evolution it has to be determinant for function. We also aimed to observe the enzyme distribution through the different taxonomic groups. We obtained a working data set with 2004 sequences. We observed that from 1804 species present at KEGG's database, 1033 (57%), distributed among the three domains of life (Eukaryotes 62%, Bacteria 60% and Archaea 25%), contain at least one gene encoding NDH-II.We further performed comparative studies using a range of bioinformatics approaches (amino acid sequence alignment, phylogenetic trees and weighted network), where different NDH-II features (number of copies *per* organism, clustering, quinone type) were analyzed. Our data provided the base to discuss the structural determinates for catalysis and substrate selectivity as well as to hypothesize an evolutionary scenario for NDHs-II.

P27-010
Alternative splicing of U2af26 and its role in circadian rhythm – a conserved function across the mammalian class

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Alternative splicing of the penultimate exon can increase the genomes coding capacity by generating a frameshift allowing translation into supposed 3'UTR. Within the mouse U2af26 gene, circadian exon skipping allows the generation of a novel extended C-terminus with homology to the drosophila clock regulator TIMELESS. This C-terminus destabilizes U2AF26 itself and the interacting core clock component PERIOD1 via proteasomal degradation. As a consequence, U2af26 knockout mice show defects in circadian gene expression and adaption to experimental jet-lag. Splicing of U2af26 is rhythmic in mouse and rat but a conserved circadian function across other mammalian species remained enigmatic. A comprehensive analysis of the last U2af26 exon revealed at least one ORF extending into the 3'UTR in each mammalian species with an annotated U2af26 gene. This includes two alternative C-termini for the human U2af26 gene; one accessible by usage of a conserved alternative 3' splice-site. Despite no or low sequence conservation, extended frames from elephant, rat, mouse and human dramatically decreased the half-life of GFP to below 3 hours. In addition, all analyzed instable C-termini – including human – destabilized the interacting PERIOD1 protein. Together these data suggest a conserved function of U2af26 in regulation of PERIOD1 stability and thereby the molecular clock across the mammalian class. We are currently investigating mechanistic principles that mediate the proteasomal degradation of the diverse C-termini. Strikingly all 61 analyzed prolonged C-termini contain exceptionally high amounts of proline, which might function as a novel signal for proteasomal degradation.

P27-011
Effects of hypoxia/anoxia on amylases activity, carbohydrate metabolism, and survival in saffron (*Crocus sativus* L.) corms

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Plants response to abiotic stresses is complex, involving regulatory network and circadian clock, and designed for the type of stress. Effects of hypoxia/anoxia by flooding were investigated in saffron corms with emphasis on α- and β-amylase activity, carbo-

hydrate metabolism, and survival. Dormant corms, and corms rooted for 3 days in normoxia, were flooded *in vitro* for 14 days; *in vitro* controls were cultivated in normoxia. Normoxic corms developed roots and shoots. They exhibited increases in α-amylase activity from 12 to 18 U/mg prot., changes in β-amylase activity from 7 to 4, then 11 U/mg prot., increases in maltose from 34 to 65 μmol/mg prot., and increases in lignin peroxidase activity from 2.6 to 12 U/mg prot. Hypoxic/anoxic corms did not sprout when dormant, and interrupted sprouting when rooted. In hypoxic/anoxic dormant corms, α- and β-amylase activity decreased, respectively, from 12 to 1.3 and from 7 to 1.8 U/mg prot.; maltose promptly increased from 34 to 47 μmol/mg prot.; lignin peroxidase activity decreased by 50%. In hypoxic/anoxic rooted corms, α-amylase went from 12 to 2.6, then 5.5 U/mg prot.; β-amylase, at 4 U/mg prot. for 10 days, decreased to 1 U/mg prot.; maltose, at 50 μmol/mg prot. for 4 days, decreased to 32 μmol/mg prot.; lignin peroxidase activity increased by 50%. Return to normoxia triggered limited root and shoot elongation, but new corms developed. Carbohydrate supply management during hypoxia/anoxia is a major challenge for plants. Here, energy conservation and a shift from growth to maintenance mechanisms allowed for plant survival.

P27-012
Cytokines, chemokines and growth factors profile in caveolin-1 transgenic mice

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Introduction: A caveolin-1 null (CAV-1 -/-) mouse model was created, to assess the role of caveolin-1 in molecular mechanisms of different diseases. Our aim was to investigate the interplay between caveolin-1 absence and modulation of plasma levels of different molecules, such as cytokine, chemokine and growth factor, using two multiplexing technologies.
Methods: Plasma from transgenic mice Cav-$1^{-/-}$ (Cav-1 KO: $Cav1^{tm1Mls}$/J) and Cav-$1^{+/+}$ (B6129PF2/J) (The Jackson Laboratory) as control were analyzed. xMAP analysis were performed on Luminex 200 and multiplex data acquisition was performed using xPONENT 3.1. Images from Proteome Profiler Array analysis were scanned using MicroChemi 4.2 and analyzed by ImageJ software.
Results: xMAP analysis showed increased values in plasma from caveolin-1 transgenic mice than in controls, with statistical significant differences ($p < 0.05$) for the following molecules: IL-1β, IL-2, IL-6, IL-8, IL-4, IL-12p70, TNFα and VEGF. Proteome Profiler analysis presented increased values in plasma from caveolin-1 transgenic mice compared to control group, with statistical significant differences ($p < 0.05$) for IL-1a, IL-6, RANTES, IL-12p70, Eotaxin and VEGF.
Conclusions: Our findings demonstrate that a panel of some above mentioned cytokines, chemokines and growth factors are closely linked to the absence of caveolin-1 in these transgenic mice. Monitoring of these molecular profile in caveolin-1 transgenic mice, may allow us to understand the involvement of caveoli-1 mutation in the molecular mechanisms of many pathological disorders.
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P27-013
Cholesterol homeostasis, drug metabolism and the liver clock interplay

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Circadian clocks are endogenous transcription-translation feedback loop oscillators driving daily rhythms in physiology. Over 3000 genes of the liver are expressed in circadian manner, including genes from cholesterol homeostasis and drug metabolism. Transcriptome studies show that different organs feature different sets of clock controlled genes with different peak phase distributions. Based on expression profiles and known cis-regulatory sites we developed a core clock model for the mouse liver and adrenal gland (Korencic *et al*., 2014). Most of the phase variability from transcriptome data was traced back to E-box and ROR-element regulations. ROR-elements and the REV-ERB/ROR systems represent a link between the circadian clock and lipid metabolism and are inter-connected to BMAL1 regulation. REV-ERBs have heme as their natural ligand, and cholesterol and other oxidized sterols bind to the activation modulator ROR. In contrast to lipid metabolism, the link of the ROR/REV-ERB system to drug metabolism is not well understood. We evaluated phase I, phase II and drug transporter genes for their circadian rhythmicity and potential regulation by REV-ERB and BMAL1. As expected, few genes are targets of BMAL1, and more of REV-ERB. The rhythmically expressed genes show a two-phase profile, with Phase II enzymes and transporters in antiphase. Model simulations of phase distributions of ROR-element regulated genes support the conclusion that REV-ERB directly regulates the drug metabolism components.

P27-014
Cryptochrome is involved in post-transcriptional regulation of metabolism and circadian clock of Neurospora crassa

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Cryptochromes (CRYs) are blue light photoreceptors that have strong sequence similarity with photolyases. They lack the conventional photolyase activity that is required for DNA repair. Instead, animal and plant CRYs function as circadian transcription regulators or photoreceptors. In this study we characterized the role of Neurospora CRY in the circadian clock and metabolism. We found that Neurospora CRY interacts with an argonaute protein and the 5'-3' exo-ribonuclease KEM1 suggesting a post-transcriptional role for CRY. This interaction was stabilized by the C-terminal tail of CRY that contains multiple arginine-glycine-glycine (RGG) repeats. Our preliminary data suggest that CRY binds to mRNAs whose products are involved in metabolism. Moreover, we found that growth of CRY mutant strains are affected depending on the provided carbon source.

Sys Biol S3, Comprehensive Models of Metabolism and Signaling

P28-005-SP
Three days of Islamic intermittent fasting: impact on repeated-sprints performance and related metabolic responses

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Background: This study examined the effects of 3-days of Islamic intermittent fasting (IF) on physical performance and metabolic responses to treadmill repeated-sprints (RS).
Methods: Twenty-one active healthy male Muslim adults (29.8 ± 5.9 years, endurance and team-sports based training 4 ± 1.5 times/week) performed 2-RS sessions [2 sets: (5 × 5-s maximal-sprints (25-s recovery/sprints), 3-min recovery/sets]. Fed/Control session (CS) and fasting session (FS assessed at the 3rd day of the 3-days IF) were counter-balanced.
Main Outcome Measures: Maximum sprinting power (MaxSP) was assessed using an instrumented treadmill. Serum lipids profile (TC, TG, HDL, and LDL), glucose, free fatty acids (FFA), insulin, cortisol, and blood lactate BLC were assessed pre- and post-exercise sessions.
Results: MaxSP decreased significantly in FS (1349 ± 59 W) compared to CS (1408 ± 57; $p = 0.011$ W). Specifically the FS showed a significant reduction in MaxSP at runs 1 and 2 of 2nd set ($p \leq 0.030$). Insulin decreased in CS post-exercise ($p = 0.030$) but not in FS. FFA were always higher in FS than control at pre- and at post-exercise ($p < 0.001$ and $p = 0.003$, respectively). HDL was higher in FS (1.32 ± 0.05 mmol/l) compared to CS (1.25 ± 0.05 mmol/l) at post-exercise (0.039). IF did not affect BLC, whereas, TG decreased both at pre- and post-exercise ($p = 0.008$, and $p = 0.012$, respectively).
Conclusion: During RS, performance is impacted in the initial runs of the second set when fasting. In intermittent fasting conditions, repeated sprints sessions are accompanied by an increased reliance on lipids metabolism through oxidation of FFA.

P28-006-SP
Modeling TNFR1 signal transduction using Petri net formalism

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Tumor necrosis factor receptor 1 (TNFR1) controls essential cellular processes like proliferation, inflammation, and cell death. Thus, strict molecular mechanisms modulate the cellular response either towards apoptosis, necroptosis or NF-κB activation. A disruption in this network can result in pathologies like chronic inflammatory diseases or cancer [1]. To elucidate the molecular regulation of these opposing cellular outcomes, we apply systems biology approaches. To overcome the challenge of missing data for signaling pathways, we choose the Petri net formalism, since it allows for a detailed representation of the molecular events and for topological network analysis [3]. Based on literature, we developed a comprehensive interaction map that describes the cellular processes of TNFR1 signaling including feedback and crosstalk mechanisms as well as the effect of different types of ubiquitination [2]. We found basic pathways which reproduce the cellular signaling cascades towards NF-κB activation, as well as apoptosis and necroptosis initiation. Additional simulation studies reveal distinct regulations like inhibitions or effects of post-translational modifications on the network's behavior.

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P28-007-SP
Sensor kinases TOR and GCN2 orchestrate translation and autophagy in response to carbon, nitrogen and sulfur supply for cysteine synthesis in plants

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In plants, cysteine (Cys) represents the first meeting point between the primary metabolism sulfur, carbon and nitrogen. Cys biosynthesis requires sulfide and *O*-acetylserine (OAS) which are the end-products of assimilatory sulfate reduction and carbon/nitrogen activation, respectively, and produced by sulfite reductase (SiR) or serine acetyltransferase (Serat). Here, we addressed the consequences of cysteine limitation by specific down-regulation of sulfide or OAS supply in genetically engineered *Arabidopsis thaliana* plants with decreased SiR (*sir1-1*) or Serat (*serat tko*) activity. Our results revealed a distinct metabolic phenotype and a specific transcriptional response between both transgenic lines. Furthermore, we demonstrate that the retarded growth of both *sir1-1* and *serat tko* is caused by decreased translation rates. Interestingly, translation in *serat tko* is arrested by induction of General Control Non-derepressible 2 (GCN2)-dependent phosphorylation of eIF2α. Instead of GCN2, decreased activity of Target of Rapamycin (TOR) plays a dominant role in the arrested translation in *sir1-1*, which further results in the decreased level of rRNA and induction of autophagy. Our results reveal for the first time that specific sensing of precursor availability for Cys biosynthesis allows plants to precisely coordinate S metabolism and C/N metabolism to orchestrate translation and plant growth.

P28-008-SP
Towards genome wide reconstruction and validation of signal transduction networks

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Large scale reconstruction of metabolic networks is a well-established process, and a substantial number of genome wide metabolic models are available. The picture is very different for signal transduction networks. While we have a few large scale curation efforts, they are all more limited in scope and typically do no support validation via simulation. The main difference between metabolic and signalling networks is the data structure: Metabolic models deal with changes in the amount of the components, while signalling models typically deal with redistribution of the components between distinct states. Therefore, signalling models often enumerate the possible states. However, this leads to a combinatorial problem that aggravates with network size and makes these methods difficult to use for genome wide reconstruction of signalling networks. Here, we propose an alternative approach. We have developed a workflow for network reconstruction, validation and iterative model refinement that is analogous to that for metabolic networks but adapted to signalling networks. It is based on our previously published rxncon framework. The rxncon language definition scales well with network size, is congruent with experimental data, and supports automatic model generation. The bipartite Boolean model corresponding to the network definition is used for model validation. The data structure enables large scale reconstruction and iterative improvement in fast and cost efficient cycles. Taken together, the proposed workflow provides an approach to tackle large signalling networks, and opens the door to genome wide signalling models.

P28-009
Regulatory actions of physiological concentrations of free amino acids

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Free amino acids are represented by a wide range of related chemical structure and metabolic transformations of compounds that form in the body fluids and tissues amino fund proved that quantification of their pool contributes to the diagnosis of various diseases, including hepatobiliary pathology, cardio-vascular and immune systems, oncological causes, cerebrovascular pathology, alcoholism and diabetes. We demonstrated that the removal of the intermediate metabolic changes can be achieved using individual amino acids and their derivatives, or a combination thereof as universal natural bioregulators – compounds that affect directly on the mechanisms of cellular metabolism in physiological (endogenous) concentrations. As a practical application of the regulatory actions of amino acids and their derivatives for the elimination of amino acid imbalance and metabolic therapy for specific indications proposes a methodology for the development of new multi-local amino acid mixtures according to the pathogenesis deterministic changes in their physiological concentrations.

Key words: free amino acids; regulatory effects; infusion solutions.

P28-010
Comparative analysis of the *nic*-gene cluster within the *Arthrobacter* genus

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The pAO1 megaplasmid of *Arthrobacter nicotinovoras* is responsible for spreading the nicotine-degrading ability to various Gram positive bacteria, such as *Rhodococus* or *Nocardioides*. The plasmid shares most of its *nic*-genes with several *Arthrobacter* draft genomes: M2012083, SJCon and AK-YN10. The current study attempts to make an evolutionary analysis of the *nic*-cluster based on the gene arrangement and collinearity. The draft genomes of several *Arthrobacter* strains were assembled on the existing final *Arthrobacter* genomes using MAUVE and aligned with Artemis. The *Arthrobacter* sp. AK-YN10 (a gift from Dr. Atya Kapley) and pAO1 strains were grown on citrate medium supplemented with nicotine. Nicotine consumption in the medium was fallowed by HPLC. The *nic*-gene cluster can be divided into five modules, each module encoding a precise step in the nicotine-pathway. For each module, a general rule can be observed: the pAO1 modules are the most complex, with a large number of genes, including transposases and insertion elements. The SJCon modules are the most simple, with a small number of ORF′s and large non-coding regions. The AK-YN10 strain is somewhere in the middle, but the five modules are spread through the genome. AK-YN10 can grow on nicotine containing media without forming the characteristic nicotine-blue pigment. HPLC analysis of the culture medium have shown that the growth is accompanied by a slow decrease in nicotine concentration after several days of cultivation.

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P28-011
Ghrelin modulates human Sertoli cells metabolism: relevance for male fertility

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Food habits and lifestyle are becoming less healthy over the years. The actual diet, poor in nutrients, is related with an increased incidence in metabolic diseases (e.g. obesity, diabetes mellitus or metabolic syndrome). Ghrelin is a growth hormone-releasing and appetite-stimulating peptide. The levels of ghrelin are reported to be inversely correlated, in most cases, with body mass index. Besides, it has been suggested that ghrelin can serve as modulator of spermatogenesis. However, the molecular mechanisms by which this hormone affects male fertility remains unknown. Herein, we hypothesize that ghrelin affects human reproductive potential by altering the nutritional support of sper-

matogenesis. The Sertoli cell (SC) metabolizes glucose to produce lactate for the developing germ cells. Thus, we cultured human SCs (hSCs) and tested the effect of different doses of ghrelin (20, 100 and 500 pM) to their glycolytic profile. Protein levels of glucose transporters (GLUT1, GLUT2 and GLUT3), phosphofructokinase, lactate dehydrogenase (LDH) and monocarboxylate 4 were analyzed by Western blot. Metabolite production/ consumption were analyzed by proton nuclear magnetic resonance and LDH activity was assessed. Firstly, we were able to identify the expression of growth hormone secretagogue receptor in hSCs. Our results provide evidence that ghrelin alters the metabolic behavior of hSCs by modulating GLUT1 protein expression, and LDH protein expression/activity and glucose consumption. Additionally, exposure to ghrelin decreased alanine and acetate production in hSCs. This is the first report illustrating that ghrelin, a hormone known to be deregulated in obesity, modulates hSCs metabolism and consequently the nutritional support of spermatogenesis.

P28-012
Tracing the presence of an enzyme essential for *de-novo* biosynthesis of NAD in the avian lineage

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NAD is a crucial cofactor in redox reactions, but is also involved in a multitude of important regulatory processes. Evolutionary studies revealed that NAD biosynthetic enzymes are very conserved among vertebrate species. However, one enzyme – the quinolinate phosphoribosyl transferase (QPRT) – that is essential for the *de-novo* synthesis of NAD from tryptophan, seems to be missing in some species. To date there is no evidence for the presence of QPRT on the genomic level in any bird, even though all adjacent enzymes of the NAD metabolic network are present. Two hypotheses could explain this observation (1) QPRT has not been identified in the avian lineage because it is encoded on a very small chromosome that is very difficult to assemble or (2) QPRT encoding genes are absent from avian genomes – and QPRT function is compensated either directly by another enzyme or due to regulatory plasticity. Here we use 48 recently sequenced bird genomes to extensively scan for the presence of QPRT. We compare our finding to other reptiles and use interspecies comparison of adjacent enzymes in the metabolic network to explain functional shifts of avian NAD *de-novo* biosynthesis. This study has several fundamental implications. First, it will reveal the importance to carefully examine database information. Second, it will shed light on the question if pathways might exist that circumvent QPRT in vertebrates. Third, it will reveal a possible genetic mechanism to cope with the malfunction and/or absence of QPRT, known to cause severe diseases in humans.

P28-013
Connecting signalling output to metabolic regulation reveals strategies of reprogramming a cellular energy homeostasis

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Reprogramming of the carbon/nitrogen-balance in plants is crucially involved in the metabolic response towards environmental fluctuations and stress conditions. This metabolic reprogramming is an output of complex biochemical regulation affecting various levels of molecular organization, such as the transcriptome, proteome and the metabolome. Numerous regulatory circuits, nonlinear enzyme kinetics and thermodynamic constraints prevent the intuitive derivation of conclusive hypotheses from such comprehensive experimental data sets. To overcome this limitation, we developed a strategy of experimental high-throughput analysis and mathematical modelling focusing on steps of metabolic reprogramming during energy depletion and carbon starvation in the model plant *Arabidopsis thaliana*. We report on a strategy for functional integration of levels of proteins and metabolites from high-throughput mass-spectrometry analysis based on network information gained by a genome-wide metabolic reconstruction. Statistical time-series analysis and mathematical modelling revealed a highly dynamic interplay between carbohydrate metabolism, the tricarboxylic acid cycle and the metabolism of amino acids being crucial for an efficient metabolic response to low energy stress. Further, the analysis of the phosphoproteome revealed a significant involvement of highly-conserved signalling components in metabolic reprogramming. Thus, we were able to directly connect the output of signalling pathways to the regulation of metabolic activity comprising rate-limiting enzymatic steps in plant primary metabolism. Finally, our results reveal evolutionary conserved strategies of metabolic regulation playing a central role in reprogramming of metabolism due to environmental changes.

P28-014
Effect of oxidative state and TNFα on ICAM-1 expression and release in intestinal myofibroblasts

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Intercellular adhesion molecule-1 (ICAM-1) is distributed and expressed on cell surface and is present in circulation as soluble form (sICAM-1), lacking the transmembrana and cytoplasmic domains. Tumor necrosis factor-α (TNFα) and radical oxygen species (ROS) up-regulate the expression of ICAM-1. Intestinal subepithelial myofibroblasts (ISEMFs) express ICAM-1 and in the literature there are not data concerning the release of sICAM-1 by these cells and the redox regulation of ICAM-1 and sICAM-1 in ISEMFs. The aim of this study was to investigate the role of oxidative state on ICAM-1 expression and sICAM-1 release in a myofibroblast cell line, derived from human colonic mucosa (18Co), stimulated or not by TNFα. The intracellular redox state was modulated by buthionine sulfoximine (BSO) or N-acetylcysteine (NAC), inhibitor and precursor, respectively, of GSH synthesis. In cells treated with BSO or TNFα, used separately or together, an increase in H_2O_2 production was measured and this was related to an up-regulation of ICAM-1 expression and sICAM-1 release. Moreover, a direct effect of TNFα on ICAM-1 expression and sICAM-1 release was observed. In fact, in these conditions, the treatment with NAC was able to restore H_2O_2 production to control values but not ICAM-1 or sICAM-1 levels. The involvement on sICAM-1 release of metalloproteinase TNFα-converting enzyme, by using its specific inhibitor, TNFα-protease inhibitor-1, was demonstrated. The results indicate that the up-regulation of ICAM-1 expression and of sICAM-1 release in 18Co depends on ROS production, but a TNFα-independent redox regulation was also demonstrated.

P28-015
Inference of signal transduction pathways from phosphorylation data to identify targets of combinatorial cancer therapy

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Over-activation of the MAPK/ERK signaling pathway can lead to uncontrolled cell growth and has been associated with many types of cancer. Detailed knowledge about the underlying network has therefore led to the discovery of potent treatments in targeted cancer therapy. However, intrinsic and acquired resistance through network rewiring corrupts their effectiveness, calling for combinatorial therapeutic interventions. The ability to design such appropriate treatments requires the identification and quantification of related kinase interactions. To this end, we conducted an experimental survey of phosphorylation states of selected kinases in different cancer cell lines and measured the response to various types of stimulations and inhibitions. However, these measurements represent the aggregate interplay between all involved kinases and do not directly quantify their direct interactions. To overcome this challenge, we propose a computational method that infers local interaction strengths between pairs of network components from global steady states. Its crucial advantage is a high practicality, as it allows for an efficient and coherent mathematical treatment of large networks, unobserved network components, and noisy data.

P28-016
Angiotensin I-converting enzyme inhibiting (ACEi) activity of oat *Avena sativa* L.) protein-derived *ex-vivo* digests

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In recent years an increase interest in food ingredients with specific biological activity and health-related functions has been observed. The best known bioactive peptides with antihypertensive properties are angiotensin I-converting enzyme [E.C. 3.4.15.1] inhibitors. Globulin and prolamin fractions from oat seeds are proteins of which the nutritional value is comparable to the proteins of e.g. milk. In this study, oat protein digests obtained by use of human gastrointestinal enzymes were analyzed for their ACEi activity. Digestion was carried out in three steps: (1) "chewing" – 3 min, (2) "stomach" with a gradual pH reduction from ~6.2 to 2.5 continuing – 2 hours, (3) "duodenal" – 1 hour (pH = 7). The resulting digests were used for ACEi peptides screening. The sample treated with gastric and duodenal juices demonstrated the highest degree of ACE inhibition (84%, IC_{50}=0.44 mg/ml). The proteins are only partially digested with pepsin in the stomach, so the sample digested by gastric juice was characterized by a lower degree of inhibition (74%, IC_{50}=5.25 mg/ml) comparing to the sample treated with gastric and duodenal juices. The degree of ACE inhibition of untreated sample was the lowest (47%, IC_{50}=27.62 mg/ml). Digested samples of oat proteins were separated using RP-HPLC-MS method. Amino acid sequences were identified using LC-MS/MS method based on *in silico* systematic screening for ACEi peptides. In oat proteins digests, the following ACE inhibitory sequences were identified: GDAP, LSP, LLP and VAV. In our studies we documented ACEi effects of oat proteins digests obtained by human gastrointestinal enzymes.

P28-017
Characterization of physiological roles of enzyme X in pancreatic β-cells *in vitro* and *in vivo*

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The X is a class of enzymes and mediates GPCR signaling pathways induced by neurotransmitters, hormones and growth factors which regulate diverse cellular processes. The X enzyme consists of four isotypes, X-1,2,3 and 4. The X-4 is highly expressed in brain and deletion of X-4 in mice causes severe ataxia and impaired visual processing. In addition, immunohistochemistry results show that X-4 is expressed in compartment positive for insulin which is pancreatic β-cells in islets. The G_q and G_{a11}, which activates X-4, mediate physiological effects in pancreatic β-cells. Therefore, the X-4 has potential of key molecule in signaling pathway in the pancreatic β-cells. However, the role of X-4 and its molecular mechanism in pancreatic β-cell has not been established *in vitro* and *in vivo*. To study X-4 function in pancreatic β-cells *in vivo*, we conditionally inactivated X-4 in islets by mating neurog3_cre mouse. The ablation of X-4 gene in mouse causes islet hypertrophy and hyperplasia in pancreas. The expansion of islets will be contributed to amount of insulin secretion. The disturbance of insulin secretion results in pathologic conditions such as diabetes mellitus, insulinoma and metabolic syndrome. Therefore, maintenance of insulin balance by regulation of insulin secretion is important and the regulation of insulin secretion is the key factor for improving different metabolic disorders. The X-4 can be identified as candidate for a therapeutic intervention.

P28-018
Oxidative stress in the kidney of adult dahl rats with salt hypertension

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Oxidative stress is enhanced in different rat models of hypertension. It seems that a major source of O_2^- in the kidney of rats with different forms of experimental hypertension is nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase. This multi-subunit enzyme catalyses the reduction of molecular oxygen and oxidation of NADPH to generate superoxide radicals (O_2^-). The aim of our study was to investigate whether salt hypertension induced in adult Dahl salt-sensitive (DS) rats is accompanied with a more pronounced oxidative stress in the kidney when compared to normotensive Dahl salt-resistant (DR) controls. NADPH oxidase activity (determined by lucigenin-enhanced chemiluminiscence assay), content of thiobarbituric acid-reactive substances and conjugated dienes (indicating a degree of lipid peroxidation damage) were evaluated in both renal cortex and medulla. High salt intake induced hypertension (213±5 mmHg) and increased relative heart and kidney weights in DS rats but did not modify blood pressure (139±2 mmHg) and relative organ weights in DR rats. The enhanced NADPH oxidase-mediated O_2^- production was detected in the renal medulla of salt hypertensive

DS rats (202469±23020 counts/mg protein) compared to both normotensive controls – DR rats fed a high-salt diet (102419±10613 counts/mg protein) and DS rats fed a low-salt diet (127389±6031 counts/mg protein). In contrast to renal medulla, there were no significant changes in the renal cortex of hypertensive animals. Nevertheless, we have not observed any signs of increased lipid peroxidation in the renal medulla or cortex of adult salt hypertensive DS rats. This work was supported by research grant 304/12/0259 (Czech Science Foundation).

P28-019
Mechanism of LPK activity regulation by intrinsically disordered region phosphorylation

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Pyruvate kinase (PK) catalyses the last step of glycolysis, transferring the phosphoryl group of phosphoenolpyruvate (PEP) to ADP so producing pyruvate and ATP. L-type pyruvate kinase (LPK) consists of 3 main domains and small intrinsically disordered N-terminal domain, which participates in regulation of LPK kinetic properties. The phosphorylation of this region on Ser12 residue significantly modifies catalytic properties of LPK and triggers off allosteric behaviour of enzyme toward substrate PEP [Faustova 2010]. Fenton & Tang 2009 suggested that N-domain interact with main body of LPK thus affecting substrate binding. We have revealed that introducing different ionic groups by mutations around the phosphorylation site was not enough to disturb the suggested interaction and couldn't lead to occurring the cooperative properties of enzyme. However, in some cases mutations decreased the enzyme affinity for PEP [Faustova 2012]. Therefore we suggested that phosphorylated and non-phosphorylated regulatory domain binds to different sites of protein main body. We used computational blind docking approach to identify possible putative binding sites in LPK subunit for peptides RRASVA and RRAS(Pi)VA. These peptides mimic non-phosphorylated and phosphorylated binding sites of intrinsically disordered region. Analysis revealed that preferable docking site for peptide RRASVA is in enzyme active centre. The docking site for phosphorylated analogue occurred preferably in C-domain, overlapping the binding site of allosteric regulator FBP [Kuznetsov 2014]. This result supports both suggestions. As the active form of LPK is tetramer, the real regulation mechanism should consider possibility that phosphorylated domain of one subunit interacts with C-domain of another subunit. Significant flexibility of intrinsically disordered domain seems to allow this inter-subunit interaction.

P28-020
90 days of human muscle rest decrease the expression of many mRNAs from glucose metabolism. Exercise partially counteracts this effect

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Skeletal muscle atrophy and strength loss induced by long-term rest are attenuated by resistance exercise. The metabolic involvement in this atrophy has not been well studied in such long periods of rest. The aim of this study is to monitor and compare the expression of enzymes that regulate glucose metabolism in human muscle by determining changes in mRNAs of subjects who are immobile for 90 days and others who follow the same pattern but performing a concentric-eccentric exercise every two days. 21 participants performed 90 days of bed rest. Group 1: (BR) 12 participants rested. Group 2: (BRE) 9 participants completed a concentric-eccentric resistance protocol. Muscle biopsies were obtained before and after finish the experience. Muscle glycogen synthase (GS), muscle glycogen phosphorylase (GPh), hexokinase 2 (HK), phosphofructokinase (PFK-1), citrate synthase (CS) mRNAs were assessed by qRT-PCR using a relative standard curve and normalized by GAPDH and 18S rRNA. BR caused a decrease in GS, GPh, HK and CS mRNA being significant in HK and CS. However, BR showed an important increase of PFK-1 mRNA. In the other hand, exercise training increased expression of all mRNAs being particularly significant the GPh and HK. In summary, the decrease in energy metabolic capacity provided by the oxidation of glucose that occurs during prolonged immobility muscle degeneration corresponds to a decreased activity of regulatory key enzymes, correlating with decreased expression at the gene level. This effect is partially counteracted by resistance training. Funded by European Space Agency (ESA 12RL000214).

P28-021
Role of nitric oxide and CD2BP3 adapter protein on human sperm motility

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Nitric oxide as a modulator of several physiological processes and is involved on different sperm functions including motility. Since CD2BP3 adapter protein takes part in the regulation of cytoskeleton stability and dynamics, it is assumed that it can regulate the sperm motility. In present study we have investigated the concentration of NO metabolites and expression of CD2BP3 in human sperm cells. Sperm samples were obtained from 20 normozoospermic fertile men and 20 patients with excretory toxic infertility. It was shown that changes in NO_2^- concentrations correlate with the development of many pathological conditions, including asteno- and teratozoospermia. It was established that NO_2^- concentration in the sperm cells was not significantly changed in patients with oligozoospermia, but positively correlated with the development of astenospermia. A significant negative correlation was evident between NO_2^- concentrations and sperm motility in patients with excretory toxic infertility. It was found that CD2BP3 adapter protein associated with proteins of cytoskeleton is expressed in sperm cells. Results of immunofluorescence analysis indicate that signal is concentrated in the neck and the tail of spermatozoa containing a large number of motor fibers. It was shown that electrophoretically slower migrating form of CD2BP3 adapter proteins are not detected in sperm with impaired mobility. These data suggest that NO concentration and CD2BP3 expression have a potential pathogenetic implication in reduction of sperm motility.

P28-022
Chrysin attenuates liver fibrosis and hepatic stellate cell activation through TGF-β signaling pathway

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Chrysin is a natural flavonoid present at high levels in honey, propolis and has been found to possess antioxidant, anti-inflammatory and anti-cancer properties. In the present study, we investigated the antifibrotic effect and mechanism of action of chrysin in a mouse model of carbon tetrachloride (CCl_4) -induced liver fibrosis. Experimental fibrosis was established by CCl_4 intraperitoneal injections of mice for 7 weeks. Mice were orally treated with 3 doses of chrysin (50, 100 and 200 mg/kg) and with vehicle as a control. The degree of hepatic fibrosis was determined by hematoxylin&eosin and Fouchet-Van Gieson staining, along with ultrastructural changes assessed by electron microscopy. The expression and specific hepatic distribution of transforming growth factor-b1 (TGF-β1), SMAD2/3, alpha-smooth muscle actin (α-SMA), tissue inhibitors of metalloproteinases (TIMP) were determined. Hepatic fibrosis decreased markedly in CCl_4-treated animals following treatment with flavonoid, compared with control. Treatment of chrysin significantly inhibited the expression of TGF-β1 in dose-dependent manner. Also, chrysin administration successfully decreased hepatic fibrinous deposits and restored histological and ultrastructural architecture. Our results suggest the therapeutic effects of chrysin in CCl_4-induced liver fibrosis by promoting HSC inactivation and down-regulation of fibrogenic stimuli, with strong enhancement of hepatic regenerative capability.

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P28-023
Magnetic photons of homeopathic remedies cured rheumatic disease according to biochemical pathways

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Magnetic photons with resonance frequencies in the MHz region had been detected in highly succussed homeopathic remedies by two resonance methods. This fact led to the conclusion that high potencies of substrates, inhibitors and enzymes of the pathological pathways could regulate them by the resonance principle according to the homeopathic law of similars. 15 rheumatic patients suffered from various symptoms: exhaustion, weakness of the muscles with delayed reactions and pains in all muscles during movement, accompanied by high blood pressure and obesity. These symptoms indicate a low bioavailability of the neurotransmitter acetylcholine. In some cases the activity of cholinesterase (EC 3.1.1.7) splitting acetylcholine into the acetyl-residue and choline was very high. It was supposed that the activities of lipase (EC 3.1.1.3) and glycolysis were low. For the synthesis of acetylcholine substrates, inhibitors and enzymes in LMK-potencies had been applied:

1) to get choline by the splitting of lecithin: Lipasum, Lecithinum, Cholinum, Phosphorus;

2) to get acetyl-coenzyme A from glycolysis and fatty acid metabolism: Glucosum, Glycerinum, ATP, and Acetyl-Coenzyme. A.

3) for the regeneration of acetylcholine receptor and acetylcholine-esterase at the post-nervesynapsis: Naja trip, its venum contains cholinesterase and cobrotoxine – irreversible inhibitor of acetylcholinereceptor- Atropinum – its reversible inhibitor- and its substrate Acetylcholine, Dendroaspis polylepis – venom of black mamba/ irreversible inhibitor of acetylcholine-esterase;

4) for the function of the nerv-channels:Natrium chloratum, Kalium carbonicum, Calcium phosphoricum and Magnesium phosphoricum. All remedies were given every other day. After two months the patients moved without having pain and cholinesterase became normal.

P28-024
Application of the correlations between the interfacial tensiometry and biochemical parameters of the animal blood for comprehensive diagnostics

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The development and application the "integrative" approaches and methods for animal blood diagnostics are both of fundamental and applied importance. There were some attempts (prior to our work) to estimate a static surface tension of human serum or plasma samples. Recently the most powerful techniques of the dynamic surface tension (DST) measurements has been developed and successfully applied for biological liquids. The main aims of the work are the following: to study the DST and biochemical parameters of the animals serum; to obtain the correlations between theses parameters in order to prove the availability of such method for animal blood diagnostics. The biochemical (proteins, lipids, etc.) and DST (σ_1, σ_2, σ_3 at 0.1s, 1s, 10s) parameters were obtained for correlation analyses. For goats: the strong positive correlation (SPC) found between σ_1, σ_2, σ_3 and glucose levels; whereas strong negative correlation (SNC) found between σ_3 and protein (chloride) levels. For cattle: SPC found between σ_1 and protein (triglycerides) levels, σ_2 – sodium level, σ_3 – chloride level; SNC found between σ_1, σ_2 and chloride level, σ_3 – sodium level. There were also some middle or weak correlations found. In the veterinary science and practice such correlations are important for the estimation of the organism physiological status, for general inspections of cattle before vaccination (immunization) or slaughter, for "quick separation" of healthy and ill animals in the case of infection, etc. The author is thankful to Dr.I.Milaeva, Dr.E.Zarudnaya, Dr.N.Dovshenko. This work was supported by the Russian Scientific Foundation (grant 14-16-00046).

P28-025
Identifying *cis*-acting sugar response elements in promoter of genes that facilitate glucose signaling in *Arabidopsis*

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Over the years, a number of genes involved in juvenility and floral signal transduction, has been found to be transcriptionally, regulated by sugars. However, little is known about the transcrip-

tional mechanisms which underlying these responses. This is due to the capacity of sugars to act as nutrients, osmotic regulators and signalling molecules. *Cis*-acting sugar regulatory elements are important molecular switches involved in the temporal and spatial expression of a dynamic network of gene activities. This network controls hormone and abiotic stress responses, and developmental events such as juvenility and floral induction. In this study, we have examined expression levels and promoter features of different genes encoding proteins that have been implicated as targets of glucose signal transduction pathways, and might participate in juvenility and floral induction. Identifying the functionally active sugar response elements in the predicted promoter regions of genes that undergo glucose induced transcriptional regulation will lead us closer to understanding these signal transduction mechanisms.

P28-026
Study of *Brachypodium distachyon* and local breed soft wheat varieties tolerance to adverse environmental factor

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The Republic of Kazakhstan is one of the world leading countries in production of trade wheat grain, and the problem of lands salinity here is quite acute. Proline is one of the most widely distributed natural osmolytes, which is accumulated in plants during their protection against various abiotic factors. *Brachypodium distachyon* is a widely recognized model plant, closely related to wheat. The aim of our study was to evaluate the content of proline and soluble protein in *Brachypodium* and local breed soft wheat varieties (Shagala and Kazakhstanskaya 3) under standard 2% NaCl salinity. Experimental data on Shagala variety have shown 3 times increase in proline content under salinity for seedlings (namely, 126.53±0.011 mg/g from 45.21±0.02), and 5 times increase in such for roots; thus leading to a conclusion that under salinity prolin is mostly accumulated in seedlings, rather than in roots. However, we got an opposite picture for Kazakhstanskaya 3: 9 times proline increase in seedlings (169.00±0.03 mg/g), with only 3 times increase in roots (16.65±0.05 mg/g). In *Brachypodium* proline content under salinity in seedlings raised up to 101.00±0.03 mg/g, in roots 16.50±0.05 mg/g; absence of change in proline content in seedlings and roots has been observed. Content of soluble protein in *Brachypodium* is higher (0.460±0.002 mg/ml) in comparison with such of Kazakhstanskaya 3 and Shagala (0.179±0.01 and 0.188±0.01 mg/ml, correspondingly), using microbiurete method by *Bailey*. Experimental data allowed to place them in the following order of salt tolerance *Brachypodium* < Shagala < Kazakhstanskaya 3.

P28-027
Collagen I induces TNF-α production and down-regulation of IRF4 to regulate the activation of dendritic cells

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The activation of dendritic cells (DCs) play a role to regulate the immune response. Inflammatory mediators such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β and lipopolysaccharide (LPS) are also known to activate DCs. We have previously shown that collagen I enhances the maturation and function of DCs. Here we investigated the involvement of TNF-α on the collagen I-induced DCs activation. The of neutralization of TNF-α inhibited collagen I-induced IL-12 secretions by DCs. Additionally, we observed suppression of collagen I-induced co-stimulatory molecules expression along with down-regulation of genes involved in DCs activation pathway. Furthermore, TNF-α inhibition upon collagen I stimulation up-regulated the expression of interferon regulatory transcription factor IRF4, when compared to collagen I only treated cells. Collectively, our data demonstrate that collagen I induce TNF-α production, which is crucial for the activation and function of DCs, through down-regulation of IRF4, and implicates the importance in development of anti-TNF-α therapeutics for several inflammatory diseases.

P28-028
Luteolin attenuates adipocyte-derived inflammatory responses via suppression of NF-κB/MAPK pathway

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Inflammation of adipocytes has been a therapeutic target for treatment of obesity and metabolic disorders which cause insulin resistance and hence lead to type II diabetes. Luteolin is a bioflavonoid with many beneficial properties like antioxidant, antproliferative and anti-cancer. To elucidate the potential anti-inflammatory response and the underlying mechanism of luteolin in 3T3-L1 adipocytes we stimulated 3T3-L1 adipocytes with the mixture of TNF-α, LPS and IFN-γ (TLI) in the presence or absence of luteolin. Luteolin opposed the stimulation of inducible nitric oxide synthase (iNOS) mRNA and protein expressions and NO production by simultaneous treatment of adipocytes with TLI. Also, it reduced the mRNA expression of pro-inflammatory genes like COX-2, IL-6, and resistin and also the chemokine, MCP-1. This inhibition was associated with suppression of IκB-α degradation and subsequent inhibition of NF-κB p65 translocation to the nucleus. In addition, luteolin blocked the phosphorylation of ERK1/2, JNK and also p38 MAPKs. These results illustrate that luteolin attenuates inflammatory responses in the adipocytes through suppression of NF-κB and MAPKs activation, suggesting that luteolin may represent a therapeutic agent to prevent obesity-associated inflammation and insulin resistance.

P28-029
CrossHub: cross-analysis of TCGA RNA-Seq, miRNA-Seq, methylation and mutation data

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The Cancer Genome Atlas Project (TCGA) is the largest resource in the field of molecular oncology. It accumulates genomic, transcriptomic and methylomic data for more than 15 cancers. We developed the CrossHub software (available at https://

sourceforge.net/projects/crosshub/) which allows to perform cross-analysis of these data enabling new insights into mechanisms of carcinogenesis. This software provides various types of correlation analyses. First, CrossHub enables correlation analysis of TCGA RNA-Seq and miRNA-seq data coupled with miRNA target prediction performed with several algorithms (TargetScan, mirSVR, PicTar, DIANA microT) and catalogue of validated miRNA targets (miRTarBase). This suggests CrossHub as a powerful instrument for prediction of miRNA targets playing a role in the development of various cancers. The second feature of CrossHub is the correlation analysis of gene expression level and methylation status of either single CpG sites or whole CpG-island. CrossHub also helps to identify driver mutations in non-coding regions: it evaluates correlations between the presence of mutations in gene promoter region and gene expression as well as associations between mutations (either in coding or non-coding regions) and tumor size, grade, and prognosis. Thus, CrossHub outlines three most common reasons of tumor suppressor gene inactivation: it allows to link CpG-island methylation, miRNA interference and mutations in the non-coding regions to the gene expression alterations as well as identify associations between mutations and tumor pathomorphological characteristics. This work was supported by the Russian Foundation for Basic Research (grants 15-04-08731 a, 14-04-32084 mol_a, and 15-04-06198 a) and RAS Presidium Program "Molecular and Cellular Biology".

P28-030
Mitochondrial dysfunction in patients with HIV infection

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Development of potent active antiretroviral therapy (ART) has revolutionized the treatment of HIV infection. An increasing number of therapeutic drugs have been implicated in targeting HIV and causing mitochondrial dysfunction, which likely contributes to some of the adverse effects and organ toxicity associated with these drugs. Because mitochondria play an important role in the apoptotic pathway, the activation of one results in the destruction of hepatocytes via apoptosis. The aim of the paper was to assess whether antiretroviral regimes are able to diminish apoptosis. In our study, mitochondrial apoptotic caspase cascade has been evaluated in 80 patient with HIV-infection and treated by antiretroviral drags (NRTI+NNRTI predominantly). Level of cytochrome C, BAX, apoptosis regulators BCL-2 and BAX, caspases 1, 3, 9 in blood samples was measured by a commercial enzyme-linked immunosorbent assay (ELISA). The means and standard deviations were calculated and the regression test and Student's *t*-test were performed.Our results suggest that HIV decreases the production of molecules involved in marking the cell for apoptosis, giving the virus time to replicate and continue releasing apoptotic agents and virions into the surrounding tissue. In patients administered ART, activity apoptotic factors was significantly different that in HIV-patients, who not received ART, but with a broad spectrum. This finding support various mechanisms of mitochondria apoptotic cascade alteration in presence/absent antiretroviral agents and clarifies the involvement of apoptosis in the pathogenesis of HIV infection and side-effects (especially hepatotoxicity via mitochondrial dysfunction) of treatment with different schemes of ART.

P28-031
Radiotherapy-related changes in serum profile of lipids are primarily associated with a type of acute toxicity; comparison of radiation-induced effects in patients with prostate cancer and head and neck cancer

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Partial body irradiation during radiotherapy affects different molecular components of blood. Here we characterized changes of lipid levels in human serum samples induced by radiotherapy. We also compared their effects between patients with prostate cancer and head and neck cancer. 129 patients with prostate adenocarcinoma and 66 patients with head and neck squamous cell carcinoma were enrolled into the study. Patients were subjected to IMRT using 6MeV photons. Blood samples were collected from each patient in three points of time: pre-treatment (A), within-treatment (B) and post-treatment (C). The samples were analyzed using MALDI-ToF spectrometer. Obtained data was subjected to statistical analysis. The Wilcoxon signed rank test was used for verification whether observed differences in abundances were significant. Several spectral components changed their abundances significantly between compared time points. The most changes were noticed while the treatment was carried out. Most of them were reversing during the follow-up, yet several changes could be still detected one month after the RT was ended. We noticed that the amount of altered lipid levels in serum was higher in case of HNSCC patients than for PC patients. The level of RT-induced changes in serum lipidome profiles was associated with intensity of acute radiation toxicity, which was apparently higher in HNSCC patients. Risk and intensity of acute radiation response could be monitored by molecular profiling of lipid fraction of serum in cancer patients undergoing IMRT. This work was supported by the European Community from the European Social Fund within the INTERKADRA project UDA-POKL-04.01.01-00-014/10-00 (to MR).

P28-032
Metabolic state modulates the intracellular localization of aldolase B and its interaction with liver fructose-1,6-bisphosphatase

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Understanding how glucose metabolism is finely regulated at molecular and cellular levels in the liver is critical for knowing its relationship to related pathologies, such as diabetes. In order to gain insight the regulation of glucose metabolism, we studied the liver expressed isoforms aldolase B and fructose-1,6-bisphosphatase (FBPase-1), key enzymes in gluconeogenesis, analyzing their cellular localization in hepatocytes under different metabolic conditions and their protein-protein interaction *in vitro* and *in vivo*. We observed that glucose, insulin, glucagon and adrenaline dif-

ferentially modulate the intracellular distribution of aldolase B and FBPase-1. Interestingly, the *in vitro* protein–protein interaction analysis between aldolase B and FBPase-1 showed a specific and regulatable interaction between them, whereas aldolase A (muscle isozyme) and FBPase-1 showed no interaction. The affinity of the aldolase B and FBPase-1complex was modulated by intermediate metabolites, but only in the presence of K+. We observed a decreased association constant in the presence of adenosine monophosphate, fructose-2,6-bisphosphate, fructose-6-phosphate and inhibitory concentrations of fructose-1,6-bisphosphate. Conversely, the association constant of the complex increased in the presence of dihydroxyacetone phosphate and non-inhibitory concentrations of fructose-1,6-bisphosphate. Notably, *in vivo* fluorescence resonance energy transfer studies confirmed the interaction between aldolase B and FBPase-1. Also, the co-expression of aldolase B and FBPase-1 in cultured cells suggested that FBPase-1 guides the cellular localization of aldolase B. Our results provide further evidence that metabolic conditions modulate aldolase B and FBPase-1 activity at the cellular level via the regulation of their interaction, suggesting that their association confers a catalytic advantage for both enzymes (FONDECYT1141033).

P28-033
Stearyl alcohol, one of the most effective lipase-super-inducers, not only induces the expression of virulence related genes but also induces the production of polyester in *Ralstonia* sp. NT80

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Extracellular lipase activity from *Ralstonia* sp. NT80 is induced significantly by fatty alcohols such as stearyl alcohol [1)]. We found that when lipase expression was induced by stearyl alcohol, a 14-kDa protein (designated EliA; effector protein of lipase induction) was produced concomitantly and abundantly in the culture supernatant [2)]. Comparison of secreted proteins from NT80 cells grown with or without stearyl alcohol revealed that stearyl alcohol induced expression not only of lipase but also of hemolysin-coregulated protein (Hcp) and nucleoside diphosphate kinase (Ndk), which are involved in the virulence of pathogenic bacteria. Expressions of these secreted proteins were induced at the transcriptional level. Stearyl alcohol also induced synthesis of poly hydroxyl butyrate (PHB), known to be produced by several bacteria, and affected the shape of cells as well. These responses of NT80 cells to stearyl alcohol required the secreted protein EliA. The concentration of the stearyl alcohol in the culture supernatant of the DeilA cells did not decrease whereas that in the wild type cells evidently decreased during the course of growth. These results suggest that EliA is essential for cells to respond to stearyl alcohol and it plays an important role in the recognition and assimilation of stearyl alcohol by NT80 cells.

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P28-034
Long-chain alkane degrading *Acinetobacter* sp. BT1A from petroleum contaminated Soil

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The aim of this study is to perform the isolation of petroleum-degrading bacteria from oil-contaminated soils. A bacterial strain BT1A belong to *Acinetobacter* genus was isolated from petroleum contaminated soil in Diyarbakır petroleum field. Morphological, physiological and biochemical characterization of bacterial strain were carried out. According to 16S rRNA gene sequencing analysis, BT1A was found to be closely related to *Acinetobacter baumannii*.The bacterial strain BT1A was found to use crude petroleum as carbon and energy sources in order to grow.With the aliphatic hydrocarbons, growth was seen only in the long-chain alkanes tested (tridecane, pentadecane and hexadecane). No growth was recorded in the short-chain alkanes (hexane) tested.Among the long-chain alkanes tested, hexadecane was the most preferred. GC-MS analysis showed that BT1A was able to degrade 83% n-alkanes in the crude oil in 7 days, which means that it metabolises the crude oil.

P28-035
Thermo-alkaliphilic strains producing some industrial enzymes, isolated from Sorgun Hot Spring in Turkey

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Four different novel thermo-alkaliphilic bacteria were isolated from soil and water samples of a hot spring of Sorgun, Turkey. The isolated bacterial strains were identified morphologically, biochemically and molecularly with the aid of 16S rDNA sequencing. All bacteria showed their optimum growth at alkaline pH (7.0–12.0) and grew maximally at different temperature (40–70 °C) in the thermophilic range. The strains grow on different mono- and polysaccharides such as glucose, galactose, lactose, maltose, fructose, sucrose and starch and on proteinaceous substrates such as ammonium sulphate, peptone, yeast extract, tryptone and casein. It was determined that isolated strains have ability of producing some important industrial enzymes such as a-amylase, protease, b-galactosidase and lipase.

P28-036
Oxidative stress in the brain of dahl rats with salt hypertension elicited in adulthood

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It is accepted that oxidative stress participates in both human and animal models of hypertension. Hypertension has a neurogenic basis and the brain plays an essential role in the control of arterial blood pressure. The major source of reactive oxygen species (ROS) is activated nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase. The aim of this study was to investigate whether salt-sensitive hypertension induced in adult Dahl salt-sensitive (DS) rats was accompanied by a more pronounced oxidative stress in the brain compared to normotensive Dahl salt-resistant (DR) controls. NADPH oxidase activity (determined by lucigenin-enhanced chemiluminescence assay) and a degree of lipid peroxidation monitored as thiobarbituric acid-

reactive substances (TBARS) formation (determined by fluorescent method) and conjugated dienes were evaluated in two brain regions containing either hypothalamic paraventricular nucleus or rostral ventrolateral medulla. High salt intake induced hypertension and increased relative heart weight in DS rats but did not modify blood pressure and relative heart weight in DR rats. Salt-loaded DS and DR rats did not differ in NADPH oxidase-dependent production of ROS, TBARS content or oxidative index in either part of the brain. In addition, high-salt diet did not change significantly any of these brain parameters. Our findings suggest that there are no signs of enhanced oxidative stress in the brain of Dahl rats with salt hypertension induced in adulthood. This work was supported by research grant 304/12/0259 (Czech Science Foundation).

P28-037
Potential of urines fluorescent fingerprints for detection of metabolic changes of various animal species

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Urine is one of the most easily available biological fluids. It consists of a number of intrinsic fluorescent compounds – fluorophores. The quality and quantity of these fluorophores vary in different biological materials, and many fluorescent molecules are associated with cardinal metabolic pathways and can indicate a variation in metabolism. It is known that cardinal metabolic pathways are the same or very similar in different species, but the expression of genetic information, the regulation of metabolism and the preference for collateral or minor paths resulting in metabolites content in the body fluids can vary. Whereas gene and protein sequences vary between species, many metabolites are conserved between species, so that the fluorescent metabolome of one species can easily be compared with that of another. The native fluorescence of urine from 8 species of mammals (a total of 137 animals) was analysed without any added reagents (except water) using modified synchronous fluorescence spectra. The results show a specific composition of fluorophores for the tested species, enabling one animal to be distinguished from another with high significance.

P28-038
Calmodulin in the black tiger shrimp, *Penaeus monodon*

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Calmodulin (CaM) is one of well-known calcium (Ca^{2+}) binding protein that plays a crucial role in signal transduction. It was shown that CaM gene showed a high transcription level in hemocyte of bacterial infected shrimp, indicating involvement in the shrimp immune response. In this study, CaM gene (*Pm*CaM) and recombinant protein (r*Pm*CaM) of *Penaeus monodon* was characterized and its function on shrimp immunity was also determined. Firstly, tissue distribution was performed. Also, to examine an effect of *Pm*CaM gene silencing, RNA interference was carried out. The result revealed that the *Pm*CaM silenced shrimp was susceptible to *Vibrio harveyi* infection and hemolymph phenoloxidase activity was decreased. By 2-DE analysis, the protein profiles of hemocyte of the silencing shrimp showed down-regulation of glyceraldehydes-3-phosphate dehydrogenase, oncoprotein nm23 and twinstar. The result showed that the Ca^{2+} binding could induce a conformational change of r*Pm*CaM protein. Furthermore, to identify CaM-binding proteins that could involve in shrimp immune response, protein pull-down assay and LC/MS/MS were performed. The result revealed transglutaminase, elongation factor 1-alpha, elongation factor 2 and actin have been found. However, only the first three proteins contained a predicted CaM-binding site. From all of the investigations, the results implied that *Pm*CaM might play a crucial role in shrimp immunity.

P28-039
Proteomics and metabolomics in early diagnosis and monitoring of patients with chronic kidney disease

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Introduction: Vascular calcifications (VCs) represent significant predictors of cardiovascular mortality in patients with chronic kidney disease (CKD). VCs are considered an active process with a variety of proteins involved in kidney-bone-vascular axis. The present study aims to assess the relationship between bone/vascular modifications and circulating level of 10 biomarkers in CKD patients.
Material and method: Multiplex assay was performed on 109 serum samples (88 CKD – stages II-IV, not undergoing dialysis and 21 controls) to analyse the level of 8 molecules (osteoprotegerin, osteocalcin, osteopontin, FGF-23, PTH, IL-6, IL-1β, TNFα) performed on Milliplex MAP Human Bone Magnetic Bead Panel. Fetuin A and calcitriol were assessed using Quantikine ELISA Human Fetuin A and EIAab General Calcitriol ELISA kit.
Results: Increased levels of molecules involved in mineral metabolism were correlated with high levels of pro-inflammatory cytokines in CKD versus controls. Level of FGF-23, a novel marker of bone disease in CKD has been shown to correlate with vascular calcifications. Reduced serum level of fetuin-A, inhibitor of pathologic calcification, was associated with CKD. Calcitriol levels were decreased in CKD patients versus control. A positive correlation between biomarkers level and the stages inside the CKD cohort has also been observed.
Conclusion: Circulating biomarkers assessment for early diagnosis of VCs in CKD through advanced proteomic approaches will allow developing further clinical strategies in the benefit of patients. The configuration of a specific biomarker panel would improve the CKD early diagnosis, prognosis and monitoring.
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P28-040
Modulation of MAPK and NFkB signaling pathways by TiO_2 nanotubes Ti-modified surface

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Inflammatory mediators produced through inflammatory processes play an important role on pathological evolution of several chronic diseases. During inflammation, activation of the signaling pathways of MAPK and NF-kB families lead to the expression of inflammatory cytokine genes. Ti coated with TiO_2 nanotubes (Ti/TiO_2) proved to down-regulate the secretion of pro-inflammatory mediators but the mechanism involved is still unclear. In this context, the aim of the study was to investigate the activation level of signaling pathways in RAW 264.7 macrophages induced by Ti/TiO_2 surface comparatively to commercial pure Ti (cpTi). The activation of p38, ERK, JNK and IKK-β kinases was determined by assessing the phosphorylation status using ELISA technique, in the presence or absence of lipopolysaccharide (LPS). The obtained data showed that macrophages exposure to LPS resulted in increased phosphorylation levels of all analyzed kinases. Also, the contact between cells and Ti/TiO_2 lead to a significant reduction in the expression level of all MAPK phosphorylated forms and in the activation level of IKK-β as compared with cpTi. Moreover, the translocation of NFkB-p65 subunit in the nucleus was emphasized by immunofluorescence studies. The fluorescent images revealed the nuclear translocation of p65 in LPS treated-macrophages. A significant reduction of p65 nuclear accumulation on nanotubular surface was noticed. These results suggest that MAPK and NFkB family proteins may constitute major components involved in alleviating the macrophage inflammatory response to TiO_2 nanotubes modified surfaces.

P28-041
Comparative analysis of the effectiveness of sample preparation methods of biological samples for «shotgun» proteomic analysis

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The methodology of «shotgun» proteomic analysis is sequential implementation of the range of various procedures: protein extraction, HPLC and mass-spectrometry analyzes of peptides and results analyzing using special software. Problems of bad reproducibility of this technique are due to the first steps of the method, especially with sample preparation, which is hardly could be automated. That's the reason why our work includes system analysis of the effectiveness of the most common methods used in proteomics analyses for protein extraction from biology samples. Experiments were carried out on model proteins (such as myoglobin and BSA) and bacterial cells – *Pseudomonas aeruginosa*. The efficiency of the methods was evaluated by the comparison of protein amount after extraction with its original concentration in biosample and by the detected diversity of the bacteria's protein profile. For the quantitation determination Bredford method was used. For the proteomic analyses HPLC-Agilent 1290 with quadrupole TOF mass-detector was used. Data processing were held using Spectra Mills software. The features of widely used proteomics methods were established: amount of extracted proteins for model proteins as well as for the bacteria cells, possibility of using this method with membrane proteins, diversity of detected proteins in shotgun analyses. It was the first time the results of the comparative analysis of methods for detecting total protein fractions on the example of the individual model proteins and real biological object – *Pseudomonas aeruginosa* cells were obtained. Obtained results can help to choose correct method for forthcoming shotgun analyses taking into account its specificity.

P28-042
Computational determination of selenoprotein inhibitors

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Despite two cases of selenium toxicity report in 2006, selenium (Se) is seen as a trace element exhibiting several biological functions by catalyzing a variety of enzymes. They are known to catalyse redox reactions, participate in peroxide degradation, feature in antioxidant defense mechanism and control intracellular redox potential as well as influence thyroid hormone metabolism in humans. Most important biological functions of selenium are attributed to selenoproteins. Selenoprotein W and small thioredoxin-like mammalian selenoproteins may serve to transduce hydrogen peroxide signals into regulatory disulfide bonds in specific target proteins while recently engineered crystal structure of selenoprotein X is known to be human methionine-R-sulphoxide reductase. We present a structure-based drug design protocol and apply it to the study of selenium and selenoprotein beginning with the crystal structure 3MAO and retrieved from the protein databank. Similarly, five other biomolecular structures were considered in the study and we were able to screen a virtual compound library in view of an *in silico* structure-based drug design for potential inhibitors of this enzyme. The inhibitors that fitted best into the active site of this crystal structure during docking were carefully superimposed and used to construct the superligand which was in turn used to build pharmacophore. It is on this basis that we build a compound library to propose new chemical entities (NCEs) which are likely to be next generation inhibitors of this selenoprotein. It is hoped that this may perhaps be the first stage towards elimination of disease-causing agents in humans.

P28-043
Genome mining approach to secondary metabolism research: Biosynthesis of Ochratoxin A in *Aspergillus westerdijkiae*

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Ochratoxins are some of the most abundant mycotoxins known to contaminate cereals and are hazardous to public health. Details are still missing about the exact biosynthetic and regulatory mechanism for this potent carcinogen. Fungi belonging to the genera *Aspergillus* and *Penicillium* are the major producers of Ochratoxins in the natural environment. Since, genome mining in secondary metabolism (SM) research has proved to be quite useful in recent times; we sequenced the whole genome of *Aspergillus westerdijkiae CBS112803* which is known to be a major producer of Ochratoxin A (OTA). In this study we have identified the presence of more than 70 predicted SM gene clusters in the *A.westerdijkiae* genome including a putative OTA biosynthetic cluster. Here, we present the results that validate the function of the putative OTA cluster with a series of gene knockout experiments and propose a

possible biosynthetic mechanism of this toxin biosynthesis pathway. Regulation of secondary metabolism is another aspect that is complex and less understood. A number of SM gene clusters remain cryptic under laboratory conditions. While, few of these clusters involve one or more transcription factors and other regulatory genes, some function under the influence of a universal regulator. Here, we report the role of a bZIP transcription factor associated to the OTA cluster, acting as an activator of OTA synthesis in *Aspergillus westerdijkiae CBS112803*. Our studies have confirmed that the deletion of this gene not only eliminates OTA synthesis but is also found to affect other phenotypes such as pigmentation and spore formation.

P28-044
Functional expression of a novel indigenous Endo-beta 1,4- glucanase gene in *Apis mellifera*

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Apis mellifera is an insect of immense economic importance lives on rich carbohydrate diet including cellulose, nectar, honey and pollen. The carbohydrate metabolism in *A mellifera* has not been understood fully, as there are no data available, on the functional expression of cellulase gene. A dissection of Apis genome had revealed that there is one gene present for the expression of endo-beta-1,4-glucanase, for cellulose hydrolysis. In the presented work, functional expression of endo-beta-1,4 glucanase gene is reported. Total soluble proteins of honey bee were isolated and were tested cellulose hydrolyzing enzyme activity, using carboxy-methyl cellulose, as substrate. *A mellifera* proteins were able to hydrolyze carboxy-methyl cellulose, confirming its endo- type mode of action. Endo beta-1,4 glucanase enzyme was only present in the gut tissues, no activity was detected in the salivary glands. The pH optima of the enzyme was in the acidic pH range of 4-5-5-0, indicating its metabolic role in the acidic stomach of *A mellifera*. The reported enzyme is unique, as endo-beta- 1,4glucanase was able to generate non reducing sugar, as an end product. The results presented, are supportive to the information that the honey bee is capable of producing its novel endo-beta-1,4 glucanase. To our knowledge there is no report on the functional gene for cellulose in honey bee and the metabolism of carbohydrate is not completely understood. The information presented, could be helpful, in understanding, the carbohydrate metabolism in *A mellifera*.

Struct Biol S1, Mechanisms of Membrane Transport

P32-005-SP
Studying HIV-1 envelope lipid environment using photoactivatable lipids

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The HIV-1 envelope protein (*env*) is involved in the fusion between the virus and the host cell, a key process in virus infectivity. *Env* is incorporated in the nascent virion's lipid bilayer, but the recruitment mechanism is yet unknown. The elucidation of *env* recruitment and its lipid environment would point out new therapeutic targets to hamper virus entry and the spread of the infection. Knowledge acquired in this work would aid in the generation of immunogens against different envelope epitopes. It has been suggested that the targeting of *env* to budding regions occurs at lipid *raft*-like domains. Therefore, the aim of this work is to study the lipid environment of *env* in a cellular and viral context, and the role of other viral proteins in this sorting. Viral proteins expressing cells were fed with radioactively labeled photoactivatable lipids ([^{3}H]-photo-cholesterol, [^{3}H]-photo-sphingosine, [^{3}H]-choline, 10-ASA) and the specific protein-lipid interaction was studied in the cells and in the produced virus. Our results suggest that *env* is associated with cholesterol and sphingolipids both in the cellular and viral membranes. This association also exists at a cellular level when only *env* is expressed. Therefore *env* seems to be associated to *raft*-like lipids independently of other viral proteins. This biochemical tool provides us the lipid environment of specific proteins. We also plan to apply it to study the interaction of lipids with different *env* mutants, other viral proteins and even cellular accessory proteins present in the budding site that may play a role in *env* recruitment.

P32-006-SP
Fish-mammalian GLUT4 chimeric proteins as tools for studying GLUT4 trafficking and endocytosis

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Glucose transporter 4 (GLUT4) plays a key role mediating glucose uptake in adipocytes. The translocation of GLUT4 from intracellular compartments to the plasma membrane (PM) as well as its endocytosis are major events in this process. Our group has identified a fish GLUT4 isoform (btGLUT4) that differs from mammalian GLUT4 in motifs reported to be involved in endocytosis and intracellular retention and that can be used as a tool to study GLUT4 endocytosis and trafficking. In order to study GLUT4 endocytosis and trafficking in adipocytes, we stably-expressed btGLUT4, rat GLUT4 and two rat GLUT4 chimeras consisting of the ratGLUT4 backbone with exchanged intracellular regions of btGLUT4 (i.e. N-terminus and cytoplasmic loop) in 3T3-L1 cells. We analyzed the surface levels of the different GLUT4 constructs and their rate of endocytosis, in the absence or presence of insulin. Furthermore, in order to elucidate the endocytic routes of the various constructs, we determined their internalization rate while separately inhibiting clathrin-mediated, cholesterol-dependent and caveolar endocytosis. Our data suggest that rat GLUT4 internalizes through clathrin- and cholesterol-dependent endocytic mechanisms while btGLUT4 only internalizes through the clathrin-mediated pathway. However, the internalization route used by each construct seems to vary in the absence or presence of insulin. Our results also suggest that abrogation of caveolin-1 may unlock or promote endocytosis of rat GLUT4 through a faster yet uncharacterized pathway. Furthermore, both the N- and L-termini appear to be important for GLUT4 endocytosis and trafficking during basal and insulin-stimulated conditions.

P32-007-SP
Functional reconstitution of a type I secretion system into nanodiscs

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Type I secretion systems can be found in Gram-negative bacteria and allow the translocation of proteins and peptides in one step

across both membranes without the occurrence of periplasmic intermediates. The haemolysin A (HlyA) secretion system in *E. coli* consists of the ABC transporter haemolysin B (HlyB), the membrane fusion protein haemolysin D (HlyD) and the outer membrane factor TolC, which is recruited upon substrate interaction in the cytosol. Additionally to the canocial motif of ABC transporters, HlyB comprises a cytosolic N-terminal domain, a so-called C39-peptidase-like domain (CLD) whose presence is crucial for secretion *in vivo*. Substrate interaction has been confirmed but its precise role in the secretion process is still unknown. The membrane fusion protein HlyD is thought to seal the pore between the inner membrane complex and TolC. Interestingly, a small cytosolic domain present at the N-terminus is indispensible for substrate secretion *in vivo* suggesting more than mere sealing function of the protein. We are able to homologously overexpress and purify all components of the HlyA type I secretion system. By reconstituting HlyB into nanodiscs, which are small lipid patches surrounded by a membrane scaffold protein (MSP), we have a powerful tool at hand to functionally characterise the ABC-transporter and the role of the CLD in its natural lipid environment. Co-reconstitution with HlyD is attempted to unravel its so far unknown role in protein secretion and its influence on the activity of HlyB in presence and absence of the substrate HlyA.

P32-009
Estrogenic regulation of Na^+-dependent bicarbonate transporters from SLC4 family in human Sertoli Cells

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The formation of competent spermatozoa is a complex event that depends on the establishment of adequate environments throughout the male reproductive tract, in which the maintenance of proper ionic contents in the luminal milieus plays a crucial role. HCO_3^- is essential not only to ionic homeostasis but also to pH maintenance along the male reproductive tract. Herein we determined the effect of E2 on the expression/functionality of sodium dependent HCO_3^- transporters from SLC4 family in human Sertoli cells (SCs). All the studied transporters (NBCn1, NBCe1 and NDCBE) were expressed in human SCs. We localized NBCn1 and NBCe1 on the basolateral portion of membrane of human SCs, while NDCBE was detected on the apical region of membrane of the polarized human SCs. Previous studies support an association of 17β-estradiol (E2) levels with modulation of specific ion transporters expression. In E2-treated human SCs (100 nM) we could observe an increase in NBCn1, NBCe1 and NDCBE protein levels, as well as altered intracellular pH and transcellular transport. E2-treated SCs presented also a significant perturbation of ATP-induced short-circuit current and significant alterations on intracellular pH shift when DIDS the inhibitor. Overall, we report a relation between increased E2 levels and the expression/function of NBCn1, NBCe1 and NDCBE in human SCs, providing new evidence on the mechanisms by which E2 can regulate SCs physiology and consequently spermatogenesis, with direct influence on male reproductive potential.

P32-010
Dipole modifiers affect channel-forming activity of amyloid and amyloid-like peptides

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We have studied the influence of dipole modifiers on the steady-state transmembrane current induced by amyloid and amyloid-like peptides in planar lipid bilayers. Virtually solvent-free bilayer lipid membranes were prepared from negatively charge mixtures of lipids in 0.1 M KCl (pH 7.4) using monolayer-opposition technique. We have found that the addition of dipole modifier phloretin to the membrane bathing solutions leads to an increase in the multichannel activity of amyloid β-peptide fragment 25-35, [Gly^{35}]-amyloid β-peptide fragment 25-35, prion protein fragment 106–126 and amyloid-like peptides myr-BASP1 (1–13), myr-BASP1 (1–19) and GAP-43 (1–40). Comparing the results of measurements of the dipole potential of membranes and the influence of dipole modifiers (flavonoids and styryl dyes) on the channel-forming activity of amyloid and amyloid-like peptides we have concluded that the observed effect of phloretin is not caused by the changes in the membrane dipole potential. Using of different fragments of amyloid β-peptide, various presenilins, fragments of prion and neuronal proteins BASP1 and GAP-43 we have proposed that the increase steady-state peptide-induced transmembrane current at the addition of phloretin resulted from the electrostatic interaction between the positively charged channel-forming agent and negatively charged dipole modifier. The results obtained by electron microscopy have demonstrated that the interaction influence on peptide oligomerization. The study was supported in part by RFS (14-14-00565), RFBR (14-04-31738), SP-69.2015.4 and SS-1721.2014.4.

P32-011
Periplasmic binding protein AccA from *Agrobacterium tumefaciens*

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Agrobacterium tumefaciens is a pathogenic soil bacterium causing the crown gall disease, characterized by tumour formation. The virulence of the bacteria is brought by the presence of the virulence plasmid pTi. The infection mechanism is well known and composed of 4 steps: 1) The wound on the plant activates *A. tumefaciens*' virulence. 2) T-DNA, a piece of the pTi is transferred to the plant's cell and integrated to its genome. 3) The plant secretes hormones, triggering the tumour formation, tumours which are colonized by *A. tumefaciens*. The plant also secretes Agrocinopine that is used as energy source by the bacterium. 4) Agrocinopine also activates quorum sensing mechanisms in *A. tumefaciens* leading to spreading of the pTi to non-virulent bacteria. Agrocinopine is imported by Acc system. Acc is composed of an ABC transporter and a periplasmic binding protein. The PBP AccA recognizes Agrocinopine. AccA also is responsible for the import of Agrocine 84, a lethal toxin, produced by another bacteria *A. radiobacter* K84. The subject of my thesis is the understanding of the interaction specificity of the PBP AccA with Agrocinopine and Agrocine 84, thus combining structural and biochemical studies of the complexes formed by AccA with its ligands. We determined the 3D structure of a PBP with its opine ligand. We also determined the structure in complex with the toxin Agrocin 84 allowing us to characterize the interaction with each ligand. Combined with biochemical studies, we revealed the interactions involved in the binding of the antagonist ligands leading to their import.

P32-012

Type IV secretion system coupling proteins, the role of the transmembrane domain

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Type IV secretion systems (T4SS) are macromolecular transport systems related to bacterial conjugation and virulence processes. The study of these systems has been intensified in the last years, due to their implication in pathogenic processes of bacteria and in the spread of antibiotic resistance genes. One of the most important proteins of these systems is the coupling protein (T4CP), which is essential during the conjugative process. Besides connecting the relaxosome with the secretion system T4CPs are molecular motors that could pump the DNA into the inner membrane. $TrwB_{R388}$, which is composed of a small transmembrane domain (TMD_{TrwB}) and a voluminous cytosolic domain (Cit_{TrwB}), is the most studied protein of this family. Previous studies in our group indicate that TMD of TrwB could play a regulatory role in the *in vivo* function of this protein. The aim of this work is to further study the role of the TMD as the regulatory element in T4CPs to establish a general molecular mechanism for these proteins. To do so, different members from the T4CP family and chimeric proteins constructed with domains of different T4CPs are being studied. In particular, mating assays were performed to study *in vivo* activities and interactions between homologue systems. Additionally, overexpression of the membrane proteins was optimized and purification protocols are being developed. Finally, the *in vivo* localization of the different proteins is being studied by confocal microscopy.

P32-013

Maturation of endothelial Weibel-Palade bodies: Analysis of trafficking routes to Weibel-Palade Bodies

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Vascular endothelial cells play a key role in the control of blood vessel homeostasis which is mainly achieved by the activity of endothelial Weibel-Palade Bodies (WPB), large secretory organelles that store bioactive molecules to be released upon stimulation. Upon blood vessel damage or local inflammation, the WPB are triggered to exocytose and release their contents such as the haemostatic protein von Willebrand Factor (VWF), the leukocyte receptor P-selectin and the tetraspanin CD63, which are required for wound healing and the initiation of inflammatory reactions, respectively. The correct maturation of WPB is critical for their function as regulated secretory organelles and depends on the proper targeting of WPB content proteins. WPB initially form at the trans-Golgi network (TGN), but maturation of WPB also depends on other organelles such as endosomes which deliver CD63 and P-selectin to WPB. However, trafficking routes, transport machineries and mechanisms that deliver proteins from other organelles to WPB are not well characterised. In order to identify the molecular machinery operating on the endosome-to-WPB transport pathways, we set up an endosome-WPB-fusion assay in a cell free system using purified WPB and endosomes. Furthermore, the protein and lipid composition of WPB which are still incompletely defined are analysed to characterise the potential contribution of certain key lipids and proteins in the maturation process.

P32-014

Comparative analysis of the activity of MDR pumps in *Salmonella enterica* using different indicatory compounds and methods of assay

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Multidrug resistance (MDR) caused by efflux pumps is one of the most common reasons of bacterial resistance to antibiotics. It is important to assay the efficiency of efflux pumps in bacteria and to identify the selectivity of these pumps. In our study we used well-known efflux pump substrates – ethidium (Et^+), tetraphenylphosphonium (TPP^+) and Nile red (NR) as the indicatory compounds. The main distinctness of Et^+ is the binding this indicatory ion to DNA leading to the increase of the fluorescence of studied samples. TPP^+ does not bind to intracellular structures and leaks out of deenergized cells. These two lipophilic cations and lipophilic stain NR were simultaneously used as agents competing for the interaction with efflux pumps. Beside Et^+ fluorescence measurements we applied potentiometric analysis using selective electrodes to determine Et^+ and TPP^+ uptake by the cells. Phenylalanyl-arginyl-β-naphtylamide (PAβN) was used to evaluate the input of RND-family pumps in total efflux activity of the cells. Antibiotic polymyxin B (PMB), EDTA and heat treatment were used to permeabilize and deenergize the cells. Our experiments demonstrated the important role of the assay conditions on the efflux pump activity. pH, ionic strength, temperature of the incubation medium considerably affected the efficiency and kinetics of the efflux. The level of aeration of *S. enterica* cell suspension also affected the efficiency of the pumps.

P32-015

Lipid dependent activities of cell-free expressed MraY translocase homologues

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Rapidly spreading multiple antibiotic resistances request new approaches for the screening of chemotherapeutic compounds e.g. targeting the bacterial cell wall precursor formation. The first membrane bound step of cell wall biosynthesis is catalysed by the integral membrane protein MraY containing ten transmembrane segments. Expression of MraY homologues from human pathogens in conventional cellular expression systems is highly challenging and the enzymes were not available for biochemical characterization. We have developed efficient cell-free expression protocols for the preparative scale production of a number of MraY homologues. The expression efficiency was dependent on the design of the mRNA and on the translation initiation. The quality of the synthesized enzymes was further strongly modulated by modifications of the hydrophobic environment, by selecting conditions for the post-translational solubilization and by screening of the lipid composition in preformed nanodiscs. The specific activity of the produced MraY samples was analyzed by *in vitro* lipid I formation. We demonstrate that cell-free expression strategies as well as the determined optimal solubilization conditions are specific to the individual MraY homologues. The *B.subtilis* MraY can be synthesized as a stable enzyme with

high activity in a variety of different conditions, whereas enterobacterial MraY homologues from numerous pathogens were inhibited by detergents and high quality protein samples could only be produced in presence of specific compounds. The complete biosynthetic pathway starting from UDP-N-acetylglucosamine precurosr to lipid II formation could be reconstituted with cell-free expressed proteins and will provide the basis for developing new drug screening platforms in defined environments.

P32-016
Structural investigation into the comprehensive mechanism of concentrative nucleoside transport

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Nucleoside transporters (NTs) are very important in humans, and play vital roles in nucleic acid synthesis, energy metabolism and a host of physiological processes involving regulation of intra- and extra-cellular concentrations of purine and pyrimidine (deoxy) nucleosides. Furthermore, it possesses a wide range of potential applications in the development of drugs, especially for antiviral and anticancer drugs. To date, two main families of membrane nucleoside transporters have been identified in mammalian cells, including the concentrative nucleoside transporter (CNT) and the equilibrative nucleoside transporter (ENT) families. In the former family, concentrative transport of nucleosides is energized by transmembrane sodium and/or proton gradients, whereas in the latter bidirectional nucleoside transport is driven solely by the concentration gradient of the nucleosides across the membrane. However, our understanding of the molecular mechanisms of nucleoside transport remains limited. The only known structure of a CNT is VcCNT from *Vibrio cholera* in an inward-facing and partially occluded conformation. At present, we are attempting to resolve the nucleoside transport mechanism by capturing and analyzing the different conformations likely to be involved in the translocation cycles of different bacterial CNTs. Meanwhile, by comparing sodium-driven transporters such as VcCNT and homologous proton-driven transporters such as NupC from *Escherichia coli*, we aim to illustrate the basis for the differing cation selectivities of CNTs. This work should help to elucidate the molecular mechanisms of concentrative nucleoside transport by a structural approach, not only in the bacterial transporters but also in their physiologically and medically important counterparts in humans.

P32-017
MacAB efflux system of *Serratia marcescens* as a potential protective system against oxidative stress

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Bacterial resistance to antibiotics is one of the major problems in the world. The most important in the appearance of bacterial antibiotic resistance is understanding and investigation of its molecular mechanisms. Bacteria genus of *Serratia* is opportunistic and antibiotic resistance pathogens with increased clinical significance. Efflux systems of *Serratia marcescens* involved in an excretion of a wide range of antibiotics. Analysis of genome sequence of *S. marcescens* allowed discovering a new ABC-type efflux system. This system has a high homology to MacAB system of *E. coli*. Special characteristic of MacAB efflux system of *S. marcescens* consists in defending against reactive oxygen species (ROS) addition to participating in antibiotics excretion. Goal of this research was an investigation of resistance of wild type (w.t.) and mutant Δ macAB (m.t.) *S. marcescens* to ROS. Resistance of both strains to hydrogen peroxide (HP) was explored. HP presence in the medium led to m.t. cell death and w.t. viability. Co-cultivation of both strains resulted in the emergence of resistance of m.t. to HP. W.t. supernatant provided a clear protective effect for m.t. in the presence of HP. Thermostability and sensitivity to proteinase K treatment of w.t. supernatant metabolites allowed suggesting that protective compounds have a protein essence. Thus, macAB efflux system of *S. marcescens* plays a crucial role in a cell defense against ROS and its absence prevent to extracellular protective metabolites formation. This work was funded by the subsidy of the Russian Government to support the Program of Competitive Growth of Kazan Federal University.

P32-018
Acetazolamide, an inhibitor of carbonic anhydrase, supresses photophosphorylation and stimulates light-induced ATP hydrolysis in isolated spinach chloroplast

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The chloroplast CF_1CF_o-ATPase/synthase is located in energy-transducing thylakoid membranes of chloroplasts where it catalyzes light-induced ATP synthesis and ΔμH+ generating ATP hydrolysis. It has a membrane sector (CFo) attached to a membrane extrinsic oligomeric complex (CF1), that contains the catalytic sites for ATP synthesis and hydrolysis and noncatalytic (regulatory) sites. The noncatalytic sites can bind some oxyanions (bicarbonate, sulfite, borate etc.), activating CF_1-ATPase. We have shown recently (Semenikhin & Zolotarova, 2014) that both CF_1CF_o complex and its isolated catalytic part, factor CF_1, are able to accelerate the prosess of interconversion of carbonic acid forms: $CO_2 + H_2O \Rightarrow H^+ + HCO_3^-$, ie to exhibit carbonic anhydrase activity. The aim of the present work is studying the effect of acetazolamide, an inhibitor of carbonic anhydrase, on the rate of photophosphorylation and the light-induced ATP hydrolysis in isolated spinach chloroplasts. The rate of ATP synthesis was determined by hexokinase method in chloroplast suspension illuminated for 2 min in the presence of electron acceptors. The amount of ATP was determined enzymatically using glucose-6-phosphate dehydrogenase and NADP. Formed in the reaction NADPH was measured by fluorescent method. The amount of Pi released in ATPase reaction of thylakoids was determined by colourimetric method after illumination of the chloroplast suspension for 2-5 min. The data show that acetazolamide inhibits light-induced synthesis and stimulates ATP hydrolysis suggesting participation of carbonic anhydrase activity in transmembrane proton transfer coupled with ATPsynthesis/hydrolysis in thylakoid membrane of chloroplasts.

P32-019
Reconstitution of vesicle priming for Ca^{2+}-triggered millisecond exocytosis through chemical clamp-mediated control of SNARE zippering

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Neurotransmitter release at the synapse is mediated by SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor)-driven fusion of synaptic vesicles with the presynaptic membrane. Vesicles in the readily releasable pool (RRP) are primed to undergo fast fusion in the active zone, and Ca^{2+} influx triggers neurotransmitter release within a millisecond. Partially zipped SNARE complex has long been assumed to play a critical role in achieving the extraordinary speed and synchrony of neuroexocytosis. However, it has not been possible yet to reproduce such a fusion-competent partially zipped state, from which physiological concentration of Ca^{2+} can stimulate fusion pore formation at a millisecond time scale, casting doubt on the zippering hypothesis. Here we developed a reversible chemical clamp to control SNARE zippering and membrane fusion, and recapitulated the primed state which undergoes rapid exocytosis upon Ca^{2+} influx. Myricetin, which binds to and arrests half-zipped SNARE complex, clamped membrane fusion at the hemifusion state. Subsequently, when the bound myricetin was enzymatically lifted to resume the completion of SNARE zippering, the majority of vesicles synchronously underwent full lipid mixing and opened fusion pores within 30 ms upon influx of physiological 10 μM Ca^{2+}. These results suggest that vesicles in RRP are primed to undergo Ca^{2+}-triggered release by clamping half-zipped *trans* SNARE complexes at the hemifusion state.

P32-020
Comparative study between mammal and plant GPI modification mechanism

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GPI (glycosylphosphatidylinositol) is a kind of glycolipid which can anchor proteins to the cell membrane. GPI-anchored proteins (GPI-AP) are known to localize themselves to the microdomain on the cell membrane, called raft, via the Endoplasmic Reticulum (ER). GPI-APs have two signal sequences, signal-peptides (SP) and GPI-attachment signals (GPI-AS). These are N-terminal ER localization sequences and C-terminal signal sequences for GPI modification, respectively. Human GPI-APs are closely related to incurable human disorders including cancer, Parkinson's disease and bovine spongiform encephalopathy. Plant proteins which have similar GPI modification systems as mammal GPI-APs, can translocate to the cell wall or the raft region on the cell membrane. However, because few plant proteins have been isolated as GPI-APs, the GPI modification mechanism is not clarified. CEBiP (Chitin Elicitor Binging Protein), one example of cell membrane localized plant GPI-AP, is known to bind chitin which is major ingredient of fungus and then activate defense reaction called elicitor. Based on this information, plant GPI modification of plant protein was compared with that of human modification to clarify the plant GPI modification mechanism in this study. Our previous experiment indicated that native CEBiP SP did not work in HeLa cells. Therefore, the subcellular localization of the mutant CEBiP gene, which replaced CEBiP SP gene by human prion SP gene, expressed in HeLa cells, was analysed. The mutant CEBiP protein was observed by confocal laser microscope after immunostaining method for visualizing the protein by fluorescence. The molecular weight of the CEBiP mutant was estimated by Western blotting.

P32-021
In vivo reconstitution of a cytochrome b_{559} like structure with a truncated N-terminus α-subunit

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The cytochrome b_{559} protein has two subunits, α and β. Both subunits from *Synechocystis sp.* PCC 6803 and *Thermosynechococcus elongatus* have been cloned and overexpressed in *Escherichia coli* and *in vivo* reconstitution experiments have been performed. Formation of homodimers in bacterial membrane was only observed in the case of β-subunit but not with the full length α-subunit. *In vivo* reconstitution of an α-homodimer was possible using a chimeric N-terminus truncated before the isoleucine 17 (chimeric I17), eliminating a short amphipathic α-helix that lays on the surface of the membrane. Overexpression and *in vivo* reconstitution in the bacteria was clearly demonstrated by the brownish color of the culture pellet and the use of a monoclonal antibody against the maltoside-binding protein. A simple partial purification after solubilization with Triton X-100 confirmed that the overexpressed protein complex corresponded to the maltoside-binding protein-I17 cytochrome b_{559} like structure. The features of the new structure were determined by UV-Vis, electron paramagnetic resonance and potentiometry. Our hypothesis about the amphipathic α-helix was confirmed by making several mutants with different length of the N-terminus domain. Our data also showed that Cyt b_{559} maturation occurred through the three step model: incorporation of the protein subunits within de membrane, recognition of the subunits as homo- or hete-rodimers, and finally incorporation of the heme group.

P32-022
Cellular uptake mechanisms and activity of novel polyprenyl-based anionic DNA lipoplexes

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Lipofection is one of the most commonly used method of transfection. However, the multiple mechanisms by which these processes occur are largely unknown. The aim of our study was to investigate the lipofecting activity and cellular uptake pathways

of novel anionic polyprenyl-based DNA-lipoplexes. Unique negatively charged lipoplexes containing polyprenyl derivatives – trimethylpolyprenylammonium iodides (PTAI) with different length of polyprenyl chain (7,8,11,15 isoprene units) and co-lipid DOPE (dioleylphosphatidylethanolamine) developed in our laboratory represented high lipofecting activity for DNA and shRNA delivery without significant side effects on cell physiology. Moreover they exhibited no haemolytic activity against human red blood cells. The size of lipoplexes was composition-dependent, with lipoplexes 200–300 nm, except PTAI-15-based lipoplexes (426-485 nm). All of them were within a range of 200–500 nm size that was shown to determine cell entry via caveolae-mediated endocytosis. Uptake mechanisms were verified by uptake inhibition assay. Caveolae-mediated endocytosis may be the reason of high lipofection efficiency due to the prolonged existence time of the lipoplexes in early endosomes thus avoiding rapid degradation. There was also a portion of bigger aggeragates identified for PTAI-15-based lipoplexes (4.1–5.1%, 5100–5500 nm) that correlated with the loss of lipofecting activity upon storage which was characteristic only for PTAI-15-based particles. These findings can be useful for optimization of lipoplexes composition to enhance efficiency of lipofection. Work supported within grants: UDA-POIG.01.03.01-14-036/09-00 co-financed by European Regional Development Fund and BMN14/2014 Grant for Young Scientists of Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University.

P32-023
Alternative import routes into peroxisomes of *Saccharomyces cerevisiae*

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The posttranslational import of nearly all peroxisomal matrix proteins is mediated by peroxisomal targeting signals (PTS). Under oleate-induced growth conditions, the translocation of PTS1 enzymes is enabled by the import receptor Pex5p, which binds its cargo protein in the cytosol and transports it to the peroxisome. There, the receptor inserts into the peroxisomal membrane and together with its docking partner Pex14p forms a transient protein-conducting pore. Genome sequencing revealed the existence of a paralogous gene with unknown function in baker′s yeast. The gene product YMR018wp exhibits a significant structural similarity with Pex5p, including the presence of characteristic protein interacting motifs. Therefore, YMR018wp might acts as a second PTS1-receptor, which adds to the system depending on environmental conditions and which might be specific for a subset of peroxisomal enzymes. Until now, two yeast PTS2 proteins are known. First, the oleate-inducible Thiolase Fox3p. The PTS2 of Fox3p is recognized in the cytosol by the soluble import receptor Pex7p, which functions in concert with its co-receptor Pex18p. The latter binds the PTS2-receptor/cargo complex and is essential for its transport and docking to the peroxisomal membrane. The second PTS2 protein is the glycerol-producing enzyme Gpd1p that is present in peroxisomes as well as in the cytosol under osmotic stress conditions. No peroxisome-related function of Gpd1p is known so far. Two possible mechanisms are currently considered for the regulated import of Gpd1p into peroxisomes: a controlled exposure of the PTS2, e.g. by phosphorylation or expression of co-receptor as Pex21p as Gpd1-specific factor.

P32-024
Functional characterization of the peripheral peroxisomal membrane protein Pex17p

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A special feature of peroxisomes is their capability to import folded, even oligomeric proteins. Peroxisomal matrix proteins contain a peroxisomal targeting signal (PTS), which is recognized by import receptors in the cytosol. The receptor cargo complex is targeted to the peroxisomal membrane and binds to the docking complex, which consists of Pex13p, Pex14p and Pex17p. Pex5p together with Pex14p form a highly dynamic and transient pore, which facilitates cargo-translocation. Pex13p and Pex14p provide binding sites for the import receptor but also contribute to pore formation. However, the function of Pex17p in peroxisomal protein import remains elusive. Here we performed two-hybrid analyses to elucidate the minimal region of Pex14p for its interaction with Pex17p. We compared isolated Pex13p- and Pex14p-complexes from *pex17Δ* and wild-type cells. Deficiency in Pex17p results in the lack of a high molecular weight Pex13p- and Pex14p-containing complex. Moreover, Pex5p associates with complexes isolated from both wild-type and *pex17Δ* cells, but with a lower efficiency. This observation correlates well with a decreased amount of cargo-protein Mdh3p in the complexes. The data indicate that Pex17p plays a role in membrane docking of the cargo-loaded receptor and assembly of the peroxisomal translocon.

P32-025
Structure–function analysis of a putative kinase, involved in the regulation of the Type Three Secretion System in *Shigella flexneri*

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The Type Three Secretion System (T3SS) is used by many Gram-negative bacteria for infection of the host. It is a macromolecular complex spanning both bacterial membranes, delivering effector proteins to the host cell. A putative kinase might be involved in the regulation of the T3SS mechanism of *Shigella flexneri*. We are interested in understanding its role in T3SS-mediated *Shigella* infection. Pull-down analysis revealed the presence of the putative kinase in *Shigella* strain M90T. The *Shigella* gene knockout exhibited reduced invasiveness to 40% in HeLa cells. For biophysical analysis we designed a fusion construct that leads to strongly increased yield of soluble protein and improves crystallization probability. The fusion-protein was successfully purified using immobilized ion and size exclusion chromatography. We aim to solve the crystal structure of the putative kinase and perform mutagenesis in order to analyze the reaction mechanism. Furthermore, substrates of the kinase need to be identified to perform functional studies.

P32-026
Effect of (-)-roemerine on the RND-type efflux pumps of *E. coli*

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In Gram-negative bacteria, antibiotic efflux is a major mechanism of bacterial resistance. Amongst the five major superfamilies of bacterial efflux transporters found, the resistance-nodulation-cell division superfamily, RND, type antibiotic efflux pumps is the major pump responsible for extrusion of a wide range of substrates in *Escherichia coli*. In this work, the transcriptional regulation of the components of the two RND-type efflux pumps, AcrEF-TolC and AcrAB-TolC have been investigated in response to 1-hour treatment with the plant alkaloid (-)-roemerine. The high antimicrobial activity of this alkaloid on different microorganisms makes it a significant target. Hence results obtained provide an insight to the extrusion of this alkaloid, which may contribute to the understanding the role of efflux pumps in the development of resistance to (-)-roemerine. This work has been supported by TUBITAK-MAG Project with the number 113M052. NBG was supported by TUBITAK-BIDEB Fellowship.

P32-027
Regulation homeostasis of metals in plants

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Precise control of metal concentrations in cell compartments was made possible by the evolution of regulatory systems that (Clemens 2003). An important role in metal homeostasis is to add a non-protein amino acid called nicotianamine (Douchkov 2005). Thus, the main role of nicotianamine is the adaptation of plants to the new, less favorable conditions, resulting from the deficiency or excess of microelements. In the experiments conducted to date indicate that the expression of extra copies of the gene in a plant NAS noble tobacco and tomato ordinary affects the downloading and translocation of Zn and Cd in the plant. An important issue is to determine the extent to which the introduction into the genome of plants (tomato) gene from *Arabidopsis halleri* AhNAS2 modify the collection, accumulation and distribution of Cd in the tissues of vegetative and fruit. Particularly important is to determine whether expression of AhNAS2 modifies Cd translocation from root to shoot. Answering this question on the basis of the results of research under my thesis. The research in this thesis was conducted using soil as the medium for plant growth. They were so experimental conditions close to natural. It follows that an additional important aspect of the research a comparison of the results of experiments conducted on plants grown in hydroponic and soil. These results confirm the previously described assumptions. Plants with NAS gene showed a tendency to the accumulation of Zn and Fe. Moreover, the plants with the NAS gene showed a tendency to limit uptake Cd.

P32-028
Liposomes for photodynamic therapy of vitiligo via pilosebaceous route

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Vitiligo is an acquired depigmentory skin disorder in which pigment producing cells (melanocytes) are absent. It affects 0.1 to 4% of the population worldwide and is emotionally and socially devastating. Well established treatment modalities in today's therapeutic armamentrum have their own side effects and failure. Photodynamic therapy (PDT) an entirely new treatment modality which involves a photosensitizer and light. PUVA therapy used so far has marked failure in many of the clinical trials studies. Since no full therapeutic solution for vitiligo is available, PDT is the ray of hope. The aim of project was to develop and investigate the therapeutic efficacy of liposomes to produce immediate repigmentation (by melanin) along with correcting the cause simultaneously. Topical route has been chosen to directly target the diseased site and to minimize the systemic toxicity. Methoxsalen-melanin loaded liposomes were prepared by a lipid cast film method and were characterized *in-vitro* for their shape, size, percent antigen entrapment, Skin permeation and stability. Fluorescence microscopy was carried out to confirm the uptake of bilosomes. The *in-vivo* part of the study comprised of Induction of vitiligo by mushroom tyrosinase intradermally & photodynamic studies with different formulations. Cosmetic disfiguration and psychological sequel underlines the impact of vitiligo. Immunization with mushroom tyrosinase resulted in discoloration of the areas showing its effectiveness in inducing vitiligo. Sustained pigmentation resulted after application of formulation was suggestive of cure with fast repigmentation. Thus, pilosebaceous route is effective in targeting follicular melanocytes. Toxic manifestations of methoxsalen were also subsided when delivered in liposomes.

P32-029
A palmitic acid functionalized with a maleimide group is used to recruit SH-containing peptides to lipid and biological membranes

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In this study, we present a novel and easily applicable approach to recruit sulfhydryl-containing biomolecules to membranes by using a palmitic acid functionalized with a maleimide group. A main advantage of the assay is that it can also be conducted with preformed biological membranes. To demonstrate the applicability of our approach, we performed fluorescence spectroscopy, lifetime measurements, and microscopy characterizing the binding of a Rhodamine-labeled peptide to lipid and cellular membranes. Our assay enables new possibilities for preparing biologically active liposomes and manipulating living cells.

P32-030
Molecular mechanism of Mg-ATPase activity

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Mg-ATPase is very important in living organisms. To better understand the molecular mechanism of Mg-ATPase activity we applied the method of kinetic analysis of multi-sited enzymes systems; this is a suitable approach used for kinetic investigation of multi-sited enzyme systems. The study of Mg-ATPase has demonstrated: 1) It is a multi-sited enzyme system whose functional unit is minimum a dimmer; 2) Its substrate is MgATP, while free ATP and Mg^{2+} appear to be the enzyme modifiers with a dual

effect; 3) The enzyme system for MgATP has at least three sites, i.e. the essential activatory, full inhibitory and partial effect modifiers; 4) Mg-ATPase carries out Mg^{2+} transport through the $1Mg^{2+}$:1ATP stochiometry. Based on the results of these analyses, the kinetic scheme for Mg-ATPase has been developed.

P32-031
Bacterial synthesis of Intracellular Palladium Nanoparticles

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Bio-Palladium nanoparticles (Pd-NPs) provide novel catalysts for green chemistry applications. However, it has remained equivocal whether these palladium particles could also be synthesized within the bacterial cell cytoplasm which requires a recognition and uptake system for Pd, a non-essential metal. This study utilizes Gram negative (*D. desulfuricans*) and Gram positive (*B. benzeovorans*) bacteria for the synthesis of intracellular Pd-NPs. Bacteria were grown according to published methods and subsequently to obtain the cells which were then used to reduce Na_2PdCl_4 solution (Pd (II)) at the expense of hydrogen and formate as electron donors to make Pd-NPs by both bacteria. This study aims to apply high resolution STEM (Scanning Transmission Electron Microscrocopy) coupled with a HAADF (High Angle Annular Dark Field) detector with EDX (Energy Dispersive X-ray Spectrometry) to visualize and characterize intracellular Pd-NPs using "Imagej"software for image processing and analysis of Pd-NPs. Preliminary studies using flow cytometry confirmed cellular integrity and metabolism during the process of Pd (II) uptake and its reduction into Pd-NPs. The Pd-NPs were small and largely monodispersed, with sizes ranging from 0.2 to 8 nm (from H_2), and from 9 to 12 nm (from formate) with occasional larger particles. In *B. benzeovorans*, single crystalline octahedral structures with {111} facets were synthesized while multiple twinned structures were found in *D. desulfuricans*. This study provides unequivocal evidence for the intracellular synthesis of palladium NPs, as compared to those localized in the cell walls and surface layers *via* extracellular mechanisms.

P32-032
Water and electrode potential effect on the structure and function of tethered bilayer membranes probed by vibrational spectroscopy

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Tethered bilayer membranes serve as a useful model for studies of interaction of peptides and proteins with biological membranes as well as for construction of biosensors. Function of such membrane depends on both the structure of self-assembled monolayer used to tether lipidic layer to the metal surface and the structure of the phospholipids bilayer. In this work surface enhanced Raman and reflection absorption infrared (RAIR) spectroscopies were used for structural analysis of tethered bilayer membrane and adsorbed peptides. We focused on the analysis of water and electrode potential induced conformational changes in the structure of the membrane anchoring monolayer formed from short chain hydrophilic 2-mercaptoethanol molecules and long chain hydrophobic thiols WC14 [20-tetradecyloxy-3,6,9,12,15,18,22-heptaoxahexatricontane-1-thiol, C14(myristoyl)] adsorbed on gold substrate. Water-induced formation of clusters of long chain anchoring thiols was detected by spectroscopy. Drastic electrode potential-induced changes in the orientation of monolayer were observed. Observed alterations in structure of anchoring monolayer were found to be essential for the function of membrane. The interaction of beta amyloid peptide oligomers prepared by different protocols and having different aggregation level with tethered bilayer membranes was probed by vibrational spectroscopy.

P32-033
Biophysical properties of neuronal cells are gravity dependent

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Gravity sensing is well examined in living organisms. Along with the different organisms, the mechanics of the gravity perceiving systems vary (e.g. the vestibular organ of vertebrates, statocytes in plants). Single cells also react to changes in gravity, but there are many open questions about the molecular mechanisms. Additionally, when looking at biological systems as excitable media under the aspects of non-linear thermodynamics, they fulfill all prerequisites and therefore should be depending on small external forces including gravity. This extends to all levels of organization, down to cellular membranes. Consequently we have investigated the response of neuronal cells to conditions of variable gravity and we found that these cells react to changes in gravity. Here, we especially show that the resting potential and the fluidity of cell membranes is gravity dependent. In addition we also investigated if the actin cytoskeleton has an effect on membrane properties. The experiments have been performed with a high throughput plate reader, which was adapted to microgravity conditions. The fluorescent dye Di-4-Anepps was used to monitor the electrical properties of SH-SY5Y cells and DPH (1,6-Diphenyl-1,3,5-Hexatriene) was used to investigate membrane fluidity.

Struct Biol S2, Channels and Transporters

P33-004-SP
Serotonin transporter associated protein complexes – new insight into transporter activity regulation and trafficking

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The serotonin transporter (SERT) functions in high-affinity serotonin uptake and is the primary target for commonly used antidepressant drugs. To gain novel insight into SERT regulation, we conducted a comprehensive proteomics screen to identify components of SERT-associated protein complexes in the brain combining three independent approaches, namely affinity purification, GST pulldowns, and yeast two-hybrid screens. Identified

SERT associated proteins are highly enriched in synaptic vesicle membrane proteins as well as proteins involved in energy metabolism and ion homeostasis. Using subcellular fractionation we show that SERT is indeed associated with purified synaptic vesicle fractions, but more strongly enriched in fractions containing vesicles and membrane fragments of larger size (>100nm diameter). Following up on hypotheses emerging from our proteomics approach, we also studied the regulation of SERT activity by selected interacting proteins in more detail *in vitro* and *in vivo*. One such protein is the Gαq subunits of heterotrimeric G proteins. We found that in Gαq knockout mice, SERT activity is increased in distinct brain regions. Furthermore, we show that members of the proteolipid protein family, i.e. stress-regulated glycoproteins M6a and M6b, directly interact with SERT and alter transporter function. In mice SERT activity is increased in M6a/M6b double, but not single knockouts. Interestingly, with both knockout models, Gαq and M6a/M6b, we uncovered, rather unexpectedly, gender differences in SERT activity regulation, which may be relevant to depression and other mood disorders, as these disorders are more common in women.

P33-005-SP
Influence of membrane cholesterol in the molecular evolution and functional regulation of TRPV4

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TRPV4 is involved in several physiological and sensory functions as well as with several diseases and genetic disorders, though the molecular mechanisms for these are unclear. In this work we have analyzed molecular evolution and structure-function relationship of TRPV4 using sequences from different species. TRPV4 has evolved during early vertebrate origin (450 million years). Synteny analysis confirms that TRPV4 has coevolved with two enzymes involved in sterol biosynthesis, namely MVK and GLTP. Cholesterol-recognizing motifs are present within highly conserved TM4-Loop4-TM5 region of TRPV4. TRPV4 is present in lipid raft where it co-localizes with Caveolin1 and Flilipin. TM4-loop4-TM5 region as well as loop4 alone can physically interact with cholesterol, its precursor mevalonate and derivatives such as stigmasterol and aldosterone. Mobility of TRPV4-GFP depends on membrane cholesterol level. Molecular evolution of TRPV4 shared striking parallelism with the cholesterol bio-synthesis pathways at the genetic, molecular and metabolic levels. We conclude that interaction with sterols and cholesterol-dependent membrane dynamics have influence on TRPV4 function. These results may have importance on TRPV4-medaited cellular functions and pathophysiology.

P33-006-SP
The role of the MIM complex in the biogenesis of mitochondrial outer membrane proteins

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Mitochondrial proteins are synthesized on cytosolic ribosomes and imported into the organelle via different proteinaceous machineries. The translocase of the outer membrane (TOM complex) forms the entry gate for the majority of precursor proteins. Subsequently, the precursors are sorted into the different subcompartments like the inner membrane, intermembrane space and matrix. Even outer membrane proteins with β-barrel structure are first transported across the TOM machinery and then inserted into the outer membrane via the sorting and assembly machinery (SAM complex). In contrast, only little is known how outer membrane proteins with α-helical membrane anchor reach their final destination. Here, we report a central role of the mitochondrial import machinery (MIM complex) for the membrane insertion and assembly of outer membrane proteins with an α-helical membrane anchor. The MIM complex consists of Mim1 and Mim2. Both subunits are required for complex stability and function. The MIM complex promotes the biogenesis of single as well as of multispanning α-helical proteins. Taken all together, two protein translocases mediate sorting of outer membrane proteins: the SAM and the MIM complex.

Struct Biol S3, Protein-Mediated Membrane Deformation and Penetration

P34-005-SP
Structural and physicochemical studies of the fusion mechanisms and assembly of Hepatitis C virus

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The Hepatitis C virus (HCV) is the major cause of viral hepatitis, becoming a worldwide health problem. Current approved antiviral therapies are not completely effective. In order to contribute with the rational development of new antiviral therapies, our group used structural and physicochemical approaches to better understand the fusion mechanisms and assembly of HCV. We studied the interaction between membrane biomimetic models and a fusion peptide candidate, $HCV_{421-445}$, present in HCV E2 glycoprotein, and small regions of the HCV core protein (HCVcp), comprising the peptides 22–39, 50–67 and 85–102. With this aim we used different techniques such as dynamics light scattering, isothermal titration calorimetry and fluorescence microscopy. The peptide $HCV_{421-445}$ showed better interaction with membranes containing negatively charged lipids, and the interaction was favored in acidic pH. Circular dichroism (CD) and nuclear magnetic resonance analysis showed that this peptide is unstructured in solution and it gains helix content when in the presence of micelles. Our results strongly suggest that $HCV_{421-445}$ participates in the HCV entry process. The assembly process was investigated in the absence or in the presence of non-specific nucleic acids. The HCVcp peptides 22-39, 50-67 and 85-102 do not prevent the formation of nucleocapsid-like particles by truncated HCVcp. The peptide 50-67 was the only one able to interact with DNAs. CD and fluorescence spectroscopy data showed that the peptide 85-102 adopted an alpha-helix structure in n-octylglucopyranoside and sodium dodecyl sulfate micelles with partial internalization of its tryptophan residues. These findings present new approaches to understand the HCV assembly process.

P34-006-SP

Lipid interactions of integral membrane proteins: Rapid evaluation by a synthetic biology approach

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Cell-free expression has provided a breakthrough for the reliable and fast production of membrane proteins in even preparative scales. Numerous recent examples of the structural and functional characterization of a diverse variety of integral membrane proteins demonstrate the potential of this new technology platform. Membrane protein production in synthetic cell-free environments follows reliable and standardized protocols. However, the functional folding and stability of membrane proteins is often highly susceptible to the expression environment and specifically defined conditions are required. The open accessibility of synthetic expression reactions perfectly meets these requirements and allows to design individually adjusted hydrophobic environments. While many membrane proteins fold in presence of diverse artificial environments such as detergent micelles or amphipols, others are only stable in presence of particular lipids. The combination of cell-free expression with the nanodisc technology provides excellent synergies for the characterization of such difficult membrane proteins. We will present data on the biochemical characterization of G protein-coupled receptors and of prokaryotic lipid-I translocase enzymes that have been co-translationally solubilized in a variety of different environments. Both protein classes are problematic to synthesize in classical cellular expression systems and they are of high pharmaceutical interest. The analysed parameters cover (i) insertion efficiencies into supplied artificial bilayers, (ii) functional activity and variation of kinetic parameters in different environments and (iii) membrane protein stability and potentials for subsequent structural approaches.

P34-007-SP

Effect of 3′,6-diNonylneamine, an amphiphilic aminoglycoside derivative, on *Pseudomonas aeruginosa's* shape and membrane integrity

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Amphiphilic aminoglycosides derivatives targeting the bacterial cell wall or cell membrane have been used for the last decades for their efficacy even on multi drug resistant strains. In this perspective, we previously synthesized a variety of amphiphilic neamine derivatives and studied their efficacy on *Pseudomonas aeruginosa* strains. Among these derivatives, 3',6-dinonylneamine (3',6-diNn) had the ability to bind to lipopolysaccharides, and to negatively charged lipids (cardiolipin and phosphatidylglycerol) of the inner membrane. Therefore, membrane protein functions could be altered as it is known that they are specifically localized in cardiolipin enriched domains. This study investigates deeper into the effect of 3',6-diNn on the cells shape, membrane and membrane protein functions trying to elucidate it's mode of action. By time lapse studies, we demonstrated that cells treated with this neamine derivative lost their rod shape, and decreased in length in a concentration and time dependent manners. Moreover, duplicated cells were unable to undergo scission. We also showed that 3',6-diNn had a mode of action different than that of colistine and gentamicin. At high concentration, this derivative induced bacterial membrane deformation. Having this effect on bacterial membrane's integrity, we analyzed how this deformation could affect membrane proteins by analyzing the bacterial growth rate and the respiratory chain activity. Results showed a bacterial growth reduction, and a disturbance of the respiratory chain. Taken together, our results enlighten the mode of action of the 3',6-diNn and focus on the efficacy of aminoglycosides derivatives through targeting membranes integrity and disturbing membrane protein functions.

P34-008

Effects of employment of distinct strategies to capture antibody on antibody delivery into cultured cells

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The characteristics of antibody delivery into cultured HeLa cells were examined by using two delivery systems. Both systems used a cell-penetrating peptide as a tool for intrusion of an antibody into the cells, but either a "protein A derivative" or "hydrophobic motif" was employed to capture the antibody. When we examined the uptake of the Alexa Fluor-labeled antibody by these two systems, both systems were found to effectively deliver the antibody into the cultured cells. However, when we compared the amount of antibody delivered by these systems with the amount of transferrin uptake, the former was 10 times smaller than the latter. The lower efficiency of antibody delivery than transferrin uptake seemed to be attributable to the involvement of the antibody delivery reagent which failed to catch antibody molecule. This interpretation was validated by an experiment using a larger amount of antibody, and the amount of antibody delivered by the "protein A derivative" system under this condition was determined to be 13 ng proteins/10^5 cells. The antibody delivery achieved by the "protein A derivative" or "hydrophobic motif" showed two differences, i.e., a difference in intracellular distribution of the delivered antibody molecules and a difference in the fluorescence spectrum observed with cellular lysates. Possible reasons for these differences between the two delivery systems are discussed.

P34-009

Mechanism of nanoparticle deposition on polystyrene latex particles revealed by electrokinetic, AFM and SEM measurements

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Deposition of positive amidine latex particles (98 nm in diameter, A100) on negative polystyrene latex particles (820 nm in diameter, S800) was studied by SEM imaging, micro-electrophoretic and the concentration depletion methods involving AFM. The residual concentration of the positive latex in the mixture acquired after deposition on the negative latex was determined via the indirect procedure. The number of deposited positive latex particles was evaluated by a direct counting procedure exploiting the SEM images. The role of ionic strength, varied between 10^{-4} to 10^{-2} M, was systematically studied. This allowed

one to calibrate results obtained by measuring the electrophoretic mobility of large latex particles covered by a controlled amount of the positive latex. The electrophoretic mobility *vs*. coverage dependencies were quantitatively interpreted in terms of the 3D electrokinetic model previously used for planar interface. This allowed determination of the coverage of nanoparticles on latex carriers under *in situ* conditions. Additionally, the maximum coverage of the positive latex was determined via AFM where the kinetics of the residual latex deposition on mica was measured. The maximum coverage monotonically increased with ionic strength attaining 0.52 for 10^{-2} M, NaCl. This effect was interpreted in terms of reduced electrostatic repulsion among positive latex particles and theoretically accounted for by the RSA model. The obtained results have significance for basic science enabling one to properly interpret nanoparticle and protein monolayer formation at colloid carrier particles. Additionally, a robust method of preparing enzymatic micro-reactors and supporting catalyst beds based on protein and nanoparticles system can be envisaged. This work is financially suported by the PRELUDIUM 2013/11/N/ST4/00981.

P34-010
Revealing human Fb monolayer conformations at different pHs

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Adsorption mechanism of fibrinogen on mica at different pHs is studied using colloid deposition and the streaming potential measurements. The human fibrinogen monolayers on mica are produced by a controlled adsorption under diffusion transport at pH. Initially, the electrokinetic properties of these monolayers and their stability for various ionic strengths are determined. It is shown that at pH=3.5 fibrinogen adsorbs irreversibly on mica for ionic strength range of $4x10^{-4}$ to 0.15M. At pH=7.4, a partial desorption is observed for ionic strength below 10^{-2}M. This is attributed to the desorption of the end-on oriented molecules whereas the side-on adsorbed molecules remain irreversibly bound at all ionic strengths. The orientation of molecules and monolayer structure is evaluated by the colloid deposition measurements involving negatively charged polystyrene latex microspheres. An anomalous deposition of negative latex particles on substrates exhibiting a negative zeta potential is observed. At pH=3.5 measurable deposition of latex is observed even at low ionic strength where the approach distance of latex particles exceeded 70 nm. At pH=7.4 this critical distance is 23 nm. This confirms that human fibrinogen monolayers formed at both pHs are characterized by the presence of the side-on and end-on oriented molecules that prevail at higher coverage range. It is also that positive charge is located at the end parts of the αA chains of the adsorbed fibrinogen molecules. Therefore, one can conclude that the colloid deposition method is an efficient tool for revealing protein adsorption mechanisms at solid/electrolyte interfaces.

P34-011
Survival analysis of CKD patients' erythrocytes in ammonium medium

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The changes in the structure of cell membranes are one of the important mechanisms for the development and progression of chronic kidney disease (CKD). The survival functions of erythrocytes were compared in two groups: the 1st – healthy people (n = 32); the 2nd – patients with chronic kidney disease stages 3, 4, 5 (chronic renal failure 1, 2, 3) (n = 34). RBCs were incubated in ammonium medium for 15 minutes. Evaluation of hemolysis was carried out by the determining of MCV. Due to changes in the membrane ion channels activity erythrocytes volume increased smoothly at first, and then declined sharply. Minute, during which there was a sharp decrease in MCV, was marked like the point of the cell death. The Kaplan-Meier estimator was used for the analysis. The application of the log-rank test revealed statistically significant differences in the survival functions of erythrocytes in compared groups (p = 0.009). According to survival table erythrocytes of healthy people can survive for 6 minutes in ammonium medium. On the average this time is 4–5 minutes. Survival analysis of erythrocytes of patients with CKD showed that their erythrocytes are more rigid and can survive for 11 minutes in ammonium medium, on the average 5–6 minutes. This testifies to the deep structural and functional changes in erythrocyte membranes of patients with CKD 3, 4, 5.

P34-012
Functionalization of quantum dot-plasmid dna conjugate with a cell-penetrating protein

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YopM protein of *Yersinia* is an acidic protein that contains multiple leucine-rich repeat (LRR) motifs. These motifs are important for interaction of YopM with certain proteins. It has been reported that YopM suppresses inflammatory response. YopM is also able to penetrate the cell barrier and localize in the nucleus [1]. Cell penetrating and nuclear-localizing properties of YopM attract interest for gene delivery. We aim to increase the transfection efficiency of an existing quantum dot-plasmid DNA conjugate[2] by using the cell and nucleus penetrating properties of YopM. For this aim, CdTe/CdS nanocrystals were synthesized by one-pot synthesis method. One-pot synthesis were improved to synthesize water-based thiol-covered CdTe nanoparticles[3]. Plasmid DNA(gWIZ-GFP) including green fluorescent protein (GFP) gene fragment was labeled by quantum dot via peptide-nucleic acid(PNA)(H_2NCO-TCTCTCTC-OOO-JTJTTJTJT) recognition site[4]. YopM protein was purified by Ni-chelate affinity chromatography. The purified recombinant YopM protein was inserted into this conjugate via a second PNA(SH-C6-TTCCTTCC-OOO-JJTTJJTT) recognition site. Functionalization of quantum dot with plasmid DNA was confirmed by agarose gel electrophoresis. YopM has not been attempted for use as a cell penetrating agent in gene delivery studies before. Therefore, the developed conjugate holds promise for gene delivery studies.

Keywords: gene delivery, cell penetrating protein, quantum dot

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P34-013
Bispecific antibodies that cross-neutralize two Ebola virus strains

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Ebola viruses are associated with frequent outbreaks of highly lethal hemorrhagic fevers, including the currently ongoing outbreak in western Africa, which exhibits unprecedented magnitude and geographic spread. All five Ebola viruses (Zaire, Sudan, Bundibugyo, Tai Forest, and Reston) express a single glycoprotein (GP) on their surface which is solely responsible for host cell attachment and entry. Broadly neutralizing antibodies (Abs) or Ab-like molecules that can interfere with viral entry aspects such as attachment, uptake or membrane fusion represent a promising strategy for therapeutic intervention.[1,2] However, most neutralizing Ebola virus Abs, target one specific antigen on the surface of GP, which varies highly among the five Ebola virus species, rendering these antibodies narrowly strain specific. To address this problem, we created a set of bispecific Abs that combine the specificity against two different Ebola virus species in one molecule. Our data shows that bispecific Abs in which IgGs targeting the GP of Sudan served as scaffolds onto which an antigen-binding domain of a Zaire-GP targeted antibody is genetically engrafted, exhibit strain cross-reactivity capable of neutralizing the two different viral strains at nanomolar concentrations. Thus, these bispecific Abs bear the potential to serve as post-exposure therapeutics in cases where the infecting species of Ebola virus is unknown, since Sudan and Zaire together account for more than 90% of all Ebola virus outbreaks.

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P34-015
Analysis of the antimycotic effect of yeast killer toxin zygocin

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The increase in local and systemic fungal infections and also in antifungal drug resistance is one of the major concerns in clinical medicine. Unlike bacteria, eukaryotic yeast and fungal cells are closely related to mammalian cells and, therefore, the treatment of mycosis is often accompanied by many adverse side-effects. Furthermore, most of the common antimycotics either target fungal ergosterol synthesis (which to a big extent reflects mammalian cholesterol biogenesis) or interfere with yeast or fungal cell wall components, however none of these drugs efficiently kills a broad spectrum of pathogenic yeasts and fungi. In addition the molecular mechanisms of yeast cells' adaption processes leading to antimycotic insensibility are poorly characterized. A promising candidate as potential antifungal is the killer toxin zygocin secreted by the spoilage yeast *Zygosaccharomyces bailii*. This monomeric toxin possesses an unusual wide killing spectrum against various human as well as plant pathogenic yeasts and fungi, including *Candida albicans and C. glabrata*. In this study the biochemical and structural properties of zygocin and its effects on mammalian cell lines will be further characterized.

P34-016
Discovery of a non-cationic cell penetrating peptide derived from membrane-interacting human proteins and its potential as a protein delivery carrier

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Cell penetrating peptides (CPPs) are peptides that can be translocated into cells and used as a carrier platform for the intracellular uptake of cargo molecules. Subject to the source of CPP sequences and their positively charged nature, the cytotoxicity and immunogenicity of conventional CPPs needs to be optimized to expand their utility for biomedical applications. In addition to these safety issues, the stability of CPPs needs to be addressed since their positively charged residues are prone to interact with the biological milieu. As an effort to overcome these limitations of the current CPP technology, we isolated CPP candidate sequences and synthesized peptides from twelve isoforms of annexin, a family of membrane-interacting human proteins. The candidate screen returned a CPP rich in hydrophobic residues that showed more efficient cellular uptake than TAT-CPP. We then investigated the uptake mechanism, subcellular localization, and biophysical properties of the newly found CPP, verifying low cytotoxicity, long-term serum stability, and non-immunogenicity. Finally, model proteins conjugated to this peptide were successfully delivered into mammalian cells both *in vitro* and *in vivo*, indicating a potential use of the peptide as a carrier for the delivery of macromolecular cargos.

Poster Session 2
Tuesday 7 July & Wednesday 8 July
08:30–19:30, Foyer Convention Center
Gen Ex S4, RNA Processing and Modifications

P05-003-SP
Sequestering and protein cofactor competition regulate a multifunctional RNA helicase in different pathways

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DEAD/H-box RNA helicases play key roles in all major pathways of RNA metabolism by regulating the structure and dynamics of RNA-protein complexes. A rapidly increasing number of RNA helicases are implicated in several distinct cellular processes, however, the modes of regulation of such multifunctional RNA helicases and their recruitment to different target complexes have remained unknown. Here we identify the orphan G-patch protein Cmg1 as a novel RNA helicase cofactor that alone does not contact RNA, but stimulates the RNA binding and ATPase activity of the DEAH-box protein Prp43. Cmg1 was found to localise to the cytoplasm and to the intermembrane space of mitochondria. Furthermore, overexpression of Cmg1

promotes apoptosis while its deletion increases cell survival, indicating that Cmg1 is a new pro-apoptotic factor. Prp43 predominantly functions in ribosome synthesis and nuclear pre-mRNA splicing, and our data demonstrate that in apoptosis Prp43 is no longer able to interact with RNA. Moreover, different G-patch protein cofactors compete for interaction with Prp43. Changes in the expression levels of Prp43-interacting G-patch proteins modulate the cellular localisation of Prp43 causing accumulation of the helicase in the cytoplasm or nuclear splicing speckles. G-patch protein overexpression also leads to defects in ribosome biogenesis that are consistent with the withdrawal of the helicase from the pathway. Together, these findings suggest that the interplay of cofactors and the sequestering of a helicase are novel means to regulate the activity of multifunctional RNA helicases and their distribution between different cellular processes.

P05-004-SP
RPB1 foot mutations demonstrate that post-transcriptional regulation depending on Rpb4 plays a major role controlling the environmental stress response in *Saccharomyces cerevisiae*

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RPB1 mutants in the region corresponding to the foot of the enzyme, affect assembly of the complex by altering the correct association of Rpb6 and of the Rpb4/7 dimer. Assembly defects alter transcriptional activity and the amount of enzyme associated with the genes. Global transcriptional analysis of foot mutants shows the activation of an environmental stress response, ESR, that occurs at permissive temperature under optimal growth conditions. Our data indicate that ESR occurring in foot mutants depends on post-transcriptional regulation mechanism. Notably, these mechanisms are dependent on Rpb4-mRNA imprinting. Moreover, we propose that under optimal growth conditions, Rpb4 serve as a key to globally modulate mRNA stability and to coordinate transcription and decay. Taken all these together our results suggest that post-transcriptional regulation plays a major role controlling ESR.

P05-005-SP
Targeted modulation of alternative splicing by TALE-directed chromatin editing

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Pre-mRNA splicing primarily takes place co-transcriptionally enabling the transcription machinery and the chromatin environment to influence alternative splicing decisions. It has been previously shown that certain histone modifications correlate with exon inclusion rates; however, the mechanistic details are still elusive. In addition, most studies used global knockdown or overexpression of histone modifying enzymes or small molecule inhibitors to perturb the chromatin state genome-wide before assessing alternative splicing changes. This approach is prone to be influenced by secondary effects due to global chances in the transcriptional program. Here, we employ a targeted approach asking to which extend local modulation of histone modifications can affect inclusion rates of a selected alternative exon. We made used of transcription effector-like activator (TALE) domains which can be programmed to bind unique DNA sequences in alternative exons and fused them to histone modifying enzymes. Alternative splicing changes of target exons were assayed by RT-qPCR and histone modifications were monitored by ChIP. We found that targeting H3K9 methyltransferases, Suv39H1 and G9a, to the EDB exon of human fibronectin increased exon inclusion rates, whereas H3K36me3 methyltransferases, SETD2 and ASH1L, had no significant effect on inclusion of the same exon. This experimental system allows further elucidations of the mechanistic principles how chromatin effects alternative splicing.

P05-006-SP
Structural dynamics of H/ACA ribonucleoproteins studied by single molecule FRET spectroscopy

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H/ACA RNP complexes catalyze the modification of target ribosomal and spliceosomal RNAs in a sequence-specific manner. This is achieved by assembling a pseudouridine synthase, three auxiliary proteins and a guide RNA to form the core H/ACA complex *in vivo*. The target RNA is recruited via base-pairing to the guide RNA, and the target uracil is modified to its isomer pseudouridine. Despite a body of structural data on H/ACA RNP complexes from various organisms, information on structural dynamics throughout the cycle of assembly, substrate recruitment and catalytic turnover are sparse. Single-molecule spectroscopy in combination with fluorescence resonance energy transfer readout (smFRET) provides powerful means to study structural dynamics in RNA and RNP complexes. For this technique, RNA and proteins are covalently labeled with fluorophores and analyzed using fluorescence microscopy. Single molecule analysis provides data on conformational changes including kinetic parameters without ensemble averaging. Here, a first approach to study structural dynamics in H/ACA RNA and RNP complexes will be presented. We use splinted ligation of chemically modified RNA to introduce fluorophores into the guide RNA. This is complemented with introduction of non-natural amino acids via amber suppression for site-specific modification of proteins. We have reconstituted catalytically active full RNPs labeled with FRET pairs and show that this approach correctly reports on RNA-protein distances. In addition, we show that the guide RNA undergoes conformational changes when binding to the RNP.

P05-007
Assembly of complex ribozymes from short RNA oligomer pools

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There is compelling evidence for a primordial biology in which RNA was the central biomolecule responsible for information storage and catalysis. Key components of this 'RNA world' would have been ribozymes that were capable of catalysing their

own replication. Maybe the closest analogue of primordial replicases are RNA polymerase ribozymes (RPRs) about 200 nucleotides (nts) long, which are capable of templated synthesis using nucleoside triphosphates as substrates. However, non-enzymatic polymerisation of RNA from all four natural nucleotides yields RNA oligomers barely exceeding ~20 nts. Moreover, replication of long RNAs is complicated by the related problems of inhibitory template secondary structures and the high stability of RNA duplexes longer than 20–30 nts. There is thus a compositional as well as a conceptual gap between the primitive pools of RNA oligomers and the phenotypically complex ribozymes likely to be required for self-replication. Working backwards from one of the most advanced RPRs, we show efficient assembly of RPR function from mixtures of oligomers no longer than 30 nts. We discover that some physicochemical processes can be potent drivers of this RPR assembly reaction. Critical among these are eutectic ice conditions, which not only enhance the replication activity of RPRs but also allow more primitive ribozymes to harness prebiotic feedstock molecules and synthesize short oligonucleotides. Our work sketches a pathway by which RNA self-replication could have emerged from primitive oligomer mixtures and how the size of nascent RNA genomes could have been uncoupled from the limits associated with primordial RNA replication.

P05-008
Prototype tool for dsRNA manipulation in sequence-specific manner

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Existing RNA-modifying tools are poorly described compering to ones available for DNA, thus hampering the studies of RNA structure and function. Ribonucleases (RNases) that could serve as RNA-specific counterparts of restriction endonucleases would facilitate various *in vitro* and *in vivo* analyses. Many RNases that cut RNA internally exhibit substrate specificity, but their target sites are usually limited to one or a few specific nucleotides in single-stranded RNA, and often in a context of a particular three-dimensional structure of the substrate. Thus far, there is no known sequence specific RNase that could cleave double-stranded RNA. During our studies we investigated one of RNases able to cleave dsRNA. For this approach we used primer extension method and we developed method for Next Generation Sequencing of the ends generated by the endonucleolytic cleavage of the substrate dsRNA. Analysis of the sites cleaved by this enzyme in limited digest of bacteriophage Φ6 dsRNA by these two methods led to identification of a preferred target sequence. Based on obtained results we tested our predictions and performed kinetic analysis on a set of different sequences derived from the Φ6 genome. Here, we present evidence for a purely sequence-dependent cleavage of long dsRNA by the investigated RNase. We have also determined that the loop 5b-6, a distinctive structural element in Mini-III RNases, is crucial for the specific cleavage. We thus postulate the sequence-specific RNase as a good prototype tool for molecular biology applications in studies of RNA structure and function.

P05-009
Domain organisation and functional analysis of small RNA methyltransferase HEN1

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Small, 21-33-nucleotide RNA molecules are essential for post-transcriptional gene regulation in eukaryotic organisms including humans. All types of small interfering RNAs (siRNAs) and microRNAs (miRNAs) in plants, piwi-interacting RNAs in animals require 2'-O-methylation on the 3'-terminal nucleotide for their stabilization. This specific modification is carried out by the S-adenosyl-L-methionine-dependent small RNA 2′-O-methyltransferases, which are widely distributed in all biological kingdoms except archea. The best studied representative of them is *Arabidopsis thaliana* HEN1. Analysis of tertiary protein structure revealed that *Arabidopsis* small RNAs methyltransferase consists of five domains. To elucidate experimentally the function of each domain, miRNA/miRNA* and siRNA/siRNA* binding analysis, steady-state and pre-steady-state kinetic studies of truncated variants of methyltransferase and HEN1 mutants with point mutations were done. The obtained data indicate that: the methyltransferase domain of HEN1 is important for methyl group transfer; the first double-stranded RNA-binding domain is required for substrate recognition and its tight binding; the second double-stranded RNA-binding domain is an essential factor decelerating the decay of ternary complexes after methylation reaction. Similar binding and methylation parameters observed with siRNA and miRNA substrates suggest that the HEN1 does not encompass any domain necessary for distinguishing two types of small non-coding RNAs *in vitro*. As the central part of HEN1 is not responsible for the interaction with substrates, it was supposed that this part can be important for binding others biogenesis proteins in plants. This hypothesis was confirmed by data obtained using electrophoretic mobility shift assay, yeast two-hybrid system and pull-down method.

P05-010
Oligomerization and phosphorylation of RNA binding proteins in the assembly of stress granules

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RNA-Binding Proteins (RBPs) shuttle between the nucleus and the cytoplasm, coordinating the life of mRNAs. RBPs associate with the nascent mRNA to form highly dynamic RiboNucleoProtein (RNP) complexes that determine the transcript processing, its localization and how efficiently is translated or degraded (reviewed in [1]). A powerful way of silencing mRNA translation is by rapidly assembling mRNA molecules with their associated RBPs into aggregate-like structures such as the Stress Granules (SGs) [2]. TIA-1 and HuR RBPs comprise three RNA Recognition Motifs (RRMs) that bind to DNA/RNA molecules. Additionally, TIA-1 presents a C-terminal Prion Related Domain (PRD). Consolidation of both TIA-1 and HuR outside the nucleus and their co-localization inside pathological SGs impair their own physiological post-transcriptional regulation, causing the neurodegeneration associated with these proteins [3,4]. This transition towards a pathological RBP aggregation within SGs may be upper-regulated post-translationally, mainly by RRM module phosphorylation [5,6]. Our hypothesis, which presents a

novel paradigm in neurodegenerative research, suggests that SG nucleation may be initiated and/or stabilized by TIA-1/HuR RRM domains since these domains often form oligomers and many PRD-lacking RBPs are included in SGs.

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P05-011
Structural and functional analysis of the N-terminal helicase-associated region of the spliceosomal Brr2 protein

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Pre-messenger RNA (mRNA) splicing, an essential step in gene expression, is catalyzed by the spliceosome, a multi-megadalton ribonucleoprotein machinery. Unlike other macromolecular machines, none of the building blocks contains a preformed catalytic center. The Brr2 helicase is a key player in the catalytic activation process. Recent crystal structures provided insights into the molecular architecture and regulation of Brr2′s helicase region. Brr2 consists of two tandem helicase cassettes. Interestingly, only the N-terminal cassette shows unwinding activity, while the C-terminal cassette is thought as an intramolecular modulation device. While the helicase core is functionally and structurally well addressed, little is known about the structural organization and function of its N-terminal region comprising ~400 residues. Here, we present an atomic-resolution crystal structure of a PWI-like domain within the N-terminal region of Chaetomium thermophilum Brr2 and a low-resolution structure of the yeast protein showing the PWI-like domain in context of the helicase region bound to a fragment of Prp8, a key regulator of Brr2′s helicase activity. We found that the PWI-like domain of Brr2 does not interact with nucleic acids like other canonical PWI domains. We addressed the function of the N-terminal region by sequential truncations and tested the effects on Brr2 *in vitro* and *in vivo*. Our data suggest a self-inhibitory function of the N-terminal region, interestingly mainly by interactions to the unwinding inactive C-cassette. We found, that the N-terminal region is crucial *in vivo*, underlining the importance of this self-inhibitory.

P05-012
Towards a role of the B complex-specific protein FBP21 during splicing

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In the process of protein expression in eukaryotic cells, non-coding elements have to be excised from the pre-mRNA in a process called pre-mRNA splicing. It is catalyzed by the spliceosome, a highly dynamic megadalton machinery which assembles in a stepwise manner on the pre-mRNA. Many protein components are exchanged during the splicing cycle and are sometimes specific for a certain step in splicing. The spliceosomal protein FBP21 was found as a pre-catalytic B complex-specific protein. It activates pre-mRNA splicing and localizes to nuclear speckles. Molecular details of how FBP21 is involved in splicing remain elusive; however, FBP21 has been put into context with alternative splicing of clinically relevant targets such as vascular endothelial growth factors. In previous work in our group, we could show that FBP21 interacts with several spliceosomal core and accessory proteins containing proline-rich sequences via its tandem WW domains and possibly other partners via its structured matrin-type zinc finger. ITC and NMR showed that the tandem arrangement of the WW domains and the multivalency of the proline-rich ligand both contribute to an apparent affinity enhancement, which could be of importance for the dynamics of splicing. More recently, we identified spliceosomal binding partners in a comprehensive yeast-two-hybrid screen, which mainly included proteins playing a role in the transition from complex B to the catalytically activated complex B*. Additionally, we were able to confirm some of the potential interactions *in vitro*. FBP21 may thus act during catalytic activation of the spliceosome which may also modify alternative splicing.

P05-013
Splicing in two yeasts is predominantly co-transcriptional and can already be detected close to the 3′ splice site

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Pre-mRNA splicing refers to the removal of introns from pre-mRNA and can take place during transcription (co-transcriptionally) or post-transcriptionally after transcript cleavage and polyadenylation. The co-transcriptionality of splicing can provide the basis for functional coupling to transcription. Terminal exon pausing of RNA polymerase II, which was previously identified in *S. cerevisiae*[1], is one example for such coupling. It remains unknown when and where splicing can take place during endogenous gene transcription. We study co-transcriptional splicing in the two distantly related yeasts *S. cerevisiae* and *S. pombe*, which have very similar genome and gene sizes, but distinct gene architectures. We purify nascent RNA engaged in transcription from the chromatin-enriched fraction after cellular fractionation and determine *S. pombe* co-transcriptional splicing levels by nascent RNA-Seq. We found that half of the introns are spliced to 73% or more co-transcriptionally. In other systems – *S. cerevisiae*, humans, and fly – a similarly high fraction of co-transcriptional splicing was described previously[2]. To measure the actual position of co-transcriptional splicing along endogenous genes we develop a deep sequencing strategy called Single-molecule intron tracking (SMIT), which allows us to quantify nascent RNA splicing relative to the position of RNA polymerase II. Data for endogenous genes in *S. cerevisiae* suggest that splicing can be accomplished as soon as the RNA exits the catalytic center of RNA polymerase II.

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P05-014
Bis(phosphorothioate) cap analogues as a tool to enhancing a translational capabilities of therapeutical mRNA

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mRNA-based therapies such as anti-cancer immunotherapies or gene therapies receive more and more attention as a new potential option of medical treatment. One limiting obstacle for *in vivo* and therapeutic applications of mRNAs is their instability in cellular environment. Therefore, mRNA-based therapies need simple methods of mRNA modification which would improve stability but not interfere translation process. One approach to stabilize mRNA molecule is to modify its 5′-end. The effect of many various chemical modifications of 5′-cap has been studied. As a continuation of our previous research[1-3], we explored the impact of bis(phosphorothioate) modification on stability and translatability of capped-mRNAs. Here, we present an efficient synthesis method of bis(phosphorothioate) cap analogues and their biological properties. The results revealed efficient recognition by eukaryotic translation Initiation Factor 4E (eIF4E), resistance to decapping by hDcp2 enzyme and efficient translation of capped mRNAs in Rabit Reticulocyte Lysate and human immature Dendritic Cells. As high expression in hiDCs is an important factor for mRNA-based immunotherapies, new cap analogues appear as a potential tool to boost therapeutic properties of antigen-encoding mRNAs.

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P05-015
Study on HAX-1 protein and it's impact on transcriptome and mRNA turnover in the cell

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It is proposed that HAX-1 protein is engaged in many cellular processes including apoptosis, cell migration and adhesion. It also binds mRNA and probably plays a role in transcripts localization. We established stable cell lines with silenced expression of *HAX-1* gene as well as control cell lines using HeLa, HEK293, HS578T, MCF-7 and MDA-MB-231 cells. Through such a diversity of models we have the opportunity to study HAX-1 protein function in various types of cells (epithelial and mesenchymal-like). To examine the impact of HAX-1 expression changes on transcriptome, we used microarray-based approach. To obtain results related to specific cell functions, microarray data were searched against KEGG Pathway Database and Gene Ontology Database. We also conducted qPCR experiments to confirm microarray outcomes. A complementary approach was employed to identify new RNA binding partners of HAX-1. In order to do that we utilized CRAC, a novel technique based on UV-cross-linking of RNA-protein complexes and purifying them. We used stable cell lines with induced overexpression of *HAX-1* gene. The gene was engineered with adding 5' and 3′ sequences, responsible for protein N′- and C′-tagging after the translation. These tags were necessary in the CRAC protocol that we followed. It was confirmed that Cterminal part of HAX-1 molecule is essential for RNA binding. CRAC results from MiSeq, identifying HAX-1 protein RNA binding partners, require further detailed analysis. To conclude, we confirmed that HAX-1 protein has an impact on transcriptome and mRNA turnover in selected cell lines.

P05-016
Functional characterisation of the human rRNA methyltransferase WBSCR22

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Many cellular RNAs require modification of specific residues for their biogenesis, structure and function. Ribosomal (r)RNAs are extensively modified co- and posttranscriptionally during ribosome synthesis. Besides pseudouridinylation and 2′-O-methylation that are mediated by snoRNPs, rRNAs contain a variety of base methylations catalysed by stand-alone methyltransferases. However, the cellular functions of most human rRNA methyltransferases are still poorly investigated. WBSCR22 (Williams-Beuren-Syndrome Critical Region 22) contains a S-adenosylmethionine binding site and belongs to the family of Rossmann-fold methyltransferases, but up to date no methyltransferase activity has been reported. Here we demonstrate that impaired 18S rRNA maturation upon depletion of WBSCR22 is caused by the nuclear accumulation of 3′-extended 18SE pre-rRNA intermediates, which we map by deep sequencing. Furthermore, we show that WBSCR22 is an active RNA methyltransferase *in vivo* and that it mediates the N^7-methylation of G1639 in the 18S rRNA. Interestingly, the catalytic activity of WBSCR22 is not required for 18S pre-rRNA processing, implying that the key role of WBSCR22 in 40S subunit biogenesis is independent of its function as an RNA methyltransferase.

P05-017
Enzymatic modification of the 5′-cap in eukaryotic mRNAs enables labeling by click chemistry

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Recent studies demonstrate that a large fraction of mRNAs is localized to distinct subcellular compartments enabling spatial and temporal control of gene expression.[1] Efficient labeling of eukaryotic mRNAs with small organic reporter molecules would provide a way to detect endogenous mRNA and is therefore highly attractive. We established a chemo-enzymatic approach for enzymatic site-specific transfer of a reactive moiety to the 5'-

cap typical of eukaryotic mRNAs and further derivatization using click reactions.[2] The *Giardia lamblia* trimethylguanosine synthase 2, which catalyzes the methyl transfer from *S*-adenosyl-L-methionine to the N^2-atom of 5'-caps,[3] was engineered to install alkene, alkyne, azido or 4-vinylbenzyl groups on eukaryotic mRNAs.[2,4,5] Alkyne and alkene functionalities gave access to label RNA caps with fluorophores using Cu(I)-catalyzed azide-alkyne cycloaddition and thiol-ene click reaction.[2] By introducing an azido moiety, bioorthogonal labeling via strain-promoted azide-alkyne cycloaddition was achieved.[4] 4-Vinylbenzyl-modified caps could be converted in a tetrazine ligation or photo-click reaction generating turn-on fluorophores that are highly attractive for cell applicabilities.[5] In the long run, labeling of eukaryotic mRNAs in living cells in combination with modern sensitive live-cell imaging techniques could open up new possibilities for investigation of transport mechanisms, dynamics, but also misregulation of subcellularly localized mRNAs.

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P05-018
Expression of potential target genes regulated by miR-373 in patients with laryngeal squamous cell carcinoma

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MicroRNAs (miRNAs) are small, noncoding RNA molecules that emerge as important regulators of cancer-related processes. Laryngeal squamous cell carcinoma (LSCC) is very common malignant neoplasm of the head and neck. The alteration of miRNA expressions in LSCC still remains unclear. miR-373 was first investigated as a potential oncogene intesticular germ cell tumor to promote cell proliferation and carcinogenesis of primary human cells. By TargetScan database, we determined a miR-373 target site in the promoter of cell adhesion molecules E-cadherin and CD44. In the present study, we aim to investigate the relationship between miR-373 and target genes expressions in laryngeal cancer tissues. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was used to characterize the expression patterns of miR-373 and its target genes (E-cadherin and CD44) in 15 paired samples of laryngeal squamous cell carcinoma and adjacent noncancerous larynx tissues. Our results showed that the expression level of miR-373 was downregulated in the laryngeal cancer tissue compared with that in adjacent normal tissue. Similarly, E-cadherin and CD44 genes were significantly downregulated between the patient and control groups. This is the first report that indicates the existence of differences in miR-373 and its predicted genes expression in patients with laryngeal cancer. These findings might provide the basis for deep understanding of laryngeal cancer and further molecular experiments.

P05-019
The human spliceosomal protein-protein interaction network

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Splicing decisions are mainly controlled by the pre-mRNA sequence, its inherent signals and the spliceosomal protein complement. One of the major difficulties in the functional characterization of the spliceosome arises from the dynamic interactions between its sub-complexes and the huge number of proteins that participate in this procedure.The aim of this study is the *in silico* reconstruction of the protein-protein interaction (PPI) network of the human spliceosome. We created a dataset including all the proteins that have been isolated as subunits of the human spliceosome and/or its subcomplexes, based on all the relevant publications. Direct PPIs between spliceosomal components were retrieved from the human interactome knowledge base, PICKLE enhanced with PPIs for the *D. melanogaster* and *C. elegans* orthologous to human spliceosomal proteins from the DroID and Worm Interactome Database. The acquired data and the supporting publications were thoroughly evaluated, manually, in order to create a final set of confidently direct PPIs. The proteome of the human spliceosome contains 630 proteins, 60% of which can be integrated to specific spliceosomal sub-complexes. The reconstructed spliceosomal PPI network, which follows the power law distribution, consists of 457 nodes and approximately 1600 edges. Almost 25% of the proteins-nodes have been associated with genetic diseases, making the reconstructed network a valuable platform for suggesting documented functional relationships between genes, diseases and network topology.

P05-020
Effects of Epidermal Growth Factor (EGF) on CAIX and CAXII Expression In MG-63 Cells

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Carbonic anhydrases catalyse the reversible reaction from H_2O and CO_2 to HCO_3^- ions. Carbonic Anhydrase IX and Carbonic Anhydrase XII are member of the carbonic anhydrase family. Carbonic Anhydrase IX and Carbonic Anhydrase XII are in cell membrane. Carbonic Anhydrase IX expressed in solid tumor cell. CAIX expression in many cancer types is associated with disease processes. Elucidation of CAIX regulation associated with cancer would be a new approach in cancer diagnosis. Epidermal growth factor (EGF) is a growth factor that stimulates cell growth, proliferation, and differentiation by binding to its receptor EGFR. Human EGF is a 6045-Da protein with 53 amino acid residues and three intramolecular disulfide bonds. The aim of this study was to investigate the CA IX and CA XII mRNA expression levels in osteosarcoma cells (MG-63) treated with EGF. Therefore, MG-63 cells were treated by EGF for different time intervals, namely 1h, 3h, 6h, 24h, 48h and 72h. EGF upregulates CA IX mRNA expression mainly at 1h, 3h and 6h. EGF upregulates CA XII mRNA expression mainly at 3h and 6h. Different concentration of EGF on CAIX cells were also investigated and optimum dose for maximum expression was determined.

Key words: Carbonic anhydrase IX, Carbonic anhydrase XII, MG-63,EGF

P05-021
Identification of 5′-capped RNAs in bacteria

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RNA plays a central role for many cellular processes. Increasing number of RNA modifications correlates very well with the manifold functions of RNA. Until now, more than 150 chemical modifications of RNA have been discovered. Recently our group showed that nicotinamide adenine dinucleotide (NAD) is covalently attached to the 5'-end of several regulatory sRNAs and sRNA-like 5′-terminal fragments of certain mRNAs. NAD-capped RNAs were identified by NAD captureSeq, a chemoenzymatic capture approach in combination with next-generation sequencing (NGS). During the course of this work, *Escherichia coli* Nudix phosphohydrolase NudC has been found to hydrolyze the phosphoanhydride bond of the NAD-moiety resulting in 5'-monoposhorylated RNAs. We exploited this feature to develop a second-generation NAD captureSeq method. The approach relies on NudC-treatment of dephosphorylated total bacterial RNA. The generated 5'-monophosphorylated RNAs to which a biotinylated adapter was ligated, were captured and enriched on streptavidin beads, prior to reverse transcription. Reverse transcription was either performed with specific primers, or random hexamers. The cDNA was PCR-amplified and analyzed by NGS and bioinformatic means. We intend to apply the second-generation NAD captureSeq approach to identify RNAs, which are 5'-modified with other residues.

P05-022
The impact of oxidative stress on protamine 1 and 2 transcripts contents in human spermatozoa from smokers and nonsmokers

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Background: A proper protamination process is essential for sperm chromatin maturity and DNA integrity. Alterations in these sperm nuclear proteins were observed in smoker men.
Objectives: To evaluate the correlation between cigarette smoking, semen quality and protamines mRNAs ratios in smoker′s patients.
Methods: The present prospective study including the sperm from 123 men; 64 smokers and 59 non-smokers whose wives attending assisted reproduction and andrology laboratory. Quantitative real-time polymerase chain reaction (RT-PCR) for protamines 1 and 2 were evaluated in all ejaculates; sperm purification followed by mRNA extracted, reverse transcribed and then quantitative RT-PCR using specific primer pairs for protamine-1 and protamine-2. All samples were evaluated according to the World Health Organization guidelines.
Results: Protamine 1 mRNA levels in smokers (22.05 ± 2.64) were significantly higher (p=0.050) than that of nonsmokers (21.10 ± 2.96), besides, protamine 2 mRNA levels in smokers (19.80 ± 2.80) were significantly lower (p=0.001) than that of non-smokers (21.99 ± 3.24). P1/P2 mRNA ratios in non-smokers samples (0.96 ± 0.07) shows significant differences (p=0.001) compared with those in smokers (1.12 ± 0.11). P1/P2 mRNA ratios was negatively and significantly correlated with semen volume (r=-0.285, p=0.001), sperm count (r=-0.239, p= 0.008), and normal morphology (r=-0.286, p=0.001).
Conclusions: These dataset supporting the idea that smoking negatively affect sperm and serve as new evidence for the hazardous effect of smoking on men fertility. Further, alteration ratios in protamine transcripts in smoker men may serve as a marker for men fertility.

P05-023
Effects of Bisphenol A on the intraprostatic regulation of 5α-Reductase type 3 transcription by testosterone

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Background: 5α-reductase (5α-R) is a key enzyme for prostate physiopathology. Three 5α-R isoenzymes have been identified so far. The role of 5α-R1 and 5α-R2 in prostate diseases is well-known, whereas 5α-R3 is currently being investigated. We have previously demonstrated that 5α-R1 and 5α-R2 are positively regulated by testosterone (T). Recently, studies *in vitro* have indicated that T regulates 5α-R3 transcription in a cell type-specific manner and that this regulation is androgen receptor (AR)-dependent. On the other hand, increasing data indicate that the endocrine disruptor Bisphenol A (BPA) acts as an antiandrogen, binding to the AR and interfering with AR-mediated transcriptional activities.
Objectives: (i) Examine *in vivo* regulation of 5α-R3 transcription by T, and
(ii) determine whether BPA interferes with this regulation.
Methods: Adult male Wistar rats were used for these experiments. The experimental groups were: Castrated rats (C), Castrated rats treated subcutaneously (s.c.) with 500 μg of T propionate for four days (C+T), Castrated rats injected s.c. with 25 μg BPA/Kg/d 30 min before T administration (C+T+BPA25). Rats were euthanized 30 min after the last T injection and mRNA levels were measured by absolute qRT-PCR in ventral prostate tissues.
Results: Castrated rats treated with T significantly increased 5α-R3 transcripts ($P < 0.05$). BPA-treated rats exhibited a higher increase of 5α-R3 transcripts in comparison to unexposed rats.
Conclusion: Our results shed light on intraprostatic 5α-R3 regulation and suggest that: (a) *in vivo* T regulates positively 5α-R3 transcription in normal prostactic tissue, and (b) BPA regulates positively 5α-R3 perhaps independently of AR.

Gen Ex S5, Non-Coding RNAs in Gene Regulation

P06-005-SP
The activated androgen receptor regulates WNT/TCF7 through mediation of microRNA-1

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The WNT family of signaling proteins has pivotal roles in multiple developmental processes and tumor progression. An inverse

relationship between androgen receptor (AR) activity and WNT signaling was observed in advanced prostate cancer; however, modulation of AR/WNT crosstalk that leads to metastatic prostate cancer is unclear. Our recent report showed that activated AR increases microRNA (miR)-1 expression. Herein, we showed that progressive prostate cancer cells were associated with decreased AR-regulated miR-1 and increased WNT/TCF7. Our results demonstrated that the activated AR induced miR-1 reduced WNT/TCF7 levels in prostate cancer model systems. miR-1 directly binds to the 3' untranslated region of *TCF7* and regulates the stability of *TCF7* messenger RNA in the manner of AR activation. Relationships among the AR, miR-1, and TCF7 were also confirmed in clinical dataset and specimens. We anticipate that the induction of WNT/TCF7 results in increased prostatic bone metastasis that is linked to dysregulation of the AR signaling pathway through inactivation of miR-1.

P06-007-SP
Shifts in non-coding RNA expression profile distort the set of nuclear envelope proteins and affect the nuclear-cytoplasmic transport

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Aberrant nuclear morphology and impaired nuclear transport are typical for tumor cells. Our investigation aims to identify in what way shifts in expression of non-coding RNA, especially microRNA, can contribute to these abnormalities. MicroRNA targets within gene transcripts were predicted *in silico* using TargetScan software. We found that transcripts of genes encoding the nucleoporins NUP35/50/153/210, POM121, SEH1L, RAE1, RANBP2, nuclear transporters KPNA1/2/3/4/6, KPNB1, IPO7, TNPO1, NXF1, EIF4E as well as nuclear lamina proteins CBX5 (HP1), LBR, LMNB2, SYNE1/3, AKAP1, SUN1, BANF1 (BAF) carry highly conserved sites for microRNAs miR-15/16, miR-17/17-5p, miR-26, miR-31, miR-122, miR-125, miR-128, miR-143, miR-144, miR-145, miR-148/152, miR-185, miR-204, miR-205, miR-320, down-regulation of which is necessary for cancer cells. This mechanism can underlie overexpression of above-mentioned genes (in particular *KPNA2, KPNB1, EIF4E, RANBP2*), which is typical for transformed cells and predetermines nuclear accumulation of some transcription factors, e.g. NF-kB, as well as higher level of nuclear transport. However, microRNAs, hyperexpression of which is essential for abnormal proliferation and survival of cancer cells, can silence genes encoding some nuclear lamina proteins. We found highly conserved sites for microRNAs miR-21, miR-19 and miR-181 in *LEMD3 (MAN1)* gene transcript. Also, miR-181 can silence gene *LBR* encoding lamin B receptor. MicroRNAs miR-19 and miR-221/222 can target genes *SUN1* and *SUN2*, respectively. Because the above mentioned genes encode proteins forming the link between the lamina and nuclear envelope, silencing of these genes may entail nuclear lamina disorganization and heterochromatin disruption, contributing to genome instability and to overall derepression of chromatin in tumor cells.

P06-008-SP
microRNAs as effectors regulated by androgen receptor in prostate cancer

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Background: The recent evidence of a specific microRNA (miRNAs) signature associated with prostate cancer (PCa) tissues as well as cell lines, together with miRNAs involvement in antiandrogen therapy resistance highlights the need of new insights into androgen receptor (AR) mode of action in the intricate regulatory network including AR, miRNAs and their down-stream target genes.
Methods: AR regulated miRNAs/miRNA host genes were screened through microarray expression profiling and ChIP-seq. To verify the presence of AR binding sites in the candidate miRNAs/miRNA host genes, ChIP combined with PCR was performed in DUCaP cells following 1 h stimulation with a synthetic androgen. Real-time PCR was used to evaluate miRNAs/miRNA host genes expression after 24-48 h androgen stimulation or 24 h treatment with the antiandrogen MDV3100. Validation of putative down-stream target genes of the selected miRNAs was assessed transfecting miRNA mimics/antagomiRs for 48 or 72 h. Additionally, 41 matched cancer-benign tissue samples from primary tumors were analyzed by means of qPCR.
Results and conclusions: AR binds and regulates miR-22, miR-29a and miR-17-92 cluster after androgen stimulation. Interestingly, in the PCa cell lines harboring AR miR-22 and miR-29a basal expression is lower. *In vivo*, miR-22 and miR-29a levels are reduced in the cancerous tissue compared to the benign counterpart in line with their impairment of oncogenic pathways via targeting LAMC1 and MCL1. To summarize, this work highlights the importance of miRNAs and AR interplay in PCa and suggests a potential tumor suppressive role of these miRNAs. Supported by the PhD Program MCBO of FWF.

P06-009
MicroRNA control of protein expression noise

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MicroRNAs repress many genes in metazoan organisms by accelerating mRNA degradation and inhibiting translation, thereby reducing the level of protein. However, microRNAs only slightly reduce the mean expression for most targeted proteins, leading to speculation about their role in the variability of protein expression, or noise. Here we use mathematical modeling and single cell reporter assays to show that microRNAs – in conjunction with increased transcription – decrease protein expression noise for lowly expressed genes, but increase noise for highly expressed genes. Genes that are regulated by multiple microRNAs show more pronounced noise reduction. We estimate that hundreds of (lowly expressed) genes in mouse embryonic stem cells have reduced noise due to substantial microRNA regulation. Our findings therefore suggest that microRNAs confer precision to pro-

tein expression and thus offer plausible explanations for the commonly observed combinatorial targeting of endogenous genes by multiple microRNAs as well as the preferential targeting of lowly expressed genes.

P06-010
Analysis of oligoribonucleotides influence on the expression of interferon-stimulated and NF-κB-target genes in mice influenza model

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Natural and synthetic oligoribonucleotides (ORN) are known to posses antiviral and anti-inflammatory properties. In this respect, the aim of our research was to study the influence of ORN on the interferon-stimulated (ISG) and NF-κB-target genes expression of the innate immunity system in mice influenza model using RT-PCR method.

Results: Significant increasing of the *ifnα, ifnβ* and *ifnγ* genes expression was observed in virus-infected mice. But the ORN injection into mice for prevention and for treatment reduced these indexes. We observe the decreasing *ifnα* and *ifnβ* genes expression in 3 times and *ifnγ* – in 1.3 times when ORN were injected for treatment. The expression of *mx1* gene increased more than in 200 times in group virus-infected animals. But, when ORN were injected to animals infected with influenza virus for treatment, the *mx1* gene expression reduced on 30%. The *oas* gene expression decrease on more than 30% in the group of ORN-treated mice. The injection of ORN for treatment leads to the decreasing of *rnasel* gene expression in 1.4 times, compared with the virus-infected animals. It was shown that ORN influence the expression of the protein components of the transcription factor NF-k-B. The ORN injection into mice for prevention and for treatment reduced the expression of nf*κbia* gene in 2.8 times and *nfκb1* – in 3.6 times. Thus, in all animal groups, infected with influenza virus and ORN-treated, we observed the significant changes in the expression of ISG and NF-κB-target genes.

P06-011
Dissecting the role of microRNAs and their therapeutic potential in Alzheimer's disease

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A pivotal role for microRNAs (miRNAs) has been proposed in aging and neurodegeneration based on studies demonstrating that several miRNAs significantly change their expression during senescense. In this context, the identification of "miRNA signatures", may help gain new insights into the mechanisms of neuronal loss and lead to the discovery of new therapeutic targets in Alzheimer's disease (AD). Hence, this study aims to modulate the levels of selected miRNAs predicted to target proteins involved in AD. Through the use of bioinformatic tools, we have identified miRNAs predicted to bind with high affinity to the 3'UTR of APP and BACE1. We performed a biochemical validation of these binding sites in HT-22 neuronal cells, using a luciferase reporter assay and miRNA mimics. The results demonstrated that several of the selected miRNAs bind to APP and BACE1 mRNAs. Additionally, a decrease in APP and BACE1 mRNA levels was observed upon transfection of HT-22 and HEK-293 cells with lentiviral constructions containing the selected miRNAs sequences. Since we observed that miR-31-5p targets both APP and BACE1, simultaneously, we developed a lentiviral platform to overexpress this miRNA *in vivo*, though stereotaxic injection in the hippocampus of the 3xTg mouse model of AD. Our preliminary results show that miR-31-5p is able to diffuse efficiently in the mouse hippocampus and decrease the levels of human APP and mouse BACE1 mRNA *in vivo*. Given the high conservation of these miRNAs across species, we believe this work will support new diagnostic and therapeutic avenues to treat AD.

P06-012
Impact of small RNA molecules derived from the 5′ ends of tRNAs in cell function

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tRNA fragments (tRFs) form a new class of small non-coding RNAs derived from precise processing at the 5′ or 3′ end of mature or precursor tRNAs. These molecules have been detected in response to cell stress and reported to act as negative regulators of cellular translation in stress response [1]. More recently, 29–33 nt 5' tRFs have been detected in immune cell-derived vesicles [2] and were found to be expressed in response to viral infections by syncycial respiratory virus [3], HBV and HCV [4]. The mechanisms underlying the biogenesis of these molecules and their function are still under investigation. To investigate how the expression of 5' tRFs influences cell metabolism and gene expression processes, we have generated cell lines over-expressing these molecules by stable transfection of the Gly (GCC) 5' tRF sequence downstream of a Pol III promoter. Here we present a detailed analysis of the impact of the expression of this tRF in cell proliferation and metabolism.

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P06-013
MiR-486 and miR-92a identified in HDL subfractions discriminate between stable and vulnerable coronary artery disease patients

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MicroRNAs (miRNAs) are small non-coding RNAs implicated in the regulation of numerous genes, including those involved in coronary artery disease (CAD). We aimed to identify miRNAs in

the sera and lipoproteins (Lp) from CAD patients associated with CAD vulnerability. A CAD-focused screening array using 84 miRNAs was used to assess serum miRNAs distribution from 54 CAD patients presenting stable angina (SA), unstable angina (UA), one month after myocardial infarction (MMI) and 11 control subjects (CS). Screening analysis showed that miR-122, miR-486, miR-92a have the highest expression in CAD patients. These 3 miRNAs together with other 3 miRNAs (miR-125a-5p, miR-146a, miR-33a) known to be implicated in lipid metabolism, were selected and individually analyzed in sera and Lp using TaqMan miRNA assays and real-time PCR. All analyzed miRNAs had higher levels in CAD patients than in CS sera, but they did not discriminate between the CAD groups. Using a binary logistic regression model, we obtained a significant association of serum miR-486 and miR-92a levels with CAD vulnerable groups. Further, miRNAs were analyzed in Lp isolated from sera by density gradient ultracentrifugation. The selected miRNAs were identified primarily in high density lipoproteins (HDL). Specifically, miR-486 prevailed in HDL_2, while miR-92a prevailed in HDL_3, both having the highest levels in UA and MMI groups (versus CS). In conclusion, we identified 2 miRNAs in CAD patients' sera, whose distribution in HDL subfractions can discriminate between stable and vulnerable CAD patients.

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P06-014
Integrated miRNA profiling of estrogen receptor-positive breast cancer cell MCF-7

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Purpose: MicroRNAs (miRNAs) are small RNAs play a prominent role in a variety of physiologic and pathologic biologic processes. Changed miRNA expression has been found in many cancers, including breast cancer. The present study aimed to investigate the alterations in breast cancer cell MCF-7 miRNAs from 4 to 48 hours.

Methods: The expression profiles of 84 miRNAs in MCF-7 cell were evaluated using high-throughput real-time quantitative polymerase chain reaction. Total RNA was isolated from the MCF-7 cells and cDNA was synthesized. RNA was isolated using the High Pure miRNA Isolation Kit (Roche). The BioMarkTM 96.96 Dynamic Array (Fluidigm Corporation) for real-time qPCR was used to simultaneously quantite the expression of 84 miRNAs. Statistical analyses were performed using the Biogazelle's qbase PLUS 2.0 software.

Result: Our results demonstrated that statistically significant differences were detected in 35 miRNAs investigated between the groups. Total 28 miRNAs were down-regulated and 7 miRNAs up-regulated in MCF-7 groups in different time period (4h, 6h, 12h, 24h, 48h) comparing with control groups (fold regulation < 2, >2, $p< 0.05$). The most down regulated miRNAs were seen at 6 hours (25 down versus 1 up regulation). Effected miRNAs target genes were related with pathways such as PI3 kinase/AKT, ErbB, GnRH, Wnt, pathways in cancer, MAPK and mTOR.

Conclusion: This study demonstrates that altered miRNA expression pattern is involved in time-dependent in MCF-7 cells. The results provide a comprehensive view of the function of differential expression miRNAs related to breast cancer and may be helpfull for the further studies.

P06-015
A systematic approach to identify novel microRNAs without reference genome sequences in non-model organisms

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MicroRNAs (miRNAs) are small single-strand non-coding RNAs with about 21 nucleotides long on average found in plants, animals, and some viruses, functioning in post-transcriptional regulation of gene expression via mRNA cleavage or translation inhibition. Because it is difficult to detect miRNAs systematically by traditional experimental techniques, next generation sequencing (NGS) is applied to explore novel miRNAs in either model or non-model organisms. Therefore, computational methods play important roles in identification of novel miRNAs. Recently, some machine learning-based approaches are developed to identify novel miRNAs from NGS data. However, most of them essentially require precursor/genomic reference sequences to identify novel miRNAs, especially focusing on pre-miRNA identification. Owing to these requirements, non-availability of genomic sequences becomes a limitation in miRNA discovery in non-model organisms. It is necessary to develop a systematic approach to identify novel miRNAs without reference genome sequences. Based on our statistical analysis results, 5'-U, the different read counts between read-pairs, and the pairing structure of small RNA sequencing library are useful for miRNA model training. These features were selected when performing support vector machine (SVM) to develop a novel miRNA prediction model and analysis system. In this study, an effective miRNA prediction model was constructed to identify novel miRNAs from any small RNA sequencing data with no reference genome sequence, and will be very helpful in non-model species.

P06-016
miRNA mediated mechanisms of Trastuzumab and lapatinib treatment in breast cancer

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Breast cancer is a life-threatening disease with varied molecular features. Trastuzumab and lapatinib are frequently used therapeutic agents to inhibit HER2-mediated signaling, which has a role in approximately 25% of breast cancers. Clinical studies suggest that they may exhibit synergistic anti-tumor activities. However, the molecular effects underlying their complementary mechanisms of action are still needed to be examined. Recent progress in research has showed that miRNAs regulate multiple molecular pathways in breast cancer. A major discovery is their ability to mediate therapeutic actions by targeting genes that are important for drug function. The aim of this study is to reveal common miRNAs responsive to trastuzumab and lapatinib treatment in SKBR3 cells, which are representing HER2+ breast cancer, to identify miRNA-mediated mechanism and target gene signatures in drug function. Responsive miRNAs were determined by microarray profiling and two data sets were intersected to find out common miRNAs for both drugs. The targets of common miRNAs were provided by miRNA-target prediction databases and characterized through pathway enrichment analysis and functional annotation. 11 miRNAs were found to be common in both drug treatments. Combined enrichment analysis of their targets showed that ubiquitin mediated proteolysis was one of the most significant predicted pathways, while phosphoprotein and biological adhesion were among the highly clustered

functional categories. According to our results it may be suggested that miRNAs might be key players to explain the synergistic effect of two drugs and they can regulate the complementary mechanisms of action through ubiquitin mediated proteolysis and cell migration pathways.

P06-017
Two-level inhibition of *galK* expression by Spot 42: degradation of mRNA mK2 and enhanced transcription termination before the *galK* gene

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The *Escherichia coli*, *gal* operon has the structure *Pgal – galE – galT – galK – galM*. Spot 42, a small RNA, is known to downregulate *galK* expression. Of the six mRNA species produced by the *gal* operon, three species, mK2, mK1, and mM1, include the *galK* open reading frame and the Spot 42 binding site. The Spot 42 binding site resides at the 5' end of mK2 and at a cistron junction in the middle of mK1 and mM1. We find that Spot 42 downregulates the production of these mRNAs by two different mechanisms: 1) degradation of mK2 and 2) enhancement of transcription termination at the cistron junction between *galT* and *galK*. Spot 42-mediated degradation of mK2 is the major cause of *galK* downregulation and exclusively occurs in the early exponential growth period. Additionally, Spot 42-mediated enhancement of transcription termination at the end of *galT* leads to lowered production of mK1 and mM1. A molecular mechanism is proposed to explain how Spot 42 enhances Rho-mediated transcription termination at the end of *galT*.

P06-018
Antioxidant effect of Lithium is regulated by microRNA-34a in SH-SY5Y cells

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Neurodegenerative diseases are characterized by slow progressive loss of neurons in the central nervous system and their pathological mechanisms remain uncertain. Today, several neuroprotective agents are being investigated with the purpose of slowing or preventing further cell loss. Lithium is used as a treatment agent for a wide range of psychiatric and neurological conditions. Previous studies suggested that lithium has neuroprotective effects against a variety of insults, but the mechanisms of the neuroprotective effect of lithium have not been fully clarified. SH-SY5Y cells were pretreated with various concentrations of lithium at different time points. First we analyzed the effects of lithium treatment on brain-derived neurotrophic factor (BDNF), apoptosis related genes (Bcl-2, Bax) also, NF-E2-related factor 2 (Nrf2) transcription factor and its target genes' expression with real-time PCR. Our results showed that, lithium induces Bcl-2 and BDNF expression while decreasing Bax mRNA levels. More importantly; lithium induces nuclear translocation of Nrf2 and activates Nrf2 transcription factor, besides up-regulates HO-1, GCS and NQO1 expressions. Secondly, we examined role of apoptosis related microRNA, miR-34a, in the protective effect of lithium and we found that, lithium-mediated neuroprotection had significantly reduced after *miR-34a overexpression*. To confirm this relationship, we investigated the expression levels of miR-34a-target genes and we found that, miR-34a over-expression significantly reduced the mRNA levels of BCL-2, NRF2, and BDNF in SH-SY5Y cells. In conclusion, our results provide insight into the pathways mediating the antioxidant effects of lithium.

P06-019
miR-29b is a highly promising molecular marker for breast cancer progression

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Aberrant levels of microRNAs expression contribute to the molecular complexity of breast cancer. The aim of the present study was to analyze the expression of miR-29b in 81 malignant and 33 benign breast tumors, so as to explore its clinical value. Toward this direction, total RNA was extracted, polyadenylated, and reversely transcribed to cDNA from tissue specimens. Subsequently, a highly sensitive quantitative real-time PCR protocol was developed and miR-29b levels were then estimated by applying the $2^{-\Delta\Delta C}{}_T$ method by using *RNU48* as a reference gene. The relative quantification units measured for miR-29b were finally subjected to comprehensive statistical analysis in order to assess their relationship with the clinicopathological features of samples analyzed. So far, miR-29b expression was found to be slightly upregulated in benign breast tumors compared to the malignant ones. Additionally, our results strongly suggest that miR-29b is a highly promising marker for staging of breast cancer as its levels were significantly correlated (r_s=-0.259, p=0.020) with the diameter of the malignant tumors analyzed, and with their primary tumor (T) classification according to TNM staging system, as attested by both Kruskal-Wallis (p=0.049) and Jonckheere-Terpstra statistical tests (p=0.016). Further work is ongoing in order to investigate the significance of miR-29b as a survival factor, thereby establishing its clinical value in breast cancer.

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P06-020
Secondary structure of mature miRNAs suggests therapeutic approach

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The generally accepted model of the miRNA-guided RNA downregulation proposes that mature miRNA targets mRNA in a nucleotide sequence-specific manner. The structure of RNA determines its resistance to nucleases and function. Using specific nucleases, T1, V1 and S1, as well as NMR, UV/Vis and CD spectroscopies, we found that miR-21, miR-93 and miR-296 can adopt hairpin and/or homoduplex structures. Their structure suggests that miRNA structure may direct its specificity, also beyond the miRISC, which indicates that miRNAs are even more sophisticated regulators, that it was previously expected. Aiming at

understanding of molecular basis of gliomagenesis, based on microarray analysis, we provided a comprehensive overview of miRNA signature in malignant gliomas and proposed a set of miRNAs, which may be biomarkers of the malignant brain tumors and the targets of their therapy. Invariably for many years, the glioblastoma therapy includes surgical reaction and adjuvant radiotherapy and chemotherapy. Therefore there is a constant need for new therapeutic approaches. We designed anti-miR-21, -10b and -15b hammerhead ribozymes, for inactivation of both miRNA and their precursors. They specifically and highly effective hydrolyze both miRNAs and their precursors, and thus lower oncomiRs level in the cells.

P06-021
Homo sapiens exhibit a distinct pattern of CNV genes regulation: an important role of miRNAs in expression plasticity

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Gene expression regulation is a complex and highly organized process involving a variety of genomic factors. It is widely accepted that differences in gene expression can contribute to the phenotypic variability between species during evolution and their interpretation can contribute to the understanding of the physiologic variability. Copy number variations (CNVs) and miRNAs are two major players in the regulation of expression plasticity and may be responsible for the unique phenotypic characteristics observed in different lineages. We have previously demonstrated a close interaction between these two genomic elements contributing to the regulation of gene expression during evolution. This work describes a comprehensive analysis of the molecular interactions between CNV and non CNV genes with miRNAs and other genomic elements in eight different species presenting the unique nature of human CNV genes regulation in relation to the other species. By using genes with short 3' UTR that abolish the "canonical" miRNA-dependent regulation, as a model, we demonstrate a distinct and tight regulation of human genes that might explain some of the unique features of human physiology. In addition, comparison of gene expression regulation between species indicates a significant difference between humans and mice possibly questioning the effectiveness of the latest as experimental models of human diseases.

P06-022
The interaction between heat shock response and small RNA biogenesis in *Drosophila melanogaster*

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Once cells are subjected to stress they must re-establish their gene expression pattern to form an adaptive state as well as ensure the restoration of the original cellular homeostasis after stress termination. This may partly be achieved through miRNA that controls the expression of a large number of genes. Moreover, transposable elements expression can be also altered upon environmental change leading to derepression of transposons. It remains unclear how, under stress conditions, small RNA-machinery is modulated. The aim of this study was to explore the features of the regulation of miRNA and piRNA levels upon heat shock (HS) exposure as well as reveal a role of a major stress protein HSP70 in the formation of presumably adaptive small RNAs expression profile in *Drosophila melanogaster* after HS. We analyzed miRNA expression after HS exposure and demonstrated that HS results in rather similar pattern of miRNA expression in all strains investigated so far. We speculate that HS does not lead to the induction of miRNA expression and, hence, probably the regulation of miRNA levels occurs post-transcriptionally and reflects the cellular changes in gene expression program. Although, piRNA-machinery seems to be rather stable to HS, such impact can modulate the expression of certain germline piRNA-clusters. Our data indicates that HS forms a certain expression level of miRNA thereby maintaining a proper gene expression pattern which is a key feature of organismal adaptation to stress conditions. We also discuss a possible involvement of *hsp70* in the normal functioning of RNA-interference machinery under stressful conditions.

P06-023
CircRNAs and human diseases

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Circular RNAs (circRNAs) represent a class of widespread non-coding RNAs that regulate gene expression. They share a stable structure and tissue-specific expression and other features. Harboring microRNAs (miRNAs) competition sites, some circRNAs may act as competing endogenous RNAs. For example, *CDR1as* sponging miR-7 is related with pathogenesis of lung cancer, breast cancer, glioma, and amyotrophic lateral sclerosis and so on. cANRIL affects atherosclerosis through Polycomb protein family members. With an overwhelming number of identified circRNAs in humans, in-depth study of the structure and function of circRNAs may not only prompt us to disclose mechanisms of some diseases, but also provide a new direction for the prevention, diagnosis and treatment of disorders.

Mem Biol S4, Extrinsic and intrinsic regulation of cellular growth control

P11-003-SP
Structural insights into conformational changes of Arp2/3 complex, induced by ligand binding

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Actin nucleation is one of the key control points in cellular regulation of actin cytoskeleton dynamics. The Arp2/3 complex is an evolutionarily conserved actin nucleator that binds to the side of an existing actin filament and polymerizes a new daughter branch. We used single particle electron microscopy to compare the structures of Arp2/3 complexes bound by inhibitory ligands: GMF, Coronin, and Arpin. Each inhibitor appears to have a distinct binding site on Arp2/3 complex, yet they each cause the complex to adopt 'open' nucleation-inactive conformations. Binding of GMF induced two distinct and novel forms of the open conformation of Arp2/3 complex, possibly consistent with it having separate binding sites on Arp2 and Arp3. Binding of Arpin induced the standard open conformation of Arp2/3 complex, and tagging Arpin revealed that it may also bind near or on Arp2 and Arp3, consistent with its competitive interactions with VCA

for binding Arp2/3 complex. Additionally, we identified that Arp2/3 activator Abp1 may bind near Arp3 and induce the 'closed' primed for nucleation conformation of the complex. Overall, these results reveal similarities and differences in the mechanisms of the three inhibitors. Coronin and Arpin both induce a similar open/inactive conformation; yet have highly distinct binding sites on Arp2/3 complex. In contrast, while GMF and Arpin have neighboring binding sites on Arp2/3 complex, and both compete for binding with VCA, they induce distinct inactive conformations, pointing to differences in their functions. This work was supported by RSF grant (#14-14-00234) to O.S.

P11-004-SP
Allosteric regulation of insulin receptors by membrane lipids

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A number of studies reported an association of lipid alterations and insulin sensitivity, e.g., high cholesterol and glycolipid GM3 correlating with insulin resistance. However, the underlying modulatory mechanisms remain to be shown. Insulin acts through its receptors (IRs), which are membrane-embedded type II receptor tyrosine kinases. It is conceivable that their function is modulated by lateral localization to membrane domains and thus their interactions with the lipidic environment. Owing to their complexity and dynamics, it remains challenging to unambiguously show direct lipid-protein effects in living cells. Therefore, recombinant IR isoforms A and B were affinity-purified and reconstituted into proteoliposomes (i.e., lipid vesicles) with various lipid compositions to screen for effects of different membrane physicochemical properties. Affinity-purified IR excels in purity and exhibits insulin-dependent activation. Proteoliposomes were controlled for proper protein transmembrane insertion and orientation, for vesicle integrity and leakage. We present here a platform, which allows the screening for various effects such as IR activation by different ligands in dependence of specific lipids as well as IR modulation by proteins in the context of distinct lipid environments.

P11-005-SP
Cyclin-dependent kinase 5 is involved in pleiotrophin-induced endothelial cell migration

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Cyclin-dependent kinase 5 (CDK5) is a serine/threonine kinase that requires the regulatory subunits p35 or p39 for activation. CDK5 plays an important role in neuronal migration and neurite outgrowth and there are studies showing its implication in tumor growth and angiogenesis. Pleiotrophin (PTN) is a heparin-binding growth factor that induces cell migration in neuronal, cancer and endothelial cells through its receptor protein tyrosine phosphatase β/ζ (RPTPβ/ζ) and $\alpha_v\beta_3$ integrin leading to activation of c-Src kinase, β_3 Tyr773 phosphorylation and activation of ERK1/2. In the present study, by using immunoprecipitation/Western blot analyses, proximity ligation assays and direct measurement of the kinase activity we showed that PTN increased CDK5 kinase activity and its interaction with p35. Down-regulation of CDK5 by siRNA abolished PTN-induced endothelial cell migration. We also observed that PTN-induced CDK5 activation seemed to be independent of $\alpha_v\beta_3$ but dependent of RPTPβ/ζ expression. Moreover, activation of c-Src kinase was involved in CDK5 activation, while pharmacological inhibition of CDK5 did not affect PTN-induced β_3 Tyr773 phosphorylation and ERK1/2 activation. Collectively, these data suggest that CDK5 is a significant regulator of the PTN/RPTPβ/ζ signaling pathway that contributes to PTN-induced endothelial cell migration.

P11-006-SP
Three to stick with: Interactions of the Bazooka PDZ domains with cell–cell junction molecules

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Almost all cells in the human body display some kind of polarity. This polarity ranges from morphologically highly polarized cells, such as neurons or epithelial cells, to asymmetric round cells, such as rolling macrophages inside blood vessels. Studies of the mechanisms underlying the establishment and maintenance of cell polarity have revealed the PAR complex (PARtitioning defective) as a key player. This complex comprises Par3, Par6 and atypical protein kinase C (aPKC), with Par3 being the central scaffolding protein. Par3 contains three PDZ (postsynaptic density protein-95 kDa/ Disk-large/ Zonula occludens 1) domains that interact with numerous ligands and thereby organize polarity and cell junction complexes. PDZ domains usually bind the C-termini of their ligands via a β-sheet augmentation. It has been reported that the Par3 PDZ domains interact with several proteins involved in cell-cell junction formation, such as cadherins, nectins and JAMs. However, to date there remains a lack of structural data concerning the three PDZ domains of Par3 and their interactions with these ligands. In our work, we focus on the *Drosophila* Par3 homolog Bazooka (Baz) and its interactions with different ligands in the context of cell-cell junctions. To this end, we applied a combination of x-ray crystallography and NMR spectroscopy in order to elucidate the structure–function relationship between Baz and its ligands. Our findings will offer the potential to further investigate the link between cell polarity and cell junctions.

P11-007
Heavy metal resistance of *Bacillus subtilis* AG4 isolated from the Sotk Gold Mine in Armenia

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In some environments, such as the mines and ores, heavy metal concentrations are exceeding the lethal limit for most living organisms. However, bacteria highly adapted to the response of long term stress conditions have evolved elaborate metal resistance mechanisms. The present work concerns growth response, heavy metal accumulation ability and the expression of the *copA* and *nikA* genes of *Bacillus subtilis* AG4 isolated from Sotk Gold Mine in the presence of Cu(II), Ni(II), Zn(II) and Cd(II) metals. The results indicate that *B. subtilis* AG4 showed high resistance to Ni(II) and Cu(II) (up to 4.5 mM concentrations) but was

more sensitive to Cd(II) and Zn(II) (up to 0.5 and 1 mM concentrations, respectively). The concentration of the complex metal ions in the medium was found to be optimal for bacterial grow at 16 μM Cu(II), 17 μM Ni(II), 10 μM Cd(II) and 15 μM Zn(II). Strain AG4 showed a strong ability to accumulate Cu(II) and Zn (II) (up to 7 and 3 mg/g of wet weight, respectively). *B. subtilis* strain AG4 was found to harbor the *nikA* and *copA* Ni(II) and Cu(II) resistance genes. The highest expression of the *nikA* and *copA* genes, assessed using RT-qPCR, was observed in the presence of Cu(II), Ni(II), Cd(II) and Zn(II) in the growth medium. The results indicate that *B. subtilis* strain AG4 has potential for biotechnological and bioremediation purposes. The work was partially supported by ANSEF-2015 microbio-3869 and CPEA-2011/10081.

P11-008
Identification of multiple phosphoforms of the Lymphocyte Phosphatase Associated Phosphoprotein (LPAP) by site-directed mutagenesis and mass spectrometry

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Background: LPAP (Lymphocyte Phosphatase-Associated Phosphoprotein) is a transmembrane protein with unknown function that is tightly associated with the phosphatase CD45. There is evidence that phosphorylation status of LPAP undergoes changes after lymphocyte activation. This indicates that LPAP may be involved in the regulation of immune response. However, the information about the identities of LPAP phosphorylation sites is limited. Our aim was to investigate LPAP phosphorylation in rested and activated lymphocytes, identify individual LPAP phosphoforms, and determine possible transitions between them.
Methods: LPAP phosphorylation was determined by electrophoretic mobility shift assay in SDS-PAGE, phosphate affinity electrophoresis (Mn^{2+}-Phostag SDS-PAGE), Pro-Q Diamond phosphoprotein gel staining, Differential Gel Electrophoresis (*DIGE*), and tandem mass-spectrometry. Site-directed mutagenesis was used to define the contribution of individual phosphorylation sites in total phosphorylation. Wild type and mutated LPAP were either transiently expressed in HEK293T cells or stably transduced in T cell line CEM-CCRF. Endogenous LPAP in CEM cells was knocked out by bacterial endonuclease Cas9.
Results: 2D electrophoresis showed that in CEM cells at least five different phosphoforms existed. Using NetPhos software, we predicted 11 the most probable sites of LPAP phosphorylation. These sites were mutated to alanin in order to determine their impact on protein phosphorylation. LPAP transiently transfected in HEK293T cells was phosphorylated only on the Ser153, whereas LPAP in CEM cells was phosphorylated on additional sites, Ser99 and Thr113. The phosphorylation of Ser99 was decreased after cell activation.

P11-009
Activity of Akt/mTOR pathway depends on type and time of hypertensive stimuli in the heart

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Hypertension induces biomechanical stress which causes pathological left ventricular hypertrophy by reactivation of fetal genes in the heart. Akt signaling plays crucial role in the development of physiological and pathological cardiomyocyte hypertrophy by activation of mTOR pathway followed by elevation of protein synthesis. We used two animal models of pathologic hypertension: Spontaneously Hypertensive Rats (SHR), in which hypertension develops gradually, with age, and rats after abdominal aortic banding (AAB), in which hypertension is caused rapidly, by a surgical procedure. Phosphorylation levels of Thr308 and Ser473 of Akt were increased in SHR rats indicating increased kinase activity, whereas Akt phosphorylation was decreased in AAB group. Downstream targets of Akt, GSK-3 and FOXO1, were highly phosphorylated in SHR whereas their phosphorylation was decreased in AAB rats reaffirming regulation by Akt. Both Akt and GSK-3 as well as another kinase – ERK1/2 activate mTOR pathway, whereas AMPK inhibits mTOR activity. The levels of phosphyralotion of Akt, GSK-3 and ERK1/2 were significantly increased in SHR rats preventing inhibition of mTOR pathway by AMPK. Opposite, in AAB group phosphorylation levels of AKT, GSK-3 and ERK1/2 were decreased allowing AMPK to downregulate mTOR activity. This results in increased phosphorylation of mTOR downstream kinase – S6K in SHR but not in AAB group. This study clearly indicates that activity of Akt/mTOR pathway in hypertrophied cardiomyocytes is highly dependent on the origin and period of biomechanical stress, suggesting that only long-term, gradual increase of hypertension in SHR rats mobilize Akt/mTOR pathway in cardiac remodeling. NCN grants: UMO-2011/01/D/NZ3/04777, UMO-2014/13/B/NZ4/00199

P11-010
Three roles of survivin in differentiation and malignant transformation

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Survivin, member of inhibitor of apoptosis (IAP) protein family, is expressed in most tumour cells and is considered to be promising therapeutic target. In addition to inhibition of apoptosis, it regulates proliferation and promotes migration. We aimed to discover when survivin expression is linked to lack of apoptosis, cell migration or proliferation. We used canine kidney epithelial MDCK cells as model of a differentiated cell type. As model for malignant transformation we used ts-Src-transformed canine kidney MDCK cells which, when cultivated at 40.5 °C, behave as normal epithelial cells, whereas after shift to 35 °C, Src tyrosine kinase is activated and transformation process begins. MDCK cells and Src-MDCK cells were forced to grow in suspension (1D) by adding beta 1 integrin antibody into culture medium. Survivin was not expressed in MDCK cells and consequently, cells went to apoptosis. In contrast, Src-MDCK cells were proliferating and formed large cell clusters, survivin being heavily expressed. In 2D environment, survivin was expressed both in MDCK cells and Src-MDCK cells. There was no apoptosis and

cells were not able to migrate. Instead, both cell types kept proliferating even though non-transformed cells showed differentiated phenotype. In 3D matrigel, MDCK cells form cysts with single cell layer surrounding lumen. Lumen formation correlated with initial expression of survivin and its downregulation when cyst is differentiated. Upon shifting the Src-MDCK cells from 40.5 °C to 35 °C, Src was activated within one hour and survivin expression took place in two hours when the cells started migrating towards lumen.

P11-011
Screening of antibiotic producing actinomycetes from the sediments of undisturbed forest areas of Asella, Ethiopia and its hyper activity after mutation

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Wide and uncontrolled usage of antibiotics has made the pathogens to become resistant to currently used antibiotics. There is an urgent need for development of a new drug or a highly active molecule for controlling antibiotic resistant strains. In this study 32 strains of *Actinomycetes* were isolated and subjected to primary screening by giant colony method against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Aeromonas hydrophilia*. The secondary screening was carried out by fermentation process. Antibacterial activity was evaluated by well plate method. The extract of isolates was subjected to well plate method against pathogenic bacteria. *B. subtilis* and *E. coli* was highly inhibited by R1 isolate. Other isolates showed limited inhibition of bacteria. The R1 isolate was mutated by UV irradiation. The mutants differed from the wild parent in reduced growth rates, changes in the shape and size of the colony, sporulation level, antibiotic activity and variation in the color of the mycelium. *Bacillus subtilis* and *E. coli* was highly inhibited by AK1 isolate. Comparatively the zone of inhibition was higher with *Actinomycetes* AK1 inoculated plates. The secondary metabolite production was enhanced by UV mutagenesis when compared to wild type. *Actinomycetes* may produce different molecule that can inhibit different types of pathogens, however efforts like strain development can be done to produce new bioactive components against multidrug resistant bacteria.

P11-012
The cytotoxicity of different PMMA/ Hydroxyapatite nanocomposites

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Poly(methyl methacrylate) (PMMA) is a polymer that has been used in dentistry and orthopedic applications for more than 50 years. Nanohydroxyapatite is a well known biocompatible particle and it has high compression resistance. Hydroxyapatite addition to the PMMA increases the bioactivity because of the chemical nature of hydroxyapatite which is similar to the bone. In this study, PMMA polymers with different molecular weights were used to produce PMMA/Hydroxyapatite nanocomposite films with the help of twin-screw extruder. Our nanocomposites were composed of 1, 2.5 and 5 % (w/w) nanohydroxyapatite fillers. After the syntheses of the nanocomposites, the XRD and FTIR-ATR analyses were done. The residual monomers can be released from the polymeric matrix and they can migrate into the bloodstream. Therefore, we have determined the cytotoxic effects of our composites on lymphocytes by acid phosphatase assay and tryphan blue exclusion method performed by live cell imaging system (JuLI).

P11-013
Influence of snake venom Phospholipase A_2 on RPE-1 cells – multiple biological roles of $sPLA_2$

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Secreted phospholipases A_2 ($sPLA_2$, EC 3.1.1.4) catalyse the hydrolysis of the sn-2 ester bond of 1,2-diacyl-3-sn-phosphoglycerides in a Ca^{2+}-dependent manner, releasing lysophospholipids and free fatty acids (mediators involved in membrane damaging, cell proliferation, inflammation and apoptosis). Snake venom $sPLA_2$s affect different type of tissues and provoke neurotoxicity, myotoxicity, cardiotoxicity, nephrotoxicity, anticoagulant effects, hemolytic activity, inflammation, etc. Our interest has been focused on the toxic effects of snake venom $sPLA_2$ on retinal pigment epithelium (RPE) playing a key role in photoreceptor homeostasis. We investigate the effects of vipoxin $sPLA_2$ subunit on RPE-1 model cell line. Vipoxin is the main neurotoxin in the venom of *Vipera ammodytes meridionalis* snake. It is a heterodimeric protein composed of a basic and toxic GIIA $sPLA_2$ subunit and an acidic, enzymatically inactive and nontoxic subunit associated spontaneously in a tight complex. Vipoxin subunits were separated and purified using ion-exchange chromatography on Mono S column. We use MTT and comet assays to elucidate cyto- and genotoxicity, and actin fluorescence staining to detect cytoskeleton rearrangements induced by the toxic $sPLA_2$ subunit. Our results suggest dynamic changes in plasma membrane morphology and cell metabolic activity, actin cytoskeleton rearrangements and generation of double-strand DNA brakes in RPE-1 cells. We assume that the products of $sPLA_2$ enzyme activity have their own impact on cell survival pathways.

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P11-014
Aquaporin-1 plays important role in proliferation by affecting cell cycle progression

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Aquaporin-1 (AQP1) has been associated with tumor development. In this study, we investigated how AQP1 may affect cell proliferation. Specifically, the proliferative rate of adult carotid body (CB) cells, known to proliferate under chronic hypoxia, was analyzed in wild-type (AQP1 +/+) and knock out (AQP1 -/-) mice. Animals were kept in normoxia or exposed to hypoxia while BrdU was administered. The number of TH^+, $BrdU^+$ and TH plus BrdU double-positive cells was evaluated by immunohistochemistry. Lower numbers of total $BrdU^+$ and TH-$BrdU^+$ cells were observed in AQP1 -/- mice, indicating some role for AQP1 in the proliferation of CB cells. Then, by flow cytometry, cell cycle state and proliferation of PC12 cells with stable overex-

pression of AQP1 (PC12-AQP1) were compared to those of wild-type cells (PC12-Wt). Cell cycle state in the presence of sodium butyrate and nocodazole, and cell resistance to apoptosis by annexin V staining were also analyzed. Higher cell proliferation and percentages of cells in phases S and G2/M, as well as lower numbers of apoptotic cells were seen in the PC12-AQP1 cell line. Western blot analysis showed higher expression of cyclin D1 and E1 in PC12-AQP1, and microarray analysis revealed changes in many cell proliferation-related molecules, including, Zeb 2, Jun, NF-kβ, Cxcl9, Cxcl10, TNF, and the TNF receptor. Overall, our results indicate that the presence of AQP1 modifies the expression of key cell cycle proteins apparently related to increases in cell proliferation. This contributes to explaining the presence of AQP1 in many different tumors.

P11-015
Ouabain and marinobufagenin binding induce different conformations of Na,K-ATPase

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Na,K-ATPase, an ubiquitous ion pump, provides active transport of Na^+ and K^+ across plasma membrane in all types of animal cells. Moreover Na,K-ATPase is a receptor selectively responding to the changes in endogenous CTS level. Ouabain and marinobufagenin are cardiotonic steroids (CTS), which specifically inhibit Na,K-ATPase activity. These CTS inhibit transport function of Na,K-ATPase in cells in the same concentrations, however they induce cell death characterized by different values of IC50. The reason for their different physiological effect is still not clear. Applying fluorescence measurements, isothermal titration calorimetry (ITC) and molecular modelling we have shown that binding of ouabain and marinobufagenin cause different structural changes in Na,K-ATPase. Using fluorescence labeling we have shown that binding of both CTS with Na,K-ATPase shift conformation of Na,K-ATPase closer to the E1-state, and marinobufagenin induces more significant response than ouabain. Ouabain and marinobufagenin inhibit Na,K-ATPase hydrolytic activity with similar IC_{50} value (~1μM). However using ITC we observed a 17-fold higher affinity for binding of Na,K-ATPase in the E2P state to ouabain compared to marinobufagenin. The binding of ouabain to the enzyme is enthalpy-driven. In contrast, marinobufagenin binding has a reduced enthalpic contribution and a larger entropic component. According to the ITC data both CTS bind to the same site. Molecular modeling predicts that ouabain is located deeper inside the binding site than marinobufagenin. According to our data ouabain and marinobufegenine may induce different conformations of Na,K-ATPase which supporting binding of different proteins to the enzyme. Supported by the Russian Scientific Foundation (grant #14-14-01152).

P11-016
Oxidative stress and cell death are enhanced by N-3 pufa membrane incorporation in breast cancer cells

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Epidemiological studies highlight the correlation between the long chain n-3 polyunsaturated fatty acids (n-3 PUFAs) and a reduction of cancer, suggesting a protective n-3 PUFA effect. The n-3 PUFAs have been shown to improve the efficacy of various cancer prevention, chemotherapy drugs and radiation against cancer. The potential mechanism comprises mainly alterations of cellular lipid composition and metabolism, which might consequently modulate membrane properties and functions, eicosanoid productions, and modulation of different signaling pathways related to cell growth and death. The goal of the study was to investigate the effects of n-3 PUFA (EPA and DHA) incorporation in two lines of human breast cancer cells characterized by different expression of ER receptor. After treatments, PUFAs were partially metabolized from both cell lines and were incorporated in membrane phospholipids with different specificity, especially in lipid rafts. The n-3 PUFA incorporation increased the membrane unsaturation degree, decreased the sphingomyelin and cholesterol content, and altered the Glutathione Peroxidase (GPx), Reductase, Catalase activity, GSH and Malondialdehyde (MDA) content. By using the annexin-V staining we confirmed that DHA and EPA induce apoptosis and in addition we found that caspase-8, an effector caspase, is activated in n-3 PUFA treated cells. In conclusion we speculate that in breast cancer cells DHA and EPA incorporation alters membrane organization and might increase ROS accumulation in or near the plasma membrane especially lipid rafts where the assembly of the death inducing signaling complex (DISC) and the subsequent activation of apoptosis take place.

P11-017
The α1 subunit of Na^+/K^+-ATPase is a key component in the osmotic adaptive response of nucleus pulposus intervertebral disc cells

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Hyperosmotic conditions are an everyday experience for intervertebral disc cells *in vivo*. Here we assessed the high osmolality-induced transcriptional changes of bovine nucleus pulposus cells *in vitro* using whole-genome arrays. A 5- and a 24-h hyperosmotic treatment led to the differential expression of >100 and 200 genes, respectively, including nine genes encoding transporters (SLC4A11, SLC5A3, ATP1A1, SLC38A2, KCNK17, KCTD20, KCTD11, SLC7A5 and CLCA2). Differences in the transcriptional profile of these selected genes were validated by qRT-PCR in 2D and 3D cell cultures, under hyperosmolar salt and sorbitol conditions, revealing the presence of a common triggering signal for osmotic adaptation. The key signaling molecules p38 MAPK and p53 were demonstrated to differently participate in the regulation of the aforementioned transporters. Finally, siRNA-mediated knocking-down of each one of the three transporters with the highest and sustained over-expression (i.e. SLC4A11, SLC5A3 and ATP1A1) had a distinct outcome on the transcriptional profile of the other transporters, on p38 MAPK and p53 phosphorylation and consequently on cell cycle progression. The inhibition of ATP1A1 had the most prominent effect since its knocking-down under hyperosmotic conditions inhibited SLC4A11 mRNA expression, abrogated p53 phosphorylation and the p53-dependent activation of the G1 cell cycle checkpoint, enhanced p38 MAPK phosphorylation and thus amplified the p38 MAPK-mediated G2/M block. Overall, ATP1A1 loss-of-

expression resulted in an additive to that of high osmolality anti-proliferative effect, providing evidence for a central role of this pump in the osmoregulatory response of nucleus pulposus intervertebral disc cells.

P11-018
Analysis of the molecular mechanisms involved in the control of lung fibroblasts growth during exposure to silicon-based quantum dots

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Different types of nanoparticles, including quantum dots (QDs), have become powerful tools in various biomedical applications. It is necessary to test their toxicity using *in vitro* systems or animals in order to characterize their potential human health effects. Because almost all of the *in vitro* reports include an analysis for short periods of time, the present study aims to provide new useful insights regarding the influence on cellular growth triggered by long-time exposure to silicon-based QDs. The effect of QDs on cell membrane was evaluated in the presence of fetal bovine serum by measuring the pressure/area isotherms obtained using a Langmuir-Blodgett trough. The protein expression was established by Western Blotting and the telomeres length was assessed using Southern Blotting during the 9-month exposure of MRC-5 lung fibroblasts to QDs. Our results revealed a strong effect of protein corona adsorbed on the QDs surface in order to maintain the integrity of phosphatidylcholine layer which is a major component of cell membrane. The increase in p53 and apoptosis-inducing factor protein expression highlighted the activation of apoptosis after the 7th week. The role of autophagy pathway in the regulation of cellular growth during the exposure was suggested by the up-regulation of Beclin-1 and LC-3 protein levels. In order to maintain a cell proliferation rate near to untreated cells, the expressions of heat shock proteins 70 and 90 were increased. Finally, the lung fibroblasts activated survival signaling pathways to counteract the induction of apoptosis and autophagy and to control cellular growth during QDs exposure.

P11-019
UPARANT inhibits vascular endothelial growth factor-induced migration and angiogenesis via the VEGFR2-dependent pathway in human retinal endothelial cells

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Diabetic retinopathy (DR), a sight-threatening microvascular complication of both type-1 and type-2 diabetes, is the leading cause of blindness worldwide. Although the cellular and molecular bases of DR are only partially understood, it is evident that diabetic chronic hyperglycemia and hypoxia cause retinal angiogenesis and increased retinal vascular permeability [1]. Ultimately, they involve the angiogenic signalling systems such as vascular endothelial growth factor (VEGF)-VEGF receptors. Anti-angiogenesis treatment has been proposed as an important strategy for proliferative DR. UPARANT is a urokinase receptor-derived peptide inhibitor of VEGF-driven angiogenesis [2]. We investigated the anti-angiogenic activity of UPARANT *in vitro* on primary human retinal endothelial cells (HREC) used as model of blood-retinal barrier function. Our results show that UPARANT is able to restore TEER values and claudin-1 expression (indicators of the barrier function) that were decreased by $VEGF_{165}$ treatment. UPARANT also inhibits *in vitro* the VEGF-dependent HREC invasion and migration without affecting cell proliferation. VEGF mRNA expression level is also decreased by UPARANT after VEGF autocrine stimulation. Moreover, at a molecular level, UPARANT inhibits the VEGF-induced VEGFR2 phosphorylation and VEGFR2-mediated MAPK signaling pathway in HREC. The present study demonstrates that UPARANT represents a promising new therapeutic agent for the treatment of angiogenesis-related diseases.

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P11-020
N-Glycosylation as determinant of Epidermal Growth Factor Receptor conformation in membranes

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The epidermal growth factor receptor (EGFR) regulates several critical cellular processes and is an important target for cancer therapy. Structural analysis of growth factor receptors in their membrane environment is key to understanding their functions that are vital to the development and survival of organisms. High structural flexibility and post-translational modifications of the full-length receptors, however, hinder structural analysis at high resolution. Here we present atomistic MD simulations of the monomeric N-glycosylated human EGFR in biomimetic lipid bilayers that are in parallel also used for the reconstitution of full-length receptors. This combination enabled us to monitor and experimentally validate the influence of N-glysycylation on EGFR structure and the structural consequences of specific lipid–protein interactions, such as the inhibitory action of the ganglioside GM3.

P11-021
The alkaloid (−)-roemerine blocks carbohydrate uptake in *Escherichia coli*

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A dramatic increase in multi-drug-resistant bacteria constitutes a serious bottleneck in the treatment of bacterial infections. The quest for new antibacterials is a promising strategy to eradicate the resistance mechanisms of those bacteria. In this work, the potential of the plant alkaloid, (-)-roemerine, was investigated as an antibacterial drug against *Escherichia coli*. Under (-)-roemerine treatment, proteomic analysis clued the changes in membrane integrity, with up-regulation of the outermembrane protein

ompX and the type-1 fimbrial protein, fimA. The involvement of these membrane proteins in pathogenicity forced this work towards membrane integrity. Interestingly, further studies showed that the two transporters of the outermembrane, ompF and ompC, also involved in glucose uptake, were down regulated. Along with the down-regulation of these non-specific solute porins, the down regulation of malE and malM proteins of the maltose operon and mglB of glucose and galactose uptake suggested that (-)-roemerine leads to shortfall of intracellular carbon sources and eventually causes bacterial death. This work has been supported by TUBITAK-MAG Project with the number 113M052 and NBG was supported by TUBITAK-BIDEB fellowship.

P11-022
Thymic Stromal Lymphopoietin (TSLP) and its receptor as targets for the development of anti-inflammatory and anti-leukemic inhibitory agents

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Thymic Stromal Lymphopoietin (TSLP) is an interleukin-7-related cytokine expressed in epithelial cells and keratinocytes. It plays a central role in the pathology of inflammatory allergic disorders as well as in other pathologic conditions such as leukemia and solid cancers. The activated TSLP receptor (TSLPR) is formed by ligand-induced heterodimerisation out of the specific TSLPR α chain and the IL-7 receptor α chain and signals via the JAK/STAT pathway. Because of its involvement in various diseases, the TSLP/TSLPR system is a potentially interesting therapheutic target. We have explored possibilties to specifically block TSLP-induced receptor activation both for the human and the murine cytokine (mTSLP and hTSLP) by means of (i) recombinant ligand binding receptor exodomains, (ii) functional antibodies to both receptors and ligands and (iii) TSLP variants. These agents were analysed for biological activities and inhibitory properties employing various cellular models such as novel TSLP-responsive reporter cell lines and primary cells of human and murine origin (e.g. dendritic cells and long term cultures from leukemia patients). Recombinant TSLPR exodomains proved as competitive inhibitors of TSLP activity. Monoclonal antibodies could by isolated which are able to block TSLPR activation and intracellular signaling. Based on structural considerations and mutational analysis, TSLP variants with antagonistic properties were identified. These approaches are systematically further extended and exploited.

P11-023
Silencing of Carbonic anhydrase 9 and Tetraspanin-8 caused decrease at invasion capacity of human Pancreatic Carcinoma (PANC-1) cells

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Carbonic anhydrases (CAs) (EC 4.2.1.1) are zinc-containing metalloenzymes that catalyze the hydration of CO_2 molecule and dehydration of HCO_3^- ions. Carbonic anhydrase 9 is expressed in many solid tumors and plays a significant role in tumor acid-base homeostasis. Depletion of CA9 gene expression or inhibition of its catalytic activity shown to retard tumor growth in murine models and reduce metastasis.Tetraspanins are integral membrane proteins playing a role as organizers of multimolecular complexes in the plasma membrane. The human tumor-associated antigen CO-029 (TSPAN8) is a monoclonal antibody-defined cell surface glycoprotein and described as metastasis-promoting in several tumor systems. In this study, we aimed to evaluate the effects of silencing genes, CA9, and TSPAN8, on the invasion properties of human pancreatic carcinoma (Panc1) cells. Therefore, cells were transfected with specific siRNAs with siRNA transfection method along with control siRNAs for non-specific targets. Upon silencing, mRNA and protein assays show that the expression of CAIX and TSPAN8 were decreased compared to control cells. siRNA transfected cells were subjected to cell cytotoxicity assay at different time points in order to see if the silencing result in an proliferative effect. In addition, the metastatic and proliferative profiles of CAIX and TSPAN8 were determined after in order to 72 and 92 hours by matrigel and clonogenic assay.

P11-024
Effects of high doses of IL-2 on the inhibition of cervical cancer cells proliferation

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The function and structure of the IL-2 receptor (IL-2R) has been well characterised in lymphocytes, and its function as a necessary signal for cell proliferation has been solidly established. The IL-2R has been found to be expressed on non-haematopoietic cells, especially on several types of tumour cells. Recently, we have shown that cervical cancer cells express this receptor and that IL-2 induces their proliferation. However, the role of the signal transduction events in cervical cancer cells is not yet fully understood. We show that in IL-2R expressing cervical cancer cells at low doses of IL-2, the constitutive phosphorylation of JAK3 and STAT5 increases in the tumour cells and decreases in normal lymphocytes, while the opposite occurs at high doses of this growth factor. Using high doses of IL-2, we found that the activation of JAK3 and the proliferation of cervical cancer cells were inhibited. We further analysed its effect on the cell cycle and our results show that IL-2 induces a growth arrest but not cell senescense. We also found that IL-2 has an effect on the expression of genes related to the cell cycle. We think that these negative effects on cell proliferation could be used to develop strategies to treat cervical cancer or other solid tumours bearing IL-2R. Research supported by PAPIIT, DGAPA-UNAM (IN222915).

P11-025
Characterization of B16 F10 cells in culture by dielectrophoresis

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It was observed that cells changes their electrical properties throughout cellular cycle. This phenomenon can be studied using dielectrophoresis (DEP). DEP occurs when a polarizable particle, e.g., a cell, is suspended in a non-uniform electric field. The cell will experience a force, either attractive ($DEP_{positive}$) or repulsive ($DEP_{negative}$) according to the orientation of the cellular electrical momentum with respect to the field gradient vector. B16F10 mel-

anoma cells were cultured for 24, 48 and 72 h starting from the same clone. Washed 3 times and resuspended in Mannitol solution (300 mM, 10–20 μS/cm), the cells were exposed to DEP field (AC 2–4 V_{pp}) within a DEP chamber (triangulated electrodes, 1 μm thickness, 100 μm long edge and gap). Images were acquired before and after turning on the DEP field set at certain frequency (1–22 kHz). The ratio of cells experiencing $DEP_{positive/negative}$ was calculated for each frequency. It was observed that the percentage of $DEP_{positive}$ cells is increasing with culture time. It is presumed that this behavior occurs due to a decrease of membrane general capacitance while the cellular surface changes with time spent in culture.

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P11-026
Toll-like receptor-4 (TLR4) in the expression of ICAM-1 and VCAM-1 in cardiac fibroblasts and myofibroblasts

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Toll-like receptor-4 (TLR4) is crucial for the initiation and resolution of the inflammatory response to the occurrence of damage. After activation increases secretion of proinflammatory cytokines that promote increased recruitment and adhesion of immune cells to the site of injury. This receptor has been studied and characterized in immune cells that infiltrate the heart tissue after cardiac infarct. However, TLR4 has not been extensively studied in resident cardiac cells, such as cardiac fibroblasts (CF) and cardiac myofibroblasts (CMF). The aim of this study was to evaluate in primary culture of CF and CMF rat heart: a) the expression of adhesion proteins ICAM-1 and VCAM-1 and b) the possible signal transduction pathways involved in its expression, by action of lipopolysaccharide (LPS). For test this, CF and CMF were stimulated with LPS (1 ug/ml, 2-48 h) in the presence or absence of inhibitor TLR4 signaling (TAK241, 1 uM). Levels of ICAM-1, VCAM-1, NFkB, JNK, ERK1/2, p38 and Akt were assessed by Western blot. In conclusion, we found that LPS increased ICAM-1 and VCAM-1 expression in both cell types, but is greater in CMF compared to CF. We also found that JNK is involved in increased expression of VCAM-1 in CF and ERK1/2 and JNK participates in the expression of ICAM-1 in this cell type. Supported by FONDECYT 1130300 and FONDAP 15130011 (to LG and GD).

P11-027
Putrescine defect leads to G1-phase cell cycle arrest by methylglyoxal accumulation

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Polyamines protect the protein glycation in cells against an advanced glycation end product precursor methylglyoxal, which is inevitably produced during glycolysis, and the enzymes that detoxify this a-ketoaldehyde have been widely investigated. However, non-enzymatic methylglyoxal-scavenging molecules have not been sufficiently studied either *in vitro* or *in vivo*. We preliminarily observed that the putrescine auxotrophic odc^- cells exhibited a significant growth defect and underwent G1-phase cell cycle arrest, which was rescued by exogenous putrescine. Moreover, cellular glutathione and methylglyoxal were decreased or increased in odc^{oe} and odc^- cells, indicating that putrescine might act as a cellular methylglyoxal scavenger. Using UV-VIS spectroscopy, we found *in vitro* formation of a Schiff base complex comprising putrescine and methylglyoxal and confirmed this formation using liquid chromatography-mass spectrometry. The isolated putrescine-methylglyoxal Schiff base complex was calculated to have molecular masses ranging from 124 to 128. Based on this novel finding, we showed that the cellular putrescine-methylglyoxal Schiff base complex decreased proportional to the level of endogenous or exogenous putrescine in odc^- cells. Taken together, our experimental evidence suggests that methylglyoxal accumulation in putrescine-depleted cells might be a primary factor for cell growth. Therefore, methylglyoxal functions as a signal molecule through reciprocal interactions with putrescine in *Dictyostelium*.

P11-028
EBR promotes p53 independent apoptosis in colon carcinoma cell lines

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Epibrassinolide (EBR) is a polyhidroxylated sterol derivative and member of brassinosteroids, with well known growth promoting roles in plant growth. Due to its structural similarity to steroid hormones, EBR effect has been investigated in mammalian cells. Our previous data suggested that EBR exerts an apoptotic effect on cancer cells without affecting normal epithelial cells. We recently showed that EBR triggers apoptosis in a p53-independent manner in different cancer cells. However the exact molecular mechanism is still being investigated when cells do not express p53. There are various mechanisms identifying the p53 bypass in the apoptotic induction. One of the possible mechanisms is the inactivation of glycogen synthase kinase 3 beta (GSK3b) which abolishes cell growth in the absence of p53. Therefore in our study we investigated the role of GSK3b in EBR treated HCT 116 (wild type), HT-29 (mutant p53) and HCT 116 $p53^{-/-}$ cells. We determined that all cell lines underwent apoptotic cell death via dephosphorylation of AKT at Ser473 by causing alterations in the phosphorylation status of GSK-3b at Ser9 in all colon carcinoma cells after EBR treatment. On the other hand we found that hypophosphorilation of GSK3b was accompanied with the induction ER stress in all colon cancer cell lines. Since, the inhibitory effect of phosphorylation on Ser9 domain of GSK3b is involved in ER stress induction, our findings suggest that EBR triggers ER stress-mediated apoptosis via GSK3b in colon carcinoma cell lines.

Mem Biol S5, Lipid Signaling & Dynamics

P12-005-SP
Cooperation of CD14 and PIP5-kinase Iγ in PI (4,5)P_2 generation during stimulation of cells with LPS

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Activation of macrophages by lipopolysaccharide (LPS) requires interaction of CD14 protein with signaling receptor TLR4. We found that 10–100 ng/ml LPS triggers a bi-phasic generation of PI(4,5)P_2, a plasma membrane lipid involved in TLR4 signaling. The aim of these studies was to decipher the role of CD14 in LPS-induced PI(4,5)P_2 elevation. Immunoelectron microscopy combined with quantitative image analysis revealed that after 5–10 min of LPS stimulation, when the first peak of PI(4,5)P_2 generation occurred, CD14 underwent prominent clustering in the plane of the plasma membrane. This was concomitant with accumulation of PI(4,5)P_2 at CD14 clusters since as many as 42%–46% gold particles marking PI(4,5)P_2 co-localized with CD14 in LPS-stimulated cells in comparison to 23% in unstimulated cells. This co-localization was transient and after 60 min only 30–35% of PI(4,5)P_2 labels coincided with CD14. Generation of PI(4,5)P_2 and its co-localization with CD14 were inhibited by an antibody blocking binding of LPS to CD14. PIP5-kinase Iγ, one of three PIP5-kinase isoforms generating PI(4,5)P_2, also co-localized with CD14 clusters. The kinase was enriched Triton X-100 insoluble (DRM) fraction of cells containing also majority of CD14 and the newly generated PI(4,5)P_2. Silencing of PIP5-kinase Iγ abolished LPS-induced PI(4,5)P_2 generation, inhibited both activation of NFκB and production of pro-inflammatory cytokines by about 40%. A similar inhibitory effect was exerted by LiCl and 2-bromoplamitic acid interfering with phosphatidylinositol cycle. Taken together the studies indicate that CD14-enriched regions of the plasma membrane can serve as platforms for PI(4,5)P_2 generation which is required for pro-inflammatory signaling of TLR4.

P12-006-SP
Characterization of the Ca^{2+} and phosphoinositide-binding sites of the C2 domains of Rabphilin 3A

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C2 modules are most commonly found in enzymes involved in lipid modifications and signal transduction (PKC, phospholipases orphosphatidyl-inositol 3-kinases), and proteins involved in membrane trafficking like synaptotagmins and rabphilin 3A, among many others. Previous studies in our laboratory have shown that the C2 domain of PKCα can interact with both phosphatidylserine and PI(4,5)P_2 simultaneously, revealing an specific PI(4,5)P_2-binding site located in a polybasic region at the β3-β4 strands. In this work, we have characterized the molecular mechanism of Ca^{2+} binding to the C2A and C2B domains of rabphilin 3A. In addition, we have also shed light into the molecular mechanism of interaction of these domains with phosphoinositides. To address these questions we solved the 3D structure of the C2A domain of rabphilin 3A by X-ray crystallography in complex with PI(4,5)P_2, IP_3or Ca^{2+}. ITC and FRET studies of WT and mutant proteins revealed the molecular determinants of these interactions in both C2A and C2B domains of rabphilin 3A and demonstrate that in spite of sharing a collection of critical residues, each one possess differential aminoacids that confer them special abilities.Taken together, these results allow us to propose a molecular mechanism to explain the specificity of each particular C2 domain-membrane interaction.

P12-007-SP
New insights into the underlying mechanisms of Niemann-Pick disease type A/B

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The Niemann-Pick disease type A/B is caused by a mutation in the acid sphingomyelinase gene (SMPD1). It leads to the accumulation of the lipid sphingomyelin (SM) and causes lethal, neuronal problems. As the SMPD1 interferes with the sphingolipid pathway we investigated the underlying mechanisms in a SMPD1 KO mouse model. We used a LC-MS/MS approach to analyze the lipid levels in SMPD1 KO mice. As expected, SM levels were increased while phosphatidylcholine (PC) levels were decreased. Ceramide (Cer), the direct product of SMPD1, remained stable if not slightly increased. Moreover, sphingosine (Sph) was elevated and not decreased compared to WT mice. MS analysis in SMPD1 KO animals also revealed a metabolite, Deoxy-sphinganine (DOX-Sa), which was significantly lower in WT animals. *In vitro* experiments with J774 mouse macrophages showed that DOX-Sa significantly reduced the activity of SphK2, which might account for the increased Sph levels seen in the SMPD1 KO liver. As DOX-Sa has been reported to be synthesized in the *de novo* sphingolipid synthesis pathway if the serine-palmitoyl-transferase (SPT) incorporates alanine instead of serine, we fed SMPD1 KO mice with serine. Serine supplementation significantly decreased the DOX-Sa levels in SMPD1 KO mice. Still, the activity of the SphK2 could not be restored *in vivo*. We conclude that DOX-Sa alone is not responsible for increased Sph levels and decreased SphK2 activity found in SMPD1 KO mice. Altogether, this work indicates that the SMPD1 KO not only affects SM and Cer, but also other enzymes and lipids of the sphingolipid pathway.

P12-008-SP
SNX9 regulates focal adhesion disassembly during cell migratio

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The turnover of focal adhesions is essential for normal cell migration and tumor cell invasion, but the underlying mechanisms are still incompletely understood. We previously showed that the turnover of focal adhesions occurs through dynamin 2- and clathrin-dependent endocytosis of integrins from focal adhesions. Here, we report on the role of the endocytic adaptor protein Sorting nexin 9 (Snx9) in focal adhesion disassembly. Knockdown of Snx9 with short interfering RNA causes a severe defect in focal adhesion turnover, which results from the inhibition of beta1 integrin endocytosis. We further show that Snx9 is necessary for the recruitment of dynamin 2 to focal adhesions before adhesion turnover. Snx9 also recruits N-WASP and the

actin nucleation complex ARP2/3 to adhesion sites suggesting that Snx9 thereby stimulates actin assembly at focal adhesions to drive integrin endocytosis. Snx9 localization to focal adhesions, in turn, is dependent on PI4,5P_2 synthesis by the Type I phosphatidylinositol 4-phosphate 5-kinase beta (PIP5K1b). Notably, Snx9 is known to interact with PIP5K1b. Moreover, Snx9-PIP5K1b complex formation has been shown to stimulate PIP5K1b activity leading to increased PI4,5P_2 synthesis. These results suggest that Snx9 engages in a positive feedback loop with PIP5K1b to locally upregulate PI4,5P_2 synthesis for integrin endocytosis and focal adhesion disassembly. Together, our findings suggest that Snx9 plays an important role in cell motility control via regulation of focal adhesion disassembly.

P12-009
Corrective effect of N-stearoylethanolamine on pancreas phospholipid imbalance in rats with obesity-induced insulin resistance

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Disturbances of phospholipid (PL) composition triggered by dyslipidemia influence the membrane fluidity that affects activity of membrane-bound insulin receptor. Earlier, we showed that N-stearoylethanolamine (NSE) – minor lipid with membrane stabilizing properties, compensated the lipid imbalance under different pathologies. Therefore, the aim of our study was to investigate the NSE effect on pancreas phospholipid composition in rats with obesity-induced insulin resistance (IR). We determined the PL content of pancreases taken from rats of 4 groups: «Control», «NSE», «IR», «IR+NSE»; first two groups were fed with regular chow (4% fat), other groups were given HFD (58% fat) for 6 months. At the end of the experiment «NSE», «IR+NSE» rats were given per os water suspension of NSE (50 mg/kg daily) for 2 weeks. The conformation of the IR existence was based on the results of glucose and insulin content assays. The pancreas lipid assay from rats with IR showed increased total lipids level, whereas total PL content was reduced, primarily by considerable decrease of PC, PE, PI, PS level. Furthermore, we found reduced content of SM in IR rats that was accompanied by a decrease of free cholesterol level. The NSE administration to IR rats normalized the pancreas free cholesterol content and total level of PL, by a significant increase of the content of the main individual PL (PC, PE, PS, SM) in comparison with IR rats without NSE treatment. Therefore, NSE corrected the phospholipid imbalance in pancreas tissue, normalizing fasting insulin level and restoring insulin sensitivity under obesity-induced IR state.

P12-010
Fasting-induced changes of hepatic lipid and carbohydrate stores in the absence of GLUT2

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Metabolic adaptations to fasting rely on appropriate glucose sensing networks involving GLUT2 activity. Counter-regulatory glucagon secretion and increased food intake following a glucoprivic stimulus are blunted in mice lacking whole body GLUT2 expression. Glucagon promotes the release of fatty-acids (FA) from adipose stores to the liver, stimulates hepatic VLDL secretion and glycogenolysis. To investigate how fasting-induced dynamics of intra-hepatic lipids and carbohydrates was altered by glucose sensing derangements, we performed non-invasive magnetic resonance spectroscopy measurements *in vivo* in the liver of GLUT2-ko mice and controls. Intra-hepatic glucose levels were extremely high in GLUT2-ko mice and despite their decrease with fasting they were still abnormally elevated after a 24h-fast (~50mM). Relative to controls, GLUT2-ko mice showed higher levels of mono-unsaturated FA and conversely very low levels of poly-unsaturated FA in the fed state. Interestingly, the contribution of poly-unsaturated FA sharply increased with fasting in GLUT2-ko mice, what probably reflects the mobilization of FA from adipose stores. Nevertheless, the overall hepatic lipid content remained unchanged in GLUT2-ko mice with fasting, whereas it doubled in controls. Such observation indicates a higher turnover of hepatic lipids in the former case. In sum, we detected dynamic changes in hepatic lipid content (controls) and poly-unsaturation degree of FA (controls and GLU2-ko) that provide non-invasive evidence for utilization of lipids as energy fuels with fasting in both models. We conclude that the mobilization of lipid stores with fasting is preserved under faulty glucose sensing and that lipids are the preferred energy fuels in this case.

P12-011
Quantitative analysis of dynamic palmitoylation in human T cells

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Palmitoylation is the addition of 16-carbon palmitate groups to cysteine residues; once lipidated, proteins localize to membrane-ordered "lipid rafts" in the plasma membrane. In recent years, the reversible nature of palmitoylation has led the identification of regulatory palmitoylation cycles in a variety of cell types, with palmitoyl groups being added by DHHC palmitoylacyltransferases at the ER and Golgi and being removed by thioesterases at the plasma membrane. While several key players during TCR signaling in T cells are known to be palmitoylated, it has been traditionally assumed these proteins are constitutively lipid-modified when exiting the Golgi. However, the possible role of dynamic palmitoylation-depalmitoylation cycles has not been addressed, at a global level, as a means of localizing TCR signaling proteins following antigen stimulation. In this way, palmitoylation could possibly play a role similar to phosphorylation in spatiotemporally regulating proteins important for proper signaling function. Here we couple an established enrichment method of isolating palmitoylated proteins with isotopic labeling and quantitative mass spectrometry, allowing a global view of dynamic palmitoylation following TCR stimulation. We report a pool of palmitoylated proteins found only after TCR stimulation, as well as a large pool of novel, previously unreported palmitoylation candidates.

P12-012
Regulation and signaling mechanism of cancer cell migration by TGF-Beta receptors and ceramide metabolism

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Recent studies indicate that ceramide species play diverse biological functions including, skin barrier function, liver homeostasis, cell death and cancer pathogenesis, highlighting the importance of ceramide synthases (CerS) in these processes. Migration, a part of these processes, also is effected by ceramide metabolism. However, the molecular mechanism of CerS/ceramide involved is unknown. Here, we investigated the effect of CerS on migration and its related signal pathways *in situ* and *in vivo* model. Interestingly, our data show that among CerS only CerS4 is related to cell migration. Here, we also have generated CerS4$^{-/-}$ mice, and these mice were viable with no lethal tissue. Interestingly, we observed that loss of CerS4 resulted in irreversible alopecia, which was associated with hyper-proliferation and migration of keratinocytes. Mechanistically, we show that knockout/knockdown of CerS4 enhances cell migration by which ligand-independent signaling of TGFβ receptors in various cell types, including keratinocytes, MEFs, and cancer cells. Additionally, low level of TGFβR1-Smad7 interaction was found in knockdown of CerS4 cells. Moreover, we found that ceramide interact with Smad7 and interaction was decreased by knockdown of CerS4. Thus, ceramide-Smad7 binding modulates plasma membrane association of TGFβR1, and inhibits its signaling through Sonic-Hedgehog (Shh) signaling for migration. In fact, inhibition of TGFβR/Shh signaling using molecular or pharmacologic inhibitors almost completely prevented cell migration in response to CerS4 knockdown. These data suggest that CerS4/ceramide signaling plays key roles in the regulation of cell migration via controlling the TGFβR/Shh axis.

P12-013
Inhibition of ceramide synthesis as post-ischemic therapy for myocardial reperfusion injury

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Therapeutical interventions aimed at reducing post-ischemic injury may have enormous potential to improve short and long-term morbidity and mortality. Besides an array of collateral effects, reperfusion after ischemia triggers a pathological inflammatory reaction caused by several mediators including lipotoxins such as the sphingolipid ceramide. Ischemia-reperfusion (I/R) injury was shown to increase myocardial ceramide content (Beresewicz A. 2002) and pharmacological inhibition of ceramide formation to ameliorate cardiac dysfunction (Gundewar S. 2008). We recently proved that pharmacological inhibition of ceramide synthesis significantly reduces acute inflammation in lung infection (Caretti A. BBA 2014). Our present aim was to evaluate the therapeutic potential of ceramide synthesis inhibition in I/R myocardial injury in mice. After 30 minutes of left anterior descending coronary artery ligation (LAD), we performed intramyocardial injection of the ceramide synthesis inhibitor just at the beginning of 3 hours reperfusion. The treatment reduced infarct size (36% decrease), decreased ceramide content, expression of inflammatory cytokines, formation of hydroperoxides within the risk area. Finally, the treatment enhanced Nrf2 activated transcription of HO1. We concluded that inhibition of I/R induced accumulation of the stress lipid mediator ceramide during reperfusion of infarcted myocardium allows i) a decrease in tissue infarct, ii) a significant reduction in inflammatory and oxidative factors production, iii) enhanced myocardial pro-survival adaptive response.

P12-014
The Role of lipids in the intramembraneous interactions of Viral proteins

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Recent experimental data indicate that many processes during virus infections are highly cholesterol dependent. In line with that, the intrinsic cholesterol binding motif, CRAC of the HIV glycoprotein gp41 was not only demonstrated to be an indispensable determinant of the protein's lipid raft partitioning and lateral sorting in the membranes of infected cells, but in addition is was shown to influence two other important properties of the protein, oligomerization and membrane perturbation. This finding underlines the importance of protein-lipid interactions on structure and function of proteins and protein complexes. By *in silico* analysis (string alignment and blosum analysis) we identified novel CRAC motifs in several, previously described immunomodulatory interaction partners of the gp41. We hypothesize that the cholesterol, being associated with the proteins is part of the respective interaction interfaces between gp41 and its interaction partners, and significantly influences the binding affinities and as a consequence also the overall immune-modulation efficiency of gp41.

In this study we aim to investigate the impact of the CRAC motif, but also different lipid compositions of the bilayers on the various, intramembraneous activities of gp41. In particular we will focus on the interactions of the viral fusion peptide, transmembrane domain and extracellular loop-region with host proteins, such as Toll-like and T-cell receptor (TLR and TCR) that were discovered to cause immune modulation in T-cells and macrophages. Our study will shed further light on the role of lipid in the membrane-associated interactions of viral proteins and add to our understanding of pathogenic immune modulation.

P12-015
The main endocannabinoid anandamide as signaling mediator: towards the effects upon human decidualization

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Decidualization is a tipping point for successful embryo implantation and maintenance of pregnancy. During this process, endo-

metrial stromal fibroblasts differentiate into specialized decidual cells under control of ovarian steroid hormones. Disruption of decidual process predisposes to pregnancy complications, including miscarriage and poor outcome of *in-vitro*-fertilization. Recently, endocannabinoids emerged as signaling mediators in reproduction. We have previously characterized the endocannabinoid machinery in rat decidual tissue and reported the apoptotic role of anandamide (AEA) on decidual cells, thereby concurring to decidual remodeling process.

So far, the functional role of endocannabinoids in the decidualization remains undefined. Kessler *et al*. evidenced that WIN-55,212-2, a synthetic cannabinoid, inhibits decidualization and stimulates apoptosis. As the understanding of the molecular signaling network that coordinates decidualization becomes emergent, we aimed to unveil the role of endocannabinoid system towards the decidualization.

In this work, we reported the endocannabinoid levels evaluated by liquid chromatography-mass spectrometry in human primary endometrial cells, both in undifferentiated and differentiated stages. In addition, we characterized the expression of the endocannabinoid system members by quantitative Q-PCR, Western blot and immunocytochemistry. Parallel, we studied the impact of the main endocannabinoid, anandamide, during decidualization. AEA inhibited the endometrial stromal cells differentiation, by decreasing mRNA levels of decidualization-markers like prolactin and IGFBP-1. These effects were partially reversed by the cannabinoid receptor antagonist, suggesting the involvement of CB1.

Impairments of decidualization process compromises reproductive health and predisposes to pregnancy complications. Hence, our findings suggest that anandamide may impair the differentiation process and natural remodeling process occurring during this period.

P12-016
Effect of abiraterone and ionizing radiation on the glycohydrolases activities in prostate cancer cells

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Prostate cancer (PC) is the most common malignancy and second leading cause of cancer-related deaths in men. Among the different therapeutic options, abiraterone is a new promising drug recently approved for the treatment of PC, nevertheless it mechanism of action is almost unknown.

PC is characterized by "aberrant glycosylation", caused by specific glycosyltransferases and glycohydrolases present both intracellularly and at the plasma-membrane (PM) level. Interestingly, changes in the activity of different glycohydrolases have been detected in different cell lines after proton irradiation. In particular, it has been recently demonstrated that in breast cancer cells the irradiation caused the production at the PM-level of pro-apoptotic ceramide through the *in-situ* hydrolysis of complex glycolipids. Based on these findings, this study addresses whether these enzymes are a target of abiraterone and of ionizing radiations in human PC. To this purpose, androgen-sensitive and androgen-insensitive PC cell lines were subjected to treatments with abiraterone and/or ionizing radiation and the activities of different PM-associated glycohydrolases as well as the ceramide level were evaluated. Interestingly, all the cell lines tested showed a marked increase in all the PM-associated glycohydrolases as well as in their ceramide content, especially after the combined treatment with abiraterone and ionizing radiation.

These data demonstrate the involvement of the glycohydrolases in the mechanisms of abitaterone- and radiation- induced cell death in both androgen sensitive and insensitive PC cells and suggests that these enzymes, capable to evocate the production of ceramide at the PM-level, could represent new potential therapeutic targets for PC.

P12-017
Dyslipidemia pattern induced by the chronic inflammation in the rheumatoid arthritis

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The current studies on rheumatic pathology propose different dyslipidemic patterns. The relationship between the inflammatory syndrome, dyslipidemia and the risk for developing cardiovascular diseases is significant, therefore, a dyslipidemia pattern is needed to be described in patients with rheumatoid arthritis (RA). Certain research works consider the hypothesis that in RA there is an inverse association between the inflammatory markers and the lipid markers: elevated inflammatory markers are significantly associated with the high risk of cardiovascular disease (Johnsson and col., 2013), while in other studies, the reduced concentrations of total and LDL-cholesterol are paradoxically associated with an increased risk of cardiovascular diseases (Myasoedova and col., 2011).

Our research tries to determine the lipid pattern and its relation with the inflammatory syndrome in the patients with rheumatoid arthritis, analyzing the total cholesterol (TC), HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C), fibrinogen and calculating the atherogenic risk (AR = Total cholesterol/HDL-cholesterol).

The lipid profile of rheumatoid arthritis is similar with the one described in other studies, with increased concentration of TC and LDL-C and an unfavorable atherogenic risk. There are negative correlations between fibrinogen and TC, LDL-C, AR: FIB-CT ($r = -0.500$, $p = 0.018 < 0.05$), FIB-LDL ($r = -0.544$, $p = 0.009 < 0.01$), FIB-AR ($r = -0.481$, $p = 0.023 < 0.05$, confirming the hypothesis that the lipid parameters increase as the fibrinogen values decrease.

P12-018
Molecular detection of linoleate isomerase gene in lactic acid bacteria and associated CLA production

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Conjugated linoleic acid (CLA) isomers are bioactive lipids with potentially relevant benefits to human health. They are produced by certain bacteria present in foods that convert linoleic acid into CLA by the action of linoleate isomerase (LAI). The common strategy to screen CLA production in bacterial strains is focused

on the quantification of production, being a time-consuming and laborious work. The aim of the study was to evaluate the potential of utilising a molecular method to detect the presence of the LAI gene and to confirm the enzyme activity by the extension of use of linoleic acid (LA).

DNA was extracted from a panel of 12 *Lactobacillus* spp. strains. Polymerase chain reaction (PCR) and real-time PCR techniques were used to detect the presence of the LAI gene using specific primers. The specificity of qPCR amplifications was confirmed by analysis of the melting profile. In addition, the concentration of fatty acids was determined in the supernatant by gas chromatography, after aerobic incubation of the selected strains for 24 h and 48 h at 37 °C in MRS medium.

The presence of LAI gene was detected in *Lactobacillus plantarum* D36, *Lactobacillus plantarum* 229V and *Lactobacillus brevis* D24. For these strains, the substrate was reduced in more than 50% after bacterial growth when compared to the control. In contrast, LA was not significantly reduced in the strains lacking the LAI gene.

In conclusion, molecular detection of genes coding for the LAI allow a fast and cheap methodology for potential CLA producing strains.

P12-019
Effect of analysis delay on vitamin D measurement by liquid-chromatography mass spectrometry

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Background: Vitamin D plays a major role in calcium and bone metabolism and is an essential (pre)hormone involved in cell maturation and proliferation. Although vitamin D is considered as a relatively stable analyte, effect of preanalytical conditions and stability of vitamin D in serum and plasma needs to be identified clearly due to the limitations of previous studies. Our aim was to determine the effect of analysis delay on vitamin D measurement by LC-MS/MS.

Methods: Blood samples were collected and serum was seperated with centrifugation from 84 patients. 100 μL internal standard and 1000 μL acetonitrile were added to 250 μL of serum for protein precipitation, vortexed for a minute and centrifuged at 13,000 rpm for 10 min. 40 μL of supernatant was injected into HPLC analytical column for chromatography. Mass spectrometric analyses were performed using an Shimadzu LC-20-AD (Kyoto, Japan) coupled with a ABSCIEX API 3200 triple quadrupole mass spectrometer (USA) equipped with an atmospheric pressure chemical ionisation (APCI) operating in positive mode. The supernatant was stored at 2–8°C and analyzed again after 48 h to determine the effect of analysis delay.

Results: The mean values for 0.hour and 48.hour vitamin D measurements were 19.8 ± 9.86 and19.6 ± 11.4, respectively ($p = 0.425$).According to paired t-test, there was no statistically significant difference between 0.hour and 48.hour vitamin D measurements ($p = 0.083$).

Conclusion: Our findings regarding analyte stability after delayed processing demonstrated that serum vitamin D is a stable molecule up to 48 h after pretreatment.

P12-020
Fructose feeding alters fatty acid profile in offspring exposed to excess folic acid during the perigestational period

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Our group has previously demonstrated that perigestational high folic acid (HFA) exposure predisposes female offspring to an insulin-resistant state and renders them more susceptible to develop metabolic dysfunction in adulthood after a metabolic challenge with fructose feeding (1). This work aims to characterize the liver fatty acid profile in adult female offspring previously exposed to perigestational HFA and the effect of fructose feeding on it. Thus, Sprague-Dawley females were administered a dose of folic acid recommended for pregnancy (C, 2 mg FA/kg of diet) or a HFA dose (40 mg FA/kg of diet) which began at mating and stopped only at weaning. Female progeny were divided at 10 months of age into a group fed the standard rat diet (C/Std and HFA/Std) and a group fed 10% fructose in the drinking water plus the standard rat diet (C/Fru and HFA/Fru). Fructose feeding lasted from 10 to 13 months of age, when the animals were sacrificed for tissue collection.

We observed that females receiving fructose and particularly those born from HFA exposed mothers (HFA/Fru group) had a significant increase in the desaturase activity, measured as C16:1c9/C16 and C18:1c9/C18 ratios. The corresponding increments in the concentration of C16:1c9, a lipokine involved in insulin homeostasis, and C18 : 1c9, involved in membrane fluidity, suggest that a metabolic insult such as fructose induces in HFA exposed animals, a compensatory response in an attempt to alleviate the metabolic dysfunction provoked by HFA exposure and fructose feeding.

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P12-021
Activation of gelatinases plays a key role in ceramide 1-phosphate-induced macrophage migration

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The bioactive sphingolipid ceramide 1-phosphate (C1P) is implicated in inflammatory responses and was recently shown to promote cell migration. However, the mechanisms involved in cell migration are poorly described. Here we show that C1P stimulates the activity of the matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9) in J774A.1 macrophages. When looking into the mechanisms involved in this action we observed that C1P potently stimulates the phosphatidylinositol 3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MEK)/extracellularly regulated kinases (ERK) pathways in the macrophages, suggesting a possible involvement of these pathways in the acti-

vation of MMP-2 and MMP-9. Noteworthy, inhibition of these kinases with specific siRNA blocked C1P-stimulated MMP-2 and MMP-9 activity. Moreover, inhibition of MMP-2 and MMP-9 with selective inhibitors or treatment with specific siRNA potently decreased C1P-stimulated cell migration. Hence, it can be concluded that C1P promotes MMP-9 and MMP-2 activation, which act as mediators of C1P-stimulated cell migration. In addition, PI3K/Akt and MEK/ERK are important downstream effectors of this action.

P12-022
The effect of temperature on LPS-induced inflammatory cytokine production

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Lipopolysaccharide (LPS), which consists of hydrophobic lipid A and hydrophilic polysaccharides, is a major component of the outer membrane of Gram-negative bacteria. LPS acts as endotoxins and induces pro-inflammatory cytokines, including TNF-α and IL-1β. Although it has been reported that high temperature (e. g. febrile-rage temperature; 40°C) modulates inflammatory cytokine production in LPS-stimulated macrophages, little is known about the effect of low temperature on macrophage inflammatory cytokine production. In this study, we investigated the influence of low temperature on TNF-α production in LPS-stimulated J774.1 macrophages during incubation at 32 and 37°C. The release of TNF-α in culture medium was attenuated during culture at 32°C as compared with 37°C cell cultures, which caused by the down regulation of TNF-α mRNA expression. We then examined the effect of low temperature on nuclear translocation of NF-κB by LPS-stimulation. Western blot analysis revealed that NF-κB p65 in the nucleus was reduced in 32°C cells compared with 37°C cells. Furthermore, we investigated NF-κB transcriptional activities of 32°C cells and 37°C cells. In a luciferase reporter gene experiment, the 32°C cells showed decreased NF-κB transcriptional activity. These results suggest that low temperature significantly reduces LPS-induced TNF-α production by attenuating NF-κB signaling pathway.

P12-023
Effect of palmitoleic acid on the inflammatory phase of wound healing

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Introduction: A common practice in the popular cultures is to treat wounds with fatty acids. However, the mechanisms of action of these substances are unclear.
Aims: This study involved an investigation of the effects of palmitoleic acid on the inflammatory phase of the healing process.
Methods: *In vivo:* Rats were subjected to full-thickness punch biopsies on the left dorsal. Palmitoleic acid was administered topically until wound closure occurred. We analyzed the tissue by measuring the concentrations of pro-inflammatory cytokines, and VEGF prior to, immediately after, 24 h, 48 h, 72 h and 120 h after treatment. *In vitro*: The effect of palmitoleic acid on cytokine production and migration was investigated, respectively, by means of ELISA (R&D Systems, Minneapolis, USA) and chemotaxis plates (Neuroprobe, Gaithersburg, MD).
Results: The closing speed of the wounds in the treated group ($n = 10$, $p < 0.05$) was 2-folder faster. It was found that palmitoleic acid can lower the concentrations of TNF-α, IL-1β, IL-6, 1β, CINC-2α/β, MIP-1α1β and VEGF-α in the wound in the different phases of the healing process by acting as anti-inflammatory agent. In the *in vivo* assays, palmitoleic acid showed potent anti-inflammatory activity, inhibiting the release of LPS-induced TNF-α ($p < 0.05$), IL-1β ($p < 0.001$), l-selectin ($p < 0.05$), IL-61β ($p < 0.001$), CINC-2α/β, and MIP-1α ($p < 0.01$) by LPS-stimulated neutrophils.
Conclusion: The anti-inflammatory effect of palmitoleic acid accelerates wound healing. It should be noted, however, that the findings of this study do not completely elucidate the mechanisms of action of palmitoleic acid.

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P12-024
Effects of EPA and DHA on the HaCaT keratinocyte cell line

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Introduction: Keratinocytes form the outer layers of the epidermis and are important for maintaining skin integrity, participating in the immune response and healing. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) show important immunomodulatory activity.
Aims: Our purpose was to investigate the effect of EPA and DHA on the HaCaT keratinocyte cell line.
Methods: The effect of EPA and DHA (5–200 μM) on the production of cytokines by keratinocytes was investigated by ELISA. DNA fragmentation and cell membrane integrity tests were performed using flow cytometry.
Results: EPA and DHA were not toxic to HaCaT cells. EPA inhibits the release of IL-1β in a dose-dependent manner ($r = 0.94$ without LPS and $r = 0.96$ with LPS), resulting in 56% inhibition at 200 μM. EPA also inhibits the release of TNF-α by 35% at a concentration of 100 μM, by 43% at 150 μM, and by 39% at 200 μM, and of IL-8 by 72% at a concentration of 200 μM. Keratinocytes treated with DHA show a dose-dependent inhibition of IL-6 ($r = 0.99$ without LPS and $r = 0.94$ with LPS) of 88% and of IL-8 ($r = 0.95$ without LPS and $r = 0.98$ with LPS) of 61%. We also noted 53% and 46% inhibition of the release of TNF-α and L-selectin, respectively, when keratinocytes were treated with 200 μM of DHA.
Conclusion: EPA and DHA can modulate keratinocyte functions through anti-inflammatory action. Further studies to elucidate the mechanisms involved in these effects will allow for the development of drugs that can resolve abnormal wound healing processes.

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P12-025
High-throughput SAXS analysis of lipidic mesophases for structural studies of membrane proteins

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Crystallization in membrane-mimicking mesophases, such as Lipidic Cubic Phase (LCP), has proven highly successful for structural studies of challenging membrane proteins [1]. The choice of lipids available for this application is however limited. In this work, twelve *de-novo* synthesized lipids were investigated by the high-throughput small-angle X-Ray scattering (HT-SAXS) method [2]. Lipids that form LCP in a wide range of conditions, including commercial and home-made crystallization screens in the temperature range 4–40°C, have been identified. An automated analysis of SAXS curves was used to classify lipidic mesophases and determine their lattice parameters. For the lipids that formed LCP the most common occurrence was a cubic-Pn3 m phase with the lattice parameters ranging between 80 and 100 Å. Crystals of bacteriorhodopsin were obtained using one of these lipids validating their compatibility with membrane protein crystallization. Data obtained in this work will help for expand the arsenal of lipids and screens for structural studies of membrane proteins.

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P12-026
Characterization of AnNce102 and its role in eisosome stability and sphingolipid biosynthesis

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Plasma membrane organization in *Saccharomyces cerevisiae* is at least in part mediated by the tetraspan Nce102 protein and a subcortical stable structure termed eisosome. Homologues of *S. cerevisiae* eisosomal proteins are present in the filamentous fungus *Aspergillus nidulans* and are designated PilA, PilB and SurG. In conidiospores and ascospores, the three proteins colocalize at cell cortex forming stable tightly packed structures that are excluded from membrane regions of cell growth. In the present study we report that AnNce102 colocalizes with eisosomes and plays a crucial role in the stability and the number of PilA/SurG foci in the head of germlings derived from conidiospores. In addition, absence of AnNce102 results in mislocalization of PilA foci in septae, regions of cell growth. Finally, we report that PilA and AnNce102 proteins genetically interact with the YpkA kinase and are involved in a temperature-dependent regulation of sphingolipid biosynthesis.

P12-027
The polyphenol curcumin mitigates lysosomal cholesterol traffic impairment *in vitro* by promoting exosomes secretion

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Introduction: Exosomes are originated from multivesicular bodies and contribute to the secretion of endolysosome components out of the cell. Curcumin, the main polyphenol extracted from the rhizome of *Curcuma longa,* has been shown to alleviate the accumulation of different lipids in the endocytic compartment.
Objectives: To investigate the effects of curcumin on exosomes secretion in different type cells treated with U18666A to block the intracellular cholesterol trafficking.
Methods: HepG2 hepatocarcinoma and THP-1 macrohage cells were used. Exosomes were isolated and then analyzed by western-blot, electron microscopy and bead fluorescence-activated sorting. Other methods used were immunocytochemistry, DiI-LDL uptake by flow cytometry, cholesterol efflux assays and sterol analysis by GC-MS.
Results: In both cell lines, curcumin affected the size and localization of endosome/lysosomes accumulated by effect of U18666A, and reduced the cholesterol cell content. Concomitantly, curcumin stimulated the release of cholesterol and the lysosomal β-hexosaminidase enzyme, as well as the exosome markers, flotillin-2 and CD63, out to the cell. Electron microscopy studies demonstrated the presence of small vesicles similar to exosomes in the secretion fluid. These vesicles harbored CD63 on their surface, indicative of their endolysosomal origin. These effects of curcumin were particularly intense in cells treated with U18666A.
Conclusion: These findings indicate that curcumin ameliorates the U18666A-induced endolysosomal cholesterol accumulation by shuttling cholesterol and presumably other lipids out of the cell via exosomes secretion. This action may contribute to the potential of curcumin in the treatment of lysosomal storage diseases.
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P12-028
Small-angle scattering studies of phospholipids phase transition in membrane mimicking systems

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This paper shows the results of studying the lipidic phase transition in membrane mimicking systems. Temperature changes, lattice parameters, thicknesses and geometrical sizes of multilamellar and unilamellar vesicles and nanodiscs (ND) prepared from phospholipids (DMPC, DPPC) and membrane scaffold proteins (MSP1, MSP1E3D1) respectively, were measured by SAXS and SANS methods using installations YuMO (JINR, Dubna, Russia) and Rigaku (MIPT, Dolgoprudny, Russia).

It was found that the phase transition occurs at 23.5 ± 1.0°C and 42.0 ± 1.0°C for DMPC and DPPC vesicuves respectively, as in literature.

In [1,2] for DMPC/MSP1E3D1 ND in the range 22–26°C there have not been any changes over the statistic errors for the radii of gyration.

In this work the temperature range was extended at 22–35°C for DMPC and for DPPC the SAS curves were obtained at 38–50°C. It is discovered that the ND structural phase transition takes place and occurs at temperatures higher than the phase transition temperature for vesicles. However, the temperatures of ND structural phase transition differ from the result [3].

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P12-029
Regulation of astrogliocytes functions by *trans*-2-hexadecenal

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Trans-2-hexadecenal (Hex) is formed from sphingosine-1-phosphate under the action of enzyme sphingosine-1-phosphate lyase. In our earlier studies we have shown that hypochlorous acid (HOCl) formed in the halogenating cycle of myeloperoxidase causes destruction of sphingolipids with the formation of Hex. The treatment of astrogliocytes with NaOCl solution also leads to Hex formation.

It is known that Hex is bioactive signaling molecule and induces apoptosis through cytoskeletal reorganization in JNK-dependent manner in a number of human and mouse cells, such as HEK293T, HeLa, NIH3T3. It can be assumed, that in cases when the level of HOCl is significantly increased in the brain (stroke, cranial injury, tumors) the Hex formation from brain sphingolipids can occur.

In order to establish the biological role of Hex in brain, its influence on functions of astrogliocytes (glioma cells C6) was studied. It has been shown that co-cultivation of C6 cells with Hex in concentrations of 0.01–10 μmol/l during 36 h, reduces proliferative activity by 20–40%. At the same time the increase in the redox activity of cells in Hex concentrations up to 0.1 μmol/l was observed. In this case the yields of superoxide anion radicals and hydrogen peroxide are increased.

It was found that Hex modifies the redox state of the cells by influencing intracellular signaling processes involving p38-, JNK MAPK and transcription factor nFκB. Action of Hex on astrogliocytes causes reorganization of the actin cytoskeleton that is shown in modification of cells shape and reducing the amount of filopodia, the redistribution of F-actin.

P12-030
Sphingolipid destruction in HOCl-treated red blood cells

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Interest in blood sphingolipids has been broadened by the development and clinical application of the immunosuppressive drug fingolimod, which targets sphingosine-1-phosphate receptors resulting in lymphocyte sequestration. Not much is known about the metabolism of sphingolipid breakdown products. For example, sphingosine-1-phosphate lyase catalyses the final step of sphingolipid degradation, yielding 2-hexadecenal and phosphoethanolamine. It has been shown that 2-hexadecenal possesses a wide spectrum of biological activity. Furthermore, in our earlier studies has been established that the action of gamma-, UV-irradiation and HOCl on aqueous sphingolipids dispersions was accompanied by the formation of 2-hexadecenal.

In this study, new data have been obtained on the formation of 2-hexadecenal in human red blood cells which contain sphingolipids under the exposure to HOCl, which is generated in many cells in the course of MPO-dependent reactions. Blood of healthy volunteer was collected to heparin tubes and centrifuged to obtain erythrocytes and plasma. The reaction of erythrocytes with a freshly prepared NaOCl solution was performed at room temperature, in short time while stirring. To separate the aldehyde from the biological samples, we used extraction procedure. Analysis of the effect of HOCl on extract of blood erythrocytes indicates 2-hexadecenal to be formed. Numerous studies have shown that an increased production of HOCl in a living organism leads to the development of inflammatory processes and cardiovascular diseases, in particular atherosclerosis.

Therefore, it can be assumed the existence of a link between the HOCl-induced formation of bioactive 2-hexadecenal from sphingolipids and the development of a number of pathological processes.

P12-031
Functional analysis of GPI transamidase with molecular phylogenetic tree

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Glycosyl-phosphatidyl-inositol (GPI) is one of the major post-translational modifications. GPI-anchored proteins (GPI-APs) play essential roles in living cells including immunity, signaling

regulation and cell adhesion. In the Endoplasmic Reticulum (ER), GPI-attachment signals are recognized and separated by GPI transamidases (GPI modification enzymes). GPI transamidase is known as a protein complex consisting of GPI8, GAA1, PIG-S, PIG-T and PIG-U. According to the previous research, the GPI-attachment signals are separated by the GPI8 domain. However, the GPI-attachment signal recognition and separation mechanisms of the GPI transamidase have not been clarified. Therefore, to understand the molecular mechanisms, the molecular phylogenetics analysis of the GPI transamidase orthologs was performed in this study.

The dataset of GPI transamidase was extracted from the UniProt KB/Swiss-Prot protein sequence database Release 2014_11. Molecular phylogenetic tree of GPI transamidase complex was created by the neighborhood-joining method. Based on these result, GPI-APs were considered to interact with the high preservation regions of GPI transamidases.

P12-032
SREBP-2 upregulates PNPLA8 expression to increase autophagy

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Sterol regulatory element-binding proteins (SREBPs) are key transcriptional regulators of lipid metabolism. Previously we reported genome-wide ChIP-seq with isoform-specific antibodies and chromatin from select tissues of mice challenged with different dietary conditions that enrich for specific SREBPs. In this study, we show that SREBPs directly up-regulate expression of PNPLA8, an enzyme that catalyzes the hydrolysis of the sn-2 position of glycerophospholipids, in the livers from mice fed a chow diet supplemented with lovastatin plus ezitimibe (L/E) and prostate cell line. The five fold increase in PNPLA8 mRNA mediated by L/E treatment was accompanied by the induction of SREBP-2 binding to the PNPLA8 promoter detected by a ChIP assay in liver. We identified a SREBP responsive region, E-box region, CTCGAG within the first 300 upstream bases of both human and mouse PNPLA8 promoter. PNPLA8 protein was also induced in cells over-expressing SREBP-2 in culture. We also found that lipid droplets were consumed upon induction of PNPLA8 by statin treatment, which leads to accumulate autophagy puncta. The induction of PNPLA8 through SREBP-2 provides a mechanistic explanation for why statin therapy may be effective in reducing cholesterol levels in treating hypercholesterolemia.

P12-034
Impact of drying processes on the fatty acid composition of *Chlorella vulgaris*

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Healthy fatty acids (FA) composition, namely, polyunsaturated fatty acids (PUFA), is one of the most interesting characteristics of microalgae in the development of new functional food products. In order to be more easily incorporated into different formulations, drying of the microalgae biomass can be performed. Freeze-drying is the most widely utilized methodology for drying microorganisms while spray-drying can be a faster and less expensive solution. The aim of this research work was to evaluate the effects of these two drying techniques on the yield and FA quantitative and qualitative profiles of harvested *Chlorella vulgaris* biomass. Results showed that the yield of *C. vulgaris* biomass powder was of almost 100% when obtained by freeze-drying, and only 40% when spray-drying was employed. Although the highest total FA concentration was found in the freeze-dried *C. vulgaris* powders, some FA were present in higher amounts in the equivalent spray-dried powders. However the most important FA compounds for human nutrition such as C18 : 2 c9c12 (linoleic acid), C18 : 3 c9c12c15 (α-linolenic acid) and C22 : 5 n3 (docosapentaenoic acid; DPA) were higher in the freeze-dried biomass powders: e.g. DPA concentration was two-fold higher in the freeze-dried powder than in the spray-dried counterpart. According to the results from this research, when PUFA content is concerned, freeze-drying is the best method to obtain algae powders from *C. vulgaris*.

P12-035
Human steroid sulfation pathways – a biochemical perspective

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Sulfation pathways are an integral part of biochemistry; vital for blood homeostasis, bone and cartilage development, biotransformation of xenobiotics as well as cell-cell and cell-virus interaction. All human sulfation pathways require active sulfate in form of 3'-phospho-adenosine-5'-phosphosulfate (PAPS) and PAPS is exclusively provided by the two bi-functional PAPS synthases PAPSS1 and PAPSS2. As PAPS biosynthesis is energetically highly costly, PAPS synthases are subject to tight regulation at various levels; involving regulated nucleo-cytoplasmic shuttling [1] as well as stabilisation of fragile PAPS synthase proteins [2] by the atypical nucleotide adenosine-5'-phosphosulfate, an intermediate of PAPS biosynthesis [3]. Genetic defects in PAPS synthases point to an important role of sulfation also in the regulation of steroid hormone action [4]. Here we set out to analyse the differential impact of PAPS synthase isoforms on the sulfation of the steroid androgen precursor dehydroepiandrosterone using siRNA knockdown technology, protein-protein docking as well as co-localisation analysis. We provide first evidence for an isoform-specific interaction of SULT2A1 with PAPSS2, but not PAPSS1, that may be the underlying mechanism explaining why PAPSS2 deficiency results in disruption of DHEA sulfation despite ubiquitous expression of PAPSS1.

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P12-036
The membrane skeleton in lymphocyte activation – Molecular control of receptor signalling

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The cytoskeleton is a dynamic structure responsible for innumerable cellular events, including both housekeeping functions and highly specialized cell-type specific tasks of multicellular organisms. One highly differentiated biological system is adaptive immunity, composed of B and T lymphocytes that play a critical role in the fight against infections and malignancies. We investigate the fundamental role of the cytoskeleton and the functional unit it forms with the plasma membrane, called membrane skeleton, during immune response and B cell activation. Our recent published studies show that the underlying actin cytoskeleton regulates B cell receptor organization and function to the extent that signalling can occur in a complete absence of a ligand, solely upon alteration of the cytoskeleton. The molecular mechanisms of this regulation remain completely unknown. Here, we follow this up by examining the relationships of various membrane domains, B cell receptor behaviour and the underlying actin cytoskeleton to uncover the molecular principles regulating receptor activation. We apply interdisciplinary approaches, including advanced super-resolution microscopic techniques, such as single particle tracking, to gain fundamental novel understanding on the molecular mechanisms controlling receptor signalling.

P12-037
Phospholipases A_2: from membrane remodeling to the involvement in sperm motility

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One of the major causes of male infertility is asthenozoospermia which, in particularly severe cases, may even influence the pregnancy success rates following assisted reproductive techniques. PLA_2 activity in human seminal plasma is closely related to male fertility; PLA_2 deficiency in the head of spermatozoa may be one of the reasons causing male infertility. Significant differences in cytosolic PLA_2 (c-PLA_2) and its phosphorylated/activated form, calcium- independent (i-PLA_2), and secreted PLA (s-PLA_2) content and distribution in human normal and asthenozoospermic patients were evaluated. Human healthy and asthenozoospermic spermatozoa were purified, fixed for SEM, immunostained for PLA_2s, lysed for specific activity evaluations and immunoblotting. cPLA_2 was localized in heads, midpieces and tails of all spermatozoa, less expressed in the tail of asthenozoosperms. While active phospho-cPLA_2 distribution was homogeneous throughout the cell body of control-donor spermatozoa, lower levels were detected in the tails of asthenozoospermic patients, as opposed to its strong presence in heads. Low immunofluorescence signal for iPLA_2 was found in astenozoosperm, whereas sPLA_2 was significantly lower in the heads of asthenozoospermic patients. Spermatozoa with low progressive motility showed differences both in terms of total specific activity and of intracellular distribution. cPLA_2, iPLA_2 and sPLA_2 specific activities correlated positively and in a statistical manner with sperm progressive mobility both in normozoospermic men and in infertile asthenozoospermic patients. PLA_2s were expressed in different areas of human spermatozoa. Spermatozoa with low motility showed differences in total specific activity and enzyme distributions. We speculated that PLA_2 isoforms may be a potential therapeutic target for asthenozoospermia.

P12-038
The P4-ATPase TAT-5 inhibits the outward budding of the plasma membrane in *C. elegans* embryos

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Extracellular vesicles capable of intercellular signaling form by the outward budding of the plasma membrane, but the molecules that regulate budding are poorly understood. In extracellular vesicles, the outer leaflet of the membrane bilayer contains phospholipids such as phosphatidylethanolamine that are normally sequestered to the inner leaflet, suggesting a role for lipid asymmetry in ECV budding.

We show that loss of the conserved P4-ATPase TAT-5 causes the large-scale shedding of extracellular vesicles in *C. elegans* embryos. TAT-5 flippase activity is required to prevent plasma membrane budding, because the dephosphorylation mutant E246Q is unable to rescue excess budding in *tat-5* mutants. TAT-5 localizes to the plasma membrane, in contrast to its yeast homolog Neo1p that localizes to late Golgi and endosomes. Plasma membrane localization depends on the catalytic Aspartate, because the D439E mutant localizes to the endoplasmic reticulum. In contrast, the Cdc50 family of proteins is not required for TAT-5 localization or activity. Loss of TAT-5 results in phosphatidylethanolamine exposure on the cell surface, with no effect on phosphatidylserine asymmetry. TAT-5 has at least one conserved cofactor involved in budding, the Dop1p homolog PAD-1. Loss of TAT-5 also recruits membrane-sculpting proteins to the plasma membrane, such as the ESCRT complex, which regulates viral budding.

TAT-5 provides a molecular link between loss of phosphatidylethanolamine asymmetry and the dynamic budding of vesicles from the plasma membrane, supporting the hypothesis that lipid asymmetry regulates budding. The *C. elegans* embryo is an excellent genetic model system for studying the function of essential P4-ATPases *in vivo*.

P12-039
Novel aspects of the contribution by the lipid A acyl groups to Toll-like receptor 4 activation by lipopolysaccharide

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Agonistic and antagonistic LPS from *E. coli* are the most studied TLR4 ligands, however natural TLR4 ligands are definitely more divergent. We investigated biological activity of LPS from several pathogenic bacteria: *B. mallei*, *Y. pestis*, *P. aeruginosa*, *A. baumannii* and ancient *Psychrobacter* spp. Each LPS was purified by hydrophobic chromatography and chemical structure of lipid A was determined using HR-ESI or MALDI-TOF mass spectrometry. The biological activity of LPS preparations was evaluated by their ability to activate production of proinflammatory cytokines by bone marrow-derived macrophages (BMDM) from C57BL/6 mice, using TLR4-deficient BMDM as specificity control.

The lipid A structures of *A. baumannii* and *Psychrobacter* spp. were characterized by a high content of hexa- and hepta-acyl forms. The acyl chains had 12–14 carbons (C12-C14) in *A. baumannii* and C10-C12 in *Psychrobacter* spp. Lipid A of *P. aeruginosa* also had short acyl groups (C10-C12) but the degree of acylation was lower. Lipid A from *B. mallei* had a low number of acyl chains as well, however they were on the average longer (C14-C16). Wild-type and mutant *Y. pestis* strains had a more diverse lipid A repertoire of all.

The number and the length of the lipid A acyl groups correlated directly with the ability of LPS preparations to activate TNF and IL-6 production increased with the number of acyl chains and their length. Evaluation of contribution of other lipid A functional groups in progress.

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P12-040
Examination of the role of the Sphingosine Kinases in Polymicrobial Sepsis

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As sepsis remains one of the leading causes of mortality in the hospital environment, understanding its molecular mechanisms is of vital importance. Previously thought to function solely as cell membrane constituents, the current literature identifies sphingolipids as important modulators of cellular pathways. Foremost among the members of this lipid subclass is sphingosine 1-phosphate (S1P). Produced via the phosphorylation of sphingosine by sphingosine kinases (SphK1/2), S1P activates five G protein-coupled receptors ($S1P_{1-5}$) to mediate numerous physiological and pathophysiological processes.

Using the peritoneal contamination and infection model (PCI) of sepsis in SphK1/2 knock-out mice, we examined the role of S1P and more directly the roles of the SphK1/2 in polymicrobial sepsis. Ongoing experiments indicate marked reductions in plasma cytokine levels at 6 h post infection in $SphK1^{-/-}$ and at 24 h post infection in $SphK2^{-/-}$ as compared to their wild type counterparts indicating time dependent effects. Using *in vitro* and *ex vivo* models, we are currently examining the role of SphKs in different immune cell subsets (macrophages, neutrophils, T-/B-cells, endothelial cells, and dendritic cells) and their individual roles in mediating cytokine responses using an LPS model. Our current data indicate a minimal role of SphK on macrophage response in bone marrow derived macrophages under cell culture conditions. Examination into the remaining subsets is ongoing.

Together our current data indicate a role of SphKs in mediating immune responses in polymicrobial sepsis. Further analysis is under way to elucidate their exact actions and underlying mechanisms.

P12-042
The deletion of glycopeptidolipid in *Mycobacterium smegmatis* J15cs strain affects morphology and survival in host cells

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Mycobacterium smegmatis is a rapidly growing, nonpathogenic mycobacterium, and *M. smegmatis* strain mc^2155 in particular has been used as a tool for molecular analysis of mycobacteria because of its high rate of transformation. Unlike the *M. smegmatis* mc^2155 strain, *M. smegmatis* J15cs strain has the advantage of surviving for one week in murine macrophages. We clarified that the J15cs strain has deleted apolar glycopeptidolipids (GPLs) in the cell wall. The apolar GPLs were recognized by macrophages via toll-like receptor 2, but not 4. The gene causing the GPL deletion in the J15cs strain was identified. The *mps1-2* gene (MSMEG_0400-0402) correlated with GPL biosynthesis. The J15cs strain had 18 bps deleted in the *mps1* gene compared to that of the mc^2155 strain. The *mps1*-complemented J15cs mutant restored the expression of GPLs. Although the J15cs strain produces a rough and dry colony, the colony morphology of this *mps1*-complement was smooth like the mc^2155 strain. The length in the *mps1*-complemented J15cs mutant was shortened by the expression of GPLs. In addition, the GPL-restored J15cs mutant did not survive as long as the parent J15cs strain in the murine macrophage cell line J774.1 cells. The results are direct evidence that the deletion of GPLs in the J15cs strain affects bacterial size, morphology, and survival in host cells.

P12-043
The antioxidant effect of boric acid in chronic alcohol abuse

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Alcohol is a toxin that causes serious damage on many organs depending on the dose and duration of use. Chronic alcohol consumption is the most important factor that leads to cirrhosis and liver failure. In this study, oxidative stress that is generated due to chronic alcohol intake and the protective effect of boric acid was evaluated. Experimental animals were divided into four groups: Control, alcohol, alcohol + boric acid and boric acid. The levels of alcohol-induced oxidative stress indicators [malondialdehyde (MDA), total sialic acid (TSA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] were measured in liver tissues. While the MDA and TSA levels increased significantly in the alcohol group compared to the control group ($p < 0.05$, $p < 0.01$), that of the alcohol + boric acid group decreased significantly compared to the alcohol group ($p < 0.05$, $p < 0.001$). The TSA level was significantly low in the boric acid group as compared to the alcohol group ($p < 0.01$). In the alcohol group, SOD and GPx activities were significantly lowered ($p < 0.01$, $p < 0.001$), while there was an increase in that of the alcohol + boric acid group compared to the alcohol group ($p < 0.05$, $p < 0.05$). SOD and GPx activities increased significantly in the boric acid group compared to the alcohol group ($p < 0.05$, $p < 0.001$). There was no significant difference between the groups in CAT activity. Consequently, these results show that alcohol triggers membrane damage on liver and boric acid can act to increase the antioxidant mechanisms against alcohol-induced oxidative stress.

P12-044
A phosphoinositide conversion mechanism for exit from endosomes

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Phosphoinositides (PIs) are a minor class of comparably short-lived membrane phospholipids that serve crucial functions in cell physiology ranging from cell signalling and motility to their role as signposts of compartmental membrane identity [1-4]. PI 4-phosphates such as PI(4)P and phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] are concentrated at the plasma membrane and on secretory organelles such as the Golgi complex, exocytic vesicles [5], and on lysosomes [6], whereas PI 3-phosphates, most notably PI(3)P [7] are a hallmark of the endosomal system [1-4]. Directional membrane traffic between endosomal and secretory compartments therefore requires regulated PI conversion by PI metabolizing enzymes. The molecular mechanism underlying this conversion of PI identity during cargo exit from endosomes by exocytosis, a process that mediates surface delivery of proteins and lipids, is unknown.

P12-045
The autocrine/paracrine action of bone-marrow-derived mesenchymal stromal cells required SphK1/S1P/S1P1 axis

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Bone-marrow-derived mesenchymal stromal cells (MSCs) have the potential to significantly contribute to tissue healing through the secretion of paracrine factors that support proliferation and enhance participation of the endogenous stem cells in the process of repair/regeneration. However, the factors that can regulate the autocrine/paracrine action of MSCs have been poorly investigated. The aim of this study was to characterize the involvement of bioactive sphingolipids on the autocrine/paracrine signaling of MSCs. By using a biochemical and morphological approach, it was found, that sphingosine 1-phosphate (S1P), a natural bioactive lipid exerting a broad range of cell responses in many physiological and pathological conditions. For example, conditioned medium obtained from MSCs cultured in the presence of the selective sphingosine kinase inhibitor (iSK), blocked increased muscle cell proliferation caused by MSC-conditioned medium, and the addition of exogenous S1P in the conditioned medium from MSCs pre-treated with iSK further increased myoblast proliferation. We will also present data concerning the ability of S1P generation/release as well as the activation of S1P1 receptor in the regulation of MSC autocrine/paracrine functions and the important implications of these bioactive sphingoid in the optimization of cell-based strategies to promote tissue regeneration, prevent cell degeneration and influence cell engraftment.

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P12-046
The role of intra-membrane sensing in controlling membrane homeostasis

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The composition of a biological membrane is extremely complex and the mechanisms underlying its homeostasis are poorly understood. We are interested in how cellular membranes communicate their composition and maintain their identity. In the past two years we have been focusing on establishing interdisciplinary pipelines to study intra-membrane sensing processes. Using yeast genetics, reconstituted *in vitro* systems with purified components, and MD simulations we aim at a better understanding of how eukaryotic cells sense and control their content of saturated lipids. The identical strategy has been applied to the unfolded protein response and provides intriguing insights into how this transcriptional program is controlled by the lipid environment.

P12-048
The *Tm7sf2* gene deficiency protects mice against endotoxin-induced acute kidney injury

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Cholesterol is essential for diverse cellular functions. Cellular and whole-body cholesterol homeostasis is highly controlled and maintained through a network of transcriptional programs. Cholesterol can also influence cellular susceptibility to injury. The connection between cholesterol metabolism and inflammation is exemplified by the *Tm7sf2* gene, the absence of which results in an inflammatory phenotype, i.e. NF-κB activation and TNFα up-regulation[1]. In mouse skin, the loss of *Tm7sf2* gene reduce cholesterol/cholesterol sulfate rate of synthesis following inflammatory insults and increases the susceptibility to skin papillomas formation by classical two-stage skin carcinogenesis protocol[2]. Here, by using $Tm7sf2^{+/+}$and $Tm7sf2^{-/-}$ mice[3], we investigated whether the *Tm7sf2* gene, through its role in the inflammatory response, is involved in the renal failure induced by the administration of LPS. We found that the loss of *Tm7sf2* gene results in significantly reduced blood urea nitrogen levels accompanied by decreased renal inflammatory response and neutral lipid accumulation. At molecular level, we observed that the *Tm7sf2* insufficiency leads to FXR activation which, in turn, antagonizes NF-κB signaling/iNOS expression and SREBP-1c mediated lipogenesis, leading to a reduced renal damage. These results suggest a pivotal role for *Tm7sf2* in renal inflammatory and lipotoxic response under endotoxemic conditions.

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P15-007
Discovering a disease associated biomarker "ANXA4" by proteome profiling: moving toward an understanding of tumor progression

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We aimed to identify potential biomarker involved in HCC progression. We analyzed human clinical samples of HCC (n = 15) and fibrotic liver (n = 15) with respected control. A comparative proteomics approach was utilized to identify the differentially expressed proteins. Significantly altered liver proteins were identified by MS/MS in HCC relative to fibrotic liver and control. Of the identified, annexin A4 (ANXA4) is significantly implicated in HCC related to fibrotic liver. Annexins are implicated in several disease processes including tumor development and progression, its over expression and localization in the HCC patients is further validated by western blot and immunohistochemistry. After validated current finding, we applied *in silico* analysis to integrate the data generated from proteomics technologies. We extend this current understanding to demonstrate the significantly induced phosphorylation and S-nitrosylation signals by insilico study, suggesting the involvement of ANXA4 in cancer progression. Moreover, we revealed interacting partner of ANXA4 which include heat shock beta 1 protein (HSPB1) and beta actin like protein (ACTB). These interacting partners are bestowed with critical capabilities, namely apoptosis, cell cycling, anticoagulation, cell motility and stress resistance that together demonstrate their possible role in cancer progression. Overall, our results shed new light on the potential biomarker ANXA4 that are used for early diagnosis, prognosis prediction, and personalized treatment of HCC.

P15-008
Design, cytotoxicity and toxicity of new thiophene and thieno[2.3-*b*]pyridine derivatives

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Newer thiophene derivatives **3a–f** were synthesized by using the w-cyanoacetophenone derivatives **1a–c** with elemental sulfur and either of malononitrile or ethyl cyanoacetate . Compounds **3a**, **3c** and **3e** reacted with ethyl cyanoacetate to give the amido derivatives **4a–f** . Compounds **4a–f** underwent ready cyclization in sodium hydroxide to give thieno[2,3-b]pyridine derivatives **5a–f**. Compounds **5a–f** underwent [4 + 2] cycloaddtion to produce thequinoline derivatives **7a–f**. All the products were assayed for antitumor activity towards human cancer human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast (WI38) cell lines. Excellent antitumor activities were shown by compounds **3c**, **3d**, **4b**, **5b**, **8c**, **8d,** 9a, **9c**, **9d**, **11d** and **15d** where they exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range. Compounds7b and 14b showed the maximum inhibitory effect and these are much higher than the reference CHS 828.

P15-009
The apoptosis inducing effects of new flavanone derivatives in human prostate cancer cell lines

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Background: The goal of cancer therapy is to kill cancer cells. Many drugs currently used in anti-cancer therapy kills target cells by induction of apoptosis, which is characterized by a discrete set of biochemical steps and morphological changes. Apoptosis is characterized by morphological changes including cell shrinkage, membrane blebbing, chromatin condensation, and nuclear fragmentation. A family of cystein-dependent aspartate-directed proteases called caspases, are considered the engine of apoptosis. As well, inhibition of the poly (ADP-ribose) polymerase (PARP) protein, involved in multiple DNA repair pathways, may enhance cytotoxic chemotherapeutic agents

Methods: The growth inhibitory activities of two synthetic dichloroflavanone derivatives were evaluated for inducing mechanism of cell death in PC-3 and LNCaP human prostate cancer cells. Fluorochrome staining (acridine orange/ethidium bromide double staining) and TUNEL (TdT-mediated dUTP Nick-End Labeling) assays were used to identify kind of cell death. Caspase-3 activity was evaluated using a colorimetric caspase assay

method. To determine cleavage of PARP in apoptosis, immunoblot analysis was used.

Results: According to the results of this study, flavanone inhibited proliferation of cultured PC-3 and LNCaP human cancer cells by inducing apoptosis that were characterized by morphological changes, DNA fragmentation, caspase 3 activation. The synthetic falvanone derivatives also inhibited the PARP and interfere with DNA repair, which have been studied as potential cancer therapeutics in some cancers.

Conclusion: The results of the present investigation showed the cytotoxic activity of the synthetic compound in PC3 and LNCaP cells occurs via apoptosis and could be subjected to further studies.

Keywords: Anticancer, Flavanone, Apoptosis

P15-010
Bioorthogonal enzymatic cleavage of protection groups for prodrug activation

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The triggered release of bioactive compounds through the cleavage of protection groups is a powerful tool for applications in chemical biology (uncaging) and medicinal chemistry (prodrug activation). Here, we report enzyme-catalyzed bioorthogonal chemistry, namely the selective enzymatic cleavage of tailored protection groups. Genetically modified P450 BM3 monooxygenases are employed as catalysts both *in vitro* and *in vivo* for the uncaging of ether-protected fluorescent substrates with high activity, while at the same time orthogonality between different mutants is achieved. Our findings indicate that it is not always necessary to start with the creation of new mutant libraries by directed evolution to find suitable variants: screening existing collections with outstanding activity/selectivity profiles highly accelerates the screening process. Our enzyme-catalyzed bioorthogonal chemistry allows the use of engineered proteins for applications in imaging techniques or for the generation of bioactive compounds at their site of action, ultimately allowing the development of enhanced systems for targeted prodrug delivery.

P15-011
Therapeutic potential of fisetin and identification of its mechanisms in action in chronic myeloid leukemia and acute promyelocytic leukemia cells

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Flavonoids prevent the initiation, promotion and progression of cancer by modulating various signaling pathways. Fisetin, a natural flavonol, is a promising chemopreventive/chemotherapeutic agent and studied on several cancer types but not on CML and APL. Moreover, there is no information about the precise mechanisms by which fisetin exerts its antileukemic effects. We aimed to determine its mechanisms of action in CML and APL cells by biochemical and genetic approaches. The growth inhibitory and apoptotic effects of fisetin were evaluated by MTT assay, analysis of mitochondrial membrane potential (MMP) and caspase-3 enzyme activity, annexin-V/PI staining and cell cycle analysis. Genome-wide microarray (Illumina) analysis was performed. Gene clustering and pathway analysis were performed with GENOME STUDIO Software (Illumina), KEGG and Ingenuity Pathway Analysis (IPA) software, respectively. Fisetin inhibited cell growth and induced apoptosis through the loss of MMP, caspase-3 activation and cell cycle arrest for both leukemias. In fisetin treated HL60 and K562 cells, JAK/STAT pathway was common altered pathway together with decreases in the expression of STAT5A, STAT5B, STAT3 and JAK1 genes. Fisetin treatment in APL cells also modulated MAPK kinase and PI3K/AKT pathways and cell cycle related events while causing changes in p53 pathway, c-kit signaling and various apoptosis related events in CML cells. We determined the molecular mechanisms by which fisetin exerts pleiotropic effects on CML and APL cells. This study illuminates biological pathways affected by fisetin while identifying candidate genes that might be targeted for CML and APL therapy if supported with *in vivo* studies.

P15-012
AS_2O_3 induce epigenetic modification in NB4 cell line

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AS_2O_3 effectivly induces complete clinical and molecular remissions in APL patients and triggers apoptosis in APL cells. The effect induced by AS_2O_3 is also associated with extensive genomic-wide epigenetic changes with large-scale alterations in DNA methylation. The aim of this study was toinvestigate the AS_2O_3metabolism in association withfactors involved in the production of its methylated metabolites in NB4 cell line. In this study, we used high performance liquid chromatography (HPLC) technique to detect AS_2O_3metabolites in APL-derived cell line, NB4. The effects of AS_2O_3on glutathione level, S-Adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) levels were examined. We also studied the expression levels of arsenic methyltransferase (AS3MT) and DNA methyltransferases (DNMT1, DNMT3a and DNMT3b) by real-time PCR. Our results show that after entry ofAS_2O_3into thecellit is converted intomethylated metabolites, mono-methylarsenic (MMA) and dimethylarsenic (DMA). Glutathione (GSH)production was increasedin parallel withthe methylated metabolites formations. AS_2O_3treatment inhibited DNMTs (DNMT1, DNMT3a and DNMT3b) in dose- and time-dependent manners. SAH levels in AS_2O_3-treated cells were increased; however, SAM levels were not affected. Collectively, the continues formation of intracellular methylated metabolites, inhibition of DNMTs expression levels and increase of SAH level by arsenic biotransformation presumably would affect the DNMTs-methylated DNA methylation in a fashion related to the extent of DNA hypomethylation. Production of intracellular methylated metabolites and epigenetic changes of DNA methylation during AS_2O_3metabolism may contribute to the therapeutic effect of AS_2O_3in APL.

P15-013
Nanomedicine and drug delivery: enhancing nanoparticle efficacy through knowledge of their intracellular fate

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Nanoparticles (NPs) are particles with sizes in the nanometre scale which show high potentiality as diagnostic and therapeutic tools for the delivery of small molecule drugs and biologics to a wide range of tissues and cells. Despite the wide application of NPs in various technologies and clinical trials, their mechanisms of intracellular interaction are not well understood. It is currently believed that NPs enter cells by endocytosis, and irreversibly populate lysosomes resulting in the destruction of the sensitive compounds they are meant to deliver.

We have designed a strategy to systematically discover the cellular machinery associated with NP internalisation and trafficking. Using fluorescently-labelled synthetic nanoparticles, we have employed a high content screening (HCS) microscopy approach to quantify their accumulation and distribution in cells. This has been achieved by using advanced automated image analysis tools, which allow unbiased assessment of intracellular NP distribution, in combination with RNA interference (RNAi), allowing us to analyse the relevance of several thousand genes to NP uptake and trafficking.

Our genome-wide down-regulation screen (21,000 targets) has revealed members of GTPase families, plasma membrane receptors and cytoskeletal components as key regulators of NP transport from the cell surface to lysosomes. Our study provides an innovative experimental approach in the nanotechnology field providing information about how NPs are controlled in the intracellular environment, thereby opening new avenues for the design of more effective drug delivery vehicles.

P15-016
Nucleolipid-based Ru(III) complexes as new anticancer agents

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In order to achieve more efficient and selective anticancer therapies, several drug delivery systems have been designed to ensure the protection of the active principle and the realization of passive or active targeting[1]. In this context, we have developed new nanovectors[2] – consisting of differently decorated amphiphilic nucleolipids self-assembled in stable and biocompatible liposomes – uploaded with AziRu, a ruthenium-complex inspired to NAMI-A, drug under clinical trials. Although they are rapidly hydrolyzed under physiological conditions, ruthenium compounds have emerged as promising alternative to platinum-based drugs[3], and the inclusion in nanocarriers should improve their stability. Ru-nanocarriers activity was evaluated by *in vitro* bioscreens on a panel of cancer and normal cells lines, showing IC_{50} values 10–20 fold more effective than AziRu used as control, result of a higher cellular uptake due to nanovectorization. In addition, they are about 50-fold more effective on cancer than healthy cells, supporting the hypothesis that ruthenium is converted in the active species (Ru^{+2}) exclusively in cancer cell environment. Fluorescence microscopy demonstrated that Ru-nanocarriers are efficiently and rapidly incorporated in cancer cells. FACS analysis, DNA fragmentation assay and Western blot, performed to evaluate the molecular mechanism of action, showed both apoptosis and autophagy pathways activation. Taken together, these findings suggest that the combination of innovative nanobiotechnological platforms, such as nucleolipid nanovectors, with promising metal-based drugs as Ru-complexes, might represent an effective strategy to target cancer cells.

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P15-017
Effects of a fullerenol/doxorubicin nanocomposite on the heart tissue of healthy rats

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Fullerenol is a 60-carbon molecule in the form of a buckyball functionalized with 24 hydroxyl groups. Good water solubility enable its use in biological applications. This assembly holds potential for delivery of anticancer drugs such as doxorubicin. Our previous research had shown fullerenol's cardioprotective and hepatoprotective activity during doxorubicin treatment. In the past three decades doxorubicin has been a first line cancer chemotherapeutic, but its systemic cytotoxicity limits its clinical use. Taking into account good features of both substances our research team came up with the idea to make a fullerenol/doxorubicin nanocomposite. Firstly, we examined its stability under different environmental conditions using variety of analytical methods. After that we showed its cytotoxicity against different malignant cell lines. In this particular research, our aim was to find out what were the effects of our nanocomposite on the heart tissue of healthy rats in comparison to doxorubicin alone. After the 24 h-treatment, adult male Wistar rats were sacrificed and hearts were collected and stored for further analysis. Ultrastructural study revealed that the nanocomposite is less harmfull to the heart tissue compared to doxorubicin alone. Considering the ability of doxorubicin to induce oxidative stress as well as apoptosis, and considering that fullerenol mitigates both, we had chosen to monitor gene expression of certain enzymes involved in antioxidant defense and apoptosis. We had also performed certain biochemical blood tests. All together, our results have shown that the fullerenol/doxorubicin nanocomposite induces less damage to the hearth tissue in comparison to doxorubicin alone.

P15-018
Singlet oxygen and flavin-binding fluorescent proteins: a deadly tandem in LOV

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Fluorescent proteins (FP) and super-resolution microscopy have become essential tools for advanced research in the biomedical sciences. Both discoveries, recognized with the Novel Prize (2008 and 2014 respectively), have revolutionized the study of bioprocesses at the subcellular level. The development of novel FPs capable of generating reactive oxygen species (ROS) upon light illumination has enabled their use in a new whole set of applications ranging from chromophore-assisted light inactivation (CALI) to assess protein function, photodynamic therapy (PDT) for the treatment of cancer diseases or novel imaging techniques such as STED.

Rational design of the light, oxygen, voltage (LOV) domain of flavoproteins led to miniSOG, the first flavoprotein succeeding in generating singlet oxygen ($^1O_2$). Our study focus on the development, characterization and *in vitro* application of novel Flavin-binding Fluorescent Proteins (FbFPs). In a germinal work, we reported the $^1O_2$ photosensitization properties of the Leu30Met mutant of the Pp2FbFP from *Pseudomonas putida*. The main result is that Pp2FbFP Leu30Met outperforms the capacity of miniSOG -the reference sensitizing flavoprotein- by a three-fold factor, with a ϕ_Δ value of 0.09 ± 0.01.

Recently, a palette of new mutants has been developed and their characterization is currently ongoing. Selected proteins have been expressed in *E. coli* and cell death could be induced in a light-dose dependent manner. Thus, genetically-encoded photosensitizers arise as a powerful tool to be exploited for CALI as well as in cancer and antimicrobial PDT. In this respect, the potential applied in conjunction with antibodies or to assess cellular death mechanisms is very promising.

P15-019
Modified PNAs for splice blocking

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Several improvements have been made in the field of antisense technology. Among a variety of reagents PNAs (peptide nucleic acids) show favourable characteristics. Introduction of functional side chains into the PNA backbone further improved the properties of these antisense molecules making them promising candidates for therapeutic applications.

Antisense molecules can block translation and also interfere with mRNA splicing. In order to test and quantify the effects of antisense molecules on splicing, we developed a reporter assay for cell culture cells. The assay is designed in a way that functional luciferase is produced only if splicing at a specific site is blocked, in absence of functional antisense molecules the cells therefore produce only background signals. The splice sites can be exchanged, thus allowing optimisation for different target genes.

For initial testing we chose Wnt signalling. Activation of this pathway is seen in a variety of cancers. ß-Catenin is one of the major players of the pathway making it an interesting potential target. Our aim is a knock down creating a dominant negative version of the protein. Targeting the C-terminus of the protein leads to a shortened non-functional version, which however can compete with the fully functional protein. Based on cell culture experiments with truncated ß-catenin versions the splice donor of exon 13 (human ß-catenin) was chosen as target for the dominant negative antisense strategy. This approach will be compared with PNAs targeting the translation start as well as a splice donor of the N-terminal exon 4.

P15-020
Regulation of cathepsin B activity with nitroxoline derivatives

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Dysregulation of expression and activity of cathepsin B is associated with a variety of pathological processes including cancer. Cathepsin B differs from other cysteine cathepsins in possession of exopeptidase and endopeptidase activity. The latter is associated with its pathological role in extracellular matrix degradation and consequently tumor invasion and metastasis. In this case its increased activity could be balanced by exogenous inhibitors. Several groups of exogenous inhibitors have been identified, but none of them have been introduced into clinical practice due to low bioavailability and off-target effects. As potent selective reversible non-covalent inhibitor of cathepsin B nitroxoline was recently identified. We develop and tested new nitroxoline derivatives based on crystal structure of nitroxoline-cathepsin B complex. First, we evaluate their potency and selectivity against cathepsin B endopeptidase and exopeptidase activity. Further, for compounds that displayed improved inhibition constants compared to nitroxoline we performed cell-based assays to evaluate the impact on cell invasion in 2D and spheroid based 3D cell invasion models, as well as the impact on extracellular and intracellular degradation of extracellular matrix.

In regulation of cathepsin B activity inhibitors demonstrated different potency and selectivity. 2-{[(8-hydroxy-5-nitroquinoline-7-yl)methyl]amino}-acetonitrile was identified as the best performing inhibitor with much lower constant of inhibition than nitroxoline. It appeared also as selective and reversible inhibitor of cathepsin B endopeptidase activity and as efficient inhibitor of degradation of extracellular matrix and tumor invasion. It is therefore a promising new inhibitor of cathepsin B and could be considered as a candidate for further testing.

P15-021
Two directions of targeted destruction of cancer cells

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Currently destruction of the target cancer cells actively studied in two directions: (i) by method of photodynamic therapy (PDT) and (ii) by acting on receptors of cancer cells leading to prevention of their dimerization. (i) As a damaging agent in a method of PDT are used photosensitizers (usually porphyrins). Photosensitizers accumulate selectively in tumors and upon illumination promote generating of reactive oxygen species that result to the destruction of cancer cells. In Armenia were synthesized more than 100 different cationic porphyrins and metalloporphyrins. We showed that cationic porphyrins and metalloporphyrins

showed high efficacy in PDT as *in vitro*, as well *in vivo*. The highest efficiency demonstrated the Zn-containing metalloporphyrins. (ii) The epidermal growth factor receptor (EGFR) is a membrane-spanning protein, as a result of its over expression and deregulation goes an aggressive tumor growth. Together with scientists from the University of Nantes we have shown that some small compounds [non-peptide compound nitro-benzoxadiazolyl (NBD)] may purposefully bind to dimerization domain sEGFR. This causes allosteric activation of receptor, promotes the formation of stable dimers and launching of oncological process. On the other hand we showed high affinity of cationic porphyrins with a number of proteins, as well as with low molecular weight compounds. It allows assuming that by target action of porphyrins (by complexation with EGF or a molecule type NBD) with the extracellular dimerization domains I and III of EGFR and by photodynamic illumination, the reactive oxygen species can cause destruction of the domains, prevent the dimerization process and cancer launch.

P15-022
Targeting cathepsin B in the tumour microenvironment by inhibitory DARPins

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Cathepsin B is a lysosomal cysteine protease involved in tumour cell invasiveness and angiogenesis. While normally the localization of the protease is confined to the endo-lysosomal vesicles, in tumours it is secreted to the membrane or the extracellular space by tumour as well as stromal cells, such as tumour-associated macrophages, fibroblasts and endothelial cells.

Pharmacological inhibition of cathepsin B by small-molecule inhibitors was shown to inhibit tumour growth and metastasis in animal models, and tumour-specific up-regulation of cathepsin B has been explored for diagnostic purposes as well as targeted drug delivery.

We propose that small protein binders such as DARPins offer a great opportunity for design of highly selective reversible cathepsin B inhibitors that could be applied with dual therapeutic mode of action – as cathepsin protease inhibitors and as a targeted drug delivery platform. These engineered proteins have several key characteristics that allow demanding chemical or biochemical modifications without affecting the binder activity, namely the small size, high stability and ease of site-specific labelling.

Here we present the selection and characterization of two inhibitory DARPins with high affinities for human and mouse cathepsin B and no detectable affinity for highly homologous cysteine cathepsins. We used a combination of competition assays and enzyme kinetic studies to characterize the binding, and we confirmed the results with solved crystal structures of the complexes. Furthermore, both DARPins successfully inhibited cathepsin B in human and mouse cancer cell lines, which suggests they are suitable candidates for further drug delivery development.

P15-023
Experimental regulative effect of selenium compunds on the glioblastoma multiforme cells – *in vitro*

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Glioblastoma multiforme (GBM) is caused by the central nervous system-derived glial cells and is the most common form of primary brain tumor. Our aim was to investigate the regulative *in vitro* effects of selenium on human glioblastoma multiforme cells.

This is the first study to examine SeMet effects on cell growth and death in GBM cell lines GMS-10 and DBTRG-05MG. Here both cell lines were used as a model to examine the proliferation, cytotoxicity, DNA fragmentation and apoptosis of the selenomethionine treated and non-treated cells groups in order to analyse an alternative regulative effect on glioblastoma cells *in vitro*. The selenium derivative compound selenomethionine's regulative effect was assessed by WST-1 and lactate dehydrogenase (LDH) tests, respectively. For DNA fragmentation we used cell death ELISA kit and the apoptosis was determined by Annexin V staining.

According to our results, cells respond to seleno-L-methionine (SeMet) in a dose-dependent and time-dependent manner for both cell lines examined. Incubation of the cells with 50 and 100 μM SeMet for 24 h increased proliferation. Also, SeMet induced apoptosis after 72 h incubation with GBM cells, in addition to its reduction of the cellular proliferation. Our results suggest that SeMet may be key target for future GBM therapeutic approaches.

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P15-024
Antitumor viral protein variant selectively cytotoxic for cancer cells when exogenously added

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Here we describe the production of viral protein variants for their potential use as antitumor drugs. Most of these variants are prone to aggregate and we characterized them by different biophysical methods such as dynamic light scattering, circular dichroism and transmission electronic microscopy. Interestingly, one of them, named LEP50, does not aggregate and behaves as a monomer in solution. This variant is not only cytotoxic when the gene is transfected into tumor cells but also when it is exogenously added. This cytotoxicity is selective for cancer cells. The monomeric nature of this variant has allowed us to characterize it structurally. Therefore, its ^{1}H-^{15}N-heteronuclear bidimensional NMR (HSQC) spectrum has been completely assigned. Our results suggest that this viral protein variant is a good antitumor lead candidate and shed light on the structure and function of this protein.

P15-026
Understanding the mechanism of dendrimer adsorption onto oppositely charged surfaces using surface plasmon resonance and quartz crystal microbalance techniques

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Dendrimers are fascinating hyperbranched polymers, which belong to the multifunctional, well-defined and nano-sized compounds. Due to their unique properties and specific structure they have been considered to be one of the most promising groups, that could revolutionise medicine. At present, dendrimers are very popular in many areas of research: drug delivery, gene delivery, cancer-targeting therapy and diagnosis.

One of the most studied dendrimers is polyamidoamine, PAMAM, a dendrimer containing primary amine groups in the outermost layer. The physicochemical properties of 6th-generation poly (amidoamine) G6 PAMAM dendrimers have been investigated using different techniques such as surface plasmon resonance (MP-SPR) and quartz crystal microbalance (QCM-D). They are powerful methods that enable highly sensitive, qualitative, real-time, label-free and noninvasive detection of macromolecular interactions. We investigated how dendrimers adsorbed from aqueous solution onto SiO_2 surface. This phenomenon strongly depends on pH of the electrolyte solution that influences swelling of the PAMAM films (the lower pH, the stronger the swelling). This is a consequence of spatial relocation of the dendrimer amide groups due to the interactions of the positively charged amines with the oppositely charged condensed counterions and the penetrating water molecules. Comparing the results obtained from MP-SPR and QCM-D allows the estimation of the water content of the film. These results are essential for designing an alternative scheme for drug and gene delivery.

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P15-027
Towards small molecule-based targeted delivery to immune cells

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In cancer therapy conventional, systemic application of pharmaceutically active small molecules and biologics often results in lack of selectivity and nonspecific toxicity. Although passive targeting of nanocarriers increases penetration of the diseased tissue, cell-specific delivery would greatly increase the therapeutic efficacy of many drugs. In particular, targeted delivery of tumor antigens using nanoparticles to immune cells has gained momentum in cancer immunotherapy. C-type lectins are cell surface receptors on immune cells involved in the regulation of antitumor response and consequently harbor great potential for targeted delivery approaches. These receptors recognize carbohydrate structures on pathogens and trigger internalization of the cargo. Therefore they represent receptors for antigen delivery and processing. Here, we explore several mammalian cell lines as model systems to investigate C-type lectin receptors for small molecule-based liposomal delivery *in vitro*. Interestingly, cell-type specific characteristics regarding expression levels and the occurrence of intracellular receptor pools were observed. With these models in hand we can now investigate the endocytic mechanisms as well as their relationship to the nature of the nanocarrier systematically.

P15-028
S1103Y-SCN5A alterations in tumors and normal tissues of patient with colorectal cancer

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In recent works, ion channels and transporters have emerged as novel mechanisms driving the carcinogenesis. A novel hypothesis of metastasis called "CELEX" (for cellular excitability) is based upon concerted expression of voltage-gated ion, particularly Na+ and K+, channels during cancer progression. The aim of our study depend on results of previous ones was to assess S1103Y-SCN5A alteration in the patients with colorectal cancer.

A total of 60 paraffin-embedded colorectal cancer specimens were obtained from department of pathology in Cerrahpasa Medical Faculty. Also a total 60 paraffin-embedded normal tissue was used from same cases as a control group. Ten-micrometer-thick tissue sections were placed on a glass slide and stained with HE. DNA was extracted from the tissues with 100 μL of extraction buffer at 55°C over night. The tubes were boiled for 10 min to inactivate the proteinase K. The S1103Y genotype was determined by PCR amplification of SCN5A exon 18, restriction enzyme analysis and gel electrophoresis. PCR reactions were performed with sense primer 5′AGGGTCTGAAACCCCCAG GGTCA3′and antisense primer5′CCAGCTGGCTTCAGGGA CAAA3′. Restriction enzyme analysis was performed using 1 μL of PCR product, 1 μL of enzyme digestion buffer, 2 U BseRI. The reaction mixture was incubated at 37° C for 2 h, followed by 65° C for 20 min. Digested samples were separated on a 3% agarose gel.

In this study, we explore S1103Y-SCN5A mutations in the colorectal tissues, not only tumors but also normal. On the other hand, much more work is required for the association Na+ channels with cancer progress.

P15-029
Interferon regulatory factor 5 as a therapeutic target in Hepatitis C virus-associated hepatocellular carcinoma

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Chronic inflammation associated with HCV infection is implicated to promote cirrhosis and hepatocellular carcinoma (HCC), but the molecular players and signaling mechanisms which contribute to this process largely remain elusive. Interferon regulatory factor 5 (IRF5) is a multi-faceted protein with critical role in virus-, IFN- and DNA damage-induced signaling pathways. Of note, is its well documented role in several inflammatory disorders including lupus and recent emerging evidence for IRF5 function as a tumor suppressor molecule. Given the relevance of both inflammation and cancer to HCV infection, it is very intriguing that IRF5 expression and signaling in context of HCV infection has not been investigated to-date. Here, we present evidence for the first time for modulation of IRF5 expression and signaling during HCV infection. Employing human hepatoma cells autonomously replicating HCV RNA, we demonstrate down-regulation of IRF5 expression at the mRNA and protein levels. Notably, we reveal the clinical significance of IRF5 to HCV from immunofluorescence (IF) staining of human tissue array specimen depicting dramatic down-regulation of IRF5 pro-

tein in control liver versus HCV-HCC. We further noted that IRF5 exhibits distinct punctate like perinuclear localization in HCV replicon cells. Functional studies revealed that ectopic IRF5 expression is detrimental for the translation of HCV proteins and the replication of its RNA. These findings suggest that IRF5 may function as a negative effector of HCV replication and pathogenesis and may thus be a promising target in HCV-associated hepatocellular carcinoma.

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P15-030
The probable molecular pathways of antitumor activity of fenugreek (*Trigonella foenum graecum L.*) *in vivo*

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Recent studies show that fenugreek has growth-inhibiting activity against some kinds of tumors *in vitro*. However, the precise mechanism of antitumor action of fenugreek is still unclear. The aim: to study the possible mechanisms of antitumor activity of fenugreek in tumor-bearing animals.

Materials: We used the Wistar female rats with intracranial grafted C6 glioma; non-inbred female rats with subcutaneously grafted Guerin carcinoma and substrains, resistant to doxorubicin and cisplatin; CDF1 female mice with intraperitoneally grafted ascites L1210 lymphoid leukemia. The animals of experimental group were administered the fenugreek powder (250 mg/kg of body weight) from the day of tumor grafting up to the end experiments. All experiments were carried out accordingly to the rules of local Ethic Committee.

Results: It was found that fenugreek decreased the level of malone dialdehyde in liver (37–63%), kidney (21%) and heart (33%); generation of superoxide anion-radicals reduced in kidney (16–23%) and liver (11–41%). It was shown that fenugreek improved hematological parameters – especially, increased the erythrocytes (29–30%) and hemoglobin (35–37%) level. We found that fenugreek increased level of global DNA methylation in tumor cells. Also, fenugreek decreased level of PA: putrescine (30–77%), spermidine (11–26%) and spermine (12–24%); diminished level of the p50 and p65 NF-κB and level c-myc, bcl-xl and cox-2. These biochemical data are in a good agreement with the tumor growth retardation (25–48%).

Conclusions: Thus, obtaining results suggest that the mechanisms of antitumor action of fenugreek may be mediated by NF-κB-dependent signal pathways and its influence on DNA methylation and PA synthesis.

P15-031
Hydroxyapatite/poly(lactic-co-glycolic acid)/doxorubicin coatings for the prevention of bone cancer relapse at the bone-implant interface

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The aim of this study is to bring a novel and sustainable solution for the patients affected by bone cancer, by optimizing the surface of an endoprosthesis in order to prevent bone cancer relapse. Thin coatings based on hydroxyapatite (HAP), poly(lactic-co-glycolic acid) (PLGA) and doxorubicin (DOX) were prepared by Matrix Assisted Pulsed Laser Evaporation Tehnique (MAPLE). The prepared thin coatings were characterized by TEM, SEM, XRD, SAED, AFM and FT-IR. Biological characterization consisted in the *in vitro* (by using stem cells, osteoblasts and osteosarcoma cells) and *in vivo* (on mice, up to 21 days) qualitative and quantitative evaluation of the toxicity of the prepared thin coatings. The prepared surfaces triggered pronounced anti-proliferative effects against osteosarcoma cells providing new efficient route to control bone cancer relapse with minimum side effects compared with classical chemotherapy.

P15-032
The oxidative stress generated in mice spleen by polymeric micelles coated SPIONS

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SPIONS are used for medical purposes, when conventional therapies show limited efficacy for diagnosis, treatment and theranostics. They can be used as contrast agents in MRI and also in other biomedical applications.

The aim of this *in vivo* study was to evaluate oxidative stress changes in mice spleens, generated by SPIONS coated with polymeric micelles, and establish a toxicological profile. The mice were divided into three groups and injected intravenously into the tail veins with three suspensions of nanoparticles: 0.7% sodium chloride – the control, and the other ones containing 5 mg Fe/kg body weight, respectively 15 mg Fe/kg body weight. At one, two, three, seven and fourteen days after the treatment administration, the levels of some oxidative stress biomarkers:

reduced glutathione (GSH), malondialdehyde (MDA) and advanced oxidation protein products (AOPP) were evaluated.

In the case of GSH concentration, a significant increase was observed after one day exposure, whereas after 3 days a significant decrease for both doses occurred. The AOPP levels increased only after the first and second days of exposure. The MDA concentrations raised significantly for both doses in the first two days, then decreased after 3 days at control level and slightly increased again in a non-significant way after 7 and 14 days.

In conclusion, SPIONS encapsulated in polymeric micelles induced oxidative stress at splenic level, which was well counteracted for the lower dose and to a certain extend for the higher one. Taking into account these minimal damages, these nanoparticles could be used as contrast imaging agents.

P15-033
Scorpion toxins at rescue: insecticidal peptides with anticancer activity

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Scorpions are one of the most ancient groups of terrestrial arthropods. During 400 million years of evolution they developed an effective hunting weapon, their venom. Venom produced by Buthidae scorpions contains diverse insecticidal peptides with neurotoxic activities. Unexpectedly, some of them exhibit anticancer properties. The most studied peptide with antiglioma activity is chlorotoxin (CTX) from *Leiurus quinquestriatus*.

Insecticidal peptides I5A, I4, and MeuClTx-1 from *Mesobuthus eupeus* venom are homologous to CTX (>68% sequence identity) and possess the CSα/β motif typical of scorpion neurotoxins. Even though they were purified a long time ago, their anticancer properties were not elucidated. Here we report that I5A inhibits C6 glioma cells invasiveness, while the other two peptides fail to show significant inhibition. Rhodaminated I5A specifically binds to glioma cells and consequently demonstrates endosomal localization similarly to CTX. Given that no cytotoxic or cytostatic effect for human cells and no toxicity for mammals were found, fluorescent derivatives of I5A may be used to develop novel agents for glioma diagnostics and therapy.

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P15-034
Characterization of a new DNA aptamer selected against STAT5B, a protein involved in leukemias

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STAT5a/b are common transcription factors that play an important role in haematopoiesis and immune cells development. During immune response, they directly convert the signal promoted by cytokines or growth factors into transcription activity of particular genes. Disruptions occur when Stat5a/b are inappropriately phosphorylated: They promote the transcription of anti-apoptotic and/or proliferative genes, thus leading to leukemia and solid tumors. Consequently, they should constitute a prime target to therapeutic intervention. We have previously selected an aptamer against STAT5B by SELEX approach. In this work, we study the selected aptamer effect on chronic myeloid leukemia cell line KU812 proliferation by trypan blue exclusion test. Results show that the aptamer inhibits the cell growth in a dose-dependent and time-dependent manner. The aptamer apoptosis-inducing effect was performed and confirmed by TUNEL assay. Changes in the protein level of phosphorylated STAT5 and its target genes were also analysed by western blot after cells transfection. A decrease in STAT5 phosphorylation suggests that the aptamer interacts with the STAT5 phosphorylation domain which leads to a down-regulation of anti-apoptotic STAT5 target genes.

SELEX: Systematic Evolution of Ligands by EXponential Enrichment.

P15-035
A novel 3D cell culture system for *in vitro* evaluation of anticancer compounds

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Recently, a number of approaches have been developed to generate 3-dimentional (3D) cell culture models to mimic *in vivo* environments for cancer studies; e.g. scaffolds, microcarriers, and spheroids. However, many challenges remain, such as applying them into high throughput screening (HTS) systems and improving the efficiency of anti-cancer drug discovery. In this study, we developed a novel 3D cell culture medium using a polymer, FP001 which has the ability to form cancer cell spheroids in uniform and appropriate size. 3D cell cultures system using ultra-low attachment multi-well plates in combination with FP001 exhibited >3-fold increase in the number of A549 cells after 5-day culture as compared to that without FP001. The positive effect of FP001 was applicable to a wide variety of cancer cell lines and was clearly beneficial for HTS. As for the cell proliferation of HeLa and A549, the 3D culture system was more sensitive to AKT inhibitors and MEK inhibitors, compared with that employs 2D monolayer culture condition. In conclusion, we established a novel method for the 3D culture of cancer cells under low attachment condition by using FP001, which was available for HTS and showed high sensitivity to molecularly-targeted drugs, EGF signal inhibitors. The approach using FP001 would facilitate the development of novel models for *in vitro* evaluation of anticancer compounds.

P15-036
Is Vitamin D_3 has any effect on the proliferation of colorectal cancer (HCT116) cells?

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The effects of Vitamin D on cell cycle and apoptosis is a remarkably growing topic. Different groups analyzed different treatment

time-intervals to test the Calcitriol's effect on breast(MCF7) and prostate(MDA-MB-435) cancers, and osteosarcoma(LNCaP) cell lines and indicated the inhibiton of cell growth by Calcitriol in these cancer cell lines. However,there is a lack of studies in different cancers. This preliminer study was designed to investigate the possible selective effects of active form of vitamin D_3(1,25-dihydroxyvitaminD_3[Calcitriol]),on cell proliferation and viability in HCT116 colon cancer cells(ATCC/USA).The determination of effective duration-time and doses of Calcitriol(AbCamPlc./Cambridge,UK,Code:ab141456) was performed according to cell growth by Methyl-Thiazol-Tetrazolium-Assay which was verified by Trypan-Blue-staining.0–500 nM Calcitriol treatment was analyzed for 24,72 and 120 h. A modest effect on growth inhibition was detected in 24 h. Besides,in 72 h-treated-cells the first remarkable effect was seen at 20 nM,and higher doses and 120 h-treatment inhibited cell growth more efficiently. On the other hand,protein isolations and quantifications were done from the lysates of 24 h 100/500 nM Calcitriol or ethanol(control)-treated HCT116 cells. VDR,c-Myc,β-Catenin and β-Actin expression levels were analyzed by Western-Blot-Technique and it was found that VDR levels were increased with the dose of Calcitriol used. Besides while β-catenin levels were modestly decreased c-Myc levels were significantly decreased at 500 nM of Calcitriol. Our results in HCT116 cells were convenient to many studies which indicated the inhibitory effect of Calcitriol on cell growth in various cancer-cell-lines. As a conclusion,since this vitamin was shown to selectively inhibited cell growth on different cell lines,it has the potential to become an effective anti-cancer drug supplement in the future.

P15-037
Proteomic investigation into GM2 extract from *Grangea maderaspatana* (L.) Poir. Induced Apoptosis and cell cycle arrest in the MDA-MB-468 human breast cancer cell line

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Breast cancer is a key disease affecting women's health worldwide. MDA-MB-468 breast cancer cell line (ER^-, PR^-, $HER2^-$) is mostly associated with poor recognition by molecular targeted-therapeutic approaches. The anticancer activities of GM2 ((-)-7-α-hydroxyfrullanolide) from extract of *Grangea maderaspatana* has been used on various types of cancer cell lines. However, its activity against MDA-MB-468 has not been determined. We screened for the cytotoxic activity of GM2 on MDA-MB-468 by MTT assay. The apoptotic cell pattern and DNA distribution in cell cycle were investigated by flow cytometry. To identify the cytotoxic mechanism, proteomic technique was used to identify either up or down-regulated protein expression. The results showed that GM2 had high cytotoxicity (IC_{50} = 2.29 μg/ml) with moderate selectivity index (SI = 3.97). GM2 induced cell death occurred via apoptosis and cell cycle arrest at the G_2/M phase in a dose-dependent manner. From the proteomic results, the major proteins involved in three biological process including apoptosis: Caspase-7, BCL2/adenovirus E1B 19 kDa protein-interacting protein 3; cell cycle: serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha, testis mitotic checkpoint BUB3 and; cell signaling: 14-3-3 epsilon, NADPH oxidase activator 1. All these proteins had increasing expressions in GM2 treated MDA-MB-468 compared with control group. Hence we have shown that GM2 extract possessed anticancer properties and induced apoptosis and cell cycle arrest. This basic knowledge will hopefully lead to anticancer drug development and therapeutic application in breast cancer patients.

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P15-038
Combined effect of Cetuximab and Stabilized-Ag ion solution on epirubicin-resistant human Non-Small Cell Lung Cancer (NSCLC) comparing with parental cells

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This study aimed to assess the antitumor effect of cetuximab (C225, Erbitux_, a chimeric anti-epidermal growth factor receptor (EGFR) monoclonal antibody) combined with stabilized-Ag ion solution on epirubicin-resistant and parental human non-small cell lung cancer (NSCLC).

The antitumor activity of cetuximab and stabilized-Ag ion solution were evaluated with CellTiter-Blue^R Cell Viability assay. R-H1299 cells viabilty were found higher than the parental cells against cetuximab's cytotoxicity at IC_{50} cetuximab concentration for 72 h, while parental cells were found more sensitive. Against stabilized-Ag ion solution cytotoxicity also R-H1299 cells were found resistant than parental H1299 cells. For both cells, cytotoxicity was dependent upon the concentration of the cetuximab and stabilized-Ag ion solution. The most combined cytotoxic effect was found for resistant cells IC_{50} cetuximab + IC_{10} stabilized-Ag ion solution combination. Caspase-3 activity of H1299 cells was measured with ApoTox-Glo0™ Triplex assay by a fluorescent microplate reader after treated with cetuximab (IC_{50}) alone and- combined with stabilized-Ag ion solution concentration which has maximum cytotoxic effect with cetuximab (IC_{50}) for 72 h. For both cells, combined treatment of cetuximab with stabilized-Ag ion solution caused incresing caspase-3 activity 1.5 times higher than cetuximab treatment alone and 3 times higher than control.

Antitumor activity of combined cetuximab with stabilized-Ag ion solution was found higher than cetuximab treatment alone.

P15-039
The role of (poly)sialic acid during meningeoma progression

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Sialic acids (Sia) represent a family of sugar acids, which are located on cell surface as terminal sugar of glycans and are involved in a variety of cell-cell interactions. Due to its negative charge, Sia is involved in the regulation of cell interaction and adhesion. Increased sialylation leads to decreased adhesion and increased migration. Polysialic acids (PolySia) are long homopolymers of Sia and are mostly bound to the neural-cell-adhesion-molecule (NCAM). PolySia is known to interfere with

NCAM-mediated adhesion. This causes flexibility, which is important during development of brain and during learning and memory. PolySia was also detected in malignant tumours such as neuroblastoma. High concentrations of PolySia are associated with decreased adhesion, higher metastasis rate and bad prognosis.

One of the most frequent tumours in brain (up to 20%) is the meningioma. Meningioma is a slowly growing tumour based on the cap cells of arachnoidea. It is mostly benign and WHO classified I. In some rare cases, meningiomas become atypical or anaplastic and grow more aggressive, faster and infiltrative (WHO classified II or III). The prognosis is mostly good in low-grade tumours. Higher grades have worse prognosis.

Most brain tumours express NCAM and PolySia. However, meningioma is not well characterized. Here, we present data on the expression of NCAM in different meningioma samples from neurosurgery and in different meningioma-derived cell lines (HBL52 as grade I, IOMM-LEE and KT21 as grade III cell lines). Furthermore, we compared adhesion and proliferation on NCAM and several extracellular matrix proteins.

P15-040
Biosilica nanovector from diatomite for siRNA transport in cancer cells

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Diatomite is a natural porous biomaterial of sedimentary origin, formed by fragments of diatom siliceous skeletons, called "frustules". Due to large availability in many areas of the world, chemical stability, and non-toxicity, these fossil structures have been widespread used in lot of industrial applications, such as food production, water extracting agent, production of cosmetics and pharmaceutics. However, diatomite is surprisingly still rarely used in biomedical applications. In this work, diatomite nanoparticles for small interfering ribonucleic acid (siRNA) transport inside human epidermoid cancer cells (H1355) were exploited. Nanometric porous particles were obtained by mechanical crushing, sonication, and filtering of micrometric frustules. Morphological analysis performed by dynamic light scattering and transmission electron microscopy revealed a particles size included between 100 and 300 nm. siRNA bioconjugation was performed on nanometric fragments by silanization and poly D-Arg peptide functionalization. *In-vitro* experiments showed very low toxicity on exposure of the cells to diatomite nanoparticle whereas confocal microscopy imaging performed on cancer cells incubated with fluorescent siRNA conjugated nanoparticles demonstrated a cytoplasmatic localization of vectors. Furthermore, the release profile in solution of siRNA, conjugated with diatomite, showed an initial burst phase followed by slow and sustained release phase. Gene silencing by delivered siRNA is also demonstrated. The results obtained endorse diatomite nanoparticles as innovative nanocarriers for siRNA transport in cancer cells and provide a new basis for the development of unique tools for the delivery of antitumoral molecules to cancer cells.

P15-041
C_{60} fullerenes modify protein tyrosine phosphorylation patterns in normal and transformed T cells

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C_{60} fullerenes are nanodimensional molecules, which due to their small size and hydrophobicity can incorporate into cell membranes and specifically interact with cell proteins, thereby exerting a variety of biological effects. Since phosphorylation of proteins on tyrosine residues plays a crucial role in the malignant transformation, the aim of the present work was to study the effects of pristine C_{60}fullerenes on the phosphotyrosine patterns in normal and transformed T lymphocytes treated with different apoptosis-inducing agents.

Preincubation of rat thymocytes with C_{60}fullerenes (10^{-5}M) for 1 h significantly reduced cytotoxic effects of such apoptosis-inducing agents as staurosporine, cytosine arabinoside and hydrogen peroxide. By contrast, C_{60} fullerenes did not protect human T lymphoma Jurkat cells from death induced by these agents. Investigation of cellular phosphotyrosine patterns demonstrated, that the main immunoreactive bands detected with anti-phosphotyrosine antibodies correspond to proteins with *Mr* 17, 30, 50, 72 and 100 kDa in thymocytes and 17, 26, 30, 50, 55, 70, 90 kDa in Jurkat cells. In both cell types the intensity of phosphorylation of almost all phosphotyrosine-containing proteins was decreased after incubation with all the apoptosis-inducing agents studied. The cell death inducers effect on protein tyrosine phosphorylation in thymocytes was modifided in the presence of C_{60}fullerenes.

In conclusion, the selectivity of C_{60}fullerenes effects in normal and transformed T cells might be helpful for the development of the complex approaches to therapy of T lymphomas.

P15-042
Numerical features of the mechanisms of cancer cell death triggered by homologous cationic peptides

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Cytolytic peptides from venom of insects and snakes are toxic to mammalian cells, including cancer ones. Such peptides can be either linear, or contain disulphide bonds. Latarcins (Ltc) from spider venom are linear ones. Their counterparts, cardiotoxins (CTs) from cobra venom, are beta-structural toxins, stabilized with 4 disulphide bonds [1]. We have elucidated structural properties of Ltc and CTs in model membranes and mechanisms of their antibacterial and anticancer activities. Antibacterial effect of these peptides is caused by the plasma membrane permeabilization of bacteria. Hydrophobic characteristics of the peptides, F-score for Ltc [2] and HTL-score for CTs [1], correlate positively with their activity [3]. If membrane deterioration is involved in toxin-induced death of cancer cells, the correlation remains positive. An alternative mechanism involves capture of the toxins inside the cells, followed by their interrogation into cell metabolism. This manifested by negative correlation of activity/hydro-

phobicity. We assume, this relationship may be used for facile control of the mechanism of cancer cell death, when arrays of homologous peptides are tested.

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P15-043
Cytotoxic activity of novel acridine-thiazolidinone agents: DNA binding properties, topoisomerase I inhibition activity of (2Z)-3-(acridine-9-yl)-(diphenylhydrazin-1-ylidene)-1,3-thiazolidine-4-ones

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Acridine derivatives are a well-known group of multi-target anticancer agents which interact with DNA at a fundamental level and inhibit the activity of topoisomerase enzymes. The versatile thiazolidinone scaffold has featured in a number of clinically used drugs, and thiazolidine compounds have seen use as antineoplastic agents with a broad spectrum of antitumour activity against a variety of human cancer cells. In this study, a series of new acridine derivatives were synthesized and their binding with calf thymus DNA was investigated using instrumental techniques including UV-Visible spectroscopy, fluorescence spectroscopy and circular dichroism spectroscopy. The results confirmed the interaction of the new compounds, derivatives **1–3**, with calf thymus DNA in both UV-Vis and fluorescence applications. The binding constants were found to be in the range from 0.75×10^{-5} to 0.80×10^{-4}/M. CD spectroscopy results revealed the presence of conformational changes in B-DNA upon interaction with these agents. Topoisomerase I relaxation assays were also carried out and the results confirmed the inhibition of topoisomerase I activity at concentrations of 60 and 80 μM. The synthesized compounds **1–3** were tested against human leukemic cancer cell line HL-60 using different techniques such as MTT assay, the detection of mitochondrial membrane potential, cell viability measurements and cell cycle distribution analysis after 24, 48 and 72 h incubation.

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P15-044
Characterization of Solid Lipid Nanoparticles to improve liposoluble drug delivery

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Many promising drugs are often rejected because of their poor bioavailability due to their low water solubility, poor absorption or cell membrane permeability. Therefore, improving drug stability in aqueous dispersions could increase their efficiency and reduce solvent-related degradation. In this regard, Solid Lipid Nanoparticles (SLN) are one of the most promising nanocarriers for controlled drug delivery because of their multiple advantages. They are able to incorporate hydrophilic and lipophilic drugs, present no biotoxicity and drug release is controlled thanks to their solid core. Moreover, their most important characteristics are their ability to pass through some biological barriers and their tendency to accumulate in solid tumour environments. This phenomenon, called "Enhanced Permeability and Retention", could be a potential tool for tumour-targeted drug delivery.

In the present work we have characterized different SLN composed of long-chain saturated fatty acids, Epikuron 200 (mostly phosphatidylcholine), and bile salts. Different systems were prepared, and characterized in order to determine z-size, polidispersity index, z-potential and transition temperature of the core lipid. The capacity to incorporate non-polar drugs and the ability to internalize hydrophobic compounds into cell cultures was also studied. Our results suggest that one of the studied SLN composition present good particle size, polidispersity index and stability. Besides, this SLN composition could be internalized by fast diffusion mechanisms suggesting that these compositions could reach solid tumours by enhanced permeability.

P15-045
Targeting mitochondrial citrate transport in cancer

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Cellular metabolic alterations in cancer cells are among the hallmarks of the cancer which is one of the most important medical problems of our age. Increased rate of glycolysis and synthesis pathways are among these alterations. Novel studies suggest that proteins playing a role in these pathways such as ATP-citrate lyase (ACLY), acetyl-CoA carboxilase and fatty acid synthease have high activity in cancer cells. However the role of citrate transport protein (CTP) which enables transportation of citrate from mitochondria into the cytoplasm where it plays a critical role for fatty acid synthesis as a source for cytoplasmic acetyl-CoA, is rather unknown. In this study the importance of citrate transport protein for cellular processes is examined by its inhibition in breast cancer cell lines via siRNA or chemical inhibition.

In this study CTP was inhibited in MCF-10A cells which represents normal epithelial cells, MCF-7 cells which represents less-aggressive breast cancer and MDA-MB-231 cells which represents metastatic breast cancer was. Efficiency of the inhibition was demonstrated via western blotting and the alterations in cytoplasmic citrate levels were detected via spectrophotometry. Cell viability was assessed by crystal violet assay while the alterations in apoptosis, necrosis and cell cycle regulation were evaluated via flow cytometry. Autophagy was assessed both by fluorescence microscopy and flow cytometry. Lastly the effects of the inhibition on acetylation of histones, an epigenetic regulation process which requires cytopalsmic acetyl-CoA, were detected

spectrophotometrically. During the experiments ACLY was also inhibited along with CTP and the results were evaluated comparatively.

Obtained results suggest that the proteins of interest are expressed in all cell lines and siRNA treatment can effectively silence proteins. An association between basal cytoplasmic citrate levels and the aggressiveness of the cancer was determined, and these levels were reduced partially by siRNA treatment and effectively by chemical inhibition. According to the data obtained from viability experiments inhibition of CTP and ACLY inhibit cancer cell viability without affecting normal cells. This inhibition is more evident in cells treated with chemical inhibitor. No alteration in apoptosis, necrosis, cell cycle or autophagy was detected. Lastly inhibition of proteins was also detected to inhibit histone acetylation which was more prominent in cells treated with chemical inhibitor in accordance with previous experiments.

Data obtained throughout this study demonstrates the importance of CTP and ACLY for cell viability and histone acetylation, and there processes can be interfered with by inhibiting those proteins. High potency of chemical inhibition compared to RNA interference suggests the importance of extracellular citrate as a source of cytoplasmic citrate and that this pathway is effected by chemical inhibition. However effectiveness of the inhibition of both proteins on cancer cells without affecting normal cells demonstrates that targeting these proteins is advantageous for cancer therapy strategies.

Keywords: ATP-citrate lyase; cancer metabolism; cytoplasmic citrate; citrate transport protein; histone acetylation; SLC25A1

P15-046
L-asparaginase from *Pisum sativum* L. and its application as an effective drug in cancer treatment

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Enzymes are in huge demand as chemotherapeutic agents against many terrible diseases. The enzymes can diminish the ability for cancer cells to attach to healthy organs or tissue. L-asparaginase (EC 3.5.1.1) is the enzyme that catalyses the hydrolysis of the amide group of L-asparagine releasing L-aspartate and ammonia. L-asparaginase found to be very promising agent in the treatment of acute lymphoblastic leukemia (ALL) and other kinds of cancer. Normal tissue can synthesize L-asparagine but the cancer cells, particularly malignant and carcinoma cells require external source of L-asparaginase for their growth and multiplication. In the presence of L-asparaginase, the tumor cells deprived of an important growth factor and they failed to survive. Thus the enzyme L-asparaginase can be used as a chemotherapeutic agent for the treatment of ALL (mainly in children) as a potent antitumor or anti-leukemia drug. Moreover application of L-asparaginase in the food industry for the elimination of cancer-causing acrylamide from baked food has been one of the eminent discoveries of modern time. Thus a lot more is needed to investigate about this astounding enzyme.

The L-asparaginases of *Erwinia* and *E. coli* have been reported for many years as effective drugs in the treatment of acute lymphoblastic leukemia. Their main side effects are anaphylaxis, pancreatitis, diabetes, leucopoenia, neurological seizures and coagulation abnormalities. Hence an attempt has been made to find out novel sources of this enzyme from plants.

P15-047
Histone deacetylase inhibitors, EX527 and AGK2, suppress cell proliferation and migration by inhibiting the HSF1/Hsp27 pathway

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Histone deacetylase (HDAC) plays crucial roles in many biological processes, including cell proliferation, differentiation and apoptosis. It has been considered as a potential therapeutic target for various cancers. In this study, we demonstrated that EX527 and AGK2, class III HDAC inhibitors, suppressed the proliferation of HeLa cells and caused G1 phase arrest by inhibiting the expression of Cdk6 or Cdk4. Agar colony formation assay revealed that EX527 and AGK2 decreased colony formation in soft agar and cell migration in a dose-dependent manner. Furthermore, EX527 and AGK2 pre-treatment inhibited the expression of HSF1 and Hsp27 and reduced the phosphorylation of heat shock-induced HSF1. Sirt1 overexpression reversed the effects of EX527 and AGK2 on HSF1 and Hsp27 expression and increased the cell migration levels. Overall, these results indicate that EX527 and AGK2 suppresses cell growth and migration by inhibiting the HSF1 and Hsp27 pathway.

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P15-048
Polymorphisms in the *TOX3* gene and hormone receptor status of breast cancer in Kazakh women

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TOX3 encodes a nuclear protein belonging to the high mobility group family and might act as a transcription factor. *TOX3* was one of the first breast cancer (BC) regions to be identified through GWAS in populations of European and East Asian origin, however, it still was unclear whether the same SNPs are associated with risk of BC in Kazakh population.

In the present case-control study 340 Kazakh female BC patients and 344 cancer-free controls were recruited to investigate the involvement of three SNPs in *TOX3* (rs8051542, rs12443621, rs3803662) in BC risk. Additionally, subtypes of BC, stratified by estrogen receptor (ER±), progesterone receptor (PR±) and human epidermal growth factor receptor 2 (HER2 ±) status were estimated. Pearson p-value, odds ratio, 95% confidence interval tests were applied to data analysis.

No significant differences were found in alleles and genotypes distributions at rs8051542, rs12443621, rs3803662 variants in *TOX3* between the patients and control groups. However, significant association with BC was revealed for rs8051542 after differentiating patients according to ER±, PR± and HER2 ± status of tumors. The T allele was associated with ER+ ($p = 0.049$, OR=1.34; 95%CI:1.00–1.79) and PR+ ($p = 0.018$, OR=1.45; 95% CI:1.06–1.96) BC carriers. Also, the T allele can be considered as a risk factor in ER+/PR+/HER2-luminal type of tumor ($p = 0.035$, OR=1.47; 95%CI:1.03–2.11). All investigated groups were in Hardy-Weinberg equilibrium.

The obtained results allow us to consider the T allele of rs8051542 as a marker of BC risk in the Kazakh population with predictive value, restricted to ER, PR and HER2 status of the tumor.

P15-049
Improved anti-tumor activity of cytostatic drugs functionalized magnetite nanoparticles without application of high amplitude alternating magnetic fields

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Nanotechnology offers a viable solution to reduce the side effects that may appear after chemotherapy. Doxorubicin and Gemcitabine functionalized magnetite nanoparticles were prepared by co-precipitation method and further characterized by XRD, SEM, HR-TEM, SAED, AFM, DTA-TG. The anti-tumor effect of the obtained systems has been evaluated up to 72 h on G2929 osteosarcoma cells using *in vitro* tests to estimate the osteoblasts viability, alkaline phosphatase activity and the level of reactive oxygen species (ROS). The cell viability decreased in a time-dependent manner for Dox@Fe_3O_4 and Gem@F_3O_4 systems with a significant higher percent compared with the simple drugs. These two nanosystems induced ROS accumulation and decreased the ALP activity in human osteoblasts proving their strong cytotoxic effect. Also, a higher accumulation of Dox@-Fe_3O_4 was evident in the cytoplasm and nuclei compared with simple doxorubicine. Designed MNPs were evaluated on mice and on chick chorioallantoic membrane model; the vital organs had no nanoparticle accumulations, with the exception of spleen where black-brown deposits have been found. The spleen is a heavily vascularized organ, involved in the storage of blood cells, nanoparticles being transported here through the macrophages. These functionalized MNPs triggered pronounced anti-proliferative effects against osteosarcoma cells providing new efficient cancer treatment options without application of any alternative magnetic field.

P15-050
NDRG1 as a marker gene for accute hypoxic oxygenation conditions in the brain tumor environment

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NDRG1 is a member of the N-myc downregulated gene (NDRG) family. Its induction occurs via diverse physiological and pathological conditions Hypoxia represents a common feature of solid tumors. In our study, differences in NDRG1 expression between different WHO grades of astrocytic tumors were comparatively examined *in vivo* in human low-grade astrocytoma (WHO grade 2) and glioblastoma (GBM), (WHO grade 4) at both the protein and mRNA level by Western blot analysis and semi-quantitative RT-PCR, respectively. Furthermore, the same proteins were determined *in vitro* in U373, U251 and GaMG human GBM cells using the same methods. HIF-1α protein and mRNA regulation under hypoxia was also determined *in vitro* inU251, U373 and GaMG cells. This regulation was shown at the same levels *in vivo* in human low-grade astrocytoma (WHO grade 2) and glioblastoma which showed a higher NDRG1 over-expression level in glioblastoma than in low-grade astrocytoma. siRNA- and iodoacetate (IAA)-mediated downregulation of NDRG1 mRNA and protein expression *in vitro* in human GBM cell lines showed a nearly complete inhibition of NDRG1 expression when compared to the results obtained due to the inhibitory role of glycolysis inhibitor IAA. Hypoxia responsive elements (HREs) bound by nuclear HIF-1α in human GBM cells *in vitro* under different oxygenation conditions showed an O_2 concentration dependent binding behaviour of HIF-1α. Results of these series of analysis have proven that NDRG1 represents a diagnostic marker for brain tumor detection, due to the role of the acute hypoxic oxygenation conditions in regulating this gene in the tumor microenvironment.

P15-051
Hypoxia induced CA9 targeting via different alternative approches including sulfonamide derivative compounds in human brain cancer *in vitro*

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HIF-1α regulated genes are mainly responsible for tumour resistance to radiation- and chemo-therapy. Among these genes, carbonic anhydrase isoform IX (CA9) is highly over expressed in many types of cancer especially in high grade brain cancer like Glioblastoma (GBM). Inhibition of the enzymatic activity by

application of specific chemical CA9 inhibitor sulphonamides (CAI) like Acetazolamide (Aza.), the new sulfonamide derivative carbonic anhydrase inhibitor (SU.D2) or indirect inhibitors like the HIF-1α inhibitor Chetomin or molecular inhibitors like CA9-siRNA are leading to an inhibition of the functional role of CA9 during tumorigenesis. Human GBM cells were treated under *in vitro* hypoxia (1, 6, or 24-hrs at 0.1%, O_2). Aza. application was at a range between 250 and 8000 nM and the HIF-1a inhibitor Chetomin at a concentration range of 150–500 nM. Cell culture plates were incubated for 24-hrs under hypoxia (0.1% O_2). Further, CA9-siRNA constructs were transiently transfected into GBM cells exposed to extreme hypoxic aeration conditions. Aza. as well as SU.D2 displayed inhibitory characteristics to hypoxia induced CA9 expression in the four GBM cell lines for 24-hrs of hypoxia (0.1% O_2) at concentrations between 3500 and 8000 nM, on both the protein and mRNA level. CA9-siRNA experiments confirmed these results. Aza., SU.D2, Chetomin or CA9-siRNA possesses functional CA9 inhibitory characteristics when applied against human cancers with hypoxic regions like GBM. They can used as alternative or in conjunction with other direct inhibitors possessing similar functionality and can be used ın the development of an optimized therapy in cancer treatment.

P15-052
Cross-talk between GHRH and EGFR in triple-negative breast cancer cells

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Growth hormone-releasing hormone (GHRH) and epidermal growth factor receptor (EGFR) are promoters of cell proliferation, migration and adhesion in breast cancer. Triple-negative breast cancer, which lacks estrogen receptor alpha and progesterone receptor expression and HER2 overexpression, accounts for 10%–20% of all breast cancers. The aim of this work was to study the cross-talk between the signaling pathways in which are involved both type of receptors in triple-negative breast cancer. Therefore, we analyzed *in vitro* effect of GHRH on the activation of EGFR and several elements implicated in such an effect. For this purpose, a triple-negative breast cancer MDA-MB-468 cell line was used. We obtained that phosphorylated EGFR (p-EGFR) levels were enhanced after cell incubation with GHRH with the highest expression at 45 min. The response to GHRH was mediated by specific binding of the neuropeptide to GHRH receptors since cell pre-incubation with MIA-690 blocked GHRH-induced EGFR tyrosine phosphorylation. Furthermore, protein kinase inhibitors (H89 for PKA and PP2 for Src kinase) and specific inhibitors of metalloproteinases (GM6001 for MMPs and TAPI-1 for ADAMs) were able to block GHRH-mediated effects at 45 min on p-EGFR, respectively. The results shed light on the mechanisms of action of GHRH and the inhibitory effect of its antagonist in triple-negative breast cancer. These findings support the merit of further studies on the potential usefulness of GHRH-R antagonists and anti-EGFR targeted therapies in triple-negative breast cancer.

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P15-053
On-line SAW-biosensor-mass spectrometry as a powerful tool for studying biological complexes

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Bioaffinity interactions play a key role in all mechanisms of cellular life. Biosensors are powerful tools for the detection and quantification of biomolecular interactions such as antigen-antibody, protein-peptide, etc. A principal limitation of biosensors is the lack of chemical structure information of affinity-bound ligands. Przybylski et al. developed an on-line bioaffinity-mass spectrometry system using a surface-acoustic wave biosensor (SAW-biosensor) and an MS interface. This system provides the simultaneous affinity detection, quantification and mass spectrometric structural characterization of affinity-bound biopolymers.

We report the study of different types of biological complexes: antigen-antibody (VEGF-Avastin), protein-receptor (EGF-EGFR) and protein-drug complexes (EGF-peptides or molecules). VEGF (vascular growth factor) is implicated in breast cancer, rheumatoid arthritis, diabetic retinopathy and age-related macular degeneration. Anti-VEGF therapies involve monoclonal antibodies such as Avastin. EGF (epithelial growth factor) is a growth factor that stimulates cell growth, proliferation and differentiation by binding to its receptor EGFR. Therapies against EGF-EGFR binding have been developed for lung and colon cancer.

In VEGF-Avastin complex, affinity binding constants (K_D) were determined for complexes of Avastin with a fragment of VEGF and GST-VEGF, which are in the nM and μM order, respectively.

In EGF-EGFR and EGF-drug systems, a wide range of concentrations of EGFR, peptides or drugs were used to determine the respective affinity binding constants (K_D), which are in the nM order for EGF-EGFR system and in the mM order for complexes of EGF with peptides and molecules.

These results demonstrate on-line SAW-biosensor-MS as a powerful tool for structural and quantitative analysis of biopolymer interactions.

P15-054
Serum NEDD9 levels may have prognostic roles in patients with gastric cancer

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Neural precursor cell-expressed, developmentally downregulated 9 (NEDD9), a member of Crk-associated substrate (CAS) family, is highly expressed in multiple cancer types and involved in cancer cell adhesion, migration, invasion. The prognostic value of NEDD9 has been evaluated before; its expression predictor for poor prognosis of cancer patients. The objective of this study was to determine the clinical significance of the serum levels of NEDD9 in gastric cancer (GC) patients.

A total of 68 patients with a pathologically confirmed diagnosis of GC were enrolled into this study. Serum NEDD9 concentrations were determined by the solid-phase sandwich (ELISA) method. Twenty-eight healthy age- and sex-matched controls were included in the analysis. The median age at diagnosis was 60 years, Forty-nine (72%) patients were male and cardia was

the most common tumor localization (n = 37, 77%). The most frequent histologic subtype was adenocarcinoma (n = 45, 66%). Liver was the most common metastatic site in 32 patients with metastasis (n = 14, 21%). Sixty-one percent of 23 metastatic patients who received palliative chemotherapy were chemotherapy-responsive. The median serum NEDD9 levels of GC patients was significantly higher than controls (1339.51 versus 1187.91 pg/ml, p = 0.02). There was no significant difference according to known disease-related clinicopathological or laboratory parameters (p > 0.05). Serum NEDD9 levels had a significant impact on progression-free survival (p = 0.04). On the other hand, serum NEDD9 levels showed no significantly adverse effect on overall survival (p = 0.50).

Serum NEDD9 levels are elevated in GC patients and have an unfavorable prognostic value. However, it has no predictive role on chemotherapy response.

P15-055
Effects of novel gene delivery vector systems based on poly(vinyl benzyl trimethylammonium chloride) on A549 cell line

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Polymer-based gene delivery systems are safer, less pathogenic and less immunogenic alternatives to viral systems. In this work we are focused on effects of novel homopolymers based on vinyl benzyl trimethylammonium chloride as gene delivery vector systems in a mode A549 human lung cancerous cell line. Cells are incubated with DNA/polymer complexes (polyplexes contain salmon sperm DNA) at a wide range of N/P (amino-to-phosphate groups) ratios and concentrations for 6 h. Using MTT assays, trypane blue and methylene blue staining, we investigate cytotoxicity of polyplexes, whereas their behaviour into cells during five days period was investigated by microscopy observations.

Using MTT assay, we found very low/non toxicity of both the pure polymer and polyplexes at various N/P ratios in the concentration range 5–50 μg/ml of. At the same time, a partial permeabilisation of cellular membranes was detected. Our results suggest absorption on the cell surface and entering of polyplexes into about 50% of the cells. Additionally, 48 h–72 h after treatment, the polyplexes showed movement out of the cells, probably forming exosomes. The cell number dramatically decreased and the cell morphology was affected.

Our data suggest successful internalization of the studied polyplexes; however, they stay stable for several days into cytoplasm. For delivery of DNA it is critical to develop less stable polymer particles. We conclude that these nanosized complexes are promise materials to transport biological molecules and particular for gene therapy for treating a wide range of diseases.

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P15-056
Reversion of glioblastoma stem-like cells chemoresistance by adenosine A_3 receptor blockage

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Introduction: Glioblastoma Stem-like Cells (GSCs) have been associated with the multiple drug resistance (MDR) phenomenon on Glioblastoma multiform (GBM) because of the expression of high levels of MRP1 transporter. Previously, we demonstrated that MRP1 can be regulated through the purinergic signaling in differentiated glioblastoma cells. Our aim was to determinate *in vitro* and *in vivo* the chemosensitizer effect of the inhibition of the adenosine A_3 receptor ($ADORA_3$) activity on GSCs.
Methods: GSCs of U87 (human) and C6 (rat) were treated with the selective antagonist of $ADORA_3$ MRS1220 in combination with vincritine, an anti-tumor drug substrate of MRP1. The expressions of MRP1, $ADORA_3$, Bcl-2 and stem cells markers were analyzed by FACS, IF, IHC and western blot. The MRP1 activity was assessed by CFDA-retention assay. Cell viability was evaluated by MTT assay. MRS1220 was tested *in vivo* in subcutaneously inoculated C6 and U87 GSCs in rats and NOD/SCID mice, respectively.
Results: MRS1220 decreased the expression (U87 20.8 ± 0.1; C6 20.1 ± 8.6) and activity (U87 3.57 ± 0.52 fold; C6 3.95 ± 0.73 fold) of MRP1. GSCs incubated with MRS1220 + vincristine decreased the cell survival (U87 58.1 ± 5.1%; C6 49.8 ± 2.8%). *In vivo* assays demonstrated that MRS1220 + vincristine were able to mediate regression of tumors following seven days of treatment. Immunohistochemical analysis showed that tumors treated with MRS1220 + vincristine showed decreased expression of Bcl-2, GFAP and nestin.
Conclusion: GSCs can be chemosensitized through blockade of $ADORA_3$, by decreasing MRP1 extrusion activity, thus representing a new therapeutic alternative for GBM.

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P15-057
Reduced expression of RNF43 is associated with the presence of somatic mutation and poor prognosis of cholangiocarcinoma patients

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Ring finger Protein 43 (RNF43) encodes an E3 ubiquitin-protein ligase that negatively regulates Wnt/β-catenin signaling pathway. RNF43 reduces Wnt signals by selectively ubiquitinating frizzled receptors, thereby targeting these Wnt receptors for degradation. It has been shown that RNF43 is frequently mutated in cholangiocarcinoma (CCA). In this study, we determined RNF43 expression in CCA tissues and demonstrated the correlation between RNF43 expression and RNF43 mutation status, RNF43 polymorphism, clinicopathological features and prognosis of CCA patients. We found that RNF43 had a reduced expression in CCA. This reduced expression was correlated with the presence of somatic mutation. In addition, overall survival was also worst in patients with low expression of RNF43. However, there

was no statistically significant association of RNF43 expression and any clinicopathological features or RNF43 polymorphism (RNF43 rs3744093 and rs2257205 genotypes) respectively. These results indicate that RNF43 mutation might cause down regulation of the expression of RNF43 and RNF43 may play a crucial role during development and progression of CCA.

P15-058
Optimization of novel benzothiophene-3-carboxamide inhibitors of Aurora kinases

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Aurora kinases A and B are regulating several steps of normal cell division. However, several experimental data indicates that overexpression of Aurora kinases fosters malignant transformation, while their inhibition induces apoptosis. In human tumours Aurora gene amplification and/or protein overexpression are indeed common and correlate with poor prognosis. On the basis of these observations several small molecule inhibitors of Aurora kinases were developed in the last decade, but most of them failed in clinical experiments, so there is still no such drug in the market.

Therefore, we decided to look for new, ATP analogue Aurora kinase inhibitors based on previously unused scaffolds. To achieve this, we screened the Nested Chemical Library© of Vichem Ltd. using an *in vitro* recombinant Aurora A kinase assay. The best identified compound was a benzothiophene-3-carboxamide derivative. Applying this core structure, further 35 analogues were synthesised, showing even better enzyme inhibition. A subset of them also inhibited cell viability equipotent to VX-680 and MLN8054. According to flow cytometry experiments, this effect was due to the generation of abnormal, multinucleated cells and apoptosis induction as in the case of VX-680 or MLN8054 treatment. On the molecular level our compounds effectively reduced Aurora A and B autophosphorylation and phosphorylation of the dedicated Aurora B substrate histone H3 as well.

In all, we successfully developed unique, benzothiophene-3-carboxamide based inhibitors which attenuated Aurora kinase function and perturbed cell division the same way as other known Aurora kinase inhibitors do, giving promising new molecules for further preclinical evaluations.

P15-059
Loss of antiproliferative response attributed to ablated glucocorticoid receptor function in mouse skin carcinogenesis is compensated by N-bromoamine taurine

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Glucocorticoids (GCs) are steroid hormones used in clinical practice as anti-inflammatory agents. In cancer management, they are often included in combination regimens to enhance efficacy of anticancer agents and/or to mitigate chemotherapy-induced side effects. GCs exert their effects by activating glucocorticoid receptor (GR), a pleiotropic transcription factor with tumor-suppressive function. However, in practice, GC use has raised concerns, due to both suspected tendency of cancer cells to develop resistance to GCs and to possible positive correlation of GCs to skin carcinogenesis. These concerns further highlight the emerging need for finding alternative options to GCs, especially in GC-resistant cancers. To address these issues, we used a well-established mouse skin carcinogenesis study model comprising of a number of cell lines which represent distinct, progressive stages of the full range of skin carcinogenesis. Using MTT assays, western blot and immunofluorescence, we estimated the GC responsiveness in correlation with the GR status and localization. DNA binding activity of GR was estimated by EMSAs, whereas its functionality was tested by luciferase assays and endogenous expression of representative antiproliferative targets. We found an early insensitization of cells to GCs starting from papilloma stages and retained throughout the most aggressive stages. This is attributed to dysfunctional GR, which appears overexpressed, DNA binding-competent, but transactivation-incompetent from papilloma to squamous and finally to spindle cell lines. This loss of antiproliferative response of mouse cancer skin cells is bypassed by N-bromoamine taurine, a new generation, NSAID, small molecule investigational drug which is used interchangeably to GCs in several anti-inflammatory conditions.

P15-060
Identification and validation of angiotensin II type 1 receptor as a possible anti-cancer target in neuroendocrine tumours

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Neuroendocrine tumours (NETs) have been found to overexpress somatostatin receptors (SSTRs). This enabled the development of somatostatin analogs utilized for diagnostics and therapeutics. However, around 30% of all NETs do not respond to somatostatin based approaches, leading to an interest to find and characterize alternative cell surface targets.

A clear response of neuroendocrine cell lines to angiotensin II (ATII), the natural ligand of the angiotensin II type 1 receptor (AGTR1), was observed in a screening approach using different cell-based assays. This resulted in further experiments elucidating the role of ATII and AGTR1 in NETs.

First, quantitative real-time PCR revealed significantly elevated AGTR1 mRNA levels in neuroendocrine tumour tissue (n = 72) compared with healthy control tissue (n = 13). For the following establishment of autoradiographic protein detection and based on mRNA expression analysis, two AGTR1-positive (BON, H727) and two AGTR1-negative (LCC18, QGP-1) NET cell lines were chosen. Radioactive binding assays identified specific binding sites for ATII on BON and H727 cells respectively, with an affinity at nanomolar concentrations and a density between 50 and 200 fmol/mg protein. *In-vitro* receptor autoradiography using tumour xenografts of BON and H727 cells confirmed these data. Finally, patient samples will be tested for their AGTR1 protein expression. In addition, altered functional consequences of AGTR1 overexpression, e.g. in proliferation, migration, metastasis and secretion are under investigation.

AGTR1 overexpression was shown in various cancers and anti-hypertensive drugs that are already clinically used, might be

repositioned. Therefore, AGTR1 may be considered an interesting molecule for therapeutic approaches in NETs.

P15-061
Apoptosis induction of 2H-chromene derivatives on human breast cancer cells

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Background: Breast cancer is the leading cause of cancer deaths in the world wide.

The development of chemotherapy for breast cancer has been limited by the toxicity of treatment. The efforts to obtain compounds with a better activity profile that led to a chemotherapy drugs generation characterized by improved potency and absence of adverse effects is necessary.

Methods: A series of 2H-chromene derivatives were screened for their cytotoxic activity against three human breast cancer cell lines including MCF-7, T-47D and MDA-MB-231 by standard 3-(4, 5-dimethyl thiazol)-2,5-diphenyl tetrazoliumbromide (MTT) assay. Apoptosis, as the mechanism of cell death, was investigated morphologically by acridine orange/ethidium bromide staining and TUNEL (TdT-mediated dUTP Nick-End Labeling) technique. Caspase-3 colorimetric assay was used to determine the increase in caspase-3 activity in early stage of apoptotic cells.

Results: All compounds showed significant cytotoxic activity with inhibitory concentration (IC_{50}) values in the micromolar range. Further biological assessments including flowcytometric

analysis, AO/EB staining, TUNEL and caspase-3 activation assays, revealed that the selected two derivatives of 2H-chromen led to the induction of apoptosis through the activation of caspase-3 in breast cancer cell lines.

Conclusion: Cytotoxic and apoptotic effects of these compounds in human breast cancer cells indicated that some derivatives of 2H-chromens could be an excellent candidate for further pharmacological studies to discover effective anticancer agents.

Keywords: Chromen, Anticancer, Apoptosis

P15-062
Expression profiling of apoptotic proteins and their induction by Bcl-xL inhibitors in endometrial cancer cells

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Defects in apoptotic pathways can promote cancer cell survival and also confer resistance to antineoplastic drugs. The purpose of this study was to quantitate the expression of human apoptosis associated genes (93) in tissue from patients with endometrial carcinoma (ECa) and control patients without malignancies. We estimated also a cytotoxic effect of Bcl-xL inhibitors (ABT-737 and BH3I-1) in the human endometrial adenocarcinoma cell lines (Ishikawa and MFE-280). Furthermore, we investigated the mRNA expression levels of apoptotic genes in control and treated cells using TaqMan® Human Apoptosis Array. Between the carcinoma group and controls, 8 potential apoptotic genes were higher level, more than 2.0-fold difference. In contrast, cell lines treated with inhibitors to demonstrate changes in the levels of 52 genes. ECa are relatively resistant to ABT-737 and BH3I (individually), but we detected a lower resistance of Ishikawa to both inhibitors. Bcl-2, Bcl-xL, Bax, Mcl-1 and p53 protein expression was analysed by Western blotting. We found increased ration of Bax/Bcl-xL in treated MFE-280 cells. The expression of Bcl-2 did not change significantly in both cell lines treated by Bcl-xL inhibitors. In conclusion, our array results point to the importance of apoptotic pathways during the formation of endometrial carcinoma and also suggest that some members of the Bcl-2 family of proteins are modulated by ABT-737 and BH3I-1 molecules.

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P15-063
Assessment of breast cancer and melanoma cells transmigration through blood–brain barrier by electron microscopy

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Cerebral metastasis is an important issue in practical oncology. Brain metastases are derived from a primary tumor originate from another tissue, therefore mechanism of interaction between tumor cell and the blood–brain barrier (BBB) is important to be identified. The mechanisms of transmigration of different metastatic cells through the BBB and central nervous system (CNS) colonization are still unclear. For this study we used an *in vitro* experimental model based on the culture of cerebral endothelial cells with the 4T1 mouse breast cancer cell line and B16/F10 mouse melanoma cell lines. The interaction between the tumor cells and BBB were analyzed by transmission electron microscopy. We observed that the tumor cells are able to adhere to endothelial monolayer, followed by formation of protrusions and transmigration from the luminal to the basolateral side of the endothelial monolayers. Electron microscopy analysis showed that tumoral cells are able to migrate through the paracellular pathway, by disruption of the interendothelial junctions. Further studies are necessary to establish a way of interaction between the metastatic tumor cells and the blood–brain barrier, this provides important hints to guide us to anticancer therapies at the central nervous system level.

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P15-064
Investigation of BAG-1's effect in the regulation of autophagy

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BAG-1 (Bcl-2 associated athanogene-1) is an anti-apoptotic protein found in humans that belongs to the BAG-protein family. BAG-1 has three major isoforms that are translated through the alternative initiation sites, and these isoforms form various complexes that enable BAG-1 to be involved in various cellular processes mainly related with apoptosis, cell proliferation, metastasis, cell migration, hormone action and autophagy. To

date, some of the BAG-1 interacting partners were determined, and one of the well known interaction partner of BAG-1 is Bcl-2. Beclin-1 is a protein that has a central role in autophagy during periods of cell stress and extinguishes during the cell cycle. Beclin interacts with the anti-apoptotic Bcl-2. In this study, we aimed to understand the role of BAG-1 through the interactions of Bcl-2 and Beclin-1 in the regulation mechanisms of autophagy in MCF-7, MDA-MB-231 and MCF-10A cells. We observed that overexpression of BAG-1 leads to the upregulation of autophagic proteins like Atg7, Atg16 and Atg5. Also Beclin-1 showed an enhanced expression with BAG-1 overexpression. In conclusion, we think that BAG-1 as an anti-apoptotic protein may have role in the regulation of autophagy through its interactions with Bcl-2 and Beclin-1.

P15-066
Yersinia enterocolitica strains of different bioserotypes and genotypes exhibit inhibitory potential on papain-like cysteine proteases

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Cysteine proteases are hydrolases with the catalytic cysteine residue in the enzyme's active site. The most abundant cysteine proteases belong to the clan of papain-like enzymes (CA) and share a common fold with papain. They comprise cathepsins, lysosomal peptidases, which play multiple physiological roles, including: intracellular protein turnover, phagocytosis, antigen processing and bone resorption. On the other hand, overexpression and hyperactivity of various cathepsins may contribute to development of different pathologies, such as: osteoporosis, rheumatoid arthritis, atherosclerosis and cancer. Therefore, there is a need for finding or designing the effective and selective cathepsin inhibitors to be used in targeted therapies. Cysteine protease inhibitors have been identified in several microorganisms. In our research, we investigated whether *Yersinia enterocolitica* may produce such inhibitors. *Y. enterocolitica* is a Gram-negative enteropathogenic coccobacillus. Pigs are its main reservoir and it may cause a zoonotic disease (yersiniosis). We have chosen *Y. enterocolitica* strains isolated from aborted fetuses and sows. They had previously been classified into different biotypes and serotypes. Firstly, we carried out further characterization of the strains by pulsed-field gel electrophoresis. This technique revealed high genetic polymorphism among the strains. Subsequently, we identified inhibitory potential of the cell extracts of selected strains on papain and human cathepsin L (bovine cathepsin B was not affected). Some of the bacterial culture media also inhibited papain. Furthermore, we examined the effect of culture conditions on inhibitor and endogenous cysteine protease production. Initial purification of the inhibitor together with evaluation of its nature and binding mode were also undertaken.

P15-067
Do serum nectin-2 levels have a prognostic effect in patients with colorectal cancer?

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Nectins are a family of integral protein and immunoglobulin-like cell adhesion molecules involved in the formation of functioning adherence and tight junctions. Aberrant expression is associated with cancer progression, apoptosis and cell proliferation but little is known how these effects change in cell behavior. The objective of this study was to evaluate the serum levels of nectin-2 in regard to diagnostic, predictive and prognostic value in colorectal cancer (CRC) patients.

One hundred forty CRC patients were enrolled into this study. Pre-treatment serum nectin-2 levels were determined by enzyme-linked immunosorbent assay (ELISA) method. Age- and sex-matched 40 healthy controls were included in the analysis.

The localization of tumor in majority of the patients was colon (n = 81, 58%).The number of patients who received neoadjuvant treatment was 37. Of the patients who received palliative treatment, 24 had oxaliplatin whereas 22 and 9 had irinotecan and FU/capecitabine, respectively. Thirty-six and 15 of the patients who received targeted therapy had bevacizumab and cetuximab, respectively. The baseline median serum nectin-2 levels were significantly higher than in the healthy control group ($p < 0.001$). However, known clinical variables including response to CTX were not found to be correlated with serum nectin-2 concentrations ($p > 0.05$). Patients with elevated serum nectin-2 concentrations had significantly unfavorable PFS compared with those with lower levels (median 5.8 v 9.1 months, respectively, $p = 0.04$). On the other hand, serum nectin-2 levels showed no significantly adverse effect on OS ($p = 0.19$).

Serum levels of nectin-2 may have diagnostic and prognostic roles in patients with CRC.

P15-068
5-aminolevulinic acid-based photodynamic therapy procedure affects matrix metalloproteinase 2 activity in surviving SW620 cancer cells

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Photodynamic therapy (PDT) is a promising anticancer approach, utilising destructive capability of Reactive Oxygen Species (ROS), generated by the light-sensitive chemical agents – photosensitizers. Activation of photosensitizers by light of specific wavelength, as well as preferential accumulation of these agents in tumour cells, makes PDT a very precise tool targeting favourably abnormal tissues. PDT response may be induced by more than one cellular mechanism. Among numerous effects, PDT putatively influences motility of cancer cells and their metastatic potential, what can be related to the alterations in matrix metalloproteinase 2 and 9 (MMP-2 and MMP-9, respectively) expression levels. MMP-2 and MMP-9 are both secretory proteolytic enzymes, involved in the breakdown of extracellular matrix (ECM) components and the regulation of cell-cell and cell-ECM adhesion. Due to the fact that ROS may participate in the activation of MMP-2 and MMP-9 zymogens, the relation between activity of these enzymes and PDT procedure still remains unclear.

Here we report the effect of 5-aminolevulinic acid-based photodynamic treatment on the overall secretory potential and MMP-2 activity in SW620 colorectal adenocarcinoma cell line. As determined by BCA assay, PDT procedure causes significant increase in protein secretion during the first hours after treatment. This is in relation with higher activity of extracellular MMP-2, revealed by gelatine zymography. Population of surviving cells, obtained after consecutive 48 h of cultivation, presents

further loss of proteolytic potential, while the overall secretion level reaches the steady state.

P15-069
Intensification of extranuclear effects of cisplatin promotes cytotoxicity towards drug-resistant leukemic cells

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Cytotoxic effects of the anticancer drug cisplatin (cis-Pt) are realized not only at the nuclear level, but also by extranuclear mechanisms. We have shown that treatment with cis-Pt in a doses 1–5 μg/ml has no effect on viability of leukemic cells resistant to anticancer drug (L1210R), but was cytotoxic both towards rat thymocytes as precursors of normal lymphoid cells and towards leukemic cells, sensitive to drug (L1210S). Using the fluorescent probes DCF-DA, indo-1 and TMRE it was confirmed that the early cytotoxic effects of cis-Pt were realized by intense ROS production, cytosolic Ca^{2+}($[Ca^{2+}]_i$) increase and mitochondrial membrane potential ($\Delta\psi_m$) dissipation. The study indicates that L1210R cells are characterized by underload Ca^{2+}-store of endoplasmic reticulum, reduced $\Delta\psi_m$ and decreased efficiency of mitochondria as compared to L1210S, i.e. by remodulation of pathways leading to Ca^{2+}-dependent mitochondrial way of apoptosis.

To reduce cytotoxic effect of cis-Pt in normal lymphoid cells and to reinforce it in resistant leukemic cells the representative of carbon nanostructures fullerene C_{60} was used. C_{60} is shown to be an efficient free radical scavenger, but after photoexcitation C_{60} is able to generate ROS with high quantum yield. C_{60} appears to prevent cis-Pt-induced ROS production in thymocytes, but not in leukemic cells. When cis-Pt treatment of L1210R cells was combined with UV/Vis photoexcitation of accumulated C_{60} significant increase in ROS production, $[Ca^{2+}]_i$ and decrease of cell viability were observed comparing with separate treatment. These data indicate that intensification of extranuclear effects of cis-Pt promotes cytotoxicity towards drug-resistant leukemic cells.

P15-070
ACE2 associated with pulmonary inflammation and MMPs activities in acute lung injury by bleomycin treatment

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Anigotensin converting enzyme (ACE)/Ang II axis in renin-angiotensin system (RAS) contributes to idiopathic pulmonary fibrosis. ACE2, an ACE homologue, can degrade Ang II to Ang-(1–7) and limit Ang II accumulation, and play roles on pathological RAS-induced pulmonary fibrosis. Therefore, we investigated whether ACE2 plays protective effect against bleomycin-induced acute lung injury. C57BL/6 (WT) and ACE2-KO (knock-out) mice were applied. The mice were given a single dose everyday of bleomysin-aerosol inhalation (6 mg/kg; an anti-cancer drug) and physiological changes and survival of mice were monitored. The mice also were sacrificed for lungs isolation after 4 days treatment. After bleomysin-aerosol inhalation, a severe increase in resting respiratory and heart rate was found in ACE2-KO mice compared with those in WT mice. ACE2-KO mice stared to die after 4 days and without survival after 8 days treatment; whereas, no WT mice died during a period of 8-day treatment. Compared to WT mice, bleomycin-treatment could markedly increase pulmonary ACE, MMP-2 and MMP-3 activities, but decrease TIMP-2 and TIMP-3 activities in ACE2-KO mice. Pathological findings, including infiltration of BWCs and alveolar damage combined with higher immunokines levels were observed in ACE2-KO mice. Above pulmonary damages and MMPs/TIMPs changes in ACE2-KO mice received bleomysin-aerosol inhalation can be effectively attenuated by Lenti-ACE2 (recombined lentivirus that can overexpress ACE2). ACE2 deficiency may promote bleomycin-induced pulmonary inflammation, and further lead to abnormal lung MMPs/TIMPs activities, the enzymes involving tissue fibrotic process. It implies that ACE2 dysregulation may play pivotal roles in process of acute lung injury induced by bleomycin.

P15-071
Apoptotic genes expression in human neuroblastoma cells after apoptotic inhibitors treatment

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Neuroblastoma is an aggressive childhood tumour of the sympathetic nervous system. Resistance to therapy of high-risk neuroblastoma is a major obstacle to successful treatment. The aim of our work was to study response of human neuroblastoma cell line (SH-SY5Y) to apoptotic inhibitors *in vitro*. ABT-737 is selective anti-apoptotic Bcl-2 family member's inhibitor, while MIM-1 is Mcl-1 inhibitor molecule. Viability of cells and response to apoptotic inhibitors ABT-737 and MIM-1 was assayed biochemically by cytotoxic methyl-thiazol tetrazolium (MTT) assay. Neuroblastoma cell survival refers their different sensitivity to inhibitors treatment (individually). In second part of our study were quantitate differences in gene expression patterns by micro fluidic array technology. TaqMan® Human Apoptosis Array provided quantification of human apoptosis associated genes expression (n = 93) in SH-SY5Y cell line between apoptotic inhibitors treated groups (individually) and intact control group. We identified potential apoptotic genes (n = 13) that exhibited more than 2.0-fold difference in their expression level. Bcl-2, Bcl-xl, Bax, Mcl-1 and p53 protein expression was analysed by Western blotting. Identification of differentially expressed human apoptosis associated genes in SH-SY5Y cell line could help us to understand mechanisms of apoptotic inhibitors in neuroblastoma cells. Recognizing of neuroblastoma growth inhibition biological attributes still remains one of the most challenging questions worldwide.

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P15-072
Potential role of NLRX1 as a tumor suppressor and a predictor of sensitivity to oncolytic viruses

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NLRX1, a mitochondrial NOD-like receptor protein, contributes to inflammation, although its roles in cell death, metabolism and

tumorigenesis are poorly understood. NLRX1 affects activities of mitochondrial Complex I and Complex III and assists in maintaining homeostasis of ATP levels following treatment with TNF-α. A forced expression of NLRX1 compromises growths of cancer cells *in vitro* and suppresses tumorigenicity suggesting a tumor suppressor role. Different human cancer cell lines were examined for expression levels of NLRX1, tested for tumorigenesis in nude mice and for the sensitivity to a set of human non-pathogenic enteroviruses and mammalian orthoreovirus T1L. Overexpression of NLRX1 delayed tumor growth in nude mice while NLRX1 knockdown produced aggressively-growing tumors. We found that expression levels of NLRX1 correlate with sensitivity to the oncolytic viruses, both *in vivo* and *in vitro*. As NLRX1 is a component of antiviral innate mechanisms that are frequently lost in cancer cells we consider its potential usefulness as a prognostic marker for a prediction of therapeutic responses to oncolytic viruses.

P15-073
Ibuolcydine sensitizes human hepatocellular carcinoma cells to TRAIL-induced apoptosis via calpain-mediated Bax cleavage

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Tumor necrosis factor-related apoptosis-induced ligand (TRAIL) induces apoptosis selectively in cancer cells without affecting the majority of normal human cells. However, hepatocellular carcinoma (HCC) cells often display resistance to TRAIL-induced apoptosis. Ibulocydine (IB) is an isobutyrate ester prodrug of novel synthetic Cdk inhibitor and has an activity against Cdk7 and Cdk9. In this study, we show that treatment with subtoxic doses of IB in combination with TRAIL displays potent cytotoxicity in TRAIL-resistant HCC cells. Combination of IB and TRAIL was found to synergistically induce apoptosis through activation of caspases, which was blocked by a pan-caspase inhibitor (zVAD). Interestingly, the combination treatment induced cleavage of Bax, which was translocated to the mitochondria upon induction of apoptosis. Furthermore, the down-regulation of Bax expression by small interfering RNA effectively induced a reduction of cell death and loss of mitochondrial membrane potential (MMP) when cells were treated with IB and TRAIL. Finally, pretreatment of Hep3B cells with the specific calpain inhibitor effectively blocked IB plus TRAIL-induced cleavage of Bax and apoptosis. Although the expression of Mcl-1 and survivin were reduced by IB plus TRAIL, overexpression of Mcl-1 and survivin did not block cell death induced by the co-treatment. Collectively, our results demonstrate that IB increases TRAIL sensitivity of HCC cells via mitochondria signaling pathway by calpain-induced cleavage of Bax, suggesting that combined treatment with IB and TRAIL cells may offer an effective therapeutic strategy for HCC.

P15-074
Evaluation of the major capsid protein of trichodysplasia spinulosa-associated polyomavirus as a carrier for target epitopes

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Trichodysplasia spinulosa-associated polyomavirus (TSPyV) is a recently discovered human polyomavirus associated with a rare proliferative skin disease. The major capsid proteins VP1 of different polyomaviruses have been successfully expressed in yeast *Saccaromyces cerevisiae* and shown to self-assemble to virus-like particles (VLPs). Recombinant VLPs are highly immunogenic due to their repetitive structure and the capability to activate antigen-presenting cells. Therefore, the VLPs may represent a promising carrier for target epitopes to enhance their immunogenicity.

The aim of the current study was to evaluate the VP1 protein of TSPyV as a new platform for insertion of B cell- and T cell-specific epitopes. Chimeric VLPs harbouring selected foreign epitopes at certain surface-exposed positions of VP1 protein were constructed and produced in *S. cerevisiae*. In parallel, hamster polyomavirus VP1 protein harbouring the same epitopes was used. The immunogenicity of different chimeric VLPs was evaluated in a mouse model. The chimeric TSPyV VP1 protein-based VLPs induced a strong antibody response and T cell response against the inserted epitopes.

In conclusion, TSPyV VP1 protein represents a promising carrier for protein engineering and design of novel immunogens with a potential application as prophylactic and therapeutic vaccines.

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P15-075
Expression of GS28 in colorectal carcinoma tissues

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GS28 (golgi SNAP receptor complex 1) is involved in ER-golgi transport of proteins synthesized in ER, but almost unknown in another role. We observed the decreased expression of GS28 in ischemic hippocampus of rat brain. In this study, we examined the expression of GS28 in colorectal carcinoma tissues (n = 200). Formalin-fixed, paraffin-embedded tissue blocks were used to observe the GS28 expression by immunohistochemistry. Two independent pathologists who were blinded to the clinical information performed semiquantitative scoring of immunostaining. Records of patients' clinicopathological characteristics and follow up data were reviewed. The relationships between GS28 expression and clinicopathological parameters were analyzed. GS28 expression is increased in colorectal carcinoma tissues compared with adjacent normal tissues. The progressive expression of GS28 was significantly associated with the higher stage of colorectal carcinoma ($p = 0.034$), but independent to the lymph node invasion or distant metastasis. The relationship between GS28 and

EGFR expression is also observed ($p = 0.097$). In cell culture experiments with HCT116 (colorectal carcinoma) cells, GS28 siRNA-transfected (K/D) cells showed an increased cytotoxicity in cells treated with oxaliplatin, compared with that of control cells. Our data suggest that GS28 is a potential biomarker in colorectal carcinomas.

P15-076
Cotyledon extract of *Vatica diospyroides* Symington type SS induces apoptosis in colorectal cancer cells

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Vatica diospyroides Symington (VDS) extracts have been previously demonstrated anticancer properties in a series of breast cancer cell lines. In the present work, *in vitro* cytotoxic properties of the cotyledon extracts of VDS type SS on inhibition of human colorectal cells (PMF-ko14 and HCT116) proliferation were performed by using MTT assay. Based on apoptotic rate determinations, half IC_{50}, IC_{50} and 2-fold IC_{50} dose levels were used in Annexin V-FITC/PI binding analysis using FACS method. The acetone (ACC) and methanolic (MEC) extracts of cotyledon were highly active against both PMF-ko14 (IC_{50} = 3.25 and 4.84 μg/ml, respectively) and HCT116 (IC_{50} = 3.2 and 5.19 μg/ml, respectively) cell lines. After 24 h of treating, significant apoptosis induction was observed with the most extracts in a dose-dependent manner. The ACC extract at IC_{50} produced the lowest dead (10.2%) and highest apoptotic (79.2%) PMF-ko14 cells via apoptosis. Meanwhile, apoptotic rate in HCT116 cells had no significant different among concentrations of this extract used. Treating with MEC extract, the population of PMF-ko14 cells in viable stage increased (37.6 to 46.9%) whereas apoptotic cells declined (55.6 to 47.5%) continuously, with increasing dose level. Interestingly, the highest apoptotic cells (97.1%) found in IC_{50} MEC-treated HCT116 cells. The results indicated that both acetone and methanolic extracts of VDS type SS cotyledon induced apoptosis in PMF-ko14 and HCT116 cells. This suggests that the extracts may provide active ingredients for targeting colorectal cancer therapy.

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P15-077
Carbonic Anhydrases IX and XII as anticancer targets and their inhibitors

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Carbonic Anhydrases (CA) catalyze CO_2 hydration to bicarbonate and protons, participate in a number of essential or pathological biochemical processes such as respiration, pH regulation, calcification, gluconeogenesis, ureagenesis, lipogenesis, electrolyte secretion and tumorigenesis. Humans contain 12 catalytically active isoforms. Numerous CA inhibitors have been used as drugs against glaucoma, as diuretics and neural diseases. CA isoforms IX and XII are highly expressed in tumor tissue acidifying the environment of the cancerous cells and promote their survival and metastatic invasiveness. Their selective inhibition could potentially be helpful in the treatment of solid hypoxic tumors.

A series of fluorinated benzenesulfonamides with substituents on the benzene ring were designed and chemically synthesized. Human CA IX and CA XII were affinity purified from mammalian cell cultures and *E. coli*, respectively. Compound binding studies were performed using the fluorescent thermal shift assay, isothermal titration calorimetry and the inhibition was determined by the stopped-flow CO_2 hydration assay. X-ray crystallographic structures of the compounds bound to CA II and chimeric CA IX were determined. Bulky ortho substituents fit to the hydrophobic pocket in the active site of CA IX but not CA II. The strongest inhibitor of human CA IX achieved the observed affinity of 50 pM. However, the high affinity diminished selectivity and the compound which showed the best balance between affinity and selectivity properties bound with 1 nM affinity to CA IX. Compound effects in cells are being tested and their suitability for development into drugs is being evaluated.

P15-078
Evaluation of the biocompatibility of Gd-lymphotropic nanoparticles on RAW 264.7 cell line

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The safety of contrast agents is a major concern in clinical applications. The aim of our study was to investigate the biocompatibility of Gd-lymphotropic nanoparticles (HGdDOTA and GdDOTP) in murine macrophages cell line (RAW 264.7) used for lymph nodes MR imaging.

RAW 264.7 cells were exposed for 6 and 24 h to different concentration of Gd-nanoparticles (1, 2.5, 5 μM) in order to assess RAW 264.7 cell viability by MTT, Sulforhodamine B and LDH assays. For oxidative stress assessment, reactive oxygen species (ROS) generation, reduced glutathione (GSH) and malondialdehyde (MDA) levels were measured in untreated and treated cells with 1 and 5 μM Gd-nanoparticles for 6 and 24 h.

Exposure for 6 h to 1 and 2.5 μM Gd-nanoparticles did not affect the normal state of murine macrophages.

Our results showed that cell viability decreased after 24 h of exposure to 5 μM HGdDOTA respectively GdDOTP by about 24% and 44 % compared to control. No significant changes were observed in LDH activity. ROS generation in RAW 264.7 cells was increased only after 24 h by 21% and 76% for 5 μM HGdDOTA, respectively GdDOTP treatment, compared to untreated cells. In addition, GSH concentration decreased significantly by 20% for both types of nanoparticles after 24 h. Also, MDA level increased after 24 h by 20% and 36% in 5 μM HGdDOTA and GdDOTP-exposed cells, respectively, which can be correlated with the decrease of GSH level.

These data demonstrate that HGdDOTA nanaparticles present a higher biocompatibility compared to the GdDOTP ones.

P15-079
Identification of a novel class of lysosomotropic REV-ERB antagonist as an innovative anticancer strategy

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Inhibition of autophagy process is emerging as a promising anticancer strategy. We recently reported that the circadian nuclear receptor REV-ERBβ plays an unexpected role in sustaining cancer cell survival when autophagy flux is compromised and it can be a useful pharmacological target for an innovative combined anticancer strategy. Indeed, we identify a novel lysosomotropic REV-ERBβ ligand (ARN5187) with a dual inhibitory activity toward REV-ERB-mediated transcriptional regulation and autophagy, which showed a higher *in vitro* anticancer activity than the clinically relevant autophagy inhibitor, chloroquine (CQ), against breast cancer BT-474 cells.

By structure activity relationship (SAR) study we generated ARN5187 analogues with improved *in vitro* anticancer activity against a number of human tumor tissues cells. In particular, we identified compound **30** with a ten-fold higher cancer cytotoxicity compared to the hit. Biological evaluation of 30 revealed that the improved potency was mainly related to an increased antagonistic activity against REV-ERBβ. **30** decreased the viability of different tumor tissue cells at concentrations from 5 to 50 times lower than CQ, while it did not affect the viability of normal HMEC cells.

Our data strongly support that a combined autophagy and REV-ERBβ inhibitory activity may be a suitable novel anticancer strategy and identified a novel class of dual REV-ERBβ/autophagy, which provide a valuable scaffold for progressing new multi-target anticancer agents.

P15-081
miR-3158: a TAp73-induced target which inhibits epithelial-mesenchymal transition through downregulation of vimentin

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p73 is a *p53* family member that synthesizes the full-length TAp73 and the N-terminal-truncated DNp73 isoforms. Strikingly, TAp73 isoforms inhibit all demonstrated hallmarks and enabling characteristics of cancer, including invasion and metastasis. Anti-metastatic effects of the other two members of p53 family, i.e. p53 and p63, are mediated by crucial microRNAs (metastamiRs). However, to date, there are no published reports on p73-induced metastamiRs. In this study, using wound healing assays and Western blot, we showed that TAp73 isoforms differentially inhibit cell migration *in vitro* and alter levels of prometastatic EMT markers. To test whether this effect is miRNA-mediated, we searched for miRNAs that are upregulated upon TAp73 induction, using high-throughput approaches. We found that TAp73 isoforms induce a set of miRNAs with putative prometastatic targets. One member of this set, namely miR-3158-5p, was identified as a direct TAp73 target, using ChIP and luciferase assays. miR-3158-5p was under-expressed in breast cancer cell lines with high invasive potential compared to cell lines with low invasive potential. Importantly, miR-3158-5p downregulates the prometastatic molecule vimentin and inhibits epithelial-mesenchymal transition and invasiveness *in vitro*. Ongoing experiments are anticipated to validate other prometastatic targets of miR-3158-5p, as well as to fully elucidate the role of this yet uncharacterized miRNA in inhibition of aggressive and metastatic characteristics *in vivo*.

P15-082
Serum MCP-1 in pancreatic adenocarcinoma

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Chemokines may control the macrophage infiltrate found in many solid tumors. Monocyte chemotactic protein-1 (MCP-1/CCL2) plays a key role in the recruitment and activation of monocytes during inflammation. MCP-1 is small chemotactic protein that has been found in several kinds of tumor tissue samples and functions as key regulator of cancer progression. This study was conducted to investigate the serum levels of MCP-1 in patients with pancreatic adenocarcinoma (PA) and the relationship with tumor progression and known prognostic parameters.

Thirty-five patients with PA were investigated. Serum samples were obtained on first admission before treatment and follow-up. Both serum MCP-1 levels were determined using enzyme-linked immunosorbent assay (ELISA). Age and sex matched 32 healthy controls were included in the analysis. The median age at diagnosis was 61 years, range 38–84 years; 21 (60%) patients were men. The tumor was located in the head of pancreas in 24 (69%) patients. Median progression-free survival (PFS) and overall survival (OS) of the whole group were 13.7 ± 2.3 weeks (95% CI=9–18 weeks) and 48.0 ± 12.8 weeks (95% CI=23–73 weeks), respectively.

The baseline serum MCP-1 levels were significantly higher in patients with PA than in the control group ($p = 0.02$). Moreover, serum MCP-1 levels were significantly higher in the patients with low albumin and platelet levels ($p = 0.04$ and $p = 0.05$, respectively). However, serum MCP-1 had significantly affect on neither PFS nor OS survival ($p = 0.20$, and $p = 0.49$, respectively).

Although serum levels of MCP-1 assays were found to be diagnostic value, no predictive and prognostic value was determine in PA patients.

P15-083
Effects of GHRH on the regulation cycle of prostate cancer cells

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Growth hormone releasing-hormone (GHRH) is a hypothalamic peptide implicated in the progression of malignancy in various tumours. The balance between proliferation and death is crucial in a tumorogenesis process. In this regard, cell cycle and apoptosis control the number of cells and remove damaged cells. The aim of this work was to study the involvement of GHRH on cell cycle and apoptosis in prostate cancer (PCa). We used androgen-independent prostate cancer cells PC3. GHRH-treatment (0.1 μM) for 8 h caused a decrease in the number of cells in G1 phase (28%) and an increase in the G2/M phase (27%). These results above were accompanied by a decrease in the expression

of p21 mRNA (27%), p53 mRNA (15%) and p53 protein (30%). Furthermore, the treatment with GHRH for 2 h cause a decrease and an increase of Bax (pro-apoptotic) and Bcl2 (antiapoptotic) protein levels, respectively. These data showed a reduction in the ratio of both molecules (Bax/Bcl2), which means a decrease of apoptotic process. In conclusion, GHRH treatment helps to cell cycle activation and causes an anti-apoptotic effect in advanced PCa cells. The current findings shed more light on the involvement of GHRH on cell cycle and apoptosis, as signalling pathway in these prostate tumoral process.

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P15-084
Polyelectrolyte nanocapsules as a drug carrier for targeted cancer therapy

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The development of effective targeted therapies for cancer treatment is the one of the most important challenges of today's science. Among various approaches to targeted drug delivery to pathological sites in the body, two seem to be the most advanced – passive targeting (EPR effect), based on the longevity of the pharmaceutical carrier in the blood and its accumulation in pathological sites with compromised vasculature, and active targeting, based on the attachment of specific ligands to the surface of pharmaceutical carriers to recognize and bind to pathological cells.

Nanocapsules have the high application potential in medicine since they can be used as drug delivery systems as they can penetrate the cell membrane. Moreover, they can be functionalized to achieve targeted drug delivery.

The aim of our work was to develop the method of preparation of loaded nanocapsules and their surface modification for passive targeting. Nanocapsules containing anticancer drugs (e.g. Curcumine, Paclitaxel), were prepared by direct encapsulation of emulsion droplets in polyelectrolyte multilayer shell. The oil (chloroform) cores stabilized by an AOT/PLL (Poly L-Lizyne) interfacial complex were encapsulated with shells formed by layer-by-layer adsorption of polyelectrolytes using biocompatible polyelectrolytes. The average size of the obtained capsules was 100 nm. Surface of obtained capsules were modified by pegylation for passive targeting as the copolymer PGA-g-PEG was used as the topmost layer of capsules shell. *In vitro* anti-cancer activity of the nanoencapsulated anticancer drugs on CT26 CEA – mouse colon cancer cells were evaluated.

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P15-085
The integrated analysis of gene expression profiles related with aquired cisplatin resistance

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Acquired resistance to platinum-based antitumor agents such as cisplatin is a major barrier to the effective chemotherapy of many solid cancers. The aim of this study was to identify differentially expressed genes (DEGs) and analysis biological processes related to aquired cisplatin resistance in ovarian cancer. Using the integrative meta-analysis of expression data software tool, we performed a cross-platform meta-analysis of publicly available microarray datasets related to aquired cisplatin-resistant ovarian cancer. Then we conducted enrichment analyses and pathway analysis by using Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO). Protein-protein interaction (PPI) network analysis was performed using CYTOSCAPE software. We identified meta-DEGs between aquired cisplatin-resistant ovarian cancer cells and their parental cell lines. Interestingly, many of them were not involved in individual DEGs. The up-regulated gene with the largest effect size was H2AFZ, which is a highly conserved variant of histone H2A. The up-regulated gene with the smallest P-value was UBC, which is a polyubiquitin precursor. The down-regulated gene with the largest effect size was ALG5. Among the GO terms associated with the set of DEGs, the most significantly enriched was"mRNA metabolic process". PPI network indicated that UBC had a high degree and participated in many interactions. In conclusion, our meta analysis provide a comprehensive view of gene expression patterns associated with acquired cisplatin resistance in ovarian cancer and new insights for further investigation.

P15-086
Importance of HGMB1 serum levels in breast cancer patients

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Breast cancer affects ~ 12% women worldwide and results in 14% of all cancer-related fatalities.

HMGB1 is a nuclear protein that can bind and bend DNA. The unraveling of the post-translational modifications has led to a better understanding of the mechanism of its translocation and its function within the immune system. So HMGB1 is important in the development and survival of tumours.

We determine the serum levels of HGMB1 in the pathologically verified breast cancer patients (n = 95) before treatment and compare this results with the healthy controls (n = 30).

Our study group consists of 95 breast cancer patients in the University of Istanbul, Institute of Oncology and 30 healthy subjects who were blood donors undergoing regular physical and laboratory examinations with no evidence of any disease. The serum levels of HMGB1 were measured by enyzme-linked immunosorbent assay (ELISA). 95 cases of breast cancer were enrolled in the study. The mean (629,621), standard deviation (204,639), median (84,000) values in the breast cancer patients and the mean (48,200), standard deviation (10,480), median (10,480) values in the healthy controls were calculated by using SPSS software (SPSS 16, Chicago, IL, USA). Serum HGMB1 ($p = 0.00$) levels were significantly higher in patients wih breast cancer than the healthy controls. Statistical significance was determined with the Mann-Whitney U test.

Our data indicate that HGMB1 can be used as a diagnostic parameter for breast cancer. We believe HGMB1 can be a useful marker for clinicians to help decide the diagnosis of breast cancer.

P15-087
Deregulation of histone acetyltransferases (HATs) and deacetylases (HDACs) in urothelial carcinoma

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Background: Histone acetyltransferases and deacetylases are essential factors in gene regulation. HDACs are moreover well-established as cancer drug targets. The GNAT family of HATs (GCN5/PCAF) and the Class I HDACs 1, 2 and 3 are often deregulated in urothelial carcinoma (UC) tissues and cell lines. We evaluated the potential of specific targeting these enzymes for arresting proliferation and enhancing apoptosis of UC cell lines.
Material and method: We conducted siRNA-mediated knockdown of GCN5 or PCAF in 3 UC lines and of individual class-I HDACs in 5 UC lines. Levels of HATs, HDACs and marker proteins were determined by western blotting and qRT-PCR. Cellular effects were analyzed by ATP assay, colony forming assay, caspase assay and flow cytometry.
Results and discussion: Efficient siRNA-mediated knockdown reduced proliferation and inhibited clonogenic growth depending on cell line and HAT/HDAC, with knockdown of HDAC3 and double knockdown of HDAC1/2 usually most efficient. Western blotting suggested that siRNA-mediated knockdown of HDAC1 and HDAC2 may be counteracted by compensatory upregulation, but no compensation was observed for the HAT pair. Flow cytometry revealed slight increases in the sub-G1 fraction indicating induction of apoptosis after GCN5/PCAF and HDAC1/2 double and HDAC3 knockdown. Accordingly, caspase activity increased after PCAF or HDAC1/2 double knockdown.
Conclusion: Specific down regulation GCN5/PCAF reveals a potential for targeted therapy comparable to that of Class I HDAC in UC cell lines. Developing drugs that target individual HATs, as already available for HDACs, seems therefore promising for treatment of UC and other cancers.

P15-088
Strong down-regulation of tumor suppressor genes RB1 and CTDSPL is associated with aberrant expression of cell cycle regulation genes in non-small cell lung cancer

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Development of anti-cancer drugs involves searching for new targets for combined targeted therapy or gene therapy. The first step in this research direction is a comparative analysis of expression of genes involved in a complex network of interactions in tumors. RB1 protein, a key regulator of cell cycle, can be activated by another tumor suppressor CTDSPL/RBSP3, small CTD-serine phosphatase. In order to verify their functional connection we tested two sets of paired specimens representing two histologic subtypes of non-small cell lung cancer (NSCLC): adenocarcinoma (ADC) and squamous cell carcinoma (SCC). These two tumor suppressor genes were down-regulation simultaneously in the majority of NSCLC samples (75%, 18/24). In case of ADC, the mRNA level of both *RB1* and *CTDSPL* was lower in metastatic samples compared to non-metastatic ones ($p < 0.05$). Advanced quantitative expression analysis of 84 genes (Human Cell Cycle Regulation Panel, Roche) revealed dysfunctions of $p16^{INK4A}$-Cdk/cyclin D1-RB1 and $p53/p21^{Waf1}$ pathways in NSCLC. Over-expression of many genes from the panel was stronger in ADC compared to SCC and more pronounced at late stages. Twenty five genes (survivin, cyclins, Ser/Thr-protein kinases, transcription factors, phosphatases, etc.) that revealed the strongest expression gain (up to 100-fold) may be potential targets for combined targeted therapy of NSCLC and perspective markers for monitoring of disease progression. Our results concerning incremental inactivation of *RB1*, *CTDSPL* and other cell cycle control genes are in agreement with the continuum model for tumor suppression.

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P15-089
Investigation of inhibitory effects of 4-arylycoumarin derivatives on human glutathione s-transferase

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Cancer is one of the most leading causes of death and is projected as the primary cause of death in the future. Chemotherapy is the treatment of choice for many types of tumors; however it has some clear limitations, one of the major problems in the treatment of human cancer is the development of intrinsic or acquired resistance of malignant cells against chemotherapeutic agents. Therefore, it is very important to find replacements for drugs previously used or find suitable chemomodulators in order to reverse drug resistance.

The most important mechanism that involved in drug resistance is increased Glutathione S-transferase (GST) expression which inactivates anticancer drugs. GST enzymes, especially GSTpi isoenzyme, inactivate chemotherapeutic substances by conjugating them to glutathione. Various GST inhibitors have been synthesized and used to prevent drug resistance in patients undergoing treatment with chemotherapeutic agents. However, most of the existing inhibitors are either too toxic *in vivo* or are only effective *in vitro*. Considerable research has been conducted to identify naturally occurring plant polyphenols as potent inhibitors of GST activity. However, there are very few studies reporting the effects of coumarins on the activity of human GSTs.

In this study, in order to evaluate potential GST inhibitors as adjuvant to overcome chemotherapy resistance, we synthesized and investigated the effects of hydroxyl and/or methoxy substituted 4-aryl coumarin derivatives on the activities of human placental GST enzyme using 1-chloro-2,4-dinitrobenzene as a substrate. We identified 5,6-dihydroxy-4-(3,4-dihydroxyphenyl) coumarin as the most effective GST inhibitor with an IC_{50} value of 19,22 μM.

P15-090
The double-stranded RNA-binding protein DGCR8A, a major component of the microprocessor complex, bears anti-proliferative properties in cancer cells

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Introduction: MicroRNAs (miR) have been identified as a major force in cancer cell control. Long primary miRs are transcribed by an RNA polymerase and processed to precursor miRs. Subsequently, pre-miRs were exportated to cytoplasm and formation of mature miRs occurs. Processing of primary miRs in the nucleus is mediated by the microprocessor complex, comprising the RNAse endonuclease Drosha and the double-stranded RNA-binding protein DGCR8. In this study human DGCR8 was cloned and cellular functionality was characterized concerning to proliferation properties in prostate cancer (PC) cells.
Materials and methods: DGCR8 and shRNA directed against DGCR8 were cloned by standard techniques. Alterations in cellular growth were assessed by cell counting in absence and presence of anticancer drugs. miRs and proteins were analyzed by quantitative RT-PCR and Western blotting.
Results: Overexpression and knockdown studies in PC cells showed that DGCR8 is an anti-proliferative factor. Interestingly, modulation of DGCR8 protein was not accompanied by a shift of mature miR-1 and miR-21, which were utilized as model miRs for microprocessor complex activity. Basal expression levels of DGCR8 were modulated by all three drugs used in this study.
Conclusion: Notably, our data point at antiproliferative properties of DGCR8 in PC cells, even though we could not detect alterations in mature miR levels. Moreover, DGCR8 protein is involved in drug dependent cellular response and may play critical role in chemoresistance mechanisms. Thus, restoration of DGCR8 protein levels may offer new therapeutic approaches in anticancer therapy.

P15-091
ER resident protein expression increases upon Bag-1 overexpression in breast cancer cells

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Bag-1 (Bcl-2 associated athano gene-1) is a member of an anti-apoptotic Bag family that acts as an adaptor protein to regulate a wide variety of cellular processes, including proliferation, survival, transcription, apoptosis, tumorigenesis and motility. To perform these functions Bag-1 has three functionally distinct isoforms [Bag-1L (p50), Bag-1M (p46), Bag-1S (p36) and a minor isoform (p29)] that can interact with a diverse array of molecular targets such as Hsp70/Hsc70 molecular chaperones, components of the ubiquitylation/proteasome machinery, Bcl-2 apoptosis regulator, Raf-1 kinase, nuclear hormone receptors and DNA. In human malignant cells, Bag-1 induces expression of distinctive set of proteins to modulate cell survival. It is known that, in breast cancer cells, the expression of Bag-1 is enhanced. Yet, pathways involving Bag-1 or induced by Bag-1 expression are not understood clearlyIn this line, our research aims to reveal Bag-1 role in carcinogenesis related cell survival pathways. For this purpose, proteomic analysis based experiments were performed to find out the expressional differences between the proteomes of Bag-1 overexpressed and endogenously Bag-1 expressed normal and malignant breast epithelial cells, respectively. The identified differences led us to the idea that Bag-1 directs cell to survival by causing ER stress throgh the upregulation of critical ER-resident proteins.

P15-092
Magnetically responsive polyelectrolyte nanocapsules as carriers of therapeutic compounds

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In the past decades nanoencapsulation of therapeutic agents became highly promising field in nanomedicine. Nanocapsules are typically particles with sizes ranging from ~ 10 to ~ 100 nm consisting of colloidal core and thin shell. Nanocarriers enhance solubility of lipophilic, poorly water-soluble, or even water-insoluble drugs. Such carriers can be easily modified and functionalized to target and accumulate only in pathologically changed tissues/cells therefore improves therapy efficiency (e.g. cancer) in the same time reducing the side effect. The layer by layer technique (LbL) is a convenient method to form multilayer coverage on colloidal cores by sequential adsorption of charged species like polyelectrolytes, nanoparticles, proteins, organic complexes. Functionalization of nanocarriers by magnetic nanoparticles can be achieved by direct encapsulation of particles in liquid core or incorporation of it in polyelectrolyte shell.

The aim of this work was to develop the method of preparation of magnetically responsive, loaded nanocarriers. Nanocapsules liquid cores composed of hydrophobic phase (chlorophorm, toluene) and stabilized by surfactants (e.g. AOT) were prepared using low-energy nanoemulsification. Capsules' shells were formed by the LbL technique using biocompatible polyelectrolytes (Poly L-lysine as the polycation and Poly Glutamic acid as the polyanion). The model lipophilic drug, β-carotene and Fe3O4, was successfully encapsulated in the liquid core, moreover, magnetic nanoparticles were embedded into the polyelectrolyte multilayer shell. Toxicity test of synthesized magnetic nanocapsules were performed. This magnetically responsive drug nano delivery system may be a promising platform for future targeted therapies (e.g. cancer) or other biomedical applications (e.g. diagnostics).

P15-093
Increasing oncolytic potentials of viruses through optimization of codon usage characteristic to cancer cells

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Translation rates and correct folding of nascent protein chains heavily depend on existing repertoires of tRNAs representing synonymous codons. Dramatic differences in the repertoires are observed not only in distinct species, but also depending on physiological conditions of cells, cell cycle stages and proliferation rates. Assuming that during evolution viruses are adapting to conditions of the host we suggested that polioviruses, which in humans replicate in lymphocytes of intestinal Peyer's patches and

motor neurons, would have codon usage approaching the tRNA repertoires of non-dividing cells, while attenuated vaccine strains that were selected by multiple passages in cell culture would evolve toward a different codon usage characteristic to proliferating cells. As the bioinformatics analysis has confirmed the idea, we decided to optimize artificially poliovirus type 1 to make its codon usage more appropriate for the repertoires present in fast-growing cancer cells. We synthesized the optimized poliovirus genome and tested replication and oncolytic properties of the virus in different types of normal and tumor cells. We conclude that the approach could be further applied for the generation of new safe oncolytic strains belonging to other viral families.

P15-094
The protective role of chlorophylline-Cu complex on N-methyl-N-nitrosourea induced breast cancer model in Spraque Dawley Rats: glutathione and DNA damage levels

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Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Investigation shows that glutathione and related enzymes especially GST P1-1 are often overexpressed in tumor cells and are regarded as a contributor to their drug resistance. The GST P1-1 isozyme of glutathione S-tranferases family (GST) is thought to play an important role in cancer progression and resistance to chemotherapy because it is frequently overexpressed in cancer cells and drug-resistant tumors. Chlorophlline is one of these inhibitors and its inhibitor and antioxidant effect was investigated on chemically-induced breast cancer model in this study.

N-methyl-N-nitrosourea (MNU) was used for inducing carcinogenesis in female Sprague-Dawley rats. Their weight and tumor diameters were measured throughout 5 months. At the end of the study, all animals were sacrificed.

GST activities, Glutathione levels and DNA damage were investigated in liver and tumor tissues. While an important change in GST activities for all groups were not observed, we found that regenerated GSH levels with Chlorophylline treatment compared with control and MNU groups. Besides we determined that chlorophylline significantly reduced DNA damage.

Conclusion: The use of antioxidant molecules should be discussed in cancer therapy even if the GST inhibitor.

Keywords: Chlorophylline, DNA Damage, Glutathione, Glutathone S-transferases, Breast Cancer, N-methyl Nitrosurea

P15-096
Oncolytic activity of non-pathogenic human enteroviruses in humanized sublines derived from rat glioma cells C6

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Tumor cells lacking normal anti-viral response system are excellent targets for oncolytic viruses, and this property could be used for cancer therapy. Besides, the efficiency of virotherapyvaries substantially because of individual differences in cancer cases. The presence of host cell receptors used for virus entry on cancer cells may serve as an important determinant of sensitivity to virotherapy. Cell lines derived from rats are resistant to human enteroviruses as they do not express the appropriate receptors. The goal of the study was to develop a model of humanized cell line of rat glioma C6 by a transduction of human cDNAs for receptor proteins CD155, CD55 and CXADR. The receptor proteins fused with green or red fluorescent proteins and luciferase were transduced to a set of human cells as well as to rat C6 glioma cell line using lentiviral vectors, their expression on plasma membrane was visualized, and the permissiveness to a set of human enteroviruses was tested. We found that individual sensitivity of cancer cell lines to viruses strongly correlated with the relative expression level of the appropriate surface receptors (CD 155, CD55 and CXADR). Humanized rat glioma(C6)cell lines acquire different levels of sensitivity depending on the type of the transduced receptor. We conclude that the cell line could serve as a useful preclinical model for studying oncolytic activity of human non-pathogenic enterovirusesin the well-characterized rat models, both *in vitro* and in animal tumors.

P15-097
Generation and characterization of intracellular nanobodies to trace dynamic changes of endogenous vimentin in living cells

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Vimentin has become an important biomarker for epithelial-mesenchymal transition (EMT), a highly dynamic cellular process which is tightly associated with tumorigenic events and the development of metastasis. Here we report on the development of novel vimentin specific binding single domain antibodies, referred to as nanobodies. We demonstrate the application of these nanobodies in different biochemical and cellular assays. Most importantly, we generated intracellularly functional vimentin chromobodies by combining the nanobodies with fluorescent proteins. Using a cancer relevant cellular chromobody model in combination with automated high content imaging we were able for the first time to visualize and monitor dynamic changes of vimentin upon extrinsic or intrinsic stimuli. This versatile approach enable now detailed studies to evaluate the function of vimentin in the process of tumorigenesis and for the development of novel therapeutic compounds affecting EMT.

P15-098
Effect of the bioactive components of *Salvia absconditiflora* on gene expressions of HepG2 cell line

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The liver is the principal organ of metabolism in the body. It has specialized tissues consisting of mostly hepatocytes that regulates a wide variety of biochemical reactions. Liver disease caused about 10% of all human population. There are around 900 species of Salvia species, 95 of which are represented in Turkey consumed as herbal tea. They have been used since ancient times for more than 60 different diseases such as cancer, aging and etc.

Salvia absconditiflora is one of the endemic Salvia species grown in Central Anatolia, Turkey.

In this study, *S. absconditiflora* leaves collected in 3 months (April–May–June) were extracted with methanol. Antioxidant activities, total phenolic (TPC) and flavanoid contents (TFC) of *S. absconditiflora* leaves were characterized. Antioxidant activity results showed no difference compared to the months. In respect to the TPC and TFC, methanol extract of April showed the highest amount. Rosmarinic and Caffeic acid are the most abundant substances measured by RP-HPLC. Cytotoxic effects on HepG2-cell lines were examined via XTT colorimetric method and IC_{50} values were calculated. The lowest IC_{50} values for 48 h incubation are obtained for April. Effects of *S. absconditiflora* methanol extract on the expression of phase I and phase II detoxification enzymes in HepG2 cells were investigated with q-RT-PCR technique. Treatment of HepG2 cells with Salvia extracts induced the expression of certain CYPs (3A4, 1A1, 1A2, 2E1) but no differences were observed in GSTs (GSTM1 and GSTP1).

These results may have implications on the drugs which are co-administered with herbs like Salvia.

P15-099
Permeability of membranes is more susceptible to hyperthermia in cancer cells as compared to normal cells lines

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Hyperthermia is promising modality for cancer treatment that requires more detailed knowledge on molecular and cellular processes for rational development of treatment protocols. It is important to establish thresholds for thermal damage of various structures in human tissues that vary among tissue species as well as among normal and diseased tissues. Comparison of thermal susceptibility of biomolecules has revealed that among all cellular components, plasma membrane (PM) is the most sensitive to heating. The response of other cellular membranes was not yet more thoroughly studied in this respect.

The aim of this study was to compare the effect of 30 min hyperthermia (42°C) on permeability of PM and inner mitochondrial membrane (IMM) in Chinese hamster ovary (CHO), rabbit myoblasts, murine liver cancer (MH22A), human pancreatic carcinoma (PANC1) and human primary pancreatic adenocarcinoma (BXPC3) cells. The effect of hyperthermia on PM and IMM permeability was evaluated by fluorimetry and fluorescence microscopy analysis after staining cells with propidium iodide and JC-1 dye. The results showed that increase of temperature from 37 to 42°C increased permeability of PM and IMM (by 13% and 27%, respectively) in cancer cells stronger than in normal cells. The results of our study demonstrate for the first time that inner IMM is cellular component that is even more sensitive to hyperthermic treatment than cellular PM. Another important observation was that both PM and IMM in cancerous cells was more sensitive to the damaging effect of hyperthermia in comparison to the same membranes in normal cells.

P15-101
Effects of alcohol consumption on DMH-induced rat colon cancer

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Alcohol drinking is a potential risk factor for lifestyle related diseases like liver disease, cardiovascular disease and various cancer. Although alcohol is responsible for consider morbidity and mortality, an epidemiological study explains cardioprotective effect of low to moderate alcohol intake. Our objective was to investigate the effect of low to high alcohol intake on DMH-induced rat colonic cancer.

Material and method: 40 Male F344 rats were randomly divided into 4 groups: I group (water), II group (1% ethanol), III group (2% ethanol)and IV group (5% ethanol). All rats of 4 group were permitted free to access to water and various ethanol, and fed MF diet during the experimental. They were injected 1, 2-dimethylhydrazine (DMH, 20 mg/kg/wt) once a week for consecutive 8 weeks from 5 weeks of age. All the rats were sacrificed at the end of week 28 and colon were removed for examination of the number of aberrant crypt foci(ACF) by methylene blue staining. The tissue section was histopathologically examined for adenoma and adenocarcinoma of the colon, and immunohistochemically was studied with PCNA for proliferation index of tumor cells

Result and conclusion: Low 1% ethanol I group compared with control group, 2% and 5% ethanol group showed statistically lower number of adenoma and adenocarcinoma of the colon. I group compared with other group showed statistically lower number of well differentiated and moderately differentiated adenocarcinoma. The J-shaped relationship between alcohol consumption and colon cancer risk was confirmed in DMH-induced rat colon tumor of low taking alcohol group.

P15-102
Drug delivery to human endothelial and glioblastoma cells by poly(methacrylic acid)-graft-poly(ethylene glycol)-coated magnetic nanoparticles

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Although magnetic nanoparticles are non-invasive agents that have been initially developed for magnetic resonance imaging, recent advances suggest that they may be used for cell-specific targeting and drug delivery. Most of such efforts have focused on cancer chemotherapeutics, in order to increase efficacy and/or limit general toxicity. In the present work, we developed a series of hybrid magnetic drug nanocarriers (MDNs) via a self-assembly process of poly(methacrylic acid)-graft-poly(ethyleneglycol methacrylate) of various polyethyleneglycol molecular weights and graft densities. The MDNs produced display small hydrody-

namic diameter (50–100 nm), excellent stability in high ionic strength media and high drug loading for doxorubicin. These MDNs were tested for their uptake by cells, as well as biocompatibility, toxicity and effective drug delivery in both human endothelial and glioblastoma cells. None of the tested empty MDNs was toxic to cells; nevertheless, their interactions with cells displayed significant differences, as far as uptake of the MDNs is concerned. In all cases, though, their uptake by glioblastoma cells was significantly higher that the uptake of the same MDNs by endothelial cells. The doxorubicin loaded MDNs decreased glioblastoma cell growth to a significant degree, suggesting that they adequately release the drug when in the cell environment. Collectively these data suggest that MDNs warrant further exploitation for selective targeting of cancer cells.

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P15-103
Targeted near-infrared imaging of breast cancer xenografts using optimized CMKLR1-targeted peptide probes

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With cancer being still one leading cause of death, there is an urgent need for personalized diagnostics and therapies. Knowledge about molecular properties as overexpression of certain receptors is thereby offering a promising tool for targeted imaging and tumor selective treatment of cancer cells. One challenging target is the chemokine-like receptor 1 (CMKLR1), with its peptide ligand chemerin being an encouraging molecular entity for probe optimization.

Highly specific and affine peptide ligands for CMKLR1 were obtained by substitution of wild type chemerin-9 and analysis of structure-activity relationship. In consequence, we designed peptide conjugates with different linkers and fluorophores to gain novel probes for tumor targeting. The combination of near-infrared dye labeled peptides and an established tumor model in immunodeficient nude mice enabled tumor-specific imaging *in vivo*.

Our novel peptides demonstrated significantly improved properties compared to chemerin-9 concerning biological activity, affinity and metabolic stability. Beside other tumor entities like esophageal cancer, we could show chemerin receptor overexpression in the mamma carcinoma cell line Du4475. After establishment of the breast cancer model along with target negative tumors, near-infrared optical imaging revealed a strong target specific accumulation of our probes in mamma carcinoma tissue within twenty-four hours. Further expression analysis of the *ex vivo* tissue confirmed their target selectivity.

We found CMKLR1 to be overexpressed in the breast cancer cell line Du4475. With this, we had a model endogenously expressing our target to evaluate optimized chemerin peptides as stable ligands with high affinity for potential imaging applications such as mammography and intraoperative imaging.

P15-104
Activation of Beta-cateni/c-Myc signaling pathway by HN1 promotes growth and metastasis of Hepatocellular carcinoma cells

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Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide having high mortality. Previously, we found that HN1 is strongly associated with patient's survival in HCC. However, the biological function of HN1 on cell growth and metastasis in HCC cells remain largely unknown. The purpose of this study is to investigate the underlying molecular mechanism by which HN1 regulates metastasis in HCC cells (HepG2 and SNU 368). Silencing of HN1 significantly decreased the invasion of HCC cells by wound healing and mitrigel invasion assays. The mRNA levels of vimentin, beta-catenin, and uPA were significantly decreased in HN1shRNA HCC cells. Silencing of HN1 also significantly reduced protein levels of uPA, c-Myc, cyclin D1, p-beta-catenin. In addition, silencing of HN1 inhibited the growth of human HCC tumors in a xenograft mouse model. Therefore, our results suggest that HN1 regulates the migration and metastasis of HCC cells mediated through beta catenin and targeting HN1 may constitute a therpeutic strategy for HCC.

P15-106
Naïve and genetically-modified hMSCs exhibit anti-proliferative effects on human cancer cells

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The discovery that mesenchymal stem cells (MSC) bear the unique property of tropism towards tumors *in vivo* has rejuvenated the area of cancer cytotherapy. Genetically modified MSC (GMSC) acting as carriers for antitumorigenic molecules, and naïve MSC (nMSC), have been used against cancer, with strategies based on the cells directly or their paracrine effects. GMSC have shown good efficacy in preclinical models but their use has associated safety risks. The use of nMSC is controversial, as in some studies they have been reported as detrimental for tumor development, while in others, they have been found to support tumor growth, for reasons that remain unknown. We hypothesize that the outcome of cancer cytotherapy is dependent on the combination of MSC population and cancer cell type target, and the dynamics of their interaction. Here, we evaluated the paracrine effects of various MSC populations on the growth of four archetypal cancer cell lines in a comprehensive culture setup which included indirect co-culture and 3D culture. We then examined the transcriptome of two of these cell lines, on which the antitumorigenic effect was most prominent, to compare the expression pathways and regulatory networks mediating the latter. Global mRNA and microRNA array analysis of cancer cells revealed that the anti-tumorigenic effects are target-dependent, e.g. mediated by different pathways (metabolic, cell cycle VS complement, innate immune response) and are initiated by the same MSC secretome-derived stimuli. Analysis revealed a small set of genes and microRNAs which were expressed in common and comprise putative cancer markers/drug targets.

P15-107
Carbon nanotubes for efficient mitochondrial tumor targeting

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Cancer is uncontrollable growth of cells which are devoid of apoptosis. We developed a novel strategy ie Ligand mediated tumor targeting via carrier systems. Multiwalled Carbon nanotubes (MWCNTs) were used as it directly enters into the cell without passing through endo-lysosomes, large inner volume, distinct inner and outer surfaces & have ability to enter the cell by spontaneous mechanism. Thus, proposed work envisages Rhodamine-123 conjugated Paclitaxel loaded *functionalized*-CNTs to provide enhanced cell permeation in order to enhance mitochondrial availability of Paclitaxel.

The raw MWCNT were procured and purified, oxidized & then conjugated with rhodamine-123 by carbodimide method. The MWCNT's were characterized *in-vitro* for shape & size by Scanning(SEM) & Transmission Electron Microscopy(TEM), FTIR analysis, X-ray diffraction and zeta potential determined. Stability studies were performed at exaggerated conditions along with Hemolytic Toxicity Study. The Cell Cytotoxicity Study-MTT Assay was done using Hela cell lines. Mitochondrial localization was determined by CLSM study. The *in-vivo* part of the study comprised of determining the distribution of drug in various organs by fluorescence microscopy.

The Rhodamine-123 conjugated MWCNTs were prepared and characterized. The CNTs showed high paclitaxel loading, sustained release, and excellent biocompatibility as evident by *in-vitro* drug release and low hemolytic toxicity. MTT assay against HeLa cell lines suggested the potential anticancer activity of the developed system. CLSM study suggested that mitochondrial specific localization of Rhodamine-123 conjugated MWCNTs in HeLa cells.

Thus, Rhodamine-123 conjugated Paclitaxel loaded *f*-CNTs system has potential to provide an enhanced cell permeation and mitochondrial localization for effective tumour chemotherapy.

P15-108
Serum profile pattern in prostate cancer by proteomic analysis

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Background: Prostate cancer is the most common cancer in men and the second most frequent cause of cancer death. Proteomic approaches are continuing to make headways in cancer research by helping to elucidate complex mechanism for new biomarkers that could be translated to clinical applications.

Material and methods: Surface Enhanced Laser Desorption/Ionization-Time of Flight-Mass Spectrometry (SELDI-ToF MS) and two-dimensional gel electrophoresis (2-DE) were used to perform the proteomic biomarkers profile from samples. In the ProteinChip SELDI system, twenty-five serum samples from 15 prostate cancer and 10 controls were applied to 3 types of protein chips: CM10 (weak cation exchanger), Q10 (strong anion exchanger), IMAC30 (immobilized metal affinity).

Results: The proteomic spectra obtained were compiled, normalized, and mass peaks with mass-to-charge ratios between 2 and 50 kDa identified. Peak information were analyzed using univariate statistics, 20 significantly different protein peaks were detected, with AUC values ranging 0.750–0.930 and p values lower than 0.01.

The comparison of the protein profile between prostate cancer and control by 2-DE showed several differentially expressed proteins. These results confirm those obtained by SELDI-ToF-MS analysis.

BIOMARKER PATTERNS Software (BPS) was applied to generate multiple biomarker correlation with clinical phenotype and accurate and reliable predictive models.

Conclusion: Using two different proteomic techniques we have demonstrated that, the majority of the differentially expressed protein peaks detected by SELDI-ToF-MS and of protein spots revealed by 2-DE analysis can be considered discriminating markers of prostate cancer potential therapy.

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P15-109
The sensitivity of neuroblastoma cells to binase toxic effect depends on the expression of KIT oncogene

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Ribonucleases (RNases) are promising agents for use in anticancer therapy. RNases with potential antineoplastic properties were found in fungi, bacteria, plants and animals. An important property of antitumor RNases that can be potentially utilized for medicinal application is their ability to induce cell death by apoptosis. However, despite a large number of studies of the cytotoxic effect of RNases on cells, key elements of the signaling network and the mechanism of apoptosis induction are poorly understood. The object of our study is the microbial RNase *Bacillus intermedius* – binase.

Previously, we have shown that sensitivity of the acute myogenic leukemia Kasumi-1 cells to binase toxic effect depends on the expression of KIT and AML1-ETO oncogenes. We have hypothesized that the presence of such oncogenes in various tumor cells will determine their sensitivity to binase toxicity. It is known that neuroblastoma (NB) cells are frequently detected to be KIT positive, which causes their drug resistance. In this work we have studied cytotoxic effects of binase on three NB cell lines: SK-N-SH, SH-SY-5Y and SK-N-AS. We have shown that expression of the KIT oncogene in these cells is different. We have also demonstrated that the increasing level of KIT oncogene expression in NB cells leads to the rise of toxic effects of binase on these cells. These data allow to view binase as a perspective therapeutic for treatment of the KIT positive cancer cells.

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P15-110
Polylectrolyte oil-core nanocarriers of up-converting $NaYF_4$:Tm^{3+},Yb^{3+} nanocrystals for enhanced delivery and bioimaging in human ovarian carcinoma (SKOV3) cells

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There is increasing attention in development of theranostic nanocarriers that are intended to deliver separately hydrophobic drugs (mainly cytostatics for the anticancer therapy) as well as imaging agents (organic dyes, quantum dots or up-converting nanocrystals) to the target cells.

Thus, the present contribution deals with encapsulation of Tm^{3+} and Yb^{3+} co-doped $NaYF_4$ NCs in three types of multilayer nanocapsules obtained by layer-by-layer coating of silicone-core polymeric nanoparticles stabilized by cationic dicephalic-type surfactant, i.e., N,N-bis[3,3-(dimethylamine)propyl] dodecanamide dichloride, $C_{12}(DAPACl)_2$, via different, i.e, standard (PSS/PDADMAC), sugar-based (DEX/CHIT) and pegylated (PGA-*g*-PEG) polyelectrolyte shells. The biological potential of the obtained nanosystems was evaluated in cytotoxicity studies as well as in imaging of their intracellular distribution upon well characterized human cancer cell line – ovarian carcinoma (SKOV3) – and normal human vaginal fibroblasts (HVFs). DLS measurements confirmed the nanoparticle diameter below 200 nm, while AFM and TEM – its shape, morphology and the NCs encapsulation. Doppler electrophoresis provided a highly positive ζ-potential and colloidal stability. The fabricated long-lasting nanosystems exhibited good luminescence properties – under the 980 nm excitation, infrared and blue up-conversion emissions centered at 800 and 480 nm were observed. The performed studies point out to a opportunity for the development of complex theranostic modalities – a platform by co-encapsulating hydrophobic cytostatic agents and labeling $NaYF_4$:Tm^{3+},Yb^{3+} NCs within the oil-core compartment of the nanocapsules – that would allow investigation of penetration and localization of the multifunctional nanocarriers in various cancer cells.

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P15-111
Targeting the breast tumor in mouse model using undifferentiated mesenchymal stem cells and VEGFR-expressing endothelial-like cells

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Stem cell therapy of breast cancer is promising in controlling micro-metastasis and tumor progression. Stem cells with proliferation and differentiation potential such as mesenchymal stem cells (MSCs) are implicated in tumor control. However there are controversies in targeting tumors with either differentiated cells or undifferentiated progenitor MSCs. It is assumed that expression of vascular endothelial growth factor receptor (VEGFR) in cells at early stage of differentiation induction can help directing cells to the tumor site with abnormal capillary network. In the present study a mouse model of breast cancer were used to investigation of the efficiency of MSCs and endothelial cells derived from them to control angiogenesis-mediated tumor growth. The results of tumor size monitored for 4 weeks after tumor induction, suggesting that both MSCs and endothelial-like cells are involved in breast tumor regeneration and suppression. This was consistent with pathological characters in experimental groups of mice treated with either MSCs or endothelial-like cells in comparison with untreated group. In conclusion, using of VEGFR-expressing cells in the host tissue is an approach to target solid tumors with abnormal angiogenesis.

P15-112
A novel immunotherapeutic and anti-cancer drug GA-40

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Search nontoxic naturally-occurring substances that can cause selective destruction of cancer cells directly or by activating antitumour immunity are the two major strategies for development anti-cancer drug discovery. As a result of such works novel immunotherapeutic and anticancer drug GA-40 was created. Drug is a standardized complex of multiple peptides obtained from plant, widely used for medical cancer treatment since old times in Georgia. *In vivo* and *in vitro* preclinical studies and clinical trials of GA-40 shows, that it is not toxic and has no contra-indications, and is completely safe for the patients. It was shown that GA-40 has a direct apoptotic effect in malignant tumor cells and unlike chemical preparations has no negative effect on the normal cells. GA-40 direct action induces differentiation of human myeloid leukemia cells (HL-60) into non-growing mature granulocytes. GA-40 by its direct action on mononuclear cells causes the activation of anti-tumor cellular immunity. GA-40 activates cytotoxic-T cells, macrophages, production of Tumor Necrosis Factor and Interferon-γ, which play important role in the destruction and selectively remove cancer cells by the way apoptosis. GA-40 inhibits the release of vascular epithelial cell growth factor (VEGF) by cancer cells and the development of new blood vessels the process of revascularization in malignant neoplasm's preventing tumor growth and spread of metastases. GA-40 shows high antioxidant activity.

P15-113
Antioxidant activitis of *Salvia fruticosa* and its effects on HT-29 cell line

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Many epidemiological studies have revealed that there is a strong correlation between consumption of polyphenol-rich foods or beverages and the prevention of certain diseases such as cancers, cardiovascular diseases and aging. Phenolic compounds are abundant in all plants, therefore they form an integral part of the human diet. Salvia species, commonly known as sage, have been used since ancient times for more than 60 different ailments ranging from aches to epilepsy. There are around 900 species of Salvia, 95 of which are represented in Turkey including *Salvia fruticosa*.

In this study, $DPPH^{\bullet}$ and $ABTS^{\bullet}$ radicals scavenging activities, total phenolic and flavanoid contents of water extract of *S. fruticosa* was determined by spectrophotometrically. Rosmaniric acid, caffeic acid, gallic acid, syringic acid, quercetin and t-resveratrol contents of water extract of *S. fruticosa* were determined by using RP-HPLC. Cytotoxic effect of the extract on HT-29 adenocarcinoma cell lines was examined via XTT colorimetric and Trypan Dye Exclusion cell viability assay. Effects of the extract on the expression of phase I and phase II detoxcification enzymes in HT-29 cell line were investigated with q-RT-PCR technique.

Turkish endemic sage, *S. fruticosa*, is reported to be a promising medicinal plant, it has the potential to be used as adjuvant with chemoterapeutic agents to overcome the drug resistance occuring during chemotherapy. Further investigations are ungoing to reveal its bioactive components and their beneficial activities in biological systems.

P15-114
Demonstration of apoptosis via TUNEL assay and Codon 72 Polymorphism of p53 gene of MCF-7 and MDA-MB-231 cell lines upon treatment of Doxorubicin

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Apoptosis is programmed cell death which is characterized by morphological alterations such as shrinkage of cell and nucleus, roundation of cells, chromatin condensation and nuclear fragmentation as well as biochemical alterations which includes caspase activation, protein cleavage, DNA breakdown and cell outer membrane modification that results in phagocytic recognition. DOX is a potent chemotherapeutic drug approved by FDA which is clinically applied to treat various types of cancer including breast cancer. In the present study, drug-sensitive and drug-resistant breast cancer cell lines MCF-7 and MDA-MB-231 respectively were treated with 2 doses of DOX (200 nM and 800 nM) or not treated with the drug at all as control. After 48 h of incubation, TUNEL assay was performed with the aim of examining apoptosis via DNA fragmentation. It was demonstrated that both of the doses of DOX induced apoptosis in MCF-7 cells *in situ*. When compared to the control, effect of the drug was more significant with the dose of 0,8 μM. As for MDA-MB-231 cells, apoptosis was not triggered significantly with the dose of 0,2 μM and triggered slighty with the dose of 0,8 μM of DOX. Finally, in order to investigate codon 72 polymorphism profile of p53 gene, standard PCR was performed to amlify the p53 gene with DNA samples of both cells. FnuDI restriction enzyme was established to recognize and cut CGC but not CCC. It was demonstrated that these cell lines have different poymorphism profiles.

P15-115
Approaches to Multiple Sclerosis therapy by selective autoreactive B-cells depletion

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Multiple Sclerosis (MS) is a dreadful disease associated with inflammation in the central nervous system white matter and is thought to be mediated by autoimmune processes. Clonal expansion of B-cells, their antibody products, and T-cells, hallmarks of inflammation in the central nervous system are found in MS. Today, MS is usually treated with glatiramer acetate (Copaxone), injections of cytokines (IFNγ) and various monoclonal Abs (Natalizumab, Rituximab, etc.), and orally administered low-molecular-weight chemical drugs. Despite the variety of therapeutics available against MS mostly of them affect overall balance in patient's immune system. Development of novel more specific approaches to MS treatment is of high importance in modern pharmaceutics. We tried to create more selective and effective way to eliminate pathogenic B-Cells. We have designed therapeutic molecules based on immunodominant peptides of the myelin basic protein (MBP) fused with Fc domain of antibody.

We demonstrated functional activity of designed Fc-MBP chimeric molecules *in vitro* and showed that obtained fusion molecules were recognized by antibodies produced by autoreactive lymphocytes.

Further, on animal model of MS – experimental autoimmune encephalomyelitis induced in SJL/J mice, we provide selective elimination of autoreactive B-cells *in vivo*, by injection of the recombinant Fc-MBP molecules. Moreover, we found that injection of Fc-$MBP_{82–103}$ fusion molecule lead to depletion of $MBP_{82–103}$ specific B-cells and suppress formation of autoreactive B-cells population toward C-terminal part of MBP.

Our data suggest that Fc-MBP molecules are best choice for pathological B-cells depletion due to the excellent balance between their legibility and cytotoxic properties.

P15-116
Identification and characterization of small molecule inhibitors targeting DNA polymerase gamma for the treatment of cancers deficient in mismatch repair

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Synthetic lethal interactions between mutated oncogenes or tumor suppressor genes with molecules involved in the DNA repair pathways can be therapeutically exploited to preferentially kill cancer cells. Recently it was demonstrated that DNA polymerase gamma (POLG) inhibition in MLH1-deficient cells or tumors, which are defective in mismatch repair (MMR) displays synthetic lethality. Germline mutations in the MLH1 gene predispose to hereditary nonpolyposis colorectal cancer. MLH1 acts as tumor suppressor protein where tumor cells can have complete loss of MLH1 function, whereas normal cells retain at least one functional allele. Although MMR is involved in repair of base mispairs arising during replication, it also plays a role in the repair of oxidative damage induced DNA lesions. These lesions are repaired mainly by base excision repair (BER) pathway. POLG is involved in the mitochondrial BER and in mtDNA replication. Synthetic lethality of MLH1/POLG led to the accumulation of 8-oxoguanine in mtDNA. Thus, the inhibition of POLG may have a role for the selective treatment of cancer arising from MMR deficiency with MLH1 mutation. Therefore, to identify and characterize inhibitory molecules specific to POLG, we performed *in silico* screening of in-house and commercial small mol-

ecule libraries encompassing drug-like compounds. We demonstrate that two lead molecules, Obcun19 and Phany28, directly bind POLG by surface plasmon resonance analysis. These compounds were selectively lethal to HCT116 colon cancer cells lacking functional MLH1 by real time cellular analysis. They are under further investigation for the development of new anticancer agents.

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P15-117
Production of sugar-1-phosphates using nucleoside phosphorylases

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Nucleosides and their analogues are well known for their antiviral, antibacterial and anticancer activities. The multistep and highly expensive chemical synthesis process hinders their wide spread as therapeutic agents, as well as limiting their use in biological trials. Chemo-enzymatic methods are being used as the new approach for synthesis, due to their relatively low cost, high selectivity and being environmentally friendly. Nucleoside Phosphorylases are employed in the enzymatic synthesis of the nucleoside analogues. In the presence of phosphate, they catalyse the reversible phosphorylation of nucleoside to form nucleobase and a pentofuranosyl-1-phosphate (PF-1-P). To produce nucleoside analogues a one-pot synthesis is applied using two different enzymes, a sugar donor and a base or a base donor. As both enzymes compete for the PF-1-P intermediate the final nucleoside yield of the product might be decreased. The availability of the PF-1-P as a starting material for the nucleoside synthesis would increase the final product yield and allow for better optimization. In 2011, a chemo-enzymatic based method was developed using the *E. coli* NPs yet it was not active against some PF-1-P. Thermostable NPs were cloned, expressed and purified. These NPs showed high activity towards different modified PF-1-Ps. The availability of these NPs raised the idea of enzymatic synthesis of PF-1-P as a mean to improve the production of nucleoside analogues. Ribofuranose-1-Phosphate, 2'-deoxyribofuranose-1-Phosphate, Arabinofuranose-1-Phosphate and other modified PF-1-P were synthesized. Sugars were salted and purified. The nucleoside final yield increased by almost 50%, in some reactions, upon using the PF-1-P as the starting material.

P15-118
UV-Vis absorption studies of serum albumin binding with poly(D,L-lactide) nanospheres stabilized with Cremophor EL and loaded with hydrophobic cyanines

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The half-life of nanocarriers in the circulation and their biodistribution after parenteral administration are associated with the ability of plasma proteins adsorption. Unlike opsonins i.e. fibrinogen or immunoglobulin, serum albumin act as disopsonin and play important roles in protecting nanoparticles from opsonization. Nanocarriers coated with albumin do not undergo uptake by macrophages which phenomenon benefit prolonged half-life in the circulation system. The aim of this work was the UV-Vis absorption studies to evaluate the ability of bovine serum albumin, BSA, to biocomplex formation with nanospheres fabricated on a poly(D,L)-lactide (PLA) base by nanoprecipitation method. The Cremophor EL-stabilized (PLA)-nanospheres were loaded with hydrophobic cyanine-like dyes, zinc phtalocyanine (ZnPc) or indocyanine IR-780. The affinity changes of Cremophor EL/PLA/water nanospheres to BSA, predicted from nanocarriers geometry, remained in the order: Cremophor EL/PLA/water+ZnPc > Cremophor EL/PLA/water+IR-780. The dissociation constants K_D values 8.32 and 10.08 μM determined for the ZnPc or IR-780 nanospheres, respectively, confirms the differences in the ability to BSA adsorption. More stable nanocarrier-albumin bioconjugates longer remain in the circulation and can more efficiently reach the target cells. The nanospheres with ZnPc form the most durable complexes with BSA and may have the longest life-time in blood, that is, their distribution in the body may be more favorable than nanospheres Cremophor EL/PLA/water + IR 780.

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P15-119
VEGF and PDGF-AA over-expression have a functional role in promoting prostate cancer progression in rats

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Chronic inflammation of the prostate gland in men is characterized by cell infiltrates with an increased risk of prostate cancer. Taking this into account, our main aim was to determine if the levels of proteins, the vascular-endothelial growth factor (VEGF a potent mitogenic and angiogenic protein) and the platelet-derived growth factor-AA (PDGF-AA an essential autocrine regulator for VEGF expression) play key roles in prostate cancer. For this purpose, prostatic tissues were taken from healthy rats, a histological analysis was performed and the expression of both proteins was analysed. Rats were divided into three groups. The first comprised 6 animals with a body weight of 187.4 ± 9.8 g (young adult rats). The second group consisted of 6 rats with a body weight of 371.8 ± 21.8 g (adult rats). And the third group was made up of 5 rats with a body weight of 671.8 ± 21.8 g (old rats). The histological analyses showed the presence of spontaneous prostatitis in the adult rats, prostate cancer in old rats was confirmed whereas none of the young adult′s analysis revealed prostate inflammation. In addition, increasingly high levels of proteins, PDGF-AA and VEGF were detected by Western blot. Results show that the VEGF increases about 50% in adult rats and rises to 85% in old rats while the PDGF-AA increases about 80% in adult rats and rises to 130 % in old rats These results indicate that the levels of both cytokines can be associated with prostate cancer and could potentially be used as biomarkers in prostate cancer detection.

P15-120
Production of antileukemic L-asparaginase by filamentous fungi isolated from Brazilian Savanna

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Microbial asparaginase has attracted considerable attention since the demonstration that has ability to hydrolyze the amino acid asparagine and, consequently, their antitumor activity. The reduction of plasma levels of asparagine leads to the inhibition of the leukemic cells' protein synthesis, causing its death by apoptosis. Some species of filamentous fungi are producers of enzymes including asparaginase. Brazil is internationally recognized for its rich and exuberant biodiversity, being a promising source of new enzymes and metabolites of therapeutic value. In this regard it is relevant to the search for new sources of L-asparaginase, seeking to maximize the chances of success in the therapeutic process; modulation of improving affinity binding site for the enzyme performed by the optimization of treatment time intervals and doses, and even reduction of toxicity related. In the present study, 41 fungal strains were isolated from soils and plant materials collected from different parts of Brazilian Savanna. Screening for production of L- asparaginase was performed in a Petri dish containing modified Czapek Dox medium (MCD), pH 6.2 and supplemented with phenol red (0.009%), which was used as an indicator of L-asparaginase production. Among the 41 fungi strains isolated and tested, 22 showed the formation of a red halo, possible indicative of enzyme production. These promising strains were cultivated in liquid medium and the high asparaginase activity was identified in broths fermented by two endophytic fungi isolated from Savanna plants (not yet identified), and one isolate of soil, *Aspergillus sydowi*.

P15-121
Targetomics, microarray-based screening for the identification of new drugs against *Leishmania braziliensis*

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Targetomics is an approach for identification of new targets specific in diseases such as cancer, malaria and leishmaniasis. One group of these targets are heat shock proteins (HSPs) belonging to the chaperone machinery which are involved in ATP-dependent protein folding. HSP inhibitors hinder proteins folding, which may lead to malfunction activities in cells with oncogenic or pathogenic potential. The aim of our study is to identify novel inhibitors against HSPs in different host cells. These novel inhibitors are geldanamycin derivatives and bypassed from chemo-biosynthetic synthesis using a block mutant from *Streptomyces hygroscopicus*.

To study putative drug binding on Hsps, a miniaturized protein microarray was developed which allows to monitor binding of fluorescent-labeled ATP and displacement by inhibitors on spotted purified full length HSPs under parallelized conditions and consumes only subnanolitres volumes for a single spot. ATP binding was studied for several HSPs obtained from *Leischmania braziliensis* HSP83 (Lb_HSP83), human HSP90α (HS_HSP90α), a plant HSP83 and some bacterial Hsps. We can show that ATP binding was competed by different inhibitors with IC_{50} values in the range of 2 nM to 0.1 μM and Z-factor between 0.50 and 0.75. Our investigation about sixty different possible inhibitor candidates demonstrate that several geldanamycin derivatives including HK568j2, HK571d, HK581F10 and Geldanamycin semisynthetic derivative 17AAG have a high affinity to Lb_HSP83 and HS_HSP90α. The idea of this concept is to select pathogenic specific inhibitors by systematic analysis of the inhibitor pattern.

P15-122
Boosting NAD(P)$^+$ biosynthesis with NAD(P)$^+$ intermediates and monitoring mitochondrial NAD(P)$^+$/NAD(P)H pool by means of fluorescence-based techniques could be a strategy for preventing and treating woman's cancers

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NAD(P)H autofluorescence has wealth information on mitochondrial conditions since autofluorescence chromophores are related to mitochondrial functions, metabolic activities (Schuchmann et al, 2001; Niesner et al, 2004; Skala et al, 2007; Schweitzer et al, 2007; Yu and Heikal, 2009; Gukasyan and Kao, 2009) and bioenergetics.

Based on the central involvement of metabolic tumour mitochondrial alterations in cancer, therapeutic normalization of tumour cell metabolism might interfere with the expansion of residual and breakthrough clones. Brunhilde Felding-Habermann and colleagues, in their experimental work used an elegant approach based on expression of the yeast NADH dehydrogenase Ndi1 in human tumor cells to investigate a cause-and-effect relationship between aberrant mitochondrial complex I activity and malignant progression in breast cancer. These researchers analyzed metabolic alterations caused by mitochondrial complex I malfunction and translated the information gained into novel therapeutic approach against breast cancer progression. Current standard of care for cancer patients relies primarily on chemio-and-radiation therapies aimed to killing the tumour cells. Evolutionary models predict that selective pressure imposed by these approaches causes survival of resistant clones that eventually reactivate the disease (Merlo et al, 2012). Accordingly a combination of standard therapy con NA(P)$^+$ precursor treatment (i.e. NMN) may halt cancer progression and prevent relapse. Thus, a new age for cancer therapy will be inaugurated and the old coenzyme NAD(P)$^+$ will assist the medical community for a novel approach on 4P Medicine (viz Personalized, Predictive, Preventive and Participatory).

P15-124
Synthesis of sugar-modified nucleoside analogues by novel N-deoxyribosyltransferases – from screening to application

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Modified nucleosides are well known drugs due to their antiviral, antibacterial or anticancer activities. The state-of-the-art chemical synthesis process is often inefficient due to a number of side products and in many cases low final product yields are observed. Chemo-enzymatic methods are a new approach for synthesis of modified nucleosides due to relatively low costs, high selectivity and the environmentally friendly aspect. Several classes of enzymes are currently used for the enzymatic synthesis of nucleoside analogues including nucleoside phosphorylases (NPs) or N-deoxyribosyltransferases (NDTs). For the production of sugar-modified nucleoside analogues the use of NDTs seems to be very promising as they show a higher promiscuity for modifications in the 2′ or 3′-position of the nucleoside. In a Parallel-Screening-and-Process-Development (PSPD) approach the expression of novel NDTs from mesophilic and thermophilic microorganisms in *Escherichia coli* was optimized directly with different fed-batch type of fermentation protocols (EnPresso®). The screening included various tags for purification and the activity analysis on different 2′-modified nucleoside analogues.

P15-125
The role of antiviral innate immunity mechanisms abrogation in the acquisition of sensitivity to oncolytic viruses

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A loss of innate mechanisms of antiviral defense is one of the hallmarks of cancer. Cancer cells tend to abrogate functions that are important for the organism but impose a burden for the propagation and expansion. As the result, cancer cells are generally more sensitive to viruses providing a basis for oncolytic virus therapy. Antiviral mechanisms are complex and act differentially in response to different viral families, while inactivation of the mechanisms may occur in different component of the pathways. To study variations in the acquisition of sensitivity of cancer cells to different viruses we created cell models in which components of antiviral mechanisms were blocked by RNA interference. The affected genes related to mechanisms of IFN induction (MDA-5, RIG-1, TLR3, MAVS, IRF3) and IFN response (IFNAR1, STAT1, IRF9). Testing virus sensitivity was carried out either in the absence, or after treatment with INF. Knocking down the genes in different cell lines correlated with changes in specters of sensitivity to certain entero, reo, rhabdo and paramixoviruses. We conclude that molecular genotyping of individual tumors might form a powerful personalized approach toward choosing the most efficient type of oncolytic viruses suitable for the patient.

P15-126
Curcumin induced-apoptotic cell death in GH overexpressed MDA-MB-231 breast cancer in time dependent manner

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Curcumin, a polyphenolic substate isolated from *Curcuma longa*, has therapeutic efficiency in frequently seen cancers such as colon, prostate, melanoma, lung and breast cancer cases. A pituitary-derived hormone; Growth hormone (GH), induces postnatal breast gland development and the upregulation of GH overexpression was determined both in serum levels of acromegaly patients and breast tumor samples. In this study, our aim is to evaluate the potential apoptotic efficiency of curcumin in wt and GH+ MDA-MB-231 breast cancer cells. For this purpose, PC3.1 plasmid with GH gene insert was transfected by liposomal agents in MDA-MB-231 breast cancer cells and cells with forced GH expression have been selected by Neomycin (G418). Both wt and stably GH expressing MDA-MB-231 breast cancer cells were treated with increasing concentration of curcumin for 24 h and 48 h. According MTT cell viability results, GH+ MDA-MB-231 cells showed a resistant profile against curcumin treatment for 24 h [wt (56 %) versus GH+ (70 %)], but this resistance was accomplished following 48 h curcumin treatment [wt (50 %) versus GH+ (57%)]. Moreover, it was shown that 20 mM curcumin treatment inhibited the cell proliferation in time dependent manner in both wt and GH+ MDA-MB-231 cells. Although GH overexpression induced cell growth and leads a more aggressive profile compared to wt MDA-MB-231 cells, time dependent curcumin treatment overcome this resistance and induced apoptotic cell death *via* suppression of autocrine GH expression in MDA-MB-231.

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P15-127
Synthesis and biological evaluation of a novel series of Cyanoacrylamide derivatives as anticancer therapy

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Antitumor evaluation was performed on a novel series of acrylamide incorporated benzo[*d*]thiazole compounds. The structure of these compounds was confirmed by different spectral tools analysis. Concerning the antitumor activity, the two molecules **5** and **6** exhibit strong cytotoxic effects against human breast cancer (MCF7). Human liver carcinoma (HEPG2), and prostate cancer (PC3). It is believed that the cytotoxic effect of these compounds was attributed to different cellular pathways including tyrosine kinase inhibition (Gazit *et al*, 1996), tubulin polymerization inhibition, and cell death via apoptosis. On the other hand, they showed much less toxic effect against the normal HBF4 (normal melanocytes) cells. Also we are still trying to study apoptotic effect, cell cycle arrest, and DNA fragmentation and P53 expression as molecular tools to know the inhibition mechanism of the new drugs. Also, it is expected that all new synthesized compounds will exhibit a promising antibacterial activity through binding to sterol components of cells or alteration in cell permeability and cell death (Ambisome, Astellas Pharma US, Inc.). Antiviral assay will be tested for all synthe-

sized compounds as a future study against *vesicular stomatitis virus (VSV)*.

P15-128
Application of innovative drugs against tumor

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It is an ultimate trend to face tumors by using new components. This strategy is applying in numerous labs in many countries of the world.

Especially the natural compounds are the preferred ones. Compared with synthetic drugs the natural ones demonstrate a higher anti-tumorigenic performance and less side effects.

These drugs activate numerous stress pathways in cancer cell and lead it toward capitulation. I administered indole-3-carbinol, a not well known drug, extracted from broccoli, in glioblastoma multiform cells. I took some interesting results like: due to the application of indole-3-carbinol in glioblastoma multiform, their cell vitality is reduced. The causes are the cell cycle arrest at G_0G_1 stage and necrotic cell mortality.

Combination of drugs is an optimal solution. I tried to combine indole-3-carbinol with temozolomide. I obtained satisfactory results. The synergism between the two drugs lowered cell vitality compared with those samples where was applied a single drug.

I believe that in a close future the tumor will be healed. Studying the effects of new drugs in cancer cells is a promising manner to strike on target. Previous works have shown that drug administering gives some hopeful outcomes. It is needed to continue the studies in this field to achieve a proper result.

P15-129
Synthesis of novel peptidyl platform multitargeted anticancer drugs and its immobilisation on Ag-, Au- and Pt-functionalized magnetic silica nanoparticles

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At present, effective anticancer substances designing is one of the major problem modern medicine. Current anticancer therapy is based on cytotoxic substances that affect biochemical mechanisms of the cell by means of interfering with its bioactivities, consequently preventing its growth and ultimately destroying it. So one of the major disadvantages of such drugs is its insufficiency of their unspecificity and low targeting properties.

One of the ways to solve this problem is to design the "multitargeted drug platform" based on combining of various types anticancer drugs in one compound. In our research, we performed the synthesis of novel peptide-based anticancer drugs by attaching of several known namely Camptothecin (DNA Intercalator) and Chlorambucil (DNA alkylator) to specific carrier though the amino acid linkers to provide a drug delivery platform. Next this platform was conjugated with a specific peptide carrier which binds to Somatostatin receptor, because the latter is overexpressed in almost 60% of solid tumors.

It is known, that Ag-, Au- and Pt-functionalized silica nanoparticles have wide applications in many fields of research, in particular, for cancer treatment. So the next step was to synthesize and characterize such nanoparticles wich could be used as delivering agents.

We also modified nanoparticles by immobilizing of magnetic nanobeads onto them, resulting polyfunctional drug-delivery system, which successfully used to load previously-obtained multitargeted compound.

We suppose that such model that we propose, will help to improve pharmacological activity of drugs by optimizing pharmacokinetic properties (adsorption, distribution, metabolism and excretion) and drug release control and some others.

P15-130
Targeted delivery of therapeutics using genetically engineered commensal bacterial protoplast-derived nanovesicles

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With increasing incidents of patients diagnosed with cancer every year, the technologies for drug delivery have also improved to help patients undergoing chemotherapy. However, despite the advancement of the delivery techniques, targeting the chemotherapeutics to solid tumor in order to increase the drug efficacy and reduce the undesirable adverse effects, still remains as a challenge. The complication arises mainly by the difficulty in the molecular manipulation and conjugation of bio-specific molecules to the vehicles. Therefore, here in this study, we newly developed nanovesicles derived from genetically engineered safe commensal bacterial protoplast having no outer membrane components as the next-generation targeted drug delivery system.

To prepare bacterial protoplast nanovesicles, protoplasts expressing tumor-targeting moiety on the surface were serially extruded through nano-sized membrane filters. Electron microscopy images revealed that the prepared protoplast nanovesicles had vesicular structure of around 100 nm in diameter. Western blotting analyses showed that the protoplast nanovesicles expressed tumor-targeting moiety on the surface without any toxic bacterial components. Moreover, when loaded with chemotherapeutics, the protoplast nanovesicles were specifically targeted to the cancer cells *in vitro* and *in vivo via* receptor-mediated endocytosis and induced dose-dependent cytotoxicity. Furthermore, drug-loaded nanovesicles efficiently prevented the growth of tumor when administered to tumor xenograft mice models without adverse effects.

Taken together, the results obtained herein suggests a strong potential of protoplast nanovesicles as the advanced targeted drug delivery vehicle that is both safe and effective. This study has the potency to optimize and contribute to the development of future chemotherapy.

Chem Biol S3, Functional Glycobiology – from Mechanism to Disease

P16-003-SP
Biosensing of intact glycosylphosphatidylinositol-anchored proteins in serum as biomarkers for stress-induced diseases

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A novel "phenomenological" approach may lead to biomarkers for the prediction of stress-induced disorders with higher infor-

mative value than traditional phenotypic, peptidic and genotypic ones. It relies on the demonstration and biophysical characterization of glycosylphosphatidylinositol-anchored membrane proteins (GPI-APs) associated together with phospholipids within extracellular complexes (ECGAPP). ECGAPP have been shown to be released from the surface of relevant cells through non-classical secretory mechanisms in response to metabolic stress (e.g. diabetes) and are assumed to differ in level, morphology and biophysical properties. However, ECGAPP in body fluids of diabetes patients have not been studied so far, presumably due to conceptual restrictions and technological challenges.

A novel type of chip-based biosensor will be used for detection and biophysical characterization of ECGAPP relying on the generation of horizontal surface acoustic waves (SAW) within the gold surface of a microfluidic four-channel chip. Any interaction of (macro)molecules with the gold surface will result in corresponding changes in frequency and amplitude of the SAW.

These alterations reflect mass loading (binding of ECGAPP) to and biophysical properties (size/shape of ECGAPP) at the chip surface. Preliminary data indicate that signatures recorded in course of successive binding of ECGAPP to the chip surface *via* specific interaction with alpha-toxin (detection of GPI-APs) and annexin (detection of phospholipids) significantly differ between plasma from normal and diabetic mice. Albeit SAW biosensing *per se* does not enable the delineation of the type of ECGAPP, the SAW summation/subtraction signatures will be characteristic for the overall amount/biophysics of the ECGAPP in a given sample.

P16-004-SP
Interaction analysis between sugar chain and aromatic residue in mammalian protein

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Glycosylation, one of the PTMs, is known to affect protein folding, protein functions and enzyme activities. In O-glycosylation, various sugar chains are attached to motif residues (usually Ser or Thr) by glycosyltransferases in the Golgi body. However, consensus sequences for protein glycosylation have not been clarified completely. Lectins, carbohydrate binding proteins, have been found in various organisms. The significance of aromatic residues in binding domains of lectins to bind carbohydrate has been pointed out in the previous reports. Interaction analysis between a carbohydrate and an aromatic residue in lectin is useful for clarifying glycosylation mechanism. Therefore, correlation between an aromatic residue and a sugar chain within a unit ball around three-dimensional recognition motifs of each glycosyltransferase was analyzed.

In this study, to find three-dimensional recognition motifs based on each sugar type, the three-dimensional coordinate data of atoms in amino acids around the O-glycosylation site were analyzed. Mammalian protein data with O-glycosylation was extracted from the Uniprot KB/Swiss-Prot 2014_01 and the Protein Data Bank release 2014_01. Amino acid propensities which are depending on each sugar type within a unit ball in which center was an O-glycosylation site were calculated. Correlation between sugar chain and aromatic residues within a unit ball was analyzed. The existence of the aromatic residues depending on each sugar type was found through comparing amino acid propensities in each sugar type. Environment factors around glycosylation sites based on each sugar type were discussed in this study.

P16-005-SP
Analysis of GOLPH3 depletion on protein glycosylation in human glioblastoma multiforme T98G cells

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Glioblastoma multiforme (GBM) is the most common and most aggressive malignant primary brain tumor in humans. Despite enormous advances in our understanding of this type of brain cancer, little is known about the different aspects of the secretory pathway in GBM tumorigenesis, and in particular the contribution of the Golgi apparatus is poorly understood. GOLPH3 is a highly conserved phosphoprotein of the Golgi apparatus. Importantly, GOLPH3 is overexpressed in several tumor types, including breast, lung, melanoma, ovarian, prostate, and GBM, but its function in malignant cells is not well known. GOLPH3 is a peripheral membrane protein of the trans-Golgi network that has been implicated in several cellular functions, such as in the transport of Golgi carbohydrate transferases, or in the maintenance of the structure of the Golgi apparatus through its interaction with the actin cytoskeleton. To understand the possible role of GOLPH3 in GBM tumorigenesis, we analyzed the effect of GOLPH3 depletion in the GBM cell line T98G. Depletion of GOLPH3 by RNAi resulted in impaired glycosylation of a variety of proteins, and in a distinct change in morphology of T98G cells, as well as reorganization of the actin cytoskeleton. Depletion of GOLPH3 also resulted in a different steady-state distribution of several transmembrane glycoproteins, suggesting an effect on protein trafficking. We propose that overexpression of GOLPH3 plays a critical role in the tumorigenic phenotype of T98G cells by means of altering the functionality of glycoproteins.

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P16-006-SP
Nanoscale self-assembled multivalent (SAMul) heparin binders: promising clinical tools

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Our work develops self-assembling nanoscale systems able to interact with biological polyanions. Heparin, an anionic polysaccharide, is an anti-coagulant widely used during major surgery, which requires neutralisation after use in order for clotting to begin (*Chem. Soc. Rev.* 2013, *42*, 9184–9195). Currently, protamine is used for this in the clinic although not without allergic responses in a significant number of patients. Through biomimetic design, we aim to develop highly tuneable and degradable self-assembling multivalent (SAMul) nanosystems as protamine alternatives (*Chem. Int. Ed.* 2012, *51*, 6572–6581; *Chem. Sci.* 2014, *5*, 1484–1492).

This talk will present our latest SAMul nanostructures, which demonstrate – using data from our novel Mallard Blue heparin binding assays (*J. Am. Chem. Soc.,* 2013, *135*, 2911–2914; *Chem. Commun.*, 2013, *49*, 4830–4832) – that the morphology of the self-assembled nanoscale structures can profoundly affect their binding behaviour, for example in different media. Additionally, synthetically-straightforward modifications to the monomer units can influence the overall binding preferences of the resulting SAMul assemblies. For example, we have designed SAMul ligand

arrays able to preferentially interact with either heparin or DNA. Excitingly, the facile tuneability offered by this approach gives great potential to provide chemical tools able to probe or intervene in biological systems, whilst also demonstrates the acute sensitivity of biological polyanions to the molecular structure of the binding unit.

We hope that by beginning to understand biological polyanion binding in this way, a clinically relevant alternative to protamine will emerge from this SAMul approach.

P16-007
Distribution of myophosphorylase in muscle development using zebrafish as a research model

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In zebrafish (*Danio rerio*) we can distinguish three isoforms of glycogen phosphorylase: liver, muscle and brain/heart.

The skeletal muscles isoform, myophosphorylase, is involved in the first step of glycogenolysis. It catalyzes the disconnection of glucose subunits from the glycogen chain. The main task of the glycogen accumulated in a skeletal muscle is to provide them sufficient quantity of energy substrates during the intense physical activity. Myophosphorylase is expressed in zebrafish muscles at the early stages of development, which suggests that it is important in vertebrate myogenesis. A deficiency of myophosphorylase in a human organism causes glycogen disease type V (McArdle's disease). Affected patients suffer from muscles pain, exercise intolerance combined with early fatigue.

We choose the zebrafish as a model in our research because it shares many histological similarities with mammals. At the ultrastructural level zebrafish muscles are almost identical to human. Moreover the zebrafish and human myophosphorylase gene shows high sequence similarity. The goal of our studys is to gain knowledge about myophosphorylase distribution in skeletal muscle during early development. In our study, we also show the influence of the physical activity on the myophosphorylase level. The knowledge about myophosphorylase enzyme distribution and its level in skeletal muscles will help in designing the accurate therapy in human diseases, such as McArdle syndrome.

P16-009
Glycosaminoglycans – from abstract knowledge to the use of knowledge in clinical medicine

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Factual abstract knowledge of biochemistry can be used in different fields of clinical medicine. It became clear because of three clinical problems learning: vertabral disease osteochondrosis (OCh), reparation of the acute myocardial infarction (AMI) and neoadjuvant polychemotherapy (NPChT) efficiency evaluation in ovarian cancer (OC) at III-IV stages. It's known that cartilage, intervertabral disks, cardiac valves and other organs include connective tissue (CT). It has glycosaminoglycans (GAG).Chondroitinsulfates (Ch-S) are one of the 7 types of GAG. In the 1960s OCh was treated by the injection of plant enzyme, papaine, to destruct the defected disk and to form strong comissure between the vertebras. It was clinically stated the higher the level of Ch-S concentration was, the grater the effect of OCh treatment. When CT is repaired the content of CT-macromolecules comes to the norm. It has also been revealed that the decrease in the Ch-S concentration in the blood shows the formation of CT-rib in myocard that is the sign of the reparation of the AMI. Thus, it should be noted that the change of Ch-S content in blood helps us to monitor the level of ANI healing. As known, patients are often prescribed NPChT to treat OC at the late stages. After the NPChT (1–6 courses) it becomes possible to make an operation to delete the original tumor in OC patients. It was found that the changes in level of total GAG and GAG-fractions help to find optimal number of NPChT courses for every patient, correlate with ultrasonic and morphological parameters.

P16-010
Structure and specificity of lectin from bacterium *Burkholderia pseudomallei*

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The bacterium *Burkholderia pseudomallei* is a human pathogen causing febrile illness called Melioidosis which is endemic in East Asia and north Australia. The pathogen is found in contaminated water and soil. Due to the bacterial resistance to antibiotics, the treatment is often very problematic and mortality rate is about 40%.

The carbohydrate structures located on the cell surface serve as recognition sites between a pathogenic bacterium and a host cell. Lectins are capable of specific and reversible binding to carbohydrate moieties. This carbohydrate-mediated recognition plays an important role in the ability of pathogenic bacteria to adhere to the surface of the host cell during the first phase of their infection of the host.

A new lectin from *B. pseudomallei* was identified, which did not show any significant sequence similarity to the known proteins. The gene coding the lectin was cloned and protein expression and purification were successfully optimized. Surface plasmon resonance (SPR) and titration microcalorimetry (ITC) were used to characterize the interactions between the lectin and saccharides. Both methods revealed the lectin ability to bind d-mannose and mannosylated derivatives. The structure of the protein was solved using X-ray diffraction and showed a novel fold of bacterial lectins. The lectin is present as a monomer in solution as determined using analytical ultracentrifugation, which was also confirmed in the crystal structure. According to its structure and sequence, the protein belongs to a not yet described family of lectins.

P16-011
Expression of *Schistosoma mansoni* Sm21.7 protein in *Pichia pastoris* and the subsequent immune response in mice

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Schistosomiasis mansoni, caused by *Schistosoma mansoni*, is endemic to the Egypt. Several vaccine candidates have been identified and tested in different animal models, but it is still unclear which

will be optimal for testing in the field. Therefore, new antigens and strategies are necessary for vaccine development. This study was performed to evaluate the efficacy of Sm21.7 protein expressed in *Pichia pastoris* as a candidate vaccine and the subsequent immune response in mice. The Sm21.7 gene was produced into *E. coli* and *Pichia pastoris*. The Sm21.7 gene was amplified and subcloned into the expression vector pPICZa-B and transformed into *Pichia pastoris* X-33 or pET3a and transformed to *E. coli*. The Sm21.7 gene was expressed and secreted into the medium and its molecular weight was about 21.7 kDa as determined by SDS-PAGE. Western blotting showed that the protein had a high specificity against mouse-anti-*Sm* 21.7 monoclonal antibody and rSm21.7 had a promising immune reactivity. The results of the immuno-protective experiments revealed that the worm reduction was 28.1%, 32.4%, and 36.9%, respectively. The number of eggs in liver tissue was reduced by 37.0%, 44.2%, and 45.1%, respectively. The results showed that, the molecular vaccine of Sm21.7 could partially induce resistance to the infection with *S. mansoni* in C57 BL/6 mice. In addition to reductions in worm viability, worm fecundity and egg hatching ability were observed following challenge infection in the immunized group. In conclusion, the recombinant protein *Sm*21.7 has promising immunological potentials for further approach to the diagnosis and development of molecular vaccine.

P16-012
Sialic acid – risk marker for diabetes complications; Modifications according to gender and age in patients with type 2 diabetes

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Sialic acid (*N*-acetyl neuraminic acid) is a marker of the acute phase response and is a recently investigated potential risk-marker for diabetes complications. One of the many unpleasant aspects of diabetes are the numerous complications that can arise from this metabolic disorder.

The objective of this study was to observe the modification of sialic acid concentration depending on the blood glucose level, gender and age of type 2 diabetics with various microvascular complications and presence of cardiovascular pathology.

We investigated serum sialic acid and glucose levels in 42 subjects. Patients were grouped as Control and T2DM in which were used criteria based on gender and age.

There were significantly elevated levels of serum sialic acid and blood glucose in diabetics compared to Control subjects ($p < 0.001$). We also observed in both genders a progressive increase in sialic acid concentration directly proportional to the level of blood glucose and age. The two variables sialic acid and glucose are correlated: the correlation coefficient $R = 0.883$ has an associated probability $p < 0.001$, a value that indicates a strong positive correlation between the dependent variable sialic acid and independent variable glucose.

We conclude that development of type 2 diabetes can occur in younger age groups due to inadequate diet and lifestyle. Therefore elevated serum sialic concentration is dependent on the duration and quality of diabetes management which determine the development and severity of complications such as retinopathy, nephropathy and cardiovascular disease in Romanian type 2 diabetics.

Keywords: diabetes, glucose, sialic acid

P16-013
New potential drugs with multiple therapeutically effects obtained from small sea fish

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Based on an original technology, a bioactive concentrate has been obtained from small see fish, rich in sulfated glycosaminoglycans, essential aminoacids, essential fatty acids (ω3, ω6) and trace elements (Ca, Na, K, Fe, Mg, Se, Ni, Cu, Si). The results of *in vitro* and *in vivo* experiments prove that the pharmacochemical actions of this bioactive extract have clear therapeutical value, such as: the inhibition of the activity of hyaluronidase, elastase and collagenase – enzymes involved in the degradative processes of the bone, cartilaginous and connective tissues, thus contributing to the reconstruction of the extracellular matrix; it also lowers inflammation and proves an antiproliferative effect to malign transformed cells (JURKAT) by inhibiting DNA synthesis during cell cycle division. The *in vivo* administration of this bioactive extract to carbon tetrachloride intoxicated rats stimulates the activity of hepatic catalase and inhibits the lipid peroxidation in a dose-effect manner, thus contributing to the reduction of free radical activity and of the harshness of diseases that involve their formation. This bioactive concentrate of marine origin can be formulated either as a drinkable or injectable solution, or in the form of gels or capsules, thus obtaining new drugs for therapeutic applications which require safe alternative for patients that undergo multiple treatment (e.g. osteoarthritis, diabetes, hypertension, hyperlipidemia, etc.).

Keywords: glycosaminoglycans, therapeutic applications, new drugs, small sea fish

P16-014
Effect of Fabaceae (*Galega officinalis L.*) consumption on levels of blood glucose, lipids and Lipoproteins in streptozotocin-induced diabetic rats

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Diabetes is the most common endocrine disease which blood sugar and fat increases followed. The aim of present study was to assay ethnopharmacological effects of Galega officinalis consumption on levels of blood glucose and lipids in streptozotocin-induced diabetic rats. 40 male rats, weighing 200 ± 20 g and 9 to 10 weeks old, were obtained from the animal breeding center of University. The rats were randomly divided into 4 equal groups of 10 animals including: (a) – normal control, (b) – normal rats treated with extract, (c) – diabetic control, and (d) – diabetics treated with extract. For induction of diabetes, after 15 h fasting,

the rats were intraperitoneally injected with streptozotocin at a dose of 60 mg/kg body weight, freshly dissolved in distilled water (5%). Animals with fasting blood glucose of 120 to 250 mg/dl were considered diabetic. Results showed a significant difference among animals of groups 3 and 4 with control group during 3rd week. Results showed that blood glucose level on weeks 3 and 6 in groups 3 and 4 was higher than control group significantly. Increased cholesterol level in group 3 was observed on weeks 3 and 6 compared with prior the study. A significant increase in serum triglycerides was observed on weeks 3 and 6 in group 3 compared with prior the study. Measurement of HDL has revealed that this parameter in rats of group 3 decreased significantly in compared with prior the study and LDL levels were increased in rats of group 3 in compared with control group.

P16-015
Specific expression of O-glycoprotein glycans in cholangiocarcinoma cell lines

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Protein glycosylation is the most common posttranslational modifications in mammalian cells. It is involved in many biological pathways and molecular functions. Aberrant protein glycosylation may be associated with the disease processes, including cancer. We have identified and quantified the glycan structures of O-linked glycoprotein from Cholangiocarcinoma (CCA) cell lines and compared their profiles with normal biliary cell line by nanospray ionization-linear ion trap mass spectrometry (NSI-MS^n). Five human CCA cell lines, K100, M055, M139, M213, M214 and the normal biliary cell line, MMNK1 were characterized. The results showed that the O-linked glycan profiles of the CCA cell lines and the normal biliary cell line were consisted of tri- to hexa-saccharide with the terminal galactose and sialic acid; $NeuAc_1Gal_1GalNAc_1$, $Gal_2GlcNAc_1GalNAc_1$, $NeuAc_2Gal_1GalNAc_1$ $NeuAc_1Gal_2GalNAc_2$ and $NeuAc_2Gal_2GalNAc_2$. All five CCA cell lines showed a similar glycan profiles with the normal biliary cell line, but with the different in their quantities. The $NeuAc_1Gal_1GalNAc_1$ is the most abundant structures in poorly differentiated adenocarcinoma (K100; 57.0%), moderately differentiated adenocarcinoma (M055; 42.6%) and squamous cell carcinoma (M139; 43.0%) while moderately to poorly differentiated adenocarcinoma (M214; 40.1%) and adenosquamous cell carcinoma (M213; 34.7%) are dominated by $NeuAc_2Gal_1GalNAc_1$. These results suggested the differential expression of the O-linked glycans in different histological types of the cancer. Interestingly, all five CCA cell lines are abundant with the O-linked glycans with the terminal sialic acid, suggesting the important role of sialic acid in the cancer cells. These glycans structural analyses may provide important information leading to the development of disease-related glycoprotein of CCA.

P16-016
Lectin activity among *Phaseolus vulgaris* cultivars

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Since recently to generate highly effective pharmaceuticals lectins extracted from various plant sources are investigated for anticancer, antimicrobial, and immunomodulatory properties. The most abundant source of lectins is generally plants, and especially the phabaceous species. In order to pave way towards implicating bean lectins, more profound knowledge related to medical and biological research on biological activities and effect on human body of lectins diagnostic and therapeutic preparations are required. These investigations are of high scientific and practical meaning for Kazakhstan, as mobilization of domestic plant resources, and perspective common bean accessions. As shown in our experiments, four cultivars have revealed rather high lectin activity. Highest activity is intrinsic for the seeds of these cultivars. Based on the dynamics of lectin accumulation in different organs of common bean cultivars with high lectin activity have been determined for the first time among Kazakhstan, Russian and other foreign brands. By descending order of lectin activity, organs of common bean may be arranged as follows: seeds, stems, leaves, and roots. Lectin isolation method has been modified to study common bean brands forementioned. During the step of lectin extraction buffer volume as time of elution should be increased. The yield of lectins from bean seeds depending on the genotypes has ranged between 13 and 39 mg/100 g.

The data obtained could be used in the future for the development of the lectin isolation technology, studying and modeling of their effect on cells as subsequent design of pharmaceuticals with different action spectrum.

P16-017
The role of the mmp2 and mmp9 in progression of atherosclerosis with type ii diabetes mellitus patients

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İnflammatory process is essential for the initiation and progression of vascular remodeling, entailing degradation and reorganization of the extra-cellular matrix (ECM) scaffold of the vessel wall, leading to the development of atherosclerotic lesions. Matrix metalloproteinases (MMPs) are zing dependent endo-peptidases found in most living organisms and act mainly by degrading ECM components. Diabetes increase the production of matrix metalloproteinase (MMP2,MMP9) that lead to breakdown of collogen. Collogen is responsible from mechanical stability to the plaque's fibrous cap. When collogen breakdown increases and synthesis decreases, plaque may rupture more readly, a trigger to thrombus formation. So, atherosclerosis also occurs rapidly. In this study, diabetes patients, serum MMP2 and MMP9 levels are measured by elisa method and serum LDL, HDL,triglyserid,cholesterol, fasting blood glucose by enzimatic method. According to our results in Group II (before treatment) and Group III (after three mounts 10 mg/day dose statin (rosuvastatin) therapy) patients, MMP2, MMP9,Lipid profile levels were higher than Group I (control Group). Although serum

MMP 2 and MMP 9 levels of Group II patients are higher than Group III patients, Group II patients have the lowest HDL levels among the other groups. All these results are not statistically signaficant,HDL levels ($p = 0.198$) and MMP2 levels ($p = 0.261$), MMP9 levels ($p = 0.228$). We are suggesting that these parameters should help in treatment and diagnosis of the diabetic and hypercholesteromic patients having aterosclerosis.

P16-019
Indoleamine 2,3-dioxygenase related metabolic effects of 3-aminobenzamide and infliximab in lung tissue of experimental colitis model

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Lung involvement due to inflammatory bowel disease (IBD) is considered to be frequent, however the pathogenic mechanism is still debatable. The rate limiting enzyme indoleamine 2,3-dioxygenase (IDO) of the kynurenine pathway is normal effectors of peripheral immune tolerance in the immunoregulatory pathway of tryptophan metabolism. Here, the presence IDO was investigated in ulcerative colitis model in rats experimentally induced by 2,4,6-trinitrobenzenesulfonic asit. Experiments were performed using five groups of rats (n = 9). Group 1: sham+saline, group 2: colitis+saline, group 3: colitis+3-aminobenzamide (3-AB, 10 mg/kg, every 12 h), group 4: colitis+infliximab (10 mg/kg, every 24 h) and group 5: colitis+ 3-AB+infliximab (24 h before colitis was formed). The same protocol was applied for 7 days. Lung cultures were incubated on specific agar media plates aerobically and anaerobically. The strain types of the grown colonies were identified by using conventional microbiological techniques and calculated as colony forming units per gram of tissue to assess the bacterial translocation (BT) in the tissues. The levels of IDO protein of the same lung tissue were tested by western blot analysis. The main findings of our study were; IDO was detectable in all groups. No BT was found in group 1, BT was found in equal numbers in group 2 and 3, in group 4, half of the samples had BT, and in group 5, no BT was found 6 of the nine samples. As a result, IDO facilitates the formation of suitable immune response by affecting the balance between immune tolerance and response.

P16-020
X-ray structure of recombinant non-glycosylated FAD glucose dehydrogenase derived from *Aspergillus flavus*

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The flavin adenine dinucleotide dependent glucose 1-dehydrogenase (FADGDH) is oxidoreductase, which acting on the CH-OH group at the 1-position of glucose. FADGDHs are able to use a variety of external electron acceptors but not oxygen. Currently, fungi-derived FADGDH is the most advanced and popular enzyme for use in the glucose sensor strips utilized for self-monitoring of blood glucose, focusing on its oxygen insensitivity and the narrow substrate specificity. Despite of the long history of fungi-derived FADGDHs, no structural information is currently available. Recently we succeeded in overexpression of recombinant non-glycosylated FADGDH derived from *Aspergillus flavus* (AfGDH) in *E. coli* [1,2]. We report here the X-ray structures of the recombinant AfGDH alone and in complex with beta-D-glucono-1,5-lactone (LGC). The structure of AfGDH showed monomer, and the subunit structure was similar to those of the so far reported fungal glycosylated glucose oxidases (GOxs), *Aspergillus niger* GOx (PDBID, 1CF3) with the r.m.s.d. of 1.7 Å and 35% identity, and *Penicillium amagasakiense* GOx (1GPE) with the r.m.s.d. of 1.7 Å and 34 % identity by Dali search, though the GOxs showed dimer. In the subunit structures of AfGDHs, the bound FAD molecules are reduced form and occupy a narrow channel. Although, most of residues are conserved with those of reported in GOxs, AfGDH lacks the residues existing closed to the 6th hydroxyl group of hexose as the bound substrate.

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P16-021
Determination of exopolysaccharide production in lactic acid bacteria isolated from Turkish local yogurt

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Exopolysaccharides (EPS) are sugar polymers those are secreted by the microorganism out of the cell. EPS have an important role for the adhesion to epithelial cells in lactic acid bateria. EPS have health effects as immunostimulant activity, antitumor activity, activation of macrophages and lymphocytes to increase their endurance, and as prebiotics. This study aimed to determine exopolysaccharides production capacity in lactic acid bacteria isolated from Turkish home-made yogurt.

All bacteria cultures were isolated from Turkish local yogurt collected from seventeen different Turkish villages. This experiment of EPS production capacity was studied by Alp (2008) using the phenol sulfuric acid method. EPS levels were measured spectrophometrically at 490 nanometer wavelength. A standard curve was drawn with glucose solutions which were prepared using varying amounts of 10–100 mg/l, was used to calculate EPS production amount.

EPS production capacities were determined spectrophotometrically and calculated. The lowest and highest amounts were calculated respectively as 36.28 and 94.86 mg/l.

Probiotic microorganisms must be resistant to stomach acid, tolerant to bile and able to keep the intestinal mucosa. Exopolysaccharides are very important for adherents to the epithelial cells on intestinal mucosa. So they can be used as a quality marker to choose a probiotic microorganism. All of our lactic acid bacteria strains showed EPS production activity at different rates. These results can be supported by resistance to stomach acid, tolerance of bile, reduction in cholesterol and bile salt hydrolase production in the future.

Keywords: Lactic acid bacteria, Probiotic, Exopolysaccharide production

P16-022

Multiple approaches to characterise α-1-acid glycoprotein glycosylation in pancreatic cancer

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Pancreatic cancer (PDAC) still shows a low survival rate mainly due to its aggressiveness and the absence of biomarkers to detect it in earlier stages. Therefore, the search of PDAC biomarkers represents an important challenge for the scientific community.

Previous results using small cohorts of patients showed that acute phase proteins were good carriers of pathological information. In this study we have analysed the glycosylation of α-1-acid glycoprotein (AGP) from healthy controls (HC), chronic pancreatitis (ChrP) and PDAC patients' sera. We purified AGP and corroborated its purity by silver staining of SDS-PAGE gels and MS. Afterwards, glycan structures were analysed before and after N-glycan release. AGP isoforms were separated by capillary electrophoresis and some of them showed significantly different proportions between PDAC and HC. Immunoassays using Aleuria aurantia lectin displayed statistically significant increased fucosylation levels of AGP and sialic acid digested AGP in PDAC patients compared to ChrP and HC, respectively. Finally, the ratio of fucosylated/non-fucosylated released AGP glycans analysed by stable isotope labelling and ZIC-HILIC-ESI-TOF-MS was different in PDAC compared to ChrP. Regarding branching and sialic acid content, no differences were obtained among groups. The methodologies performed produced complementary data and reveals the powerful of these techniques for glycan analysis. The increase in fucosylation is higher on samples from patients with an advanced stage of the pathology, suggesting that this glycoform may give an advantage to the tumour and could be useful as a diagnostic tool to achieve more specificity in tumour detection.

P16-023

Role of sialyltransferase expression in breast cancer progression and metastasis

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Tumor progression is accompanied by multiple changes of the cell surface. Tumor invasion and metastasis depend on alterations of the cell adhesion properties of the tumor to leave the primary tumor site and migrate to other parts of the body. Sialic acids are a family of nine-carbon amino sugars that occupy the terminal position of oligosaccharids in many glycoconjugates. Therefore they are one of the most abundant molecules at the cell surface and play an important role in cell adhesion and migration. In the oligosaccarides chain sialic acids can be linked to galactose or N-acetylgalactosamine in alpha-2,3 linkage or alpha-2,6 linkage and to another sialic acids in alpha-2,8 linkage. The expression of sialic acids at the cell surface is regulated by the expression of different sialyltransferases that prefer different linkages and substrates. We have analysed breast cancers samples of different grade and corresponding cell lines for the expression of 20 sialyltransferases mRNA using q-PCR to analyse their role in tumor progression and metastasis. We identified ST3Gal4, ST6GalNAc4 and ST6GalNAc2 as potential candidates. In the breast cancer cell line MDA-MB-468 the elevated expression of ST3Gal4 and ST6GalNac2 leads to a reduced cell-cell adhesion and reduced adhesion of the cells to different matrices. Therefore a switch in ST3Gal4 or ST6GalNAc2 expression in breast cancer could be a factor for tumor progression and metastasis in breast cancer.

P16-024

Efficacy and immunogenicity of an insect cell-derived virus-like particle vaccine for avian influenza H7N9 virus in mice

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On March, 31 2013 human infections with a novel avian influenza A virus, subtype H7N9, were reported by the Chinese Center for Disease Control and Prevention. As by latest reports from February this virus has been the causative agent for almost 600 human infections with a case fatality rate of about 37%. Over the past decades, there have been multiple incidences of sporadic zoonotic transmissions of H7 viruses to humans, however, those predominantly have been associated with mild disease, typically conjunctives, or even have been subclinical. Although no cases of human-to-human transmissions have been reported so far, the ongoing H7N9 outbreak is of great concern. Due to the lack of an available vaccine, avoidance of exposure to the sources of infection and careful hygiene are the only ways to prevent infection. Whether the virus could have potential to cause a pandemic is still uncertain, so attempts to generate potent vaccines are already underway. In the current work we describe the generation of a potential virus-like particle (VLP)-vaccine, consisting of hemagglutinin (HA) from the H7N9 A/Shanghai/1/13 strain and the matrix protein (M1) from a human H3N2 strain using the baculovirus expression vector system and the *Trichoplusia ni* insect cell line BTI-TN-5B1-4. Different concentrations of the immunogen were evaluated in a prime-boost versus a prime-only vaccination regimen without adjuvant in a challenge experiment with BALB/c mice. An ELISA-setup using divergent recombinant HAs as well as hemagglutination inhibition assays were used to evaluate binding properties and neutralizing capabilities to divergent H7 strains.

P16-025

Immunological biomarkers elicited in female rats administered with pro-fertility extract of *Anthocleista vogelii*

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Anthocleista vogelii Planch. is a plant that has been widely used alternatively by Traditional medicine practitioners either singly or in combination with other plant materials to treat several diseases or ailments, which include fertility problems both in male and female.

Animals were sacrificed following completion of extract administration. Blood samples from experimental animal groups were collected into potassium ethylenediaminetetraacetate (K3EDTA) tubes and plain tubes. Elucidation of the absolute counts of clusters of differentiation 4 and 8, (CD4+ and (CD8+) was performed using the Becton Dickinson's (BD)FACS Count Automated technique.

The aqueous ethanolic extract of Anthocleistavogelii caused a decrease in CD4+ and CD8+ cells counts in the female albino rats, the NAC treated followed by extract administration group compared with showed a statistically significant decrease ($P < 0.05$) in CD4+ and CD8+ counts, from (851.33 ± 96.34) to (451.00 ± 21.02) and from (1058.67 ± 93.31) to (636.00 ± 28.93) for CD4+ and CD8+ cells respectively. The extract showed a significant increase of Oestradiol concentration in the female rats, from (184.65 ± 30.06 pg/ml) in the control group to (288.29 ± 30.06 pg/ml) in the extract group.

These findings suggest that Anthocleista vogelii may have a role in creating the environment required for successful pregnancy by decreasing the ratio of CD4+ and CD8+. Therefore, this study supports the claims on the traditional use of Anthocleista vogelii to enhance reproduction in female fertility.

Keywords: ethanolic extract, Anthocleista vogelii, female fertility, CD4+ and CD8+, Oestradiol

P16-026

Serglycin promotes breast cancer cell aggressiveness via up-regulation of the expression of proteolytic enzymes and controls osteoclastogenesis

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Serglycin has been shown to associate with aggressive phenotype of breast cancer cells and confers resistance against complement system attack. In order to evaluate the role of serglycin, we stably transfected MCF-7 cells with either pEGFP-N3 vector carrying the serglycin cDNA or empty vector and compared expression profiles and properties between serglycin-overexpressing and control vector cell lines.

We noticed a significant induction in the expression of the mesenchymal markers fibronectin and vimentin and transcription factor snail-2 in serglycin-overexpressing cells that was accompanied by changes in cell phenotype such as increased membrane ruffling. Serglycin-overexpressing MCF-7 cells showed also significantly elevated expression of various matrix metalloproteinases (MMPs), such as MMP-1, MMP-2, MMP-7, MMP-9 and MT1-MMP. Serglycin isolated from breast cancer cells was found to interact with osteoprotegerin via its glycosaminoglycan chains. The presence of serglycin significantly decreased the ability of osteoprotegerin to inhibit osteoclastogenesis through binding to RANKL.

We demonstrate that serglycin promotes a more mesenchymal phenotype in breast cancer cells MCF-7 and modulates the biosynthesis of proteolytic enzymes. Furthermore, the ability of serglycin to inhibit the suppressive role of osteoprotegerin in osteoclastogenesis suggests a role for serglycin in breast cancer-induced bone disease.

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P16-027

Increased expression of serglycin in solid tumors and aggressive cancer cell lines

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Recent studies have shown that serglycin promotes the invasive potential of breast cancer cells. In the present study, we studied the expression of serglycin in various cancer cell lines and respective solid tumors.

Several tumor cell lines of lung, breast, prostate and colon cancer were positive for the expression of serglycin and an alternative splice variant lacking exon 2 as revealed by sequencing analysis. Higher levels of expression were detected in cell lines that reveal a more metastatic type as compared with less aggressive cancer cell lines. We further examined the cellular expression and distribution of serglycin in a tissue microarray that included 40 carcinomas of different grade and TNM stage, originated from colon, breast, lung and prostate, as well as normal epithelial tissues. The cellular distribution of serglycin was diffuse and almost exclusively cytoplasmic in cancer cells in all tumor samples. Notably, in some cases serglycin had also membrane localization. Serglycin was also expressed in lower levels in normal epithelia, as well as in plasmatocytes, endothelial, smooth muscle and stromal cells.

The expression of serglycin in malignant cells and the correlation of serglycin levels with the metastatic potential of the cells indicate its participation in the progression of malignancies.

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Chem Biol S5, Signal Transduction in Tumor Development, Differentiation and Immune Escape

P18-006-SP

Chronic stress suppresses autophagy and affects spontaneous differentiation of bone marrow stromal cells

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Bone marrow-derived mesenchymal stromal cells (MSCs) are multipotent cells with a high constitutive level of autophagy and low expression of CD99. Under some conditions, MSC may develop tumorigenic properties. However, these transformation-induced conditions remain largely unknown. Recently we identified association between Hsp70, a main participant in cellular

stress response and tumorigenesis, and CD99. Preliminary observations revealed up-regulation of both proteins in stressed long-term cultured MSCs. We hypothesized that CD99 is implicated in stress-induced mechanisms of cellular transformation in MSC. Hence, we investigated the effects of prolonged stress on MSCs and the role of CD99 and autophagy in their survival. We found that chronic stress factors are able to change morphology of MSCs and to inhibit spontaneous differentiation into adipocyte lineage. Furthermore, CD99 elevation and disappearance of p53 and p21 accompanied defected autophagy, which is usually associated with tumor formation. We found that inhibition of autophagy promoted cell detachment and modulated CD99 expression level whereas incorporation of CD99 recombinant protein into the cells suppressed autophagy. These results provide a model for chronic stress-induced transformation of MSCs via CD99 and thus are likely of relevance for mesenchymal tumorigenesis.

P18-007-SP
Activation and repression by oncogenic Myc shape tumor-specific gene expression profiles

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Enhanced expression of the oncoprotein Myc contributes to the formation of many tumors. Myc is a basic HLH-Leucin Zipper transcription factor and binds to thousands of promoters in mammals. In many biological systems, Myc both activates and represses gene expression. However, recent work in lymphoid cells shows that activation of Myc promotes an increase in expression of virtually all genes (Lin at al, Cell, 2012; Nie et al, Cell, 2012). To understand this discrepancy, we studied the consequences of inducible expression of Myc in human tumor cell lines (Walz et al, Nature, 2014). Changes in Myc levels activate and repress specific sets of direct target genes that are characteristic of Myc-transformed tumor cells. Three factors account for this specificity: (i) The magnitude of response parallels the change in occupancy by Myc at each promoter. (ii) Accordant to Myc′s role in gene regulation, Myc both positively and negatively affects transcription initiation. (iii) Complex formation with Miz1 mediates repression of multiple target genes by Myc.

To test the impact of Myc and Miz1 on tumorigenesis *in vivo* we started recently to investigate the Myc/Miz1 complex in a murine tumor model for Myc driven medulloblastoma. In contrast to primary neuronal cells, where Miz1 binds only to about 200 target genes (Wolf et al, Nature Communications, 2013), Miz1 and Myc co-occupy thousands of promoters in medulloblastoma. Interestingly, disruption of the Myc/Miz complex results in a massive change of the transcriptional program, shifts the identity of the tumors and leads to a strong survival benefit.

P18-008
Expression of pro- and anti-angiogenic genes in U87 glioma cells is regulated by ERN1 mediated endoplasmic reticulum stress

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The endoplasmic reticulum (ER) stress play an important role in angiogenesis activation, and is mediated by ERN1/IRE-1α, enzyme that activates genes responsible for tumor survival. Thus, blockade of ERN1 may have anti-tumor effects via angiogenesis suppression, which is regulated by growth factors, such as tissue inhibitors of matrix metalloproteinase (TIMP1, TIMP2, TIMP3), thrombospondin (THBS1, THBS2), connective tissue growth factor (CTGF), osteonectin (SPARC) and VEGFmembers – VEGF-A, VEGF-B, VEGF-C.

The aim of this study is to investigate the expression of genes involved in angiogenesis in glioma cells under hypoxia in relation to ERN1.

We used human glioma cell line U87 and its modified variant with suppression of ERN1 endoribonuclease and protein kinase enzymatic activities. Hypoxia was created with 3 % oxygen. The expression of genes was measured by qPCR and Western blot was performed to evaluate the expression of several genes on protein level.

We have shown that ERN1 blockade affects expression of anti-angiogenic factors. The expression levels of TIMP2, TIMP3, THBS1, THBS2, SPARC and CTGF are significantly increased after ERN1 knockdown. Hypoxia enhances these genes expression in control cells. However, blockade of ERN1 didn't change significantly hypoxic effect on TIMP3 expression, but eliminated hypoxic regulation of TIMP2. At the same time ERN1 knockdown caused a decrease of VEGF-A, VEGF-B, VEGF-C and TIMP1 expression. Hypoxic effect on most of genes expression is decreased or eliminated.

ERN1 knockdown affects the expression of genes that control angiogenesis as well as hypoxia. Thus, inhibiting of ERN1 activity can be a strategy for anti-angiogenic treatment.

P18-009
Dioxin receptor (AhR) transcription factor modulates hepatocytes polyploidization, stem cells maintenance and regeneration in liver mice presumaby via Wnt/Beta-catenin pathway

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Polyploidy in mammalian cells is indicative of terminal differentiation and senescence. During growth, the liver parenchyma undergoes dramatic changes characterized by gradual polyploidization, in which hepatocytes of several ploidy classes emerge as a result of modified cell division cycles.

The dioxin receptor (AhR) is required for xenobiotic-induced toxicity and carcinogenesis and for cell physiology and organ homeostasis. Interestingly, in different liver pathologies, like hepatocarcinoma, hepatocellular growth shifts to a nonpolyploidizing growth pattern, and expansion of the diploid hepatocyte population has been found to take place. It has been suggested that the polyploid genome may provide protection against the dominant expression of mutated oncogenes.

In this regard, we have seen that the vast majority of AhR knockout hepatocytes are diploid and tetraploid compared to wild type mice, which have a significantly higher percentage of hepatocytes octaploid. Moreover, the number of stem cells in AhR knock-out liver is significantly greater too, and probably for this reason, the regenerative capacity post-carbon tetrachloride treatment is also higher.

Considering the polyploid fraction alone, 20–30% of hepatocytes are binuclear in wild-type mice whereas this percentage is reduced to a half in knockout livers.

Also, we have studied the involvement of AhR in Wnt/β-Catenin Pathway which participates in the regulation of adult tissue self-renewal, stem cell maintenance and pluripotency. We have seen that wnt/beta-catenin pathway is upregulated in AhR

knockout mice. We propose that *AhR* is a novel regulator of hepatic polyploidization probably through wnt/beta-catenin pathway and it could be a marker for good prognosis in hepatocarcinoma.

P18-010
Aldehyde dehydrogenase requires dioxin receptor knock-down to promote melanoma tumorigenesis

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The dioxin (AhR) receptor regulates tumor development in a cell type-specific manner both in presence and in absence of xenobiotics. AhR expression has recently been shown to suppress primary tumorigenesis and lung metastasis in murine melanoma while, consistently, AhR levels were significantly reduced in metastatic human melanomas with respect to benign nevi lesions. Here, we have identified aldehyde dehydrogenase 1a1 (Aldh1a1), a tumor promoting enzyme, as an AhR repressed gene that mediates melanoma progression in the absence of AhR. Melanoma cells stably knocked-down for both AhR and Aldh1a1 (sh-AhR+sh-Aldh1a1) had reduced migration and invasion and lower clonogenic potential than sh-AhR interfered cells. Aldh1a1 depletion impaired the tumorigenesis and lung metastasis potentials of sh-AhR cells in immunocompetent AhR+/+ recipient mice. In agreement with the role of Aldh1a1 in maintaining an stem-like phenotype in cancer cells, sh-AhR+sh-Aldh1a1 cells had reduced expression of CD133+/CD29+/CD44+ and lower levels of the pluripotency marker Sox2. Interestingly, transient Sox2 expression increased Aldh1a1 levels and cell migration in sh-AhR but not in sh-AhR+sh-Aldh1a1 cells, suggesting the existence of a co-regulatory mechanism between Aldh1a1 and Sox2 in melanoma cells. Luciferase labelling *in vivo* confirmed that sh-AhR+sh-Aldh1a1 cells had lower metastatic potential and enhanced the survival of recipient mice with respect to single sh-AhR cells. We conclude that AhR down-modulation promotes Aldh1a1-dependent tumorigenesis in melanoma, and suggest that the association of low AhR expression with high Aldh1a1 activity could be a poor prognosis marker in melanoma.

P18-011
Treatment of certain types of carcinomas by drugs from natural source

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Mushrooms are considered as functional foods because they elicit their positive effect on human being in several ways. Fruiting bodies as well as active mycelia of Pleurotus and Ganoderma spices possesses a number of therapeutic properties like anti-inflammatory, immune-stimulatory and immune-modulatory and anticancer activity.

We modulate growth conditions of Pleurotus ostreatus and Ganoderma lucidum mushrooms cultures by treatment of mm-waves 45–53 GHz, which lead to the sharp increasing of peroxidase activity of up to 3 times and betta-glucosidase up to 2 times at the some frequencies of mm-waves, as well as obtained increasing of protein content in extracts. Obtained differences have the differently directed character and depend from frequency and time of exposition by mm-waves.

The purpose of this work is to study acting of wood-decaying mushroom culture extracts on cytophotometrical and morphmetrical indices' of each investigations tissue, by using data of known malignant tissue lines behavior of proliferation and transcription. As evidence our results extracts from culture of wood-decaying mushrooms possessive an antiproliferative activity on cancer tissues, by suppressing mitotic activity of cells of some carcinoma tissues up to 29%. As evidence our data mushrooms culture extracts from P. ostreatus and G.lucium have a suppressive action to 48 h of cultivation and almost completely suppressed mitotic activity of all investigated human carcinoma cell cultures.

P18-012
The dioxin receptor downmodulation enhances cell reprogramming of somatic cells into induced pluripotent stem cells (iPSCs)

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Cell differentiation is responsible for many of the phenotypic changes that take place during embryonic development and in the adult life and its miss-regulation is associated to diseases like cancer. Because of that, major efforts are been made to understand the mechanisms and to identify novel molecular intermediates controlling differentiation in tumor cells. The dioxin receptor AhR is a transcription factor highly conserved and recent studies have shown that it has relevant roles in tissue homeostasis besides its well-known implication in toxicity. For instance, AhR expression can either promote or inhibit cell proliferation and tumor development and

some reports also suggest that it could participate in immune cell differentiation. We have found that AhR affects reprogramming in mouse embryonic fibroblasts (MEF) from transgenic mice expressing the pluripotency genes Klf4-Sox2-Oct4- Myc (KSOM). Transient AhR knockdown (sh-AhR) increases reprogramming efficiency with respect to cells transfected with the empty vector. Thus, these MEFs display a higher ability to form alkaline phosphatase-positive iPSC clones and the expression of KSOM genes and nanog is up-regulated. On the opposite, the expression of a constitutively active receptor (CA-AhR) inhibits reprogramming. Moreover, the treatment of these KSOM-MEFs with the non-xenobiotic activator of AhR FICZ compromises seriously the reprogramming efficiency and this effect is partially abolished by using the AhR antagonist CH-223191. To further investigate this inhibitory role of AhR in reprogramming, we are also transfecting MEF cells from AhR-wt and AhR-null mice with the KSOM cocktail and quantify the formation of iPCS clones and expression of differentiation markers.

P18-013
IRF5 activates the apoptotic pathway in HCV infected hepatoma cells

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HCV is a global human pathogen that targets the liver and replicates in hepatocytes and occurs Hepatocellular carcinoma (HCC). The mechanism of HCV-associated HCC remains elusive. Therefore, it is critical to identify the molecular effectors of HCV

in order to develop novel therapeutic targets against HCV-HCC. We have focused our attention on Interferon regulatory factor-5 (IRF5) that is a multifaceted protein with critical role in virus-, IFN- and DNA damage-induced signaling pathways. Importantly, IRF5 tumor suppressor function has now been implicated in several cancers. Surprising, we know very little about its role in HCV-associated liver cancer pathogenesis. To investigate IRF5 function during HCV infection, we evaluated the regulation of different types of cytokines and chemokines by IRF5 over expression in HCV replicon cells. We also analyzed the cancer pathway mediators modulated by IRF5 in HCV replicon cells employing RT-Profiler PCR array technology. The PCR-array data was validated by western blotting. Mitochondrial membrane potential was determined with JC1 assay through flow cytometry and fluorescence microscopy on IRF5 transfected HCV-replicon cells. We demonstrate that IRF5 overexpression down-regulated anti-apoptotic proteins as Bcl-2 and Bcl-xL and up-regulated pro-apoptotic proteins as caspase-3,cyt-c,Bax,Bid. Our studies further reveal that IRF5 mediates its effect by suppressing mitochondrial membrane potential and signaling cell death pathway. These studies present evidence in support of IRF5's role as an apoptotic protein in HCV infection. The findings from this study have implications in identifying IRF5 as a novel target in HCV-associated HCC.

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P18-014
Berberine inhibits proliferation by cell cycle arrest at the G2/M phase via PI3K /Akt and p38 kinase in HTB-94 human chondrosarcoma cell line

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Berberine is a clinically important natural isoquinoline alkaloid found in many medicinal herbs. Berberine has been shown to have many pharmacological effects including antimicrobial, antitumor, and anti-inflammatory activities. However, the effects and mechanism of action of berberine have not been studied in chondrosarcoma. Therefore, the effects of berberine on proliferation in human chondrosarcoma cell line (HTB-94) were investigated. Berberine inhibited cell proliferation in a concentration-dependent manner as determined by the methyl thiazole tetrazolium (MTT) assay. Flow cytometry showed that inhibition of cell proliferation by berberine occurred via G2/M phase arrest in HTB-94 cells. Western blot analysis showed that berberine induced p53 and p21 expression and suppressed cyclin B1, cyclin dependent kinase 1 (cdc2), cdc25c, and phosphorylated retinoblastoma tumor suppressor protein (pRb) expression. In addition, berberine stimulated phosphorylation of protein kinase B (Akt) and p38 kinase. Inhibition of phosphatidylinositol 3-kinase (PI3K)/Akt with LY294002 (LY) and p38 kinase with SB203580 (SB), respectively, decreased berberine-induced p53 and p21 expression, and restored cell proliferation and expression of cyclin B1, cdc2, Cdc25c, and pRb cell cycle progression proteins. These results suggest that berberine-induced inhibition of cell proliferation by cell cycle arrest at G2/M phases was regulated through PI3K/Akt and p38 kinase pathways in HTB-94 chondrosarcoma cells.

P18-015
Fad104, a positive regulator of adipogenesis, inhibits invasion and metastasis of cancer cells through the suppression of STAT3 activity

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Factor for adipocyte differentiation 104 (*fad104*) is positive regulator of adipocyte differentiation. Previously, we showed that *fad104* regulated cell adhesion and migration. Cell adhesion and migration are considered essential for invasion and metastasis of cancer cells. Therefore, it was thought that *fad104* could regulate invasion and metastasis of cancer cells. In the present study, we elucidated the function of *fad104* in invasion and metastasis of cancer cells.

The expression level of *fad104* in highly metastatic A375SM melanoma cells was lower than that of poorly metastatic A375C6 melanoma cells. Depletion of *fad104* enhanced invasion ability of A375C6 cells. In contrast, over-expression of *fad104* attenuated invasion ability of A375SM cells. Over-expression of *fad104* decreased in the expression of *mmp2*, which is essential for invasion and its expression level correlates directly with the pathogenesis of melanoma. Moreover, *fad104* negatively regulated invasion of not only melanoma cells but also breast cancer cells. In addition, melanoma cells stably expressing FAD104 showed a reduction in formation of lung colonization compared with control cells. These results suggest that *fad104* suppresses invasion and metastasis of cancer cells. FAD104 interacted with STAT3 and partially colocalized with STAT3 in melanoma cells. Furthermore, *fad104* down-regulated the phosphorylation level and transcriptional activity of STAT3 in melanoma cells.

In summary, we demonstrated that *fad104* inhibits the invasion and metastasis of cancer cells and is closely involved in negative regulation of the STAT3 signaling pathway.

P18-016
Carboxyl-terminal of IGF-1Ec variant induces proliferation and migration of ER$^+$ breast cancer MCF-7 cells via ERK signaling

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Introduction: The potential roles of the distinct IGF-1 isoforms in human malignancies are largely unknown. Recently, the c-terminal of the IGF-1Ec variant (Ec peptide-24aa) has been suggested to have a distinct role in prostate cancer biology. Herein, we investigated potential role of Ec peptide in human MCF-7 breast cancer cells.

Methods: We generated MCF-7 cells stably overexpressing the Ec peptide (MCF-7 Ec). Using MTT and trypan blue exclusion assays along with flow cytometry we compared the proliferation rates of MCF-7 Ec cells, mock transfectants (mock MCF-7) and wild type MCF-7 cells (wt MCF-7). In addition, we investigated the migratory capacities using the wound healing/scratch assay. Expression patterns of ERα, Cdh-11 and pERK1/2 were estimated using real time qPCR and WB analysis.

Results: MCF-7 Ec cells acquired a spindle-like phenotype and possessed an increased rate of proliferation (up to 53%, 48 h), compared to mock MCF-7 and wt MCF-7 cells. Metabolic activity of the MCF-7 Ec transfectants was upregulated [up to 44% ($p < 0.03$), 24 h], whereas the distribution into the S phase of the cell cycle was marginally increased (by 10%, 24 h). Furthermore, the MCF-7 Ec cells exhibited an increased migratory potential

(by 37%, 16 h). Protein levels of ERα along with mRNA levels of Cdh-11 were upregulated by 2.21 and 2.8 folds respectively while phosphorylation of ERK1/2 was increased by 2.41 times in MCF-7 Ec.

Conclusions: Our results indicate that IGF-1 Ec peptide induces proliferation and migration of ER^+ MCF-7 breast cancer cells via ERK1/2 signaling.

P18-017
Mitochondrial dysfunction induces EMT through the TGF-β/Smad/Snail signaling pathway in Hep3B hepatocellular carcinoma cells

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Mitochondrial dysfunction has recently been found to be associated with various pathological conditions, particularly cancer. However, the mechanisms underlying tumor malignancy induced by mitochondrial dysfunction have not been fully understood. In this study, the effects of mitochondrial dysfunction on epithelial mesenchymal transition (EMT) were investigated using mitochondrial-depleted ρ^0 derived from the Hep3B hepatocarcinoma cell line. The Hep3B/ρ^0 cells displayed the EMT phenotype with more aggressive migration and higher invasiveness compared to their parental cells. The Hep3B/ρ^0 cells also showed typical expression of EMT markers such as vimentin and E-cadherin. These phenotypes in Hep3B/ρ^0 cells were mediated by increased tumor growth factor-β (TGF-β) through the canonical Smad-dependent signaling pathway. Additionally, TGF-β signaling was activated via induction of c-Jun/AP-1 expression and activity. Therefore, mitochondrial dysfunction induces EMT through TGF-β/Smad/Snail signaling. These results indicate that mitochondrial dysfunction plays an important role in the EMT process and could be a novel therapeutic target for malignant cancer therapy.

P18-018
The role of KCNMA1 in mature adipocytes

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Potassium channel, calcium activated large conductance subfamily M alpha, member 1 (KCNMA1) has the ability to integrate changes in intracellular calcium and membrane potential and plays significant roles in various physiological functions such as the regulation of smooth muscle tone, neurotransmitter release and neuronal excitability. However, little is known about the role of KCNMA1 on adipocyte differentiation.

In this study, we revealed that the expression level of *kcnma1* was drastically elevated at the late stage of adipogenesis in 3T3-L1 cells and *kcnma1* abundantly expressed in white adipose tissue, suggesting that KCNMA1 has an important role in the function of mature adipocytes. It is well known that mature adipocytes are highly sensitive to insulin. To examine whether KCNMA1 regulates insulin signaling in mature adipocytes, we next performed the knockdown experiments. Insulin-induced Akt phosphorylation in mature adipocytes was clearly suppressed by the reduction of *kcnma1* expression, whereas the level of total Akt did not differ between *kcnma1* knockdown and control cells. These results indicate that KCNMA1 contributes to the regulation of insulin signaling in mature adipocytes. We are now examining whether the channel activity of KCNMA1 is necessary to regulate the insulin signaling.

P18-019
Ceramide 1-phosphate stimulates cell migration in pancreatic cancer cells

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Pancreatic cancer is an aggressive and devastating disease that is characterized by invasiveness, rapid progression and profound resistance to treatment. Despite recent advances in surgical and medical therapy, little progress has been made to decrease the mortality rate of pancreatic cancer. It is now well established that sphingolipids are important signaling molecules for diverse cellular processes. Simple sphingolipids including ceramides, sphingosine or their phosphorylated forms sphingosine 1-phosphate (S1P) and ceramide 1-phosphate (C1P) have been involved in the control of cell homeostasis, as well as tumorigenesis. In this connection, increasing experimental evidence indicates that modulation of sphingolipid metabolism can reduce cancer cell viability, decrease tumor size, and sensitize cancer to conventional treatments. For many years, our group has focused on the role of C1P in the regulation of cell growth and survival, and more recently we discovered that C1P promotes cell migration in macrophages. In this work, we demonstrate that C1P enhances cell migration in two pancreatic cancer cell lines (PANC-1 and MIA PaCa-2). C1P-stimulated cell migration was blocked by selective inhibitors of phosphatidylinositol 3-kinase (PI3K) or Akt, and by specific siRNAs to silence the genes encoding for these kinases. MAPK pathway was shown to be also involved in C1P-stimulated cell migration. We also show in this work that C1P-stimulated cell migration is inhibited by pertussis toxin (Ptx), a potent inhibitor of Gi proteins, thereby suggesting that C1P induces cell migration through interaction with a specific Gi protein-coupled receptor.

P18-020
The level of HSF1 expression and its phosphorylation status do not correlate with migration efficiency of melanoma and breast cancer cells

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The heat shock transcription factor 1 (HSF1), the main regulator of the heat shock response, facilitates tumor progression as well as cell migration and metastasis. In the previous experiments we revealed that mutated, constitutively active HSF1 supports motility, anchorage-independent growth and *in vivo* metastasis of the mouse B16F10 melanoma cells via down-regulation of vinculin. In many tumor types HSF1 is overexpressed. It could be activated by proteotoxic stress and altered kinase signaling characteristic for cancer cells. Thus, we examined if there is any positive correlation between the level of HSF1 expression/phosphorylation and migration efficiency of cancer cells.

The research was performed on a broad panel of human melanoma and breast cancer cell lines (totally 11 lines). The level of HSF1 expressionand its phosphorylation statusat 326 or 320 serine residues (which are the most important for HSF1 activation)

and at Ser 303 (which is responsible for HSF1 repression) was assessed by Western blot. The ability of cells to migrate was examined in Boyden Chamber Assay. Although we observed differences in the level of HSF1 expression/phosphorylation and in migration efficiency, we did not notice any correlation between these values. Moreover, the level of vinculin did not correlate with migration efficiency. It indicates that other mechanisms, distinct from HSF1 activity, are more essential for cancer cell movement.

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P18-021
The role of *fad24*, a positive regulator for adipogenesis, in early embryonic development and muscle cell activation

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To elucidate the molecular mechanisms of the earliest step in adipogenesis, we isolated genes induced to express at the beginning of the differentiation of 3T3-L1 cells. Among them, *factor for adipocyte differentiation 24 (fad24)* is a novel gene. We have previously shown that *fad24* regulated mitotic clonal expansion and involved in DNA replication at the early phase of adipogenesis. Our analyses of *fad24* transgenic mice indicated that *fad24* functions as a regulator of adipogenesis *in vivo*. Moreover, the changes in *fad24* expression in injured skeletal muscle suggested that *fad24* may have roles in muscle regeneration. However, the physiological roles of *fad24* were largely unknown.

To characterize the effect of *fad24* deficient *in vivo*, we generated *fad24*-knockout mice by gene targeting. When the mice from heterozygous parents were genotyped, no *fad24*-null mutants were recovered after embryonic day 9.5 (E9.5). Although *fad24*-null embryos were detected at E3.5, none of the homozygous mutants developed into blastocysts. *In vitro* culture experiments revealed that the development of *fad24*-/- mutants arrested at the morula stage. The number of nuclei decreased in *fad24*-deficient morulae compared with that in wild-type ones, suggesting the deteriorated growth of *fad24*-/- embryos probably due to the inhibition of proliferation. These results demonstrated that *fad24* is essential for pre-implantation development into blastocysts. In addition to the examination of embryonic development, analyses of the function of *fad24* in muscle regeneration are now ongoing.

P18-022
Tetraspanin CD9 and CD82 negatively regulate epithelial-to-mesenchymal transition, anoikis resistance, and stemness of human prostate cancer cells

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Tetraspanin transmembrane protein CD9 and CD82 are known to suppress metastasis of various human cancers, partially by inhibiting cancer cell motility and invasiveness, but the mechanisms underlying their metastasis-suppressing roles are not fully understood. The epithelial-to-mesenchymal transition (EMT), anoikis resistance, and stemness are typical characteristics of cancer cells with highly invasive and metastatic potential. Therefore, we here examined the functional effects of CD9 and CD82 on EMT, anoikis resistance, and stemness of human prostate cancer cells. When CD9 was overexpressed in DU145 prostate cancer cells, E-cadherin expression levels were increased, but Snail and N-cadherin levels were decreased as compared to the parental cells. Stable overexpression of CD82 also resulted in increased expression of E-cadherin and decreased expression of mesenchymal genes. Both CD9 and CD82 also inhibited cell motility and invasiveness. Upon TGF-β_1 stimulation, CD9- and CD82-overexpressing cells did not undergo EMT-associated phenotypic change. Thus, both CD9 and CD82 appeared to suppress EMT in prostate cancer cells. Moreover, both CD9 and CD82 reduced anchorage-independent cell survival by activating caspase-mediated apoptotic pathways, suggesting that these tetraspanins attenuate anoikis-resistant survival of circulating tumor cells. Additionally, sphere formation and CD24-/CD44+ cell population of prostate cancer cells were also decreased by CD9 and CD82, which implicate CD9- and CD82-mediated inhibition of cancer cell stemness. Collectively, these results strongly suggest that both CD9 and CD82 tetraspanins suppress EMT, anoikis resistance, and stemness of human prostate cancer cells, leading to repression of metastatic potential of cancer cells.

P18-023
The inhibition of b-catenin and akt reduced the binding of Peo-1 cells to fibronectin

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The aim of this study to investigate the effects of β-catenin and PKB/AKT on metastatic ovarian cancer cell binding to fibronectin. For this purpose, the expression levels of α4β1 and αvβ6 integrin were determined using α4, β1, αv and β6 antibodies by flow cytometry on PEO-1 cells. The integrin was shown by Immunofluorescence technique. Integrin-fibronectin binding rate and the effects of inhibition of PKB/AKT and β-catenin proteins on cell binding properties are examined by using real time cellular analysis. The cells were treated with different concentration cardamonin for β-catenin and FPA 124 for PKB/AKT signal pathway. The cells were monitored using the xCELLigence system at 30-min intervals for a period of 24 h. Flow cytometry results show that expression levels of αv, α4, β6 and β1 was ordered descendingly with respect to expression level in PEO-1 cells. The results are supported with the imaging of receptor localization by fluorescent microscopy. RTCA analysis results show that 100 μM concentration of cardamonin inhibited cell binding in treated group about three folds than the control group. This difference, moreover, was found to be statistically significant (at α=0. 01). While 15 μM and 25 μM concentrations of FPA 124 inhibited cell binding, inhibition was not observed in the control cells.

Fibronectin adhesion promotes the metastatic behaviors upon the type of cancer cells through the FAK-PI3K/Akt pathway. The results obtained here also demonstrated that the inhibition of PKB/AKT reduces the binding cells to fibronectin that might promote migration and invasion of in PEO-1 cells.

P18-024
Metabolic adaptation of human bronchial smooth muscle cells to hypoxia involves HIF-1 and its regulation by CK1δ

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Airway smooth muscle cells participate in airway remodeling by being not terminally differentiated and switching between contractile and proliferating phenotypes in response to various stimuli, such as oxygen concentration. To investigate this we analyzed the response of human bronchial smooth muscle cells (HBSMCs) to hypoxia (1% O_2). Exposure of HBSMCs to hypoxia resulted to accumulation of the hypoxia-inducible transcription factor (HIF) regulatory subunit HIF-1α and induction of HIF-1 transcriptional activity. This was followed by induction of lipin 1, a HIF-1 target that catalyzes a key step in triglyceride biosynthesis, lipid droplet accumulation and stimulation of cell proliferation. These responses were significantly enhanced upon treatment with an inhibitor of CK1δ, a kinase that is known to impair HIF-1 activity. These data reveal that HIF-1 and its regulation by CK1δ play an important role in the metabolic adaptation that underlies the response of HBSMC to hypoxia.

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P18-025
Development of peptide inhibitors that target the ERK-dependent function of HIF-1α

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Hypoxia provokes a number of adaptive changes, which are coordinated at the transcriptional level by Hypoxia-Inducible Factors (HIFs). HIF-1α, the oxygen-regulated subunit of HIF-1, represents an attractive therapeutic target because of its overexpression in many cancers and its association with poor patient outcome. Apart from oxygen, HIF-1α is regulated by post-translational modifications that involve its direct phosphorylation. We have previously reported that ERK-mediated phosphorylation of HIF-1α at Ser641/643 in a 42-amino acid long domain termed ETD (ERK-Targeted Domain) promotes HIF-1α nuclear accumulation and activity by masking a CRM1-dependent nuclear export signal (NES). To extend our investigation, flag-tagged wild-type ETD or ETD variants carrying mutations either in the ERK phosphorylation sites or inside the NES were expressed in cancer cells grown under hypoxia All forms, except the phosphodeficient mutant, accumulated inside the nucleus and drastically inhibited endogenous HIF-1α phosphorylation and activity. Recombinant inhibitory ETD forms fused to the cell-permeable Tat sequence were then produced in pure form and added to the culture medium of cancer cells. We show that these peptides were able to penetrate the cells, concentrate inside the nucleus and cause mislocalization and inhibition of endogenous HIF-1α. These data offer proof-of-principle for the development of peptide inhibitors based on the ETD sequence that specifically target and impair HIF-1 phosphorylation and function.

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P18-026
Structural characterization of the aryl hydrogen receptor, a newly identified pattern recognition receptor

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The highly conserved Aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor belonging to the basic helix-loop-helix-Per-Arnt-Sim (bHLH-PAS) protein family that senses environmental toxins and endogenous ligands. While the receptor was mainly studied by toxicologists in the past due to its function in inducing detoxifying enzymes upon ligand binding its role in immune cell differentiation and modulation of immune responses has only recently attracted attention when bacterial pigmented virulence factors of different microbial species were identified as novel exogenous AhR ligands (Alves *et al.*, 2014). Receptor activation upon ligand binding leads to virulence factor degradation and cytokine and chemokine production. Hence, AhR serves as an intracellular pattern recognition receptor and bound bacterial pigments present a new class of pathogen associated molecular patterns. At the moment the molecular mechanism of how AhR senses bacterial pigments during infections is unknown. Therefore solving the AhR crystal structure should give important insides into the way of ligand binding and receptor activation. Furthermore analyzing the receptor in its inactive chaperoned complex should provide information about the regulation of the AhR pathway.

P18-027
Repression of HNF4α nuclear receptor expression promotes malignant properties of human pancreatic ductal adenocarcinoma cells

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HNF4α transcription factor is an important regulator of liver, pancreas, intestine and kidney differentiation and tissue-specific gene expression. Derangement of its expression is crucial for the development and progression of hepatocellular carcinoma (HCC); restoration of HNF4α expression in HCC cells results in partial reversion of their malignant properties. HNF4α also acts as tumor suppressor in kidney and intestinal epithelium cells.

We suppose that its deregulation plays a role in pancreatic ductal adenocarcinoma (PDAC) progression. In our previous works we have shown that abnormal HNF4α expression often occured in PDAC cells. Exogenous reexpression of HNF4α in poorly differentiated PDAC cells resulted in attenuation of their malignant potential.

The aim of the current study was to clarify the possible consequences of HNF4α repression in highly differentiated PDAC cell culture CAPAN-2. Using HNF4α-targeting shRNAs we obtained CAPAN-2 cultures with stable HNF4α knock-down. HNF4α repression resulted in downregulation of several important pancrea-specific transcription factors (PDX1, PTF1A and others), significant increase in cells' proliferation rate, colony formation ability and migratory properties.

Thus, HNF4α repression in differentiated PDAC cells considerably promotes their malignant potential. This result clearly indicates that HNF4α acts as a tumor suppressor in PDAC cells. We propose that loss of HNF4α expression may be significant molecular mechanism of PDAC tumors progression.

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P18-028
Elevated circulating endothelial-derived apoptotic microparticles are associated with tumor invasion and poor prognosis of hepatocellular carcinoma

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Background: Endothelial-derived microparticles (MPs) have been reported to be increasing in various diseases including malignant diseases. The aim of this study was to investigate the roles of endothelial-derived MPs in the progression and clinical outcome of hepatocellular carcinoma (HCC).

Methods and Results: Flow cytometric analysis demonstrated that the circulating levels of endothelial-derived activated MPs and endothelial-derived apoptotic MPs were significantly higher in HCC (n = 40) patients than in 30 age- and gender-matched normal control subjects (all $p < 0.05$). Additionally, circulating level of endothelial-derived apoptotic MPs was significantly lower ($p < 0.01$), whereas the circulating levels of the endothelial-derived activated MPs did not differ (all $p > 0.2$) in early stage versus late stage HCC patients. An increased level of endothelial-derived apoptotic MPs was significantly correlated with the presence of multiple tumors ($p < 0.01$), advanced tumor stage ($p < 0.001$), and high alfa-fetoprotein level ($p < 0.01$). Higher levels of endothelial-derived apoptotic MPs were associated with significantly shorter overall survival time ($p < 0.01$).

The matrix metalloproteinases (MMPs) are key regulators of malignant tumor invasion and metastasis. Plasma concentrations of MMP-9 were measured using the enzyme-linked immunosorbent assay. We found that endothelial-derived apoptotic MPs and MMP-9 levels were correlated in HCCs, and furthermore, the combination of endothelial-derived apoptotic MPs and MMP-9 level was associated with the survival of patients ($p < 0.001$).

Conclusions: Our results suggest that endothelial-derived apoptotic MPs may be important in tumor invasion and could be a potential predictor for the prognosis of HCC patients.

P18-029
Polymorphism in the Kaposi's Sarcoma-associated Herpes virus G-protein coupled receptor

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Kaposi's Sarcoma (KS) is the most common AIDS-related malignancy in sub-Saharan Africa and its etiologic agent is Kaposi's sarcoma-associated herpes virus (KSHV). KSHV has been classified based on variability in Open Reading Frame K1 (ORFK1) into six subgroups (A-F). Subtype B and A5 predominate in Africa. The viral G protein-coupled receptor (vGPCR) is the key molecule for the initiation and maintenance of KS. The aims of this study were to classify the KSHV isolated from the tumors, to determine sequence polymorphism in the vGPCR gene and to investigate the functional consequences of the identified vGPCR variants.

Genomic DNA was extracted and the full length of the KSHV vGPCR and ORFK1 were PCR amplified and sequenced followed by multiple sequence alignment using Clustal W tool.

The ORFK1 gene analysis (n = 103) revealed that Subtype A5 was the most common (51 samples), followed by B (42 samples), which are all subtypes prevalent in Africa. In addition, 6 isolates were European subtypes. While, viruses found in 4 patients did not cluster with any known subtypes. Multiple nucleotide sequence alignment of the vGPCR coding region (106 Samples) revealed that sequences from 104 samples were different compared to the prototype sequence. A total of 26 base pair changes were identified and 3 nucleotide changes resulted in amino acid substitution (D5E, G25E and V163A). In addition, a deletion of three base pairs resulting in deletion 14D was identified. These four variant vGPCRs have been expressed in COS cells to assess the functional consequences of these mutations.

P18-030
Hypoxia induces the expression of pro-fibrotic, EMT and fibrosis marker genes in hepatocellular carcinoma cells

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Hypoxia and its key mediators Hypoxia Inducible Factors (HIFs) are implicated in the development of liver diseases of diverse etiologies, often in interplay with inflammatory mediators. We investigated the role of hypoxia in the development of liver inflammation, EMT and fibrosis, using as a model cultures of human hepatocellular carcinoma Huh7 cells. HIF-1α protein levels were increased after incubation of Huh7 cells under hypoxia, but not in the presence of inflammatory mediators (TNFα or LPS). Inflammatory mediators upregulated the expression of the inflammation marker ICAM mRNA, while hypoxia induced the expression of pro-fibrotic (TGF-β1, PAI-1), EMT (E-cadherin, FSP-1) and fibrosis (LOX-2, P4HA1, P4HB) marker genes, a phenomenon also observed after treatment of Huh7 cells with the prolyl-hydroxylase inhibitor and HIF-stabilizer DMOG. Interestingly, the expression of proinflammatory cytokines IL-1β and IL-6 was not detectable in Huh7 cells under any of the conditions tested. Our results suggest that hypoxia and HIF play a critical and inflammation-independent role in development of EMT and fibrosis in hepatocellular carcinoma.

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P18-031
NF-kB, IkB, and EGFR behavior at early stages of a ferric nitrilotriacetate-induced renal cell carcinoma experimental model

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Renal cell carcinoma (RCC) is the most common cancer of the adult kidney and it is asymptomatic even at advanced stages, so opportune diagnosis is rare and the study of early phases is difficult. We reported that RCC tumors induced by ferric nitrilotriacetate (FeNTA) in rats are histologically similar to human neoplasm, and identified one and two months of FeNTA treatment as early stages of carcinogenesis.

Increased activity of NF-kB and decreased levels of its inhibitory protein (IkB), as well as elevated levels of EGFR, product of a NF-kB-target gene, are present in RCC human tumors; however, NF-KB involvement in the first phases of carcinogenesis is unknown. Therefore, renal statuses of these molecules after one and two months of FeNTA treatment were analyzed.

After one month, cytoplasmic and nuclear fractions showed no changes in p65 levels, but an increase in NF-kB activity, which coincided with an EGFR levels augment; however, unexpectedly, IkBa levels also raised in both cytoplasmic and nuclear fractions, suggesting that it may be playing a role other than NF-kB inhibitor. In contrast, analysis at two months revealed no changes of p65 cytoplasmic levels, but a decrease in nucleus, as well as in NF-kB activity, correlating with an increase of IkB levels, but not with the augment in EGFR, so another mechanism in receptor induction may be participating.

In conclusion, NF-kB, IkB, and EGFR behavior is differential at the early carcinogenesis stages studied, and IkB is suggested to have other biological function for the first time.

P18-032
Morphological and biochemical alterations in the spleen caused by immunomodulatory compound cucumarioside A_2-2

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Incubation of immune cells with the triterpene glycoside cucumarioside A_2-2 (CA_2-2) from Far Eastern sea cucumber *Cucumaria japonica* results in their activation. Ultimately, an activation of cellular immunity and magnification of the organism resistance to various opportunistic infections and anticancer effect is appeared under glycoside action. One of the target organs for CA_2-2 action is spleen. After CA_2-2 single *i.p.* administration the tendency to total weight and size enlargement of Balb/c mouse spleens (splenomegaly) was observed. The iba-1 positive area in white pulp was significantly reduced ($16.1 \pm 0.4\%$) compared to control animals ($18.44 \pm 0.4\%$), whereas the iba-1 stained red pulp area increased from $8.58 \pm 0.5\%$ up to $10.2 \pm 0.5\%$ ($p < 0.05$), apparently due to the migration of macrophages. Moreover, the part of splenic macrophages acquired activated phenotype characterized by hypertrophy of the cell body and processes of retraction, indicating pronounced immunomodulatory activity of CA_2-2. A detailed study of PCNA marker distribution showed that the activity of proliferative processes in the white pulp was notably intensified by the CA_2-2 application. Localization of PCNA-positive nuclei in the white pulp region, as well as their dimensional characteristics suggest that a large proportion of proliferating cells in the population belong to B-cells. Stimulation of animals with CA_2-2 leads to marked changes in the mass-spectrometric peptides/proteins profile of the spleen homogenate. The characteristic *m/z* peaks the intensity of which significantly varied after exposure to immunostimulant were revealed by MALDI-TOF-MS.

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P18-033
Pro-angiogenic activity of macrophage inhibitory cytokine-1 secreted from tumor cells under hypoxic conditions

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Angiogenesis, formation and growth of new blood vessels from pre-existing blood vessels, is involved not only in normal physiological process, but also in pathological situations such as cancer and inflammatory diseases. Macrophage inhibitory cytokine-1 (MIC-1), also known as GDF-15, NAG-1 and PTGF-β, is a divergent member of the TGF-β superfamily. It was previously shown that oxygen deprivation induced MIC-1 expression in various cancer cell types. Notably, serum levels of MIC-1 in the prostate, breast, and colon cancer patients were found to be higher than those in normal people. Therefore, we here examined functional effects of MIC-1 on tumorigenesis using a mouse tumor model. As a result, we found that tumors developed from MIC-1-overexpressing cells grew faster than those from low MIC-1-expressing cells. Importantly, high MIC-1-expressing tumors exhibited higher degree of blood vessel formation than control tumors with low MIC-1 expression. MIC-1 transgenic mouse also showed increased blood vessel development as compared to the wild-type mouse, without showing difference in lymphatic vessel development. However, administration of anti-MIC-1 blocking antibody significantly attenuated MIC-1-promoted tumor growth and angiogenesis. MIC-1-induced angiogenesis was further demonstrated in a variety of *in vitro*, *ex vivo*, and *in vivo* angiogenesis assays, where MIC-1 was found to promote angiogenesis by stimulating endothelial cells through the PI3K/Akt and ERK pathways. Although MIC-1 exerted pro-angiogenic activity comparable to VEGF, inflammatory activity of MIC-1 was much less significant than that of VEGF. Taken together, these results strongly suggest MIC-1 as a potent angiogenic factor that contributes to tumor development.

P18-034
CpG-oligodeoxynucleotide-stimulated macrophage migration

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Plasminogen activator inhibitor-1 (PAI-1) is an important factor in inflammation-induced macrophage migration. In this study, we

demonstrate the molecular mechanism associated with the regulation of PAI-1 expression and its biological significance in CpG-oligodeoxynucleotide (CpG-ODN)-stimulated mouse macrophages. It was revealed that PAI-1 expression in macrophages is highly up-regulated by CpG-ODN-stimulation *in vitro* and *in vivo*. The TLR-9-mediated stimulation of PAI-1 expression was independent of the NF-kB pathway and involved the synergistic activation of Sp1 and Elk-1 by the MEK1/2-ERK and JNK signaling pathways. The elevated PAI-1 expression resulted in significantly enhanced transmigration of macrophages through vitronectin but not through fibronectin. Experimental evidence indicated that CpG-ODN plays a role in regulating macrophage migration by stimulating the expression of PAI-1. Further investigation strongly suggested that the PAI-1-induced migration of CpG-ODN-activated cells is modulated depending on the microenvironmental extracellular matrix components.

P18-035
DNA damage signaling in mesenchymal stem cell differentiation

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Mesenchymal stem cells (MSC) are multipotent stem cells that can differentiate primarily to adipocytes, chondrocytes and osteocytes. MSCs have been isolated from human placentas and were characterized by cell surface phenotyping and functional assays. In MSCs undergoing osteogenic differentiation we have observed increased hydrogen peroxide production. Hydrogen peroxide produced by the differentiating cells caused DNA single strand breaks and lead to the activation of the nuclear nick sensor enzyme poly(ADP-ribose) polymerase-1 (PARP-1). PARP-1 activation was required for the differentiation process but in the same time suppressed cellular energetics and resulted in cell death. The MAP kinase p38 translocates into the nucleus, interacts with PARP-1 and also mediates differentiation coupled cell death. We observed that differentiation and cell death are closely coupled in this model. In fact all interventions applied (decomposition of hydrogen peroxide with catalase, PARP inhibition, PARP-1 silencing, p38 inhibition) inhibited both differentiation and cell death. The role of PARP-1 in cell death doesn't end here: as suggested by data from the Duer lab (Science 344:742–746; 2014) the biopolymer poly(ADP-ribose) (PAR) is released from terminally differentiated dead cells and is incorporated into the extracellular matrix. Experiments are under way in our lab to decipher the role of the hydrogen peroxide-DNA breakage-PARP-1 activation-differentiation/cell death pathway in other differentiation-related settings.

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P18-036
Dual role of calpains in murine mammary gland involution after lactation: involvement in pregnancy-associated breast cancer

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Post-lactational regression of mammary gland, also called involution, is characterized by extensive death of the secretory epithelium coupled with remodeling of the extracellular matrix and adipogenesis to regenerate the fat pad. Several signaling pathways have been described to regulate the whole process of involution: STAT family, NF-κB, NO, retinoids, PI3K/AKT; which are responsible of regulating different proteases such as metalloproteinases, cathepsins or calpains. ChIP/chip experiments have shown that NF-kB is a key factor regulating mouse mammary gland involution. Among NF-kB target genes calpains were up-regulated in weaned mouse mammary gland. These proteases trigger cell death, inducing membrane destabilization in several organelles and in the plasma membrane within epithelial cells. Thus, calpains play a role in programmed cell death and shedding of epithelial cells to alveolar lumen where they will be phagocytosed. On the other hand, metalloproteinases and calpains, both increased during weaning, are also involved in tissue remodeling and adipocyte differentiation, the ultimate process in the final regression of the gland. Strikingly, calpains have also been identified as key players during neoplastic transformation, enhancing tumour progression. Calpain 2 is over-expressed in basal-like cancer cell lines, its function being related to cell motility and migration. Indeed calpain-targets on plasma membrane are the same in either a physiological or a pathological model: talin, p120, cadherins and other proteins involved in cell adhesion. The de-regulation of these proteases in breast cancer will be discussed.

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P18-037
PARP-1 Expression and ERK Activation are negatively modulated by PJ-34 in an *in vitro* model of Glioma-Conditioned Blood Brain Barrier

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PARP-1(Poly(ADP-ribose) polymerase-1) inhibitors were proposed to play a protective role in many pathological conditions characterized by PARP-1 overactivation. They act by competing with NAD^+, the enzyme physiological substrate. It has been shown that PARP-1 also promotes tumor growth and progression through its DNA repair activity. Since angiogenesis is an essential requirement for these activities, we sought to determine whether PARP inhibition might affect rat brain microvascular endothelial cells (GP8.3) migration, stimulated by C6-glioma conditioned medium (CM). Through wound-healing experiments and MTT analysis, we demonstrated that PARP-1 inhibitor PJ-34 [N-(6-Oxo-5,6-dihydrophenanthridin-2-yl)-N,N-dimethylacetamide]

abolishes the migratory response of GP8.3 cells and reduces their viability. PARP-1 also acts in a DNA independent way within the Extracellular-Regulated-Kinase (ERK) signaling cascade, which regulates cell proliferation and differentiation. By western analysis and confocal laser scanning microscopy (LSM), we analysed the effects of PJ-34 on PARP-1 expression, phospho-ERK and phospho-Elk-1 activation. The effect of MEK (mitogen-activated-protein-kinase-kinase) inhibitor PD98059 (2-(2-Amino-3-methoxyphenyl)-4H-1-benzopyran-4-one) on PARP-1 expression in unstimulated and in CM-stimulated GP8.3 cells was analyzed by RT-PCR. PARP-1 expression and phospho-ERK activation were significantly reduced by treatment of GP8.3 cells with PJ-34 or PD98059. By LSM, we further demonstrated that PARP-1 and phospho-ERK are coexpressed and share the same subcellular localization in GP8.3 cells, in the cytoplasm as in nucleoplasm. Based on these data, we propose that PARP-1 and phospho-ERK interact in the cytosol and then traslocate to the nucleus, where they trigger a proliferative response. We also propose that PARP-1 inhibition blocks CM-induced endothelial migration by interfering with ERK signal-transduction pathway.

P18-038
TGFβ1-induced migration of adenocarcinoma of the lung by Smad-dependent and -independent mechanisms

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TGFβ1 concentration is enhanced in many epithelial tumors but its role during development of carcinomas remains contradictory. TGFβ1 causes diverse and sometimes opposing effects during carcinogenesis even in the same type of tissue. Whereas TGFβ1 suppresses proliferation and supports differentiation of most non-transformed epithelial cells, it promotes tumor progression and metastasis in later tumor stages. In pancreatic, colon or breast cancer this tumor promoting role is at least partially independent of Smad4-mediated effects.

Here, we compared the TGFb1-induced Smad4-dependent und -independent intracellular signaling in different tumor-derived adenocarcinoma [non-small cell lung cancer (NSCLC)] cell lines. Protein biochemical studies revealed that most of the examined cell lines express TGFβ receptor proteins type I and II as well as Smad2, 3 and Smad4. Six out of eight NSCLC cell lines responded to TGFβ1 with induction of Smad2/3 phosphorylation and activation of Smad-responsive luciferase reporter constructs. Five of eight NSCLC cell lines react to TGFb1 also with activation of further signaling pathway not related to Smad-signaling such as the MAPKs ERK1/2 and p38 and the phosphoinositol-3-kinase/AKT pathway. In contrast to other carcinoma types, in lung adenocarcinoma cell lines TGFβ1 maintain its capability to inhibit cell proliferation and gain the property to induce cell migration. Most analyzed cell lines exhibited changes in epithelial to mesenchymal transition (EMT) marker but not necessarily a complete EMT.

In summary, our data support an important role of TGFβ1 in invasive growth of non-small cell lung cancer cells.

P18-039
Role of KIT signaling in survival of neuroblastoma cells

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Protein kinases regulate activity of many cell survival pathways in neuroblastoma (NB) and are considered as promising therapeutic targets for treatment of NB. Receptor tyrosine kinase KIT expression can be detected in majority of NB cases and its overexpression is associated with MYCN amplification and unfavorable outcome. Still KIT role in NB development and maintaining cell malignancy is not clearly understood. To elucidate KIT role in NB cell survival we downregulated *c-kit* gene expression in two NB cell lines: SH-SY5Y and SK-N-AS with different c-kit expression levels (SH-SY5Y has 10 times more *c-kit* mRNA than SK-N-AS). To downregulate *c-kit* expression we used lentiviral vectors expressing short hairpin RNA (shRNA) against *c-kit* mRNA (shKIT) and nonspecific control shRNA (shSCR). We performed a transcriptional profiling to determine what signaling pathways are regulated by KIT in NB cells and contribute to cell death after *c-kit* gene downregulation, and also to identify changes in signaling pathways that may allow some population of NB cells to survive *c-kit* silencing in long-term period. We performed transcriptional profiling on a third day after transduction with shRNA lentiviral vectors, when there is no significant change in proliferation of transduced SH-SY5Y cells, on a sixth day, when cells with downregulated *c-kit* started to die, and after two weeks when we established a survived population of SH-SY5Y shKIT cells. No significant changes in proliferation of SK-N-AS cells were observed after *c-kit* downregulation and these cells were selected as control for possible of shKIT lentiviral vector nonspecific effect.

P18-040
Effect of microenvironment on Imatinib resistance of K562 cells

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Tyrosine kinase inhibitor Imatinib is the first treatment option for CML. However, leukemia cells develop resistance to Imatinib in time and this accounts for the main reason of treatment failure in CML. Various mechanisms have been purposed to be responsible for the acquisition of drug resistance. One probable mechanism is the transfer of information between cells by the factors, cytokines, chemokines and vesicles in the cancer microenvironement. Recent studies have shown that microvesicles might mediate transfer of elements which play role in malignancy such as miRNAs, proteins and DNA. In this study we treated K562s (Imatinib sensitive) cell line with medium of K562r (Imatinib resistant) cell line which was filtered with with different pore sized filters. Cell viability parameters and response to Imatinib were evaluated by MTT and flow cytometry and our results showed that the components of the microenvironment may influence drug resistance.

P18-041
CCAAT/enhancer binding protein-beta regulates HIF-1alpha expression through mTORC1 pathway

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C/EBPbeta is a transcription factor involved in cell growth, differentiation, survival, inflammation, cellular transformation and tumorigenesis. Recently, it is reported that C/EBPbeta is highly expressed in several human cancers including glioblastoma and breast cancer, especially in more invasive cancers. We observed that the depletion of C/EBPbeta by RNAi in A549 lung cancer cells caused morphological changes. Furthermore, C/EBPbeta promoted migration and invasion of A549 cells and up-regulated the proteins related to Rho family of GTPases which stimulate cancer invasion by regulating cell adhesion and the cytoskeleton. Hypoxia is known to promote the process of tumor invasion, which is mainly mediated by HIF-1alpha stabilization. C/EBPbeta was required to increase expression of HIF-1alpha at protein levels in hypoxic condition, and also promoted cell migration and invasion activities. Even though the detailed mechanism is to be determined, our results indicate that the C/EBPbeta regulates migration and invasion of lung cancer cells both in hypoxia and normoxia suggesting C/EBPbeta as a potential target for controlling cancer cell invasion.

P18-042
LAR protein tyrosine phosphatase enhances PDGF β-receptor signaling by the inhibition of G-protein-coupled receptor kinase 2

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Platelet-derived growth factors (PDGF) are well-established stimulators of normal cell function in many cell types including endothelial, fibroblast, neuron and smooth muscle cells. Interaction of PDGF with its receptor results in dimerization and trans-phosphorylation of the receptors that leads to downstream signaling events such as activation of the ERK/MAP kinases. We have previously identified a novel role for the leukocyte common antigen related (LAR) protein tyrosine phosphatase (PTP) in enhancing PDGFβR signaling through the inhibition of c-Abl activity. In this study, we have established that LAR regulates PDGF β-receptor activity and downstream signaling through inhibition of G-protein-coupled receptor kinase 2 (GRK2) phosphorylation via c-Abl. Western blot analysis of WT and LARΔP (cells with stable knockout of C-terminal LAR phosphatase domains) revealed that phosphorylation of ERK1/2, Akt and SAPK/JNK kinases was significantly reduced in the LARΔP cells confirming that LAR activity is required for the normal PDGFβR signaling. Knockdown of GRK2 from LARΔP cells restores PDGFβR signaling activity suggesting a novel mechanism whereby LAR enhances PDGFβR signaling through suppression of GRK2.

P18-043
Molecular changes of wnt signaling play important roles in astrocytic brain tumor etiology

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Molecular and genetic landscapes of human astrocytic brain tumors still need elucidation. Astrocytic brain tumors are the most common primary CNS neoplasms and are classified into four WHO grades. In the present study key players of wnt signaling, beta-catenin (CTNNB1), TCF1 and LEF1, APC and AXIN1 were investigated in the set of human astrocytic brain tumors. The investigation of beta-catenin demonstrated 10% of samples with potential activating mutations. The results on protein levels demonstrated that 50% of glioblastomas and 56% of astrocytomas showed upregulation of beta-catenin and nuclear localization was found in 52.1% of glioblastomas. Transcription factors of the wnt pathway were also upregulated. Strong TCF1 and LEF1 expression was observed in 51.6% and 71% of glioblastomas. Astrocytoma grade I showed almost opposite expression levels with weak or no expression in the 63.2% for TCF1 and 68.2% for LEF1. Statistical analysis confirmed significant differences in protein expression levels associated to 3 important values, TCF1 weak expression ($F = 2.804$; $p = 0.045$), LEF1 weak ($F = 4.255$; $p = 0.008$) and LEF1 strong expression ($F = 5.498$; $p = 0.002$) with regard to malignancy grade. Allelic losses of APC gene were frequent with glioblastomas showing 60% and diffuse astocytomas 20%. Allelic losses of AXIN1 were found in 10% of glioblastomas. In 31% of glioblastomas and 22% of astrocytomas downregulation of AXIN1 protein was detected. In 31% of glioblastomas AXIN1 was localized in the nucleus. Our findings contribute to better understanding of human astrocytic brain tumor genetic profile and suggest that molecular changes of wnt signaling play important roles in astrocytic tumor etiology.

P18-044
AMPK activation blocked oxidative damage and mitochondrial dysfunction induced by nutrition deprivation as mediated with induction of farnesoid X receptor

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AMPK acts as a key sensor of intracellular energy homeostasis and regulates cell survival or death in response to pathologic stressors. Nutrition is indispensable for cell survival and proliferation. Thus, loss of nutrition caused by serum starvation in cells could induce formation of reactive oxygen species (ROS), resulting in cell death. Serum deprivation in HepG2 cells successfully induced oxidative stress and apoptosis, as indicated by depletion of glutathione, formation of ROS, and altered expression of apoptosis-related proteins such as procaspase-3, poly(ADP-ribose) polymerase, and Bcl-2. Treatment of some AMPK activators significantly blocked these pathological changes, and also induced the expression of both farnesoid X receptor (FXR) as well as small heterodimer partner (SHP). In conclusion, beneficial compounds such as AMPK activators can protect cells against

oxidative injury and mitochondrial dysfunction induced by serum deprivation as mediated with FXR induction.

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Mol Neu S4, Molecular Architecture and Assembly of the Synapse

P23-005-SP
Overlapping functions of stonin 2 and SV2 in sorting of the calcium sensor synaptotagmin 1 to synaptic vesicles

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Neurotransmission involves the calcium-regulated exocytic fusion of synaptic vesicles (SVs) and the subsequent retrieval of SV membranes followed by reformation of properly sized and shaped SVs. An unresolved question is whether each SV protein is sorted by its own dedicated adaptor or whether sorting is facilitated by association between different SV proteins. We demonstrate that endocytic sorting of the calcium sensor synaptotagmin 1 (Syt1) is mediated by the overlapping activities of the Syt1-associated SV glycoprotein SV2A/B and the endocytic Syt1-adaptor stonin 2 (Stn2). Deletion or knockdown of either SV2A/B or Stn2 results in partial Syt1 loss and missorting of Syt1 to the neuronal surface, whereas deletion of both SV2A/B and Stn2 dramatically exacerbates this phenotype. Selective missorting and degradation of Syt1 in the absence of SV2A/B and Stn2 impairs the efficacy of neurotransmission at hippocampal synapses. These results indicate that endocytic sorting of Syt1 to SVs is mediated by the overlapping activities of SV2A/B and Stn2, and favor a model according to which SV protein sorting is guarded by both cargo-specific mechanisms as well as association between SV proteins.

P23-006-SP
Comparison of synaptic connectivity in iPSC-derived neurons from patients with schizophrenia and autism

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Neurodevelopmental disorders such as schizophrenia and autism are complex and heterogeneous diseases signified by emotional and cognitive disturbances. Both diseases share dysfunctional molecular pathways and aberrations in synaptic connectivity. Based on these findings we have analyzed induced pluripotent stem cell (iPSC) – derived neurons from patients with schizophrenia and autism to reveal basic mechanisms of cognitive dysfunction. Differences in gene expression were investigated at the transcriptome level to identify signaling pathways linked to synaptic alterations.

Fibroblasts of patients and healthy individuals were reprogrammed into iPSCs via retroviral transduction and characterized by the analysis of stem cell markers (e.g. SSEA4 and Tra-1-81). Subsequently, iPSCs were differentiated into neural progenitor cells and finally into neurons. Successful terminal differentiation of iPSCs into functional neurons was assured through immunostaining for neuronal marker proteins like b-III-Tubulin and assessment of electrophysiological properties. Similarities and differences in gene expression of neurons derived from patients with schizophrenia or autism were analyzed at the transcriptome level. Neurons of both diseases showed down-regulation of genes related to synaptic connectivity. Accordingly, immunocytochemical staining revealed significant reduction of synaptic marker densities in cultures of iPSC neurons derived from both patients with schizophrenia and autism which points towards shared disease characteristics with respect to synaptic wiring.

In conclusion, iPSC-derived neurons from patients with schizophrenia and autism showed common features at the transcriptomic and immunocytochemical level. The results suggest that the *in vitro* system is applicable to investigations of synaptic deficits associated with cognitive impairments as observed in schizophrenia and autism.

P23-007-SP
Diffusional spread and confinement of newly exocytosed synaptic vesicle proteins

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Neurotransmission relies on the calcium-triggered exocytosis of synaptic vesicles (SVs) with the presynaptic membrane near active zones (AZs) followed by compensatory endocytic retrieval of SV membranes. Whether newly exocytosed SV proteins are recaptured immediately for rapid endocytosis or diffuse away from AZs is unknown. Here we studied the diffusional fate of newly exocytosed synaptic vesicle (SV) proteins in hippocampal neurons by high-resolution timelapse imaging. Newly exocytosed SV proteins rapidly dispersed within the first seconds post-fusion until confined within the presynaptic bouton. Rapid diffusional spread and confinement was followed by slow reclustering of SV proteins at the periactive endocytic zone. Confinement within the presynaptic bouton was modulated by SV protein association with the endocytic machinery to limit diffusional spread of newly exocytosed SV proteins. These data suggest that diffusion and axonal escape of newly exocytosed vesicle proteins are counteracted by the endocytic machinery together with a presynaptic diffusion barrier.

P23-008-SP
Regulation of PSD-95 MAGUK scaffold assembly

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Membrane-associated guanylate kinases (MAGUKs) are a family of multi-domain proteins defined by the minimal presence of PDZ, SH3, and GK-like domains. These domains allow MAGUKs to engage in diverse protein-protein interactions and thus serve as central players in membrane-associated scaffolds that mediate cellular signal transduction. A subset of MAGUKs is highly expressed in dendritic spines and has an established role in regulating synaptic transmission and plasticity. These functions

rely substantially on their direct association, via classical ligand-PDZ domain interactions, with transmembrane AMPA receptor complexes at the post-synaptic membrane, and on the parallel association of other MAGUK domains with numerous proteins within the spine. We have observed that protein-protein interactions of the prototypical synaptic MAGUK PSD-95 are dynamically regulated. In particular, binding of a ligand to the PDZ domain within the MAGUK core (PDZ3-SH3-GK) results in conformational changes in the molecule that have direct influence on the oligomerisation properties of PSD-95 itself, and also on its binding affinity for a subset of regulatory interacting proteins that have been shown to bind distal regions of PSD-95. Our aim is to identify the structural and molecular basis underlying the conformational dynamics of MAGUKs. We investigate how these induced conformational and architectural changes regulate MAGUK scaffold formation, stability and function. Thus our study provides a basis for future investigations into the nature of post-synaptic protein network regulation and receptor clustering.

P23-009
Glutamate concentration at hippocampal excitatory synapses: establishment by deterministic dynamical modelling

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Synaptic transmission (ST) strongly depends on characteristics of its biochemical participants: rates of channels opening and closing, equilibrium constants and others. The experimental establishment of their values often represents a complicated task and the results of value determination may belong to the wide range for different experimental methods. Construction of ST mathematical models may provide the new solutions of this problem. In this work we present the application of such approach that is based on previously elaborated deterministic model of ST [1]. We modified this model in accordance with peculiarities of receptor-inhibitor interaction and simulated the inhibition of ST mediated by AMPA receptors, antagonist being 6-cyano-7-nitroquinoxaline-2,3-dione. The experimentally measured inhibition curve of field excitatory postsynaptic potentials is determined by association and dissociation rate constants for antagonist and concentration of neurotransmitter in the synaptic cleft [2]. The first two parameters are known values; therefore, one can derive the concentration of neurotransmitter in the synaptic cleft by fitting the model simulation to the experimental results. Obtained peak value of neurotransmitter concentration in the synaptic cleft is 0.2 ± 0.05 mM. Furthermore, this mathematical approach enables to calculate the concentration of neurotransmitter in synaptic vesicle: 40 ± 7 mM. Now we are carrying out analogous analysis with inhibitors aimed at another stages of ST.

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P23-010
Analyzing the interplay between MuSK dependent signaling and the cytoskeleton during neuromuscular synapse formation

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The formation and maintenance of the neuromuscular synapse (NMS) are crucially linked to signal transduction events induced by the receptor tyrosine kinase MuSK. MuSK becomes autophosphorylated and initiates its kinase activity in response to motorneuron-derived agrin. Activated MuSK phosphorylates downstream targets to induce a signaling cascade driving presynaptic and postsynaptic differentiation characterized by the clustering of acetylcholine receptors (AChRs). Impaired MuSK function results in acute neuromuscular deficiencies as shown during myasthenia gravis or more severely in respiratory failure and perinatal death in *MuSK* deficient mice.

We have used a quantitative mass spectrometry method to identify and investigate the phosphoproteomic map of MuSK signaling. We identified a total of 10183 phosphopeptides of which 203 were at least 2-fold up/down regulated. Regulated phosphopeptides were classified into four different clusters according to their temporal profiles. Within these clusters we found an overrepresentation of specific protein classes associated with different cellular functions. Particularly, we found an enrichment of regulated phosphoproteins involved in posttranscriptional mechanisms and in cytoskeletal organization. Due to the indispensable role of the cytoskeleton in AChR clustering, we have focused our efforts on regulated phospho-targets with cytoskeletal functions. Our aim is to silence targets in differentiated myotubes using RNAi and to determine their role during MuSK signaling, AChR clustering and NMS formation. For that, we developed TET-ON muscle cell lines for subsequent Doxycycline-inducible miRNA expression. With these studies we expect to unravel the so far poorly understood interplay between cytoskeleton rearrangements and NMS formation.

P23-011
FGF22-induced activation of the PI3K/Akt and Erk signaling pathways in the hippocampus

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The information that flows between neurons is crucial for proper brain functioning. Particularly, in the hippocampus the correct assembly of synapses is dependent on the ability of the neuron to undergo structural and functional changes. Along this process, a diverse set of molecules will influence when and where synapses are formed, establishing synaptic specificity. The fibroblast growth factor 22 (FGF22) is a presynaptic organizing molecule in the CNS, regulating the formation of glutamatergic synapses. Dysregulation of FGF22 signaling during development has been proposed to increase vulnerability to neuropsychiatric disorders, including epilepsy. However, the signaling pathways activated in response to this neurotrophic factor are not clear. To contribute to the basic understanding of synaptogenesis we investigated the signaling pathways that are activated in response to FGF22 stimulation. We found that FGF22 induces robust activation of the PI3K/Akt and MEK/Erk pathways in hippocampal neurons in culture. Moreover, inhibiting any of these pathways with the cor-

responding pharmacological inhibitors blocks FGF22-induced synaptogenic effect, and the same was observed in neurons transfected with shRNA against Akt and Erk. PI3K/Akt and Mek/Erk signaling pathways are known for their role in neuronal survival, axonal growth and branching. Here we demonstrate that these pathways can also regulate synaptogenesis and might have an important role in brain connectivity.

P23-013
Adenosine A_1 and A_{2A} receptor heterotetramers simultaneously bind to G_i and G_s protein

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G-protein-coupled heteromers serve as unique protein complexes that allow cells to sense the environment in a variety of ways. The dynamics and structural characteristics underlying their functional diversity are not known. Studying the model heteromer of adenosine A_1 and A_{2A} receptors, we show here by single particle tracking experiments, that heteromers can form dynamic but stable heterocomplexes. Using biophysical energy transfer techniques and single molecule microscopy, together with molecular models of protein oligomerization, we provide experimental evidence to support a model of these A_1-A_{2A} receptor complexes to be heterotetramers formed by two transmembrane helix-4-interacting A_1 and A_{2A} homodimers bound together via transmembrane helix 5. The resulting non-square heterotetramer forms a complementary interface that can simultaneously accommodate two separately bound abg heterotrimeric G proteins (G_s and G_i) only if the g but not a subunits face the inside of the heterotetramer.

P23-014
Role of the Lipocalin-2 in the structural plasticity of neurons

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The re-arrangement of neuronal networks plays a fundamental role in learning processes, as well as in many pathological states. The structural changes in neuronal networks may rely on modifications of dendritic arbor and/or morphological changes of the dendritic spines that harbor excitatory synapses. Recent studies imply Lipocalin-2 (Lcn-2) in the morphological alterations occurring in neurons. The lack of the Lcn-2 influences dendritic spines density and shape under stress conditions and causes alterations in the complexity of dendritic tree. In this study we further characterize the Lcn-2 mediated structural changes of the hippocampal neurons. We have checked the effect of the Lcn-2 on the on development of dendritic tree. The Lcn-2 was added to the rat neuronal hippocampal cultures on 6 day *in vitro*. After 8 days of incubation we performed morphometric analysis of dendritic trees. The Lcn-2 treatment increased a number of secondary dendrites and extended total dendrite length. We have also checked if the Lcn-2-mediated change of dendritic spines shape occurs immediately after Lcn-2 administration. The GFP-labeled neuronal cells were visualized in the confocal microscope upon 10, 20 and 30 min from the Lcn-2 addition. The 20 min incubation with Lcn-2 was sufficient to cause elongation and thinning of the spines that had the length-to-width ratio smaller then median. Altogether, these results show that Lcn-2 can alter the complexity of dendritic tree and exert short-term effects on the dendritic spine shape of hippocampal neurons.

P23-015
The neuro-cardiac interaction defines an extracellular microdomain required for neurotrophic signaling

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Purpose: Cardiac activity is tuned by sympathetic ganglia neurons (SGNs), whose survival depends on neurotrophins released in low amounts by the myocardium. This study aims to determine whether specific cellular structures are present at the SGN-cardiomyocyte (CM) contact site, investigate the role of SGN/CM contact in NGF-mediated signaling.
Methods and results: Immunofluorescence on mouse heart slices showed close association between SGNs and CMs and enrichment of NGF receptor (TrkA) at the contact site, supporting that specialized and locally organized signaling domains exist (neuro-cardiac junction, NCJ). We tested the functional role of the NCJ in NGF-mediated prosurvival signaling. NGF expression by CMs was silenced in co-cultures and caused 66% decrease of neuronal density, suggesting that SGNs depend on NGF released by CMs. NGF uptake was observed only in processes contacting NGF-overexpressing CMs, supporting that the NCJ is required for neurotrophin-mediated signaling. Consistently, cultured SGNs in contact with CMs survived NGF withdrawal, whereas neurons alone treated with CM-conditioned medium did not survive because of the very low NGF concentration (0.13pM). Conversely, NGF concentration at the contact site was estimated by using the TrkA inhibitor K252a and resulted about 1000-fold higher, supporting that the NCJ allows amplification of intercellular NGF signaling. Immunofluorescence on mouse heart slices showed dystrophin accumulation at the NCJ, and consistently, mdx mice showed 74.36% decrease of cardiac innervation, supporting that dystrophin plays a key role in cardiomyocyte-neuron communication.
Conclusions: Taken together, our results suggest that NGF-dependent signaling to the neurons requires a direct and specialized interaction with myocytes.

P23-016
Functional analysis of the Shank/ProSAP N-terminal domain (SPN) of Shank3

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Shank/ProSAP proteins are major scaffold proteins of the postsynaptic density; mutations in the human *SHANK3* gene are associated with intellectual disability or autism spectrum disorders. We have analyzed the functional relevance of several *SHANK3* missense mutations affecting the N-terminal portion by binding assays in heterologous cells and by expression of wild type and mutant Shank3 in cultured neurons. Postsynaptic targeting of recombinant Shank3 was unaltered. We observed that

several mutations affected binding to interaction partners of the Shank3 ankyrin repeat region (ARR). One of these mutations, L68P, improved binding to the ARR. L68 is located N-terminal to the ARR, in a highly conserved region which we have identified as a novel domain termed the Shank/ProSAP N-terminal (SPN) domain. We showed that the SPN domain folds autonomously and interacts with the ARR in an intramolecular manner, thereby restricting access of either Sharpin or alpha-fodrin. The L68P mutation leads to unfolding of the SPN domain, thereby disrupting the intramolecular interaction and exposing the Shank3 ARR to its ligands. On the other hand, the R12C mutation does not interfere with binding of the SPN domain to the ARR. Here we further characterize the function of the SPN/ARR unit of Shank3. Using site directed mutagenesis and binding assays, we identify residues which are present in the interface between SPN and ARR domains. In addition we analyze the effect of overexpressing wt and mutant forms of Shank3 in primary hippocampal neurons on formation and composition of the postsynaptic density via immunochemistry.

P23-017
Structural and functional characteristics of xenapses – a novel model system for synaptic transmission

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Understanding the detailed molecular underpinnings of synaptic transmission is one of the key problems in molecular neurobiology. However, existing experimental systems for studying live synapses have some limitations: the small size of CNS synapses, random orientation in space, clear distinction of pre- and postsynaptic processes. In order to separate pre- and postsynaptic processes and to investigate presynaptic mechanisms alone we grew neurons on microstructured glass coverslips, functionalized with synaptic cell adhesion protein Neuroligin 1. It triggers formation of presynaptic sites on microstructured host sites, which we thus call xenapses. We found that they are formed exclusively by axons, contain several normal active zones facing the coverslip, and harbour hundreds of synaptic vesicles. Our conditions predominantly facilitate growth of GABA-ergic synapses. Experiments with calcium sensors, FM dyes and endogenously expressed pHluorin constructs have shown, that these xenapses respond on stimulation and are functionally normal. Fast synchronous single vesicle fusion events on single action potentials can be monitored by TIRF microscopy.

We suggest that xenapses will open the possibility to study presynapse formation, presynaptic calcium signalling and dynamics of exo- and endocytosis under controlled conditions.

P23-018
JNK phosphorylation of post-synaptic scaffold proteins

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The c-Jun N-terminal kinases (JNKs) are stress-activated serine-threonine kinases that have recently been linked to various neurological disorders. In patients with intellectual disability (ID), we detected *de novo* truncations in the CNS-expressed *MAPK10/JNK3* gene, highlighting an important role for JNK3 in human brain development. To further elucidate the function of JNK3 in the brain, we searched for neuronal interaction partners and novel phosphorylation targets. We identified several novel JNK3 interaction partners, including the synaptic membrane-associated guanylate kinase (MAGUK) PDZ-domain proteins SAP102 (involved in ID) and PSD-95, the Shank proteins (involved in autism), as well as other neuronal post-synaptic scaffolding proteins.

We have been able to identify the precise site of JNK phosphorylation in the disease-associated PDZ-domain scaffolding protein SAP102, and with custom phosphorylation-site specific antibodies, we are examining the effects of JNK-mediated phosphorylation on SAP102 in primary hippocampal rat neurons. We also use viral-mediated gene transfer of tagged phospho-mimicking/phospho-deficient expression constructs and subsequent FRAP experiments to explore how the mobility of these novel JNK targets is regulated. Additionally, we are investigating the molecular properties of the interaction between JNKs and the MAGUK protein family. Given the location of JNK docking and phosphorylation of these post-synaptic scaffold proteins, specific protein-protein interactions and subsequent signalling may also be affected by JNK. Our data on novel synaptic JNK targets, together with the fact that JNK3 has been implicated in neurodevelopmental disorders, provide the impetus for further studies on novel functions of JNK3 in neurons.

P23-019
Morphine alters laterality index for distribution of biogenic amines in lobes of cerebral cortex

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Morphine is one of the most important and widely used opioid for the treatment of chronic and acute pain: the very wide inter-individual variability in the patients' response to the drug may have behavioral derivations. In experiments on rats we study the effect of acute morphine intoxication (i.p. 10, 20 and 40 mg/kg body weight) on the contents, measured by high-performance liquid chromatography (HPLC), of 3,4-dihydroxyphenylalanine, epinephrine, norepinephrine, 5-hydroxytryptophan, 5-hydroxytryptamine, 3,4-dihydroxyphenylacetic acid, dopamine and h*omovanillic acid in* symmetric part of frontal, parietal, occipital lobes of cerebral cortex. Changes of laterality index ($Li=(Q_{LH}-Q_{RH})/(Q_{LH}+Q_{RH})$, Q_{LH} – left lobe concentration, Q_{RH} – right lobe concentration) were dose-dependent and characterized by the time of intoxication (from one to six hours).We believe that a deep understanding of this mechanism, from physical, biochemical and genetic points of view, could improve morphine administration by helping decrease adverse reactions and customize patient pain therapy.

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P23-020
JNK-associated scaffold proteins and their role in the development and function of neurons

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We are interested in how signaling through the c-Jun N-terminal kinase (JNK) family of proteins influences the development and function of neurons. We have shown that aberrations of the brain-expressed JNK3 are linked to neurodevelopmental disorders, and JNK signaling abnormalities have been observed in mouse models for related diseases. We have demonstrated that disease-associated mutant proteins exhibit loss of classical kinase

activity but are able to bind a subgroup of known JNK scaffolding proteins. This data, together with the fact that JNKs exhibit high basal phosphorylation in neurons, provided the impetus to search for novel neuronal JNK substrates. We have combined the results from a recent computational study (in which JNK-docking sites in the human genome were predicted) with data from large scale phospho-proteomic studies designed to identify neuron-expressed phosphoproteins, in order to identify novel JNK-interacting proteins. The aim of the project described here is to contribute to our understanding of the neuronal function of two of these proteins, namely CNKSR1 and CNKSR2, both of which were recently implicated in neurodevelopmental disorders. We have identified novel interaction partners for CNKSR proteins and we are currently investigating the role of CNKSR1 and CNKSR2 as scaffolds for JNKs and other selected synaptic regulatory proteins and receptors, in order to elucidate the mechanisms by which alterations in CNKSR-function result in defective neurological development.

Mol Neu S5, Control of Neuronal Function by Regulating Protein Homeostasis

P24-003-SP
Vaccinia-related kinase 2 controls eukaryotic chaperonin TRiC/CCT stability by inhibiting Ubiquitine-specific protease 25

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Molecular chaperones monitor the proper folding of misfolded proteins and function at the first line of defense against mutant protein aggregation in neurodegenerative diseases. The eukaryotic chaperonin TRiC is a potent suppressor of mutant protein aggregation and toxicity in early stages of disease progression. Elucidation of TRiC functional regulation will enable us to better understand the pathological mechanisms of neurodegeneration. We have previously shown that Vaccinia-related kinase 2 (VRK2) downregulates TRiC protein levels through the ubiquitin-proteasome system by recruiting the E3 ligase COP1. However, although VRK2 activity was necessary in TRiC downregulation, the phosphorylated substrate was not determined. Here, we report that Ubiquitine-specific protease 25 (USP25) is a novel TRiC interacting protein that is also phosphorylated by VRK2. USP25 catalyzed deubiquitination of the TRiC protein and stabilized the chaperonin, thereby reducing accumulation of misfolded polyQ protein aggregates. Notably, USP25 deubiquitinating activity was suppressed when VRK2 phosphorylated the Thr^{680}, Thr^{727}, and Ser^{745} residues. Impaired USP25 deubiquitinating activity after VRK2-mediated phosphorylation may be a critical pathway in TRiC protein destabilization.

P24-004-SP
Dysfunction of PLC-gamma1 contributes to the development of neuropsychiatric disorders

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Neurotrophin factors activate PLC-γ1 through Trk receptors, a family of three receptor kinases that have been implicated in the regulation of cell survival, proliferation, the fate of neural precursors, axon and dendrite growth and patterning, and membrane channels in the brain. PLC-γ1 has been implicated in brain diseases, such as Parkinson's disease, epilepsy, limbic epileptogenesis, and bipolar disorder. However, the *in vivo* role of PLC-γ1 has not been clearly demonstrated. We used conditional gene targeting in mice to eliminate the PLC-γ1 in forebrain. Forebrain-PLC-γ1 knockout mice display hyper-locomotor activity. In addition, these mice show abnormal behaviors including reduced social interaction and decreased social communication. They also exhibit impaired context-dependent spatial memory. In mEPSC and mIPSCs recording, PLC-γ1 deletion has no effect on excitatory synaptic transmission. However, mIPSC frequency, but not amplitude, is substantially decreased. These results suggest that the imbalance between excitation and inhibition in PLC-γ1-deleted hippocampus contributes to abnormal behaviors. In addition, deletion of PLC-γ1 results in impaired LTP dependently on TrK B receptor activation. Molecular studies revealed that BDNF/TrkB signaling is impaired in PLC-γ1 deleted hippocampus. Taken together, our findings demonstrate a critical role of PLC-γ1 for BDNF/TrkB signaling activation and neuropsychiatric functions.

P24-005-SP
Unfolded Protein Response in Parkinson's disease: a new neuroprotective role for Glutathione S-Transferase pi

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Parkinson's disease (PD) is characterized by selective loss of dopaminergic neurons of the substantia nigra pars compacta, and by accumulation of misfolded proteins. Evidence from studies in human PD brain indicates that endoplasmic reticulum (ER) stress is a common feature of the disease placing ER dysfunction as an early component of PD pathogenesis. Moreover, the presence of misfolded proteins triggers a cellular stress response in the ER called the Unfold Protein Response (UPR).

Glutathione S-transferase pi (GSTP) is a phase II drug metabolizing enzyme that catalyzes the conjugation of reduced glutathione to electrophilic groups on substrate molecules playing an important defensive role against the accumulation of reactive metabolites that contribute to neuronal damage. We have shown that *in vivo* GSTP mediates MPTP-induced cellular stress response by controlling c-Jun N-terminal kinase activity, and that *Gstp* null mice are more susceptible to MPTP-induced neurotoxicity.

In this study, we investigated the UPR activation in the brain of C57BL/6 wild-type and Gstp KO mice under sub-acute administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), as a model of PD. Mice were also treated with tauroursodeoxycholic acid, a chemical chaperone that modulates ER adaptive capacity.

The relative concentration in ER stress responsive genes and the expression levels of UPR-related proteins was estimated by Western blot analysis of midbrain and striata tissue extracts.

Our results provide new insights into the role of GSTP in the ER-stress cellular response, unravelling a new mechanism contributing to GSTP-elicited neuronal protection.

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P24-006-SP

Regulation of SH3 domains in intersectin 1 modulates its function in the synaptic vesicle cycle

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Vesicular cargo proteins and excessive membrane material need to be recycled locally at the chemical synapse in order to allow for rapid and repeated release of neurotransmitters. This is facilitated by a series of successive processes, including vesicle endocytosis and clustering, that are collectively referred to as the synaptic vesicle cycle. A large multi-domain scaffolding protein that acts as a backbone in several steps of the vesicle cycle is intersectin 1. The five SH3 domains of this protein are involved in a large set of interactions with various synaptic proteins associated with different steps of the vesicle cycle. Combining biophysical experiments (including nuclear magnetic resonance spectroscopy and mass spectrometry) with *in vivo* studies in the lamprey giant synapse, we show that the interactions of individual SH3 domains (engaging canonical and non-canonical epitopes) happen in a regulated manner and lead to dynamic exchange of binding partners during the cycle. For example, the intersectin 1 SH3A domain is impaired by an intramolecular interaction that is regulated by phosphorylation and the local concentration of its interaction partners. The vesicle clustering protein synapsin could be shown to be the main target of this autoinhibitory mechanism that is modulated by the inclusion of a neuron-specific alternative exon in the domain. Thus, we describe a neuron-specific molecular switch between the endocytic and the vesicle clustering mode of action of intersectin 1.

P24-008

Alterations in functional status of rat brain mitochondria under circadian rhythm disorders

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The aim of our work is to study the changes of intensiveness of oxidative processes developed against the background of the stress caused by isolation and violation of circadian cycle, as well as to study the alterations of the energetic metabolism and to ascertain the relation of mitochondrial permeability transition pore – MPTP with these processes. We have studied the influences that prolonged isolation and disruption of the circadian cycle have on behavioral activity and hormonal status among animals. It has been showed that such conditions result in development of stress, and decreases occur in the functioning the creatine/phosphocreatine (Cr/CK/PCr) cycle, which contributes to the preservation of energetic homeostasis. The was found out that under the stress the quantity of nitric oxide in brain mitochondria gets increased by 65%. It is accepted that the excessive increase of nitric oxide becomes the cause of nascence of peroxynitrite, which, in its turn, is an indicator of activation of oxidative stress. The obtained results have shown us that as a result of the long-term stress in the cells there are activated the oxidative processes which can be caused by the alterations of Ca^{2+}-induced messenger system. Under such conditions there was determined the alteration of permeability of mitochondrial membranes, which is an important factor initiating apoptosis. According to our opinion, as the answer to the pathological processes induced by long-term psycho-emotional stress, there occurs the increase in permeability of mitochondrial membranes, which ought to be conditioned mostly by *in vivo* activation of MPTP.

P24-009

Neuroprotective effect of Mycophenolate mofetil against Tacrolimus induced brain failure in rats

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The brain is one of the most vulnerable organs affected by Tacrolimus (TAC) toxicity. In the present study the effect of Mycophenolate mofetil (MMF) on TAC-induced neurotoxicity in male Wistar rats was examined. The experiment was carried out on rat model, animals were gaved with TAC and MMF alone and combined for 24 h. It was found that TAC caused significant neurotoxicity as indicated by the changes in acetylcholinesterase (AChE) activity, increase the oxidative stress markers [lipid peroxidation (MDA) and protein carbonyl (PC)], a decrease of various antioxidant enzymes levels, namely superoxide dismutase (SOD), catalase (CAT). The results showed that the treatment of MMF at 50 mg/kg BW (Body weight) exposure effectively decrease oxidative stress damage and augments antioxidant defense in brain tissue. In addition, the MMF treatment minimized the brain injury via influencing the activation of AChE. The obtained results suggested that MMF is a promising target for neuroprotection against brain disease. The protective effects of MMF mediated by regulating the oxidant and antioxidant status in brain tissue of rats. Moreover, this work defines another mechanism of biological activity of MMF by increasing the AChE activity.

Keywords: Tacrolimus, mycophenolate mofetil, combination, brain, oxidative stress, rats.

P24-010

Orexin--CRF1-sigma-1 complexes as targets for cocaine

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Release of the neuropeptides corticotropin-releasing factor (CRF) and orexin-A in the ventral tegmental area (VTA) play an important role in stress-induced cocaine-seeking behavior. We provide evidence for pharmacologically significant interactions between CRF and orexin-A that depend on oligomerization of CRF1 andorexin OX1 receptors. CRF1R-OX1R heteromers are the conduits of a negative crosstalk between orexin-A and CRF as demonstrated in transfected cells and in the VTA, where they significantly modulate dendritic dopamine release. The cocaine target sigma σ1 receptor (σ1R) also associates with the CRF1R-OX1R heteromer. Cocaine binding to the σ1R-CRF1R-OX1R complex promotes a long-term disruption of the orexin-A-CRF negative crosstalk. Through this mechanism cocaine sensitizes VTA cells to the excitatory effects of both CRF and orexin-A, thus providing a mechanism by which stress induces cocaine seeking.

P24-011
Blood-Brain barrier differences between white and grey matter

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The blood-brain barrier (BBB) separates the brain parenchyma from the circulating blood. Its main cellular components are brain endothelial cells interconnected by a continuous line of tight junctions and surrounded by pericytes and astrocytic end-feet. BBB functions are selectively altered in the grey or white matter in various diseases of the central nervous system (CNS). We aimed to identify specific structural and molecular differences between white and grey matter BBB using *in vitro* and *in vivo* models.

Our western-blot results revealed higher alpha-catenin, lower occludin and equal beta-catenin and claudin-5 expression in endothelial cells isolated from the white matter compared to those form the grey matter. We observed different expression patterns of two astrocytic markers: GFAP staining was more pronounced in the white matter than in the grey matter, while AQP4 showed an equal distribution along the vessels in both grey and white matter. No major ultrastructural differences could be observed between capillaries of the white and grey matter as assessed by transmission electron microscopy.

Different expression of endothelial tight junction proteins and astrocytic markers might determine why white and grey matter BBB reacts differently in diverse diseases of the CNS.

This work was supported by grants from the strategic grant POSDRU/159/1.5/S/133391 within the project "*Doctoral and Post-doctoral programs of excellence for highly qualified human resources training for research in the field of Life sciences, Environment and Earth Science*" co-financed by the POSDRU Program 2007–2013.

P24-012
Design and synthesis of novel 2-pyrazoline analogues and their hMAO inhibitory activities

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Monoamine oxidase (MAO) is a key enzyme which is responsible for the oxidative deamination of xenobiotic amines and monoamine neurotransmitters. The enzyme exists in two isoforms, MAO-A and -B. MAO-A inhibitors have therapeutic utility mainly for the treatment of depression, whereas MAO-B inhibitors are used for the treatment of Parkinson's and Alzheimer's diseases. MAO-A inhibitors are effective antidepressants, but their use has been limited by some side effects mostly associated with the irreversible inhibition of MAO. Thus, design of new potent, selective and reversible MAO-A inhibitors, is of value.

It has been reported that 1,3,5-triphenyl-, 1-thiocarbamoyl- and 1-acetyl-3,5-diaryl-4,5-dihydro-1H-pyrazole derivatives have potent MAO inhibitor activity. Bearing in mind the above considerations and our previous researches concerning to the synthesis of novel selective MAO inhibitors, we report here the synthesis, docking studies and hMAO inhibitory activities of some new 2-pyrazoline derivatives. Chemical structures of the compounds have been elucidated by their IR, ^{1}H NMR, Mass, and elementary analysis data. All the compounds were found to be selective and reversible inhibitors towards hMAO-A. Compound **3,** which carries a 2-hydroxy-5-chlorophenyl group at the 3rd position and 4-methoxy phenyl group at the 5th position of the 2-pyrazoline ring, showed the highest hMAO-A inhibitory activity with a K_i value of 5.00 $\pm$ 0.10 nM. The selectivity index of compound **3** was calculated as 4.71 x 10^{-5}. Newly synthesized compounds were docked computationally to the active site of the hMAO-A and -B forms, and the data indicated a significant correlation between the docking results and the experimental ones.

P24-013
Discontinuous morphine administration evokes reliable changes in the neuroactive amino acid pools and biogenic amines in rat brain regions

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The rodent model of the discontinuous morphine intoxication was worked out, based on the 1, 2, and 3 cycles of the intraperitoneal morphine administration to rats (twice daily for 4 days, 30 mg/kg during the 1st cycle, 40 mg/kg during the 2nd and 3rd cycles) that was followed by the 3 days' morphine free periods. The influence of discontinuous morphine treatment was studied on the levels of biogenic amines (serotonin, dopamine, norepinephrine), their metabolites, and some neuroactive amino acids in cerebral cortex, midbrain and striatum of rats. The changes in the indices of serotoninergic system, contents of free amino acids in the brain regions varied depending on the duration of morphine administration and the brain region tested. The 14 days' discontinuous morphine intoxication was accompanied by the reliable increase in the levels of serotonin and its metabolites in midbrain and striatum, and that effect was attenuating while the number of drug exposure periods raised. Metabolic affects of the 21 days' discontinuous morphine intoxication manifested as total level amino acids reduction, as well as aromatic, neurotransmitter, and excitatory amino acids; while biogenic amines and their metabolites levels had no significant changes.

Therefore, we can propose that the changes observed were likely to reveal the disturbances in the protein homeostasis in the brain during morphine intoxication and a hidden ability of nervous tissue to generate the symptoms of abstinence syndrome after morphine cessation.

P24-014
Purified calpain hydrolyses the hexapeptide analogue of C-terminal fragment of Substance P

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Calpains are ubiquitous family of Ca^{+2} dependent thiol poteases being inhibited with endogenous protein: calpastatin. Calpains play the crucial role in an apoptose, neoplastic and Alzheimer disease, prion and NMDA neurotoxicity.

An ubiquitous undecapeptide neurohormone substance P (SP) might inhibit the apoptosis of some neurons and inhibit calpain (s).

We did not find out the hints on the interrelations of calpain (s) and substance P.

Long time ago we studied the degradation of analogues of C-terminal fragment of SP within different areas of rat brain .We elaborated the simple. sensitive assay for enzymes splitting the peptide-based on the differences of electric charge of products with pH -without columns.

Now I applied such a method to prove if and how the purified calpain hydrolyzes the hexapeptide-the analogue of C-terminal SP fragment: pyroglutamyl^{6}[^{125}I-tyrosyl^{8}] SP_{6-11} (JP), 2000 Ci/mmol. I have found it is the case indeed.

The purified calpain(exactly: 80kD calpain subunit: Sigma) splits preferentially 9–10 but hardly 7–8 peptide bond. The hydrolysis of JP with Sigma calpain was carried on in Tris-HCl pH 7.4 with β-mercaptoethanol with $CaCl_2$ or versenate (EDTA). The inhibitors applied: SH agents; calpain inhibitory peptide and purified Sigma calpastatin .

Ca^{+2}-dependent JP hydrolysis was totally inhibited with the thiol reagents and with calpain. The similar activity against JP was found within rat heart (homogenate) and brain.

Till now nobody showed the degradation of subtance P (fragments/analogues) with calpain. My discovery seems to be significant in pathophysiology of brain.

P24-015
Implication of the Na^+/Ca^{2+} exchanger to the fine tuning of the neurosecretory process of GABA

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The main goal of the present research was to elucidate mechanisms underlying the modulatory effects of presynaptic glutamate receptors on the presynaptic release machinery. We characterize the events induced by glutamate receptors agonists and antagonists in isolated hippocampal and cortical nerve terminals by analyzing following parameters i) evoked secretion of [^{3}H]GABA from nerve terminals; ii) involvement of synaptic vesicles in the release process; iii) a level of the plasma membrane potential.

The results demonstrate that glutamate receptor-induced modulation of the strength of synaptic response was due to increasing the release probability of synaptic vesicles. Our date allow to consider that activation of presynaptic glutamate receptors stimulates not only a fast synchronous vesicle fusion, but also a delayed asynchronous exocytosis as a result of inducing spontaneous fusion of synaptic vesicles with the presynaptic membrane. It could be suggested the following mechanism that leads to the glutamate-induced asynchronous exocytosis: Na^+ influx via the activated glutamate receptor channels, as well as glutamate transporter, leads to the increase in cytosolic Ca^{2+} due to reverse operation mode of the plasma membrane Na^+/Ca^{2+} exchanger. This conclusion is based first on the finding that Na^+/Ca^{2+} exhanger (NCX) inhibitor benzamil attenuates the amount of released synaptic vesicles upon glutamate stimulation. The second argument comes from the experiments where intracellular Ca^{2+} chelator BAPTA was used. In such conditions, when intracellular Ca release was blocked, the asynchronous exocytosis was failed to occur.

P24-016
Sulforaphane counteracts neurodegeneration induced by glycative stress in SH-SY5Y cells

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Glycation, an endogenous process that leads to the production of advanced glycation end products (AGEs), plays a role in the etiopathogenesis of several neurodegenerative diseases such as Alzheimer's disease (AD). Methylglyoxal is the most potent precursor of AGEs and high levels of methylglyoxal have been found in the cerebrospinal fluid of AD patients. Methylglyoxal may contribute to AD both inducing extensive protein cross-linking and as mediator of oxidative stress. Aim of this study was to investigate the role of sulforaphane, an isothiocyanate found in Cruciferous vegetables, in counteracting methylglyoxal induced damage in SH-SY5Y neuroblastoma cells. Data demonstrated that sulforaphane protected cells against glycative damage by inhibiting the activation of caspase-3 enzyme, reducing the phosphorylation of MAPK signaling pathways (ERK1/2, JNK, and p38), reducing oxidative stress and increasing intracellular GSH levels. For the first time we demonstrated that sulforaphane has a pivotal role in methylglyoxal detoxifying system increasing the expression and activity of glyoxalase I. Sulforaphane modulated brain derived neurotrophic factor and itsreceptor Tropomyosin kinase B, whose dysregulation is related to AD development. Moreover, sulforaphane was able to revert the reduction of glucose uptake caused by methylglyoxal. In conclusion, sulforaphane demonstrated a pleiotropic behavior thanks to its ability to act on different cellular targets, suggesting its potential role in preventing/counteracting multifactorial neurodegenerative diseases such as AD.

P24-017
New mechanisms of receptor-based pharmacological effects of regulatory peptides

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Pharmacological effects of many drugs is based on modulation of ligand–receptor binding functional activity. Semax, Selank and proglyprol are novel peptide drugs with a broad range of activities in central nervous system. Semax demonstrates good results for the treatment of cognitive disorders, Selank possesses strong anxiolytic properties, tripeptide Pro-Gly-Pro (proglyprol) able to protect various cells from noxious factors. The specific binding of labelled Semax and of peptides PGP and HFPGP (which are the stable Semax metabolites) to various rat brain areas was investigated and it was found that all of these compounds had different binding sites. The purpose of this study was to identify new mechanisms of receptor-based pharmacological effects of peptides Semax, Selank and proglyprol (PGP), as well as to develop more effective peptide compounds by conjugation with bioactive lipids. The joint action of specific ligands of some crucial neuroreceptor systems in the system of peptide + non-peptide allosteric modulator was investigated. For this purpose, some different tritium-labeled ligands with high molar radioactivity were prepared, which allowed to study the influence of peptides and some of their synthetic derivatives within wide concentration range (from picoM to microM) on the specific binding of labelled ligands to GABA(A), glutamate, vanilloid, dopamine, TRH (thyrotropin-releasing hormone) receptors and other of rat brain cells plasma

membranes. We showed that peptides investigated able to modulate GABA, Glu and etc. specific binding. As a result of the study, several pharmacologically important structures were identified as basic candidates for the creation of new drugs.

P24-018
The effect of the *Cyperus rotundus* terpen, alpha cyperone, on the Polymerization of Microtubules, *in vitro* as an indicator of memory

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The rhizomes of *Cyperus rotundus* (Cyperaceae) have been used in Asian traditional medicine for the treatment of several diseases. However, few studies have investigated the biological activity and molecular mechanism of action of α-cyperone, a major compound in the rhizomes of *Cyperus rotundus*, representing about 20% of the total essential oil. α-cyperone might interact with cellular proteins and modulate their functions, but the main target of this terpenoid and the other compounds of *Cyperus rotundus* have not been discovered yet. Microtubular proteins are one of the most important proteins inside the cells and have several functions in nearly all kinds of cellular processes. The aim of this study was to investigate whether α-cyperone affects on memory or learning process in brain due to polymerization of microtubule.

The result of this investigation demonstrated that α-cyperone increased tubulin polymerization and microtubule nucleation rate. α-cyperone would be able to participate in cell signaling. So it would be suggested that α-cyperone could improve memory and the rate of learning and may prevent and of improving Alzheimer's Disease.

Keywords: Cyperus rotundus, alpha cyperone, tubulin, microtubule, memory.

P24-019
Thrombin mediates migration of SK-N-SH cells via PLC, Ca^{2+}, CaMKII, PKCa, and NF-kB-dependent matrix metalloproteinase-9 expression

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Background: Matrix metalloproteinase-9 (MMP-9) plays a crucial role in pathological processes of brain inflammation, injury, and neurodegeneration. Thrombin has been known as a regulator of MMP-9 expression and cell migration. However, the mechanisms underlying thrombin-induced MMP-9 expression in human neuronal cells remain unclear.

Materials and methods: SK-N-SH cells were used in this study. The effects of thrombin on MMP-9 expression were determined by gelatin zymography, real-time PCR and promoter assay. The involvement of signaling components in these responses were investigated by using the selective pharmacological inhibitors and transfection with siRNAs. Intracellular Ca^{2+} concentration was measured by using the Ca^{2+}-sensitive dye Fura-2/AM. Cell migration was evaluated with monolayer wound healing assays.

Results: We demonstrated that thrombin induced the expression of MMP-9 and migration of SK-N-SH cells which were inhibited by pretreatment with the inhibitor of Gq-coupled receptor (GPAnt2A), PC-PLC (D609), PI-PLC (Et-18-OCH_3), calmodulin [calmidazolium chloride (CaMI)], CaMKII (KN62), PKC (Gö6976 and GF109203X), p38 MAPK (SB202190), JNK1/2 (SP600125), NF-kB (Bay11-7082 and Helanalin) and transfection with siRNA of PKCa, JNK, p38 MAPK, or NF-kB (p65). In addition, thrombin-induced elevation of intracellular Ca^{2+} concentration was attenuated by PPACK (a thrombin inhibitor). Thrombin further induced CaMKII phosphorylation which was inhibited by Et-18-OCH_3, CaMI and KN62. Thrombin also induced PKCa-dependent p38 MAPK and JNK1/2. Finally, we showed that thrombin enhanced p65 phosphorylation.

Conclusion: These results concluded that thrombin activated PLC/Ca^{2+}/CaMKII, PKCa/p38 MAPK and JNK1/2 leading to NF-kB activation and ultimately induced MMP-9 expression associated with migration of SK-N-SH cells.

P24-020
Rat brain proteome changes induced by cute and chronic stress

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Stress is a set of non-specific reactions on the impact of unfavorable factors. These non-specific reactions, are accompanied by changes in the neuro-endocrine functioning, and are known to be non-specific basis for many diseases. So far as stress reactions are triggered and regulated by the nervous and endocrine systems, detailed information about the processes occurring in the nervous system can open up possibilities to minimize the negative effects of stress in the nervous system and the whole organism. Therefore in this study we used rats as a model for understanding cute and chronic stress-induced proteom changes in brain tissue.

Material and methods: We performed a wide range of experiments concerning the analysis of protein profile of rat brain. In the current work we used homogenates of different rat brain regions provided by the Institute of biochemistry of biologically active compounds, National Academy of Sciences of Belarus. Total protein was extracted from the tissue homogenate with methanol-chloroform method and analyzed by «Shot-gun» proteomic approach.

Results: We developed optimized approach for the isolation of proteins from the aggregate of the rat brain tissues. Qualitative and quantitative differences of protein profile in different regions of rat brain obtained from experimental stressed animals. The obtained results could be important for establishment of mechanisms of pathological processes, owls occurring in the brain under stress.

P24-021
Neuropeptides, age and food availability affect the level of sugars in the haemolymph of tenebrionid beetles

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Neuropeptides are multifunctional group of signaling molecules which regulates almost all of physiological processes in animals body. In insect, based on structural and functional similarities 32 families of neuropeptides have been distinguished. They regulate crucial physiological processes, such as development, reproduction, feeding, circulation and homeostasis of metabolites in the

haemolymph. In beetles, the largest insect order physiological properties of neuropeptides are largely undiscovered. Here, we report the metabotropic action of certain neuropeptides on the regulation of free sugar levels in the haemolymph of two beetles *Zophobas atratus* and *Tenebrio molitor* together with changes in the free sugar levels observed in various developmental stages and food availability.

Reversed phase high pressure liquid chromatography (RP-HPLC) was used to determine the amount of carbohydrates in the haemolymph of beetles. The major identified sugar was trehalose. Apart from trehalose different classes of sugars – glucose, saccharose and polyoles were also identified. We investigated the effect of the endogenic neuropeptides from pyrokinin family Tenmo-PK-1 (HVVNFTPRLa), Tenmo-PK-2 (SPPFAPRLa), Tenmo-PK-3 (HLSPFSPRLa) and Zopat-PK-1 (LPHYPRLa) on the glucose, trehalose, saccharose and polyoles concentrations. Tested peptides caused various effects in the haemolymph of the larvae including hypertrehalosaemia. Moreover, the concentrations of sugars differ significantly in the haemolymph of larvae, pupae and imagoes and change after period of starvation.

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P24-022
Myelin basic protein binds the Von Willebrand domain of ubiquitin receptor Rpn10 to enable ubiquitin-independent proteasomal degradation

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Here, we studied proteasome-mediated degradation of myelin basic protein (MBP), one of the major components of the myelin sheath of neuronal axons in the central nervous system. The absolute majority of cellular proteins are degraded by the 26S proteasome only after their ubiquitination. In our previous study we showed that MBP is hydrolyzed by the 26S proteasome without ubiquitination both *in vitro* and in mammalian cells, however, mechanism of this process was not completely resolved. To explain the mechanism of ubiquitin-independent degradation of MBP, firstly, we showed that MBP, similarly to polyubiquitinated proteins, might interact with hRpn10, but did not bind second ubiquitin receptor hRpn13 or shuttle protein hHR23a, a member of Ubl-UBA family. Further determination of hRpn10 domains involved in MBP binding revealed that in contrast to UBL, classical UIM ligand, interaction of MBP with hRpn10 is mediated through cooperative interaction with both VWA and UIM domains. Our studies demonstrated that VWA effectively inhibited proteasome-mediated MBP hydrolysis both *in vitro* and *in vivo*, whereas UIMs along with hHR23a-UBL were significantly less efficient. We also specified if MBP has a distinct region responsible for its proteasome-mediated degradation. Truncation of any of three MBP fragments did not result in decreased MBP degradation by proteasome. Finally our data suggest that VWA is primary site for MBP binding, whereas UIMs rather assists it in this process.

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P24-023
Epigenetic effect of Trichostatin A on attenuating neuroinflammation and cognitive dysfunction in septic mice

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During sepsis, excessive cytokine release by microglia causes cognitive dysfunction and behavioral changes. Activated peripheral innate immune system stimulates cytokine secretion in the central nervous system, which induces cognitive function. Trichostatin A (TSA) modulate cytokine synthesis and release through epigenetic mechanism by inhibiting histone deacetylases (HDACs). We investigated the epigenetic effect of TSA on neuroinflammation and cognitive dysfunction in lipopolysaccharide (LPS)-induced septic mice. ICR mice were injected with vehicle or TSA (0.3 mg/kg). For septic induction, they were injected with saline or *Escherichia coli* LPS (1 mg/kg) on hour later. In the TSA-pretreated mice, microglial activation was lower, anhedonia did not occur, and LPS-induced cognitive dysfunction (anorexia, weight loss, and social withdrawal) were attenuated. Moreover, mRNA expression of HDAC2, HDAC5, indoleamine 2,3-dioxygenase (IDO), TNF-α, MCP-1, and IL-1 in the brain of septic mice and in the LPS stimulated BV-2 microglial cells was lower. TSA diminished inflammatory responses in the septic mouse brain and modulated the cytokine-associated changes in cognitive function, which might be specifically related to the epigenetic effect by reducing HDAC2 and HDAC5 expression.

Sys Biol S1, Interspecies Communications

P26-003
Adaptation and communication – the keys for survival in bacterial world

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Bacteria are single-cell organisms, which often act in collaboration and behave more as an integrated community than as isolated individuals. As such they need to synchronize, which is possible due to secretion of quorum sensing compounds. Amongst several signals to which bacteria respond as a community are biochemical aggressions by antimicrobials. In this case, signaling regularly leads to an altruistic behavior by most of the cells within the population, aiming overall species conservation.

In the search for mechanisms for survival and communication within the microbial world, we have studied the secretion pattern of bacteria known to be able to strive on and overcome extreme conditions, both nutritional and abiotic stress imposed. *Pseudomonas spp* are known for their potential to tolerate toxic conditions and even strive on them. Therefore, they were selected as targets for our approach. Environmental strains were isolated and characterized according to their resistances profile and secretion performance aiming at strong secretors, with success. From high to low molecular weight proteins and even peptides, a promising diversity of molecules secreted to the environment was found, at the individual cells expense, which occurs as an environment/ intraspecies/ interspecies communication survival or domination strategy.

Analysis of such proteins activity showed a significant protease-like action, which may be responsible for the "toxic" compounds inactivation or even recycling. By means of combining systems biology and global differential proteomics analysis we expect to further identify eventual signals, players, effectors, and even extrapolate networks, both intracellular and secreted, in the near future.

P26-004
Petri net based description and analysis of the autophagy of the bacterial pathogen *Salmonella*

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Antibacterial autophagy plays an important role in the clearance of intracellular pathogens like *Salmonella* [1]. *Salmonella* that intrude the host cytosol are targeted with ubiquitin for the autophagic degradation [2]. To understand the biological system of autophagic capturing of the pathogen *Salmonella*, we developed a mathematical model.

Our mathematical, semi-quantitative model of the antibacterial autophagy is based on recent literature that structurally describes the processes of *Salmonella* ubiquitination and the recognition of the autophagy receptors. We applied the Petri net formalism [3], using the freely available software tool *MonaLisa* [4]. We found basic functional modules, which describe different pathways of the autophagic capturing of *Salmonella*. The model provides the basis for the integration of further quantitative data.

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P26-005
Association of circulating Adiponectin and Leptin levels with medullary thyroid cancer

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Introduction: Adipokines are bioactive proteins that mediate metabolism, inflammation and angiogenesis. The Changes in the secretion of Adiponectin and Leptin as an important Adipokines in the serum may be associated with disorders such as obesity, cancer and metastasis. Thyroid cancer is the most important type of endocrine cancers. Therefore investigating the relationship between serum levels of Adiponectin and Leptin in thyroid cancer can be considered. The purpose of this study was to assess Adiponectin and Leptin levels in the medullary thyroid carcinoma with incentive of pursuing a new tumor marker.
Materials and methods: This research was based on case-control study, including 45 patients with medullary thyroid cancer (21 men and 24 women) and 45 healthy controls (24 males and 21 females). Adiponectin and Leptin levels were measured by ELISA method in both groups. Height and weight were measured and body mass index (kg/m2) was measured. The normal distribution was checking and The mean level of Adiponectin and Leptin between two groups compared by independent t-test using statistical software (SPSS).
Results: The obtained data showed that Adiponectin and Leptin levels did not significant difference between medullary thyroid carcinomas and healthy group. Also there was no correlation among age and body mass index and the disease (Table1).
Conclusions: These results indicate that change in the level of Adiponectin and Leptin do not play an important role in diagnosis, confirmation or risk factor in medullary thyroid cancer.
Keywords: Adiponectin, Leptin, Medullary Thyroid Carcinoma, Body Mass Index (BMI)

P26-007
Don′t stress out – linking bacterial quorum sensing with stress response in *Saccharomyces cerevisiae*

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Quorum sensing (QS) is a concentration dependent cellular signaling mechanism employed by most bacteria and some fungi. In the past, research on QS focused mainly on its role as an intra-species signaling pathway that regulates certain behaviors and phenotypes. However, in recent years more studies have shown its importance as an interspecies communication pathway. There is mounting evidence that QS molecules, commonly known as autoinducers can be detected not only within species, but can also regulate phenotypes in members of different species. As the human body is host to vast numbers of bacteria from a variety of different species, which can be synergistic or pathogenic in nature, of particular interest in this context is the QS mediated interaction between bacteria and eukaryotic cells. We have started to examine the yeast *Saccharomyces cerevisiae* (baker′s yeast). When exposed to a variety of QS molecules (QSMs) from different bacteria and from *Candida albicans* we found that certain particular QSMs induced a specific stress response in the yeast. mRNA micro array experiments confirmed and strengthened these data, showing a unique and specific expression pattern that differed significantly from the response to previously described yeast stress factors. We are currently aiming to identify and characterize the yeast receptor for this signaling molecule.

P26-008
The regression analysis for interfacial tensiometry data of natural milk

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The use of regression analysis for interfacial tensiometry data, obtained by the measurement of dynamic surface tension (DST)

in biological liquids (for example, serum, milk, etc.) is important for humans and animals disease diagnostics. Using this mathematical method one can traces the influence of individual milk components on the DST at different times (σ_0, σ_1, σ_2, σ_3) and angles (λ_0, λ_1).

We investigated 115 milk samples of black and white breed cows by tensiometer BPA-1P (Germany). The fat and protein content measured by infrared optical analyzer Bentley-150 (USA).

The following regression equations for fat and protein content in the milk were obtained:

$[fat] = 5.85 + 0.071 \times \sigma_0 - 0.094 \times \sigma_1 - 0.16 \times \sigma_2 + 0.17 \times \sigma_3$

$[protein] = 3.59 - 0.027 \times \lambda_1$

These equations were formed as a result of regression model application and its simplification within a "predetermined error" by deleting variables significantly affecting. The obtained p-parameters for fat and protein are the following: 0.000032 and 0.032 (reliability criterion $p < 0.05$). The resulting regression equations can be used to determine the protein and fat in the milk of cows according to DST data without biochemical analysis.

Thus, the application of regression and correlation analysis for biological systems enabled to create reliable regression model. These results can be used for better understanding of the individual components impact to the complex adsorption processes in biological liquids.

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P26-009
Conformational epitopes of *Candida albicans* β-1,2 mannan revealed by monoclonal antibodies and their reactivity to *Salmonella choleraesuis* and *Salmonella infantis*

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C. albicans is a polymorphic fungus that may be present in humans. Early diagnosis and treatment are important in candidemia treatment. Because anti-fungal drug resistance is becoming a major concern within this period, it is necessary to develop of new antifungal agents. Many of the biological functions associated with patogenicity and virulance of *C. albicans* are related to its cell wall structure. This cell wall structure, which is formed by mannan, is responsible for the serologic reactions. Mannan structure includes α-1,6, α-1,2, α-1,3 ve β-1,2 linkages and similar linkages are present in strains of *S. choleraesuis* and *S. infantis*.

We are to investigate the expression of mannan structure which is present in *C. albicans* by using monoclonal antibodies and to identificate their interaction with the bacterial strains such as *S. choleraesuis* and *S. infantis*.

In our studies, the high specific murine monoclonal antibody-2B7 against to *C. albicans* cell wall was obtained. It was observed that this mAb formed a cross-reacted with strains *S. choleraesuis* and *S. infantis* which contained similar cell wall to the *C. albicans*. ACMK-1 (Matriks Biotek®, Türkiye) mAb used in studies is specific to mannan β-1,2 bonds that existance on the surface of *C. albicans* yeast form. Although ACMK-1 and 2B7 mAb define mannan structure, because their epitope specialities are different while 2B7 mAb reacted with *S. choleraesuis*, it did not react with the same microorganism.

Because we are always in contach with *Candida* species in nature and human flora, it is very important to identify antigenic structures of this microorganism and reveal their interaction with other bacteria strains.

P26-010
Characterization of *Listeria monocytogenes* strains isolated from food processing plants

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Listeria monocytogenes is opportunistic foodborne bacterial pathogen that represents an important hazard to human health because it is capable of causing listeriosis mainly in newborns, elderly, immuno-compromised individuals, and pregnant women. Contamination of food products could be the result of *L. monocytogenes* persistence in the food processing plant.

In the present study, genetic variability of *L. monocytogenes* strains from dairy and meat processing plants were studied. Total amount of 42 *L. monocytogenes* strains were isolated from different places of food production plants and their genetic variability was assessed by PFGE, PCR-serotyping and MLST. The serovar 1/2a was the most frequent with the 62% prevalence followed by 4b, 1/2c and 1/2b. The PFGE profile 9 belonging to serotype 4b was by eight strains the most frequent type. It was isolated from one meat production plant during several independent sampling. The PFGE profile 2 was detected in seven strains in the same factory. The PFGE 2 strains were positive to ECIII marker and therefore belonged to epidemic clone III previously associated with the food-borne outbreaks. Pophage inserted into comK site was another common property of PFGE 2 strains which could enhance their environmental persistence. Our results emphasize the importance of environmental monitoring to identify potential contamination sources and transmission routes, particularly of *L. monocytogenes* persistent strains in food production chain.

Sys Biol S4, Functional Networks Regulating Cellular Stress Response and Ageing

P29-003-SP
A microfluidic platform for high-resolution imaging of single yeast cells with versatile environmental control

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Long-term culturing and analysis of growing cell populations with single-cell resolution is a key technology to advance our knowledge about cellular variability in stress response, about stochasticity in gene expression, cellular aging processes, and similar. As cell heterogeneity is inherent to any cell population, ensemble measurements do not allow for addressing these questions since they can conceal important differences in the individual cellular dynamics.

We developed a microfluidic platform for time-lapse microscopy of yeast cells to monitor dynamic changes in single cells. We show that *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* can be imaged inside the chip over the course of several days. We characterized our culture system with respect to the availability of nutrients and potential stress that may be exerted on the cells. We show measurements of cellular stress markers of

single *Saccharomyces cerevisiae* cells in response to rapid changes in the concentration of glucose and sodium chloride.

Our microfluidic platform is intended to enable researchers that are not being experts in microfluidics to carry out single-cell experiments. Our chip can be easily fabricated by PDMS replica molding from a multilayer SU-8/silicon master. Setup of the chip and cell loading takes one hour, and up to twelve yeast strains can be investigated in parallel. The cellular environment can be perturbed by media switching within one second.

We believe that our platform can be widely applied for the analysis of yeast cells and other unicellular organisms.

P29-004-SP
Angiogenin-mediated cell-autonomous translational control under endoplasmic reticulum stress attenuates kidney injury

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Endoplasmic Reticulum (ER) stress is involved in the pathophysiology of renal diseases and aging, but the molecular basis of its biological effects in the kidney remain to be established. Angiogenin (ANG) is a stress-activated and secreted ribonuclease that cleaves transfer RNAs and produce stress-induced tRNA fragments (tiRNA) that inhibit protein synthesis.

In the present study, we have analyzed the mechanisms and biological functions of ANG synthesis and secretion by human kidney epithelial cells during the Unfolded Protein Response (UPR) by combining *in vitro* (human epithelial kidney cells), *in vivo* (wild type or ANG -/- mice) and human models of kidney injuries associated with ER stress to explore the molecular basis underlying the regulation of ANG through the UPR and characterize how this regulation promotes cellular adaptation during ER stress.

Our results indicate that ANG is a critical regulator of the stress response integrated to the UPR, which plays a critical role in tissue adaptation in response to kidney injury. We show that ANG participates to translation attenuation in ER-stressed cells through an original process of RNA interference which thus expands UPR-induced mechanisms for the reduction of protein flux into the ER and comes in addition to the previously described phosphorylation of eIF2a, regulated IRE1-dependent decay of RNAs, and selective mRNA release from the ER.

In conclusion, ANG is secreted by the stressed kidney epithelium during the UPR, protects against UPR-induced apoptosis, reduces protein synthesis by promoting tiRNA production and increases tubular inflammation.

P29-005-SP
The crosstalk between NF-kB-dependent and HSF1-dependent pathways in response to heat shock

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The signaling pathways depending on NF-κB and HSF1 transcription factors are essential components of cellular response to stress. NF-κB regulates transcription of genes responsible for immune response, inflammation, and cell survival. HSF1 activates expression of cytoprotective heat shock proteins (HSPs). These two cellular responses to stress interfere with each other. Heat shocked cells do not exhibit NF-κB induction in spite of the cytokine stimulation. The main aim of this work was to find out the time window in which NF-κB is effectively blocked after heat stress.

Activation of HSF1-dependent signaling after hyperthermia as well as activation of classical NF-κB-dependent signaling after TNFα cytokine stimulation was analyzed in cancer (A549 and U2OS) and noncancerous (GM07492) cell lines. We found that heat shock resulted in a blockade or time delay in the phosphorylation of p65 (the most common NF-κB subunit) at Ser536, and expression of NF-κB target genes. What is interesting, we found that cytokine treatment led not only to HSF1-Ser303/307 phosphorylation (which is responsible for HSF1 repression), but also to HSF1-Ser326 phosphorylation, which is indispensable for HSF1 activation. To investigate dynamic responses to different stimuli by single-cell imaging we constructed cells expressing p65-EGFP and HSF1-dsRed fusion proteins. We observed creation of stress granules containing HSF1-dsRed after heat shock, and inhibition of p65-EGFP nuclear translocation after stimulation by TNFα when cells were pretreated by hyperthermia.

The NF-κB pathway inhibition by elevated temperature can last for several hours after heat treatment. The explanation of this phenomenon is the subject of our further work.

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P29-006-SP
Histone methyltransferase SUV49H1 is associated with protein kinase CK2 inhibition-mediated senescence in human cancer cells

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We have previously reported that protein kinase CK2 downregulation induces premature senescence in colon cancer HCT116 cells. Reactive oxygen species (ROS) play an important role in CK2 inhibition-mediated senescence (CIMS). ROS levels increase in CIMS, and ROS elimination prevent CIMS. p53 and $p21^{Cip1/WAF1}$ are downstream effectors of ROS to induce CIMS. Both histone deacetylase SIRT1 and the PI3K-AKT-mTOR pathway are involved in CIMS. Senescence is characterized by several molecular and cytological markers including formation of specialized domains of facultative heterochromatin, called Senescence associated heterochromatin foci (SAHFs). SAHFs result from condensation of individual chromosomes into isolated heterochromatic domains. In the oresent study, CK2 down-regulation

promoted histone H3K9 tri-methylation and SAHFs formation. Especially histone methyltransferase SUV39H1 was involved in the formation of SAHFs during CIMS. In contrast, CK2 downregulation decreased H3K9 di-methylation and expression of histone methyltransferases SETDB1, GLP and G9a. mTOR inhibitor rapamycin and antioxidant repressed the formation of SAHFs. Taken together, these results suggest that CK2 downregulation induces SAHFs formation through mTOR-ROS pathway in senescent cells.

P29-007
Replicative senescence of budding yeast starts after only a few divisions: the roles of mitochondria

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Mitochondrial dysfunctions accompany ageing process in a wide variety of organisms and one of the consequences of such dysfunctions is a decline of the cellular stress response. Studying *Saccharomyces cerevisiae* cells' mitochondria, we noticed that a significant percentage of cells in logarithmic cultures contained two subpopulations of mitochondria with noticeably different transmembrane potential. Surprisingly, the proportion of such cells increased with replicative age. At the same time, we found that the cells with 2–3 scars demonstrated higher stress resistances than the daughters or the old (more than four divisions) mother cells. We reasoned that the heterogeneity in mitochondrial transmembrane potential is likely to cause malfunction of retrograde signaling. It appeared that deletions of mitochondria-to-nucleus (retrograde) signaling genes, *RTG1* or *RTG3*, further decreased the stress resistances of older mother cells (more than four divisions) without any significant effect on the younger ones. At the same time, retrograde signaling is not the only system that seems to be altered in yeast older mothers. We found that *HO* expression is repressed in cells with more than four bud scars, which points that aging represses mating type switching. Together these facts support the idea that, similar to the cells of higher eukaryotes, a decrease in adaptation to changing environment is an early manifestation of yeast replicative aging.

P29-008
Distinct outcomes of Charcot-Marie-Tooth (CMT)-causing point mutations in *Drosophila* small heat shock protein Hsp67Bc

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Small heat shock proteins(sHSPs) are molecular chaperones abundantly present in leaving organisms, involved in development and sub-cellular protein homeostasis(both: in normal and in stress conditions). Moreover, mutations in sHSPs coding genes cause disorders affecting mainly muscle and nerve function.

Drosophila melanogaster Hsp67Bc gene encodes an ortholog of human HSPB8, known for its role in autophagy and implication in CMT syndrome. In 3rd instar larva Hsp67Bc is expressedin both the cytoplasm and in the sarcomeresof body wall muscles. The sarcomeric localization includes Z-disk and M-line, whereas the cytoplasmic fraction of Hsp67Bc accumulates at neuro-muscular junctions(NMJ) and at periphery of nuclei. To investigate impact of CMT-causingmutations on muscle function we used inducible, GFP-tagged Hsp67Bc with mutated positive residue known to cause CMT and other pathological conditions. Hsp67Bc R126E substitution resulted in nuclear localization of the protein and reduced contractility of muscle fibers. However, the sarcomeric organization was unaffected, and sarcomeric distribution of the mutated form remained 'wild type'-like. Second substitution, Hsp67BC R126N, enhanced formation of large heterogenic aggregates and showed irregular sarcomere distribution. Similarly to first mutation, it did not affect sarcomere structure nor localization of endogenous Hsp67Bc. Behavioral mobility tests supported these results. Intriguingly aggregate prone form of Hsp67Bc kept its ability to localize at the NMJ sites, similarly to the endogenous protein, suggesting potential function of sHSPs in synapse formation and/or stabilization. Altogether, substitution with differently charged amino-acids led to different phenotypes, suggesting distinct mechanisms underlying pathological changes caused by different R126 CMT-mutations.

P29-009
Study of protein *S*-nitrosylation and its role in plant development and pathogenesis

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Nitric oxide (NO) plays key roles in many plant physiological processes and has functions in responses of plants exposed to abiotic and biotic stress factors [1]. NO signaling is mediated through post-translational modifications of target proteins. The most significant is *S*-nitrosylation, a reversible attachment of a NO moiety to thiol group of cysteine residues. Regulation of many important plant proteins points to emerging role in the plant hormone signal transduction, regulatory function in the activity of antioxidant enzymes, induction of apoptosis and control of carbohydrate metabolism in the cell [2].

This work highlights the role of *S*-nitrosylation during the development of three tomato genotypes (*S. lycopersicum* cv. Amateur, *S. chmielewskii*, *S. habrochaites*), and in biotic stress conditions in the pathogenesis of *Phythopthora infestans*. Using the ozone based chemiluminescence method S-nitrosothiols (SNO) and nitrite content were assayed. *S*-nitrosylated proteins were subjected to biotin switch technique, purified using neutravidine-agarose and were analyzed using LC-MS/MS. The moderate susceptible and highly resistant genotype, *S. chmielewskii* and *S. habrochaites,* showed increased SNO content during leaves development. Significant differences in S-nitrosylated proteins and their modulation during the plant development and pathogenesis were observed in all genotypes. The obtained results provide valuable insight into the role of *S*-nitrosylation during plant development as well as various stages of *Phythopthora* pathogenesis.

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P29-010
Estrogens down-regulate RANKL/OPG ratio and sclerostin levels in starvation-induced apoptosis in osteocytes

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Osteocytes control bone remodeling through the expression of receptor activator kB ligand (RANKL), osteoprotegerin (OPG) and sclerostin. RANKL/OPG ratio is indicative of osteoclastogenic activity, and sclerostin activates bone resorption. Osteocyte apoptosis has been related to osteoclastic activation. Osteoporosis due to estrogen loss has been also related to increased oxidative state. Previous studies performed in MLO-Y4 osteocyte like cells undergone starvation-induced apoptosis, that mimics apoptosis due to bone microdamage, demonstrated an increase in H_2O_2, RANKL/OPG ratio and sclerostin levels. These events were related to increased oxidative state and activation of JNK and ERK1/2. 17β-estradiol inhibited apoptosis and JNK but not ERK1/2 activity and decreased only in part H_2O_2 levels.

The aim of the study was to evaluate 17β-estradiol role on expression and release of RANKL, OPG and sclerostin levels in osteocytes undergone to starvation-induced apoptosis. A relationship with oxidative stress and MAPKs activation was also studied.

Preliminary results show that in MLO-Y4 cells 17β-estradiol significantly inhibited the increase in RANKL expression and release values in apoptotic cells. Whereas, 17β-estradiol prevented only in part the remarkable decrease in OPG expression observed in apoptotic cells. No OPG release was possible to detect in our experimental conditions. 17β-estradiol significantly lowered RANKL/OPG ratio which increased in apoptotic cells. 17β-estradiol inhibited also sclerostin expression. 17β-estradiol effect seems to be related to non-redox regulated mechanism of JNK activity, indicating that estrogen may inhibit the osteoclastogenic activity induced by osteocyte apoptosis through a mechanism not related to changes to oxidative state.

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P29-011
Age-related changes in antioxidant enzyme activities

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Purpose: The aim of this study was to determine the effect of oxygen free radicals due to aging of the antioxidant enzymes and have evaluated aging changes in antioxidant enzyme activities.

Materials and methods: In this study, we determined erythrocyte superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities, and malondialdehyde (MDA) and reduced glutathione (GSH) levels, to evaluate age-related changes in healthy subjects. 84 healthy subjects were divided into four groups: 2–11, 12–24, 25–40 and 41–69 years of age.

Results: No statistically significant differences in SOD enzyme activities, GPX enzyme activities or GSH levels were found among the groups. A statistically significant difference in CAT activity was found between the groups 12–24 and 25–40 ($p < 0.05$), but no statistically significant difference was observed between other groups. When the MDA levels of groups 12–24, 25–40 and 41–69 were compared to group 2–11, a statistically significant difference was found ($p < 0.0001$), but when groups 12–24, 25–40 and 41–69 were compared to each other, no statistically significant difference was found.

Conclusion: The results show that CAT and SOD activities and GSH and MDA levels are affected in aging. Therefore, we recommend that lipid peroxidation may have a role in the pathophysiological alterations of aging.

P29-012
Induction of endoplasmic reticulum stress by sodium metabisulfite in rat liver and its attenuation by Ghrelin

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Sodium metabisulfite is used as a preservative in many food preparations but can oxidize to sulfite radicals initiating molecular oxidation. Ghrelin is a peptide hormone primarily produced in the stomach and has anti-inflammatory and anti-oxidant effects on gastrointestinal and cardiovascular systems. This study was performed to elucidate the effect of ghrelin on sulfite-induced endoplasmic reticulum (ER) stress and caspase activation in rat peripheral organs. Xanthine oxidase (XO), xanthine dehydrogenase (XDH) enzyme activities, ER stress markers [phosphorylated PKR-like ER kinase (pPERK); C/EBP-homologous protein (CHOP)], caspase-3, -8, -9 activities, nuclear factor kappa-B (NF-κB) levels were determined in liver, heart and kidney of rats treated with sodium metabisulfite and/or ghrelin for 5 weeks. Sodium metabisulfite treatment significantly elevated XO activity, induced expression of GRP78, CHOP and increased caspase-3, -8 and -9 activities in liver but had no significant effect in heart and kidney. Ghrelin treatment decreased XO activity to baseline levels and attenuated ER stress and caspase activation in liver tissue of sodium metabisulfite treated rats. In conclusion, metabolism of sodium metabisulfite in liver tissue increased XO activity, induced ER stress and caused caspase activation which was attenuated by ghrelin treatment. Ghrelin′s hepatoprotective effect could be through modulation of XO activity.

P29-013
Putative targets for extending lifespan and healthspan in mice

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Mouse lifespan and healthspan are influenced by mitochondrial function, which is in part reflected by immune system and other blood parameters.

Some of these parameters are known biomarkers of ageing. We will describe novel mouse models we generated to study mitochondrial influence on ageing and how they may be employed to define biomarker-based intervention targets.

Specifically, we have generated a series of 16 conplastic strains carrying distinct mtDNA mutations on the same nuclear genome background.

To study the effect of mtDNA variants on lifespan and age-related phenotypes we established a large colony of 8 conplastic

strains (N = approximately 60–70/sex/strain). We then evaluated a panel of mitochondrial, cellular and tissue functions at different ages as well as lifespan of each strain, with a focus on blood-based parameters.

We identified life-extending mutations and investigated their mitochondrial and functional consequences.

Moreover, we established a data analysis pipeline that takes blood-based parameters, evaluates their role as biomarkers of ageing, and yields hints for intervention targets when integrating public data with the data inferred from mouse strains.

P29-014
Comparative proteome analysis of differentially expressed proteins in serum of *Hevea brasiliensis* from *Phytophthora* resistant (BPM24) and susceptible (RRIM600) clones

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The worldwide demand of natural rubber, *Hevea brasiliensis*, was increasing. The rubber latex contains numerous biological-active molecules discarded as waste in the rubber industry. Especially, some abundant proteins found in non-rubber constituents of *Phytophthora* tolerant clone BPM24 compared to susceptible clone RRIM600 might be involved in defense mechanism and antifungal activity. Comparative proteomic analysis of serum from BPM24 and RRIM600 clones was performed by two-dimensional gel electrophoresis. Relative quantification analysis and tandem mass spectrometry (nanoLC-ESI-MS/MS) were utilized to identify proteins. 1-D and 2-D Western blot analysis was used to validate mass spectrometric data. Moreover, the functional activity assay of β-1,3-glucanase and chitinase was investigated by AZCL-β-glucan and 4-nitrophenyl n-acetyl-D-glucosaminide as suitable substrates, respectively. Quantitative intensity analysis coupling with nano-LC-ESI-MS/MS revealed 16 forms of 12 proteins that were significantly up-regulated (more than 2.0-fold), whereas 20 forms of 16 proteins were significantly down-regulated in the tolerant clone BPM24. The altered proteins play important roles in plant defense and carbohydrate metabolism including β-1,3-glucanase and chitinase which was found to be glycoprotein. 1-D and 2-D Western blot analysis confirmed the up-regulation of β-1,3-glucanase and chitinase. Moreover, endoglucanase and exochitinase activity of clone BPM24 were found higher than that of RRIM600. Based on mass spectrometric data coupling to the functional activity, the induction and differential expression of several proteins in rubber latex may be associated with the tolerance and response of clone BPM24 to infection of *Phytophthora spp*. The activity of β-1,3-glucanase and chitinase may synergistically contribute to enhance fungal tolerance in para rubber tree.

P29-015
Antioxidant effects of peptidylprolyl cis-trans isomerase from *Pyropia yezoensis* against hydrogen peroxide-induced oxidative stress in hepatocytes

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Reactive oxygen species (ROS) are residual metabolites generated from the cellular metabolism of living cells. Seaweeds are exposed to sunlight and oxygen, which can lead to the formation of ROS. However, the absence of oxidative damage in their structural and functional components suggests they have an efficient antioxidant defense system. For this reason, several seaweed extracts have attracted increasing scientific interest and been examined to identify new and effective antioxidant compounds. In this study, we describe the expression and purification of peptidylprolyl cis-trans isomerase (PPI) from *Pyropia yezoensis*. We also describe the antioxidant activity of PPI against oxidative stress in hepatocytes. Chang and HepG2 cells expressing recombinant *P. yezoensis* PPI exhibited reduced H_2O_2-mediated ROS formation. When cells were treated with 1 mM H_2O_2, the expression levels of catalase (CAT), Cu/Zn superoxide dismutase (SOD), Mn SOD, and glutathione peroxidase (GPx) were significantly diminished relative to those in control cells. However, treatment with PPI potently and dose-dependently induced the expression of antioxidant enzymes. Both the mRNA and protein expression of CAT decreased in Chang cells, whereas GPx expression increased in a concentration-dependent manner. On the other hand, the mRNA and protein expression of CAT was increased while that of GPx was decreased in HepG2 cells. These enzymes are regarded as the first line of the antioxidant defense system against ROS generated during oxidative stress. Accordingly, our data imply that recombinant PPI regulates the expression of antioxidant enzymes at both the transcriptional and translational levels.

P29-016
Role of BAG3 on the nuclear shuttling of HSF1 under heat stressed conditions

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BAG3, a co-chaperone protein, is induced by stressful stimulus, such as heat shock and heavy metal and regulates cellular adaptive responses against stressful stimuli by regulating proliferation, apoptosis, cytoskeleton organization and autophagy. Considering the association of BAG3 with cellular stress, we investigated the molecular inter-relationship between BAG3 and HSF1. Under the heat stressed condition, BAG3 expression was rapidly induced in HeLa cells. Interestingly, upon heat stress, BAG3 translocates from the cytoplasm to the nucleus through an interaction with HSF1. Overexpression of BAG3 induced the rapid export of HSF1 to the cytoplasm during the recovery period, and subsequent decrease of Hsp70 promoter activity. In accordance with these results, BAG3-specific siRNA down-regulates the level of nuclear HSF1, confirming that BAG3 affects nucleocytoplasmic shuttling of HSF1. $Hsf^{-/-}$ mouse embryonic fibroblast (MEF) cells shows that the translocation of BAG3 upon heat stress is not affected by the absence of HSF1, suggesting that BAG3 is a key player for the BAG3-HSF1 nuclear translocation. Considering that HSF1 is a promising target for cancer therapy, it will be of great interest to investigate the molecular mechanism of HSF1 nuclear translocation more thoroughly.

P29-017
Molecular mechanisms of toxin-antitoxin regulation: the deceiving simplicity

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The bacterial stress-response mechanism involves the activation of the toxin-antitoxin modules. When activated, the toxin HigB2

form the higBA2 module cleaves the translating mRNA molecules thereby halting the protein synthesis and leading to a persistent bacterial phenotype. Here, we studied how the higBA2 module form the human pathogen Vibrio Cholerae is regulated. At the protein level the activity of the HigB toxin is controlled by a bimodular antitoxin protein HigA2. The N-terminal intrinsically disordered domain folds upon binding the toxin, while the C-terminal DNA-binding domain is involved in the transcriptional repression. The antitoxin's modular architecture is crucial for emergence of a unique regulation mechanism where the transcription depends on the molar toxin/antitoxin ratio. At low toxin/antitoxin ratios antitoxins repress the transcription, however binding of toxins elevates the repression at high toxin/antitoxin ratios. The observed anticooperativity of the regulation is explained at the molecular level by a pair of novel antitoxin-operator and HigBA2 complex-operator crystal structures. Decreased affinity of the HigBA2 complex for the operator steams from an unfavorable conformational change of the complex which is induced by strong DNA bending. The proposed model may be a paradigm for the regulation of simpler, one-operator TA modules and provides basis for understanding the regulatory circuits with a ratio-dependent input function.

P29-018
Investigation of free radical metabolism in septic rat's liver tissues treated with lipopolysaccharide; effect of vitamin D

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Sepsis, a multiple organ dysfunction syndrome, is a common cause of morbidity and mortality in the intensive care unit. LPS-induced excessive immune response is associated with hypovolemia, shock and multi-organ damage in sepsis. Excessive immune response causes multi-organ damage by increasing oxidative stress. We aimed to investigate the effects of VitaminD on free radical metabolism in LPS injected rats.

24 female wistar albino rats were divided into 4 groups. 1) Control, 2) Sepsis, 3) Sepsis+vitamin D, 4) Vitamin D. Sepsis was induced with single intraperitoneal injection of LPS *E. coli*. Vitamin D was given 2 mg/kg via gavage (in oil) for 3 days. Rectal body temperature was measured in rats. Liver function tests (AST, ALT) were analysed. Tissue catalase, superoxide dismutase, glutathione peroksidase and glutathione-S-transferase levels were analysed kinetically. Rat liver tissues were analysed histopathologically.

SOD and GSH-Px activities were not significantly different between the groups. CAT activities were depressed in all groups compared with the control group, this inhibition was mainly watched in the sepsis+vitamin D group. GST activities were depressed in sepsis ve sepsis+vitamin D group. AST and ALT levels were elevated in sepsis and sepsis+vitamin D group. While control and vitamin D groups showed normal histological structure, inflammatory cell infiltration and necrosis were seen in sepsis and sespsis+vitamin D groups.

In conclusion, we found that vitamin D treatment in sepsis has no protective role against hepatotoxic effects on liver. However treatment vitamin D in sepsis has an inhibitory effect on antioxidant enzymes which use H_2O_2 and GSH metabolism.

P29-019
Determining the amount of ellagic acid extracted from Eregli (Ottoman) strawberry and histopathological evaluation of possible protective effect of ellagic acid in streptozotocin-induced diabetic rat

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The Eregli strawberry (Ottoman) is a kind of highly sensitive and flavored strawberry species because of its own ellagic acid that gives strawberry its distinctive odor. The purpose of the study is to evaluate possible protective effects of ellagic acid extracted from the Eregli strawberries against β-cell damage from streptozotocin (STZ) induced diabetes in rats. The strawberries were harvested then pressed by a homogenizer and analyzed with a High-performance liquid chromatography method. Thirty-two wistar albino rats weighing between 170 gr and 220 gr were divided into four gruups; A (control), B (diabetic), C (diabetic + ellagic acid from Eregli strawberry) and D (diabetic group + commercial ellagic acid). Ellagic acid obtained from the Eregli strawberries and commercial were administered by gavaj for 1 week prior to STZ administration, and the possible protective effects of ellagic acid against β-cell damage from STZ induced diabetes in rats were evaluated. Pancreatic β-cells were examined by immunohistochemical and routine light microscopy methods. There is determined 0.0341 ± 0.0007 mg/ml ellagic acid in 100 gr strawberry. Islet cell degeneration and weak insulin immunohistochemical staining was observed in rats with STZ-induced diabetes. Increased intensity of staining for insulin, and preservation of β-cell were apparent in the ellagic acid-treated diabetic rats. These findings suggest that both of the ellagic acid treatment exerts therapeutic protective effect in diabetes by preserving pancreatic β-cell integrity. Consequently, ellagic acid may be clinically useful for protecting β-cells.

Keywords: Eregli (Ottoman) strawberry, ellagic acid, diabetes mellitus, streptozotocin, b- cell morphology, rat

P29-020
Alterations of creatine levels in rat brain under stress conditions long-term social isolation

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Stress is one of the main problems of the modern society. There are several data that show increase in free creatine levels under various neuropathological conditions. The main reason for this is high amount of ROS, that induces interchange of octameric mitochondrial creatine kinase into dimmeric, thus blocking creatine phosphorylation.

Experiments were conducted on adult, male rats (150 ± 10 g) that were kept into individual cages for 30-day, while control animals were together. Creatine concentration was measured by creatine colorimetric/fluorimetric assay kit (Biovision Inc., USA). GATM, GAMT and CrT ELISA kits were obtained from MyBioSource Inc., USA. All other materials were from Sigma-Aldrich, USA.

Primarily it was measured alteration in creatine amount in brain samples under long-term social isolation. From the results

it is seen that creatine concentration was increased for about 37%. To clarify the reason for such changes it was measured amount of GATM, GAMT and CrT, that were decreased, proving that under the stress conditions increase of creatine isn't up to the activation of synthesizing pathways nor by the up-regulation of transport mechanisms.

Additionally it was monitored activity of Cr/PCr/CK system, and it was shown that the activities of creatine kinase isozymes are decreased and the amount of PCr is fallen down too.

To sum up all the obtained data, it could be easily proved that under long-term social isolation the amount of free creatine is increased, that underlines pathological influence of stress on brain, but the exact reason of such changes is still under investigation.

P29-021
Thioredoxin -an integrator parameter for pathogenic mechanisms involved in pediatric nonalcoholic fatty liver disease

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The study aimed to investigate some systemic antioxidant, immune and inflammatory markers in pediatric nonalcoholic fatty liver disease (NAFLD). We measured as blood antioxidants: the serum thioredoxin, the erythrocyte superoxid-dismutase activity (SOD), as immune parameters: IgM, IgG, IgA, complement C3, C4 and circulating immune complexes, liver kidney microsomal type 1 antibodies (LKM1-antibodies), anti-smooth muscle antibodies (ASMA) and as inflammatory markers: C reactive protein (CRP), fibrinogen, leptin. Fifty-nine obese children (10–16 years) with NAFLD and thirty age and sex matched healthy children, were involved. Immunoprecipitation, spectrophotometry and ELISA methods were used. Pearson correlations were calculated. Liver ultrasounds were used to select children with NAFLD. In the NAFLD children versus the healthy ones, higher values were measured for SOD activity ($p < 0.01$), for inflammatory markers ($p < 0.01$), for complement C3,C4 ($p < 0.001$) and lower values for thioredoxin (7.4 ng/dl versus 17.8 ng/dl, $p < 0.02$). Serum levels for ASMA and LKM1 antibodies, markers for autoimmune hepatitis, were similar in the studied groups. In NAFLD children, thioredoxin was positively correlated with IgG and IgA ($r = 0.27$ and $r = 0.32$, respectively, $p < 0.05$), with fibrinogen and leptin ($r = 0.46$ and $r = 0.31$, respectively, $p < 0.05$) and negatively correlated with C4 complement ($r = -0.34$, $p < 0.05$). Other calculated correlations (for $p < 0.05$) were between: C3 complement and CRP ($r = 0.31$), leptin and ASMA ($r = -0.27$), In conclusion, serum thioredoxin represents a link between inflammation, oxidative stress and innate immune response in pediatric NAFLD. The unchanged serum level of autoantibodies rules out the overlap syndrome with autoimmune hepatitis.

P29-022
The sublethal effects of etofenprox on zebrafish (*Danio rerio*)

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Zebrafish (*Danio rerio*), model organisms on ecotoxicological studies, were used to determine the sublethal effects of etofenprox on aquatic ecosystems. Etofenprox (2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzylether), a non ester synthetic pyrethroid, can enter water bodies directly by pest control programs or indirectly through rain water and surface run off. 1/10 (8 μg/l) and 1/100 (0.8 μg/l) of 96 h LC50 value were applied for 48 and 96 h to zebrafish. Control groups were also conducted under same conditions. After 48 and 96 h fish samples were taken under ice anastesia for histologic and DNA analysis. Seven fish samples were fixed in 10% buffered formalin for each group for histologic analysis. Routine histologic procedures (dehydration in alcohol series, cleared in xylene, embedded in paraffin, sectioned and stained with H&E.). To evaluate the DNA /RNA oxidative damage a total zebra fish was homogenizated for DNA isolation, then hydrolized and damage was measured by commercial kit as EIA. Hyperemia, epithelial lifting on the secondary lamella of the gill tissues; hyperemia, picnosis and hydropic degeneration on the liver tissues; edema and tubuler degeneration on the kidney tissues and hyperemia on the brain tissues were observed after exposed to two different sublethal etofenprox concentrations. DNA–RNA damage as 8-hydroxy-2'deoxyguanosine (pg/ml) was statistically significantly increased at low doses of 48 and 96 h exposed groups, however no difference was observed for high doses of both hours in both groups compared to their controls. Etofenprox was found to be very highly toxic to zebrafish, a non-target organism, even in sublethal concentrations.

P29-023
Inhibition of a protein kinase C (PKC)-phospholipase D (PLD)-protein kinase CK2 (CK2) network stimulates cellular senescence through reactive oxygen species (ROS) generation

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Cellular senescence is involved in regulating the aging process and acts as a barrier against cell immortalization and tumorigenesis *in vivo*. Cellular senescence can be divided into two different types; replicative and premature senescence. Replicative senescence is an irreversible cell growth arrest state triggered by telomere attrition after a finite number of cell divisions. Premature senescence can de induced various forms of stress such as reactive oxygen species (ROS) and oncogenic activation. Our data showed that downregulation of protein kinase CK2 (CK2) or PLD induced premature senescence in both normal lung fibroblast IMR-90 cells and colon cancer HCT116 cells. The ROS-p53-$p21^{Cip1/WAF1}$ pathway played an important role in senescence mediated by inhibition of CK2 and phospholipase D (PLD). In addition, protein kinase C (PKC) was also involved in cellular senescence. PKC positively regulated the activity of CK2 and PLD. CK2 inhibition downregulated FoxO3A, which was a transcription factor for expression of anti-oxidant proteins, through

activation of the phosphatidylinositol 3-kinase (PI3K)-AKT pathway. Taken together, these results suggest that the PKC-PLD-CK2 network modulates cellular senescence in a FoxO3A- and ROS-dependent manner.

P29-024
Effects of Monosodium glutamate on MDA, GSH and SOD concentrations in liver tissue of neonatal rats

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Monosodium glutamate (MSG) is one of the most well-known and most widely used flavor enhancers in the world. Previous studies have reported that the use of high doses of MSG caused neuroendocrine abnormalities, and is the cause of neurodegeneration, neurotoxicity and oxidative stress in different organs. In this respect, we aimed to investigate effects of MSG using the parameters of oxidative stress on neonatal male Wistar rats are used as an experimental model system. In this context, neonatal male Wistar rats were divided into four groups; control (n = 6), MSG1 rats (n = 6, 50 mg/kg/day), MSG2 rats (n = 6, 100 mg/kg/day), MSG3 rats (n = 6, 200 mg/kg/day). A total of 8 intraperitoneal applications were made with an interval one day. Rats were decapitated after injections, and absorbance of superoxide dismutase (SOD) and glutathione peroxidase (GSH) as antioxidant enzymes, and malondialdehyde (MDA) as a marker of lipid peroxidation were measured using spectrophotometric methods (MDA: 532 nm., SOD: 560 nm., GSH : 412 nm.) in the liver tissue. According to one way ANOVA analysis, there is a statistically significant differences between groups (control, MSG1, MSG2 and MSG3) and MDA ($p < 0.05$), SOD ($p < 0.05$) and GSH ($p < 0.05$). Further analyses showed that there is a positive corelation between MDA concentration and MSG doses. However, concentration of SOD and GSH showed negative relationships with MSG doses.

P29-025
Ginsenoside Rb1 rescues anxiety-like responses in a rat model of post-traumatic stress disorder

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Single-prolonged stress (SPS), a rat model of post-traumatic stress disorder (PTSD), induces alterations in the hypothalamic-pituitary-adrenal axis. A widely used traditional anxiolytic is Korean red ginseng, whose major active component is ginsenoside Rb1 (GRb1). However, the efficacy of GRb1 in alleviating PTSD-associated anxiety-like abnormalities has not been investigated. The present study used several behavioral tests to examine the effects of GRb1 on symptoms of anxiety in rats after SPS exposure and on the central noradrenergic system. Male Sprague Dawley rats received GRb1 (10 or 30 mg/kg, i.p., once daily) during 14 days of SPS. Daily GRb1 (30 mg/kg) administration significantly increased the number and duration of open arm visits in the elevated plus maze (EPM) test, reduced the anxiety index, increased the assessment, reduced grooming behaviors in the EPM test, and increased the time spent in the center of an open field after SPS. The higher dose of GRb1 also blocked SPS-induced decreases in hypothalamic neuropeptide Y expression, increases in locus coeruleus tyrosine hydroxylase expression, and decreases in hippocampal mRNA expression of brain-derived neurotrophic factor. These findings suggest that GRb1 has anxiolytic-like effects on both behavioral and biochemical symptoms similar to those observed in patients with PTSD

Keywords: Post-traumatic stress disorder, Single prolonged stress, Anxiety, tyrosine hydroxylase, ginsenoside Rb1

P29-026
Skin fibroblast pro-fibrotic and pro-inflammatory responses to advanced glycation end products: networks contributing to age-related diseases

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Advanced glycation end products (AGEs) were reported to accumulate in long-life protein, to interact non-specifically with dermal fibroblasts and to be involved in the process of skin ageing, and impaired wound healing in diabetics.

Human foreskin fibroblasts (CCD-1070Sk) were exposed to 50, 100 and 200 μg/ml glycated BSA (AGEs-BSA) or control BSA for 12 and 24 h. Transforming growth factor-beta 1 (TGF-β1) gene and protein expression increased significantly after 12 h of exposure, while after 24 h the increases were moderate. The 200 μg/ml AGEs-BSA dose increased the gene and protein levels of collagen I and III after both intervals analysed, particularly after 12 h of AGEs-BSA exposure. The gelatinase MMP-9 protein expression increased in a dose dependent manner, while the gelatinolytic activity was equally increased at all the doses applied and exposure intervals. In addition to this profibrogenic changes, a pro-inflammatory context was also observed, as IL-2, IL-6, IL-8, TNF-α levels in cell culture medium increased over 1.5 fold after 12 h. After 24 h exposure, IL-6, GM-CSF and TNF-α increased over 2 fold. The expression of IL-6 and GM-CSF were dependent on pro-fibrogenic factor TGF-β1 signalling, as treatment with anti-TGF-β1 antibodies inhibited their expression by 0.5 fold, while IL-8 increased by 1.6 fold.

In conclusion, fibroblasts exposed to AGEs are stimulated to produce extracellular matrix proteins in a pro-fibrotic context, while TGF-β1 pro-inflammatory signalling pathway is central in inducing chronic inflammation, a major risk factor underlying aging and age-related diseases.

P29-027
Transcriptomic study of the heat shock response mechanisms of *Asterias rubens* starfish

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Echinoderms are used as experimental models for gerontology research due to their slow aging and longevity (up to 30 years) and outstanding potential for regeneration. We performed high-throughput transcriptome sequencing (75 bp, paired-end) of tube feet of *A. rubens* (one of the most abundant echinoderm species) under normal and heat shock conditions using Illumina GAIIx.

Tissues of tube feet of normal adults (2 samples) and adults after heat shock (3 samples –30-min at 25°C, 27°C, 29°C) were used. We generated cDNA library containing expressed sequence tags (ESTs). A total of 61273037 (GC-content –40.8%) and 73538249 (GC-content –42.0%) reads were obtained from high-

throughput sequencing for normal conditions (samples #1,#2); 74525446 (GC-content – 40.4%), 73810609 (GC-content – 40.2%) and 15202358 (GC-content – 63.1%) for different stress conditions (samples #3,#4,#5). For assembly we estimated a length of k-mer via kmergenie software. We obtained optimal k-mer lengths equal to 19, 17, 21, 25 and 21. The total number of transcripts was 102169 (N50 – 536) for sample #1, 131085 (N50 – 223) for #2, 88712 (N50 – 925) for #3, and 77810 (N50 – 1243) for #4. The max length of transcripts was 22697 bp for #1, 8564 bp for #2, 18067 for #3, and 24412 for #4. Thus, we obtained ~ 1200–1500 gene transcripts. Transcriptome of echinoderm *Strongylocentrotus purpuratus* was used as a reference. The analysis allowed us to identify several candidates for differentially expressed genes in *A. rubens*. In future, we are planning to test the differentially expressed genes using qPCR.

P29-028
Analysis of the expression dynamics of 29 stress-response genes of *Drosophila melanogaster* in response to low doses radiation

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The hormesis is a stimulating effect of low dose stressors without its disruptive action. Previous study has shown that 40 cGy dose radiation exposure of *D. melanogaster* wild type strain *Canton-S* increases life expectancy. But this hormetic effect was absent in flies with mutations in *FOXO, Tefu, mei-41,* and *p53* homologues. The study of an impact of four different stressors including 20 cGy dose radiation revealed genes differentially expressed in response to more than one stressor action. This fact can confirm a nonspecific mechanism of stress response. The expression of these genes including *p53* and *FOXO* was analyzed using qPCR in response to impact of low dose radiation (5,10,20,40 cGy) within 72 h. In this way we identified the expression dynamics of 29 genes (*CG6295, CG18180, CG42751, Clock, Cyp4e2, Cyp6a20, Fer3, FOXO, GstE3, hpo, Hsp70, Hus1-like, JNK, Keap1, mei-9, mei-41, mus209, Mus309, p53, Per, Rad54, SOD, SpnB, Tefu, Wr, CG13323, Brca2, CG6675, CG9360*) immediately after radiation exposure and 6,244,872 h later. Gene expression profile was different in males and females. *SpnB, mei-9, mei-41, Cyp4e2* genes, involved in DNA repair and response to various stresses, were overexpressed in males after 48 h or more after radiation exposure. This fact may point out their late transcriptional activation in response to radiation stress. Gene expression dynamics of *CG18180* gene, involved in immune response, was different in males and females. This distinction may play a key role in hormetic effect of low dose radiation in females, that is absent in males.

P29-029
Cyclic tensile stress of human annulus fibrosus cells induces MAPK activation: involvement in proliferative status and pro-inflammatory gene expression

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The intervertebral disc (IVD) is normally subjected to a variety of stresses, among them being mechanical loads. Especially in the area of annulus fibrosus (AF), cells are experiencing predominantly tensile forces. Mechanical stress is generally considered as one of the major causes of IVD degenerative disorders, although in some cases – depending on its intensity – it may exert anabolic effects. Accordingly, aim of the present work was the *in vitro* study of human AF cells' behaviour in response to cyclic tensile stress (CTS), using a cell-stretching device allowing for the regulation of both magnitude and frequency of the strain.

CTS was found to induce in a magnitude-, frequency-, and time-dependent manner the phosphorylation of all three classes of mitogen-activated protein kinases (MAPKs), i.e. extracellular-signal regulated kinases (ERKs), p38, and c-Jun N-terminal kinases (JNKs). Phosphorylation was induced immediately following CTS application and remained slightly elevated compared to control up to 24 h later. No significant effects were detected on cell proliferation, as well as, on the expression of genes involved in extracellular matrix synthesis and catabolism, possibly indicating a capability of AF cells to adapt to mechanical stress. On the other hand, CTS only at the higher magnitude tested stimulated cyclooxygenase-2 gene expression, an effect reversed completely in the presence of p38 and ERK inhibitors, and partially by a JNK inhibitor. Hence, MAPK activation in response to CTS may serve as a biosensor mechanism, capable of responding to intense mechanical stress by the induction of proinflammatory gene expression.

P29-030
The role of oxidative stress in the lung toxicity depending on alpha amanita

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Objective: Mushroom poisoning is seen in the months which are rainy in our country and all over the world and severe poisoning can be mortal.

We aimed to investigate of changes of lung oxidant/antioxidant system parameters [superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), total antioxidant status (TAS), total oxidant status (TOS) and malondialdehyde (MDA)] by Alfa-amanita poisoning.

Methods: The study was performed on 37 mice. Four groups of BALB/C male mice were randomly categorized. First group (n = 7) was control. Second group (n = 10) was injected 0,2 mg/kg alpha amanitin intraperitoneally. Third group (n = 10) was injected 0.6 mg/kg alpha amanitin intraperitoneally. Fourth group (n = 10) was injected 1 mg/kg alpha amanitin intraperitoneally. After then all groups received diet and water *ad-libitum*

for 48 h and mice were decapitated. We measured SOD, GSH-Px and CAT activities and MDA, TAS and TOS levels in lung tissues.

Results: SOD, GSH-Px, CAT, TAS, TOS and MDA values measured in the lung tissues were meaningful between the groups statistically.

SOD, GSH-Px, and CAT activities increased compared to the control group. TAS levels decreased compared to the control group. MDA and TOS levels were increased compared to the control group.

Conclusions: The results of our study strongly support the role of increased oxidative stress in the acute lung injury in the alpha amanitin intoxication.

Keywords: alpha amanitin, lung tissue, oxidative stress, antioxidant system

P29-031

The role of the alternative pathway of respiration in wheat seedlings (*Triticum aestivum* L.) in the condition of inhibition of cytochrome pathway under the influence of high temperature

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Alternative oxidase (AOX) is a mitochondrial terminal oxidase in the respiratory electron transport chain that catalyzes the oxidation of ubiquinol, reducing O_2 to H_2O. Antimycin A (AA) was used as a modulator of alternative respiration on the background of inhibition the activity of cytochrome pathway which inhibits the cyclic electron transport in chloroplasts and mitochondria. High temperature is one of the abiotic stressors that effects on plant growth, productivity, and quality. Many abiotic stressors increase the production of reactive oxygen species (ROS) that can lead to the oxidative destruction of the cell. The aim of present investigation was to determine the total content of ROS, the content of lipid peroxidation product malondialdehyde (MDA), the concentrations of pigments (chlorophyll a, b and carotenoids), the ratio of different forms of photosynthetic pigments and the maximum efficiency of PSII F_v/F_m parameters in wheat seedlings under the influence of short-term (1 h) and long-term (24 h) influence of high-temperature (42°C). The objects of studies were first leaves and coleoptiles of wheat seedlings *Triticum aestivum* L. The data revealed that heat stress caused a significant increase in the total ROS content and MDA concentration. The presence of AA and stimulation of the alternative pathway decreased the content of total ROS, lipid peroxidation and changed the content of photosynthetic pigments in wheat seedlings under high-temperature. The functioning in plant AOX is able to be included in the regulation of energy accumulation and protect chloroplasts in stress conditions.

P29-032

The effects of alpha-amanitin on oxidative stress parameters in cardiac tissue

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Objectives: The diagnosis and treatment of Amanita mushroom poisoning is a difficult problem for physicians in Turkey. Degenerative lesions of several tissues, such as liver, heart and lung occur in exposure of various doses of alpha-amanitin.

The aim of this study was to measure the oxidant/antioxidant system parameters which reflect cardiotoxic actions in mice of alpha-amanitin.

Methods: 37 BALB/C male mice were divided into four groups. The first three groups, composed of 10 animals in each, were treated alpha amanitin intraperitoneally at doses of 0,2, 0,6 and 1 mg/kg, respectively. The 7 animals of the fourth group were used as control. Mice were decapitated 48 h after injection. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), total antioxidant status (TAS), total oxidant status (TOS) and malondialdehyde (MDA) were measured in cardiac tissues.

Results: SOD, GSH-Px, and CAT activities increased in alpha-amanitin administered groups compared to the control group. Whereas we found decreasing TAS levels in alpha-amanitin administered groups compared to the control group. MDA and TOS levels were increased compared to the control group.

Conclusions: Our results indicate that oxidative damage mechanisms are important in the cardiotoxicity caused by alpha-amanitin.

Keywords: alpha amanitin, cardiac tissue, oxidative stress, antioxidant system

P29-033

Influence of polymorphisms *CdxII* e *EcoRV* of vitamin D receptor on recuperation of burned patients

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Vitamin D is a fat-soluble compound that has biological effects (modulates cell metabolism) through binding to vitamin D receptor (VDR). VDR presents polymorphisms in vitamin D efficiency that can vary in a population. Some studies show colecalciferol supplementation efficiency, because it increases mineral metabolism (calcium and phosphorous homeostasis) control and mitigates the inflammatory process, a complicating factor observed in patients that suffer burns. The VDR polymorphisms *CdxII* and *EcoRV* were detected in patients that suffered burns and compared with hospitalization time, infection development and mortality. Patients admitted to the burn unit of Bauru State Hospital were monitored during recovery for age, sex, body surface burned and infection. DNA extraction was performed by leucocyte "salting-out" method through blood samples. The association between polymorphisms and hospitalization time was analyzed by linear regression, while logistic regression was utilized to evaluate infection development and mortality. In all cases 5% statistical significance was adopted. Of the 81 patients analyzed, eleven died while 70 were discharged. Only 30 showed some type of infection and 51 presented none. Average of hospitalization time was ~ 26 days. After analysis, a relation was not established between hospitalization time, mortality and infection with polymorphisms *CdxII* and *EcoRV*.

P29-034
Oxidative/nitrosative stress and endoplasmic reticulum stress in ischemic acute renal failure

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Ischemia-reperfusion (IR) is the most common reason of acute renal failure (ARF), known as ischemic ARF. This syndrome is associated with high levels of morbidity and mortality. IR-induced cell injury involves complex interrelated mechanisms. As the result of previous studies, it has been indicated that hypoxia, oxidative stress (OS), nitrosative stress (NS) and endoplasmic reticulum (ER) stress play significant role in the occurrence of IR-induced cell injury. Although the interactions among these pathways have been investigated in several studies, it has not been clearly explained which is the initiator or major factor on IR-induced cell injury yet. A better understanding of the molecular mechanisms of ischemic ARF may contribute to improve efficient therapeutic strategies.

In this study, we aimed to investigate the crosstalk among OS, NS and ER stress in IR-induced renal cell injury. For this purpose, ischemic ARF model has been established by using human proximal tubular kidney cells (HK-2). Then, the interactions of OS, NS and ER stress under normoxia and hypoxia-reperfusion conditions were determined by time-course experiments using immunoblotting technique.

The results revealed that; (1) hypoxia and reperfusion lead to increase in OS, NS and ER stress in HK-2 cells, (2) it has been determined that OS markers were triggered as soon as hypoxia and reperfusion occurs, whereas the NS and ER stress markers were increased in a later stage, (3) ER stress related unfolded protein response (UPR) activation may be as an adaptive mechanism under hypoxia-reperfusion conditions in response to elevated OS and NS stress.

P29-035
In vitro investigation of toxicity and specific activities of mud extracts

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Mud-extracts represent valuable therapeutic adjuvants and therapeutic alternatives to synthetic medicines; especially in chronic diseases, such as arthritis, knee osteoarthritis. The use of mud-extract, contributes to a long term stability of therapeutic effects, thus avoiding common inconveniences of conventional medicines, like installation of therapeutic resistance and adverse effects. Active fractions obtained from mud were investigated using *in vitro* methods regarding cytotoxicity and therapeutic efficacy. The real effects of mud-bath applications on the inflammatory process are still not clarified.

Methods: Cytotoxicity-testing was performed *in vitro* using ATCC-CRL-9855 cell cultures, in standard conditions and at different times of exposure at concentrations of 300, 150, 75 and 37.5 μg/ml, using the MTS and LDH assays. Anti-inflammatory effects: based on the preliminary data, anti-inflammatory action was expected to be present in the mud fractions; cytokine measurements were performed by Luminex-xMAP technology.

Results: Cytotoxicity tests: 9 investigated assays did not express significant cytotoxic effects (in LDH and MTS assays) over the concentrations ranging 37.5 to 300 μg/ml. One of the extract expressed a dose dependent cytotoxicy, yet the loss of cell viability leads to an estimate of CT50 at a value higher than 1 mg/ml.

Anti-inflammatory effects: The mud extracts were demonstrated to modulate cytokine release, generating profiles that are characteristic to marked anti-inflammatory effects.

Conclusions: Using a combination of *in vitro* assays, mud extracts could be classified and ranked for their cytotoxiciy and specific activity, providing an effective screening system for the discovery of potential therapeutic compounds.

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P29-036
Cellular rejuvenation and ageing-proteome by Ginsenoside 20(S)-Rg3

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Aging is a multifactorial process resulting from the accumulation of cellular damage over time, leading to physiological deterioration, increased mortality and eventual death. Ginseng is well known in herbal medicine as a tonic and restorative agent. The main molecular ingredients responsible for the actions of ginseng are the ginsenosides (also called ginseng saponins), which are amphiphilic molecules comprising a hydrophobic backbone of aglycone (a hydrophobic, four-ring, steroid-like structure) linked to hydrophilic carbohydrate side chains. In previous studies for ginsenoside Rg3, its functions are known to be sodium channel inhibitor in brain disease, anti-angiogenesis effect in diabetic disease, and various anti-cancer activities. However, the effects of ginsenoside Rg3 on the aging/rejuvenation are not reported yet. The senescence associated-β-galactosidase (SA-β-gal) activity was dramatically decreased in 20(S)-Rg3-treated human dermal fibroblasts (HDFs) compared to non-treated old HDFs. Moreover, the ginsenoside 20(S)-Rg3 altered numerous aging factors involved in the maintenance of mitochondrial function. To identify the 20(S)-Rg3-induced rejuvenation in HDFs, we analyzed the label-free quantitative proteome in time-dependent proteomic profiles after the treatment of 20(S)-Rg3 to old HDFs. Nano-UPLC-high definition mass spectrometry (HDMSE) revealed the crosstalk with respect of cellular assembly and organization, free radical scavenging and small molecule biochemistry. Among the identified proteins, we concentrated largely in the expression patterns and associated networks of mitochondrial function. It is suggested that the ginsenoside 20(S)-Rg3 can defense aging-associated mitochondrial events and the ginsensoside 20(S)-Rg3 affects the rejuvenation potency by a disclosed molecular mechanism.

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Sys Biol S5, Systems Biology in Stem Cells

P30-003-SP
Stem cells loaded nanobiohybrids for efficient chronic wounds healing

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The currently increasing interest in cellular delivery of various drugs in medicine leads to the development of novel tissue engineering(TE) approaches. Clay minerals possess excellent properties and promise for controlled release, thus giving rise to good perspectives for TE, pharmaceutical and medical applications. Modern strategies in TE applications involve the design of biohybrids obtained by preseeding biomaterials with undifferentiated cells to regenerate damaged tissues. Due to their particular secretory profile, adipose-derived stem cells (hASCs) enhance the healing process in a paracrine manner, thus stimulating the recruitment of endogenous stem cells and furthermore, promoting their differentiation towards the required lineage. In this context, the aim of our work was to develop a smart bacterial polyester/LDHs dressing loaded with hASCs, designed to improve the impaired healing process. Consequently, this study reports on the synthesis and characterization of biocomposite systems based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate)(PHBHV) and modified layered double hydroxides(LDH-SDS) loaded with hASCs. A series of three different compositions was studied in terms of: (i) physico-chemical characterization by FTIR, TGA and DSC, (ii) exfoliation properties and morphological structure by XRD and SEM and (iii) biocompatibility evaluation by fluorescent microscopy and spectrophotometry. Although fluorescent labeling of actin filaments showed that hASCs displayed a normal morphology in contact with all the tested biomaterials, MTT and LDH spectrophotometric assays revealed significant differences between the samples in terms of viability, proliferation and cytotoxic potential. Hence, only the composition with the highest concentration of LDHs could be considered for further *in vivo* studies.

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P30-004-SP
Effect of chromium complexes with flavonoid quercetin on the adipogenic process

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Obesity is a global public health concern as prevalence rates continue to increase, especially among children. At cellular level, a major obesogenic process is adipogenesis, the differentiation of the stem cells into mature fat storage cells. Modern approaches spot the adipocytes in the heart of a dynamic signaling network. The unbalanced secretion of the white adipose tissue leads to its dysfunction which potentially links obesity with the metabolic syndrome or even diabetes. Some bioactive compounds and trace elements hold enormous potential in regulating adipocyte's metabolism. Quercetin, the most commonly consumed dietary flavonoid, is known as a potent anti-obesity agent involved in the activation of AMPK, while chromium acts by activating the insulin receptor, with high significance in glucose homeostasis and insulin sensitivity. In this context, the aim of our study was to investigate the anti-adipogenic potential of an original chromium complex with quercetin upon adipogenic committed adipose-derived stem cells (hASCs). After sinthesys, the chemical structures of the molecules were confirmed by NMR and the DL50 were determined *in vitro* on hASCs. The anti-adipogenic potential of the least toxic complex was assessed on hASCs, during three weeks of its administration in an adipogenic medium. Oil Red O staining of the intracellular lipid droplets and the imunofluorescent labelling of perilipin revealed that the chromium complex of quercetin significantly inhibited the adipogenic process as compared to quercetin alone. Consequently, the quercetin complex with chromium could be further employed in *in vivo* studies on animal models.

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P30-005
Thin coatings based on biocompatible silver nanoparticles deposited by advanced laser processing for improved surfaces resistance to microbial biofilms

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The aim of this research was to obtain an improved coating for medical devices exhibiting higher resistance to bacterial and fungal colonization, by the superficial modification of some indispensable devices used in the current medical care – such as central venous catheters, urinary catheters, nasogastric tubes and gastrostomy tubes. The innovative aspect of our study consists in using the novel and versatile MAPLE (Matrix Assisted Pulsed Laser Evaporation) technique in order to functionally modify the concerned medical devices, by depositing thin inorganic layers of silver nanoparticles on their surface.

Silver nanoparticles were prepared and further characterized by FT-IR, DTA-TG, TEM, SAED and EDS. Nanoparticles were used to create a thin uniform surface by advanced laser processing in order to obtain different medical surfaces with a high resistance to microbial colonization and biofilm development. The prepared surfaces were characterized by SEM, TEM, IR Microscopy, AFM, XRD. The biocompatibility of the obtained coating was investigated by *in vitro* (on osteoblasts and stem cells) and *in vivo* (on mice, up to 21 days) assays. The interaction of the

obtained layers with the Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*) bacterial and fungal (*Candida albicans*) strains was investigated using a static model of biofilm development for five days. The promising results revealing the long term resistance to microbial colonization and the good biocompatibility prove the usefulness of MAPLE technique for optimizing wide-use medical care devices, the considered principles being possible to be extended for obtaining bioactive and nanostructured surfaces for other various medical applications.

Struct Biol S2, Channels and Transporters

P33-008
The permeation of small inorganic ions and metabolites through VDAC is mediated by a charged-brush mechanism

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The voltage-dependent anion channel (VDAC) is a key element of the exchange of metabolites and ions between the mitochondrion and the cytosol as it forms the major transport pathway for these compounds through the mitochondrial outer membrane. Evidence provided by numerous studies has also promoted the idea that VDAC acts as a regulator of essential mitochondrial functions.

In this study, using a combination of molecular dynamics simulations, free-energy calculations, and electrophysiological measurements, we investigated the transport of phosphate ions and anionic metabolites. In dramatic contrast to monovalent anions (Cl^-, H_2PO^{4-}), we show that the permeation of divalent phosphate and phosphate metabolites such as ATP and AMP involves binding sites along a specific translocation pathway. We also find that the *in-silico* mutation of basic residues shaping the main binding site impacts the permeation of the divalent phosphate anion and modify its energy landscape. Our simulated permeation events also evidence that a "charged brush" mechanism involving a few flexible and long basic side chains facilitates the passage of anions throughout the pore. Our data are also in agreement with the decrease in VDAC conductance measured in the presence of ATP or AMP.

Altogether our study proposes that VDAC has the capacity to use different structural and physicochemical features of its pore to permeate different types of anions. This enlightens VDAC proposed role as a dynamic regulator of mitochondrial functions.

P33-009
Purification of MCT8 for structure determination

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The most important plasma membrane transporter for thyroid hormones (T3, T4) is monocarboxylate transporter 8 (MCT8). Inactivating mutations in the *MCT8* gene lead to severe psychomotor retardation. The disease is called Allan-Herndon-Dudley Syndrome, a X-linked mental retardation syndrome. Affected patients cannot walk, stand, or speak, and suffer from abnormal thyroid hormone levels with coexisting high T3, but low T4.

T3 is an important regulator of development and metabolism and sets the basal metabolic rate.

MCT8 belongs to the major facilitator superfamily of transmembrane transporters and is predicted to have 12 transmembrane domains with intracellular N- and C-termini. In an effort to understand the substrate specificity of MCT8, we recently published a homology model on the basis of *Escherichia coli* glycerol phosphate transporter (GlpT), which is supported by biochemical evidence. Still, we need an experimental structure of MCT8 in one or several conformations to understand its function. We want to crystallize MCT8 and subsequently solve its crystal structure by X-ray diffraction. While several prokaryotic transporter structures have been solved, only few human transporter structures are available.

Up to now, we achieved a first and promising purification of human MCT8 overexpressed in *E. coli*. Purification included membrane isolation, solubilisation of membrane proteins, and subsequent metal-affinity chromatography. The aim of these preliminary purifications is to increase the expression rate of MCT8 in *E. coli*, and to optimize purification. Subsequently, several key factors for the crystallisation need to be determined as functionality of the purified protein, stability, and accessibility of the overexpressed full-length MCT8.

P33-010
Endogenous calcium channels formed by Orai proteins in HEK293 cells

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Activation of cell surface receptors, which are coupled to phospholipase C -mediated signaling pathways, results in Ca^{2+} release from intracellular stores and activation of plasma membrane Ca^{2+} influx channels. In nonexcitable cells, two distinct pathways for calcium influx have been identified: the receptor-operated pathway, and the store-operated (SOC) pathway, which is activated when intracellular calcium stores are depleted. Depending on cell type, SOC channels vary in biophysical characteristics and modes of regulation, indicating that different proteins may be involved in forming calcium channels in the plasma membrane and/or in regulating SOC channels activity. Orai and TRPC proteins are the most probable components of native SOC. Previous studies at a single-channel level have allowed us to demonstrate the existence of three types of calcium channels in the plasma membrane of HEK293 cells: I_{min}, TRPC1- formed I_{max}, and TRPC3-containing I_{NS}. But it remained unknown which of these channels are Orai-containing. To resolve this issue, we performed single-channel analysis in HEK293 cells transfected with plasmid coding for dominant-negative forms of Orai1 (E106Q) or Orai3 (E81Q). The involvement of Orai proteins in the SOC influx was initially evaluated using the Ca^{2+} imaging method based on Fura-2 fluorescence. Further, we performed single-channel experiments to evaluate the effects of Orai mutants on different types of native calcium channels previously detected in HEK293 cells.

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P33-011
Insights into proton translocation in cytochrome cbb_3 from large scale MD simulations

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Cytochrome c oxidases (CcOs) are large membrane protein complexes found in bacteria and the mitochondria of eukaryotes which catalyse the reduction of oxygen to water and couple the energy of the reaction to proton translocation across the membrane. These are divided into three distinct families, A-, B- and C-type, and although much is known about the mechanism of action of the A-type CcOs, B- and C-type mechanistic features are still poorly understood as well as their role in the evolution of respiratory reductases. In contrast with the A-type CcOs, which have 2 proton channels, B- and C- type present a single proton pathway to the active site. Furthermore, C-type exhibits some unique features as high catalytic activities at low oxygen concentrations and nitric oxide reduction under anaerobic conditions, and have some structural resemblance to *bona fide* nitric oxide reductases (NORs).

In this work we report the results of large-scale all-atom molecular dynamics simulations of the C-type CcO containing all the core subunits (cbb_3 from *Pseudomonas stutzeri*)[1]. We analyse in detail the residues essential for the proton pumping and O_2 reduction in the active site, and model the effect of mutations experimentally shown to affect the enzymatic activity. We also look into the structural features that might differentiate C-type CcOs from the A-type family and clarify their possible evolutionary connection to NORs.

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P33-012
Regulation of epithelial chloride transport by tyrosine phosphorylation

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In a recent study we reported the regulation of the chloride channel CFTR by a novel WNK4/SYK signaling pathway regulating the amount of CFTR at the cell surface. Tyrosine kinase Syk was shown to phosphorylate CFTR and promote its removal from the plasma membrane. In order to study whether Syk may also operate in the regulation of other ion channels or co-transporters, their protein sequences were inspected for the presence of this consensus motif. Among 20 different transport proteins, the Syk motif was identified in the sequence of only two renal co-transporters for electrolyte homeostasis and blood pressure regulation.

Recombinant fragments of both channels were produced and found to become phosphorylated by Syk in *in vitro* phosphorylation assays. We then asked whether Syk can modulate their expression at the cell surface. Cells were incubated under different osmotic chloride conditions and analysed by biotinylation of cell surface proteins in the presence or absence of Syk and WNK4. Cells exposure to low Cl^- hypotonic stress medium led to increased amount of one of the channels detected at the cell surface and expression of Syk wt decreased its amount at the cell surface. On the opposite, cell exposure to a hypertonic solution of sorbitol or expression of Syk showed an increase in the amount of the other channel the cell surface.

Together, our studies elucidate a novel pathway contributing to dynamic chloride transport regulation and have implications for treatment options in diseases like hypertension, cystic fibrosis and obstructive pulmonary disease.

P33-014
Functional mapping of an Arginine cluster of the potassium inward rectifier channel Kir6.2 regulated by a fused G Protein Coupled Receptor

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Ion channel-coupled-receptors (ICCRs) are created by physical and functional link of a GPCR C-terminus to the Kir6.2 ion channel N-terminus. In ICCRs, the ion channel acts as a G protein-independent sensor of the GPCR activity. Thus, the electrical signal generated by the ion channel is directly linked to ligand binding on the GPCR.

In order to understand and decipher the molecular mechanisms involved in the GPCR-evoked regulation of the channel, structure-function studies of the domain linking the GPCR and the ion channel have been conducted. This domain is crucial for ICCR function and its length affects channel regulation in terms of signal amplitude and signs. Interestingly, 2 ICCRs, having identical linker length but 9 residues differences at the fusion point, showed different phenotypes: one functional, one inactive (no channel regulation). The inactive ICCR is characterized by the lack of residues 26 to 34 in the channel N-terminus containing 5 arginines. We functionally mapped these arginines and identify specific residues essential for ICCR function.

In physiological conditions, SUR/Kir6.2 channels (K-ATP channels) result from the association of two different proteins that assemble to form a large octameric complex. Involvement of the Kir6.2 N-terminal domain in functional coupling with the SUR protein cannot be easily studied in natural K-ATP channels, since mutations in this domain could affect both physical and functional interaction with the channel subunit. The ICCR technology provides a unique method to decode the mechanisms of the complex regulation of the Kir6.2 channel by its physiological partner, the sulfonylurea receptor SUR.

P33-015
Biophysical analysis of Channelrhodopsin variants

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Channelrhodopsins are photoreceptors located in the eye-spot of green algae, which cause phototactic responses depending on light conditions in the surrounding.

Channelrhodopsin-2 from *Chlamydomonas reinhardtii* (*Cr*ChR2), a light-gated cation channel, is used in the neurophysiological field to optically control cellular processes. In biophysics as well as in optogenetic investigations the shortened membraneous part of *Cr*ChR2, which is sufficient for channel formation, is used. The C-terminal part, which naturally exists in the algae, is mostly ignored. As so far no clear functional role of this substantial protein mass has been identified, we addressed the role of this large soluble C-terminal extension.

Channelrhodopsin-1 from *Chlamydomonas augustae* (*Ca*ChR1) is yet another promising optogenetic tool. To understand which amino acids are involved in the process of channel opening upon light activation, we created variants of *Ca*ChR1 for biophysical investigations. Here, especially we focused on the role of cysteines.

P33-016
Role of Sec16A in the unconventional protein secretion pathway

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Deletion of phenylalanine at position 508 in the cystic fibrosis transmembrane conductance regulator (ΔF508-CFTR) protein, the most common form of disease-causing CFTR mutant, results in protein misfolding and deficiency of CFTR trafficking to the cell surface through the conventional Golgi-mediated exocytosis. It has been shown that ΔF508-CFTR can be rescued to the cell surface through a Golgi by-pass unconventional secretion pathway induced by ER stress or GRASP overexpression. However, how misfolded ΔF508-CFTR can leave the endoplasmic reticulum (ER) membrane in this pathway is unknown. Therefore, we examined the early secretory pathway of the GRASP-dependent unconventional secretion of ΔF508-CFTR. The first step of early secretory pathway occurs at specialized sites on the ER, so called ER Exit sites (EREs). The biogenesis of EREs in the conventional Golgi-mediated secretion involves Sec16A, the scaffolding protein for COPII assembly. Conventional sectretion of wild-type CFTR to the cell surface was abolished by silencing of Sec16A by Sec16A specific siRNA. Interestingly, depletion of Sec16A inhibited the ER stress-induced or the GRASP-mediated surface expression of ΔF508-CFTR, indicating that Sec16A also plays a role in the unconventional protein secretion pathway. These findings imply that Sec16A is a critical component of EREs in the unconventional protein secretion pathway as well as conventional secretion.

P33-017
Redirecting iron pathways in the ferritin nanocage

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Ferritin, the iron storage protein, has a nanocage hollow structure resulting from the self-assembly of 24 independent subunits. The formation of a caged iron biomineral is driven by enzymatic reaction occurring at ferroxidase centers in the central part of catalytically-active subunits, where Fe^{2+} is the reaction substrate. To this purpose, Fe^{2+} needs to be translocated through the protein cage. Two different types of channels pierce the ferritin nanocage, in correspondence of C3 and C4 symmetry axes. The polarity across the channels controls the directional Fe^{2+} fluxes towards the catalytic center. Ferritins from different species use different channels as iron entry routes. In animal ferritins the C3 pores have been identified as the entry ion channels coupled with the ferroxidase reaction. Here, we have analyzed the functional significance of the Fe^{2+} pathway imposed by this iron route. Changing the electrostatic properties of the residues at the inner edge of each channel, we can selectively activate/deactivate Fe^{2+} routes, modulating the rate of iron oxidation at the catalytic sites. The observed directionality of the iron route from C3 pores to the ferroxidase centers appears to be more the result of an evolutionary selection than a mechanistic requirements; the coupling between the ferroxidase reaction and C4 channels entry points resulted at least equally efficient for the catalytic purposes. Besides shedding light on basic aspects of the ferritin chemistry, this work provides the proof of concept for the use of modified channels to facilitate inclusion of different cargos for biomedical applications.

P33-018
The effect of voltage-gated sodium channel on matrix metalloproteinase expression and activity in human breast cancer cells

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Cancer is one of the biggest health problems of the modern world. Voltage-gated Na^+ channels (VGSCs) mediate both transient and persistent Na^+-influx into cells, to enable generation and propagation of action potentials in "excitable" and "non-excitable" cells. Surprisingly, functional VGSCs are also upregulated in various human cancers, and promote metastatic potential *in vitro* and *in vivo*. In this study, to explore the mechanism of VGSC-mediated invasion potential, we aimed to determine the functional effects of VGSC activity on the expression/secretion of matrix metalloproteinases (MMPs) in breast cancer cells.

Experiments were carried out on strongly metastatic VGSC-expressing human breast cancer cell line, MDA-MB-231. To test our hypothesis, MDA-MB-231 cells were incubated in serum-free medium with or without TTX (30 μM) and Ranolazine (5 μM) for 12–24–48 h. Real Time PCR and gelatin zymography were performed to analyze MMP-2 and MMP-9 gene expressions and activity levels, respectively.

Blockage of total VGSCs activity with TTX reduced MMP-2 mRNA expression in a time dependent manner and increased MMP-9 mRNA expression. Ranolazine had no effect on two groups. In gelatin zymography, both TTX and ranolazine increased proMMP-2 and proMMP-9 levels compared to untreated-control group in a time dependent manner.

This is the first systematic investigation of a possible functional association between VGSC and MMP expression/activity in a strongly invasive/metastatic cell line. The identification of the mechanism will contribute pharmacological approaches to inhibiting not only VGSC but also its downstream effectors and this will provide a powerful approach to molecular-based anti-metastatic therapy.

P33-019
The diversity of light-driven proton pumps and their conversion into proton channels

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Microbial rhodopsins are integral seven-transmembrane proteins which bind covalently all-*trans*-retinal as light sensitive chromophore. They are subdivided into sensory rhodopsins, ion channels and ion pumps. Light-driven ion pumps transport protons, sodium or chloride across the plasma membrane against their electrochemical gradient. Bacteriorhodopsin (BR) from *Halobacterium salinarum* is the most notable proton pump and transports protons out of the cell. More recently proton pumps have been

employed in neuroscience as optogenetical tools for silencing of neuronal activity by hyperpolarization or as voltage sensors.

It was generally assumed that all light-driven microbial proton pumps behave basically in the same manner like bacteriorhodopsin. We analyzed a variety of proton pumps using two-microelectrode voltage-clamp measurements (TEVC) of *Xenopus leavis* oocytes. We have found that the naturally occurring proton pumps show different behaviors at high electrochemical load, i.e. low extracellular pH and negative voltage. Photocurrents of Bacteriorhodopsin and the Coccomyxa-Rhodopsin (CsR) from the eukaryotic microalga *Coccomyxa subellipsoidea* are always outward directed and inactivate at high load. In contrast, the rhodopsins from *Exiguobacterium* sibiricum (ESR) and from the cyanobacterium *Gloeobacter violaceus* (GR) show inward directed photocurrents at high load. The rhodopsin Arch3 from the archaeon *Halorubrum sodomense* is well established as optogentical tool and shows weak inward directed photocurrents at high load. We have used CsR for an efficient mutagenesis study and identified key determinants for the directivity and the power of pumps. Mutations at position R83 and Y57 converted CsR into an operational proton channel with inward or outward rectification depending on the replacement.

P33-020
Identification of gates of the potassium inward rectifier Kir6.2 channel controlled by regulatory membrane proteins

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Inward rectifier potassium Kir6 channels are the pore-forming subunit of the ATP-sensitive potassium channels (K_{ATP}). Two isoforms of Kir6 have been reported: Kir6.2 and Kir6.1.The physical and functional association of four Kir subunits with four sulfonylurea receptors (SUR1 or SUR2) form an hetero-octameric, the K_{ATP} channels.

The precise molecular mechanisms of the allosteric regulation of Kir6.2 by SUR are still unknown due to the complex relationship of the physical and functional interaction of the two proteins. Thus the gates regulated by SUR are not identified. Crystallographic structures and functional characterizations of potassium channels demonstrate the presence of two gates in the transmembrane domains (the selectivity filter and the "A" gate at the cytoplasmic interface) and a third gate in the cytoplasmic domain of Kir channels (the G loop gate).

To identify the gates under control of SUR, we exploited a unique artificial K_{ATP} channel created in our group by fusing G protein-coupled receptors (GPCRs) to the Kir6.2 Nterminus. In this fusion proteins called Ion Channel-Coupled Receptors (ICCR), Kir6.2 gating is modulated by the GPCR conformational changes through the linker domain. The two proteins being covalently linked, this system is independent of physical interferences and facilitates the interpretation of structure-function results aiming at deciphering the molecular mechanisms of Kir6.2 regulation. With the objective of identifying Kir6.2 gates regulated by the fused GPCR, we developed an original approach based on a functional mapping of gates with an agonist-inhibited ICCR. Unexpected, results demonstrated that several gates could be involved suggesting a concerted mechanism.

P33-021
Time-resolved spectroscopic characterisation of channelrhodopsin-1 from *Chlamydomonas augustae*

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Channelrhodopsins are photoreceptors found in green flagellate algae, where they cause phototactic response. Electrophysiological experiments showed that they act as light-gated cation channels when heterologously expressed in mammalian cells. Due to this function these cation channels are meanwhile used in the new field of optogenetics, where specific nerve cells are activated upon light excitation. Many important applications followed, like mapping of brain circuits and research on the understanding of neurodegenerative diseases such as Parkinson's disease. Although channelrhodopsins are already widely-used in neurophysiological applications, the mechanism how these proteins transfer ions upon light activation is still not clarified in detail.

In this study we investigate the function of the red-shifted channelrhodopsin-1 from *Chlamydomonas augustae* (*Ca*ChR1). We want to understand the processes leading to the opening of this channel, which include isomerization of the retinal after light excitation and proton transfer reactions from the Schiff base which is protonated in the ground state. Therefore, we apply time-resolved spectroscopic methods to determine and compare the intermediate states on a time scale from 100 ns to 10 s. Site-directed mutagenesis, H_2O/D_2O and $H_2O/H_2{}^{18}O$ exchange were used to assign vibrational bands of specific amino acids, of the retinal and of dangling water molecules. Time-resolved IR experiments on *Ca*ChR1 shows large negative bands in the amide I and amide II region which revealed conformational changes of the protein backbone on a very early time scale.

P33-022
Effect of Ca^{2+} ions on Bestrophin-1 interaction with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine in surface films

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Bestrophin-1 (Best1) is a transmembrane multifunctional protein, expressed in the plasma membrane of retinal pigment epithelium. Best1 may acts as Ca^{2+} activated chloride channel or/and regulator of voltage-gated Ca^{2+} channels. Its interactions with the plasma membrane lipids are important for the conformation, oligomerization and functional activity. Studies in this field have not been performed so far as the protein was not purified to homogeneity. Our group published an original methodology for isolation and purification of sufficient quantities of functionally active human recombinant Best1 from stably transfected MDCK cells.

Our interest has been focused on the effect of Ca^{2+} ions on Best1 interactions with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in Langmuir monolayers since it is the most abundant phospholipid of animal cell membranes.

The π/A (surface pressure/area) isotherms and compression/expansion isocycles of Best1, POPC and Best1/POPC monolayers were recorded in absence and presence of Ca^{2+} in the subphase. The effect of Ca^{2+} on the morphology of monolayers was observed by Brewster angle microscopy (BAM).

Our study shows that the incorporation of Ca^{2+} in the subphase does not change the shape of π/A isotherms but decrease the mean molecular area of Best1, POPC and Best1/POPC monolayers. These results correlate well with BAM images representing that the presence of Ca^{2+} induces the formation of lipid/protein macromolecular aggregates (Best1/POPC clusters) during monolayers compression. We assume that Ca^{2+} ions play role for interaction of Best1 with POPC at physiological conditions in the cell.

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P33-024
A defect of paclitaxel uptake in *SLCO1B3* polymorphisms

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Background: Paclitaxel is anti-cancer drug and used in various cancer type. *SLCO1B3* is known as paclitaxel uptake transporters. They are located in hepatobiliary systems and affect drug concentration of blood by promoting biliary secretion.
Methods: We identified *SLCO1B3* polymorphisms frequenctly observed in Asians and paclitaxel uptake activity of each *SLCO1B3* polymorphisms was measured using oocytes systems.
Results: *SLCO1B3* c.334 T>G and c.699 G>A polymorphisms were selected for candidate. They are known as high linkage disequillibrium status in Asians. First, rosuvastatin, representative substrate of *SLCO1B3* was measured, and c.699 G>A variant and c.334 T>G / c.699 G>A double mutant expressing oocytes showed decreased transport activity. Likewise, paclitaxel transport activity was significantly decreased in c.699 G>A variant and c.334 T>G / c.699 G>A double mutant expressing oocytes compared to wild type *SLCO1B3* oocytes. In contrast, *SLCO1B3* c.334 T>G variant expressing oocytes showed no functional change, suggesting that c.699 G>A variant is functionally important polymorphism for paclitaxel transport.
Conclusions: *SLCO1B3* c.699 G>A variants may predict drug response such as toxicity and play important role in individualizing chemotherapy.

P33-025
D-glucose and insulin regulate the activity of equilibrative nucleoside transporters in renal glomerular cells

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Introduction: Diabetic nephropathy (DN) is a devastating kidney disease whose pathogenesis remains to be elucidated. Elevated levels of extracellular adenosine have been associated with DN progression. The major regulatory mechanism of adenosine availability involves the activity of the equilibrative nucleoside transporters (ENTs). Because the nucleoside uptake activity is lower in glomeruli from DN animals, we aim to evaluate the effect of D-glucose and insulin on ENTs.
Methods: Adenosine uptake ([^{3}H]adenosine, 60s, 22°C) was assayed in purified glomeruli from rats and primary cultured podocyte and mesangial cells preincubated with 5 mM or 25 mM D-glucose for 24 h, and exposed to 10 nM insulin for 8 h. ENT1-mediated transport is sensitive to 1 μM NBTI whereas ENT2 is to 2 mM hypoxanthine. Plasma membrane and intracellular proteins were fractionated by the biotinylation method and ENT1 and ENT2 contents were quantified by western blot. The adenosine amount was quantified by HPLC.
Results: High D-glucose concentration decreases the activity of ENT1 and ENT2 in glomeruli, podocytes and mesangial cells. Furthermore, insulin was able to reverse this effect restoring the activity of these transporters to baseline levels. There were not changes in ENT1 or ENT2 contents upon treatments using insulin and D-glucose. Conversely, we saw that these stimuli differentially regulate the plasma membrane localization of these proteins. The levels of extracellular adenosine were increased when the uptake activity of ENTs was reduced.
Conclusions: Increases in extracellular adenosine availability could be sustained by internalization and lower ENTs activity triggered by high D-glucose levels and insulin deficiency as in DN.

P33-026
Acidic pH effect on electrophysiological behavior of a new chloride channel in endoplasmic reticulum

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Introuduction: Earlier studies indicate pH importance in cellular function and organelles stability. Normal pH is essential for many important functions of endoplasmic reticulum such as Ca^{2+} homeostasis, protein folding, vesicle loading and conduction of vesicles in right destination. Chloride channels are involved in cytoplasmic and luminal pH regulation.
Methods: L-*a*-lecithin was extracted from fresh egg yolk and then utilized to form artificial bilayer lipid membrane in a 150 μ m diameter hole. Rough microsomes derived from RER of rat hepatocyte and Fusion of the vesicles was initiated by gently touching the bilayer. After recording in normal pH, record was repeated in acidic pH throe adding HCl in cis envirement. Data were analyzed by PClamp9.
Results: Our results demonstrated that the channel conductance was aproximatly 350 pS in 200 mM KCl cis/50 mM KCl trans. The channel open probability (Po) appeared voltage-dependent at -50 to +50 mV and has lower amounts with increase in positive voltages. The I-V curve of this channel was linear. Channel conductance and Po were decreased in acidic pH.
Conclusion: Our results suggest that this new chloride channel may be involved in many physiological functions of ER and could be one of important drug targets in some diseases such as cancer.
Key words: pH, electrophysiology, rough endoplasmic reticulum, chloride channel.

P33-027
Comparison of the expression and functionality of P2X7 receptors sensitive to ATP and Bz-ATP activation at different cell lines

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P2X7 receptor is a homotrimeric ion channel with two transmembrane domains and a large extracellular ATP-binding domain. Gating of P2X7 can be induced by ATP and its Bz-ATP analog. The P2X7R were found predominantly on cells of hematopoietic lineage [Surprenant et al. (1996) *Science,* 272, 735], including macrophages and lymphocytes. P2X7R is also expressed in cells of other types, including epithelia, endothelia, and neurons in CNS [Surprenant, North, (2009) *Annu Rev Physiol*, 71, 333] and its properties differ in a cell type-dependent manner: the membrane permeabilization to large molecules (up to 900 Da, e.g. fluorescent dyes) does not occur in all cells expressing this receptor. The nature of the response is partly dependent upon the activator agonist concentration used, there might also be cell type-dependent differences in the molecular composition of the P2X7 receptor complex.

We compared the expression level of P2X7R in several cell lines, and its functional properties, by means of uptake of the molecules up to 900 Da. The analysis has shown the presence of P2X7 receptor in all tested cell lines at different levels and the levels of the P2X7 mRNA do not correspond to the protein level. The pore activity of the receptors was investigated by measurement of the uptake of ethidium bromide following the ATP or Bz-ATP-assisted induction, and the results were observed by fluorescence microscopy.

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P33-028
Refolding of small monomeric outer membrane proteins

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Outer-membrane proteins of gram-negative bacteria play pivotal roles in their interaction with the environment. Therefore, these sturdy proteins represent interesting study objects. The small monomeric ß-barrel outer-membrane proteins AlkL (*Pseudomonas oleovorans*), OmpW (*Escherichia coli*), TodX (*Pseudomonas putida*) and OprG (*Pseudomonas aeruginosa*) are possible FadL-type transporters for hydrophobic substances. Although structurally well characterized, their transport characteristics are far less well known. Unfortunately, reconstitution experiments for functional characterization typically require high amounts of concentrated protein.

As with most membrane proteins, native expression of these four proteins suffers from low yields and tedious downstream processing. Leader peptide removal directs the protein into inclusion bodies, which can be purified with yields of 0.1–0.2 $g/L_{culture}$ pure unfolded protein. The challenge then was to establish proper refolding conditions to revert the proteins into their functional form.

Refolding experiments were performed using the rapid dilution method in order to investigate classical refolding parameters, such as the nature and concentration of selected detergents and supplementary folding additives as well as the initial protein concentration. While AlkL and OprG folded properly over a wide range of conditions, TodX and to a lesser degree also OmpW required a more specific folding environment. For all four proteins optimal conditions were established, which yielded high refolding efficiencies (47-96%) at room temperature. The employment of folding additives, which is rather more common when refolding soluble proteins, was effective in improving the refolding efficiency especially in the intermediate protein concentration range, allowing the proteins to be refolded at concentrations as high as 0.5–1 g/L.

P33-030
Evolution of the potassium chloride cotransporter subfamily: functional analyses of basal metazoan

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The secondary active potassium chloride cotransporter (KCCs) subfamily mediates the coupled extrusion of K^+ and Cl^-. In vertebrates, they play a major role in various physiological processes such as regulation of cell volume and lowering the intracellular Cl^- concentration $[Cl^-]_i$ in neurons. Thus, binding of the inhibitory neurotransmitters GABA or glycine to their receptors, which are cognate Cl^--gated channels, results in Cl^- influx and hyperpolarization. Severe pathologies like Andermann syndrome, deafness, and epilepsy, caused by mutations in these genes emphasize their fundamental role in human physiology. Phylogenetic analyses revealed that KCC genes were established in the genome since eukaryotes. However, analyses concerning the evolutionary conservation of the transporter function and the basal physiological role of KCCs in "early" metazoan are missing so far. Therefore, I cloned and sequence KCC of the cnidarian *Hydra vulgaris* (*hv*KCC). *Hv*KCC shares a protein identity of 48% to the human KCCs. The transmembrane domains, which are important for the translocation of the ions, are the most conserved area throughout the protein (57 % protein identity to *hs*KCCs). Functional analyses of codon-biased adjusted *hv*KCC overexpressed in HEK-293 cells indicate that *hv*KCC exhibits a small transport activity which is blocked by the KCC specific diuretic furosemide. In the future, analyzes of the *in vivo* expression of *hv*KCC in *Hydra vulgaris* will provide an important hint to the basal physiological role of *hv*KCC.

P33-031
Protein translocation through mitochondrial channel: Single channel electrophysiology

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TOM Core Complex (TomCC) is high molecular mass protein complex that facilitates the transfer of nearly all mitochondrial pre-proteins across outer membrane of the mitochondria. An adequate method to study properties of these channels is electrophysiology and in particular analyzing the ion current fluctuation in the presence of permeating signal peptides. We recently investigated the temperature and voltage dependence of the membrane transport of the signal peptide pF1β through single TomCC channel. From the kinetic data obtained from our single channel measurements peptide binding could be distinguished from peptide translocation. The equivalent increase of the peptide dissoci-

ation rates with applied voltage demonstrates translocation of peptide. We further investigate the effect of modification by pegylation on peptide partition properties through the TomCC channel.

P33-032
Analysis of antiproliferative and antimetastatic effects of nNav 1.5 sodium channel and Notch-4 receptor inhibition

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Voltage gated sodium channel activity enhances cell behaviours related to metastasis, such as motility, invasion, oncogene expression. Neonatal alternative splice form of Nav1.5 isoform is expressed in metastatic breast cancers. Furthermore, aberrant Notch signalling can induce oncogenesis and may promote the progression of breast cancers. The aim of this research is the effect of the inhibition of these two molecules on the proliferation and metastatic behaviour of highly metastatic MDA-MB-231 human breast cancer cell as well as the interaction between these molecular systems. For this purpose, sodium channels were inhibited by an anticonvulsive drug phenytoin and the Notch-4 receptor signalling was inhibited by gamma secretase inhibitör, DAPT. In order to determine the individual and combined effects of these inhibitors, 4-[3-(4- iyodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3- benzene disulfonate (WST-1) test for proliferation, wound healing assay for lateral motility and zymography for **matrix metalloproteinase-9 (**MMP9) activity were performed. Finally, we found that the combined effect of DAPT and phenytoin is not as beneficial as using DAPT alone for decreasing metastatic properties of MDA-MB-231 breast cancer cells.

P33-034
VDAC activity in the presence of huntingtin proteins

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Huntington's disease (HD) is a progressive brain disorder that gradually robs affected individuals of memory, cognitive skills and normal movements. It is caused by the mutation of the gene encoding the huntingtin protein (Htt) that results in an increase of glutamine codon number above 35 and consequently in Htt with an abnormal stretch of above 35 glutamines in the N terminus (mHtt). At present it is becoming increasingly apparent that mHtt can impair mitochondrial function directly by affecting mitochondrial bioenergetics and dynamics. Thus, mitochondrial functioning appears to be affected by mHtt and the resulting mitochondrial impairments may occur early enough to contribute to mHtt-induced toxicity and HD pathogenic mechanism. Interestingly, the proposed mitochondrial targets of mHtt include processes that are known to be affected directly or indirectly by VDAC (voltage-dependent anion selective channel) located in the outer membrane of mitochondria and presently regarded as a global regulator, or governor, of mitochondrial functions. On the other hand, it is known that Htt interacts with above 200 proteins which represent a diverse array of biological functions. However, the functional relationship of Htt to mitochondria is still uncertain. Here we present our results concerning interactions between both Htt and mHtt and reconstituted human VDAC isoforms.

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P33-035
Regulation of serotonin transporter activity in animal models of peripheral inflammation – relevance to inflammation-induced depression

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Depression is a complex disorder precipitated in susceptible individuals by various stress factors. Inflammation is thought to be one such stressor, and chronic inflammatory diseases are often associated with depression. Molecular mechanisms underlying inflammation-induced depression are poorly understood. One target for immune system modulation of neuronal function is the serotonin transporter (SERT), a key regulator of serotonergic neurotransmission and a primary antidepressant target. Employing the widely used lipopolysaccharide (LPS) model of sickness and depression-like behaviour, we show that SERT activity is upregulated in the hippocampus and cortex of rats 24 h after LPS injection. The increase in SERT activity is not caused by enhanced mRNA or protein expression but due to posttranslational modification of the transporter. To complement the rather acute LPS model, we established a more clinically relevant model of chronic inflammatory diseases, namely the collagen-induced arthritis (CIA) mouse model of rheumatoid arthritis. In the CIA model SERT activity is elevated in the hippocampus but not in other brain regions. Moreover, other neurochemical changes in CIA mice were found to correlate with previously observed effects in widely used chronic stress models of depression. For example, we found that BDNF mRNA levels are reduced in the CIA animals, suggesting that this model is suitable to study molecular mechanisms underlying depression triggered by chronic peripheral inflammation. Furthermore, comparative analysis of the two animal models allows us to characterise the neurochemical and behavioural consequences of acute and chronic peripheral immune system activation, including the modulation of serotonergic neurotransmission.

P33-036
The sea anemone *Heteractis crispa* – a source of potential pharmacological agents

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Venomous *marine organisms* are a unique source of compounds acting on various biological targets involved in important physiological processes. So, sea anemones produce a huge variety of

neuro- and pore-forming polypeptide toxins, protease inhibitors, which can find wide applications in pharmacology. Such promising polypeptides are serine protease inhibitors, which, thanks to amino acid mutation at reactive site P1 position (Arg→Lys→Thr) during evolution have acquired the ability to interact with cysteine, aspartic proteases, and modulate Transient Receptor Potential (TRP) receptors and thus exhibit an analgesic effect *in vivo*.

We investigated structure-function relationships of two family representatives, so-called HC**GS**- and HC**RG**-polypeptides of Kunitz-type (with N-terminal GS and RG residues, respectively, each family of 33 polypeptides having point substitutions), which form *H. crispa* combinatorial library. Several polypeptides were obtained in the native state (In IV, InhVJ, HCRG 1, HCRG 2) or in recombinant form (HCGS 1.10, HCGS 1.36, HCRG 21). In contrast to other HCRG-polypeptides and similar to analgesic ones belonging to the HCGS family (Isaeva *et al*., 2012), HCRG 21 is characterized by Thr at P1 position. Polypeptides HCGS 1.10, HCGS 1.36, HCRG 21 demonstrated an analgesic effect *in vivo*. Electrophysiological assay of HCRG 21 on the TRPV1 receptor revealed 50% inhibitory activity (IC50 = ±10 μM). Molecular modeling (docking, mutagenesis, MD simulation) disclosed the functional significance of reactive site residues (at positions P1, 16, 17), and residues at 1, 5, 38 positions for polypeptides interacting with both biological target types.

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P33-037
Comparative analysis of Mg- dependent and Mg- independent HCO_3^-ATPases

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The comparative analysis between two enzymes, Mg-dependent and Mg-independent HCO_3-ATPases, were studied in synaptosomal and microsomal membrane fractions of albino rat brain, using the method of kinetic analysis of the multi-sited enzyme systems. Therefore, it can be inferred that Mg-dependent HCO_3-ATPase belongs to the group of "P type" transporting ATPases. Mg-independent HCO_3-ATPase with its kinetic properties may be attributed to the group of "Ecto" ATPases.

P33-038
Novel nitrate/nitrite transporter in the *Mycobacterium gilvum* Spyr1

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Microbial degradation is the major route by which Polycyclic Aromatic Hydrocarbons (PAHs) can be removed from the environment. Microbial activity is stimulated by addition of nutrients such as nitrate, which can serve as an electron acceptor under oxygen limitation conditions in contaminated soils. Nitrate is transported across the bacterial membrane by nitrate/nitrite porter proteins (NNP). Thus far, little is known about NNP genes in PAH-degrading bacteria. Genome sequencing [1] of the PAH-degrading bacterium *Mycobacterium gilvum* Spyr1 [2] revealed the existence of two putative NNP genes: *pynar* and *pynir*. Predicted gene products retain the characteristic nitrate-signature motifs (NS1 and NS2), conserved Gly and charged residues Arg within transmembrane segments 2 and 8 (R67, R268). NNP genes were cloned into an *E. coli* strain defective in all three endogenous nitrate/nitrite transporter genes (NarK, NarU and NirC). Heterologous expression of NNP genes was demonstrated by western blotting and net nitrate uptake assays were carried out. Our results indicate that *pynar* can complement the nitrate-dependent growth of the triple mutant and transport nitrate/nitrite in/out of bacteria. Mutants replacing R67 or R268 with Lys, His or Ala were found to be devoid of nitrate/nitrite transport activity.

[1] *Stand. Genomic Sci., 5:144 (2011)*
[2] *Appl. Biochem. Biotechnol.,159:155* (2009)

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P33-039
Characterization of ATP/ADP transporters (NTT) from obligate, intracellular living bacteria

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Adenine nucleotides are the major energy carriers in the cell. As their synthesis is limited to selected locations, cells rely for their passage across membranes on transporters. In mitochondria the ADP/ATP counter-exchange is mediated by ADP/ATP carriers (AACs), while in certain organisms a second, distinct system exists. These nucleotide translocator proteins (NTT) are structurally and functionally different from the AAC proteins, to which they posses no sequence similarity. They import cyotosolic ATP in exchange for ADP and phosphate in an electroneutral fashion.

NTT proteins are found in plant plastids and in the obligate, intracellular living orders of *Rickettsiales* and *Chlamydiales*, which rely on nucleotide import from the host for survival. They are also important pathogens (Epidemic typhus, Porcine proliferative Enteritis) continuing to kill more than 200.000 persons per year. The NTT proteins are absent from vertebrates, making them interesting drug targets, for which presently no inhibitors exist. At the moment the bacterial and plant NTT proteins have been, to different degrees, biochemically characterized, yet no detailed structural information is available.

The NTT proteins from a range of different bacteria could be successfully over expressed, purified and further characterized, with crystallization experiments being underway. Effects of detergent and buffer conditions and stability were assayed using a GFP tag protein. In addition the effects of mutants, targeting basic residues or testing truncations, in the plant AtNTT1 protein point to functionally relevant loop regions and residues of the protein, which can be exploited for crystallization and give further insights into the function.

P33-040
In vitro function of the human liver ABC transporter MDR3 and its extended X loop mutant

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The human multidrug resistance protein 3 (MDR3, ABCB4) belongs to the ATP-binding cassette (ABC) transporter family and is crucial for bile formation in the bile canaliculus. There it flops phosphatidylcholine from the inner to the outer leaflet of the canalicular membrane to protect the biliary ducts from the toxicity of bile salts. Mutations in MDR3 can cause dysfunction, leading to various liver diseases.

Here, we report the ATPase activity of wild type MDR3 and the Q1174E mutant, which was identified previously in a patient with progressive familial intrahepatic cholestasis type 3 (PFIC-3). We expressed different variants of MDR3 in the yeast *Pichia pastoris*, purified the proteins via tandem-affinity chromatography and determined MDR3 specific ATPase activity in the presence or absence of phospholipids.

The ATPase activity of wild type MDR3 was stimulated twofold by liver phosphatidylcholine (PC) lipids, while crosslinking of MDR3 with a thiol-reactive fluorophore blocked ATP hydrolysis and exhibited no PC stimulation. Similar, phosphatidylethanolamine (PE), phosphatidylserine (PS) and sphingomyelin (SM) lipids did not induce an increase of wild type MDR3 ATPase activity. The Q1174E mutation is located in the nucleotide-binding domain (NBD) in direct proximity of the leucine of the ABC signature motif and extends the X loop, which is found in ABC exporters. Our data on the Q1174E mutant demonstrated basal ATPase activity, but PC lipids were incapable of stimulating ATPase activity highlighting the role of the extended X loop in the crosstalk of the NBD and the transmembrane domain.

P33-041
Dodecylrhodamine and dodecyltriphenylphosphonium are substrates of yeast multiple drug resistance pump Pdr5p

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ABC-transporters extrude diverse types of xenobiotics from the cytoplasm. Alkylated lipophilic cations such as dodecyltriphenylphosphonium (C_{12}TPP) or fluorescent dye dodecylrhodamine (C_{12}R1) are potential substrates of ABC pumps. At the same time, high hydrophobicity of these compounds suggests that after the extrusion the molecules will be instantly absorbed by the plasma membrane. We suggested that in this way alkylated lipophilic cations could fill the capacity of the transporters and thus prevent the extrusion of other substrates. We asked whether C_{12}R1 and C_{12}TPP are substrates of any particular ABC-transporter of yeast *Saccharomyces cerevisiae*. We have shown that the overexpression of pleiotropic drug resistance transporter gene *PDR5* decreased C_{12}TPP toxicity and strongly facilitated the extrusion of C_{12}R1 from the cells. Reversibly, the repression of *PDR5* increased C_{12}TPP toxicity in yeast and completely prevented the extrusion of fluorescent dye C_{12}R1. Finally, we have shown that C_{12}TPP effectively inhibits the extrusion of C_{12}R1 from yeast cells. Together, these results point that Pdr5p is the main ABC-transporter of yeast *S. cerevisiae* responsible for extrusion of C_{12}TPP and C_{12}R1. However, the overexpression of *PDR5* was not sufficient to completely prevent accumulation of C_{12}R1 in yeast cells. Possibly, this is due to the high octanol/water partition coefficient of the compound and to the absence of hydrophobic trap for such molecules outside cultured yeast cells. As a result, the extrusion may lead to a futile cycle. We suggest that such highly lipophilic cations could be used as a platform for the development of antifungal compounds.

Struct Biol S4, Monitoring Protein Conformational Dynamics and Movement

P35-005-SP
Folding of right- and left-handed three-helix proteins

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Recently the role of a mirror image conformation as a subtle effect in protein folding has been considered. The understanding of chirality both in protein structures and amyloid suprastructures is an important issue in molecular biology now. We are the first who have investigated the relationship of the protein handedness with the rate of protein folding. Our findings demonstrate that not large three-helix left-handed proteins are less-dense packed that should result in faster folding as compared to right-handed three-helix proteins. At the same time, the right-handed three-helical proteins have higher mechanical stability than the left-handed proteins. Moreover, from our analysis we have revealed that the bacterial three-helical proteins have some advantages in the packing over the eukaryotic right-handed three-helical proteins which should result in faster folding. We have created a new server FoldHandedness. Using this server it is possible: 1) to define the regions of helices from two issues (from the pdb file and using the last version of the DSSP program); 2) to determine the handedness for any chosen three helices; and 3) to calculate the angle and sign between the chosen pairs of the helices for large proteins and complexes of proteins with DNA or RNA. The FoldHandedness server is available for users at http://bioinfo.protres.ru/foldhandedness.

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P35-006-SP
The role of surface wettability and environmental conditions in Amyloid β conformational changes

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Here we are presenting an overview on some recent results related to the amyloid self-assembly of Aβ(25–35), Aβ(1–42), Aβ(12–28) and Aβ(1–40) peptides, involved in the formation of Alzheimer's disease plaques, in presence of surfaces characterized by different wettability and under the influence of external agents

such as phospholipids, acetylcholinesterase (AChE) and curcumin. Taking inspiration from the natural features of lotus leaves, nanostructured surfaces can be used in an efficient way to manipulate matter aggregation at interfaces. The investigation was supported by a multi-technique approach based on a combination of Raman spectroscopy, synchrotron radiation μFTIR and μXRD (micro X-Ray Diffraction) using highly hydrophilic nanostructured supports. The high evaporation flux at the triple contact-line resulted in ring-like solid residues showing, for the pure peptides, a α-helical to β-sheet transition from the internal rim to the external one, probably due to the enhancement of the local concentration of the protein at the external edge of the drying drop. On the contrary, the presence of a phospholipid mixture, that mimics the phospholipid composition of the neural membrane, showed the exclusive presence of β-sheet material indicating that the presence of phospholipids plays an active role in the fibrillation process. Further, the recent extension of this characterization protocol to the influence of AChE and curcumin, two external agents known to have, respectively, an amyloid fibril enhancer and inhibiting effect, open new interesting perspectives to better understand the fibrillation mechanisms of Amyloid β peptides.

P35-007-SP
Photoactivation and signal transduction of Blue Light sensors Using FAD (BLUF)

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Blue light sensors using FAD (BLUF) are modular photoreceptor proteins found in prokaryotic and eukaryotic microbes. Their design comprises the photosensory BLUF domain coupled to various signaling output domains which make them attractive optogenetic tools. The BLUF domain undergoes a light-induced reorganization of the hydrogen bond network around their flavin cofactor. These subtle changes are sufficient to modulate the activity of its cognate effector domain and thus induce signaling in the corresponding organism or *in vitro*. Although the hydrogen bond switch is accomplished in less than one nanosecond through a proton coupled electron transfer mechanism between a tyrosine side chain and the flavin, the resulting signaling state is stable for seconds to minutes and reverts thermally to the dark-adapted state. To identify the nature of the underlying structural changes and their implication for signaling we apply time resolved ultrafast UV/vis and vibrational spectroscopy in combination with a sophisticated selective isotope and chemical labeling approach. In addition, we conduct functional studies of the biological output activity and correlate the ultrafast photoactivation events to signal transduction.

P35-008-SP
Investigating partially unfolded conformations populated by monomeric human transthyretin

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Aggregation and deposition of the homotetrameric protein transthyretin (TTR) has been linked to the onset of systemic and localised amyloidoses. It is also emerging that TTR exerts a protective role against aggregation of the Aβ peptide, a process linked to Alzheimer's disease. Although it has been shown that these two processes correlate with the ability of TTR to populate a monomeric state, a complete description of the conformational states populated *in vitro* by monomeric TTR at physiological pH is missing. We used an array of biophysical methosd and kinetic tests to investigate the folding process of monomeric TTR. Our results show that once monomers of transthyretin are released by the quaternary structure, the protein establishes an equilibrium between a set of conformational ensembles bearing different degrees of disorder [1]. Thus, a molten globular state appears in equilibrium with the fully folded monomer, whereas an off-pathway species accumulates transiently during refolding of TTR [1]. These two conformational ensembles are distinct in terms of structure, dynamics, kinetics and pathway of formation [1]. Further subpopulations of the protein fold differently due to the occurrence of proline isomerism [1]. We investigated the conformational states described above by exploiting an intramolecular Förster Resonance Enegy Transfer approach. As TTR possesses one single cysteine moiety, we labelled such residue with a probe which acts as acceptor of light emitted by tryptophan residues. This system reports on intramolecular distances and compaction of the different conformational states.

Reference

[1] Conti S. et al., Biochemistry (2014) 53(27):4381–92.

P35-009
T-cell immune suppression by the cytoplasmic tail of the HIV gp41 envelope protein: implications for a virus controlled T-cell on/off switch

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HIV infects cells by utilizing its envelope glycoprotein gp160, primarily the gp41 subunit, which mediates membrane fusion between the virus and the host cell. Interestingly, our lab discovered that gp41 harbors an array of immune modulating motifs that are exposed only during the fusion process. This is surprising as immunomodulation by the virus is usually pertained to immune activation during stages that follow membrane fusion by viral proteins other than gp41, leading to an enhanced infection. Nevertheless, suppression during initial infection stages such as membrane fusion would enable the virus to infect the cell without raising "immune system alarms" before the infection has been established. To date immune modulation is mostly pertained to Th_1 helper cells that are a major target of HIV. T-cell immunosuppression by gp41 is achieved via recognition between virus motifs and transmembrane regions of the T-cell receptor complex (TCR), thus impeding TCR complex assembly that leads to cell proliferation and pro-inflammatory cytokine secretion. Recently, the cytoplasmic tail (CT) of gp41 has been implicated as a T-cell activator following gp41 endocytosis. In contrast, we show an adjacent suppressing region in the CT that targets the TCR signaling cascade. These findings point to the CT as a possible molecular switch, presumably dividing suppression and activation of the cell by space (membrane versus cytosol) and time (entry versus later infection stages).

P35-010
Allosteric regulation of human pyruvate kinase M2

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Pyruvate kinase M2 (M2PYK) plays an important role in metabolic reprogramming of cancer cells. Here we show amino acids have different effects on M2PYK activity. Some amino acids are activators whereas some are inhibitors, although binding of both kinds stabilise M2PYK tetramer over monomer. Enzymologic and crystallographic studies show that serine activates M2PYK by binding in an allosteric pocket and stabilising its active tetrameric form. Intriguingly, unlike serine, some hydrophobic amino acids, such as phenylalanine, inhibit M2PYK by binding to the same pocket as serine does but induce a rotation of the subunits to stabilise a distinct inactive tetramer.

P35-011
Inhibitory effect of β-casein on the amyloid fibril formation of $A\beta_{1-40}$ associated with Alzheimer's disease

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Alzheimer's disease is associated with the fibril formation of β-amyloid peptide in extracellular plaque. β-Casein is a milk protein that has shown a remarkable ability to stabilize proteins by inhibiting protein aggregation and precipitation. The aim of this study was to test *in vitro* the ability of β-casein to bind the Aβ1–40, change the structure and inhibit the amyloid fibril formation in Aβ1–40.

Results from the ThT binding assay indicated that incubation of Aβ1–40 with β-casein retarded amyloid fibril formation of Aβ1–40 in a concentration dependent manner such that at 1:1 w:w ratio led to a significant reduction in the amount of fluorescent intensity. The results from transmission electron

microscopy (TEM) also showed that β-casein significantly reduced the number and size of the Aβ1–40 fibrils, suggesting that the chaperone bound to the Aβ1–40 fibrils and/or interacted with the fibrils in some way.

ANS results also showed that β-casein significantly decreased the exposed hydrophobic surface in Aβ1–40. Following an ANS binding assay, CD spectroscopy results also showed that incubation of Aβ1–40 resulted in a structural transition to a β-sheet. In the presence of β-casein, however, α-helical conformation was observed implying stabilization of the protein.

These results reveal the highly efficacious chaperone action of β-casein against amyloid fibril formation of Aβ1–40. These results suggest that *in vitro* β-casein binds to the Aβ1–40 fibrils, alters the Aβ1–40 structure and prevents amyloid fibril formation. This approach may result in the identification of a chaperone mechanism for the treatment of neurological diseases.

P35-012
pH dependent conformational variations in Major Histocompatibility Complex class II (MHC II) molecules

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Major Histocompatibility Complex class II (MHC II) molecules present antigenic peptides to $CD4^+$ T cells in the course of adaptive immune responses. For this purpose, MHC II proteins traffic through several cellular compartments that differ in their pH value, from acidic pH in lysosomes to neutral pH by cell surface. It has been proposed that the low pH of the antigen loading compartment may increase the conformational flexibility of class II proteins, facilitating both the association and dissociation of peptides. In order to determine pH sensitive regions in the MHC class II allele HLA-DR1 [Human leukocyte antigens (HLA)], we acquired HSQC NMR spectra at different pH values. With this we defined three main regions sensitive to pH changes. By using different peptides presented by the MHC II (the Influenza Hemagglutinin derived peptide and a high affinity variant of the Class II Invariant Chain peptide), we were able to show that pH sensitivity of these regions is independent of peptide pocket occupation. Moreover, Histidine residues having a pKa between 4 and 7 are likely to translate pH changes into conformational changes and might thus trigger pH-dependent functional effect. Therefore we investigated a set of Histidine mutants by NMR spectroscopy and peptide loading assays in order to link pH-sensitive regions to a functional difference in peptide exchange.

P35-013
Dynamic interaction of the signal recognition particle receptor and the translocon

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Cotranslational targeting of membrane proteins to the endoplasmic reticulum of eukaryotes or the cytoplasmic membrane of bacteria is mediated by the signal recognition particle (SRP) pathway. This is an evolutionary conserved pathway in which SRP is recruited to ribosomes synthesizing membrane proteins and targets them to the insertion pore (SecYEG translocon) in the membrane guided by an interaction with the SRP receptor, FtsY in bacteria, which is associated with SecYEG. FtsY comprises two domains: the N-terminal A domain, which interacts with both the membrane and SecYEG, and the C-terminal NG domain which interacts with the homologous NG domain of SRP. The details of the targeting process at the membrane are poorly understood and the detailed role of FtsY is unclear.

We have studied the interaction of FtsY with SecYEG at equilibrium, utilizing fluorescence resonance energy transfer (FRET). We observe that unbound FtsY assumes a closed conformation in which NG and A domains are engaged in a strong intramolecular interaction, whereas the domains come apart upon binding of FtsY to SecYEG. We hypothesize that opening up the FtsY conformation facilitates subsequent steps of ribosome targeting to the translocon.

P35-014
Inhibition of human pancreatic Islet Amyloid Polypeptide aggregation and fibril formation by the molecular chaperone Hsc70

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Protein misfolding followed by aggregation and amyloid formation is an underlying pathological hallmark in a number of prevalent diseases, including Alzheimer's, Parkinson's, and Type 2 diabetes (T2D). Epidemiological studies reveal that up to 95% of all patients with T2D show pancreatic Islet Amyloid Polypeptide (IAPP) amyloid deposits, as detected in post-mortem studies. Most importantly, IAPP aggregation has been shown to be highly cytotoxic, to play a key role in the death of β-pancreatic cells, and to correlate with the severity of the disease. Thus, inhibition of IAPP aggregation is considered to be an attractive avenue for therapeutic intervention. In this respect, molecular chaperones, known to inhibit protein aggregation and promote proper folding of proteins, may be appropriate molecules for preventing amyloid formation in T2D and in amyloidosis in general.

In this work, the effect of the molecular chaperone Hsc70 and its various structural domains on IAPP aggregation has been investigated using several biophysical and biochemical approaches. The results indicate that Hsc70 is able to completely inhibit IAPP aggregation by binding preferentially to the monomeric form of IAPP thus preventing amyloid and fibrils formation. Moreover, the isolated C-terminal peptide-binding domain (residues 386–646) of the chaperone was necessary and sufficient for the inhibition IAPP aggregation. Further structure-function relationship studies suggested an inhibition mechanism similar to that involved in the chaperone activity of Hsc70 opening the way for the design of minimal chaperone structural units able to efficiently prevent aggregation and fibril formation in T2D in particular and amyloidosis in general.

P35-015
Cation/π interaction as the catalytic mechanism found in β-amyrin synthase

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β-Amyrin, a triterpene, is widely distributed in plants and its glycosides confer important biological activities (a sweetener, licorice). bβ-Amyrin synthase is one of oxidosqualene cyclases (OSCs). Mutagenesis studies on b-amyrin synthase are very limited. This study was conducted to elucidate the function of the highly conserved W257 and Y259 residues in *Euphorbia tirucalli* b-amyrin cyclase. Few reports describing the expression levels of OSC mutants are available. In order to assess the *in vivo* enzymatic activities, the quantities of the OSC protein expressed (Western blot analyses) and the triterpene products (GC quantification) accumulated in the host lanosterol-deficient yeast host must be estimated. To address the function of these aromatic residues, the side-directed mutants were constructed. The mutation of W257 into aliphatic amino acids such as Ala, Valine and Ileu decreased the enzyme activity and conferred significantly large amounts of lupeol. However, the Phe and Tyr variants showed relatively higher activity than the aliphatic amino acid mutants. Thus, W257 residue stabilizes the oleanyl cation intermediate via cation/p-interaction. Y259 residues were site-specifically mutated into some aliphatic amino acids, resulting in the significantly decreased activity for the formation of β-amyrin, but the Phe variant afforded the equivalent activity as the wild type, this result having also given the definitive evidence for the cation-π interaction as the catalytic mechanism. The aliphatic mutants conferred significantly increased amounts of tetracyclic products, derived from dammanrenyl cation, thus Y259 residues acts to stabilize the baccharenyl cation through caton/π-interaction. The detailed experiments results are presented.

P35-017
The influence of the cytoplasmic juxtamembrane regions on the structural and dynamical properties of HER2 dimeric transmembrane domains and their connection with the activation mechanism

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Receptor tyrosine kinases play critical role in regulating cell metabolism, growth and differentiation. Activation of the catalytic domain of ErbB family members is controlled primarily by an allosteric interaction between two protein kinase domains in an asymmetric dimer, rather than by phosphorylation. Several studies of their activation mechanism led to the conclusion that this process can be initiated by dimerization of transmembrane and juxtamembrane domains of these receptors. Understanding the principles, lying in the basis of intermolecular interactions of transmembrane and juxtamembrane regions in such receptors is essential for description of the activation mechanism.

Here we investigated the spatial structure and dynamics in membrane-like environment of the HER2 transmembrane domain homodimer in junction to the juxtamembrane region using solution NMR spectroscopy. Our data revealed parallel orientation of juxtamembrane regions in this homodimer that corresponds to inactive state of receptor. The transmembrane domains interact via non-standard hydrophobic motif near the N-terminal part of the domain and this interaction is supported by apolar contacts of bulky side chains. The data about dynamics of the objects obtained by measurement of the correlation time of each amino acid residue revealed that mobility of transmembrane and juxtamembrane regions is quite close, suggesting that the juxtamembrane region is inside the micelle. This conclusion confirmed by observed intermolecular NMR contacts between amino acid residues and lipid molecules in the micelle.

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P35-018
The cellular crowding effect: Spatial and temporal variations of the excluded volume effect

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The cell is a place with unique physicochemical properties. However, effects of the crowded cellular environment, which is filled up to 400 mg/ml with macromolecules, on protein behavior, dynamics and folding are often neglected. In order to mimic such effects, polymeric crowding agents are implemented and the excluded volume theory is used to describe the results. Yet, it is unclear whether cosolutes are able to mimic the complex proper-

ties of the intracellular environment as a general description of the in-cell crowding effect is missing. Utilizing a novel FRET-based sensor we are able to show that the in-cell crowding effect cannot solely be described by the theory of excluded volume. We investigate the subcellular distribution of cellular crowding effects by a combination of fluorescence microscopy and microinjection. Additionally, we investigate how compression effects in the cell change upon osmotic stress. To further probe temporal variations of cellular crowding, we inject the sensor at different stages of the cell cycle. For the first time, the presented method allows to systematically study changes of cellular crowding within a single cell, in response to different osmotic stress conditions or during the cell cycle. Our results will prove to be a useful correlate to understand the modulation of biomolecule function by the physicochemical properties of a cell.

P35-019
Mechanistic insights into the action of a bacterial protease inhibitor

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Proteases are essential enzymes in every organism, being involved in many fundamental biological processes from nutrition, tissue remodeling to virulence. Therefore, their activity must be tightly regulated to avoid non specific proteolysis or to defend cells against proteolytic attacks, a function that may be accomplished by protease inhibitors. Contrary to the *metazoans* genomes where protease inhibitors represent 1% of the genes, their appearance in unicellular and especially prokaryotic organisms is much less common, and only few of them have been described.

In our study we describe the mechanism of action of a multi-domain, 180 kDa, alpha-2-macroglobulin-like protease inhibitor encoded in *Escherichia coli* genome, by applying biochemical and structural techniques. We demonstrate that the protein is a target for proteases of diverse catalytic mechanism and specificity that cut in an unstructured bait region. This triggers a big conformational rearrangement in the molecule from a native to an induced form, which has been extensively studied. However, the inhibitor remains monomeric, contrarily to the tetrameric state of some mammalian a2Ms, and the entrapment of the protease is necessarily accomplished by covalent binding through a conserved and highly reactive thiolester bond to a surface lysine of the protease. As a consequence, the protease becomes sterically hindered to reach globular substrates of high molecular weight, so its proteolytic activity is inhibited. Taking into account the periplasmatic localization of the inhibitor, we hypothesize that it is acting as an *E. coli* defense mechanism against invading proteases that may damage cell wall components.

P35-020
Structural characterization of intrinsically disordered protein phosducin and its complex with the 14-3-3 protein

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Phosducin (Pdc) is a conserved phosphoprotein which regulates the transduction of visual signal through interaction with the complex of β- and γ-subunits of the retinal G-protein transducin ($G_t\beta\gamma$). This interaction blocks the re-association with the α-subunit and it is controlled in a phosphorylation dependent manner at Ser54 and Ser73 of Pdc. This phosphorylation alone has only a weak effect on Pdc binding to $G_t\beta\gamma$ and the efficient inhibition of this interaction requires the 14-3-3 protein which associates with the phosphorylated Pdc and blocks its interaction with $G_t\beta\gamma$. The 14-3-3 proteins are conserved family of dimeric molecules that regulate the function of other proteins through a number of different mechanisms including modulation of structures or masking binding sites. However, the mechanism and structural function of 14-3-3 protein in the inactivation of Pdc is largely unclear.

Here we present evidence that the 14-3-3 protein sterically occludes both N- and C-terminal $G_t\beta\gamma$ binding interfaces of Pdc providing the mechanistic explanation for the 14-3-3 depedent inhibition of Pdc function. N-terminal domain of Pdc which includes both phosphorylation sites also remains in flexible state even if bound to 14-3-3 indicating transient character of this complex corresponding to apparent K_d in micromolar range as shown in previous report[1].

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P35-021
Structural study of Whirlin, a crucial PDZ containing protein involved in the mechanotransduction of auditory hair cells

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Mammals perceive sound thanks to mechanosensory hair cells in the inner ear. The eardrum produces vibrations that displace the hair cell cilia, bound together by a tight network of cadherins and scaffolding proteins. Stretching of the network is directly responsible for the opening of
an ion channel that translates vibration into electric signals transmissible to the brain. Nearly all proteins involved in this cilia-associated network contain short C-terminal motifs of interaction with PDZ domains. Two proteins of the cilia-associated network encompass PDZ domains: Harmonin and Whirlin. Both of them are multi-domain proteins including three PDZ domains. With tens of potential partners in hair cells, these two proteins most likely have a central role in connecting the extracellular protein links and the cytoskeleton. However their molecular organization and interactome have been only partially described. We focus our work on several aspect of Whirlin. The N-terminal part of the protein encompasses two PDZ domains and two HHD domains (Harmonin Homology Domain). By homology to related systems, we suspect that HHD and PDZ domain can interact. Using sequence alignment, we identified the second domain HHD downstream to Whirlin second PDZ domain, creating a symmetric organization: HHD1-PDZ1-PDZ2-HHD2. We are investigating the interplay potentially occurring between those four domains and are in the process of determining inter- and intramolecular interactions. We also document the network of interaction of Whirlin in the inner ear, and more generally of all PDZ ligand motifs present in the ear by using a new high throughput method.

P35-022
Experimental and theoretical methods as a tool for the interpretation of lysozyme immobilization at a silica surface

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Lysozyme has a very important function in the immune system, because it exhibits strong antibacterial activity against gram-positive bacteria. This property has found practical applications in the medicinal and pharmaceutical industries. For this reason, the understanding of how the protein interacts with inorganic material surfaces is of major interest in both fundamental research and applications such as biotechnology. However, despite intense studies, the mechanism and the structural determinants of the protein/surface interactions are still not fully understood.

The adsorption of lysozyme (LSZ) at a hydrophilic silica surface has been chosen as a model system. We have analysed the LYZ adsorption using Quartz Crystal Microbalance with Dissipation (QCM-D) and Multi-Parametric Surface Plasmon Resonance (MP-SPR) methods. Combinations of these complementary techniques have provided crucial information on the mechanisms behind the protein-material interactions, LSZ structural changes and biomolecular rearrangements. We have found that the pH strongly affects the effectiveness of LSZ adsorption onto the surface. The highest adsorption value was attained near the protein's Iso-electric Point. Furthermore, the data clearly indicate that electrostatic interactions are a driving force for LSZ adsorption.

Molecular Dynamics (MD) simulations suggest that LSZ adsorbs at silica surfaces using the Arg and Lys residues from the N, C-terminal face. From the MD results, and their good agreement with our experimental data, the nature of the protein-surface interactions can be elucidated.

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P35-023
Structural Insights into a novel esterase

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Chloramphenicol (Cm) and florfenicol (Ff) are broad-spectrum antibiotics that inhibit protein synthesis. Both antibiotics are a bacteriostat but differ in their chemical structure in that a fluoro group is attached to C3 of Ff. Specifically, both antibiotics prevent a formation of the peptide bond by irreversibly binding to a receptor site on the 50S subunit of the bacterial ribosome. It is well established that chloramphenicol acetyltransferase inactivates Cm by first recognizing the hydroxyl group of C3 in Cm and then specifically acetylating the hydroxyl group. Due to this specificity, Ff cannot serve as a substrate of chloramphenicol acetyltransferase. Interestingly, a novel esterase, which was recently characterized by metagenome screening, inactivates both antibiotics possibly by hydrolysis of those antibiotics, suggesting that this esterase could be a novel enzyme in inactivating both Cm and Ff. Sequence analysis indicates that its sequence is highly similar to those of microbial hormone senstive lipase. Consistent with this comparison, a newly identified esterase contains Ser156 in the GxSxG motif and Asp252 and His282, the catalytic triad conserved in the family of microbial hormone senstive lipase. In order to understand this novel enzymatic feature, we are carrying out X-ray crystallographic analysis. Our studies will provide structural insights into antibiotics hydrolysis.

P35-024
Molecular dynamics of *Mycobacterium tuberculosis* tyrosyl-tRNA synthetase with different substrates in the active site

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Tyrosyl-tRNA synthetase from *M. tuberculosis* (*Mt*TyrRS) is an enzyme that catalyzes the attachment of tyrosine to its cognate $tRNA^{Tyr}$ at the preribosomal step of protein synthesis. *Mt*TyrRS is incapable of cross-recognition and aminoacylation of human cytoplasmic $tRNA^{Tyr}$, so this enzyme is a promising target for development of novel selective inhibitors as new antituberculosis drugs. In this study, we have investigated the mechanisms of substrates interaction with the *Mt*TyrRS active site. The data of dynamic binding of substrates at the active center are important to design new enzyme inhibitors.

Complexes of *Mt*TyrRS with tyrosine, ATP and tyrosyl-adenylate were constructed by superposition of the *Mt*TyrRS structure and crystallographic structures of bacterial TyrRS. All complexes of *Mt*TyrRS with substrates were investigated by all-atom molecular dynamics simulations using the GROMACS 4.5 package with the Amber ff99SB-ILDN force field. The simulations were run for 100 ns each, at physiological conditions. All MD simulations were calculated using the MolDynGrid virtual laboratory services (http://moldyngrid.org).

It was shown the formation of network of hydrogen bonds between substrates and the *Mt*TyrRS active center, which were stable in the course of MD simulations. ATP binds in the active site both by hydrogen bonds and via electrostatic interactions with Lys231 and Lys234 of catalytic KFGKS motif. The L-tyrosine binding site in the enzyme active site is negatively charged, whereas the ATP binding site contains positive Lys231 and Lys234 residues of catalytic KFGKS motif. The occupancy of H-bonds between substrates and the enzyme evidences a significant conformational mobility of the active site.

P35-025
Generation and application of high-productive diagnostic system for detection of serum level of interferon-α

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Interferon alpha (IFN-α) is the cytokine widely used in clinic for the treatment of viral diseases. Determination of IFN-a concentration in biological liquid is very important diagnostic parameter.

In addition to affinity chromatography, Protein A *Staphylococcus aureus* (SPA) affinity-immobilized on the cellulose support via cellulose-binding domain from Clostridium thermocellum (CBD) can be used for immunodetection. Crystalline cellulose CC31 CBD-SPA micro column was used for the determination

of small amounts of antigen (human interferon-α2b (IFN- α2b)) from high dilute solutions through its accumulation on the column. On cellulose-CBD-SPA micro column were oriented immobilized specific to IFN-α2b polyclonal rabbit antibody. After that, solution contained IFN- α2b was applied to the column. Definition of immune complex was carried out with specific anti-IFN-α2b single-chain recombinant antibody (scFv), conjugated with bacterial alkaline phosphatase with enhanced catalytic properties (BAPmut). ScFv genetically fused with BAPmut are an attractive alternative to the chemical conjugates with full- length monoclonal antibodies.

The genes of fusion proteins CBD-SPA and scFv-BAPmut were designed and expressed in *E. coli* in it soluble, biologically active form. Purification and immobilization of SPA-CBD were essentially one step, thus significantly reducing the cost of production.

The major advancement of such diagnostic system consists in strongly oriented immobilization of all functional components. The combination of phage antibody, gene engineering technologies, efficient expression systems and optimal immobilization methods provides a productive diagnostics system for the detection of ng amount of target cytokines.

P35-026
Revealing adsorption mechanism of human fibrinogen on positively charge latex

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The adsorption of proteins to a charged surface is an essential aspect of the cascade of biological reactions, which take place at the surface between a synthetic material and the biological environment. One of the most important proteins is human plasma fibrinogen. This protein plays an important role in clotting and the development of the surface-induced leukocyte binding, tumor growth, fouling of artificial organs.

Fibrinogen adsorption on positively charged latex particles was studied using the microelectrophoretic and concentration depletion method based on AFM imaging. Measurements were carried out for pH 7.4 and ionic strength in the range of 10^{-3}–0.15 M NaCl. The results of these experiments were interpreted according to the three-diamensional electokinetic model. It was also determined using the concentration depletion method that fibrinogen adsorption was irreversible and the maximum coverage was equal to 0.6 mg m^{-2} for ionic strength 10^{-3} M and 1.3 mg m^{-2} for ionic strength 0.15 M. The increase of the maximum coverage was confirmed by theoretical modeling based on the random sequential adsorption approach.

These experimental results were interpreted in terms of the side-on adsorption mechanism of fibrinogen whose negatively charged core part faced the positively charged latex surface.

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P35-027
Secondary structure and calcium binding properties of C1q-like domain of otolin-1

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Otolin-1 is a short collagen-like protein from C1q superfamily. It was identified in organic matrix of fish otoliths and mammalian otoconia, which are calcium carbonate biominerals responsible for reception of linear acceleration. Mechanism of biomineralization of otolith and otoconia is yet not fully understood, but it has been shown that it is controlled by proteins.

Otolin-1 contains a collagen-like domain and a globular C1q-like domain. Similar to collagen I in bone, collagenous tail of otolin-1 provides a fibrous scaffold for synthesis of otoliths and otoconia. C1q-like domains of other proteins from C1q superfamily, which include serum C1q complex, adiponectin and collagen X, form oligomers, bind calcium ions and interact with wide variety of macromolecular ligands. High sequence similarity suggests that C1q-like domain of otolin-1 could have similar properties.

In this study, secondary structure and calcium binding properties of recombinant C1q-like domain of otolin-1 from human and zebrafish were investigated. We have shown that these domains exist as trimers in solution and that they contain high percentage of β-strands and disordered structures. We have also shown that C1q-like domain of otolin-1 binds calcium ions. These results support the idea that otolin-1 plays a major role in process of biomineralization of fish otoliths and human otoconia.

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P35-028
3D-structure and dynamics of cobra cardiotoxins: NMR and MD analyses

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Three-finger toxins (TFT) share a common spatial organization of the three beta-structural loops emerging from a global core, which is stabilized by four disulphide bonds. TFT are capable to interact specifically with receptors, e.g. neuronal nicotinic one. Another group, to which cardiotoxins (or cytotoxins, CTs) belong, does not have a protein target. Instead, CTs possess membrane-active properties, eliciting toxic effect in a panel of living cells. At present, these toxins are considered as promising cytotoxic agents in treatment of various malignancies. In this work, for the first time, we obtained totally ^{15}N,^{13}C- labeled cytotoxin I (CTI, 60 residue-long) from *N. oxiana* cobra. Production of ^{13}C,^{15}N-CTI was performed via bacterial expression. We have obtained nearly complete resonance assignment of the toxin signals and determined its spatial organization. These data were compared to those obtained for wild-type CTI (wt-CTI), purified from cobra venom and lacking the N-terminal Met-residue, which the recombinant ^{13}C,^{15}N-CTI has. This allowed us to fulfill error-free resonance assignment for wt-CTI, obtained at natural abundance of ^{13}C,^{15}N-nuclei and improve quality of the 3D-structure of this toxin. To evaluate dynamical properties of wt-CTI molecule, its MD-simulations were started from the refined structure, using several modern force fields. The 1-microsecond-long trajectories were certified via comparison of the experimental and averaged over the trajectories computed chemical shifts. The combination of the NMR and MD-data obtained on CTI can be used for evaluation of the 3D-structure and dynamics of other cardiotoxins.

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P35-029
Functional domains of lamin B receptor: Structure, dynamics and interactions

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Lamin B Receptor (LBR) is a ubiquitous integral protein of the nuclear envelope known to participate in a variety of nuclear functions, including tethering of the nuclear lamina to the inner nuclear membrane and "transient trapping" of nuclear components that are involved in chromatin remodeling and transcriptional inactivation. The nucleoplasm-facing amino-terminal part of the protein mediates most of LBR′s interactions. It harbors a well-folded 60-residue Tudor domain (TD) followed by a 40-residue region rich in Arg-Ser repeats (RS region), punctuated by multiple phosphorylation sites and a typical IDP and a 110-amino acid segment with no homologues. TD is well folded and crucial, but not sufficient for LBR function. On the contrary, RS is essential for most of LBR interactions. However, its conformational and functional properties are most likely modulated by the type and the extent of post-translational modifications. Here, we present the results of an *in vitro* and *in vivo* study of LBR regions addressing the effect of physiologically relevant post-translational modifications and inter-domain interactions on LBR structure, function and dynamics.

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P35-030
Studying allosteric transitions of the pentameric ligand-gated ion channel GLIC using site-directed fluorescence

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Pentameric ligand-gated ion channel are membrane proteins located at the post-synaptic membrane of neuronal and neuromuscular junctions. They are responsible for the transduction of a chemical message, the binding of neurotransmitters, into an electrical signal at the membrane through their channel opening. Their function can be simply described by a minimal three state allosteric model comprising of a resting-closed state, an active-open state and a desensitized-closed state. Recent crystallographic data have provided us with high-resolution structural information on several of these allosteric states and intermediates. As it is well known that X-ray crystallography provides static snap-shots of proteins extracted from their physiological membranes and trapped in a crystal, important questions remain. To what extend do these structures represent actual states existing at the membrane? And what are the molecular events leading to the transition from one state to another?

We present here the use of a fluorescent approach to study allosteric transitions of unconstrained receptors in multiple environments and with minimal structural perturbation.

We used the monobromobimane, a small fluorophore sensitive to its microenvironment, as a sensor of conformational changes in GLIC (Gloeobacter violaceus), a prokaryotic homologue of the family. Through extensive labelling targeting different regions of the protein, we collected original steady-state and real-time data on the structural reorganisations of GLIC in response to agonist applications.

P35-031
Mass spectrometry contribution to NMR protein structure characterization

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In protein chemistry the nuclear magnetic resonance is a powerful tool for defining the structure, dynamics or molecular interactions at atomic level. We used this method to design the 3D structural model of the extracellular domain of the type I C-type lectin like receptor CD302 (DCL-1). The recombinant protein was produced into inclusion bodies and refolded under non/reducing conditions with the redox system of cysteamine/cystamine. After one step purification, the protein was suitable for mass spectrometry (MS) analysis and NMR measurements (when ^{13}C, ^{15}N labeled).

The MS analysis confirmed the completely formed disulfide bonds and solved the arrangement. The order of disulfides is in agreement with the common C-type lectin fold. This information was important due to the later structure calculations from the NMR data from which the disulfide bonds could not be easily defined. The MS analysis was also used to obtain distance constraints using chemical cross-linking experiments.

The sequence-specific resonance assignment of the protein backbone was accomplished with the aid of 3D HNCO/HNcaCO, HNCA/HNcoCA and HNCACB/CBCAcoNH spectra of the uniformly $^{13}C/^{15}N$-labeled protein. Further backbone and side-chain resonance assignments were obtained using HcccoNH, hCCcoNH, hbCBcgcdHD and HCCH/TOCSY 3D experiments. Remaining resonances (unto 98% of possible) were obtained using NOESY spectra acquired for the aliphatic/aromatic ^{13}C and ^{15}N. Structural models calculated in ARIA/CNS considering the disulfide bonds and torsion angles (predicted in TALOS+) with or without inclusion of the MS derived restraints will be presented.

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P35-032
Stabilization of one domain of protein Gao by introduction of a cysteine bridge

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In this study we used an approach that allows determining in what region of the polypeptide chain of protein it is required to design a disulfide bond in order to stabilize it. In our previous paper [Melnik TN at al., *Biochemistry*. 2011] it was proposed that to search for a "weak" site in the protein, it is possible to use programs which find natively unfolded protein regions. We suggested that in structured globular proteins such programs predict not protein regions in the polypeptide chain unfolded under

native conditions, but "weakened", feebly stabilized ones. Accordingly, an artificial introduction of ss-bridges using mutations in such regions would reliably result in the protein stabilization. We have taken advantage of this approach to stabilize protein Gαo. A comparison of proteins from different organisms shows that they differ mainly in the helical domain. It was namely this domain that we decided to stabilize by introducing an SS-bridge. To this end, we determined the "weakened" region in the polypeptide chain using programs for prediction of natively unfolded regions, chose in this region such amino acids which can form an ss-bridge when substituted for cysteines, introduced a corresponding mutation, and isolated and studied the melting of the mutant protein and the wild-type protein using the microcalorimetry. The designed SS-bridge increased by 4 degrees the melting temperature of one domain of protein Gαo.

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P35-033
Importance of salt bridges in the dimer interface of *Tpv* sHSP14.3 for oligomere assembly and chaperone function

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Small heat shock proteins (sHSPs) are virtually ubiquitous stress proteins that generally act as ATP-independent chaperones to bind denaturing proteins and supress their aggregation and precipitation. sHSPs are defined by a conserved α-crystallin domain (ACD) of about 90 amino acids (which is composed of nine β-strands arranged into two anti-parallel β-sheets). The ACD is necessary for dimerization and together with the nonconserved N-terminal arm and a short C-terminal extension it is also critical for the higher-order oligomerization. The hierarchical assembly of sHSPs (monomer 12–42 kDa) into large (≥12 subunits) polydisperse oligomers is extremly dynamic and linked to their effective chaperone action. In this study, we have investigated the roles of two Arg residues, *i.e.* R69 and R81 located on the β-5 and β-6 zone of the ACD in the quarternary assembly and chaperone function of the archaeal *tpv* HSP14.3 from thermoacidophilic archeon *Thermoplasma volcanium* GSS1. Substitutions by equivalent and chemically different amino acids indicated the functional and structural importance of the ionic interactions that they participate in. Alterations at these positions of tpv HSP14.3 resulted in significant structural perturbations that was implicated by the altered monomer stability, change in distribution profile of the oligomeric intermediates and decreased or incerased substrate binding efficiencies.

P35-034
Molecular dynamics simulations of peptides containing charged aminoacid-repeats derived from intrinsically disordered protein sequences

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It is now well established that conformational flexibility, known as intrinsic disorder (ID), is much more common in biologically active proteins than previously believed. Intrinsically disordered protein regions (IDRs) and even full length intrinsically disordered proteins (IDPs), have been found in all species, are involved in many important biological functions and several are linked to major diseases. IDRs are characterized by low complexity in their aminoacid composition and exert their function by mediating protein-protein interactions often modulated by post translational modifications (PTMs) of their aminoacids.

We present here results from a large number of molecular dynamics (MD) simulations on several peptides of various lengths, containing repeats of charged aminoacids, such as polyglutamate (polyE) stretches and glutamate/arginine (ER) repeats, derived from the sequences of human IDPs. We address questions related to the conformation of such protein regions, which are valuable as a starting point towards the elucidation of the structural consequences of PTMs on aminoacid repeats of this type.

MD simulations were performed using GROMACS-4.6.3. Multiple, independent MD replicas were carried out for the polyE-containing peptides (total simulation time per peptide ranging from ~6 to ~12 μs) using implicit solvation and infinite cut-offs for non-bonded interactions, whereas explicit solvation was employed in the case of the ER-rich peptides. Multiple MD replicas for each peptide were combined and analyzed using GROMACS analysis tools and bash shell scripts we developed for this purpose.

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P35-035
Conformational dynamics of GW182 silencing domain and CNOT1 fragment as monitored by hydrogen-deuterium exchange mass spectrometry

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GW182 is one of the core components of the miRNA-induced silencing complex. It recruits the CCR4-NOT deadenylase complex to the targeted mRNA *via* interactions with CNOT1, the scaffolding subunit of the CCR4-NOT complex. Here we study the conformational dynamics of these proteins by hydrogen-deuterium exchange mass spectrometry. Two newly identified regions of the GW182 silencing domain showing evidence of local structure will be studied in more detail using spectroscopic methods. On the other hand, conformational changes of CNOT1 are studied in the presence of GW182-derived peptides.

P35-036
The LINK to regulating lysine levels in wheat

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Triticum aestivum, or bread wheat, is an important agricultural crop that plays an extensive role in the global food supply.

Wheat, amongst other cereal crops, contains low amounts of the amino acid lysine, which is essential in the mammalian diet. This limits the nutritional value of cereal crops as food sources, and consequentially significant interest revolves around the development of cereal crops with increased lysine content, including wheat. Plants are capable of *de novo* lysine biosynthesis by utilising the diaminopimelate (DAP) pathway. Lysine biosynthesis is primarily controlled by dihydrodipicolinate synthase (DHDPS), which catalyses the first committed and rate-limiting step in the DAP pathway. DHDPS is allosterically inhibited by lysine in a classical feedback manner, however, the allosteric mechanism at the molecular level is poorly understood.

The aim of our research is to investigate the lysine-induced inhibition of wheat DHDPS, and ultimately to define the molecular determinants of allosteric inhibition. In order to meet this aim, our study endeavors to express and purify recombinant *T. aestivum* DHDPS, to kinetically characterise the recombinant enzyme and to characterise the structure of *T. aestivum* DHDPS both in solution and the crystalline state. The results of this study to date demonstrate that *T. aestivum* DHDPS exists as a catalytically active tetramer in the unliganded form, but dissociates to an inactive dimer in the presence of lysine. This presents as a new allosteric mechanism, coined the *Ligand Induced dissociatioN by lysine (K)* or LINK model.

P35-037
The structural basis of the TIP49a/b dodecamerization

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Essential eukaryotic AAA+ ATPases TIP49a/b have been identified as members of several macromolecular assemblies involved in chromatin remodeling, telomerase assembly and snoRNP biogenesis. These proteins play an important role in a broad variety of vital cellular activities and are implicated in cancerogenesis.

TIP49a/b are structurally organized as three-domain proteins, which form classical AAA+ hexamers via the D1/D3 domains oligomerization. The highly flexible OB-fold-containing D2 insertion domain allosterically inhibits the proteins' ATPase activity and appears to be involved in dodecamerization of the TIP49a/b homo- and heterohexamers. Based on the dodecamer framework provided by the partially resolved full-length TIP49a/b heterohexamer X-ray structures* from *C.thermophilum*, we investigated the atomic details of the dodecameric structures that are mediated by the D2/D2 inter-ring interactions. Using molecular modeling approaches, we reconstructed the full-atom model of mixed human TIP49a/b dodecamers and analyzed the D2/D2 dodecamerization interface at the atomic level. Our analysis reveals that the "sticky" β-hairpin (Leu138-Ile159, within the OB-fold of TIP49a) from one heterohexamer is likely to form the H-bond network with the corresponding "sticky" β-hairpin (Gly159-Asp173) from the opposite TIP49b protomer in the second heterohexamer. Our MD simulations of the dodecameric TIP49a/b complex in a water environment confirmed conformational stability of these structural signatures, which may serve as the key elements for interactions of TIP49 oligomers with their target proteins *in vivo*.

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P35-038
Isolation of 10 kDa and 24 kDa fragments of fibrinogen αC-region and usage them as antigens for antibodies production to design test systems for soluble fibrin quantification

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Diagnostics of the threat of intravascular thrombus formation is a very important task in our days. The aim of the work was to develop a new isolation method of 10 kDa and 24 kDa fragments of αC-region of fibrinogen and use them as antigens for monoclonal antibodies obtaining and designing a test system for soluble fibrin quantification.

Fibrinogen was diluted in 0.05 M Tris-HCl pH 7.4 buffer, 0.2 M NaCl in concentration 10 mg/ml. Plasmin in concentration of 0.03 CU/mg of fibrinogen was added. Incubation was carried out at 37°C for 30 min then 0.1 M ε-aminocaproic acid and aprotinin 250 IU/ml hydrolyzate were added. The probes were filtered with sterile syringe filter and injected in Agilent 1100 HPLC system with the usage of a guard column and two Zorbax GF-250 columns connected in series, 0.01 M KH_2PO_4 pH 7.3 buffer, 0.14 M NaCl, 5% methanol. Fraction of 10 kDa fragment was concentrated by method TCA-DOC precipitation with acetone wash (G. Peterson, 1977). Fraction of 24 kDa was concentrated on 10 kDa molecular weight cut-off centrifugal concentrators. Both fragments were analyzed by Tricine-SDS-PAGE (H. Schagger, 2006) in 10% acrylamide/bis-acrylamide gel. The yield of electrophoretically pure 10 kDa fragment was 70% and 24 kDa – 86%. The proposed method of single-step purification can be used as antigen-preparation procedure for the development of the test system for soluble fibrin quantification.

P35-039
The effects of α-tropomyosin Arg245Gly and Glu241Leu mutants on the structural states of actomyosin during the ATPase cycle

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To investigate tropomyosin-dependent mechanisms of the actin filament activation we made two substitutions in skeletal α-tropomyosin (TM): Arg245Gly and Glu241Leu. In humans the substitutions are known to cause congenital myopathies. Using polarized fluorimetry we have studied the effects of the mutations on position of TM along filament and the spatial arrangement of actin monomers and myosin heads at various mimicked stages of the ATPase cycle in the absence of troponin. Actin, TM, and myosin subfragment-1 (S1) were fluorescently labeled and incorporated in ghost muscle fibers. The changes in polarized fluorescence during simulated stages of the ATPase cycle were measured. In the absence of S1 the mutations in TM were found to cause a movement of the TM strands towards the blocked

position switching off the filament. A multi-step shift of the wild-type TM towards the actin filament centre accompanied by an increase in the number of actin subunits switched into the "on" state, as well as the number of S1 in the strong-binding state was observed. At all stages of the ATPase cycle the Arg245Gly and Glu241Leu mutations captured TM strands near the blocked position, decreased the number of actin monomers in the "on" state, but increased the number of S1 in the strong-binding state. We concluded that mutations-associated changes in TM structure interfere with the cross-bridge cycle by slowing down the rate of S1 release from actin. This mechanism may be responsible for the muscle weakness observed in patients.

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P35-040
Interactions of Banana Lectin with Man9, toward design of the enhanced HIIV-1 entry inhibitors – *in silico* study

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The molecular basis for the use of lectins as anti-HIV agents is their ability to target multiple glycosylation sites on the virus envelope. A jacalin-related lectin isolated from the fruit of bananas (*Musa acuminata*) BanLec is a potent inhibitor of HIV replication in the low nanomolar range. Recombinantly produced BanLec revealed the same glycan specificity as the natural BanLec, and is useful model protein for design novel therapeutics. Griffithsin (GRFT) isolated from extracts of red alga *Griffithsia* sp. exerts antiviral activity against HIV-1 isolates, with EC_{50} in picomolar range. We compare mannose binding moieties of BanLec and Griffithsin by using crystal structures of Griffithsin monomer (mGRFT) bound Man9 (PDB 3LL2), mannose bound BanLec (PDB 2BMZ) and the same protein without bound carbohydrates (PDB 2BMY). BanLec appeared very similar to mGRFT, having distance between carbohydrate-binding sites (CBS) on a monomer, of about 15 Å. Man9 was docked to 2BMY and the binding mode of nanomannoside was compared with 3LL2 crystal structure. In the docked structure, termini of D1 and D3 branches occupied CBSs in 2BMY. While Tyr's 28, 68 and 110 can enhance binding affinity of nanomanoside to mGRFT out of CBSs region, in BanLec binding of Man9 is probably enhanced by Tyr83 and His84 of Asn82-Val87 loop that protrude above loops that consist of CBSs. Stability of *in silico* built Man9-BanLec complex is further evaluated by 20 ns molecular dynamics simulations using CHARMM force field, and the similarity of CBSs of two proteins was compared by GRID molecular-interaction fields.

P35-041
Understanding the catalytic mechanism of Human serum paraoxonase 1-Combined mutagenesis and Molecular dynamics study

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Human serum paraoxonase 1 (hPON1) is a multi-faceted enzyme that acts on a wide range of substrates including pro-atherogenic and pro-inflammatory molecules and neurotoxic organophosphorous compounds. The crystal structure of hPON1 is not known yet. On the basis of the crystallographic structural studies of chimeric PON1, it was proposed that active site H115 residue and polymorphic Q/R192 residues are involved in the catalytic activities and H115 substitution to any amino acid abolishes its native lactonase activity. Recent reports suggest that enzyme shows its lactonase activity in the absence of H115 residue when amino acid at 192 position changes indicating the involvement of other residue(s) in the catalytic activity. As 192 position which resides at entrance of active site, is involved in controlling the substrate specificity of the enzyme but molecular details are not known. In order to understand the role of 192 position, saturation mutagenesis at 192 position was done. The protein variants were expressed, purified and their enzymatic activities were compared. The variants exhibit significantly different enzymatic activity towards different substrate, which depend on the nature of the amino acid at 192 position as well as type of substrate. Molecular dynamic simulation studies showed that the hydrogen bonding interactions of 192 position with neighbouring residues affects the conformation of inner active site residues which control lactone catalysis in the variants. Our results suggest that proposed catalytic residues are not always needed for the activity of hPON1 indicating a reconsideration of the current model(s) of the catalytic mechanism of enzyme.

P35-042
Fusion of purple membranes with lipidic cubic phase

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Crystallization from native membranes in lipidic cubic phase (LCP) is relatively recently appeared method of crystallization[1] and allows to significantly simplify procedure of crystallization as well as to increase the yield of highly ordered crystals.

The aim of our work was to develop a method of crystallization *in meso*. We studied the mechanism of fusion of the native carrier of membrane protein with lipidic cubic phase. We used a model protein bacteriorhodopsin in purple membranes.

In this paper we report the results of two types of experiments on epifluorescence microscope using FRAP method[2] on Formulatrix FRAP tool.: membrane fusion by free diffusion, and membrane fusion as a result of mechanical extrusion. In each of types of experiments we measured diffusion parameters (characteristic time and quantity of injected protein).

It is shown that quantity of mobile fraction in lipidic cubic phase is strongly depends on protein concentration.

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P35-043
Identification and functional significance of DNAJA1 as a novel interacting partner of human transglutaminase 2

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Human transglutaminase 2 (TGM2) is a multifunctional protein crosslinking enzyme which has a large number of interacting partners contributing to its diverse biological and pathological functions. Our recent studies have aimed to identify novel interacting partners of TGM2 and explore their functional significance. We employed GST pull down assays and subsequent mass spectrometry analysis in NB4 cells and found that heat shock protein (HSP 40)/DNAJA1 binds TG2 in addition to some already known interacting partners. Since DNAJA1 and human TGM2 have been reported to be involved in various and somewhat similar physiological processes we chose DNAJA1 as one of the candidate proteins for functional analysis. We performed interaction ELISA, Biacore experiments, native gel electrophoreses and co-immuno-staining studies with TGM2 overexpressing HEK cells and confirmed that TGM2 and DNAJA1 interact with each other and they co-localize in the cytoplasm. We also used TGM2 domain mutants to determine the domains which binds DNAJA1: ELISA and Biacore experiments showed that core domain of TGM2 is the most important one in this interaction. We also established via amine incorporation experiment that DNAJA1 is a glutamine donor substrate of TGM2. The effect of DNAJA1 on TGM2 activity was also explored and the results suggest that DNAJA1 increases the crosslinking activity of TGM2. We have performed *in situ* activity measurement experiments to see this effect of DNAJA1 on TGM2 activity using DNAJA1 down-regulated HEK cells. The role of TGM2-DNAJA1 interaction on cell adhesion and migration processes is also being explored.

P35-044
Cysteine-depleted ghrelin receptor: a tool for ligand-binding investigations

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Ghrelin and its G-protein coupled receptor GHS-R1a are involved in energy homeostasis promoting increased food intake. With approximately 50 % of the maximal ligand-induced capacity, the ghrelin receptor possesses a high constitutive activity, which might be a target for new and efficient pharmaceuticals.

We aimed at generating a functional and cysteine-depleted receptor variant as a molecular tool to investigate receptor-ligand interactions *in vitro*, for instance *substituted cysteine accessibility method* (SCAM) to investigate ligand binding sites or molecular switches of activity after re-introduction of selective cysteine residues.

Single mutations for eight of the ten cysteines were replaced by isosteric exchange to alanine, serine or valine, respectively. Earlier studies highlighted the importance of the two remaining cysteines for a conserved disulfide bond. The activity was assessed by inositol-phosphate accumulation assays in response to agonist as well as inverse agonist. Ligand potency and efficacy was not significantly changed as compared to the wild type receptor. However, a reduction of the basal activity was observed in the majority of the receptor variants suggesting an indirect effect of the cysteines on the constitutive activity. Finally, we combined the single mutants to an 8x-cysteine-depleted GHS-R1a. Although this variant revealed a distinct loss of basal activity, it still shows an activation and inactivation profile that is comparable to the wild type receptor.

We now have a functional, cysteine-depleted GHS-R1a in hand which might serve as a tool for diverse biotechnological approaches.

P35-045
TIP49a protein forms active rod-like structures in solution

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The ubiquitous TIP49ab proteins belong to AAA+ ATPase superfamily and have been associated with a wide variety of essential cellular processes, including chromatin remodelling, snoRNP biogenesis, DNA damage repair, telomerase assembly and implicated in cancerogenesis. Similar to other AAA+ proteins they form ring homo-, heterohexameric and dodecameric structures as observed by protein crystallography and electron microscopy. However correlation between structure and functions of the TIP49 proteins is still unclear.

The aim of our study* was to elucidate the structures formed by the active TIP49a protein *in vitro* system similar to physiological conditions. We also studied the TIP49a mutant Y366A with expected increase in flexibility of interprotein interface area. We have established reaction conditions that stimulate TIP49a DNA-dependent ATPase activity by several times using biochemical methods. As indicated by Small-Angle Neutron Scattering (SANS) in widely used *in vitro* systems TIP49a and its mutant Y366A remain substantially aggregated and possess low ATPase activity. In contrast in the established reaction conditions TIP49a proteins were found to form ordered rod-shaped structures that could be stocks of protein hexamer rings or filament-like structures. These results indicate that the observed transition between the two conformational states is associated with change of TIP49a ATPase activity and improve our understanding of TIP49a intracellular functioning.

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P35-046
Human fibrinogen monolayers under aqueous conditions

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Fibrinogen (Fb) is one of the most abundant blood plasma protein. It plays an essential role in thrombosis, angiogenesis, fouling of artificial organs, and so forth. Therefore, not only the amount of adsorbed fibrinogen affects the processes, but also their arrangement. In our work we focused on fibrinogen adsorption on negatively charged colloidal particles using the microelectrophoretic method, and the concentration depletion method combined with AFM. The experimental data were compared with

theoretical simulations assuming a 3D adsorption of fibrinogen. It was proven that depending on pH and ionic strength, the fibrinogen molecule assumes various conformations: the expanded conformation at pH below 5.8, and the semicollapsed conformation at pH 7.4. The charge distribution over the fibrinogen molecule at pH 7.4 becomes heterogeneous and it is responsible for adsorption at pH above 5.8 on negatively charged substrates. The coverage of adsorbed fibrinogen is 3–3.5 mg/m^2 for pH 7.4 and 3.5, respectively. Additionally, a smaller amount of fibrinogen is adsorbed reversibly, depending on the bulk concentration. This produces the maximum coverage of fibrinogen equal to 5 mg/m^2 for pH 7.4 and bulk concentration about 100 mg/l. For lower pHs fibrinogen adsorbs mostly in the end-on orientation both at solid surfaces and colloid particles. On the other hand, for pH of 7.4 the most probable seems the side-on mechanism. The heterogeneous charge distribution and the end-on orientation of fibrinogen molecules promotes an irreversible immobilization of negatively charged colloid and larger micro particles on negatively charged substrates.

P35-047
Active site dynamics of flavin-dependent methylases

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Enzymatic methylation of uridyl to form (ribo)thymidyl occurs during the metabolism of DNA and RNA in all organisms. Different pathways exist, implicating thymidylate synthase ThyA that forms the essential DNA precursor thymidylate by methylating deoxyuridine monophosphate (dUMP), and the S-adenosyl-l-methionine-dependent methyltransferase TrmA, which catalyzes the formation of 5-methyluridine at position 54 of tRNA. Recently, two novel folate/flavin-dependent methylases, thymidylate synthase ThyX and tRNA methyltransferase TrmFO, were discovered. Both enzymes use CH_2-H_4folate as carbon donor and rely on an FAD/NADPH couple as reductant to form a methyl group. In ThyX, all three substrates bind in close proximity to the catalytic FAD group and we have recently demonstrated the real time active site flexibility by studying the dynamics of FAD fluorescence. In order to determine if active site flexibility is a general feature of flavin-dependent methylases, we expressed TrmFO enzymes from mesophilic and thermophilic bacteria. The tRNA substrate of TrmFO proteins is much larger than dUMP, suggesting that substantial flexibility of the active site is required for enzyme function. Different from the mesophilic enzyme, the thermophilic TrmFO purified as highly stable complex with unusual spectral properties. Mass spectrometric analysis revealed very tightly, but non-covalently, bound RNA and FAD linked to the complex. We are currently using the quenching of flavin fluorescence, together with tyrosine mutagenesis and molecular dynamics simulations, to probe the dynamic properties of the active site on different time scales in TrmFO. Our data are expected to have important implications for the role of active site flexibility in multisubstrate enzymes.

P35-048
Spectroscopic studies on the structural changes in Human Serum Albumin upon 3-Hydroxyflavone binding immobilized on Silver Nanoparticles

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The secondary structure of proteins is very important in the field of biophysics. In this study the interaction between 3-Hydroxyflavone (3-HF) and Human Serum Albumin (HSA) in lecithin lipidic bi-layers (PC) and on silver nanoparticles (SNPs) is monitored using attenuated total reflectance (ATR)-Fourier transform infrared (FTIR) and Raman spectroscopies. Variations in the amide I region in the mid infrared reveal that the β-sheet region of the HSA, which is the target binding site for 3-HF reflect the altered backbone dynamics and hydrogen bonding**.** Electrochemically induced FTIR difference spectra have been obtained in order to monitor the redox properties of 3-HF. The results are discussed in terms of functional properties of the HSA in protein-flavone-PC and protein-flavone-SNPs complexes.

P35-049
Molecular dynamics studies of the phosphopantetheine adenylyltransferase from *Mycobacterium tuberculosis* conformational changes upon ATP binding

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Phosphopantetheine adenylyltransferase from *Mycobacterium tuberculosis* (PPAT Mt) is involved in the coenzyme A (CoA) biosynthesis, catalyzing the penultimate step of the process, resulting in the formation of the dephosphocoenzyme A (dPCoA) from 4'-phosphopantetheine (PhP) and ATP. Reduction of the intracellular level of CoA prevents the bacterium growth. Therefore, PPAT is suitable therapeutic target for the rational drug design.

It is known from the of X-ray studies that when binding ligands such as ATP and dPCoA with this enzyme, the enzyme molecule undergoes significant conformational changes. But in this case we know only initial and final states of composition. In this study, we used the method of molecular dynamics to create a temporal model of the conformational changes upon ligand binding. Model of PPAT Mt hexamer in the complex with ATP was created based on known structural data with use the coordinates of unbound protein. The simulation of dynamics of molecule was carried out from initial to final state. The temporal model of conformational change was received. This information will be useful in the development of innovative anti-TB drugs. Virtual screening of the ihibitors of PPAT has been done in this study. Formulas of the potential PPAT inhibitors have been obtained.

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P35-050
QM prediction for creating a mutated antibody with desired catalytic specificity towards organophosphorus toxins

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A17 antibody was selected from the Griffin.1 library, using a biotinylated phosphonate ester. It was shown that A17 hydrolyzes paraoxon via covalent intermediate formation. However, the efficiency of interaction of A17 with paraoxon is only 1.3 M/min, that is insufficient for using this antibody as antidote. The main goal of our work is to determine the necessary conditions to improve the binding and hydrolysis reactions of organophosphate toxins by antibody A17. The proposed approach is based on a hybrid method of quantum and molecular mechanics (QM/MM) that allow us to understand the reaction mechanism. From the one hand the proper substrate positioning in active site can be improved by strong H-bond network, that allowing maximizing contacts between substrate and amino acid residues of the active site. From the other hand nucleophile formation can be improved by introducing proton acceptor residues near the active amino acid. Using the MM approach we designed 43532 structure models of virtual mutants. All mutants, which can form H-bond with substrate were analyzed using QM simulation. Substitution Ser35Arg results in the formation of a stronger H-bond network.

We confirmed that replacement Ser35Arg led to two order of magnitude of second order rate constant increasing in comparison with A17, but no hydrolysis was observed. Substitution Ser35His led to improved covalent binding of paraoxon, but did not change the rate of hydrolysis. Mutation Ser35Glu blocked interaction with paraoxon. Thus, our results are in line with our computed predictions.

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P35-052
Structural investigation of HECT-type Ub ligase intermediates by NMR spectroscopy and X-ray crystallography

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The attachment of the small protein ubiquitin (Ub) is a post-translational modification that plays a key role in a vast array of cellular processes in eukaryotes.

For the modification of a target protein / substrate a three-enzyme cascade is required: The Ub-activating enzyme (E1) activates the C-terminal glycine residue (G76) of Ub in an ATP-dependent reaction.

Afterwards, Ub is passed to the catalytic Cys of the Ub-conjugating enzyme (E2) and in the case of HECT-type ligases to the E3 itself through two consecutive transesterification-reactions.

The HECT-type E3s then transfer Ub to the target protein resulting in a isopeptide linkage between the Ub C-terminus and the ε-amino group of the lysine from the target protein.

Our aim is to investigate the strucure of the HECT-Ub thioester which is formed in the Ub-transfer reaction from the E2 to E3. Structural studies of HECT thioester intermediates are aggravated by the inherent instability of the thioester bond. To overcome this problem we mimic the natural thioester linkage through a disulfide bond. For this, we form a disulfide bond between a C-terminal mutant of Ub (G76C) and the catalytic active cysteine of the HECT-domain. This approach allows us to study the intermediates of the HECT-Ub in more atomic detail by solution-state NMR spectroscopy and by X-ray crystallography.

P35-053
Preparation and characterization of novel fluoromagnetic nanoparticles containing ligand-switching UnaG protein

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Long time the main product of oxidation heme-containing proteins bile pigment bilirubin was considered to be only as ballast product of metabolism and toxic agent. However, it was found that bilirubin is able to inhibit free radical reactions, but its regulatory function is still remains unknown. Regulatory function of bilirubin was limited only by a possible inhibition of sphingomyelinase and mediating during the expression activation of one of the cytochromes by ultrasound. Recent investigations by Japanese researchers (Miyawaki et. al., Nature, 2013) revealed an unusual effect of bilirubin. When the pigment binds with novel expressed UnaG protein, the former is able to activate the latter light emission. Thereby, a new fluorescent protein from eel revolutionized key clinical assay.

At this research, we attempted to improve the technics of holoUnaG formation; examined the effect of UnaG onto bilirubin displacing from its albumin conjugate; synthesized and characterized novel fluoromagnetic silica particles, containing ligand-switching fluorescent protein UnaG. We showed that UnaG displaces bilirubin from its albumin complex, due to very high binding constant value (bilirubin-UnaG). Synthesized magnetic silica particles become highly fluorescent when holoUnaG is attached to them.

This work was supported by bursary of the President of Russian Federation No. SP-6898.2013.4 for young scientists and graduate students engaged in advanced research and development in priority directions of modernization of Russian economy (2013–2015) and grant of the President of Russian Federation MK-287.2014.3 (2014–2015), Jewish Agency for Israel and the Government of Israel Contract grant number: 1504994 (2014–2015).

P35-054
Mechanistic insights into OTU deubiquitinase specificity

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Ubiquitination is a reversible post-translational modification with key roles in a vast range of cellular processes. The ubiquitin (Ub) signal can be very complex, and it is terminated by enzymes called deubiquitinases (DUBs).

The family of human ovarian tumor (OTU) DUBs comprises 16 active members, most of which regulate cell-signaling cascades. Our recent comprehensive study on this enzyme family (*Mevissen et al., Cell, 2013*) revealed that the majority of human OTU DUBs are linkage specific, preferring one, two or a defined subset of linkage types including largely unstudied atypical Ub chains. Biochemical and structural analyses, particularly of the smallest OTUD subfamily, uncovered four main mechanisms of linkage specificity. Thereby, additional Ub binding domains (UBDs), the ubiquitinated sequence in the substrate and defined S1' and S2 Ub binding sites on the OTU domain enable these enzymes to distinguish Ub linkage types.

Here, we present new insights into OTU DUB mechanism. Crystal structures of a catalytic OTU domain in complex with monoUb and an atypical diUb substrate bound to the active site Cys residue show unexpected large conformational changes compared to the apo structure. These structures represent the different states of the OTU DUB reaction cycle and shed light on the conformational dynamics of this enzyme in action. Furthermore, biochemical and biophysical characterization reveal key residues and regions in proximal Ub binding and enzymatic activity.

Overall, our study contributes to the mechanistic understanding of DUB specificity and activity.

P35-055
Flagellar subunits as targets for structure-based epitope discovery approaches and melioidosis vaccine development

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As targets for 3D structure-based epitope discovery for melioidosis vaccine component development, we present the crystal structures of flagellin (FliC) and the flagellar hook-associated protein (FlgK) from the Gram-negative and category B bacillus *B. pseudomallei,* for which treatment and control by antibiotics remains challenging. Both FliC and FlgK are immunoreactive against antibodies from melioidosis patients and FlgK is cytotoxic to murine macrophages. *In silico* and *in vitro* methods mapped potential epitopes to discrete FlgK domains, whereas three FliC epitope peptides were predicted to be both B and T cell epitopes by both structure-based *in silico* methods and sequence-based epitope prediction tools. When synthesized as free peptides, FliC epitopes were found to be immunoreactive against human IgG antibodies and to elicit cytokine production from human peripheral blood mononuclear cells. Two FliC peptides were found to be dominant immunoreactive epitopes, and their antibodies enhanced the bactericidal activities of purified human neutrophils. Our studies provide preliminary data that suggests the further testing of FliC epitopes and FlgK domains as potential melioidosis vaccine components.

P35-056
Crystallization and three-dimensional structure determination of phosphorybosylpyrophosphate synthetase from *E. coli*

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Phosphorybosylpyrophosphate synthetases (PRPPS, EC 2.7.6.1)) catalyze the formation of 5-phosphoribosylpyrophosphate (5-PRPP) from ATP and ribose-5-phosphate. 5-PRPP is an important cell intermediate in the synthesis of purine, pyrimidine and pyridine nucleotides as well as amino acids histidine and tryptophan**.** E. coli PRPPS which belongs to the family I of phosphorybosyl pyrophosphate synthetases, was cloned, purified and crystallized in microgravity using counter-diffusion technique. The X-ray data set from grown crystals was collected at 100 K to 2.71 Å resolution using Spring-8 synchrotron radiation facility. The X-ray structure was solved to 2.71 Å by molecular replacement using Bacillus subtilis PRPPS as a starting model. It was found that the 3D-structures of both bacterial PRPPS are very similar: in both enzymes three homodimers form a homohexamer in a propeller shape, each of six subunits consists of two domains of similar topology. Minor differences of the structures have been described. The main difference is found in the system of intersubunit contacts. Its putative influence on the stability of the enzyme molecule is discussed.

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P35-057
Understanding the unique mechanistic and cellular roles of Atlastin isoforms

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While they have some common features, membranes of different cellular organelles are very specialized environments that support particular biological functions. The endoplasmic reticulum (ER) is a prime example of this specialization as lipids form an interconnected system of cisternae, vesicles and tubules, which provides a highly compartmentalized structure for a multitude of biochemical processes. Atlastin is a dynamin-related G protein that has been identified as an indispensible component for maintaining this very specific morphology. In particular, atlastin uses GTP hydrolysis to facilitate membrane fusion events. Aberrant atlastin function has been linked neurodegenerative diseases, highlighting its important cellular function. By studying the molecular mechanism and regulation of the atlastin isoforms encoded by higher eukaryotes, we will develop an understanding of how this protein family supports ER structure and hence regulates biochemical and signaling processes of this organelle.

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P35-058
Maltose binding protein in a molten globule and in the native state

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Maltose-binding protein (MBP) is in a molten globule state at pH 3 as characterized by ANS binding [1]. DEER distance measurements of seven spin-labeled double mutants in the native state at pH 7 had shown excellent agreement with X-ray data [2]. At pH 3 corresponding DEER measurements of all the mutants yield a broad distribution of distances. This was to be expected if there is no defined tertiary structure and the individual helices pointing into all possible directions.

However, as MBP still binds maltose in the molten globule state although more weakly, the native structure must be retained at or near the active site. This has now been verified with a new set of mutants, MBP 08 – 11 (Spinlabel distances below 20 Å) as well as the corresponding single mutants in order to allow for simulation of the individual tensor values to be employed in the program DIPFIT 2 by H.J. Steinhoff [3]. Maltose-dependent open and closed forms of the two domains were confirmed in the native as well as the molten globule state via cw-EPR spectroscopy. However, the simulation of these cw data suffers from a rather large error, hence double quantum coherence (DQC) measurements are now scheduled at ACERT, Cornell University, Ithaca, New York.

P35-059
Thermal effect of rosin modified biocompatible surfactants on human serum albumin conformation

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This study presents an analysis of the thermal aggregation of human serum albumin (HSA) induced by novel rosin compounds. The aggregation process causes conformational alterations in the secondary and tertiary structures of proteins. In this study, the conversion of globular protein to amorphous aggregates was followed by spectroscopic and microscopic techniques to investigate factors that are responsible for the structural and conformational change and morphology of the proteins. Our results show that the aggregation of HSA was dependent on hydrophobicity, charge and temperature because the formation of amorphous aggregates occurs in the presence of a novel cationic rosin compound, quaternary amine of rosin diethylaminoethyl ester (QRMAE), at 40 °C and pH 7.4 (at 25 °C, there was no evidence of aggregate formation). In addition, the parent compound of QRMAE, abietic acid, and other nonionic rosin compounds [ester of rosin acid with polyethylene glycol monomethyl ether (RMPEG-750) and ester of rosin maleic anhydride with polyethylene glycol monomethyl ether (RMA-MPEG-750)] do not show this property. This work provides precise and necessary information to aid in the understanding the effects of rosin compounds on HSA. This study also provides important information for athletes, health providers, pharmaceutical companies, industries, and soft drink-processing companies.

P35-060
Structural and mutational studies of poly(3-hydroxylbutyrate) depolymerase from *Bacillas thuringiensis*

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A new intracellular poly(3-hydroxylbutyrate) depolymerase from *Bacillus thuringiensis* (BtPhaZ) has been screened for potential applications in polyester biodegradation and (*R*)-3-hydroxybutyrate production**.** Here we report the BtPhaZ structure at 1.42-Å resolution, the first crystal structure of an intracellular PhaZ. BtPhaZ consists of a canonical a/b hydrolase catalytic domain and a unique a-helical cap domain. A detailed structural comparison reveals three new conserved signatures, HG^{36}, D^{61}xxGxG and G^{248}xxD, in addition to the most conserved signature, GxS^{102}xG. The turbidimetric assay revealed that G36A and G248A displayed 5% and 23% activities of the wild type, respectively. The esterase activity assay showed that G36A displayed a 10,000-fold decrease in k_{cat}. The decreased activities of G36A and G248A may be due to unfavorable contacts with surrounding residues such as Trp101 and Cys277, respectively. The D61A mutant was expressed in inclusion body, suggesting that the extensive interactions between Asp61, and Ser40, Ser41, and Asn67 are essential for the structural integrity. Therefore, these four conserved signatures not only constitute the catalytic triad and the oxyanion hole, but also attain the active-site conformation. In addition, a putative fragment containing seven units of ethylene glycol was observed and a 3HB trimer was modeled into the active site. Many mutants have been generated, and several mutants displayed markedly decreased activities. Detailed structural comparisons reveal that various PhaZs have adopted different strategies for the biopolymer binding.

Struct Biol S5, Advances in Structural Biology – from Subcellular to Molecular Resolution

P36-003-SP
Preventing oxidative damage at the early phase: The case of glucose oxidase

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Glucose oxidase (GOx) is a homodimeric flavin-dependent enzyme which catalyzes the oxidation of β-D-glucose, by molecular oxygen, to δ-gluconolactone and hydrogen peroxide. GOx has important applications in the food and beverage, textile, glucose biosensors and enzymatic biofuel cells industries. The biggest problems with GOx industrial applications are its inherent oxidative and thermal instabilities, associated with the loss of enzyme activity during the production, storage and use of GOx-based devices. Numerous studies have been conducted in order to increase GOx enzymatic activity and thermal stability; however, to the best of the authors′ knowledge, no attempts have been made to increase the oxidative stability of this enzyme. In this study, MD simulations were used to rank the eleven methionine residues by their distance from the active site and their solvent accessibility. Based on these two parameters, four representative Met residues were chosen and mutant libraries were produced using the site-directed saturation mutagenesis in combination

with the yeast surface display method for easier screening. Compared to the wt-GOx, the catalytic activity of the majority of the mutants was reduced, however, several mutants with higher activities were also found. Overall, a twofold increase in catalytic activity and a 35% increase in oxidative stability have been achieved. Although water accessibility plays a critical role in the Met oxidation mechanism, we found that all of the Met residues were solvent-accessible enough. Therefore, the solvent accessibility rank of the methionine residues turned out to be not an important parameter.

D.P. and G.K. contributed equally to this work.

P36-004-SP
Structure of α-synuclein in human cells: a disordered monomer

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How the crowded intracellular environment affects the structural properties of intrinsically disordered proteins is poorly understood. Here, we use in-cell NMR and EPR spectroscopy to delineate atomic-resolution insights into the conformations and dynamics of the human amyloid protein α-synuclein in different mammalian cells.

We find that α-synuclein is a stable monomer that maintains its disordered state in the different intracellular environments. It adopts ensemble conformations that are slightly more compact than in buffer and poised to counteract spontaneous aggregation. N- and C-terminal α-synuclein residues engage in weak transient interactions with intracellular components, whereas its amyloidogenic NAC region is shielded from exposure to the cytoplasm. These results establish that intrinsic structural disorder is sustainable in cells and that crowded intracellular environments do not inherently promote α-synuclein aggregation.

P36-005-SP
The absolute arrangement of subunits in cytoskeletal septin filaments in cells measured by fluorescence microscop

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The septins are an essential family of filament-forming GTP-binding proteins with conserved functions in cell division. Yeast septins form octameric, nonpolar, rod-shaped complexes of about 32 nm length and assemble further into higher-order structures that perform a variety of functions in the cell cycle. While *in vitro* the assembly of complexes into filaments is quite well understood, how the complexes assemble into the higher-order structures in cells remains unclear.

Here, we used single molecule localization microscopy to visualize both termini of septin rods at nanometer resolution *in vitro* and in cells. Single septin complexes appeared as pairs of localizations around 30–35 nm apart and revealed the exact spatial orientation of the complex in space. Under *in vitro* conditions favorable to septin polymerization, we detected septin assemblies as very thin, elongated stretches of equidistant localizations both when Cdc11, the terminal subunit of the rod, and when Cdc10, the central subunit of the rod, was labeled and detected. These filaments were mostly straight and occasionally appeared bundled. In a filamentous fungus, we resolved similar localization pairs and thin filaments of equidistant localizations. Our work demonstrates that septin complexes assemble end-over-end into filaments in cells and that if paired, filaments are aligned in register.

P36-007
Aneuploidy of urethane in mouse bone marrow cells and potential recovery with lupin water extract

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The incidence of *in vivo* urethane-induced chromosomal aberrations, sister chromatid exchanges (SCEs) and aneuploidy was examined in male mice. Single oral administration by gavage with urethane (0.5 and 1 g/kg) caused a significant increase in chromosomal aberrations in bone marrow and spermatocyte cells, and statistical significant in SCE induction. The clastogenic effect observed was dose- and time- dependent. Aneuploidy was observed clearly with the high dose recording a significant value. Administration of lupin water extract at 5000 ppm/mice/day (added with the drinking water) reduced the frequency of chromosomal aberrations, but still at the significant values (P, 0.001) while that administration of lupin extract elevated the aneuploidy induced with urethane. It can be concluded that urethane is a strong clastogenic and weak aneugenic agent when administered orally and administration of lupin water extract can be elevateing the aneugenic property of urethane.

P36-008
Light harvesting of bacteriorhodopsin and bacterial reaction center in generating electrochemical energy efficiency

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Bacteriorhodopsin (bR) is a light driven proton pump that converts sunlight to chemical energy. BR is an integral membrane structured protein found in the purple membrane of Halobacterium halobium. It is composed of 248 amino acids and a chromophore in the middle which captures light. Electricity can be generated through the process of light-chemical conversion, when photons are absorbed by the chromophore, the photocycle begins. Bacterial Reaction Center (bRC) is a light driven electron transfer reaction that converts solar energy to chemical energy. BRC are integral membrane structured proteins found in the purple membrane of Rhodobacter sphaeroides. It is composed of 3 major co-factors such as bacteriochlorophylls, bacteriopheophytin and ubiquinone. Its primary mechanism is to execute photosynthesis. In this interaction, electron transfer occurs through light ejection of electron that passes through the membrane. Conversion of sunlight to chemical energy simultaneously precipitates. This research aims to compare the function and structure of bacteriorhodopsin and bacterial reaction centers, underscoring the energy generated in both membranes. Through calculating the ATP, protons and photons that cross the membrane, exact value of energy emission in the order of electron volts present the energy generated. Advantages and mechanisms of photoreactions including bioelectronic, bioenergy production in bR and bRC

will be exemplified. Energy efficiency of bR and bRC will be determined and increasing photocurrency will be investigated.

P36-009
Nanoscale structure of the BMP antagonist chordin supports cooperative BMP binding

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Bone morphogenetic proteins (BMPs) orchestrate key cellular events, such as proliferation and differentiation, in development and homeostasis. Extracellular antagonists, such as chordin, are essential regulators of BMP signaling. Chordin binds to BMPs blocking interaction with receptors, and cleavage by tolloid proteinases is thought to relieve this inhibition. A model has been previously proposed where chordin adopts a horseshoe-like arrangement enabling BMP binding cooperatively by terminal domains. Here, we present the nanoscale structure of human chordin using electron microscopy, small angle X-ray scattering, and solution-based biophysical techniques, which show that chordin indeed has a compact horseshoe-shaped structure. Chordin variants were used to map domain locations within the chordin molecule. The terminal BMP-binding domains protrude as prongs from the main body of the chordin structure, where they are well positioned to interact with the growth factor. The spacing provided by the chordin domains supports the principle of a cooperative BMP-binding arrangement in which growth factors bind to both an N- and C-terminal von Willebrand factor C domain of chordin. Using binding and bioactivity assays, we compared full-length chordin with two truncated chordin variants, such as those produced by partial tolloid cleavage. Cleavage of either terminal domain has little effect on the affinity of chordin for BMP-4 and BMP-7 but C-terminal cleavage increases the efficacy of chordin as a BMP-4 inhibitor. Together these data suggest that partial tolloid cleavage is insufficient to ablate BMP inhibition and the C-terminal chordin domains play an important role in BMP regulation.

P36-010
Binding site for mRNA on the γ-subunit of archaeal translation initiation factor 2

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The heterotrimeric aIF2, the archaeal homologue of the eukaryotic translation initiation factor 2, consists of the α-, β- and γ-subunits, and in GTP-bound form binds initiator methionyl-tRNA ($Met\text{-}tRNA_i$) on the small ribosomal subunit. The γ-subunit of aIF2 is a ribosomal GTPase containing the GTP/GDP-binding site. The aIF2γ from *Sulfolobus solfataricus* (SsoIF2γ) binds to the 5'-triphosphate end of mRNA and protects its 5'-part from degradation.

Here, we show that SsoIF2γ binds mRNAs with a triphosphorylated guanosine at their 5'-end and does not bind mRNAs starting with adenosine triphosphate. GTP and GDP compete with mRNA for binding to SsoIF2γ, contrary to ATP, UTP and CTP. Thus, mRNAs with guanosine triphosphate at the 5'-end bind SsoIF2γ specifically. Using site-directed mutagenesis we obtained SsoIF2γ mutants with a defect in mRNA binding. All changes affecting the mRNA binding were located at the canonical nucleotide-binding site of SsoIF2γ. Despite their inability to bind mRNA, the SsoIF2γ mutants were able to form a complex with SsoIF2α, and to bind GTP and $Met\text{-}tRNA_i$. The mutational analysis and competition studies between GTP, GDP and mRNA for binding to SsoIF2γ present strong evidence that the nucleotide-binding site of SsoIF2γ is involved in recognition and binding of the mRNA 5'-triphosphate end. Since binding of mRNA to SsoIF2γ is independent of nature of mRNA, the guanosine triphosphate group at the 5'-end appears to be the sole recognition motif. However, mRNA seems to form additional non-specific contacts with the protein. Molecular dynamics simulations were used to build a model of an mRNA-SsoIF2γ complex.

P36-011
Investigation of RNA-binding properties and oligomerization behavior of Sm-like archaeal proteins

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Proteins of the Lsm (Sm-like) family are presented in all three domains of life. They are defined by the ability to adopt the Sm fold, which is comprised of a 5-stranded β-sheet and an N-terminal α-helix. Despite the fact that they are structurally conserved, functions of the protein from different domains of life are dissimilar. Bacterial Lsm protein Hfq acts as an RNA chaperone to facilitate interaction of regulatory RNA and mRNA. Eukaryotic Sm/Lsm proteins are mainly scaffold proteins of spliceosomes and telomerase. Sm-like archaeal proteins are well characterized structurally and their RNA-binding ability has been proved but the functions of the proteins *in vivo* remain unknown.

In order to study functions of archaeal Lsm proteins SmAP1 from *Sulfolobus solfataricus* and SmAP from *Methanococcus jannaschii* were chosen. The proteins were isolated and purified. Crystals of proteins and their complexes with ribonucleotides were obtained. Using the approach, which was developed in our group, we determined single-stranded RNA-binding sites on the surface of the proteins.

Secondly, we have tested the ability of the proteins to form fibrils as it was found earlier for others SmAP proteins. It was shown that the both studied proteins form fibrils spontaneously as Lsm proteins from *Pyrobaculum aerophilum* and *Methanobacterium thermautotrophicum*.

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P36-012
Structural and functional characterization of the mouse inhibitory C-type lectin-like receptor

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NK cells play an essential role in reproduction or organism's defense against viral infections and tumor growth. Besides the immune response is very early, healthy tissues are considered due

to prevention of autoimmunity. These complex functions require intricate system of regulation ensured by many receptors on a cell surface. One way leading to understanding of NK cell biology is through the structure of the NK receptors, which can reveal conditions of ligand binding.

This project addresses structure of the mouse inhibitory C-type lectin-like receptor Nkrp1b (Klrb1b) using mass spectrometric techniques (disulfide bonds characterization and chemical cross-linking). Functional activity of the Nkrp1b protein was examined on murine cells derived from a bone marrow by fluorescence microscopy. Main interest is focused on the position of the loop and the stalk region in the context of whole protein structure and interaction with its binding partner.

Besides design of Nrp1b models, binding properties of several Nkrp1b forms differing in the presence of the stalk region and monomeric/homodimeric conformation were compared. These forms exhibited surprisingly distinct behavior. Based on the data obtained, our investigation will evolve towards question, whether the receptor forms monomers or homodimers as it is reported in the literature without direct experimental evidence for over 20 years.

P36-013
Regulation of mitochondria beta oxidation by non-enzymatic post-translational modifications

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Metabolic regulation is an intricate process that engages a number of cellular changes at the genomic, proteomic and metabolomic levels. Recently, it has been uncovered that non-enzymatic post-translational modifications (PTMs) such as acylation, glutarylation and succinylation are extensively found in mitochondrial enzymes where they play critical roles in the control of metabolism via sirtuin-mediated regulatory pathways. Moreover, the extent of such PTMs correlates with the cellular accumulation of intermediate metabolites such as acetyl-CoA, succinyl and glutaryl-CoA, a condition that also occurs in several disease states.

The major challenge in the field is therefore to establish the mechanisms that link such effects of non-enzymatic PTMs on proteins to processes in mitochondria.

Here we report studies aimed at filling this gap in which we investigate how glutarylation of the enzyme glutaryl-CoA dehydrogenase (GCDH) influences its structure and function.

We have observed that GCDH, which has been glutarylated by its substrate undergoes lysine glutarylation. Three sites of glutarylation have been identified by mass spectrometry, and at least one of the sites is largely regulated by Sirt5, a sirtuin that has been associated deglutarylation (Tan, M. et al. (2014) Cell Metab 19, 605–1).

We are in the process of using a combination of biophysical, biochemical and structural methods to evaluate the effects of this modification in respect to an unmodified GCDH control. Our findings do not evidence major structural alterations, but rather a substantial compromise in catalytic activity, suggesting that enzymatic function and protein-protein interactions may be regulated by this modification.

P36-014
The Red Sea brine pools as source for enzymes of scientific and biotechnological interest on the example of a novel Mn^{2+} dependent alcohol dehydrogenase

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Deep-sea anoxic brine pools of the Red Sea are considered one of the most remote and extreme environments on Earth while remaining one of the least studied. High salt concentrations (4.3 M), elevated temperatures (up to 68°C) and high metal content make them promising sources for novel enzymes with structures distinguished by evolutionary adaption. Reliable functional annotation of genomic data is the key-step in the discovery of novel enzymes. Therefore we developed a Profile & Pattern Matching algorithm to eliminate false positive annotations. Based on scientific and industrial interest 13 genes were selected and are currently expressed in halophilic expression systems.

One of the most interesting genes identified might open novel class of Mn^{2+} dependent alcohol dehydrogenases. The enzyme is extremely tolerant to different salt concentrations (ranging from millimolar to saturation) and high solvent concentrations, shows a broad substrate spectra and is stable at both, high temperatures (up to 85°C) and basic pH. More interesting is the altered activity by substitution with different metal ions, where in opposite to *in silico* predictions Fe^{2+} shows no effect on activity. The enzyme is slightly activated with Zn^{2+} but shows an activation boost with Mn^{2+}. The activity profile at different temperatures, salt, pH, aggregation temperature, hydrodynamic radius and the substrate spectra changed significantly between Zn^{2+} or Mn^{2+} addition, representing the natural environment at the brine pool of origin. Currently ongoing crystal structure determinations will help identify the structural adaption and are likely to open new routes for green catalysts.

P36-015
Crystal structure of the first bacterial diterpene cyclase and structure-based engineering of plasticity residues

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Terpene molecules represent one of the most diverse groups of natural biomolecules. Sesqui- and diterpenes are a versatile class of secondary metabolites predominantly derived from plants, marine invertebrates, fungi and some prokaryotes. Properties of these natural products include anti-tumour, anti-inflammatory, antibiotic, neuroprotective and even insecticidal activities, which makes these compounds high value commercial targets for the chemical and pharmaceutical industry. Since terpenes are difficult to access by chemical synthesis, production can be alternatively performed in engineered microorganism. Here we present the first crystal structure of a bacterial diterpene cyclase, cyclooctat-9-en-7-ol synthase (CotB2), at 1.64 Å resolution by single wavelength anomalous dispersion. CotB2 catalyses the cyclisation of linear geranylgeranyl diphosphate to tri-cyclic cyclooctat-9-en-7-ol. Subsequent oxidation of cyclooctat-9-en-7-ol by two cytochrome P450 monooxygenases leads to bioactive cyclooctatin. Plasticity

residues that decorate the active site of CotB2 have been mutated, resulting in altered, novel mono-, di- and tri-cyclic compounds.

P36-017
Structural and biochemical studies of a bacterial FIC toxin that hijacks human cellular traffic

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To invade their host and avoid from being destroyed, intracellular bacterial pathogens inject numerous effectors that exert biochemical functions to take command of host cell pathways. Membrane traffic is among the primary pathways co-opted by these toxins.

We investigate the structure and regulation of effectors from *Legionella pneumophila* (the bacteria responsible of the Legionnaire's disease, a severe pneumonia), an intracellular pathogen that establishes a vacuole where it hides and replicates in infected host cells [1]. One of these effectors is AnkX, a 900-residue protein comprised of a FIC domain followed by ankyrin repeat [2].

Most FIC domains are involved in AMPylation of target proteins using ATP as a co-factor. In contrast, AnkX uses cytidine diphosphate-choline (CDP-choline) to covalently add a phosphocholine moiety to small G proteins of the Rab family, which are major regulators of vesicular traffic in eukaryotic cells [3]. This post-translational modification impairs the coupling between the GDP/GTP and membrane/cytosol regulatory cycles of these GTPases. The structure of the FIC domain of AnkX has been solved in our laboratory and revealed how the toxin cleaves CDP-choline into CMP and phosphocholine [4].

We are now investigating how AnkX interacts with Rab GTPases and the role of membranes in this process by structural and biochemical studies, which will be presented in this poster.

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P36-018
Recombinant DMP1 protein fragment expressed in *E.coli* influences the *in vitro* crystallization of $CaCo_3$

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Thirty-seven K protein is a result of proteolytic processing of DMP1 (dentin matrix acidic phosphoprotein 1) an extracellular matrix protein taking part in biomineralization of bone and dentin. Primarily, DMP1 is localized in the nuclear compartment of undifferentiated osteoblast, later, during the late stage of osteoblast maturation, DMP1 is exported out into the extracellular matrix where it regulates the proper homeostasis of calcium phosphate and the formation of hydroxyapatite. As many proteins characterized to be engaged in biomineralization, DMP1 and it′s fragments manifest character of intrinsically disordered proteins (IDPs). It was suggested, that DMP1 can take a part during otoconia mineralization as the protein is also present in mouse otoconia at a low level. The mechanisms underlying otoconia formation and maintenance are not yet fully understood. $CaCO_3$ is the common component of all otoconia from animals but have various morphologies and crystalline structures and different protein compositions. This stress out the importance of otoconins for the proper otoconia formation.

For this study, the 37K cDNA was cloned in pQE-80L vector and expressed in *E.coli*. The protein was purified in two steps, using Talon resins and MonoQ column. The pure protein undergo the *in vitro* mineralization test. It was shown that the 37K protein influences the calcium carbonate mineralization. Obtained calcium carbonate crystals were verified by the Raman spectroscopy. The data presented here show that the 37K protein influences size and shape of calcium carbonate crystals which may be crucial for understanding the process of otoconia formation.

P36-019
Impact of disease-causing mutations on emerin architecture at the inner nuclear envelope

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Emerin is an integral protein of the inner nuclear membrane. It binds to the nucleoskeleton, thus contributing to nuclear structure. It also interacts with chromatin through the DNA binding protein BAF. It is essential for nuclear envelope reassembly after mitosis as well as nuclear response to a mechanical stress. Loss of emerin and the expression of some variants cause muscular dystrophy and cardiomyopathy. Emerin has an N-terminal globular LEM domain that recognizes BAF, a large intrinsically disordered region (IDR) that interacts with the nucleoskeleton, and a C-terminal transmembrane a-helix. We demonstrate here that the LEM and IDR fragment of emerin can form different oligomers *in vitro:* elongated dimers, fibrils, and ribbons. In parallel, Proximity Ligation Assays (PLA) revealed (ectopic) wild-type emerin-emerin proximities in cells, both in the cytoplasm and at the nuclear envelope. Emerin variants S54F, del95-99, Q133H, P183T and P183TH that cause Emery-Dreifuss muscular dystrophy show different capacities to form fibrils and ribbons *in vitro*. In cells, while del95-99 shows no major cellular localization defects, PLA highlighted a significant reduction in the proximities between del95-99 monomers at the nuclear envelope. Instead, P183T is largely clustered in cytoplasmic aggregates, and taking into account that it is less present at the nuclear envelope, proximities between P183T monomers are more frequently observed than in the case of wild-type. We conclude that the LEM and IDR fragment of emerin is capable of oligomerizing *in vitro* and that disease-causing variants del95-99 and P183T that are mutated in the IDR modify emerin architecture in cells.

P36-020
Characterization of holliday junction intermediates in the vibrio cholerae Int4 integrase site specific recombination reaction

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Site specific recombination is a crucial process for the survival, and evolution of many organisms. The tyrosine recombinase family of site specific recombinases (SSRs) performs DNA transactions through the use of an active site Tyrosine amino acid. After first strand cleavage, an exchange occurs to form a Holliday junction in the reaction synapse. This junction can proceed to recombinant products if a second round of cleavage and exchange occurs, or revert to substrate. The propensity of the junction and bound recombinases to evolve in either direction along the reaction coordinates is so far unknown. Isomerization of the junction occurs prior to, or concomitantly with this process to determine the outcome. Integron integrases (Int) mediate recombination between a double-stranded attI site, located within a chromosomal integron platform and single-stranded attC site found on mobile elements flanking gene cassettes. This results in an intermediate structure with four duplex arms known as the Holliday junction (HJ). We are studying the factors that impact the isomerization states of the junction by biochemical and structural approaches.

P36-021
Zinc-induced dimerization interface of the beta-amyloid metal-binding domain 1–16

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Oligomerization of beta-amyloid peptide (Aβ) plays crucial role in the development of Alzheimer's disease (AD). Therefore the study of AD-associated mutations and post-translational modifications of the Aβ metal-binding domain, Aβ(1–16), will help to reveal the mechanism of Zn^{2+}-induced Aβ oligomerization. We have characterized interactions of Zn^{2+} with Aβ(1–16)H6R, incorporating the H6R English familial mutation, and with isoAβ(1–16), containing isomerized Asp7. We have previously shown that isomerization of Asp7 results in Zn^{2+}-induced dimerization of Aβ(1–16). In this study, using ITC and SPR we have shown that the H6R mutation of Aβ favors this process as well. NMR experiments have demonstrated that at low concentrations Aβ(1–16), Aβ(1–16)H6R and isoAβ(1–16) form dimers with similar conformation in the presence of Zn^{2+}. At higher concentrations of Aβ(1–16) monomeric form is prevalent compared to dimeric (PDB ID 1ZE9). With increasing Aβ(1–16)H6R concentration a significant quantity of the dimer complex is formed as a result of the exclusion of His6 from the Zn^{2+}coordination sphere. This allowed us to determine an NMR structure of the Aβ(1–16)H6R dimer complexed with Zn^{2+} (PDB ID 2MGT) and to characterize the dimerization interface, which is common for the analyzed isoforms of Aβ(1–16). In the case of isoAβ(1–16) increase of peptide concentration leads to the formation of insoluble aggregates, indicating the existence of additional Zn^{2+} chelating center. QM/MM calculations showed that this center can be formed by His6 and isoAsp7. Based on these data we propose a mechanism of Zn^{2+}-induced oligomerization of Aβ(1–16) H6R and isoAβ(1–16).

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P36-022
The histology and the cytology of the Brown Adipose Tissue of the Dryomys laniger (Felten & Storch, 1968) (MAMMALIA: RODENTIA) in Hibernation

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This research had been performed on *Dryomys laniger* (*D. laniger*) specimens which were collected from their natural environment and kept under uncontrolled laboratory conditions. Brown adipose tissues of these animals were obtained by dissection of the animals. The dissection procedure was carried out in two stages. These stages were the hibernation period in winter months and the active period of the animals in summer months. The dissection in winter was performed both during the animal in hibernation and while the animal is awake. The dissected tissue specimens were prepared and photographed in order to be examined by light and transmission electron microscope. It has been determined that the mitochondria number was abundant in the brown adipose tissue cells of the active *D. laniger* but the fat droplets were rare. In contrast, it has been noticed that, the capillary blood vessels and the fat droplets were high between the cells of the brown adipose tissue of the hibernating animals. It has been observed that the fat droplets were in contact with each other and there was a loss of the cytoplasmic material and some erosions on the cristae of the mitochondria, in the cytoplasm of the animals which were awake in the hibernation months. No difference was observed in the brown adipose tissue of *D. laniger* in light microscopic level.

Key Words: Brown Adipose Tissue, *Dryomys laniger*, Hibernation

P36-023
DNA aptamers for malaria diagnosis – from crystal structure to clinical application

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DNA aptamers have the potential to disrupt medical diagnostics by replacing antibodies for molecular recognition. However, few aptamers have reached the stage of clinical application. A key challenge is discovery of suitable aptamer-target pairs and how best to link the binding event to a diagnostic signal particularly for point-of-care tests. Here, we present our work to develop DNA aptamers for point-of-care malaria diagnosis. We discovered aptamers against *Plasmodium* lactate dehydrogenase as a general Plasmodium biomarker and against histidine-rich protein 2 as a *Plasmodium falciparum* specific biomarker. We have solved the crystal structure of *Plasmodium* lactate dehydrogenase aptamer in complex with its target, making this one of the best characterized aptamer-target pairs. Furthermore, we have integrated aptamers into an assay termed aptamer-tethered enzyme capture (APTEC) which is able to diagnose malaria in stored clinical

patient blood samples. Work is ongoing using microarrays to optimize aptamer affinity and we are rapid prototyping 3D printed aptamer-enabled devices with a view to developing a point-of-care diagnostic test that is inexpensive, robust, sensitive and specific for the developing world.

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P36-025
Importance of volumetric data of the human brain structure in a *PTEN* mutation positive Bannayan-Riley-Ruvalcaba Syndrome: A methodological analysis

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Bannayan-Riley-Ruvalcaba syndrome (BRRS) is a PTEN Hamartoma Tumour Syndrome (PHTS) is caused by the mutations in the *PTEN* gene (phosphatase and tensin homolog deleted on chromosome 10, MIM 601628). The mutation characterized by common intracranial pathologies such as macrocephaly, central nervous system abnormalities and less commonly as mental retardation. Volumetric analysis on brain substructures may help the investigators to find out macrocephalia degree. We aimed to obtain volumetric brain changes on the case with BRRS using Stereological volumetric analysis. The case with BRRS was an 8-year old female, who had a recurrent facial palsy, therefore she was referred to the Neurogenetic Division, University of São Paulo-Clinics Hospital, Ribeirao Preto, São Paulo, Brazil. The volumetric data of the healthy and BRRS subjects' brain structures were compared using stereological Cavallier method. The direct measurement of head circumference of the case with BRRS was larger than the control subject. According to our data, total intracranial and corpus callosum volumes, right and left hemispheres of the cerebrum, and also lateral ventricles of the case with BRRS were also larger than the control subject. The relation between macrocephaly and any other neuroimaging volumetric abnormality or not has not been examined in the children with BRRS.

P36-026
Cell-free expression and functional characterization of G protein-coupled receptors in distinct artificial environments

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G protein-coupled receptors (GPCR) represent the dominant superfamily of surface receptors in eukaryotic cells. They perceive a broad range of ligands such as light, ions, small molecules and other proteins and have abundant roles in signal transmission and physiological regulation. Their structural and functional characterization is hence of eminent importance. Cell-free (CF) expression technology is a promising tool to address those issues, as it offers fast access, high expression yields and co-translational insertion of membrane proteins into defined hydrophobic environments (e.g. detergent micelles or lipid bilayers), in addition to bypass extensive membrane disruption procedures. We are using an *E. coli* based CF-expression system in combination with nanodisc technologies for expression and functional characterization of the thermostabilized beta-1 adrenergic receptor and the non-stabilized endothelin-B receptor in a variety of defined environments. We could show that the overall ligand binding activity of the thermostabilized beta-1 receptor is sufficient for structural approaches. We also found that insertion into nanodiscs and overall ligand binding activity of both receptors strongly depends on the overall reaction conditions and composition. Furthermore, we show first indications for functional differences in ligand binding activity of the GPCRs depending on their environment. Thus, besides large scale production of GPCRs for structural approaches, their co-translational expression into designed artificial environments offers us a new and strong tool to modulate their function and stability.

P36-027
The pH-modulation of protein-nucleic acid interfaces is analyzed by a non-invasive NMR method based on histidine imidazoles

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A useful ^2J (N-H) coupling-based NMR approach is proposed to unveil, at the molecular level, the contribution of the imidazole groups of the histidines from RNA/DNA-binding proteins on the modulation of binding to nucleic acids by pH. This method goes beyond the conventional ^{1}H-^{15}N HSQC spectra that provide information of ^1J (N-H) and allows us to precise, for the first time, pK_a differences not only between the isolated and the nucleic acid-bound protein, but also among the interfaces of RNA or DNA complexes.

Such protonation/deprotonation events have been monitored on the single His96 located at the second RNA/DNA Recognition Motif (RRM2) of T-cell intracellular antigen-1 (TIA-1) protein. The pK_as of His96 ionizable groups were substantially higher in the complexes with short U-rich RNA and T-rich DNA oligonucleotides than in isolated TIA-1 RRM2.

The methodology herein applied to determine changes in pK_a of histidine side-chains upon DNA/RNA binding shed valuable information to understand the pH effect on multi-domain DNA/RNA-binding proteins that shuttle among different cellular compartments.

References

Cruz-Gallardo I, Del Conte R, Velázquez-Campoy A, García-Mauriño SM & Díaz-Moreno I (2015) Eur. Chem. J. in press.

P36-028
Multiple pleomorphic tetramers of pore-forming thermostable direct hemolysin from Grimontia hollisae in exerting hemolysis and membrane binding

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Oligomerization of proteins into specific quaternary structures plays an important role in biological functions. In this report, we determined the crystal structures of a pore-forming thermostable direct hemolysin toxin of Grimontia hollisae at 2.3 and 1.7 Å resolution limits, respectively. The toxin crystallized in the same space group of $P2_12_12$ but with two different crystal packing patterns, each revealing three consistent tetrameric oligomerization forms designated as oligomer-I, oligomer-II, and oligomer-III. In addition to oligomer-I, which has the same orientation of C_4 symmetric conformation as the known structure of the homologous toxin of Vibrio parahaemolyticus, four toxin protomers in oligomer-II and -III were arranged in lower symmetries of C_1 and C_2, respectively. All toxin tetrameric oligomers retained a central pore with comparable depth of ~50 Å and varied in shape and size. A common motif of a toxin dimer persistently found in all structures suggests a plausible minimum functional unit within the tetrameric oligomer architecture in cell membrane binding and possibly hemolytic activity, which is consistent with the reported transmissible electron microscopy analysis results of the toxin binding mode of diagonal attachment (one protomer) or with two protomers on ganglioside G_{T1} containing liposomes. Our findings highlighted that not all bacterial toxins form a single or high symmetric oligomerization state in exerting biological functions. In Grimontia hollisae, the dynamic nature of multiple tetrameric oligomers formed by the released toxin may carve a niche for bacteria survival in harsh living environments.

P36-029
Physiological impact of brain volume differences on temporal lobe epilepsy in human

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Aim: Epilepsy is a neurological disease caused by abnormal and uncontrollable electrical firings of neurons included in the central nervous system. White matter, gray matter and other regional volume differences and atrophic changes across the brain have been reported in epileptic patients. In the present study, we aimed to evaluate the brain the volumes according to right and left sides using stereological method in cases with temporal lobe epilepsy (TLE).
Material-Method: The volumes of the left and right sides of the brain were analyzed on 9 cases with TLE and the 8 age and gender matched healthy controls on axial MRI slices using by a stereological method- the point-counting approach of the Cavalier's principle.
Results: The mean brain volume in control group was (mean±SD) 529.71 ± 56.57 and 559,89 ± 48.28 in left and right sides, respectively. The mean brain volume in patients with TLE 517.99 ± 60.22 and 545.86 ± 40.84 in left and right sides, respectively. Although the mean brain volumes were decreased in both brain sides of patients with TLE, there were no statistical differences between cases with TLE and control subjects' right and left brain volumes ($p > 0.05$; Student's T test).
Conclusion: The data obtained by this study helps us to avoid intraoperative complications in addition to the optimal facilitation of the surgical access into the deep cortical area.
Key words: Brain volume, MRI, stereology, epilepsy

P36-030
Edge strands and indents of β-sheets: A comparative analysis of sequence and structural features

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Protein aggregation is central to many disorders including Alzheimer's disease, Parkinson's disease, type 2 diabetes and ALS. Avoiding aggregation while maintaining the robust structure of β-sheets requires a delicate balance of various strategies. To achieve this goal, edge strands of β-sheets have evolved unique mechanisms as compared to the inner strands. Despite their name, many inner strands have terminal portions that resemble edge strands and stick out from inner strands as 'indents' of the β-sheet. This particular type of edge-like strands has not been extensively studied. In this work we have compared indent strands, edge strands and inner strands to identify the characteristics which differentiate indent strands from edge strands. Statistical analysis of important features such as amino acid residue preferences and interactions with other secondary structural elements was performed. While being similar to edge strands in features like length distribution, occurrence of β-bulges, negative correlation with inner strand residue preferences, backbone torsion angle distribution and overall distribution of amino acid residues, the indent strands exhibit clear differences in their preference for Gly, Ala, Pro, Ile, Phe, Asn and Glu residues. The two types of strands are also different in the interaction motifs they prefer. The results suggest that although similar, indent strands and edge strands have a few key differences in amino acid residue preferences. These differences give rise to unique features that might be important to prevent aggregation and at the same time, help maintaining the robustness of the β-sheet structure.

P36-031
Comparative analysis of cell wall composition in chilling-treated leaves of C_4 grasses: maize (*Zea mays* L.) and *Miscanthus* × *giganteus*

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Plants of sub-tropical origin with C_4 type of photosynthesis have a relatively high productivity due to their ability to efficiently bind CO_2 while minimizing adverse photo-oxydation process. Maize (*Zea mays* L.) and *Miscanthus* × *giganteus* – closely

related C_4 plants, from the same Panicoideae clade, representing the same subtype of C_4 photosynthesis (NADP-ME) are characterized by different responses to cold stress. The inhibition of photosynthesis at 14°C is observed for maize, while Miscanthus is able to maintain a high rate of assimilation under these conditions. Among the effects of low temperature, altered cell wall seems to be important for the knowledge of the stress response mechanisms. The dynamic nature of cell wall is maintained by modification of polysaccharides – the main structural components of the walls. To investigate the influence of cold on the cell wall properties we performed an analysis of cell wall composition by means of FTIR (Fourier transform infrared) spectroscopy and gas chromatography technique. Changes in the cell wall properties, mainly in polysaccharides composition, seem to be an indicator of one of the elements of the mechanism for differential response to cold of maize and Miscanthus.

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P36-032
Structural study of yeast alcohol dehydrogenase in imidazolium based Ionic liquids

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Ionic liquids (ILs) provide a new generation of solvents entirely composed of ions and are usually considered as green solvents. Biotechnological applications of ILs are currently increasing. This study aims to investigate the mechanism by which an ionic liquid may enhance the rate of biocatalysis. Enzymatic activity of Yeast alcohol dehydrogenase was measured by following the reduction of NAD^+ in different concentration of (1-butyl-3-methylimidazoliumbis(trifluoromethylsulfonyl) imide; [BMIM][NTf2]). The kinetic parameters of the enzyme (k_m, V_{max} and k_{cat}) were obtained by UV-visible spectroscopy using michaelis menten equation. Structural assessment were performed to find the structure-function relationship. The obtained results showed that [BMIM][NTf2] led to reduction of K_m and increasing the enzyme performance**.** Alcohol dehydrogenase from Yeast remain active in [BMIM][NTf2]. Moreover, structural analysis showed that the used IL brings about alteration in the secondary structure of the enzyme. The obtained results would introduce [BMIM][NTf2] as a good alternative for normal organic solvents.

P36-033
New aspects on the structure of small kinetochore-associated protein/kinastrin

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The kinetochore is a large protein complex that assembles around the centromeric chromosomal DNA and plays a central role in attachment of spindle microtubules during cell division. The human kinetochore is composed of more than hundred different proteins. Higher order organization of these proteins is without doubt critical for kinetochore function. However, there is limited information on exact arrangement and higher order structure of human kinetochore components. SKAP (small kinetochore associated protein) is one of the essential components of kinetochores and the mitotic spindle. It is required for faithful chromosome segregation, progression into anaphase and maintenance of spindle pole architecture. Despite the obvious importance of SKAP, it remains poorly characterized. As a crucial step towards better understanding of the role of SKAP within the kinetochore, we here addressed on the basis of primary structures its ability to self-associate.

Our results clearly demonstrated that SKAP molecules directly interact with each other and form homomeric complexes. Furthermore, we mapped the interaction sites of SKAP subunits. Based on the prediction of SKAP structure, we reasoned that the self-interaction could occur through the two C-terminal coiled-coil domains. Indeed, we found that the C-terminal part of SKAP, predominantly second coiled coil, was sufficient to interact with the full-length SKAP protein. Self-association of SKAP is assumed to promote kinetochore structure and organization and is therefore an important aspect for future research.

P36-034
Structure/activity relationships of negatively charged peptide nucleic acid oligomers

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Peptide nucleic acids (PNAs) are functional analogs of nucleic acids, capable of forming stable complexes with DNA and RNA. Acyclic γ-*S*-PNA derivatives have shown great promise due to their preorganized secondary structures. Among various modifications of classical aminoethylglycine (*aeg*) PNAs, modifications with negatively charged side residues have recently attracted particular attention because such PNA derivatives are structurally rather similar to native oligonucleotides.

We employed known methods to obtain a series of PNA monomers, including *aeg* (achiral uncharged*)*, γ-*S*-methyl (chiral uncharged, γ-*m*) and γ-*S*-carboxyethyl (chiral negatively charged, γ-*ce*) monomers, that were subsequently subjected to Boc-protocol solid-phase oligomerization. To analyze the impact of the negative charge and the number of chiral centers on physicochemical properties of a PNA oligomers, we designed and synthesized the following dodecamers with alternating *aeg* and/or γ-substituted chiral monomers: 5′-Gly-$T^{\gamma\text{-}ce}CAC^{\gamma\text{-}ce}CTC^{\gamma\text{-}ce}CCT^{\gamma\text{-}ce}CC$-3′ (**PNA 1**), 5′-Gly-$T^{\gamma\text{-}ce}CA^{\gamma\text{-}ce}CC^{\gamma\text{-}ce}TC^{\gamma\text{-}ce}CC^{\gamma\text{-}ce}TC^{\gamma\text{-}ce}C$-3′ (**PNA 2**) and 5′-Gly-$T^{\gamma\text{-}ce}C^{\gamma\text{-}m}A^{\gamma\text{-}ce}C^{\gamma\text{-}m}C^{\gamma\text{-}ce}T^{\gamma\text{-}m}C^{\gamma\text{-}ce}C^{\gamma\text{-}m}C^{\gamma\text{-}ce}T^{\gamma\text{-}m}C^{\gamma\text{-}ce}C^{\gamma\text{-}m}$-Gly-3′ (**PNA 3**).

Hybridization properties of the dodecamers (**PNAs 1–3**) and their sensitivities to G/t, G/a and G/c mismatches in the middle of the complementary DNA chain were studied. CD spectral data, UV-melting and J-plot analysis results suggest that **PNAs 1–3** form stable antiparallel duplexes with complementary DNA, and the **PNA 3/DNA** duplex is the most stable ($T_m \geq 82.9$°C). Oligomer **PNA 2**, composed of alternating *aeg* and γ-ce-PNA monomers, demonstrated the maximum sensitivity to mismatches and mismatch types ($\Delta T_m \geq 25$°C) under chosen conditions (10мМ Na_2HPO_4-NaH_2PO_4, 140 мМ KCl, 5 мМ $MgCl_2$, pH 7.4).

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P36-035

Inhibitory effects of ethacrynic acid on glutathione S-transferase A1-1 from *Callithrix jacchus*

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Ethacrynic acid (EA) is a potent diuretic drug and also a efficient inhibitor of glutathione *S*-transferases (GST). Glutathione reacts with this unsaturated bond of ethacrynic acid, to form the conjugate. This conjugation reaction is catalysed by glutathione S-transferases. The glutathione transferases (EC 2.5.1.18) are a family of multifunctional proteins, which act as enzymes and also as binding proteins in various detoxication processes. GSTs are believed to play an important protective role in the various tissues of animals and human by catalysing the glutathione conjugation of electrophilic drugs and metabolites.In this study, studied with the wild type A-class Glutathione transferase, from marmoset (*Callithrix jacchus*). As previously study, cDNA library from marmoset was used to amplify mrGSTA1-1 cDNA by polymerase chain reaction. The full-length open reading frame of mrGSTA1 was amplified from 2 μl of the library as a template, using Taq polymerase and the primers. Enzymes were purified from the lysate by affinity chromatography using S-hexylglutathione-Sepharose6B. The inhibitory effect of EA was analyzed with different concentrations of EA. 0, 2, 5, 7, 10, 15 μM EA concentrations were choosen. It is observed that enzyme activity was decreased while EA concentrations were increased. At 0 μM EA concentration, the specific activity of GST-A1 determined as 64,75 mmol/min/gr. But at 15 μM EA concentration, the specific activity of GST A1 was detected as 7,35 mmol/min/gr. Ethacrynic acid appeared to have about 9 times greater potency in competitive inhibiting GSH S-transferase.

P36-036

Analysis of protein aggregate content at extreme concentrations using analytical ultracentrifugation with a novel interference optics

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Analytical ultracentrifugation has been enjoying an impressive reemergence as a powerful tool for the study of size distributions and interactions of various biomolecules for about 20 years. There is a particular demand for accurate measurements of size-variants in therapeutic protein solutions for biopharmaceutical research and quality control as well.

In many cases, therapeutic proteins are formulated at high concentrations > 50 g/L, often in buffers with high amounts of sugar-based excipients. Therefore, the size distribution at those original conditions cannot be monitored using routine aggregation analysis methods. This means that size-exclusion chromatography and other workhorse techniques used in pharmaceutical industries may lead to inaccurate aggregate levels, because of necessary dilutions and buffer changes.

The present sedimentation velocity analysis of highly concentrated proteins expands the hitherto tractable protein concentration range. For the first time, the aggregation levels of soluble model proteins, such as bovine serum albumin and antibodies at concentrations of up to 150 g/L were measured in common buffers or their original formulations, respectively. Thereby, any alterations in the size distribution which may arise due to substantial dilution and solvent change were avoided.

The crucial technical challenge is the SV analysis of extremely steep, fast-moving boundaries, which are for the first time amenable to analysis using the unique in-house developed AIDA (Advanced Interference Detection Array) detector. By developing a consistent experimental design and data fit approach, we could achieve a robust quantification of soluble aggregates. Limitations of the procedure are discussed.

P36-037

Purification and characterisation of polyphenoloxidase from corn tassel

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Polyphenol oxidase (PPO) is a very important enzyme that is responsible for the enzymatic browning of vegetables and fruits, which is undesired process and need to be prevented in food technology. In this study, PPO was purified from corn tassel and some of its kinetic parametres were investigated. The optimum temperature and pH of PPO were found to be 40°C and 8.0, respectively. The Lineweaver-Burk plot analysis of the corn tassel PPO showed that the Km and Vmax values were 4,087 mM and 0,9699 mM for catechol. We also found that the enzyme was activated by glucose, fructose, ribose, sucrose, but inhibited by EDTA, SDS and sodyum azide.Electrophoresis of PPO was carrired out.

Keywords: Corn tassel, Polyphenol oxidase, Purification, Enzyme kinetics

P36-038

High-resolution atomic force microscopy of G-quadruplexes

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G-quadruplex is a prominent example of non-canonical four-stranded DNA structure formed in the G-rich regions of DNA. In this study we are gathering structural information on single G-quadruplexes and trying to determine whether the differences between assembled and dismantled quadruplexes, parallel and antiparallel, quadruplexes composed of two, three, and four G-tetrads can be determined from AFM data.

We are focusing on the structures of small G-rich ssDNA 15-30 nt long. Special methods are needed to visualize such small objects with microscopy. We use a graphite surface modified with the monolayer of amphiphilic carbohydrate-glycine molecules as a substrate and supersharp cantilevers with 1 nm tip diameter. To verify the structural information obtained by high-resolution AFM we correlate the AFM data with the data from NMR and CD spectroscopy experiments.

We have shown that ssDNA has the shape of a thread and the height of 0.4 nm when adsorbed from water. In 10 mM KCl solution ssDNA folds into a globule, the height of the globule depends on the DNA length and is about 0.5 nm.

The height of annealed two-tetrad quadruplexes is about 0.6 nm. Thus it is hardly distinguishable from ssDNA in presence of KCl. The height of three tetrads is 0.7–0.8 nm; the height of four tetrads is 0.8–0.9 nm.

Different G-quadruplexes show the ability to stack on top of each other. In form of stacks they have the height about 1.5 or 2.0 nm.

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P36-039
Characterization of polyphenoloxidase from pepper seed

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Polyphenol oxidases (PPO) are enzymes that catalyze the oxidation of phenolic compounds using molecular oxygen. The ability of PPOs to act on phenolic compounds makes them highly useful biocatalysts for various biotechnological applications. They are commonly found in animals, plants, fungi and bacterial species.

In this study, the polyphenol oxidase of peper seed was partially purified and characterised for some kinetic parametres. The activity of the partially purified polyphenol oxidase of pepper seed was measured at 420 nm, using 4-methyl catechol as a substrate. The optimum temperature and pH of polyphenol oxidase found to be 40°C and 5.0, respectively. Kinetic parametres, Km and Vmax, were calculated from Lineweaver-Burk graph.

Keywords: Pepper seed, Polyphenol oxidase, Enzyme kinetics

P36-040
rs7743761 associated with disease risk of ankylosing spondylitis in Turkish population

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Ankylosing spondylitis (AS) is a chronic, inflammatory and autoimmune disease in which the spine and peripheral joints are sore and which causes restriction of movement in axial joints. Many genetic, environmental and immunological factors have roles in the development of the disease. Prevalence of AS which causes work force loss and decrease in life quality is 0.15–0.49%. Single Nucleotide Polymorphism (SNP) are single base changes frequently observed in human genome and they are important molecular markers used for disease susceptibility, development of disease, medicine response and disease diagnosis. Disease risk and some SNPs are associated in studies carried out on different populations. Effects of these SNPs have not been evaluated in our population yet. In this study, genotyping and analyzing of rs7743761 polymorphism, associated with AS in various populations, were carried out in order to determine the genetic risk susceptibility in our population. Study conducted with 100 patient with AS and 101 controls, the iPlex® method was used in the genotyping of 7743761. The association between AS disease risk with SNP genotypes was analyzed by Backward-Wald logistic regression model. With this study, it was demonstrated that in our population there was risk association between AS and rs7743761 polymorphism ($p = 0.003$, OR= 3.493, 95%CI=1.534–7.955).

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P36-041
Association analysis between Endoplasmic Reticulum Aminopeptidase 1 (*ERAP1*) Polymorphism with ankylosing spondylitis disease risk in Turkish population

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Ankylosing spondylitis (AS) is a chronic, systemic and inflammatory disease. Single Nucleotide Polymorphisms (SNP) are important molecular markers used for disease susceptibility, development and diagnosis of disease. Research on these SNPs may provide contributions to early diagnosis of the disease before the spine deformation occurs. In this study, genotyping and analysis study of 3 SNPs (rs27044, rs27434 and rs10050860) located in endoplasmic reticulum aminopeptidase 1 (*ERAP1*) gene loci, associated with AS risk in different populations, were carried out to determine the genetic risk susceptibility in Turkish population. This study was carried out with 100 patients from Turkish population who were diagnosed with AS according to Modified New York criteria and 101 controls. Using the DNA isolated from peripheral blood samples, 3 SNPs were genotyped by iPlex® method. In association of genotypes and alleles with AS disease risk, Odds Ratio (OR) test was used ($p \leq 0.05$, OR>1). In this study, it was determined that OR value of G allele for rs27044 polymorphism was 1.23 (%95Cl=0.80–1.90) and p value was 0.776, OR value of A allele for rs27434 polymorphism was 1.32 (%95Cl=0.86–2.02) and p value was 0.208, OR value of T allele for rs10050860 polymorphism was 1.08 (% 95Cl=0.54–2.15) and p value was 0.836. With this study, it was demonstrated that there were no risk association between AS and SNPs located in *ERAP1* gene loci in Turkish population.

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P36-042
Method selection for protein extraction from FFPE tissues in the proteomics studies

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Tissue proteins play important roles in biochemical pathways. The quantitative analysis of cells and tissue proteins facilitates the understanding of molecular mechanisms that differentiate between normal and disease states.

The investigation of this protein fingerprint of cells and tissues has been termed proteomics, and a major application of the process of defining global protein expression has been the identification of specific protein biomarkers that can provide diagnostic, predictive and prognostic information, and novel therapeutic interventions.

Formalin fixing and paraffin embedding (FFPE) is the universal method for tissue preservation and stabilization prior to histological evaluation by histologists or pathologists.

FFPE tissues are highly stable, and can be stored at room temperature indefinitely. Therefore, large repositories of healthy as well as pathological FFPE tissues have been generated and stored worldwide. These FFPE samples are associated with clinical information concerning diagnosis, treatment, and outcome of the patient, and mainly serve as specimens for physiological or pathological investigation. Meantime, proteins are generally preserved for a long time even at room temperature.

Protein extraction from FFPE tissue samples using traditional extraction methods are confounded by the high degree of protein covalent crosslinking.

The results obtained by gel-free and gel-based proteomics methods.

Proteomics is the global analysis of protein expression in cells and tissues.

The global protein extraction from FFPE tissue is made with commercial or manually prepared reagents.

Temperature, pH, sonication, and chemical properties of the reagents are extremely important for optimization of the methods.

P36-043
Spin-labeled oligonucleotides – useful tool for the structural biology

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Site-directed spin-labelling with further measurement of interspin distance by pulsed EPR spectroscopy is very powerful method in studies of structure and functions of biomolecules.

In this approach two spin labels should be introduced in the structure of biological object and the value of dipolar interaction between them is measured by Double Electron-Electron Resonance (DEER or PELDOR) or Double Quantum Coherence (DQC) techniques with the high accuracy in the range of 1.5–8 nm.

The most popular approach in spin-labeling based on using nitroxide labels attached to DNA, RNA and proteins and cell systems. Basically, most of experiments using the nitroxyl radicals are carried out in frozen solutions at the temperature of 50–80 K.

Triarylmethyl (TAM) radicals – a relatively new class of spin labels, with long parameters of relaxation time, Tm. This value of Tm provides the possibility of interspin distance measurements at room temperature.

In present work for the first time we have demonstrated the room-temperature distance measurements between two TAM labels linked with two 5' ends of 10 nucleotides length DNA duplex.

This work discovers the perspectives of the combination of EPR and NMR techniques for the biomolecule structure determination at physiological conditions.

P36-044
Quantitative adsorption of IgG on colloidal particles as a new method for preparation of low-cost immunoassays

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The main aim of the study was a quantitative description of the adsorption processes of antibodies (IgG) on the surface of colloidal carriers. Latex based immunoassays for the detection of various kinds of antigens, viruses have been widely studied in the literature and the field of their applications is constantly expanding. Only quantitative approach in the preparation of such tests can reduce the cost of research involving immunoassays and makes them environmentally friendly.

Physicochemical properties of sheep polyclonalan anti-human serum albumin antibody (IgG) were studied to determine the amount of adsorbed antibody on colloidal particles surface. Monodisperse, negatively charged polystyrene latex particles, 800 nm in diameter were used as colloidal carriers. The adsorption of IgG (0.1–2 mg/l) was studied via electrokinetic measurements (micro-electrophoresis) and AFM imaging. Adsorption of proteins was carried out at pH 3.5 and ionic strength range of 0.001–0.15 M NaCl. The concentration of polystyrene latex carriers was changed in the range of 60–100 mg/l.

It was observed that the electrophoretic mobility (zeta potential) of latex monotonically increased with the IgG concentration in the suspension. The coverage of adsorbed IgG was quantitatively determined using the depletion method, where the residual protein concentration was determined by the above mentioned methods. These measurements enabled a precise determination of the monolayer coverage of IgG on polystyrene latex particles.

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P36-045
Antioxidant properties of Edremit variety green olives *(Olea europea L.)*

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In the past decade, there has been a renewed interest in studying a wide variety of food sources that show beneficial effects on human health. Olea europea L. an important agricultural crop, not only because its economic importance, but also for the nutritional values, mainly due to the fact that they are an excellent source of antioxidant compounds, and also of specific constituents such as the oleuropein in the mesocarp and seed. This current study was designed to evaluate the antioxidant capacity and total phenolic contents from Edremit variety olives at green maturity stage. The antioxidant capacity of olive was directly related to the total amount of phenolic compounds detected. Therefore, three crude extracts obtained from methanol and water extractions, were used in order to evaluate the antioxidant activities of green olives. Following extractions, total phenol contents, DPPH (2,2-diphenyl-1-picrylhydrazyl) and DMPD (N,N-dimethyl-p-phenylenediamine radical cation) were determined for antioxidant activity. Ethanol extracts of olive samples showed the strongest total antioxidant capacity using both the (DPPH) and the (DMPD) methods. In addition, all values obtained from different extracts were compared to each other.

P36-046

Treatment of the olive β-glycosidase bound superparamagnetic nanoparticles onto green table olives

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Treatment of olive β-glucosidase bound superparamagnetic iron oxide nanoparticles (SPIONs) onto gren table olives were studied. The SPIONs were prepared by co-precipitation Fe^{+2} and Fe^{+3} ions in an ammonia solution at room temperature. The β-glu that catalyses on the main olive phenolic glycosides was purified from olive fruits by hydrophobic interaction chromatography and covalently bound on to SPIONs via carbodiimide activation. The end product, enzyme bound SPIONs, was used for debittering process of the gren table olives. The enzyme bound SPIONs was immersed in the olives during 6 and 24 h for oleuropein hydrolysis. The traditional olive process (brine replacement) was also carried out for comparative purposes. And, the effects of the enzyme bound SPIONs, free enzyme and NaOH (1% w/v) on oleuropein hydrolysis were compared interms of process time. After the treatments, enzyme bound SPIONs were removed from the reaction medium by a simple magnet. In order to determine the effectiveness of the comparative methods used debittering, oleuropein analysis were performed by HPLC-DAD. It is seen that the treatment of the enzyme bound SPIONs used for green olives is more efficient than traditional methods. The enzyme can be recovered by a simple magnet and used for three times for debittering process of olives.

Keywords: β-glucosidase, superparamagneticiron oxide nanoparticles, oleuropein, debittering
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P36-047

New insights into the interaction between IQGAP1 and Rho family proteins

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The scaffolding protein IQGAP1 participates in various cellular functions such as cell-cell adhesion, cell polarization and migration, neuronal motility, and tumor cell invasion by binding to target proteins, including Rac1 and Cdc42, two members of the Rho family. To better understand the molecular basis of these interactions, we utilized in this study a novel time-resolved fluorescence spectroscopy to determine individual rate constants for IQGAP1 interaction with fourteen different Rho proteins. The results indicated that IQGAP1 binds among Rho proteins selectively to Rac- and Cdc42-like proteins only in a GTP-dependent manner. Competition experiments utilizing interacting partners of Rac1, e.g. Tiam1, p50RhoGAP, Plexin-B1, $p67^{phox}$, PAK1 and RhoGDIa, along with structural analysis, revealed two negative charged areas on the surface of Rho- and Rnd-like proteins, which might explain their inaccessible interaction with IQGAP1. The overlapping binding site of Cdc42 and Rac1 on the surface of IQGAP1 together with the kinetic details of the selective interaction of IQGAP1 with Rac- and Cdc42-like proteins suggests that these interactions are most likely mediated via the same mechanism.

P36-048

Structome analysis based on direct enumeration of virulent *Mycobacterium tuberculosis* with TEM examination of serial ultrathin sections

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Serial ultrathin sections of virulent *Mycobacterium tuberculosis* H37Rv (ATCC 27294) were examined by transmission electron microscope and "structome" analysis (i.e., the quantitative three-dimensional structural analysis of a whole cell through direct enumeration and measurement at the electron microscopic level) was performed. Five *M. tuberculosis* cells were cut into 24, 36, 69, 55, and 63 serial ultrathin cross sections, respectively. On average, the cells were 2.71 ± 1.05 μm in length, and the average diameter of the cell was 0.345 ± 0.029 μm. The outer membrane and plasma membrane surface areas were 3.04 ± 1.33 μm^2 and 2.67 ± 1.19 μm^2, respectively. The cell and cytoplasm volumes were 0.293 ± 0.113 fl (= μm^3) and 0.210 ± 0.091 fl, respectively. The average total ribosome number per cell was 1,672 ± 568, and the ribosome density was 716.5 ± 171.4/0.1 fl. This is the first report of a structome analysis of *M. tuberculosis* cells prepared as serial ultrathin sections following cryofixation and rapid freeze substitution. These data are based on the direct measurement and enumeration of exquisitely preserved single-cell structures in transmission electron microscopy images rather than calculations or assumptions from indirect biochemical or molecular biological data, and may explain the slow growth of *M. tuberculosis* and enhance understanding of the structural properties related to the expression of antigenicity, acid-fastness, and the mechanism of drug resistance, particularly in regard to the ratio of target to drug concentrations.

P36-049

Enzymatic Epoxidation of non-activated Alkanes: Unravelling the mechanism of an uncommon CH-activation

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Site-specific aerobic oxidation is a common process in nature which usually involves hydroxylation of unactivated alkane sites in a molecule by P450 monooxygenase or α-ketoglutarate dioxygenase.[1] An especially interesting case is the two stage aerobic oxidation of atropine (L-hyoscyamine) to scopolamine (both essential drugs according to the WHO listing)[2] by hyoscyamine-6β-hydroxylase (H6H), which includes a unique direct hydroxyl cyclization to an epoxide (Figure 1A).[3] Detailed structural and mechanistic understanding of this biochemical transformation, which remains unrealized in conventional chemical synthesis, offers the opportunity to develop modified enzyme catalysts to efficiently place epoxide groups on other types of chemically and pharmaceutically relevant hydrocarbons. Closely connected to this topic is the synthetically compelling idea to understand how unreactive non-activated alkanes can be transformed into valu-

able epoxi-structures using this enzyme. The talk or poster will focus on recombinant expression, crystallization, molecular modeling (Figure 1B) and substrate assay approaches to reveal significant structural and mechanistic insights into this unique enzyme.

P36-050
Software-independent display of structural features in biomolecules

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Display of molecular structure is essential for analysis of numerous features related to structure and function of biomolecules, drugs, etc. Therefore, molecular visualisation is a permanent companion of any presentation or discussion of results. Fortunately, progress in the experimental resolution of macromolecular structures at atomic level has tremendously improved our understanding of molecular organization and interaction. For many years now, software tools have existed for interactively displaying such structures using nonspecialised, affordable computers. These tools have typically consisted either on the installation of dedicated software in the user's computer (like Pymol) or on the use of a web browser that had been fitted with either a specific plug-in (like MDL Chime) or with the Java counterpart that allowed to run pages that embedded the viewer applet (e.g. Jmol). These solutions, although satisfactory, depended of configuring the users' machines, were sometimes limited to certain operating systems, and posed a burden in cases such as maintaining student computer labs. The situation has recently become harder due to increasing security restrictions on Java applets and the announced removal from some browsers of the plug-in architecture.

Along the last two years, a powerful alternative has been developed in the form of JSmol, a non-Java solution functionally equivalent to the widely used Jmol applet. As a result, web pages -including many database portals- can now display molecular structures without the need for any software other than the web browser. Additionally, this is compatible across browsers, operating systems and platforms, including tablets.

P36-051
Ubiquitin chain elongation by HECT-type ligases

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HECT-type ligases are large monomeric proteins that covalently attach ubiquitin (Ub) to Lys residues in substrates. Ubiquitination is a key regulatory mechanism in signal transduction and one of the structurally most complex post-translational modifications. Ub can be conjugated to its targets as a single moiety at one or multiple sites or as a poly-Ub chain that can be linked via any of the seven Lys residues in Ub or via the Ub N-terminus. This vast array of Ub modifications creates distinct cellular signals that control virtually all signal transduction pathways in eukaryotes.

To explore how HECT-type Ub ligases elongate Ub chains we have used solution-state NMR spectroscopy in combination with biochemical assays. Using our recently developed Met scanning approach we have determined key residues for non-covalent Ub binding on HECT domains. Furthermore, we have performed chemical shift perturbation studies using short Ub chains where we have specifally labeled individual Ub moeties. Our results provide important insights into the mechanism of Ub chain elongation and enzyme processivity in HECT-type Ub ligases.

P36-052
Combination of the yeast surface display and microfluidic approach to develop high-throughput platform for biocatalysts screening

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Design of the sequence-specific enzymes is intrigue task of fundamental enzymology and structural biology. The classic combinatorial approaches and methods are well developed for screening of biomolecules as binders but not catalysts. Here we present the high-throughput platform for biocatalysts screening with esterase, protease and phosphodiesterase activity. We developed the universal special vectors for biocatalysts gene expression on the yeast cell surface. The genetic construction with genes of butyrylcholinesterase, enterokinase and DNase I were cloned to special vectors and transformed into *Pichia pastoris* yeast cells, the encoded proteins were allowed to translate on the yeast surface. Expression on the cell surface provided relatively constant level of biocatalysts during activity analysis and screening procedures. Single cells with displayed biocatalysts were compartmentalized in the aqueous droplets of a double w/o/w emulsion with the fluorescent substrate by microfluidic technology. This process seems to be time and biocatalysts concentration depended, and in the assumption of relatively constant quantity of biocatalysts can be used not only for detect catalytic activity but also for screening clones with different level catalytic activity. Compartments containing the fluorescent product were sorted by FACS, and the cells imbedded in them, together with the gene encoding the enzyme of interest, was isolated. We successful selected the cells with desired activity from pool of ballast cells. Thus our robust method is also suitable for the high-throughput screening of biocatalysts mutant gene libraries.

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P36-053
Intermediate filament structure, assembly and nanomechanics

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Intermediate filaments (IFs) consist of two-stranded coiled coils that form anti-parallel, half-staggered tetramers. By time-lapse electron microscopy, complemented with total internal reflection fluorescence microscopy, we have investigated the *in vitro* assembly of vimentin to define the assembly pathway for vertebrate cytoplasmic IFs. Assembly is induced by change of the ionic strength and starts with the lateral association of tetramers to full-width unit-length filaments (ULFs) driven by the interaction of the basic, non-structured head domains with the acidic coiled-coil rods. In a next step, ULFs longitudinally anneal by an end-on-addition mechanism to yield filaments. This mechanism is also exhibited by muscle desmin and the epithelial keratins, whereas the nuclear IF proteins, i.e. the lamins, do not assemble into ULFs. In a next step, the subunit composition of ULFs and IFs of different IF proteins were analyzed by scanning transmission electron microscopy and cryo-electron tomography of native specimens. Depending on the ionic conditions used for assembly, on average keratin IFs harbor 8, vimentin IFs 16 and desmin IFs 24 coiled-coil dimers per filament cross-section. The formation of

ULFs was investigated further by small-angle X-ray scattering and analytical ultracentrifugation, employing a mutant vimentin variant that is arrested in the ULF state. With these data at hand, we investigated the impact of human mutations found in desmin that cause myofibrillar myopathy. Last but not least, we explored the network formation of lamin A and some of its disease variants, both *in vitro* and in cells, where they cause dramatic changes of the nuclear envelope.

P36-054
Structural and functional analysis of the Vitamin D receptor-DNA interactions

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Vitamin D receptor's (VDR) DNA-binding domain (DBD) effectively recognises and binds VDR response elements (REs). VDREs are mostly formed by two hexameric half-sites with RGKTCA (R = A/G and K = G/T) consensus organised to direct repeat with three neutral base pairs separating the half-sites (DR3). Apo VDR can occupy its REs also as a homodimer whereas holo VDR forms a heterodimer with retinoid X receptor (RXR).

To date there are three VDR-VDR-DNA and one RXR-VDR-DNA crystal structure available. The binding to VDREs depends on the nature DNA-protein interaction e.g. monomer binding contribution and number of contacts which largely depends on the RE sequence. We calculated these parameters using programs from the CCP4 structural bioinformatics suite. In addition to *in silico* analysis we have provided data for VDR-RE binding from *CYP24*, *CYP2B6* and *CYP3A4* gene promoters. An approximation of structural data with transactivation assays has been also performed.

The analysis shows 88, 85, and 83 contacts for rat *osteocalcin* (rOC) RE, canonical DR3 (cDR3), and mouse *osteopontin* (mSPP) RE respectively (cutoff 3.5 Å). The interacting surface ratio for VDR-monomer-DNA-binding contribution is (upstream: downstream) 38.5:61.5% for rOC, 46.2:53.8% for the cDR3 and a reversed ratio of 57.3:42.7% for mSPP. Interestingly, for RXR-VDR it is 56.2:44.8% with higher contribution from RXR. In cells apo RXR-VDR performs the best on *CYP24* RE. On *CYP3A4* it has higher constitutive but lower ligand effect (6 fold to 11 fold) compared to *CYP2B6*, where the ligand effect is double compared to the apo heterodimer.

FEBS Education Session

P38-001
A special study module in medical education: A model of scleroderma induced by bleomycin

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Special Study Modules (SSM) are integrated into the first three years of Dokuz Eylül University School of Medicine and are offered in four different fields: literature search, clinical research, laboratory research, and the lately-inaugurated social responsibility SSMs. We planned a SSM for six second-year students in the category of laboratory research entitled "A model of scleroderma induced by bleomycin".

The objectives of this SSM were to train the students in independent learning, the basic principles of scientific methodology and written and oral presentation of the results of scientific research (1). A work plan encompassing the SSM objectives, was prepared by the responsible tutors of the module, with the participation of the students (Data-base searching, formulation of hypothesis, defining the research plan and the time line, learning the laboratory techniques, realization of the mini-project, evaluation and presentation of the results). This SSM was carried out as a mini-research project according to the work plan. This is a pilot study. After finishing the project, the students prepared a written report and presented orally their results at the final of the SSMs period.

Finally, the student feedback results showed that the students faced, at the beginning, a bit of difficulty reading the scientific articles.However, they felt that they learned how to read and discuss the scientific articles, they were happy with the wet-animal laboratory, research skills that they acquired.End of this study, all of students said that understanding of the disease and its molecular mechanism is very important for the treatment.

P38-002
The effect of garlic (*Allium Sativum*) on lipid profile in rabbits

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Objective: This study was conducted to investigate the cholesterol-lowering property of garlic (*Allium Sativum*) in whole blood of egg yolk induced hypercholesterolemia in rabbits.
Methods: Forty rabbits of both sexes of 13.1 ± 28.4 weeks of age with average body weights of 1251.9 ± 512.2 g were used for the experiment.
Results: The TC analysis shows that there was no significant difference between the control and the treatment groups ($p > 0.05$). The HDL-Cholesterol analysis indicates no significant difference between the control and the treatment groups ($p > 0.05$) except the group that received 10% egg yolk + 2% garlic supplementation ($p < 0.05$). The LDL-Cholesterol analysis show significant difference exist between the control and all other treatment groups ($p < 0.05$) except the group that received 2% garlic supplementation where a decrease ($p > 0.05$) was observed. The results of TG analysis show no significant difference between the control and the treatment groups that received 10% egg yolk, 2% garlic or 10% egg yolk + 2% garlic supplementations ($p > 0.05$). However, there was significant increase ($p < 0.05$) in the TGs of the treatment groups that received 20% egg yolk, 4% garlic, 20% egg yolk + 2% garlic or 20% egg yolk + 4% garlic compared to the TG of the control group.
Conclusion: While egg yolk supplementation did not induce hypercholesterolemia; it was observed that garlic powder supplementation did not demonstrate significant hypocholesterolemic effect on the lipid profile of rabbits.
Key words: Garlic (*Allium sativum*), Cholesterol, Grower's mash.

P38-003
Blood-antioxidant status could be used as inclusion criteria for selecting volunteers for clinical trial of antioxidant supplement

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Difficulty in translating the beneficial effects of herbal antioxidants in clinical trials could be due to selection of volunteers of varying blood antioxidant status. Since progress of FR mediated pathogenesis is inversely proportional to decline in endogenous

antioxidant status especially the activity of blood SOD and catalase, so it can be used as inclusion criteria for selecting a dose in a clinical trial. We have made 4 independent experiments in rats, to test this hypothesis.

(1) High fructose diet (HFD) feeding initially raised these enzymes up to 50 days followed by significant decline in 80 days, with inverse correlation with serum TG.

(2) The cisplatin (CPZ) administration raised these activities up to 3 days without any rise in serum urea, which was reversed on 5th day).

(3) The streptozotocin (STZ) injection raised these enzymes in initial 3 days, followed by decline on later days with rise in blood glucose.

(4) In case of STZ induced diabetic nephropathy (DN) in 40 days experiment, the biphasic changes were observed in 25 days.

Thus, stage of disease and endogenous antioxidant status could be important factors for a trial dose, to have consistent results in clinical trials.

P38-004
Voluntary student research groups in medical education: Teaching teamwork

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Motivation is one of the most important concepts in education and is related to academic outcomes in medical students. In this study, students should be given the opportunity to join voluntary scientific research groups formed in the early days of medical faculty, were expected to stimulate the students' active participation in scientific research and to provide motivation that would facilitate the process of learning in basic medical sciences.

A voluntary student research group was founded in the Department of Biophysics. The study group consisted of 30 year 1 medical students at Cerrahpasa Medical Faculty. It was clearly stated that the goal offering them the chance to discover essential biophysical facts and basic scientific methods relevant to future research projects. At the end of the first year, all members of the group were noted to have progressed in terms of their active participation. Furthermore, self-confidence improved in all members. It was observed that this progress had an independent positive effect on the students' academic achievement in first phase medical education. In the light of these outcomes, at the beginning of the medical education experience will lead us to discover enthusiastic students earlier. Although lack of basic theoretical knowledge represents an important limitation at the beginning, motivating students to participate in scientific activities will be advantageous to their progress and trained group members will have the opportunity to participate in different research projects in future.We hope that the process will facilitate students' development into inspired scientists and/or well-trained medical doctors.

P38-005
Effective teaching and learning of biochemistry and molecular life sciences with action-oriented and e-learning approaches versus instructor-dominated lecture methods

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With globalization and enormous advancement in molecular life sciences (MLS) area, embracing science, technology, health, genomics, nanoscience, and increased demand for bioinformatics skills; different learning needs, recognition of individuals' knowledge, skills and competence, certification necessitated gaining additional qualifications more open to experiment with new innovative pedagogies. New forms of teaching, learning and assessment are explored, to guide educators and policy makers for increased student performance, facilitate transnational mobility of workers and learners to meet the requirements of supply and demand in the global labor market. From a wider perspective education and training systems should be designed to the demands of the knowledge society and MLS literacy similar to science literacy will facilitate competencies for industry, research, health sector and benefit society. The EU has taken action by Lisbon Strategy, Bologna Process, LLP and development of a European Qualifications Framework (EQF). The learning outcomes approach is adopted as the basis for comparison of qualifications systems with one another and with the EQF. The present study aims to introduce new pedagogies for biochemistry and MLS education by: incorporating blended learning (e-learning incorporated with traditional forms of instruction); informal learning; student-friendly/centered, action-oriented curriculum versus instructor-dominated lecture method; considering changing educators roles due to vast resources accessible by students via the internet; academic staff providing the conceptual content and framework of the program and students as co-producers; enhancement of communication and critical thinking skills of students making them more creative and motivated; incorporating high-quality demonstration-oriented and virtual laboratory tools approaches.

P38-006
False citations, false eponyms, history distortions – exemplified by the case of Michaelis and Menten

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Citation errors are not rare occurrences in the scientific literature (see, e.g., Sweetland JH (1989) *The Library Quarterly* 59, 291–304). They cover a wide spectrum from minor formal (F) errors (e.g., a wrong page number) to severe substantial (S) errors (factual misstatements or misinterpretations). This study deals with S-errors and their possible long-term consequences on thought and language of scientists. As example serves a case in the history of biochemistry: The rise of Michaelis-Menten (M-M) eponyms (M-M equation, M-M kinetics, etc.), caused by S-errors of Claude S Hudson in 1908/1909 and Hans von Euler in 1918 ff and their blind-faith repetition by later biochemists. By these errors also the myth of Leonor Michaelis and Maud L Menten as being ground-breaking pioneers of enzyme kinetics and the originators of the so-called M-M equation was born and propa-

gated – at the cost of the true founder of enzyme kinetics (and discoverer of the hyperbolic relationship between substrate concentration and initial reaction velocity), Victor Henri. The injustice done to Henri was felt and deplored by a number of authors; nevertheless, the predominance of M-M eponyms and the (unmerited) fame of Michaelis and Menten have so far stayed essentially unaffected. This study, extending a previous paper (Kühl PW (2003) *The Biochemist* 25(6), 6–7), provides new historical insights, e.g., that the century-old, ever-repeated objections against Henri's experimental technique (disregard of mutarotation and pH) are incorrect and untenable.

Remarks on misreferencing and misnaming in general and possible ways of avoiding or correcting them conclude this study.

P38-007
Developing scientific writing and integrating feedback for undergraduate biomedical students through mimicking the professional journal article review process

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It is critical for students to develop clear, logical and persuasive writing skills during their time as undergraduates. However, it is often the case that biomedical sciences students have little opportunity to develop their writing skills and receive feedback prior to their final year research project theses. To address this need, here we present our development of a semester-long group writing project which mimicked the professional journal article writing, submission and review process as the major continuous assessment project within a semester-long penultimate year proteomics course. In groups of three, students select a topic of interest then write a review using typical review journal guidelines. The teacher acts as editor then student peers act as blind reviewers of peer submissions. The students then revise manuscript according to editor and reviewer feedback for final submission and assessment. Thereby, the project incorporates group work, interaction with research literature, peer and teacher feedback together with formative and summative assessment. Furthermore, students gain a clear understanding of the scientific authoring and publishing process. A trial run of this assessment was run in early 2015 at the University of Hong Kong. Student comments are assessed quantitatively and qualitatively to measure the effectiveness of this approach to improve student writing.

P38-008
In silico column chromatography of protein mixtures as a learning tool

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We have developed a tool for directing students into virtual experimentation with the separation of protein mixtures using column chromatography. In addition, we include proposals for student quizzes, activities and assignments that need to be solved by using the simulator.

Educational aims include active learning, training in experimental design and an understanding of the principles involved in the technique. Among possible approaches we may list (a) illustrating the mechanism of separation on each kind of column matrix; (b) demonstrating the effect of pH on ionic exchange separation; (c) inquiry-based search of conditions for resolving a mixture, e.g. in analytical separation or protein purification.

There is a choice of 13 column matrices, including gel filtration/size exclusion, affinity and ionic exchange, with running buffers of different pH. Samples can be made up by mixing 3 proteins, either from a list of 13 known proteins or using "custom proteins" with pI and M_r provided by user. Alternatively, an unknown sample may be analysed (chosen from a list of premade mixtures with hidden composition).

The simulator displays progress of the 3 components as coloured bands moving along the column, as well as the recorded chromatogram with absorbance versus elution volume.

Software requirements: there is no need to install anything but a web browser; the tool will run in both computers and mobile devices, under any operating system. This virtual laboratory is freely offered under Creative Commons by-nc-sa Licence, within the Biomodel.UAH.es site.

P38-009
Microsatellite variability of Y-chromosome C-haplogroup of Kazakhs

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The DNA analysis of modern human populations can appreciable help ethnographers and historians confirm or refute their hypotheses. Ethnogenesis of Kazakhs is studied insufficient because the remains of the steppe nomadic culture (felt, leather, wood, and fur) degrade fast and not numerous written sources are often contradictory. The abstract presents the results of the study of microsatellite variability of the Kazakh Y-chromosomes, belonging to haplogroup C, taking into account the tribal affiliation (20 tribes from three tribal formation – Elder, Middle and Junior zhuses).

One hundred and eghity-four of 340 Y-chromosomes examined belong to haplogroup C (54.2 %). Thus, more than half of the Kazakh Y-chromosome pool is viewed in this study. Seventeen Y-STRs were typed using AmpFlSTR Yfiler PCR Amplification Kit (Applied Biosistems, USA) following the manufacturer's instructions: DYS393, DYS390, DYS19, DYS391, DYS385a/b, DYS439, DYS389I, DYS389II, DYS392, DYS458, DYS447, DYS437, DYS448, Y-GATA-H4, DYS456, and DYS438. A median joining (MJ) network and reduced median (RM) network was constructed using the Network 4.612.

We have identified five sources of genetic diversity, indicating that at least five groups representing haplogroup C participated in the ethnogenesis of Kazakhs. The first group was the basis for the formation of eight tribes from the Elder zhuz and Kerey tribe from the Middle zhuz, the second group – for formation of Konyrat tribe from the Middle zhuz, the third group – for formation of Alimuly tribe and Baiuly tribe from the Junior zhuz. The fourth and fifth group, in our opinion, characterizes the tolengits and Genghis Khan descendants, respectively.

P38-010
Maintaining the quality of experimental results when anlyzing the expression of gene expression in the hypoxic microenvironment in human brain cancer *in vitro*

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Hypoxia significantly influences the human tumor cells behavior via the activation of genes involved in the adaptation to the hypoxic stress and represents an important indicator of cancer prognosis. Clinical studies findings related to NDRG1 gene expression in brain cancer, the response of NDRG1 – mRNA and protein levels *in vitro* in cancer cells in form of reactive protein and mRNA bands were detected by autoradiography. Hypoxia Induced NDRG1 gene specimens detection approach in Brain Cancer includes: A) Experimental Detection Approach of the Experimental Results. Tumor cells were first cultivated *in vitro* and concentration in the hypoxia chamber followed by the Specimens extraction, quantification, quality control & molecular separation of tumor cells specimens. Further Protein or mRNA blotting and Hybridization with subsequent NDRG1 expression Image detection and documentation take place. Different stages of the experimental approach are repeated at least three times to have statistical significant results that are necessary for: (B) Evaluation of the Experimental Results; where first the films with the Detected results are scanned followed by the detection of the genes expression (which is in our case NDRG1 and HIF-1α gene and the house keeping genes or loading controls (β-actin and 18S RNA, respectively) measurement of intensities in the analyzed specimens with statistical analysis and evaluation of the obtained results. The approach as presented above was developed based on the practical and theoretical expreience of many years and can serve as an example approach and gurantees both the quality and significance of the detected results.

P38-011
Development of laboratory resource materials on RNA and gene expression experiments for beginners and non-molecular biology researchers

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Researchers of diverse fields (physiology, cell biology, pharmacology, biochemistry, biotechnology, environmental sciences), with basic molecular biology background, may need gene expression tools in their research to elucidate the mechanisms of cell signaling, cytokine activation, enzyme induction, and hormonal control. Since, their undergraduate knowledge and incompetency in molecular techniques requires further training to employ these molecular tools, a laboratory manual of RNA isolation (tiresome and laborious due to rapid mRNA degradation) was developed and tested as an educational material. Hence, they will be able to gain hands-on experience on the methodology before analyzing their valuable samples. Total RNA isolation methods were described in detail with a self-instructional module approach. Each step was defined and supported with visual images. A "troubleshooting" guide was provided to interpret gel visualization results, and contamination sources. Three well-established protocols namely, Trizol® reagent, hot phenol and guanidinium thiocyanate methods, were used for RNA isolation from common carp gill and liver tissues since no animal ethics procedures are required for fish as model organism. Best and consistent results were obtained with the first two methods. Separate modules were prepared for each protocol, and then submitted to expert opinion (four molecular biology experts and ten biology teachers/biologists) concerning content and suitability for use as an educational material. Data were collected by semi-structured interviews, encoded by two researchers, intercoder reliability calculated with Miles and Huberman's approach: .72, which led us to conclude that the materials were suitable for application in molecular life sciences training.

P38-012
Promoting and assessment of biochemistry laboratory education to national qualifications levels by referencing to EQF; comparing with other countries

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Higher education system is being restructured as National Qualification Framework by referencing to EQF. Four education workshops were held from 1988 to 2001 in Turkey dealing with the problems of biochemistry education of our universities, before 2012. "Workshop on Multidisciplinary Approach to Biochemistry Laboratory Education" was held on 3–4 May, 2012 in Ankara/Turkey with the scope of biochemistry laboratory culture, education, and what happened since 1988. The main outcome of the workshop was the necessity of a core programme for Biochemistry Laboratory training for all disciplines. The core programme was generated by the workshop participants/stakeholders. Besides, the need for developing new applications using alternative learning techniques and, resources to modern experimentation in teaching biochemistry laboratory at different educational establishments was discussed. When all was said and done we observed from the FEBS Congress education workshop in 2014 that there were small group discussions dealing with the same problems so as to what are the key practical skills, transferable skills, and employability prospects that molecular life science students should be. After our workshop done in 2012, we had one topic directly clarifying the core programme and other seven about the problems and future of biochemistry laboratory training. Within this framework, we will discuss similarities, pros and cons of our eight topics with the learning outcomes: skills, competencies and knowledge are required to improve molecular life sciences education for other countries. Finally positive steps have been taken to develop the ability to work with internalizaiton of scientific thinking and hence improved employment.

P38-013
Innovative approaches in the biochemistry courses for student education in veterinary medicine, zootechnology and biology

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In the last years the higher education in the Russian Federation has new directions for development and is transferring in the "multilevel complex system". The biological education is among the leaders in this connection not only in the classical universities, but also in the traditional medical high schools and colleges. The aim of our Department in the Moscow State Academy of Veterinary Medicine and Biotechnology is the formation of the specially qualified personnel for various veterinary centers (both, state and private enterprises), biochemical laboratories, research institutes, etc. The general and applied biochemistry courses have particular importance for student education in fields of veterinary medicine, zootechnology and biology. Therefore, the graduates should know the molecular mechanisms of physiological and pathological biochemical processes; use methods and approaches of the so called "physical-chemical biology" for the diagnosis, prevention of particular animal diseases and control of the animal treatment; use a wide range of knowledge to develop new diagnostic and therapeutic methods, testing of new biologically active substances and drugs; participate in solving fundamental and applied problems in veterinary medicine, zootechnology and biology. The post-graduate specialization "Veterinary Biochemistry" reflects the contemporary needs of science and practice for qualified specialists in the fundamental biochemistry, who would like to obtain particular knowledge in animal health and production. It also focuses on the use of biochemical knowledge in the frame of research project for development of high-quality, environmentally friendly animal production.

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P38-014
The effect of *Helicobacter Pylori* on serum lipid profile

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Introduction: *Helicobacter Pylori* (HP) is considered to have a role in many gastrointestinal tract and extraintestinal diseases. Dyslipidemia and dyslipidemia-associated atherosclerosis are the extraintestinal diseases that thought to be associated with *Helicobacter Pylori*. The aim of this study was to determine whether HP is an independent risk factor for dyslipidemia and dyslipidemia-associated atherosclerosis after evaluating all secondary causes of dyslipidemia.

Materials and methods: 109 patients with no risk factors for secondary dyslipidemia were selected and planned for esophagogastroduodenoscopy. Patient's Serum Total Cholesterol, Trigliserid, LDL, VLDL, HDL and Glucose were analyzed with Beckman Coulter AU2700 (Brea, CA, USA) and TSH was analyzed with Roche Cobas i6000 (Roche Diagnostics, Tokyo, Japan). *Helicobacter pylori* was investigated by the urease test and histological examination of endoscopic biopsies.

Results: Sixty-five of patients were detected positive for *Helicobacter Pylori*. There was no statistically significantly between serum lipid levels and *Helicobacter Pylori* positiveness (Total Cholesterol p:0,301, LDL p:0,446, VLDL p:0,626, Trigliserid p:0,661, HDL p:0,368).

Discussion: The results of many studies that consider the effect of *Helicobacter pylori* positiveness to serum lipid levels are not compatible with each other and the relationship has not been clearly demonstrated. Most of them investigated HP by non-invasive methods which have lower specificity and sensitivity and not considered fully the causes of secondary hyperlipidemia. In this study we investigated HP with invasive method and excluded patients with secondary hyperlipidemia.

Conclusion: *Helicobacter Pylori* has no effect on serum lipid levels and hyperlipidemia associated atherosclerosis but may have an impact on the development of atherosclerosis by other mechanisms.

P38-015
Modern biotechnologies' products & ethical issues

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By classical accounts, ethics is people relating to people in justice and love. Environmental ethics starts with human concerns for a quality environment, and some think this shapes the ethic from start to finish. Others hold that, beyond inter-human concerns, values are at stake when humans relate to animals, plants, species and ecosystems. Genetically modified foods raise ethical issues that are linked by happenstance as well as logic. Genetically modified organisms (GMOs), organisms in which genes from another organism are inserted into the targeted organism's DNA, have the potential to both positively and negatively affect the environment and human health. Crops have been modified for centuries by humans using selective breeding techniques, but GMO biotechnology is a more specific and rapid selection process. All those who are involved in developing the new technology, whether they are researchers in the public sector, in agrochemical or agricultural businesses or farmers, or food manufacturers and retailers need to recognise and accept a very broad responsibility to the public. Because of this we will try to explain in this study that, it is need to ensure the ethical concerns which are taken account of, that their new modern biotechnologies and products are safe or not for human consumption and avoid further harm to the environment.

Keywords: Environment, Ethics, Biotechnology, GMO, Human health

P38-016
Modern scientific education for postmodern subjects: bioinformatics

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One of the greatest difficulties in teaching-learning sciences refers mostly to rejecting mathematics and the effect this has on teaching chemistry and physics, and not on biology.

This romantic idea of biology leads to a high percentage of young people in our country to be interested in this field as com-

pared to the low percentages observed for chemistry, physics, and mathematics. Additionally, biology is the only scientific field in our country where the gender gap is not visible. These data suggest that inside an androcentric imaginary, in which sciences are not for women, biology, being less "scientific", is the most attractive to girls.

This scenario is the framework for one of the biggest problems in teaching elementary biology in our country: The broad developments that bioinformatics and genomics have had in the last decades are not being reflected in undergraduate programs much less in high school ones.

In these discourses young students and young teachers, who represent the new generations, are familiar with the genome, cloning, and even sequencing from media before seeing it at school, though the media promotes sensationalist views that are closer to science fiction than to the biology that has evolved incredibly fast in the last decades around the world as well as in our country.

In this paper we exam the role that bioinformatics has played in the educational scenario of a country on the periphery like Colombia, comparing it the experiences of some researchers in other similar periphery scenarios to centric knowledge-generating countries.

P38-018
Effects of endurance training on the serum levels of tumour necrosis factor-α and interferon-γ in sedentary men

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Physical activity could be considered one of the factors that affect the immune system status and function. To find the relation between exercise and cytokines, we examined the possible effects of an 8-week endurance training program on the serum levels of cytokines, including tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) in sedentary men. A total of 30 healthy young male volunteers were randomly divided into an endurance training group and a control group. The training group followed a specific exercise protocol (running on a treadmill for 15~30 min at 50~70% maximal heart rate) for 8 weeks and the control group did not participate in any exercise program. Venous blood samples were collected from both the groups 24 h before and 24 h and 48 h after the exercise. Repeated ANOVA was used for statistical purposes. The serum levels of TNF-α and IFN-γ were determined by ELISA. Significant ($p < 0.05$) and non-significant ($p > 0.05$) decreases were observed in the serum levels of IFN-γ and TNF-α, respectively, after the 8-week endurance training program. Our findings indicated that an 8-week endurance exercise may affect the serum levels of some inflammatory cytokines, suggesting the beneficial role of this training protocol in elderly population and people with certain conditions (inflammation of the vertebrae or other inflammatory diseases).

P38-019
Molecular epidemiology and clinical importance of TT virus infection in haemodialysis patients, South of Iran

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Patients on hemodialysis are considered to be at risk of infection by blood-borne viruses and a prevalence of Transfusion transmitted infection has been reported in patients on hemodialysis in many countries. According to the lack of data about the prevalence of TTV in Jahrom (a city in south-west of Iran), this study was conduted to investigate the molecular prevalence of TTV viremia among hemodialysis patients in this south-west city of Iran. In this cross sectional study serum samples from HCV and HBV negative 711 patients on maintenance hemodialysis for molecular prevalence of TT virus in south of IRAN, April, 2013. Serum samples taken before dialysis from each subject were tested for molecular and biochemical analysis. Some possible risk factors of TT virus infection including: age, gender, duration of hemodialysis treatment and serum aminotransferases (AST and ALT) levels were collected from each studied population. Data were analyzed by use of parametric and non-parametric analyses with SPSS for Windows. TTV infection was detected in 27.80% of the patients. In haemodialysis patients, no association was found between TTV infection and the demographic parameters (age, sex), but we found statistically significant difference were present between these groups for what concern time on haemodialysis therapy, ALT and AST levels. The prevalence of TTV infection among hemodialysis patients reported by other authors is similar to our or even higher. According to the finding of present study TTV is presented as one of probable agent of hepatitis in haemodialysis patients.

P38-020
Antiphosphatidic acid antibodies in patents with myocardial infarction

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Background: Myocardial infarction (MI) is associated with some factors including traditional factors and new founded ones. Many people may be somehow familiar to risk factors such as smoking, fatty diets and inactivity; but scientific researches open new windows to introduce us some new risk factors of MI containing anti phospholipid antibodies. To determine anti phosphatidicacid antibodies (A.Ph.A. IgM, IgG) as an etiologic factor in many physiological and pathological conditions, in patients with MI and healthy people.
Methods & materials: Ninety patients admitted in cardiology ward of peymanieh hospital of jahrom with signs of MI as case group were compared with 90 age and sex matched healthy people with no signs of MI as control group. Five millilitre of venous blood was collected from both groups, A.Ph.A IgG, IgM were determined by ELISA method in isolated sera.
Results: The prevalence of positive a.Ph.A IgG was seen in 7.80% cases and 4.40% control group ($p = 0.536$). Positive a.Ph.A IgM test was seen in 2.20% cases and none of controls ($p = 0.497$). No significant differences were not seen in prevalence of a.Ph.A IgG, IgM between patients ant healthy people.
Discussion: The results of this study indicate that a.Ph.A IgG, IgM are not as risk factor for MI. Further studies are recommended to explore possible role of a.Ph.A antibodies in ischemic diseases.

P38-021
HTLV-I prevalence in β-thalassemia children in Jahrom, Iran

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Background: Human T-Lymphotropic Virus type I (HTLV-I)) is the etiologic agent of two distinct human disease, adult T-cell

leukemia or lymphoma and a chronic, progressive demyelinating disorder. The aim of this study was to investigate the prevalence of HTLV-I among major β-thalassemia children in Jahrom, Iran.
Methods: This cross sectional study was carried out on 85 major β-thalassemia children, September 2014. All samples tested for HTLV-I specific antibody by ELISA method and positive samples were confirmed by Nested-PCR method.
Results: Of all 85 samples, 4 (4.706%) of them were positive for HTLV-I specific antibody. None of them, was confirmed with Nested-PCR for HTLV-I.
Conclusion: The result of this study shows that frequency of HTLV-I in Jahrom, is lower than other city of IRAN. Further studies with larger samples are recommended to determine the prevalence of this virus in other community.

P38-022
Human T-Lymphotropic virus type I/II virus among blood donors: South of Iran

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Background: Human T-Lymphotropic Virus type is the etiologic agent of two distinct human diseases, adult T-cell leukemia or lymphoma and a chronic, progressive demyelinating disorder. HTLV-II is associated with HAM (HTLV associated myelopathy), but is not known to cause leukemia or lymphoma. One of the major routes of HTLV transmission is parenteral transmission. The aim of this work was to investigate the seroprevalence of HTLV-I/II among blood donors in Jahrom city.
Methods: This cross sectional study was carried out on 530 blood donors from 2013 to 2014. All samples tested for HTLV (I or II) specific antibody by ELISA method and positive samples were confirmed by nested-PCR method.
Result: Of all 530 samples, 18 (3.4%) samples were positive for HTLV (I or II) specific antibody, Of 18 positive for HTLV (I or II) specific antibody just 1 of them, was confirmed with Nested-PCR for HTLV-I, which was a blood donor. There was not any HTLV-II positivity (nested-PCR) in the blood donors.
Conclusion: The results of this study show that frequency of HTLV-I/II in Jahrom is lower than other cities of IRAN. Further studies with larger samples are recommended to determine the prevalence of these viruses in other community.

P38-023
Association of anti-phosphatidylcholines antibodies with acute myocardial infarction

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Many factors play a role in Acute myocardial infarction (AMI). One those anti-Phospholipid (aPL) antibodies, that may act in the induction of immunological response leading to the development of AMI. Anti-Phosphatidylcholines (PC) antibody is detected in various diseases like rheumatoid arthritis, systemic lupus erythematosus and anti-phospholipid antibody syndrome. The study of anti-PC antibody in AMI might shed light on etiologic mechanisms in the pathogenesis of acute coronary syndromes. This study was designed to investigate whether prevalence of anti-PC antibodies, in patients who had AMI and to analyze their relationship with traditional cardiovascular risk factors. The prevalence of anti-PC IgG and IgM in a well characterized group of patients with AMI as a case group and in age and sex matched healthy subjects as control group. Sera from the case and the control groups were tested to evaluate the presence of IgG and IgM isotypes to anti-PC by ELISA method.The prevalence of anti-PC IgG and also IgM in the case group resulted significantly higher than in the control group with AMI ($p < 0.005$). Our findings suggest that anti-PC antibodies seemed to play a role in AMI, independent risk factors for AMI, which may represent a link between autoimmunity and atherosclerosis in patients with AMI. Further studies with bigger sample size including patients with AMI and healthy people are needed to explore the exact role of anti-PC antibodies in AMI.

P38-024
Transfusion transmetted virus in beta thalassemia children

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Background: Recently a novel DNA virus transfusion transmitted (TT virus) has been identified in Japan and shown to be associated with elevated aminotransferase s levels after transfusion. However the exact role of TTV in pathogenesis of liver disease is yet to be established. The aim of this study was to determine the prevalence of TTV in thalassemia patients and its relationship with elevated alanine-aminotransfrase (ALT) and aspartate-aminotransfrase (AST).
Methods: This cross-sectional analysis study was conducted on 452 thalassemia patients. Sera were collected from all of the patients, first ALT and AST levels were determined. Then, after DNA extraction, TTV DNA was amplified and detected using semi-nested PCR.
Results: One hundred and sixty of 452 (35.40%) samples had TTV DNA detected by PCR. From 160 TTV DNA positive, 98 (61.20%) were female and 62 (38.80%) of them were male ($p = 0.549$).The mean ALT and AST values in TTV positive group were higher than in TTV negative group, and the difference was statistically significant ($p < 0.0001$).
Conclusions: The result showed that the prevalence of TTV in thalassemia patients in Jahrom is less than other studies in Iran and the mean ALT and AST values in TTV positive individuals were about 2 times more than in TTV negative individuals.

P38-025
Insulin resistance and serum levels of interleukin-17 and interleukin-18 in normal pregnancy

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We performed this study to evaluate the role of Interleukin-17 (IL-17) and Interleukin-18 (IL-18) in insulin resistance during normal pregnancy. This descriptive cross sectional study was carried out on 97 healthy pregnant women including 32, 25, and 40 individuals in the first, second, and third trimesters, respectively, and on 28 healthy non pregnant women between the autumn of 2012 and the spring of 2013. We analyzed the serum concentrations of IL-17 and IL-18 by using the enzyme linked immunosorbent assay (ELISA). Insulin resistance was measured by homeostasis model assessment of insulin resistance equation. No significant differences between the demographic data of the pregnant and non pregnant groups were observed. Insulin resistant in pregnant women was significantly higher than the controls ($p = 0.006$). Serum IL-17 concentration was significantly different in non pregnant women and pregnant women in all gestational

ages ($p < 0.05$). Serum IL-18 level was significantly lower in subjects with first, second, and third trimesters of pregnancy in compared to non pregnant women ($p < 0.05$). No significant correlations were found between serum IL-17 and IL-18 levels with insulin resistance ($r = 0.08$, $p = 0.34$ versus $r = 0.01$, $p = 0.91$, respectively). Our data suggested that IL-17 and IL-18 do not appear to attribute greatly to pregnancy deduced insulin resistance during normal pregnancy.

P38-026
Hepatitis E virus and serum level aminotransferases in blood donors

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Background: Hepatitis E virus (HEV) infection is a self-limiting viral infection that can lead to severe complications and death. In different regions the epidemiology of this infection varies. In this study we evaluated the seroepidemiology of hepatitis E infection in Jahrom, a city in southern Iran.
Methods: This was a cross-sectional descriptive study of serum samples from 477 subjects, including 30 females and 447 males. HEV immunoglobulin G (IgG) and immunoglobulin M (IgM) were measured by enzyme-linked immunosorbent assays (ELISA). Alanine transaminase (ALT) and aspartate transaminase (AST) levels were also determined. Four hundred forty-seven subjects were male and 30 were female. Subjects were classified by age and sex.
Results: One woman (3.3%) and 25 men (5.5%) were positive for HEV antibodies (IgG and/or IgM). There was found an association between serum level of aminotransferases and seropositivity for HEV.
Conclusion: The result of this study indicates that HEV is an etiological factor for hepatitis in this area of IRAN. The cost benefit of active immunization in endemic regions should be evaluated because an outbreak could have tragic consequences.

P38-027
Prevalence of prediabetes and its association to cardiovascular risk factors

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Prediabetes is an important risk factor for the development of diabetes mellitus and subsequent atherosclerosis. The aim of this study was to determine the prevalence of prediabetes and established risk factors in a population based sample in Iran. In a cross-sectional study, participants aged ≥ 30 years selected using a multistage cluster sampling method. Firstly, subjects completed a detailed demographic and medical questionnaire (gender, age, history of diabetes mellitus and hypertension, taking hypoglycemic and antihypertensive agents and history of smoking). Then all participants were subjected to physical examination, blood lipid profile, blood glucose, anthropometric, blood pressure and smoking assessments, during the years 2009 and 2010. We analyzed the association between prediabetes and other cardiovascular risk factors using SPSS version 11.5 software. p-value <0.05 were considered to indicate statistical significance. Prediabetes was observed among 140 (15.7%) subjects, 17.5% for men and 14.2% for women ($p > 0.05$). The prediabetic subjects had higher prevalence of systolic hypertension, hypertension, hypercholesterolemia, high LDL-C and hypertriglyceridemia than did the normoglycemic group. Multivariate logistic regression analysis showed that hypertriglyceridemia and high LDL-C level were the strongest predictors of prediabetes [Odds Ratio (OR) = 2.25, 95% Confidence Interval (CI): 1.44–3.52, $p < 0.001$; OR= 2.14, CI 95%: 1.19–3.85, $p = 0.01$ respectively). The major determinants of the prediabetes prevalence were hypertriglyceridemia and high LDL cholesterol level. Therefore, community-based interventions and primary prevention strategies should concentrate on reducing serum lipid profiles for diminished in Iranian adults.

P38-028
Improving biotech education through gamified laboratory simulations

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Traditional teaching methods are dominating science education, but new IT-based approaches may provide an opportunity for increasing the skill level of students and motivate young people to pursue studies within the field. Laboratory teaching is limited by many practical barriers such as cost, safety and time, making it an especially relevant area for implementing simulations. In this study we show a 76 % increase in learning outcomes by using a gamified laboratory simulation (Labster) compared to traditional teaching and a 101 % increase when used in combination, suggesting an untapped potential for increasing the skills of science students and graduates.

Poster Session 1

LB-001
Can CD36 expression in peripheral blood mononuclear cells be use for atherosclerosis process?

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Atherosclerosis, characterized by the accumulation of oxidized plasma lipoproteins that carry cholesterol and triglycerides in the arteries, is one of the major causes of morbidity and mortality worldwide. In the atherosclerotic process, atherogenic lipoproteins which are modified low density lipoprotein (LDL) are up taken by receptor-mediated endocytosis mechanism. Cluster of differentiation 36 (CD36) is one of important scavenger receptor playing role in atherosclerotic process. CD36 mediates intake of ox-LDL by macrophages in the arteria walls and long chain fatty acids into the cells.

In the present study, we investigated CD36 mRNA expressions in both aortic tissues and peripheral blood mononuclear cells compared to each other and the effects of vitamin E on these changes in the atherosclerotic rabbit model induced by 2% cholesterol containing diet. In the cholesterol group, significant increase in the CD36 mRNA expressions of rabbit aorta and peripheral blood mononuclear cells was observed compared to control group. Our results demonstrated that the CD36 mRNA levels in peripheral blood mononuclear cells reflect the levels in aorta. In addition to the results obtained from rabbits, CD36 mRNA expressions of human PBMCs were tested in hypercholesterolemic conditions. CD36 expressions of PBMCs was found to be 37% higher in hypercholesterolemia group (plasma cholesterol > 200 mg/dl, $n = 10$) when compared to normocholesterolemic

control group (plasma cholesterol < 200 mg/dl, n = 10). Maybe in the near future; CD36 expression in PBMC might be used as a biomarker for detection of atherosclerosis.

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LB-002
The effects of bergapten on memory processes and antioxidant barrier

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Bergapten (5-methoxypsoralen, 5-MOP), a furanocoumarin found in many medicinal plants, has been used in combination with UV radiation in skin photochemotherapy for decades. Literature data have demonstrated that bergapten possesses slight antioxidative activity *in vitro* and exerts both anti-proliferative effects and induces pro-apoptotic responses in human breast cancer cells. Some studies have also reported its anticancer, antidepressant, anticonvulsant and anti-inflammatory effects. Furthermore, bergapten inhibits the butyrylcholinesterase and acetylcholinesterase activity, the enzymes that degrade acetylcholine.

The aim of the present study was to examine the effects of acute administration of bergapten on memory processes in the passive avoidance (PA) paradigm and anxiety-like behaviors in the elevated plus maze test (EPM) in Swiss mice. We also assessed the influence of this drug on the antioxidant barrier in brain.

Bergapten was purified by high-performance counter-current chromatography from dichloromethane extract of the fruits of *Heracleum leskovii* Grossh. We revealed that acute injections of bergapten at the dose 25, 50 and 100 mg/kg, i.p. improved processes of memory acquisition whereas the doses of 25 and 100 mg/kg improved processes of memory consolidation in the PA task. At the same time brain level of oxidative stress biomarkers, such as total antioxidant capacity (TAC) as well as antioxidant enzymes (glutathione peroxidases (GPx), superoxide dismutase (SOD)) activities were improved and lipids peroxidation level, measured as malondialdehyde (MDA) concentration, was decreased. The results of our research suggest bergapten to be an interesting therapeutical option in disorders with memory deficits.

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LB-003
Capsaicin induces autophagy in prostate cancer cells through reactive oxygen species generation

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Capsaicin is a major component of hot chili pepper and is responsible for the hot or burning sensation experienced on the contact of chili peppers. Several studies have shown chemotherapeutic effects of capsaicin against several human cancers . We have previously shown that capsaicin inhibits the growth of the androgen-resistant prostate cancer cells PC-3 *in vitro* and *in vivo*. In this study, we show that capsaicin induces autophagy both in androgen-sensitive and androgen resistant prostate cells. In these cells, capsaicin treatment induces an increase of the lipidated form of LC-3 (LC-3 II) which is a hallmark of autophagy. Capsaicin also causes an inhibition of Akt and S6 kinase both in LNCaP and PC-3 cells. Moreover, capsaicin triggers the production of reactive oxygen species (ROS) measured by flow cytometry in 2',7'-Dichlorofluorescin diacetate (DFCDA)-labelled cells. The increase of ROS can be detected from 1 h of capsaicin treatment and is considerably higher in androgen-sensitive prostate cancer cells than in androgen-resistant prostate cancer cells.

Treatment of cells with the antioxidant N-acetyl cysteine reduces the capsaicin-induced autophagy. Results indicate that capsaicin-induced autophagy in prostate cancer cells is mediated by ROS generation.

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LB-004
Conversion of the Akt overactivity in cancer cells into cell death signal with the mutant Bim BH3 peptide

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The Bcl-2 family proteins are central regulators of apoptosis. Bim is a potent inducer of apoptosis, while Bcl-2 and Bcl-X_L block cell death through their BH3 domain-binding capacity. Herein, we present that insertion of two mutations into the BH3 domain of human Bim can convert the prosurvival activity of Akt often hyperactivated in cancer cells into the cell death activity. It was done by creating the Akt recognition sequence (RxRxxT/SF; T/S, phosphorylation site) in the Bim BH3 domain with the introduction of I155R and E158S mutations. The mutant Bim BH3 peptide interacted with Bcl-2 and Bcl-X_L weakly (KD of ~200 nM) than the wild-type did (KD of ~10 nM). However, phosphorylation of Ser158 (Akt target residue) restores its Bcl-2 and Bcl-XL binding affinity. Crystal structure of Bcl-X_L bound to the phosphorylated mutant Bim peptide showed that phosphorylated Ser158 interacts with two Bcl-X_L residues and thus contributes the complex formation, but the unphosphorylated residue does not. In the PTEN-null PC3 tumor cells, this mutant Bim peptide exhibited cytotoxic effects, as this peptide was phosphorylated by hyperactivated Akt and then interacted with and inhibited antiapoptotic Bcl-2 and Bcl-X_L. Remarkably, the cell-killing activity of the peptide was not shown in wild-type cells with the normal Akt activity or in PTEN-expressed PC3 tumor cells where the cellular Akt activity was attenuated. These results provide a new approach for the development of a novel drug candidate that is inactive in normal cells but transformed into an apoptogenic agent in Akt-hyperactivated cancer cells.

LB-005
Next generation sequencing of ancient DNA for the identifying of the *Mycobacterium tuberculosis* genome in human remains

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Biomolecular approach, specifically the study of ancient DNA (aDNA), has enormous potential in the study of palaeopathology or the history of disease. Tuberculosis (TB) is a re-emerging infectious disease, that infects ⅓ of the world's population. If untreated, *Mycobacterium tuberculosis*, the causative agent of the disease, spreads to the bone via the blood and lymphatic systems, thus changes in the spine most often being used for diagnosis in palaeopathology.

The aim of the study was to obtain TB-positive aDNA samples from human remains from the 15th–17th centuries for studying of evolution of the causative agents of TB in Latvia.

Towards this aim, careful examination of available skeletons from a Medieval Latvian cemeteries was performed, bone samples with visible tuberculous changes were selected and used for aDNA isolation. As an initial screening for the presence of *M. tuberculosis* aDNA, the Next Generation sequencing (NGS) of the full microbiome of the samples was used by the IonTorrent technology. In parallel, PCR and sequencing targeted the repeat elements IS6110 of *M. tuberculosis* genome was performed to ensure the selection of aDNA samples for the downstream procedures aimed to identify a detailed genotype for a historic strain of *M. tuberculosis* from an individual.

Our results show that NGS technology is useful tool to study the presence of *M. tuberculosis* genome in human remains and obtained data potentially enabling comparisons between *M. tuberculosis* strains from different geographic locations and time frames.

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LB-006
Correlation between mitochondrial DNA (mtDNA) copy number and telomere length in different age groups

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Introduction: In the late years there are studies, which claim that mtDNA and telomeres are connected in the process of ageing. It has been suggested that p53 induced by telomere dysfunction and PGCs repressed by the p53 or directly by telomere dysfunction act as potential pathophysiologic mediators between telomere dysfunction and mitochondrial compromise. It has been shown that mtDNA copy number correlates with telomere length (TL) in elderly women's leukocytes (Kim et al. 2013).

Methods: One hundred and thirty samples, three age groups: 20–40; 60–89; >90 years old. Telomere length was detected using the TeloTAGGG Telomere Length Assay kit (Roche). Relative copy number was detected using qPCR, TaqMan assay. Statistical analyses – GraphPad Prism 5 Software.

Results: Relative mtDNA copy number value was different for each age group. For 20–40 age it was 1.40, for 60–89 – 1.2, for, >90 – 1.43. TL became shorter with each next age group. There was a strong correlation between mtDNA copy number and TL, when samples from all age groups were analyzed together ($p = 0.0008$). But after divided into the three groups, the correlation was not observed for the age group 60–89 ($p = 0.3261$), but the 20–40 and >90 age groups show the correlation $p = 0.0096$ and $p = 0.0184$, respectively.

Discussion: As aforementioned, in other previous studies, these results prove that for centenarians' mtDNA copy amount increases. It seems that telomere function can interact with mitochondrion function for centenarians by maintaining each other functions/interactions.

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LB-007
Pro-angiogenic functions of Arginine-Glycine-Aspartate-containing osteopontin icosamer peptide via interacting with avb3 integrin

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Osteopontin (OPN) is a phosphorylated glycoprotein and contains arginine, glycine, aspartate (RGD)-motif, through which it binds to several cell surface integrins, mediating a wide range of cellular processes, such as, the adhesion, migration, and survival. In the present study, we examined the pro-angiogenic effects of a RGD-containing 20 amino acids OPN peptide (OPNpt20). Pro-angiogenic effects of OPN icosamer (OPNpt20) was examined in HUVECs and in a rat model of focal cerebral ischemia, induced by middle cerebral artery occlusion (MCAO). We found that OPNpt20 exerts a robust pro-angiogenic effect in HUVECs, including proliferation, migration, and tube formation. OPNpt20 also induced blood vessel formation in a Matrigel plug assay in mice. However, a mutant peptide (OPNpt20-RAA), in which RGD was replaced by RAA, failed to activate all of pro-angiogenic processes, indicating that the RGD motif is required for its pro-angiogenic effect. In OPNpt20-treated HUVECs, PI3K/AKT signaling was activated. Moreover, blocking avb_3 integrin by antibody or treating OPNpt20 after pre-incubating it with avb_3 integrin suppressed OPNpt20-mediated pro-angiogenic function, indicating that OPNpt20 stimulates angiogenesis via avb_3/PI3K/AKT signaling pathway in HUVECs. Pro-angiogenic function of OPNpt20 was further confirmed in the postischemic brain, wherein significant inductions of RECA-1 immunoreactivity as well as angiogenesis-associated proteins, such as, VEGF, MMP-9, and smooth muscle actin, were also observed in cortex penumbras of OPNpt20-administered animals. Together these results demonstrate that RGD-containing OPN peptide has a robust pro-angiogenic effects and it might contribute to a robust neuroprotective effects in the postischemic brain.

LB-008
Comparative analysis of serum peptides detected in samples from healthy persons and colorectal cancer patients

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More than a million people a year worldwide develop colorectal cancer (CRC).Most of the CRC cases are sporadic, only 25% of

the patients have a family history of the disease, and major genes causing syndromes predisposing to CRC only account for 5–6% of the total cases.

The aim of the present work was a search and identification of peptide markers of CRC in sera using modern mass spectrometry techniques.

Blood sera obtained from 50 patients with CRC and 50 healthy donors (control). Serum sample of each analyzed group were fractionated using magnetic beads with weak cation exchange surface, obtained eluates were analyzed by nanoLC-MS/MS using ABSciexTripleTOF 5600. All samples were analyzed by DDA (identification of serum peptides) and by SWATH (for label-free relative quantitative mass spectrometry analyses) approaches.

As a result of LC-MS/MS analysis of sera more than 6000 unique peptides originated from the almost 1000 unique proteins were identified. Among identified peptides 786 were unique for CRC samples, and 125 of those were originated from the proteins unidentified in the control samples. For the control group there were 1075 unique peptides, 259 of which were originated from the proteins unidentified in CRC samples.

Also, our analysis allowed us to identify protein–protein interactions, responsible for various cellular processes, and to identify possible ways development of pathological states at molecular level, protein–protein interactions, responsible for various cellular processes, and to identify possible ways development of pathological states at molecular level.

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LB-009
Transcription activity of angiogenesis factors in patients with limb ischemia

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Limb ischemia is one of the angiosurgical sudden events, that result is always either a partial or complete ischemia, which threaten viability of the affected limb. The objective of presented study was to analyse the effect of ischaemia and reperfusion by measuring autofluorescence of blood and analyse IGF, HIF and VEGF molecules in the ischaemia damaged part of the patient limb blood vessel tissue by using qRT-PCR. Acute ischaemia of limbs was analysed by increased fluorescence of blood patients. Measurements of fluorescence were run using the wavelength range 450–490 nm, specific for endogenous cofactor $NADH+H^+$. Spectroscopic double peak can indicate ischaemic changes of serum because reduced pyridine nucleotide $NADH + H^+$ is formed in absence of oxygen. Comparison of mRNA expression levels of genes of IGF-2, VEGF-A, and HIF-1 in the experimental group of patients with limb ischemia compared to subjects in the control group showed that the gene expression of HIF is the most significantly increased to 197% on the level $p < 0.001$, which demonstrates a lack of oxygen – ischemia. Lack of oxygen activates the production of proangiogenic factor HIF. Increased production of HIF activates angiogenesis and VEGF, which was significant increase to 127%.

The benefit of the experimental results of changes to the presence of ischemia is that we first define spectrofluorimetrically complex biological material as serum and plasma *in vitro* by using the synchronous fluorescence fingerprint analysis, which correlate with molecular analyse results.

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LB-010
MCPIP1 is involved in regulation of IL-6 expression in hypoxia

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Hypoxia is important factor in the development of several types of malignancies including cancer. In our study we analyzed the effect of efficiency of oxygen supply on expression of selected genes involved in regulation of inflammatory pathways in Clear Cell Renal Cell Carcinoma cell line, Caki-1. We devoted special attention to MCPIP1 protein which has RNase properties and regulates the half-life of transcripts encoding pro-inflammatory cytokines.

Low oxygen supply (1%) caused a decrease of MCPIP1 protein level. HIF1α served as a positive control for hypoxia treatment and its level was significantly increased in Caki-1 cells. As shown previously in other cell types, mRNA levels for hypoxia markers: vascular endothelial growth factor (VEGF) and glucose transporter 1 (GLUT-1) were up-regulated by hypoxia in Caki-1. We noted that interleukin 6 expression was also upregulated by hypoxia. MCPIP1 is known regulator of IL-6 mRNA stability. Therefore we decided to overexpress MCPIP1 in Caki-1 cells. Increased level of MCPIP1 was correlated with decreased expression of IL-6 in both normoxia and hypoxia. Moreover MCPIP1 overexpresion completely diminished stimulating effect of hypoxia on IL-6 transcript level.

Our results indicate that MCPIP1 is directly responsible for regulation of IL-6 transcript and its downregulation in hypoxia is necessary for IL-6 mRNA level increase.

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LB-011
Switching off G-protein coupled receptor 143 (GPR143)

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GPR143 mutations result in ocular albinism type I, an X-linked form of albinism characterized by developmental eye defects. Histological analysis showed dysfunctional melanosome biogenesis resulting in macromelanosomes in skin melanocytes and retinal pigmented cells (RPE), when GPR143 is not expressed or mutated. GPR143 is mainly expressed in pigment cells and exclusively localized at membranes of melanosomes, late endosomes and lysosomes in contrast to most other GPCRs, which are located at the plasma membrane.

Since the affinity of L-DOPA, the proposed endogenous agonist (Lopez *et al.*, 2008) for the receptor is not suitable as pharmacological tool for analyzing the GPR143, we generated a

stable cell line expressing a mutant GPR143 at the plasma membrane suitable for high-throughput screening of ligands.

GPR143 has been shown to interact with β-arrestin, therefore we established a β-arrestin assay for screening of compounds. Since one hypothetical function of GPR143 is to be a sensor for melanosomal maturation, we screened for inverse agonists that "switch off" the receptor's high constitutive activity.

Here we present a library screen using the β-arrestin assay. The most potent hits were further tested in *melan-a* cells, immortal mouse melanocytes where GPR143 is regularly expressed. The natural pigmentation of *melan-a* cells was dose-dependently reduced by some hit compounds, reproducing the phenotype found in patients with loss-of-function mutations of GPR143. Since the compounds display no direct effect on tyrosinase activity, the main enzyme involved in melanin production, we hypothesize that these compounds "switch off" GPR143 activity, indirectly influencing the pigmentation pathway.

LB-012
The effect of Zidovudine (AZT) on autophagy in C2C12 myocytes

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Introduction: Zidovudine (AZT), a nucleoside reverse transcriptase inhibitor (NRTI), is currently used to prevent mother-to-child transmission of HIV-1 and is part of the WHO HIV-1 treatment guidelines. However, it has been associated with adverse reactions such as liver steatosis, cardiomyopathy and myopathy. We have previously demonstrated the effects of AZT on autophagy in primary hepatocytes which was associated with the above-mentioned side effects, manifesting as accumulation of mitochondria with increased ROS production.
Methods: Fluorescent microscopy and flow cytometry was used to measure autophagic flux in C2C12 cells. Mitochondrial mass and reactive oxygen species (ROS) were also assayed.
Results: AZT causes an accumulation of autophagosomes and mitochondria in C2C12 myocytes. It also increases ROS production over time.
Conclusion: AZT affects autophagy and mitochondria even at physiological concentrations. We would like to further validate our findings in other *in vitro* cell lines and elucidate the significance of this effect in detail.

LB-013
Iron supply to plants using an easily reducible artificial microbial siderophore

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Iron is one of essential metal elements for plants as well as animals to live. There are two iron uptake systems on plants: one is a reductive mechanism, which an iron ion is directly transported to a cell interior through an iron transporter, IRT1, after reduction of ferric species to ferrous species by a membrane-bound reductase, FRO2 (Strategy I). Another is an intact uptake mechanism, which a ferric phytosiderophore complex is transported to a cell interior through a transporter, YS1 (Strategy II). Similar system to Strategy I are also found in some microorganisms but plants cannot take up iron from ferric microbial siderophore complexes because of their highly negative redox potentials (–400 ~ –750 mV versus NHE). We had developed some artificial microbial siderophores before and found that one of them, tris[2-{(N-acetyl-N-hydroxy)glycylamino}ethyl]amine (TAGE), was more easily reducible than ferric natural siderophore complexes.[1] In this study, iron supply ability to plants by this artificial microbial siderophore has been investigated using hydroponic culture.

Giving Fe(III)-TAGE to a grape tomato that made iron-deficient, TAGE was reduced on the root of a grape tomato and the amount of chlorophyll and iron in its shoot and root was recovered, which was comparable to those of a popular chelator as iron agents for plants, EDTA. Since they have similar redox potentials (–230 mV (Fe(III)-TAGE), –200 mV (Fe(III)-EDTA)), this result suggests that Fe(III)-TAGE can give iron to a plant through Strategy I.

Reference

[1] Matsumoto K. et al., *Inorg. Chem.* **2004**, *43*, 8538-8546.

LB-014
Interleukin-6 suppresses NK cell activity in peritoneal fluid of patients with endometriosis via regulation of SHP-2 expression

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Endometriosis is known to be related to a defect in natural killer (NK) cell cytolytic activity. Additionally, the levels of inflammatory cytokines are elevated in the peritoneal fluid (PF) of women with endometriosis. However, cytokines that contributes to the decreased NK cell cytolytic activity in the PF of endometriosis patients have not been determined. Therefore, we investigated the effects of PF on the differentiation and functional activity of NK cells in patients with or without endometriosis and determined cytokines that reduce NK cell cytolytic activity in endometriosis patients. PF from patients with endometriosis suppressed the differentiation and cytotoxicity of NK cells compared with PF from controls. Increased levels of interleukin-6 (IL-6) were also found in the PF of patients with endometriosis, and IL-6 level was negatively correlated with the cytolytic activity of NK cells. Furthermore, IL-6 reduced the cytolytic activity of NK cells, concomitantly with the downregulation of granzyme B and perforin, by modulating Src homology region 2-containing protein tyrosine phosphatase-2 (SHP-2) expression. Importantly, the addition of anti-IL-6 to the PF of endometriosis patients restored the activity of NK cells.

These suggest that IL-6 plays a crucial role in the reduction of NK cell activity in the PF of patients with endometriosis by downregulating cytolytic granule components through the modulation of SHP-2 expression.

LB-015
DNA polymerase gamma polymorphisms in a healthy population

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DNA polymerase gamma (PolG) is the only DNA polymerase involved in the replication and maintenance of human mitochondrial DNA. PolG polymorphisms are associated with a wide range of mitochondrial pathologies affecting the nervous system, skeletal musculature, the liver and male reproductive organs,

many of which lead to reduced lifespan and childhood deaths. Experiments with partial PolG knock-out rat models have shown symptoms of premature aging.

The goal of our study was to screen a sample of healthy population for mutations in the exonuclease domain of the *POLG* gene to determine the prevalence of *POLG* polymorphisms in the general population. We sequenced the exonuclease domain of the *POLG* gene of 165 healthy individuals from Latvia in three age groups (20–45 y.o., 65–75. y.o. and over 85 y.o.) with no know history of neural or muscular pathologies. We also tested for heteroplasmy in the HVS I region of the mitochondrial DNA to compare the impact on mtDNA of different *POLG* polymorphisms, and determined the participants mitochondrial haplogroups.

Only one sample contained a polymorphism in a heterozygous state, corresponding to the missense mutation G268A in PolG, associated with progressive external ophthalmoplegia.

The lack of polymorphisms in PolG could be related to its important role in human cell function. Further research is required to screen the other domains of the *POLG* gene.

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LB-016
Inverse-agonistic mechanisms of thioridazine on Gi protein-coupled D_{2L} dopamine receptors

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G-protein-coupled receptors (GPCR) are the largest superfamily of the signaling molecules. The D_{2L} receptor ($D_{2L}R$) is a subclass of D2-type dopaminergic GPCR and α-subunits of their Gi proteins (Gαi) inhibit the adenylcyclase (AC) enzyme nearby. Overexpression of Gi protein-coupled $D_{2L}R$ in the mesocorticolimbic area of the brain causes schizophrenia although the downregulation or dysfunction of them in the Corpus Striatum area of the brain causes the Parkinson Syndrome through the inhibition of AC enzymes. It causes the inhibition of striatopallidal neurons, whose key role is to stop locomotor behaviors.

The activation mechanisms of Gi protein-coupled receptors by inverse-agonists are still poorly understood despite extensive work in the field. Thioridazine is an antipsychotic (or neuroleptic) drug, which is specifically used in schizophrenia and bipolar disorders. It is also an inverse agonist of D_{2L} dopamine receptor.

In this study, our aim is to elucidate the interaction mechanisms of thioridazine to design more $D_{2L}R$ specific drugs, which have less side effects. For this purpose we investigated inverse-agonistic mechanisms of thioridazine by homology modeling and explicit solvent simulations of human $D_{2L}R$ in complex with a Gi-coupled protein (including the αi, β and γ subunits). The complex receptor was implanted in a membrane system including phosphatidylcholine, phospatidylethanolamine and cholesterol developed by CHARMM-GUI membrane builder.

Our preliminary results indicate that thioridazine favors inactive-state conformation of $D_{2L}R$ by weakening specific interactions between Gα(i) and $D_{2L}R$. It possibly prevents inhibition of its effector and stops the activation of $D_{2L}R$, which have roles in psychiatric and neurodegenerative diseases.

LB-017
Apoptosis induction by 3′,4′-dibenzylflavonol in leukemia cells

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Introduction: In the present study we synthesized a series of flavonols and methylether derivatives, evaluated their effects on viability of three human leukemia cell lines and examined whether the most potent induces apoptosis.

Methods: Flavonoids were obtained by a combination of a Claisen-Schmidt condensation of 2-hydroxyacetophenones and benzaldehydes followed by a cyclization. Cytotoxicity against HL60, U937 and Molt-3 cells was evaluated by the MTT assay. Apoptosis was determined by fluorescent microscopy, DNA fragmentation and flow cytometric analysis. The cleavage of procaspases, cytochrome *c* release and the activation of the mitogen activated protein kinases were studied by western blot. Reactive oxygen species were determined by flow cytometry.

Results: A series of 74 flavonoids were obtained by organic synthesis. Cytotoxicity assays on human leukemia cells revealed that 3',4'-dibenzylflavonol was the most potent of the flavonoids assayed (IC_{50} < 1 μM) and it was 50-fold more toxic than the naturally occurring flavonol quercetin. This compound induced G_1 phase cell cycle arrest and it was a potent apoptotic inducer. Cell death was (i) mediated by the activation and the cleavage of initiator and executioner caspases; (ii) prevented by the pan-caspase inhibitor z-VAD-fmk; (iii) associated with the release of cytochrome c and with the phosphorylation of members of the mitogen activated protein kinases including p38, JNK/SAPK and ERK, and (iv) through a mechanism independent on reactive oxygen species generation.

Conclusion: 3',4'-dibenzylflavonol is a potent cell death inducer and might be useful in the development of new strategies in the fight against cancer.

LB-018
Dehydrodipeptidase 1 expression triggers invasive activity to regulate the EMT/MET switch in colorectal cancer

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Dehydrodipeptidase 1 (DPEP1/EC3.4.13.19) is a zinc-dependent metalloproteinase that the candidate novel marker of colorectal cancer based on an analysis of a gene expression microarray. However, functional roles and mechanism of DPEP1 in metastasis has not been elucidated. In this study, we showed that transcriptional and translational expression level of DPEP1 increase in stage-dependent colon tissues and cell lines, compared with non-tumor tissues. Increased invasiveness and adhesion but not cell proliferation were observed in SW480 (SW480-DPEP1) and HCT-116 (HCT-116-DPEP1) cell lines stably transfected with DPEP1 cDNA, in opposite with DPEP1 siRNA treatment. We also investigated that DPEP1-overexpressing cell lines exhibited increased metastatic activity in a xenograft nude mouse model. Interestingly, expression level of DPEP1 was decreased by TGF-b1 treatment in DPEP1 overexpressed cells but increased Leukotriene D4 (LTD4) secretion and E-cadherin such as epithelial-mesenchymal transition (EMT) regulator. Increased LTD4 in

TGF-b1-induced SW480-DPEP1 cells were increased invasiveness through attenuated GSK3b phosphorylation, b-catenin rather than TGF-b1-induced SW480 cells by Western blot analysis. TGF-b1 promoted the ubiquitination of DPEP1 and thus decreasing DPEP1 levels were facilitated epithelial-to-mesenchymal transition and the mesenchymal-to-epithelial transition (EMT/MET) switch. Taken together, DPEP1 can promote metastatic activity through TGF-b1-induced LTD4 signaling pathway and our findings may identify molecular players behind the elusive switch that drives the EMT/MET.

LB-019
Live-cell and *in vitro* analysis of p53 interactions

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Dysregulation of protein–protein interactions between the tumor suppressor p53 and its binding partners is implicated in the pathogenesis of various cancers. Here we describe novel assays for analysis of p53 interactions.

To evaluate putative inhibitors of protein-protein interactions between p53 and its negative regulators Mdm2 and Mdm4, we recently developed two comparative live-cell Fluorescent-Two Hybrid (F2H) assays. The F2H principle is based on a tethering strategy: the GFP-tagged protein (here p53) is enriched at the protein interaction platform of the engineered F2H-BHK cells and serves as bait, whereas the RFP-tagged protein serves as a prey (here Mdm2 or Mdm4). By performing p53:Mdm2 and p53:Mdm4 F2H assays side-by-side, we could evaluate the dual inhibitory activity of the previously published stapled peptides. Furthermore, since F2H allows visualization of the dynamics of protein-protein interactions, we could compare the compound's kinetics with real-time imaging. We performed a mutant analysis with F2H and showed that several Nutlin-resistant mutants of Mdm2 are sensitive to inhibition with stapled peptides sMTide-02 and sMTide-02a.

For *in vitro* validation of p53 interactions, we developed novel p53 immunoprecipitation reagents. We employed the single-domain antibody technology in conjunction with phage display to isolate two specific anti-p53 VHHs (also termed nanobodies) from immunized alpacas. When conjugated to agarose beads, these VHHs serve as highly efficient pull-down reagents (p53-Traps), specific exclusively against N- and C-terminus of p53 respectively.

Taken together, we developed a toolbox for analysis of p53 interactions both biochemically and by fluorescence microscopy.

LB-020
Active learning with clickers in small classroom in Enzymology courses

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Audience response system (ARS) or clickers are pedagogic tools to test the knowledge of students during a classroom. The students are encouraged to discuss with others their point of views to convince their neighbors to vote like them. The goals are to enhance the intrinsic motivation of students and stimulate group processes.

Here is shown the test of ARS in a small classroom in enzymology courses for undergraduate student in Life Sciences. The main qualitative results show: 1) a more interactive classroom, 2) students more interested and satisfied and 3) new questions for the teacher compared to class without ARS. As a result, students make the synthesis of the courses and understand the objective of the class. The main quantitative results show: 1) 100% of student's participation and 2) the increase of the grade compared to a classroom without ARS. Learning by their peers is here a good way to understand the link between the lesson and exercises and make positive connections between the students.

LB-021
Expression levels of microRNA genes in HCC patients

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The understanding of HCC pathogenesis is important to develop effective prevention and treatment measures of this highly malignant cancer. An early identification of changes at the epigenetic level may be beneficial in this aspect. A group of small noncoding functional RNAs (microRNAs) is one of the factors which control changes in cell phenotype at the epigenetic level The aim of the study was to identify microRNAs as prognostic molecular markers, useful in early identification of differentiation, and progression of liver tumors, the panel including 12 microRNA genes (*miR-21, miR-224, miR-34a, miR221, miR-222, miR-106, miR-303; miR26a/b, let-7 g, miR-122, miR-422b, miR-145, miR-199*)

The expression levels of 12 microRNA genes (*miR-21, miR-224, miR-34a, miR221, miR-222, miR-106, miR-303; miR26a/b, let-7 g, miR-122, miR-422b, miR-145, miR-199*) were estimated by the RQ-PCR method in paraffin specimens from 100 normal and 100 HCC patients.

An evaluation showed the highest expression level of miR 21, 224 (three times) miR 9 and 222 (two times), as compared to the controls but the expression level of miR 145, 214 was 1.5 times lower.

Conclusion: The observed changes in expression profiles of the examined microRNAs may trigger studies on bigger groups of patients and allow for identification and precise determination of liver tumor type and progression.

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LB-022
Renal cell cancer stem cells as targets for molecular medicine

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Renal cell carcinoma (RCC) represents important medical challenge, since approximately 42,000 RCC cases are diagnosed in Europe annually. As much as 30% of newly diagnosed patients are metastatic with < 2 year expected survival. One of hypotheses suggests that metastases develop from cancer stem cells (CSCs)

that are known to be resistant to chemo- and radiotherapy. In RCC stem cells have been primarily suggested as CD105(+) and RCC progenitor cells as CD133(+).

We aim to investigate presence of CD105/133 subpopulations in RCC *in vitro* model and further characterize these cells. Moreover we investigated the of abundance of RCC-CSCs in primary tumour samples.

Primary and metastatic RCC cell lines were analyzed for CD105/CD133 subpopulations. RCC-CSC candidates were screened for mesenchymal/cancer stem cell markers: CD24, CD146, CD90, CD73, CD44, CD11b, CD19, CD34, CD45, HLA-DR and alkaline phosphatase. Clonogenic potential and proliferation rate under normoxic (21% O2) and hypoxic (2% O2) conditions were verified. Activity of tyrosine kinase inhibitors (sunitinib, sorafenib, axitinib) against RCC-CSCs was measured. Finally 270 cases of RCC tumors (from nephrectomy) were screened for CD105/CD44 cells.

CD105(+) and CD133(+) subpopulations are found in primary and metastatic cells. Higher numbers of positive cells were found in metastatic cases. CD105(+) were also found in cohort of RCC patients. RCC-CSC are targeted by TKI, but this activity is modulated by oxygenation. This phenomenon may represent self-limiting mechanism of TKI activity. CD105 (endoglin) may represent ancillary treatment target in metastatic RCC.

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LB-023
Involvement of mTOR signaling and unfolded protein response in cisplatin resistance induced by hypoglycemic condition

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The critical hurdle of curing cancer and a major cause of cancer recurrence is resistance to anti-cancer therapies including chemotherapy and anti-cancer immunotherapy. One of characteristics of tumor environment is poor vascularization which results in hypoxia and nutrient deprivation. It has been reported in many studies that hypoxia promote cancer cells to acquire the resistance to various anticancer therapies. However, it still remains unclear whether and how nutrient deficiency contributes the development of resistance to anticancer therapies. In this study, A549 human lung adenocarcinoma cells were cultured in the medium containing different low concentrations of glucose or FBS for long-term periods. The number of cell cultured in nutrient-deficient conditions was decreased by 15 to 20 percent of control cell number in acute phase (until 1 to 2 weeks) and thereafter gradually recovered up to 80 to 100 percent in subacute phase (2 to 12 weeks). Protein level of TCTP, an anti-apoptotic protein, was markedly reduced in acute phase of nutrient-deficient condition and gradually restored and comparable to control cells in subacute phase. In low glucose condition, cellular stress responses such as mTOR signaling and unfolded protein response (UPR) were reduced in acute phase but markedly increased at 12 and 35 weeks. In A549 cells cultured in hypoglycemic conditions for 12 and 35 weeks, acquired cisplatin resistance was observed. Together, it was suggested that TCTP, mTOR signaling and UPR may be involved in hypoglycemia-induced cisplatin resistance.

LB-024
Biochemical investigation of radioprotective role of para Amino Propiophenone on the hormones and Alkaline Phosphatase of mouse spermatogenesis

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The side effects of radiotherapy have led to the development of radioprotectors that can be delivered before the time of radiotherapy to survive the cells. The present study has been undertaken to investigate the radioprotective effect of PAPP against radiation-induced testicular impairment in wistar mice by evaluating the values of alkaline phosphatases, FSH, LH and serum testosterone in the testes. The testis FSH, LH, alkaline phosphatase (AlP) and Serum level of testosterone were measured by an immunoenzymatic method with an ELISA reader. The results show that the amount of alkaline phosphatase has increased. However, the level of LH, FSH and testosterone has decreased following 2 Gy γ-irradiation comparing with controls ($p < 0.05$). Degeneration of Leydig cell resulted in decreased synthesis of testosterone, which in turn disturbs the process of spermatogenesis. Also Gamma radiation may be inhibited the spermatogenic process hence the unutilized alkaline phosphatase was increased in the testis.

LB-025
Molecular activity of a new potential anticancer drug OAT-449

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A number of anti-mitotic drugs induce tumor cell death through interaction with tubulin, but most of them are natural products that have complex chemical structure and poor pharmacological properties. Therefore, identification of novel, more effective microtubule-targeting compounds is particularly important. Here we describe OAT-449, a novel, low molecular weight microtubule-destabilizing compound with a relatively simple chemical structure. OAT-449 strongly inhibits polymerization of purified tubulin and disrupts microtubule formation in flow cytometry assay. OAT-449 induces cell cycle arrest at G2/M transition, leading to apoptosis in HT-29 and HeLa human tumor cells. Confocal microscopy revealed that OAT-449 disrupts cellular microtubule networks and induces cell death through mitotic catastrophe, as well as through apoptosis, with nuclear condensation and DNA fragmentation. In addition we showed efficiency of OAT-449 in activation of cell death in Multimodeling studies involving 7 different types of cancer cell lines revealed that OAT-449 induces cell death with EC_{50} values ranging from 6 to 100 nM. Taken together, these findings indicate that OAT-449 targets microtubules in different types of cancer cells and exhibits strong cytostatic/cytotoxic effects. Further evaluation of *in vivo* efficacy of this compound in animal tumor models is warranted.

LB-026
Introducing lipid rafts in pathogenic bacteria with its characterization in *Bacillus anthracis*

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Lipid rafts are dynamic assemblies of specific proteins and lipids, distributed heterogeneously on membrane. Flotillin-1, a conserved raft marker protein in eukaryotes plays a significant role in cellular processes. It comprises of characteristic N-terminal SPFH domain and C-terminal oligomerization domain with coiled-coil region. The domain showing highest identity to above SPFH has been designated as SPFH2a in prokaryotes. In this study, presence of above sequence encoded features of raft marker protein was examined in all pathogenic bacteria (PB). Analysis of 300 pathogenic strains revealed *Bacillus thuringiensis* and *Bacillus anthracis* (*BA*) appeared to be better candidates for microdomain investigation in PB.

Overwhelming threat of bioterror across the globe led us to further investigation of *BA BAS0525* encoding FlotP. *In silico* and *in vitro* analyses shows its identity to eukaryotic Flotillin-1. *In vivo* studies revealed FlotP as a membrane protein restricted to Detergent Resistant Membrane (DRM) fractions, favoring its presence in lipids and signaling proteins rich regions. Heterogeneous distribution of FlotP was observed on membrane in punctuate manner. Constitutive expression of FlotP at RNA and protein levels suggested its critical role in vital cellular processes. Simultaneously, we also observed the effect of various sterol inhibitors on membrane rigidity as well as signalling responses. All of these features cumulatively appear to favor eukaryotic microdomain kind of entity. This is the first report of any raft marker protein in PB of global concern. It is likely to provide an attractive approach to control bacterial infections by targeting lipid microdomains in Flotillin harboring pathogens.

LB-027
Phosphate starvation enhances the pathogenesis of *Bacillus anthracis*

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Identifying the factors responsible for survival and virulence of *Bacillus anthracis* within the host is prerequisite for the development of therapeutics against anthrax. Host provides several stresses as well as many advantages to the invading pathogen. Inorganic phosphate (Pi) starvation within the host has been considered as one of the major contributing factors in the establishment of infection. Here, we report for the first time that Pi fluctuation encountered by *B. anthracis* at the different stages of its life cycle within the host, contributes significantly in its pathogenesis. In this study, Pi starvation was found to hasten the onset of infection cycle by promoting spore germination. After germination, it was found to restrict growth but favored cell elongation which might be one of the many reasons for the antibiotic tolerance of the pathogen. Interestingly, phosphate starvation enhanced the pathogenicity of *B. anthracis* by augmenting its invasiveness in macrophages *in vitro*. *B. anthracis* grown under phosphate starvation were also found to be more efficient in establishing lethal infections in mouse model as well. Phosphate starvation increased *B. anthracis* virulence by promoting the secretion of primary virulence factors like protective antigen (PA), lethal factor (LF) and edema factor (EF). Thus, this study affirms that besides other host mediated factors, phosphate limitation may also contribute *B. anthracis* for successfully establishing itself within the host. This study is a step forward in delineating its pathophysiology that might help in understanding the pathogenesis of anthrax.

LB-028
Transcripts characterization, recombinant expression and protein identification of alpha-glucosidase from Dysdercus peruvianus (Heteroptera)

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Alpha-glucosidase activity is found mainly in anterior midgut (V1 section) associated with membranes of *Dysdercus peruvianus* cells. The alpha-glucosidases of V1 contents are soluble. Membrane bound alpha-glucosidases are markers of perimicrovillar membranes. This membrane ensheath the microvillar membranes of midgut cells and compartmentalize the digestive process. Given the central role of alpha-glucosidases on carbohydrate digestion the aim of this work is to know the identity and sites of expression of these enzymes. A 454 transcriptome from *D. peruvianus* midgut generated the data for alpha-glucosidase screening. Two sequences called DPAlpGlu1 and 2 were the most expressed sequences by reads counting and both were cloned in PAE plasmid. Both were successfully expressed in *E. coli* BL21 STAR cells and the recombinant DpAlpGlu2 was used to raise antibodies in rabbit. DPAlpGlu1 and DPAlpGlu2 are expressed only along the midgut (RT-PCR data). DPAlpGlu1 has a carboxyterminal transmembrane helice that is absent in the DPAlpGlu2 sequence. The abundance of reads and the presence of a transmembrane helice make DPAlpGlu1 the best candidate to be the perimicrovillar membrane marker, whereas DPAlpGlu2, the accompanying soluble enzyme. AntiDpAlpGlu2 serum recognizes a single band in a western-blot performed with soluble midgut proteins, confirming the identity of the soluble enzyme. Based on analysis of seven insects of four orders, alpha-glucosidase with carboxyterminal transmembrane helice arose more than one time in insect evolution, as judged by sequence alignments and cladogram trees. Hemipterans have at least one sequence of membrane bound alpha-glucosidase, whereas the other insect orders may have only soluble alpha-glucosidases.

LB-029
Different protein expression occurs between TKI-resistant and untreated human kidney cancer stem-like cells in normoxia and hypoxia

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Introduction: Mechanisms of resistance to tyrosine kinase inhibitors (TKIs) in renal cell carcinoma (RCC) still remain elusive. RCC cells possessing specific stemness features, i.e. CD105 surface marker expression may play significant role in tumor formation. Also, hypoxia seems to contribute to altered gene and protein expression in tumors. HKCSCs (Human Kidney Cancer Stem Cells; those cells possess a small subpopulation which is CD105+ – data from our preliminary results) which have been previously subjected to axitinib in normoxia and to sorafenib in normoxia were shown by authors to be resistant to those TKIs

in suspension cultures as well in soft-agar colony formation assay.

Materials and methods: HKCSCs (Celprogen) were cultured in StemXVivo Mesenchymal Stem Cell Expansion Medium (R&D Systems). On day 3, TKIs were added to achieve final concentration of 1.5 μM. HKCSCs were also cultured untreated in normoxia and hypoxia. Each condition was performed in triplicate. Total protein was extracted on day 6 using RIPA buffer and PIC (Phosphatase Inhibitor Cocktail, Sigma). Total mRNA was extracted using Nucleospin RNA Isolation Kit (Machery-Nagel). Label-free analysis using mass spectrometry has been performed. Subsequently, Western Blot analysis and quantitative Real-time PCR were performed using chosen antibodies and primers on the basis of MS results.

Results and discussion: Alterations of several proteins' expression between HKCSCs cultured in normoxia and hypoxia as well as between resistant and untreated cells have been shown.

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LB-030
Production and characterization of antibodies to mycobacterial lipid antigens in rabbit

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The mycobacterial cell wall is rich in lipids, and the major component is mycolic acid. Cord factor is trehalose-6,6'-dimycolate, and is correlated with the formation of serpentine cords, that is the characteristic morphology of mycobacteria. We reported that anti-cord factor antibody was significantly increased in tuberculous patients, and the antigenic epitope was mycolic acid. On the other hand, the cell-wall skeleton of *Mycobacterium bovis* BCG (BCG-CWS) is composed of peptidoglycan-arabinogalactan-mycolic acid complex, and is a candidate for therapeutic agent of bladder cancer. To clarify the features of the anti-lipid antibodies in tuberculosis, it was performed to produce anti-lipid antibodies in rabbit immunized with mycolic acid-containing glycolipids (cord factor, BCG-CWS) and BCG whole bacteria as antigen. The single-immunization with BCG-CWS and cord factor in rabbit was not enough to the production of lipid-specific antibodies, and the production of the antibodies increased by the booster after 4 weeks. As a result, anti-lipid antibodies in rabbit were produced by the immunization with mycolic acid-containing glycolipids (BCG-CWS and cord factor). Anti-BCG-CWS antibody reacted to cord factor and arabinogalactan (AG), implying that both mycolic acid and AG were epitopes of this antibody. Anti-BCG-CWS antibody was produced in not only the sera of tuberculous patients but also those of healthy donors. The immunization of BCG whole bacteria is more advantageous for anti-lipid antibodies production, compared to those of BCG-CWS and cord factor alone.

LB-031
Are primary and secondary metabolism in *Artemisia alba* moderated by the endogenous cytokinins levels *in vitro*?

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Artemisia alba is a medicinal plant distributed in Southern Europe. The addition of plant growth regulators (PGR) to the growth medium *in vitro* was shown to be a tool for affecting plant morphogenesis, physiological status, as well as for targeted production of terpernoids with plausible medicinal applications (1).

In this work we study how the applied PGR influence both the primary (photosynthesis) and secondary (terpenoids) metabolism in *Artemisia alba* cultures. For this purpose we correlated the changes in the level of the endogenous cytokinins (detected by LC/MS chromatography) with: (i) the changes occurring in the photosynthetic apparatus with an accent on the macroorganization of the pigment-protein complexes involved in the light reactions of photosynthesis (characterized by circular dichroism, flow cytometry and atomic force microscopy) and the functionality of photosystem II (judged by the rate and yield of the oxygen evolution) and (ii) the plants terpenoid profile (1).

We demonstrate that plants with altered mono- and sesquiterpenoids levels also exhibit modified thylakoid membrane morphology and functionality and identify a fraction of "swollen" thylakoids which is hypothesized to indicate an early stage of scenecence-like response. Our findings indicate that primary and secondary metabolism interrelations might be mediated by endogenous phytohormone levels (bioactive cytokinins in particular).

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LB-032
Correlation between the enzymatic and non-enzymatic antioxidant protection systems in *Artemisia alba* cultures

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Artemisia alba Turra is a fragrant shrub distributed in the southern Europe. The aerials of the plant are used in traditional medicine as a tonic and for treating intestinal disorders. In this work we study the impact of exogenously applied plant growth regulators (PGR) on the total protein profile, the architecture of thylakoid membranes and the enzyme activities in *A. alba* shoot cultures.

We have found that the aerials are rich in proteins and exhibit well developed photosynthetic (thylakoid) membrane system. The application of indole-3-butyric (IBA) acid alone led to formation of small granas and emergence of new SDS-PAGE bands in the aerials, which were absent upon application of IBA and benzyl adenine (BA). BA was found to increase the protein content in the aerial parts but to lower it in the roots and callus. The latter samples displayed marked differences in their electrophoretic profile as compared to the aerials. The native PAGE enzyme activity staining, revealed presence of antioxidant enzymes (catalase, superoxide dismutase, ascorbate peroxidase, polyphenoloxidase, peroxidase). Data are in accordance with the activities of antioxidant enzymes obtained spectrophotometrically.

In conclusion, the application of PGR in *A. alba in vitro* leads to changes in the electrophoretic profile of samples derived from the plants aerials and roots and in the thylakoid membrane morphology. The changes in the polyphenol content correlated with molecular markers of lipid peroxidation and oxidative stress, suggesting a link between the enzymatic and non-enzymatic antioxidant protection of the plant *in vitro*.

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LB-033
Identification of neutrophil elastase IL-36γ processing small molecules inhibitors

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IL-36α, IL-36β, IL-36γ are members of the extended IL-1 family that play a key role in inflammatory responses. Similar to most members of the IL-1 family, IL-36 cytokines require proteolytic processing at their N-termini for acquisition of biological activity. Upon activation, IL-36 cytokines signal through the IL-36R/IL-1RAcP complex, initiating the synthesis of a battery of pro-inflammatory cytokines and chemokines. Because IL-36 cytokines have been strongly implicated in psoriasis, strategies aimed at inhibiting their activation may have therapeutic utility in this condition. Recently, it has been found that neutrophil-derived elastase processes human IL-36γ, which increases the activity of this cytokine over 100-fold. Thus, small molecule inhibitors of neutrophil elastase may represent a promising approach for the treatment of psoriasis. To this end, we screened a library of small molecules to seek compounds capable of inhibiting the proteolytic activity of the latter protease. Using *in silico* docking analysis, we selected 149 potential elastase inhibitors. Using a synthetic substrate of elastase (Suc-AAPV-MCA), we identified a new family of inhibitors of this proteases. These compounds were subsequently tested for their ability to inhibit elastase-mediated IL-36γ processing using a $HeLa^{IL\text{-}36R}$ reporter system, which responds to active forms of IL-36 by secreting pro-inflammatory cytokines. We successfully identified a lead compound that inhibits elastase-mediated IL-36γ processing and plan to perform hit-to-lead optimization in future studies.

Key words: psoriasis, inflammation, IL-36, elastase, small molecule inhibitors

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LB-034
Syntenin silencing in cancer cells induces G_0/G_1 cell cycle arrest and downregulates the expression of CDK4, cyclin D2 and Retinoblastoma protein

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Syntenin functions as a rate limiting factor to allow the escape from degradation of syndecan heparan sulfate proteoglycans and can thereby support sustained signaling of a plethora of growth factors and adhesion molecules. Syntenin controls early developmental movements in vertebrates. In adulthood, syntenin reactivation or gain of function has been associated with the metastatic potential of melanoma and a growing number of cancers. More recently, syntenin has been reported to support breast cancer tumor growth. Here we aimed to clarify the impact of syntenin loss of function on cancer cell proliferation using cells from various origin and syntenin shRNA and siRNA silencing approaches. We found that in the mouse melanoma cell line B16F10, the human colon cancer cell line HT-29 and the human breast cancer cell line MCF-7, syntenin not solely controls migration but also proliferation and the ability to form colonies and to grow in soft agar. Using the MCF-7 cell line, we further document that syntenin controls G_0/G_1 progression and the expression of CDK4, cyclin D2 and Retinoblastoma protein. These data highlight that syntenin supports tumor cell proliferation independently of their origin and reinforce its attractiveness as potential therapeutic target.

LB-035
LRRC8 heteromers form an essential component of the volume-regulated anion channel VRAC

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Regulation of cell volume is pivotal for many cellular and organismal functions, such as during osmotic changes and cell growth, division and migration. A key player in this process, the volume-regulated anion channel (VRAC), opens upon cell swelling and conducts chloride and arguably organic osmolytes. Although VRAC has been vastly described and characterized by electrophysiological means, its molecular identity has remained unknown.

We conducted a fluorescence-based, genome-wide siRNA screen to search for genes underlying VRAC activity. Our major hit, LRRC8A, was confirmed as an essential VRAC-component by electrophysiological measurements. We could show that LRRC8A localizes to the plasma membrane and forms heteromers with the other four members of the LRRC8 protein family. Genome-edited cell lines deficient for all five LRRC8 proteins in various combinations enabled us to show that VRAC requires heteromers of LRRC8A and at least one other LRRC8. The isoform combination determined VRAC inactivation kinetics,

explaining the heterogeneity of native VRAC currents. Finally we demonstrated that LRRC8 heteromers are indispensable for swelling-activated taurine release, suggesting VRAC is identical to the volume-sensitive organic osmolyte/anion channel VSOAC, and for regulatory volume decrease.

LB-036
Inhibition of translation elongation by antibiotic amicoumacin A

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The antibacterial agent amicoumacin A (AMI) was discovered more than 30 years ago, but its mechanism of action remained unknown. Recently it was shown that amicoumacin A impairs cell growth by specific inhibiting translation. The crystal structure of bacterial ribosome in complex with amicoumacin A solved at 2.4 A resolution revealed that the antibiotic makes contacts with universally conserved nucleotides of 16S rRNA in the E site and the mRNA backbone [1]. Amicoumacin A *in vitro* slows down multiple turnover translocation and polypeptide synthesis. We have studied pre-steady state kinetics of translocation using the stopped flow technique and monitoring fluorescent reported groups on the A-site peptidyl-tRNA and on the 3'-end of the mRNA. Amicoumacin A even at relatively high concentrations (30 mkM) does not change significantly the rate of single round translocation as monitored by tRNA- and mRNA-conjugated fluorescent labels. Pre-steady state kinetics of deacylated tRNA interactions with E site of the 70S ribosome reveals that amicoumacin A facilitates E-site binding of the tRNA. The unusual mechanism of translation inhibition by amicoumacin is most probably based on the increased affinity of tRNA to the E site and on reduced dissociation rate, thus slowing down on each elongation cycle of polypeptide synthesis.

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LB-037
Molecular mechanism of decoding of tRNAs with extended anticodon

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Ribosome decode the information of the mRNA and synthetize polypeptide chain by measure of triplet codon-anticodon interactions. Mutant tRNAs containing an extra nucleotide in the anticodon loop were reported to suppress +1 frameshift mutations, but the molecular mechanism of decoding and translocation for such tRNAs with expanded anticodon remains unclear. Model tRNAs with expanded anticodons were synthetized *in vitro* by T7 RNA polymerase. tRNAs were designed on the basis of yeast $tRNA^{Phe}$, where the anticodon GAA and 3'-adjacent nucleotide were substituted with pentanucleotides GAAAA and GAAAG. Here we present the kinetic studies of partial reactions of elongation cycle with tRNAs with extended anticodons. Both tRNAs with anticodon GAAAG and GAAAA demonstrate the ability to decode codons UUUU/UUUC and complete peptide bond formation. The pre-steady state kinetics of translocation was studied utilizing the fluorescent reporters on the P-site bound deacylated $tRNA^{fMet}$ and fluorescent label on 3'-end of the mRNA, demonstrating the ability of chimeric tRNAs with expanded anticodons to complete the elongation cycle in reconstituted translation system *in vitro*.

LB-038
Molecular mechanism of tRNA interactions with dihydrouridine synthases (Dus)

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Modified nucleosides tune the structure of tRNA for optimal, high-fidelity function in translation. One of the most ubiquitous modifications is dihydrouridine (D), found in positions 16, 17, 20 and 20a of tRNAs. Until now the molecular mechanism of dihydrouridine formation and the exact mechanism of role of dihydrouridine in translation remain unknown. Recently, X-ray crystallography studies revealed the structures of DusA-$tRNA^{Phe}$ complex from *Thermus thermophilus* [1] and DusC-$tRNA^{Phe}$ from *Escherichia coli* [2]. It was shown, that Dus enzymes that modify uridines at positions 16 and 20 bind their tRNA substrates in completely different orientations. The binding modes of the two Dus subfamilies differ by a major($\sim$160°) rotation of the whole tRNA molecule, providing the basis for their distinct specificities[2].

Here, we present the results of biochemical assays and mutational analysis, showing the specificity of dihydrouridine synthase C from *E.coli* towards U16 of tRNA. We also used Molecular Dynamics (MD) to reveal structural stability and persistent contacts between DusA from *T.th.* and tRNA and for DusC from *E.coli* and tRNA. MD simulations performed at relevant temperatures (65C and 37C) show that contacts between tRNA and Dus proteins are quite rigid. Dus family proteins has low structural flexibility. C-terminal part of DusA (not resolved in crystallographic study) demonstrates binding to the anticodon loop of the tRNA, thus providing additional tRNA-protein interaction.

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LB-039
Conformational flexibility of nonamer ribonucleoprotein complexes from Influenza A virus

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The nucleoprotein (NP) of negative-sense single-strand RNA viruses is the major component of the ribonucleoprotein (vRNP)

complex, which is responsible for viral transcription and replication. NP of Influenza A virus consists of two helical head and body domains, arranged in the banana-shaped configuration. We evaluated the conformational peculiarities of wild-type NP nonamers from the influenza A/Hong Kong/1/68 (H3N2) strain and mutant (E292G) NP nonamers from cold-adapted strain using the molecular dynamics method. We found that the E292G mutation in NP may lead to changing of the vRNP nonamer planar structure both at 299 And 312 K. It may lead to change in the complex functionality and shed the light to the explanation of influenza A cold-adaptivity mechanisms. Our molecular dynamics experiments also expands recent the cryoelectronic microscopy structural data. We have proposed that the observed mutation in the position 292 of NP can contribute to the development of the genetically engineered cold-adapted influenza A virus vaccines.

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LB-040
A novel model for studying voltage-gated ion channel gene expression in reversible ischemic stroke

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Cerebrovascular diseases are still the major cause of death and disability in the worldwide. Embolism is responsible for at least 20% of all stroke and half of cerebral infarctions. The dysfunction of voltage-gated ion channels contributes to the pathology of ischemic stroke. In this study, we used novel animal models of transient ischemic attack (TIA) induced by artificial particle embolization that allow us to monitor the neurologic deficit in real-time. We then evaluated the expression of voltage-gated ion channels. We inducted TIA by using solid lipid microparticles. Rats were then subjected to neurological testing, positron emission tomography (PET) scans and Spectral Doppler. The infarction volume of brain tissue was confirmed by 2,3,5-triphenyl tetrazolium chloride (TTC) staining, and gene expression was evaluated by quantitative real-time PCR (qPCR) arrays. Rats with TIA exhibited neurological deficits as determined by negative TTC and PET findings. However, the expression of voltage-gated sodium channels in the hippocampus was significantly upregulated in the qPCR array study. Furthermore, altered expressions of sodium channel beta-subunits and potassium channels, were observed in TIA groups in different time stages. To our knowledge, this is the first report of the successful evaluation of voltage-gated ion channel gene expression in TIA animal models. This model will aid future studies in investigating pathophysiological mechanisms, and in developing new therapeutic compounds for the treatment of TIA.

LB-041
Combination therapy of acute lung injury with the heme oxygenase-1 gene and the lipopolysaccharide-binding peptide from HMGB1A

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Acute lung injury (ALI) is an inflammatory disease caused by lung infection, sepsis, trauma, aspiration, or ischemia. In this study, the combinational therapy with heme oxygenase-1 (HO-1) gene and lipopolysaccharide (LPS)-binding peptide (LBP) regions from high mobility group box-1 box A (HMGB1A) was evaluated for the treatment of ALI. As a gene carrier, deoxycholic acid was conjugated to low molecular weight polyethylenimine (PEI, 1.8 kDa). Deoxycholic acid conjugated PEI (DA-3) was characterized as a carrier of the HO-1 gene *in vitro*. Gel retardation and heparin competition assays showed that DA-3 formed stable complex with DNA by electrostatic interaction. In the transfection and luciferase assay, DA-3 had higher transfection efficiency than high molecular weight PEI (PEI25k, 25 kDa) and Lipofectamine in the L2 lung epithelial cells. These results were confirmed by the transfection assays with the HO-1 gene. In cytokine assays, the combined treatment with the HO-1 gene and LBP reduced the pro-inflammatory cytokines more efficiently that the single treatment of the HO-1 gene or LBP in the LPS activated macrophage cells. Therefore, the HO-1 gene and LBP may be useful for combination therapy for ALI.

LB-042
Combined delivery of the adiponectin gene for the treatment of acute lung injury

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Adiponectin (ADP) is a signaling molecule, which is secreted from the adipose tissue. The level of ADP in serum was decreased with increasing body fat. ADP has anti-inflammatory effect and can protect the endothelial cells. Therefore, ADP may be useful for the treatment of acute lung injury (ALI) with the anti-inflammation effect. In this study, dexamethasone was conjugated to low molecular weight polyethylenimine (PEI2k, 2 kDa) or polyamidoamine dendrimer (PAMAM, Second generation). Dexamethasone conjugated PEI2k (PEI2k-Dexa) and dexamethasone conjugated PAMAM (PAMAM-Dexa) was evaluated as a carrier of the ADP gene. Gel retardation assays showed that PEI2k-Dexa and PAMAM-Dexa formed compelxes with plasmid DNAs (pDNAs). *In vitro* transfection assays with the luciferase pDNA showed that PAMAM-Dexa had higher transfection efficiency than PEI2k-Dexa, PEI25k and Lipofectamine. The transfection assays with the ADP gene showed that PAMAM-Dexa was the most efficient carrier of the ADP gene among the tested carriers. Furthermore, PAMAM-Dexa had less cytotoxicity than PEI25k, Lipofectamine, and PEI2k-Dexa. The results showed that PAMAM-Dexa may be useful for delivery of the ADP gene for the treatment of ALI.

LB-043
Curcumin loaded DA-3 as a carrier of the antagomir for the inhibition of microRNA-21 in glioblastoma

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Glioblastoma multiform (GBM) is one of the most commonmalignant primary brain tumors.It was previously reported that microRNA21 (miR-21), one of the glioma-specific microRNA, was an anti-apoptosis factor.miR-21 reduced the expression of tumor suppressor genes such as PDCD-4 and PTEN. Antagomir against miR-21 (antagomir-21)may inhibit the action of miR-21 and reduce the growth of glioblastoma.In this study, deoxycholic acid conjugated polyethylenimine (DA-3) was used as a carrier of miR-21. DA-3 is an amphiphilic molecule with hydrophilic polyethylenimine and hydrophobicdeoxycholic acid. Therefore, DA-3 forms micelle in aqueous solution. Curcumin, which has antitumor effect, was loaded into the hydrophobic core of DA-3 micelle, producing curcumin loaded DA-3 (DA-3-Cur). DA-3-Cur formed stable complex with antagomir-21. *In vitro* transfection studies showed that DA-3-Cur increased the delivery efficiency of antagomir-21 into the C6 rat glioblastoma cells, compared with polyethylenimine and Lipofectamine. DA-3-Cur increased the intracellular delivery of curcumin, compared with curcumin only or a simple mixture of DA-3 and curcumin complex. As a result, DA-3-Cur showed higher anti-tumor effect than the controls. Furthermore, the delivery of antagomir-21 with DA-3-Cur reduced viability of the C6 glioblastoma cells. Therefore, the complex of DA-3-Cur and antagomir-21 may be useful for the treatment of glioblastoma as a combination therapy of curcumin and antagomir-21.

LB-044
Mass spectrometry-based analysis of thiol-redox and phosphorylation cross talk in human bronchial epithelial cells

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In recent years it has become apparent that reactive oxygen intermediates including hydrogen peroxide serve as essential secondary messengers in signal transduction. In the respiratory epithelium, signal transduction is modulated by hydrogen peroxide-mediated oxidation of cysteine-thiol groups in protein kinases and protein tyrosine-phosphatases, e.g. after stimulation of hydrogen peroxide production by Duox1/2. However, in epithelial cells it is largely unknown which proteins function as thiol switches and to which target proteins they do cross talk to thereby mediating alterations at the level of phosphorylation. Here, we used cysteine-reactive tandem mass tags for differential redox-labeling of proteins and enrichment of phosphorylated peptides in combination with high-resolution mass spectrometry to identify potential mediators of oxidation and their targets that show alterations at the level of phosphorylation after hydrogen peroxide signaling events. Based on our results we aim to highlight the significance of cross talk between phosphorylation and thiol-redox- modifications and the central role of hydrogen peroxide as a second messenger.

LB-045
Gene expression profiling of Cancer Stem Cells (CSCs) derived from primary and metastatic renal cell carcinoma

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Introduction: Renal cancers comprise a wide variety of histological subtypes and consider as most common malignancy of adult kidney, comprising 3% of all human cancers. The key factor for deaths in renal cell carcinoma (RCC) is the metastatic dissemination of RCC cells. One of the hypothesis suggested that metastasis occurs due to the presence of cancer stem cells (CSCs) / tumour initiating cells(TICs) which are unexposed during chemo or radiotherapy. This study was designed to identify and characterise mesenchymal stem cell marker "CD105 (endoglin)" as CSCs/TICs cells in primary and metastatic RCC cell lines. In summary, gene expression profiling of isolated CD105+ cells was constructed to distinguish the up/down regulated genes in primary and metastatic RCC.
Materials and methods: This study was conducted on primary and metastatic RCC cell lines. CD105+ cells were isolated using FITC anti-human CD105 antibody (Biolegend) through BD FACSAria II instrument. Total RNA was isolated using Total RNA Mini Plus kit from A&A Biotechnology. Total RNA was reverse-transcribed into double-stranded cDNA for hybridization experiment for Agilent microarray chips.
Results and conclusions: Our results show the presence of MSCs "CD105+ cells" population in primary and metastatic RCC cell lines. Positively isolated CD105+ cells were positive for hMSCs markers such as CD90, CD73, CD44 and negative for CD11b, CD19, CD34, CD45, HLA-DR. In addition, differences in the gene expression profiling have been reported in CSCs/TICs (CD105+ cells) isolated from primary and metastatic RCC cells.
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LB-046
Dual effect of capsaicin on lipid accumulation in HepG2 cells

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Cancer cells can reprogram their metabolism and, thus, their energy production to support the anabolic requirements associated with cell growth and proliferation. These changes constitute a fundamental adaptation of tumor cells to survive in an adverse environment. Here, we have studied the effect of capsaicin, the main pungent component in hot chilli peppers, on lipid metabolism in the human hepatocellular carcimona cells HepG2. Capsaicin has been proposed as an anti-tumoral agent because of its anti-proliferative and pro-apoptotic effect on cancer cells as well as an anti-obesity agent since dietary capsaicin prevents adipogenesis and weight gain in mice. We show that doses of capsaicin ranging from 1 to 100 μM modestly increase neutral lipid accumulation in HepG2 cells as measured by oil red staining. Similar

results were obtained when BODIPY 493/503 was used as a fluorescent dye in flow cytometry assays. By contrast, a dose of capsaicin as high as 200 μM decreased oleic acid-induced lipid accumulation in HepG2 cells. The effect of capsaicin on lipid accumulation was not altered by the antagonist capsazepine, suggesting a TRPV1-independent mechanism. The involvement of AMPK, considered as the main metabolic gatekeeper of the cell, and PPARγ was also investigated.

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LB-047
A role of pleckstrin homology-like domain family a member 1 protein in anti-GD2 ganglioside 14G2a-treated IMR-32 neuroblastoma celles

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Pleckstrin homology-like domain family A member 1 (PHLDA1) is ubiquitously expressed in a wide range of normal and cancer tissues. There is evidence showing that it might act as a mediator of apoptosis and autophagy. However, the exact biological function of PHLDA1 in neuroblastoma is unknown.

We were prompted to thoroughly investigate the role of PHLDA1 using a lentivirus vector-based RNAi approach. We observed that downregulation of *PHLDA1* expression promotes proliferation of IMR-32 cells. The rate of proliferation in *PHLDA1*-silenced cells is significantly higher than in mock shRNA-treated cells and wild type cells. We also noted inhibition of cytotoxic effect of the anti-GD2 ganglioside 14G2a monoclonal Ab in the PHLDA1-silenced cells indicating that cytotoxic effect of the mAb on neuroblastoma may be PHLDA1-dependent.

Our further studies focused on describing the effect of PHLDA1 silencing on expression of molecules involved in apoptosis e.g. caspase 3, P53, PARP and autophagy process e.g. LC3B and Atg proteins. We showed that *PHLDA1* silencing inhibits caspase-3 cleavage and PARP expression in the silenced clones when compared to the mock and the wild type IMR-32 cells. These results demonstrate that down-regulation of *PHLDA1* in IMR-32 may contribute to apoptosis resistance thus suggesting proapoptotic role of PHLDA1 in our model. Furthermore, Akt and Aurora A kinases phosphorylation was significantly increased in the silenced clones as compared to the mock and the wild type cells.

Further studies are warranted to study the mechanism responsible for PHLDA1 induction in mAb-treated neuroblastoma cells and its role in apoptosis and autophagy.

LB-048
Down-regulation of ABCA3 promotes WIPI-dependent autophagy in human osteosarcoma cells

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Macroautophagy is a lysosomal bulk degradation pathway that regulates the turnover of cytoplasmic cargo, including long-lived proteins and damaged organelles, thereby maintaining cellular homeostasis. Degradation of cytoplasmic material is mediated by cargo sequestration in autophagosomes and cargo degradation in the lysosomal compartment. Degraded monomers and energy are subsequently used for recycling purposes. The ATP-binding cassette-transporter A3 (ABCA3) was found to localize in the membranes of lamellar bodies as well as lysosomes. ABCA3 is responsible for the transport of substrates, predominantly lipids, into the inner of the organelle, however, the function of ABCA3 is insufficiently understood. In the present work we demonstrate that downregulation of ABCA3 in human U-2 OS cells leads to an elevated level of both basal and starvation-induced macroautophagy, but had no influence on chaperone-mediated autophagy. Our findings suggest a potential inhibitory role of ABCA3 in the regulatory mechanism of macroautophagy.

LB-049
Sorafenib targets mitochondrial integrity and inhibits WIPI-dependent autophagy

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By clearing the cytoplasm from damaged organelles and protein aggregates, autophagy functions as a tumour-suppressor pathway. Autophagy also contributes to tumour therapy resistance, however, molecular details are insufficiently understood. Sorafenib is a multikinase inhibitor currently employed in molecular targeted therapies in renal cell carcinoma (RCC), hepatocellular carcinoma (HCC) and also thyroid cancer. Unfortunately, acquired resistance to sorafenib treatment is unpreventable. Here, we addressed the effect of short-term sorafenib treatment on the process of autophagy using a comprehensive range of quantitative measures, including automated high-throughput imaging of WIPI1 and LC3, live-cell and electron microscopy. We found that sorafenib promotes the formation of autophagosomes, however, final autophagosomal degradation was blocked and non-productive autophagosomal structures accumulated. Interestingly, sorafenib treatment severely targeted the mitochondrial network, and damaged mitochondria also accumulated since autophagy was blocked. Hence short-term sorafenib treatment blocked autophagy and damaged the mitochondrial network, which promoted cell death. We speculate that an increase of damaged mitochondria in the context of blocked autophagy during long-term sorafenib treatment may promote the acquisition of mutations that foster the survival of therapy-resistant tumour cells.

Poster Session 2

LB-050
High-throughput identification of natural compounds that modulate WIPI-dependent autophagy in human osteosarcoma cells

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Autophagy is a lysosomal degradation pathway for organelles, proteins and lipids. Autophagy is characterized by the formation of cargo sequestering autophagosomes, double-membraned vesicles that emerge from precursors called phagophores. Phagophores are formed from unknown membrane origins but need the cradle of the endoplasmic reticulum where localized PtdIns3*P* (phosphatidylinositol 3-phosphate) production occurs during autophagy initiation. The PtdIns3*P* signal is recognized and decoded by the human WIPI (WD-repeat protein interacting with phosphoinosides) family. Due to the specific binding to PtdIns3*P* WIPI1 becomes a membrane protein of phagophores and autophagosomes, and fluorescence-based WIPI1 detection (WIPI1 puncta formation) was established to reliably assess autophagy. Here we screened a library of 133 natural compounds using human U-2 OS cells expressing GFP-WIPI1 for automated high-throughput assessments of autophagy. Subsequently, candidate screening isolates where further characterized using a broad variety of quantitative autophagy assays, including WIPI1, WIPI2, WIPI3, WIPI4, LC3 and p62 puncta formation analysis, LC3 western blotting, phospho-ULK1 and phospho-mTOR detection, long-lived protein degradation, and cell viability assays in U-2 OS cells, as well as longevity assays in *C. elegans*. With this study we identified novel natural compounds that modulate autophagy, as well as known substances, such as EGCG (epigallocatechin gallate), that induce autophagy.

LB-051
High-throughput anti-cancer compound screening for automated assessments of WIPI-dependent autophagy

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Autophagy is a highly conserved catabolic process, which sequesters cytoplasmic materials in multi-membrane vesicles, called autophagosomes, and transports the cargo to the lysosomes for degradation. Upon autophagy induction the WD-repeat protein interacting with phosphoinosides (WIPI) proteins WIPI1 and WIPI2 are recruited to phosphatidylinositol 3-phosphate (PtdIns3*P*) produced by phosphatidylinositol-3 kinase class III (Vps34) in complex with ATG14L, Beclin 1 and Vps15. At the nascent autophagosome WIPI1 and WIPI2 are considered to function as essential PtdIns3*P* effectors. Due to the specific localization at autophagosomal membranes, both WIPI1 and WIPI2 have been established to serve as marker proteins for autophagy assessments. Here we used a GFP-WIPI1 U-2 OS cell line for high-throughput screening of 42 anti-cancer compounds. Screening and subsequent verification and characterization revealed that compounds employed in molecular targeted therapies – erlotinib, gefitinib, imatinib, and sunitinib – differentially inhibited autophagy in short-term treatments. In contrast, long-term imatinib treatment led to the survival of a resistant cell population with elevated basal and starvation-induced autophagy. This suggests the possibility that i) imatinib treatment of solid tumours may initially impose autophagy inhibition that permits cell death, ii) survival of imatinib-resistant solid tumor cells is driven, at least in part, by autophagy.

LB-052
Biochemical Adaptation; A missing line in cancer research

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Tumor microenvironment is a hypoxic and acidic milieu in which nutrient availability also is restricted. In the stressful condition of tumors, biochemical adaptation allows cancer cells to proliferate. The role of biochemical adaptation in tumorgenesis has not been elucidated precisely until now because the importance of tumor environment as the inducer of biochemical adaptation has been masked owing the use of tissue culture conditions in which pH is normal without any fluctuations, also oxygen and nutrient are always in excess. evaluation of biochemical adaptation in tumors is the aim of current study.

Tumor and normal specimens were obtained directly from operating room. Kinetic assays have been done on tissues samples and Breast cancer (BC) cell lines under optimum conditions.

Different Km of the enzymes in tumor tissues could be related to high lactate level, hypoxic condition and acidic pH that could not be found in cell cultures. The different enzyme kinetic is a sign of various environment and biochemical adaptation. Cancer cells in tumor tissues exploit enzyme kinetic approach in order to adapt to the stressful tumor environment to preserve acidic tumor pH and high lactate level (by lowering LDH affinity for lactate) and to live in hypoxic condition (By increasing ME affinity for pyruvate production). In contrast, cancer cells in culture's condition do not need to exploit the enzyme kinetic approach because pH and oxygen pressure are normal. Our results confirm the biochemical adaptation is an important part of cancer cell metabolism in the stressful tumor environments.

LB-053
Studying of malignant ascites as a unique tumor microenvironment

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It is well known that single tumor consists of heterogeneous cell populations; each type of these cells has diverse cellular morphology, tumor initiation capacity or acquired resistance to anticancer therapy. Cancer cells form a complex network of interactions

between them and their local environment. Recent studies have shown that after exposure to chemo- or radiotherapy during the course of the treatment, apoptotic cells secreted a number of factors accelerating proliferation of neighboring tumor cells and contributing to their more aggressive phenotype.

The general aim of this work was to identify signaling molecules secreted by both tumor cells *in vivo* (ascites), and tumor cells *in vitro* (secretome of cell line SCOV3). We performed proteomic analysis of ascites samples from patients who had received several courses of chemotherapy prior to ascites collection; and ascites of patients with cirrhosis were taken as control samples. Functional analysis of the ascites proteome demonstrated that the major differences between cirrhosis and malignant ascites were observed for the cluster of spliceosomal proteins. This result was confirmed *in vitro* using ovarian cancer cell line. We also demonstrated that some splicing RNA detected exclusively in malignant ascites; and showed that fluorescent spliceosomal U12 snRNA was localized in the nucleus of ovarian cancer cells 72 h after adding it into the culture medium. We assume that the secreted components of spliceosome could promote cancer cell survival or metastasis by affecting cancer-specific splicing changes via a yet unknown mechanism.

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LB-054
Dynamic rearrangements of WIPI1-positive ER sections during autophagosome formation

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The process of autophagy is initiated by phosphatidylinositol 3-phosphate (PtdIns3*P*) production and dynamic membrane rearrangements that lead to the formation of autophagosomes. WD-repeat protein interacting with phosphoinositides (WIPI) members are essential autophagy-related (ATG) proteins considered to function as PtdIns3*P* effectors at the nascent autophagosome. Here, we characterized the PtdIns3*P*-dependent membrane accumulation of WIPI1 (referred to as puncta) at the onset of autophagy. We demonstrate that the induction of autophagy imposed a rapid formation of fluorescent WIPI1 puncta within the first 5 min of stimulation. Delayed in time, WIPI1 puncta formation was accompanied by the formation of large, often perinuclear WIPI1 structures. Both, WIPI1 puncta and large perinuclear WIPI1 structures colocalized with the endoplasmic reticulum (ER) and ATGs considered to function upstream (ATG14L, ATG2A) and downstream of WIPI1 (ATG12, ATG16L, LC3). By quantitative live-cell imaging (>1000 individual cells), we provide evidence that in up to 10% of WIPI1 puncta-positive cells, large perinuclear WIPI1 structures dynamically rearrange into WIPI1/p62-positive ER-arrays that we characterized further. We discuss possible interpretations for the appearance of such WIPI1-positive ER-arrays during autophagy initiation.

LB-055
Multi-functionality and mutational analysis of fructose 1,6 bis-phosphatase in *saccharomyces cerevisiae*

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Up-regulated glycolysis exhibited by tumors remained, for decades, the major focus concerning cancer carbon metabolism (Warburg *et al,* 1927), whereas the role and regulation of gluconeogenesis in cancers had been overlooked until recent years when downregulation of gluconeogenesis was linked to tumor aggressiveness through evidence that the rate-limiting enzyme fructose 1,6 bisphosphatase FBP1 is silenced in both liver and colon cancers (Chen *et al*. 2011). More intriguingly, FBP1 has been proven of central importance for maintenance of epithelial phenotype in breast cancers. (Dong *et al*. 2013) a possible role for ROS in this interaction has been postulated. (Schieber and Chandel 2013). More recently, FBP1 has been demonstrated to interfere with HIF-1α transcriptional activity in renal cancer.(Li *et al*. 2014)

Akin to the apparent multiple role of FBP1 in cancer cells, We have been able to show that FBP1 in *Saccharomyces cerevisiae* exhibits additional effects to its enzymatic activity, conferring more sensitivity to low doses of the DNA-alkylating agent MMS, and leading to more ROS production upon either MMS-treatment or chronological aging (Kitanovic and Wölfl 2006). To further investigate this phenomenon, we employed site-specific mutagenesis to carry out a mutational analysis of evolutionary-conserved residues with structural and functional significance to the yeast FBP1.

The outcome of the mutational analysis further emphasized the importance of several key-residues to the enzymatic activity. Moreover two of the examined mutants showed partial decoupling of the enzymatic activity from the sensitivity to MMS.

LB-056
Prevalence of plasmid mediated β-lactam genes amongst ESBL and CRE *Klebsiella pneumoniae* strains isolated from patients with cardiovascular disease

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Our aim was to establish the antibiotic profile of *K. pneumoniae* and the prevalence of plasmid mediated β-lactam resistance genes amongst ESBL and CRE strains isolated from patients with cardiovascular disease.

Materials and Methods: A total number of 87 *K. pneumoniae* strains, isolates form different clinical sources during 2014, was selected based on β-lactam resistance profiles. The strain identification was performed using Chromatic Mueller-Hinton, chrom ID-ESBL/ID-Carba, and Vitek-2 Compact Sistem. Antimicrobial susceptibility was established by disc diffusion and CMI methods. ESBL production was confirmed using the double discs test and carbapenemases production by modified Hodge test and E-test. PCR assays were performed using primers for plasmid mediated

antibiotic resistance genes (TEM, CTX-M, NDM, OXA-48, KPC, VIM, IMP).

Results: Out of 87 *K. pneumoniae* isolates, 26 ESBL and 28 CRE producing strains was selected. All carbapenem resistant strains was multidrug resistant, being susceptible only to colistin and intermediate to amikacin. Over 60% of all ESBL-positive and carbapenem susceptible exhibited susceptibility to quinolones, aminoglycosides and trimetoprim-sulphametoxazole. The *bla*CTX-M-like gene was identified in 95.4% (n = 83) of the isolates. The *bla*OXA-48-like gene was encountered in 83.9% (n = 73) of the CRE *K. pneumoniae* strains, as well as in most of the carbapenem susceptible ESBL producing *K. pneumoniae* strains.

Conclusion: Our results suggest a high prevalence of OXA-48-like carbapenemases in ESBL producing, carbapenem susceptible *K. pneumoniae* strains in the tested area.

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LB-057
Serum levels of induced protein-10 (IP-10) improves the predictive value of rs12979860 IL28B SNP in treatment of Chronic Hepatitis C (CHC) patients treated with combined interferon alpha-2 and ribavirin

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Background: An increased level of Interferon gamma – inducible protein 10 (IP-10) – was observed in patients with chronic HCV (CHC) non responding (NR) to treatment. Also, single nucleotide polymorphism (SNP) rs12979860, near IL28B gene was shown to be highly predictive of sustained virological response (SVR) in patients with CHC.

The aim in this study was to assess the potential predictive value of pretreatment IP-10 levels and IL 28 B genotype on the SVR in CHC Egyptian patients who underwent peginterferon α-2a/ribavirin therapy for 48 weeks.

Methods: IP-10 concentrations in serum was measured by ELISA, HCV viral load levels were assessed at 0, 12, 24, 48 and 72 weeks by qPCR, IL28B SNP rs1297860 was genotyped. Serum IP-10 levels were correlated with the clincopathologic parameters. ROC curve was established to calculate the cutoff point to discriminate between SVR and (NR patients.

Results: At cutoff value 342 pg/ml for predicting SVR we achieved a sensitivity and specificity of 80% and 46%, respectively. The distribution of SNP was CC (28.1%), CT (43.6%) and TT (28.1%) with SVR 70%, 38.7%, 50 % respectively. SVR was significantly associated with the CC genotype. We found that CT patients with IP-10 levels below 342 pg/ml had 61% SVR versus 22% with high IP-10 levels. For the TT genotype, no responders in those whom have IP-10 above 342 pg/ml while those with low IP-10 had 40% SVR.

Conclusion: Pretreatment serum IP-10 when added to IL28 genotype, the predictive value is greatly enhanced especially for CT and TT genotypes (STDF #1763).

LB-058
Phenotypic and molecular changes under reciprocal interaction of normal and renal cancer cells

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Introduction: Interleukin-6 (IL-6)was a recently characterized as pleiotropic cytokine with potential antitumor activity. On the contrary IL-6 was suggested to have a stimulatory growth effect in renal cell cancer (RCC) tumors. Obtaining primary data suggesting that IL-6 is produced at high levels by renal cell carcinoma cell lines we aim to investigate the molecular mechanisms involved in its possible role as an autocrine growth factor. We hypothesized that in metastatic clear cell RCC the complex of interleukin-6 (IL-6) and its soluble receptor (IL-6sR; complex IL-6/IL-6sR) play a key role in this process using signal transduction pathway of gp130/STAT3.

Aim of the study: Identification of the contribution IL-6/IL-6sR complex and its signal transduction pathway in the communication of renal cell carcinoma and cells of the target tissue in metastases.

Material and methods: Cell lines of healthy lungs (NL-20) – metastasis target – were cultured with conditioned medium obtained from renal cancer cell lines (Caki-2, ACHN, Caki-1). Growth rate was measured. Western Blot analysis was performed to evaluate the level of IL-6 signaling pathway molecules in post-culture medium and in the cells.

Results: Obtained results indicate that IL-6 secreted from renal cancer cell lines and proteins of its signaling pathway influence the biology of healthy cells of metastasis target organ. In turn IL-6 creates formation of microenvironment favoring tumor niche. Nevertheless alternative signal transduction pathway other than gp130/STAT3 is responsible for this phenomenon.

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LB-059
Mechanistic studies of HIV-Tat unconventional protein secretion

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Most soluble secretory proteins follow the ER-to-Golgi pathway for their transport into the extracellular space. Fibroblast growth factor 2 (FGF2), known to be involved in tumor induced angiogenesis, does not follow the classical secretory pathway but rather is directly translocated across the plasma membrane in a folded state. FGF2 is first recruited to the inner leaflet by the phosphoinositide $PI(4,5)P_2$. On the cell surfaces, binding of FGF2 to heparan sulfate proteoglycans leads to a directional transport.

HIV-Tat (transacting activator of transcription), a protein of the human immunodeficiency virus type 1 (HIV-1), is another example for an unconventionally secreted protein. HIV-Tat is required for up-regulating viral gene transcription. Besides its important intracellular function, HIV-Tat is probably also important for the viral spread.

Interestingly, in a previous study HIV-Tat secretion was shown to be dependent on the interaction with $PI(4,5)P_2$ similar

as it is the case for FGF2. Thus FGF2 and HIV-Tat may share similarities in their export mechanism.

In order to gain mechanistic insight, comparing studies of FGF2 and HIV-Tat were performed. Using model membranes of different lipid composition, membrane binding, membrane insertion, conformational changes upon membrane binding and pore formation were addressed. For a deeper understanding, mutational studies of HIV-Tat were accomplished. As the main result of this study, experiments showed that for HIV-Tat membrane binding is not PI(4,5)P_2-dependent but pore formation is strictly PI(4,5)P_2-dependent, whereas for FGF2 both membrane binding and pore formation are strictly PI(4,5)P_2-dependent.

LB-060
VE-Cadherin facilitates BMP-induced endothelial cell permeability and signaling

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Endothelial cells line the lumen of blood vessels and form a semi-permeable monolayer that controls blood-tissue exchange of fluids, plasma proteins and cells. Thus, the integrity of the endothelial barrier is essential for physiological tissue homeostasis and hyperpermeability is involved in the progression of several pathological conditions, such as inflammation, atherosclerosis and cancer as plasma proteins or cells enter the surrounding tissue and lead to formation of edema, plaques or metastases respectively. Interestingly, altered bone/body morphogenetic protein (BMP) signaling has been linked to these diseases, yet their precise function in the regulation of endothelial cell permeability remains elusive.

In the present study, we investigated whether BMP signal transduction controls permeability of human umbilical vein endothelial cell (HUVEC) monolayers and demonstrate that BMP6 induces hyperpermeability by promotion of internalization and Src-mediated tyrosine phosphorylation of VE-Cadherin. Furthermore, we identify VE-Cadherin as a novel regulator of vascular BMP signal transduction and show that VE-Cadherin physically associates with the BMP type I receptor activin receptor-like kinase 2 (ALK2) and the BMP type II receptor (BMPRII) in a BMP6-dependent manner and stabilizes receptor complex formation.

Our study suggests that endothelial BMP signaling is modulated by VE-Cadherin and controls barrier function. Further studies should pave the way for novel therapies in the context of acute inflammation, atherosclerosis, metastasis and multiple other pathologies associated with increased vascular permeability.

LB-061
In vitro modelling of cell–cell interactions in metastatic renal cell cancer tumors

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At least 25–30% of patients suffering from clear cell renal carcinoma (ccRCC) at the time of first diagnosis already are metastatic. Next 20–50% of patients develop metastases within subsequent 3 years. Among those metastatic patients only 10% will survive 5 years. Lungs are most frequent organ of RCC metastases development. Understanding molecular basis of cancer spread is still fragmentary and remain elusive. Accumulating evidence indicates a crucial role in carcinogenesis must be assigned to tumor stromal cells and normal cells from metastasis target organs. This project aim was to define phenotypic and molecular changes in normal and cancers cells interacting in renal cancer metastasis.

Aim of the study: Investigation whether factors secreted by normal and cancerous cells influence biology of their interactors in metastatic RCC tumor.

Material and methods: Renal cell cancer cells (Caki-2, ACHN) and healthy lung cells (NL-20) where co-cultured. Culture with conditioned media from cancer/normal cells was analyzed as well as insert-based co-culture model. Analysis of proliferation rate was conducted with Alamar Blue Assay. Gene expression profiling was performed with SurePrint G3 Human Gene Expression 8x60K v2 Microarray Kit.

Results: Both neoplastic cells and normal cells present higher proliferation rate in co-culture when compared to 2D monoculture. Microarray results revealed significant gene expression modulation in co-cultured cells. Obtained data suggest that interaction of different cells in tumor niche is responsible for "successful" metastatic tumor development.

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LB-062
The principle of self-organization and intramolecular determination basis of genetic expression in blastomere DNA

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DNA in blastomeres of the latest stages of cleavage are already determined on their functional specializations. This stage of embryogenesis has molecular-genetic isolation, as it is self-sufficient and can fully occur in isolation – *in vitro*.

Thus, a highly regular principle of functional blastomere determination arises in the isolated system of zygote cleavage.

We believe that it is linked to molecular isolation of the universal genetics blocks – nucleotide pool of zygote. Free nucleotide pool has different isotopic forms that are unequally involved in the replication of filial DNA. In a closed system of zygote cleavage this organizes intramolecular isotopy of DNA. Our experiments have shown that forms of DNA, isotopically different by carbon, and identical by chemical structure do not methylate the same way. Thus methylation pattern is directly linked to DNA isotopy pattern, which also programmatically determines gene expression. Such intricate decision of nature permits to determine chemically identical DNA on individual genetic expression programming, thus defining functional specialization of cells of the future organism.

Exclusion of different carbon isotopes in nucleotides from metabolism leads to a stopping of embryogenesis resulting from the absence of the original cause for individuality of pattern of blastomere DNA methylation. This was confirmed by cultivating carbon-monoisotopic – stable ^{12}C isotope – plants and animals. Left without just 1 percent of stable carbon isotope – ^{13}C, these experimental organisms have lost their reproduction functions.

LB-063
Remodeling of white adipose tissue in mice through FLCN/AMPK/PGC/1α /ERRα signaling

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The tumor suppressor folliculin (*Flcn*) is a novel identified repressor of the AMP-activated protein kinase (AMPK), a key energy sensor in the cell. We generated an adipose-specific *Flcn* knockout (KO) mouse model to investigate the role of *Flcn* in whole body energy metabolism. Here we show that the *Flcn* KO mice increase energy expenditure and confer protection from high fat diet (HFD)-induced obesity. Importantly, *Flcn* deletion in the adipose tissue enhances expression and activity of AMPK, and two of its downstream effectors, PGC-1α and estrogen-related receptor alpha (ERRα), which are recognized as key regulators of mitochondrial biogenesis. Accordingly, several mitochondrial genes including an uncoupling protein (UCP1) are increased in *Flcn* KO white adipose tissue (WAT).

In vitro analysis using *Flcn* KO MEFs confirmed an increase in AMPK and PGC-1 α/ERRα activity. Indeed, ERRα transcriptional activity was significantly increased as assessed by luciferase assay and by ChIP-qPCR respectively. Importantly, this hyperactivation of ERRα was AMPK-dependent, as deletion of AMPK in MEFs blocked the ERRα's activity. Taken together, our data indicated that loss of *Flcn* in adipose tissues leads to a higher metabolic activity through AMPK/PGC-1α/ERRα pathway. As a consequence, the *Flcn* KO mice are more resistant to cold exposure associated with a higher UCP1 level and beige white adipose tissue. Thus, we revealed that loss of *Flcn* is involved in AMPK-dependent PGC-1α/ERRα axis, which leads to elevated mitochondrial respiration and browning of white adipose tissue.

LB-064
Structural and biochemical study on the Plk4-dependent scaffold switching in centriole duplication

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Centrosome duplication is the key process during cell cycle and tightly regulated by the concerted action of a number of proteins. Polo-like kinase 4 (Plk4) is a member of Polo-like kinases that plays a key role in cell cycle process. Plk4 consists of two domains: an N-terminal kinase domain and a C-terminal cryptic polo-box domain (CPB). The CPB of Plk4 is crucial for the protein function by interacting with centriolar receptor proteins such as Cep192 and Cep152. Despite its significance in the maintenance of genomic integrity, how the CPB of Plk4 regulates centriole duplication has been largely obscure. Herein, we present the cellular data showing the assembly of Cep152 around the Cep192-decorated daughter centriole and the relocalization of Plk4 from the inner Cep192-enriched ring to the outer Cep152-decorated ring during cell cycle. Crystal structures of the CPB of Plk4 alone and in complex with Cep152- and Cep192-derived fragments exhibited that those fragments interact with the CPB of Plk4 mutually exclusively to each other. Remarkably, the Cep152 (K_D of 32 nM) fragment bound the CPB of Plk4 more potently than the Cep192 (K_D of 177 nM) fragment did, and was able to effectively displace the Cep192 fragment bound to the CPB of Plk4. Together with the structural data, these results account for the effective "snatching" of the Plk4 from the preformed Cep192-decorated ring to the Cep152-enriched ring. Therefore Plk4 seems to be regulated in time and space through ordered interactions of its CPB with two scaffold proteins, Cep192 and Cep152.

LB-065
Discovery of novel competitive inhibitors of Protein Tyrosine Phosphatase Sigma

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PTPσ (protein tyrosine phosphatase sigma), one of the receptor-type PTP widely expressed in the nerve system, is the receptor of chondroitin sulfate proteoglycan (CSPG) which inhibits the regrowth of neuron after nerve injury and thus regulates nerve regeneration negatively. Previous studies have shown that the catalytic inhibition of PTPσ results in positive effects on the regeneration of neuron cells *in vitro* and *in vivo*. Therefore, PTPσ is considered as the effective target for developing drug candidates against degenerative nerve diseases. To identify novel drug candidates interfering with the PTPσ activity, we carried out structure-based virtual screening and chemical library screening. Seven PTPσ inhibitors with the IC_{50} values in the range of 5–11 μM were identified with the virtual screening-based compound search and eleven PTPσ inhibitors with the IC50 values in the range of 0.5–17.5 μM were discovered with the library screening-based compound search. Lineweaver-Burk plot and structure-based active site-docking simulation showed that these compounds competitively prevent phospho-substrate from binding to the active site of PTPσ.

LB-066
Polymorphism analysis of human growth hormone and vitamin D response elements

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The Growth hormone gene family consists of several genes related in sequence. To investigate this polymorphism existing in the growth hormone gene family whole blood was used. Proximal promoter region and exon 1 was studied. Amplification and sequencing of promoter and exon 1 was done in order to analyze the mutations present in the region. Several single nucleotide polymorphisms were found with two SNPs at -6 and -1 and involved in enhancing gene expression of GH-gene and risk of breast cancer. Moreover, the mutations were identified in the vitamin D responsive elements. Computational studies were performed to characterize the mutations identified in vitamin D response element. These mutations are important in regulating gene expression and may predict a role in Vitamin D metabolism-related diseases. The polymorphism associated with growth hormone gene family is very diverse and is related to various diseases more research is needed to elaborate the role of mutations occurring in this gene family with special reference to vitamin D Response Elements.

LB-067
Tau toxicity and rescue in cell and animal models of tau pathology

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Tau, a neuronal microtubule-associated protein, is pathologically altered in Alzheimer's disease (hyperphosphorylation, mislocalization, aggregation etc.). We study the role of Tau in neurodegeneration in AD and other tauopathies, and develop cell and animal models to observe spreading of Tau pathology, interaction with A-beta, and effects of aggregation inhibitors as therapeutic approach. This includes transgenic mice where Tau is expressed either in "pro-aggregant" or in "anti-aggregant" form. Aberrant mislocalization and aggregation of Tau, combined with loss of synapses and microtubules are hallmarks of AD. Similar features are observed in mice expressing pro-aggregant Tau, but not with anti-aggregant Tau, illustrating that the propensity for beta-structure is at the root of aggregation and pathology. Microtubules play essential roles in the maintenance of axons and dendrites as tracks for intracellular transport by motor proteins. To elucidate the cascade of events leading to microtubule breakdown we exposed wildtype and Tau knockout neurons to A-beta oligomers and analyzed changes in the Tau/microtubule system. Microtubule breakdown occurs in dendrites invaded by Tau and is mediated by spastin, a microtubule-severing enzyme. Spastin in turn is recruited to microtubules modified by polyglutamylation, mediated by translocation of the enzyme Tubulin-Tyrosine-Ligase-Like-6. Photoconversion of Tau labeled with Dendra2 reveals that missorted Tau in dendrites is newly synthesized and not derived from the axon. In absence of Tau (TauKO neurons), microtubules and synapses are resistant to A-beta induced toxicity. The results provide a rationale for microtubule stabilization as a therapeutic approach.

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LB-068
Single-enzyme experiments reveal a proton leak state in a heme-copper oxidase

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Heme-copper oxidases (HCOs) are key enzymes in prokaryotes and eukaryotes for energy production during aerobic respiration and transport protons across a membrane to generate a pH-electrochemical gradient termed proton motive force (PMF), which provides the driving force for the adenosine triphosphate (ATP) synthesis. Here we present a single-enzyme study that reveals that cytochrome bo_3 in *Escherichia coli*, a member of the HCOs and a close homologue to Complex IV in human mitochondria, can enter a rare long-lifetime leak state during which protons flow is reversed. By rapidly dissipating the PMF, we propose that this leak state enables cytochrome bo_3, and possibly other HCOs, to maintain a suitable level of PMF without having to limit its catalytic activity.

LB-069
Identification of chemokine neutraligands targeting atopic diseases

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Chemokines constitute a family of small cytokines that attract and activate leukocytes during the inflammatory response. Inspired by chemokine-clearing molecules shaped by pathogens to escape the immune system, we designed an assay devoted to the identification of molecules neutralizing chemotactic cytokines. Time-Resolved Intracellular Calcium recording, TRIC-r, is a two-step process that identifies chemokine inhibitors, and distinguishes on a kinetic basis, ligands blocking the receptor (receptor antagonists) from those blocking the chemokine (neutraligands). This technology was applied to discover neutraligands of several CC- and CXC-chemokines, including the CCR4-binding CCL17 and CCL22. Selected molecules block chemokine receptor activation by chemokines, inhibit human skin and blood cell chemotaxis and inhibit the recruitment of inflammatory cells in *in vivo* models of atopic diseases. As it is a label-free technology, TRIC-r is simple to set up and allows to rapidly identify neutraligands for numerous chemolkines and to control cell chemotaxis in normal and pathologic conditions.

LB-070
tRNA role in adaptive translation and disease

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Transfer RNAs (tRNAs) are central molecules for translation and not only a mere connecting molecule between mRNA and the synthesized protein. Here, we use different approaches, including cell-wide omics approaches, and show that tRNA abundance is dynamically regulated and tRNAs are centrally involved in adaptive translation, operating across a wide range of time scales. Mutations in the coding part of mRNA may change the usage of tRNA for a particular codon which is linked to complex human diseases. We highlight some examples of mutation-based pathologies altering the corresponding tRNA usage.

LB-071
New insight into the polymerisation mechanism of alpha-1-antitrypsin through epitope mapping of a monoclonal antibody that blocks polymerisation

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The most common pathological variant associated with α1-antitrypsin (AAT) deficiency is the Z allele (E342K) which leads to accumulation of AAT as polymers within the endoplasmic reticulum of hepatocytes predisposing to liver disease, whereas low levels of circulating Z AAT lead to emphysema by loss of inhibition of neutrophil elastase. The ideal therapy should prevent polymer formation while preserving inhibitory activity. Monoclonal antibody technology can identify interactors with Z AAT that satisfy both requirements. The novel 4B12 antibody developed in our lab binds with high affinity to AAT in its monomeric form and interestingly, blocks its polymerization *in vitro* as well as in a cell model of disease. This project aimed at mapping the epitope of the monoclonal 4B12 antibody on monomeric AAT and to (i) reveal new target sites for small-molecule intervention that may block the transition to aberrant polymers without compromising

the inhibitory activity of AAT and (ii) to further understand the mechanism of pathological AAT polymerisation which will provide new angles for drug development. We have found that (1) opening of β-sheet A to accept a β-strand is necessary but not sufficient for polymerization to occur; (2) remodeling of helix I or a nearby structural element such as β-strand 6A is a necessary step in the polymerization mechanism; (3) despite the inhibitory complex being hyperstable, local conformational change can still occur and destabilise the interaction with the protease; (4) the vicinity of helix I is a potential target for the binding of therapeutic molecules.

LB-072
Structural and molecular biology of bacterial type IV secretion systems

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Type IV secretion (T4S) systems are molecular machines used for the transport of macromolecules across the bacterial cell envelope. T4S systems are highly versatile. Conjugative T4S systems translocate DNA from a donor to a recipient bacterium and contribute to bacterial genome plasticity, spread of antibiotic resistance or other virulence trait among bacterial pathogens. In some bacteria such as *Helicobacter pylori* (Cag PI), *Brucella suis* (VirB/D), or *Legionella pneumophila* (Dot, Icm), T4S systems are directly involved in pathogenicity as they mediate the secretion of virulence factors (DNA or toxins) into host cells. The archetypal T4S system, the VirB/D system, was defined in *Agrobacterium tumefaciens* where it is naturally responsible for the delivery of the T-DNA to the plant host-cell. The *A. tumefaciens* VirB/D system comprises 12 proteins (VirB1 to 11 and VirD4). Recently, structures of large complexes formed by several of these proteins have become available shedding unprecedented light on T4S system secretion mechanism.

LB-073
MiR-19-mediated inhibition of Transglutaminase-2 leads to enhanced invasion and metastasis of colorectal cancer cells

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Background: Human Transglutaminase-2 (TG2) expression has been linked to colorectal cancer, and its functional role in the processes that drive disease appears to be context-dependant. There is now considerable evidence of a role for micro-RNAs in the development and progression of cancer, including metastasis. Using an *in vitro* model of CRC made up of the SW480 primary tumour cell lines, and the patient-matched SW620 metastatic cell line, we investigated the role of micro-RNAs in the differential expression of transglutaminase-2, and functional effects on inflammatory and invasive behaviour.
Results: Expression of TG2 correlated inversely with invasive behaviour, with knockdown in SW480 cells leading to enhanced invasion, and overexpression in SW620 causing the opposite. TG2 expression was observed at high levels in CRC human primary tumour tissue sections, but was lost in liver metastases. TG2 was identified as a target for miR-19a/b *in silico*; furthermore miR-19 expression was found higher in metastatic CRC compared to primary CRC, both *in vitro* and in tissue sections. *In vitro* miR-19 overexpression decreased TG2 expression in SW480 and increased their invasiveness through Matrigel.
Conclusions: I have found that miR-19, which is upregulated through amplification of chromosome-13 in CRC patients, is a mediator of CRC invasion through the targeting of TG2.

LB-074
Oncogenic Ras proteins in tumor cell migration and invasion

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Monomeric Ras GTPases act as molecular switches in signaling pathways important for cell growth, differentiation, and survival. Oncogenic mutant Ras proteins are commonly found in human tumors, with mutant K-Ras being most prevalent. These activating point mutations directly contribute to malignant transformation by arresting Ras in the active, GTP-bound state. Mutationally activated Ras proteins support the activation of multiple downstream signaling pathways, among which are the Raf/MEK/ERK kinase cascade, the PI3 Kinase/Akt, and the RalGDS pathway. Ras exists in four isoforms (H-Ras, K-Ras4A, K-Ras4B, and N-Ras) and although the amino acid sequences are quite similar numerous functional differences have been reported, which are dictated by the divergent C-terminal amino acid residues (the hypervariable region, HVR), targeting Ras to the plasma membrane. Functional differences between the Ras isoforms are associated with a distinct localisation in discrete plasma membrane nanodomains, mediated by the HVR and its post-translational lipid modification as well as interactions with protein factors. In pancreatic carcinoma, K-Ras is the most frequently mutated protein. We could demonstrate that in pancreatic carcinoma cells expression of oncogenic K-Ras promotes cell migration and invasion in a p38 MAPK and PI3 Kinase/Akt dependent manner. The spatial organization of K-Ras in specific nanoclusters of the membrane is highly important for K-Ras-dependent signal transduction. Galectin-3, a β-galactoside binding protein has been shown to regulate K-Ras-GTP nanocluster formation as well as signal transduction. Evidences will be presented that other Galectin family members also affect K-Ras-induced signal transduction and interfere with K-Ras-induced migration of pancreatic carcinoma cells.

LB-075
A large-scale proteomic analysis of transmembrane proteins from all cellular membranes

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About one third of eukaryotic genes code for transmembrane proteins (TMPs), which carry out significant functions, as signaling, transport, cell adhesion and various enzymatic processes. Yet, because of low expression levels of individual TMPs and especially their amphipathic nature, proteomic approaches generally lead to under-representation of TMPs in proteomic analyses.

Numerous methods aimed specifically at TMPs have been developed, however, substantial contamination by non-membrane proteins hinders this effort.

Transmembrane (TM) alpha-helices protected by the phospholipid bilayer can be isolated after complete proteolytic degradation of unprotected hydrophilic domains and all contaminating non-membrane proteins. Our current approach (based on the pioneering work of C. Wu & A. Blackler) is based on a simple enrichment of crude membrane fraction followed by opening of membrane vesicles at high pH and tryptic digestion of all accessible protein material. The "shaved" membranes are then solubilized, and TM domains are re-digested with CNBr to generate shorter peptides. The sample is delipidated and the hydrophobic transmembrane peptides are identified by LC-MS/MS. We successfully applied this method to human mantle cell lymphoma (MCL) cells and identified over 800 TMPs (over two thirds of all identifications) from plasma membrane and other organelar membranes. Most of the proteins were identified by peptides that overlapped with predicted TM domains.

Together with a quantitative approach such as SILAC, this method might serve as significant complement to standard differential proteomic methods for quantitation of previously inaccessible changes in the membrane proteome and could help complete missing data about function of certain TMPs.

LB-076
Roles of amino acid residues in the the control of enzyme activity related to allosteric activation of pyruvate carboxylase by acetyl CoA

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The activation of pyruvate carboxylase by the physiological activator, acetyl CoA, is not well understood at the molecular level. We have investigated the roles of residues in the allosteric binding site of pyruvate carboxylase in the binding of acetyl CoA and its activation of the enzyme. Mutation of Arg469 resulted in increased acetyl CoA-independent pyruvate carboxylating activity as well as bicarbonate-dependent MgATP cleavage activity in the presence or absence of acetyl CoA, but had only relatively small effects on acetyl CoA binding. Asp471 is crucial, through its interactions with Thr474 and Arg469 and the direct binding of its α-amide group to acetyl CoA, for activation of the enzyme. Mutation of this residue resulted in complete loss of acetyl CoA-induced activation of the enzyme. Neither Glu1027 nor Asp1018 is directly involved in binding acetyl CoA, but interact with residues that are crucial for acetyl CoA binding. Mutation of either residue had only small effects on acetyl CoA binding and pyruvate carboxylating activity in the presence of the activator. However, mutation of these residues did result in an increase in pyruvate carboxylating activity in the absence of acetyl CoA. In addition, the mutation of either Asp1018 or Glu1027 also resulted in increased bicarbonate-dependent MgATP cleavage activity in the presence or absence of acetyl CoA. These findings suggest that some of the interactions between residues involved in acetyl CoA binding and surrounding residues impose some kind of restriction on the structure and dynamics of the enzyme, especially in the absence of acetyl CoA.

LB-077
The student perspective on research in undergraduate education

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Accurate strategies to improve molecular life science education of freshmen students have become a great priority for universities all around the world. Many different and successful strategies, like peer reviewing, the use of clickers during lecturing, flipped classroom, massive open online coursers (MOOCs), among others, have been developed and established in some universities. Even though there is a lot of information about new technologies to teach on the internet, the majority of life science undergraduate students do not know about the existence of these new technologies to been taught.

The purpose of my talk is to give you a global idea of how students think about their life science undergraduate education. My conclusions are based on results gathered from a specific 10-question survey regarding new technologies to teach life science. Students of all countries around the world took 5 min of their time to anonymously answer this survey.

Since universities are so worried about the a modern education of life science freshmen students and eager to identify and recruit talented students in the field of research, one of the most important questions to be taken in regard are: "What skills and knowledge are expected from molecular life science undergraduates?" and "How can transferal skills and wide range knowledge been taught to life science undergraduates?"

From the students perspective based on results from a survey I present you here the balance of what is been taught and what is been expected regarding life science undergraduate education.

LB-078
Antitumor effect of vinblastine is hampered by curcumin in HeLa cervical cancer cells

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Curcumin is well-known major component of curry powder, which is a natural polyphenol product extracted from *rhizoma curcumae longae*. Vinblastine is an antitumor drug that induces tubulin depolymerization. Here, we investigated whether the antitumor effect of vinblastine is affected by tubulin association with curcumin. HeLa human cervical cancer cells were used to measure the antitumor effect of vinblastine. The percent cell survival was determined by an MTT assay. Hypodiploid cell formation was assessed by propidium iodide (PI) and/or Annexin V staining with flow cytometry. Reactive oxygen species (ROS) was measured by using DCF-DA with flow cytometry. Cell survival was reduced by treatment with vinblastine, as judged by an MTT assay and staining cells with propidium iodide, FITC-labeled annexin V, or 6-diamidino-2-phenylindole (DAPI). ROS production was decreased by the treatment with curcumin. In addition, our data show that apoptotic cell death induced by vinblastine was decreased by the pre-, co-, or post-treatment with curcumin to interact tubulin. These findings demonstrate that the competitive binding of tubulin fiber with curcumin might prevent vinblastine-induced HeLa cell apoptosis. These suggest that free tubulin dynamics play an important role in maintaining the therapeutic effects of vinblastine in tumor cells.

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LB-079
Directed evolution of GPCR-ligand analogues for development of antagonistic carrier peptides as tools in tumor diagnosis

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A successful therapy of cancer is tightly linked to early diagnosis and immediate initiation of adequate therapeutic countermeasures. One strategy of non-invasive cancer diagnosis is the targeting of tumors with molecules that specifically bind to surface structures overexpressed in neoplastic tissue. These molecular probes are generally linked to structures chelating short lived radioisotopes or fluorescent dyes to contrast tumorous structures against embedding tissue.

This strategy has been successfully employed for the somatostatin receptors type 2 and type 5 (SSTR2/5) and is reliably used in clinical diagnostics of neuroendocrine tumors (NET).

In a subgroup of NET it was previously shown that target structures can also include the G protein coupled receptors (GPCRs) for secretin and glucagon like peptide 2 (SCTR/GLP2R). The goal of this work was to create and structurally optimize peptide analogues for the ligands of SCTR and GLP2R which could be utilized as carrier peptides in molecular tumor imaging of NET. In order to enhance stability and simplify synthesis of the peptide analogues, structural determinants for the individual ligand function were assessed by single amino acid substitution and consecutive truncation variants were generated. A resulting subpopulation of ligand variants was further characterized in terms of agonistic/ antagonistic properties through functional assessment of cAMP synthesis and competition on GPCR internalization in ß-Arrestin2 translocation assays. To further evaluate comparative binding characteristics I^{125} supported radioligand binding studies were conducted. This experimental approach delivered two potent antagonistic peptide ligand variants for GLP2R and further three optimized antagonistic peptide ligand variants for SCTR.

LB-080
Hepatoprotective activities of *Hoslunda opposita* Vahl and bark of *Securidaca longepedunculata* fresen on mice

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The ethanolic extract of *Hoslunda opposite (H.O)* and *Securidaca longepedunculata* (S.L) were studied for hepatoprotective activities against albino mice with liver damage induced by 70% ethanol. Albino mice of either sex weighing between 16 and 26 g were divided into 4 groups of 10 mice each. All groups were orally administered 70% alcohol daily for 7 days. In subsequent 7 days, control was administered distilled water and other test groups were orally administered 50, 100 and 200 mg kg^{-1} body weight (b. wt.) doses of *H.O and* S.L in two different studies. Animals were weighed weekly and sacrificed after day 14. Organs were harvested, weighed and subjected to histopathologic assessment. Liver and blood samples were used for biochemical and hematological studies respectively. All mice orally administered 70% ethanol showed gross emaciation. The result of biochemical analytes of control mice administered ethanol only showed significant increased level of liver enzymes showing damage caused by ethanol. Samples from mice treated with *H.O* showed significant ($p < 0.05$) dose-dependent decrease in level of biochemical analytes indicating protection of hepatic cells. S.L also exhibited significant decrease in biochemical analytes. Haematologic studies of *H.O* and S.L showed a significant ($p < 0.05$) dose-dependent increase in some haematologic profiles. Histopathologic studies also supported that ethanolic extract of leaves of *H.O* markedly reduced toxicity of alcohol and preserved the histoarchitecture of the liver tissue to near normal better than the activities of S.L. These however suggest that both extracts act as potent hepatoprotective agents against alcohol induced hepatotoxicity.

LB-081
Investigation the potential role of FoxO members in the purvalanol and roscovitine-induced autophagy in LNCaP and DU 145 prostate cancer cells

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Autophagy is a critical process for survival or cell death, which is triggered by nutrient depletion or drug treatment. FoxO protein family members; FoxO1 and FoxO3 might promote autophagy *via* activating mTOR upstream PI3K/AKT signaling axis. Purvalanol and roscovitine are cyclin dependent kinase (CDK) inhibitors that inhibited cell proliferation and activated cell death mechanism. Recent findings showed that several CDK targets might be critical in the progression of prostate cancer. Therefore, the inhibition of CDKs via synthetic analogs gains importance in the therapy of disease. In this study, we aimed to investigate the potential targets CDK inhibitors in autophagic decision related to FoxO members in LNCaP and DU 145 prostate cancer cells.

As well as mTOR inhibition by rapamycin treatment, we found that purvalanol also increased the phosphorylation of FoxO1 at Ser256, which induced the translocation of FoxO1 into cytoplasm to trigger autophagy in DU145 cells. Drugs in the presence or absence of rapamycin triggered FoxO3a dephosphorylation at Ser 318/321 led to nuclear retention, which upregulated autophagy related genes. However, no similar effect was observed in LNCaP prostate cancer cells. Although purvalanol-induced LC3 lipidation in LNCaP cells, roscovitine was more effective to induce autophagy related genes in DU145 cells. CDK inhibitors altered FoxO1 and Atg7 interaction, which was found critical in DU145 cells.

In conclusion, CDK inhibitors purvalanol and roscovitine induced autophagy through the regulation of FoxO1 transport from nucleus to cytoplasm and nuclear retention of FoxO3 under control of PI3K/AKT/mTOR signaling axis.

LB-082
Efficient intracellular delivery of impermeable photosensitizer by DNA tetrahedron and its potential for photodynamic therapy *in vivo*

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Photodynamic therapy(PDT) is a cytotoxic treatment to kill hazardous cells such as cancer cells and pathogenic bacteria. The cell death was induced by reactive oxygen species(ROS) produced by PDT drugs called photosensitizers(PS). Among various types of PS, methylene blue(MB) has been considered one of the most

practical PS since it has decent quantum yield of $^1O_2$ generation in the therapeutic window. However, low cellular uptake is a major drawback of MB to be used as a PDT drug. To improve intracellular delivery of MB, we used DNA tetrahedron as a carrier. MB could be loaded on DNA tetrahedron and intracellularly delivered with high uptake efficiency. Evaluation of PDT effect by MB delivered with DNA tetrahedron was initially analyzed at the cellular level. Then, we also demonstrated that the delivery of MB by the DNA nanocarrier could also effective at *in vivo* level and subsequently lead to enhanced potency compared with free MB.

LB-083
Synaptotagmin-1 binds to PI(4,5)P_2-containing membranes but not to SNAREs in a physiological ionic environment

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The Ca^{2+}-sensor synaptotagmin-1 is thought to trigger membrane fusion by binding to acidic membrane lipids and to SNARE proteins. Previous work has shown that binding is mediated by electrostatic interactions that are sensitive to the ionic environment. However, the influence of divalent/polyvalent ions, at physiological concentrations, on synaptotagmin binding to membranes or SNAREs has not been explored. Here we show that binding of synaptotagmin-1 to membranes containing PIP_2 is regulated by charge shielding caused by the presence of divalent or multivalent cations. Surprisingly, polyvalent ions such as ATP and Mg^{2+} completely abrogate synaptotagmin-1 binding to SNAREs regardless of whether Ca^{2+} is present or not. Altogether, our data suggest that at physiological ion concentrations Ca^{2+}-dependent synaptotagmin-1 binding is confined to PIP_2-containing membrane patches in the plasma membrane, suggesting that membrane interaction of synaptotagmin-1 rather than SNARE binding triggers exocytosis of vesicles.

LB-084
Circulating preptin as a marker for osteoblast inhibition in rheumatoid arthritic patients treated with corticosteroids

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Preptin is a newly peptide hormone co-secreted with insulin as a regulatory element in bone metabolism with an unclear yet mechanism.

Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints.

Two classes of medications are used in treatment of rheumatoid arthritis: fast-acting (used to reduce pain and inflammation) and slow-acting drugs promote disease remission and prevent progressive joint destruction.

Corticosteroids have several adverse effects on bone metabolism. One of many is direct inhibition of osteoblast function and may be differentiation.

Aim: The aim of the present study was to assess the association of corticostroids treatment in rheumatoid arthritic patients, with circulating preptin. In an attempt to shed a light on the mechanism of induced osteoporosis in such patients.

Subjects and methods: Ninety subjects were enrolled in this study. Divided into three group: G1: Thirty RA lean patients taking DMARDs + corticosteroids. G2 thirty RA lean taking DMARDs without corticosteroids. And G3 thirty healthy weight and aged matched controls. Circulating serum preptin was measured in all groups using ELISA technique. Results showed that circulation serum preptin was elevated in patients with RA. However it was lower in G1 than in G2.

Conclusion: Results showed that preptin was affected in patients on corticosteroids when compared to arthritic patients taking DMARDs alone. This suggests that this newly discovered hormone could be considered a new marker for bone mineral density and osteoporosis.

LB-085
Expression and purification of recombinant Von Willebrand factor A1A2A3 domains

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Von Willebrand factor (VWF) is a large glycoprotein, found in plasma and platelets, synthesized by megakaryocytes and endothelial cells. The pre-pro-protein is 2813 amino acids and the mature monomer is 2050 amino acids. VWF monomer contains multiple copies of four types of domains called A, B, C and D-type domains. In order to initiate the formation of a platelet plug VWF must be assembled into large multimers. VWF undergoes post translational modifications by dimerizing through multiple intermolecular disulfide bonds and once in Golgi by forming interdimer disulfide bonds. The resulting multimers range in size between 500 to 20000 kDa. The protein dimerizes and the dimers then form a variety of disulfide crosslinked multimers with as many as 80 monomeric units, weighing more than 20 million Daltons. Studying such an enormous molecule poses special challenges. Binding sites that are independent of multimer assembly but important for the hemostatic function are located in the A1A2A3 domains of VWF. We expressed the A1, A2, and A3 domains of von Willebrand factor in a single polypeptide using Pichia Pastoris expression system. Proteins with disulfide bonds, requiring post translational modifications and glycosylation can be produced in their correctly native folded states with full function from Pichia pastoris. We purified the A1A2A3 domain using ethanol, ammonium sulfate precipitation and ion exchange chromatography. Our efforts in solubilizing the purified protein were unsuccessful more likely due to the unusual adhesive nature of the A1A2A3 domain of the VWF.

LB-086
Modulation of eukaryotic elongation factor 2 phosphorylation by muscarinic acetylcholine receptors in SNU-407 colon cancer cells

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Eukaryotic elongation factor 2 (eEF2) plays an essential role in protein synthesis by mediating mRNA translocation during the elongation step of translation. Although it has been reported that muscarinic acetylcholine receptors (mAChRs) are involved in the control of protein synthesis, the molecular mechanism underlying this process is poorly understood. Here we determined whether mAChRs modulate eEF2 phosphorylation in SNU-407 colon cancer cells. When the cells were treated with carbachol, eEF2 phosphorylation at T56 was significantly reduced in a time- and dose-dependent fashion. This carbachol effect was almost completely blocked by the muscarinic antagonist atropine, demon-

strating that eEF2 phosphorylation is specifically regulated by mAChRs. We next asked whether the activity of eEF2 kinase (eEF2K), a key enzyme responsible for eEF2 phosphorylation, is controlled by mAChRs. Carbachol treatment evoked eEF2K phosphorylation at S366 in an atropine-sensitive manner, indicating that mAChRs decrease eEF2 phosphorylation through eEF2K inactivation. When the cells were treated with U0126, a potent MEK inhibitor, the effects of carbachol on eEF2K and eEF2 phosphorylations were substantially diminished. Taken together, our results suggest that stimulation of mAChRs may lead to protein synthesis via the ERK-eEF2K-eEF2 pathway. We are currently investigating other signaling pathway(s) linking mAChRs to eEF2.

LB-087
The distinct roles of erythroid specific activators in transcription of the human adult β-globin gene

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The β-like globin genes are developmental stage specifically transcribed in erythroid cells. The spatiotemporal transcription of the β-like globin genes requires erythroid specific activators such as GATA1, NF-E2, TAL1 and Klf1. However, the roles of these activators are not clear in transcription of the human adult β globin gene. Here we have reduced the expression of NF-E2, TAL1 or Klf1 using shRNA in hybrid MEL cells containing a human chromosome 11 and analyzed the transcription of the globin genes and the binding of these activators, chromatin structure in the human β-globin locus. Knockdown of each activator did not affect the expression of other activators. Knockdown of TAL1 and Klf1 inhibited the transcription of the adult β-globin gene, whereas reduced NF-E2 expression did not affect it. The binding of other erythroid specific activators including GATA1 and DNase I hypersensitivity were decreased by TAL1 and Klf1 knockdown in the β-globin locus control region. Interestingly, the reduction of Klf1, but not TAL1, increased the transcription of embryonic ε- and fetal γ-globin genes and decreased the expression of Bcl11a and Klf3 repressing the γ-globin genes. These results indicate that NF-E2 is not necessary for the transcription of the human adult β-globin gene, and TAL1 and Klf1 contribute to the transcription in distinct manners.

LB-088
Insight into substrate-specific recognition and heat resistance of N alpha acetyltransferase SsArd1

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Nα-acetyltransferases (Nats) possess a wide range of important biological functions. The structure of Nats can vary according to the first two residues of their substrate. However, the mechanisms of substrate recognition of Nats are elusive. SsArd1 from thermophilic Achaea *Sulfolobus solfataricus*, belonging to the NatA family with preference of Ser residues, exhibits the greatest activity of acetylation at optimal temperature of 65°C. Crystal structure of SsArd1 in complex with the peptide substrate was determined to 1.84 Å. Compared the structure of SsArd1 with human Naa50p (NatE) showed significant differences in key residues of enzymes near the first amino-acid position of the substrate peptide (Glu35 for SsArd1 and Val29 for Naa50p). The biochemical data revealed that the substrate specificity of SsArd1 could be altered the substrate of NatE by a range of Glu35 mutants.

Additionally, the crystal structures of SsArd1 in different space groups indicated the loop region between β3 and β4 existing multiple conformations and forming a hydrogen bond networks via two Ser residues. Comparing with wild-type SsArd1, the variants substituted with Ala (S75A, S82A and S75/S82A) and with loop deletion had almost identical folds. Strikingly, two single-point mutants showed ~3°C decrease in melting temperature, while two other variants showed even ~7°C decrease in melting temperature, which correlated to the seriously reducing enzymatic activity. Taken together, the crystallographic studies combining spectroscopic and biochemical characterizations provide a detailed molecular basis for not only understanding the substrate-specific recognition, but also elucidating the mechanism of heat resistance of the ancient archaeal SsArd1.

LB-089
Molecular-vibration-sensitive odour coding in honeybee olfactory circuit

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The stereochemical theory of olfaction has recently been challenged by experimental observations and theoretical modelling, both of which suggest a contribution of molecular vibrations to the process of odour signal transduction. Behavioural experiments have shown that insects and humans are able to distinguish isotopomers of the same odorant with identical size and shape but different molecular vibrational modes. As an underlying mechanism, phonon-assisted inelastic electron tunnelling has been proposed. Quantum mechanical modelling has demonstrated that such a mechanism is applicable in a biological context and agrees with measured odour perception timescales. Because of the intrinsic complexity of behavioural paradigms, the involvement of molecular vibrations in the odour signal transduction process is still debated. To test the hypothesis that vibrational modes contribute to odour coding, we measured whether common and deuterated isoforms of natural odorants are coded differently in the antennal lobe, the primary processing centre of the honeybee olfactory circuit. Our results provide the first evidence that (i) different isotopomers generate significantly different odour response maps, that (ii) molecular vibrational sensibility is a general mechanism common to multiple odour receptors, that (iii) molecular vibration specificity is highly consistent among individuals, and that (iv) differences in the perceptual space between isotopomers reflect distinguishability of the measured vibrational spectra. Such findings clearly indicate a role for molecular vibrations in the ligand-receptor interaction, suggesting the involvement of phonon-assisted tunnelling in the process of odour signal transduction, which would be one of the rare manifestations of a quantum effect in a biological system.

LB-090
Effect of SLC26 anion transporters disease-causing mutations on the stability of STAS domain of *E.coli* YchM

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The human Solute Carrier 26 (SLC26) family of anion transporters consist of 10 members that are found in various organs in the body including the stomach, intestine, kidney, thyroid and the ear where they transport ions including bicarbonate, chloride and sulfate in an exchange mode. Mutations in these genes cause a plethora of diseases such as intestinal congenital chloride losing diarrhea (CCD), bone disorders including diastrophic dysplasisaa and Pendred syndrome resulting in hearing loss. To understand how these mutations affect the structures of the SLC membrane proteins and their ability to function properly, 12 human disease causing mutants from SLC26A2, A3 and A4 were introduced into the equivalent sites of the STAS domain of a bacterial homolog SLC26 protein YchM (DauA), which can be readily expressed in bacterial cells, to understand their effect on protein expression and stability. The results revealed that most mutations caused protein instability, self-association, oligomerization and increased sensitivity for degradation. The mutation A463K, equivalent to N558K in human SLC26A4, which located within alpha-helix1 of the YchM STAS domain, stabilized the protein. Circular dichroism measurements showed that most disease-related mutations decreased the helix content, whereas urea denaturation assays indicated that all mutations had more opened structures. Overall, we concluded that the disease-associated mutations destabilized the STAS domain resulting in an unfolded structure.

LB-091
Caffeine-induced alterations in human Sertoli cells metabolism and oxidative profile

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Caffeine is a widely consumed substance that has been reported to be a modulator of several cells metabolism. Since the metabolism of Sertoli cells (SCs) is essential for spermatogenesis, we aimed to study the effects of caffeine in human SCs (hSCs) metabolic pathways and hence in male reproductive health. hSCs were cultured in the absence or presence of caffeine (5, 50 and 500 μM) and its glycolytic profile was evaluated by studying glucose consumption and the production of lactate and alanine. Protein expression levels of glucose transporters (GLUT1 and GLUT3), phosphofructokinase 1 (PFK1), lactate dehydrogenase (LDH) and monocarboxylate transporter 4 (MCT4) were determined, as well as LDH activity. Besides, caffeine has demonstrated some beneficial antioxidant effects, which led us to evaluate the antioxidant capacity of hSCs, the formation of carbonyl groups and lipid peroxidation. Caffeine at the lowest concentrations (5 and 50 μM) stimulated lactate production, but only hSCs exposed to 50 μM showed increased GLUTs expression. At the highest concentration (500 μM), LDH activity was stimulated to sustain lactate production. Notably, the antioxidant capacity of hSCs was decreased in a dose-dependent way and SCs exposed to 500 μM presented a pro-oxidant potential. hSCs exposed to 50 μM presented lower protein oxidation and lipid peroxidation. Moderate consumption of caffeine appears to be safe to male reproductive health since it stimulates lactate production by hSCs, which can promote germ cells survival. Nevertheless, caution should be taken with excessive caffeine consumption to avoid deleterious effects in hSCs functioning and thus, abnormal spermatogenesis.

LB-092
Study of 25 hydroxy vitamin-D levels and Inflammatory marker in association with vitamin-D receptor *gene polymorphism* in patients with essential hypertension

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Essential hypertension is a typical example of a complex, multifactorial and polygenic trait. There are several genes, which together contribute to between 30% and 50% of the variation in blood pressure among humans. Hence determining the association of VDR gene polymorphisms with essential hypertension is expected to help in the evaluation of risk for the condition. Low 25OH-vitaminD levels are associated with high levels of high sensitive C-reactive protein (hs CRP), which decrease after 25OH-Vitamin-D administration.

In this context this study was under taken to measure 25OH-vitamin D, High sensitive c reactive protein levels and assess the association between these levels and vitamin-D receptor gene polymorphism in subjects with essential hypertension.

One hundred Essential hypertensives and 100 age, Body Mass Index (BMI) and gender matched controls were recruited from participating institution. 25OH Vitamin D levels were assessed by using High Performance Liquid Chromatography and Turbidometric method was used to estimate hsCRP. Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis was used to analyze *VDR gene polymorphism*.

There were no significant differences in age, gender and BMI of study participants. Genotype distribution and allele frequencies of VDR polymorphism differed significantly between Subjects and controls ($\chi(2)$ of 18.0; 2 degrees of freedom; $p < 0.001$). FF genotype and allele F were at significantly greater risk for developing hypertension and the risk was elevated in cases with positive family history and habit of smoking.

We conclude that, VDR gene *Fok-1* polymorphism is associated with the risk of developing essential hypertension.

LB-093
Antiproliferative effect of statins: Focus on statin transport into cancer cells *in vitro*

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Statins, widely used in clinics for treatment of hypercholesterolemia and prevention of cardiovascular diseases, are inhibitors of

3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, which is the rate-limiting enzyme of the mevalonate pathway. Statins cause depletion of both the product, cholesterol, and its intermediates, farnesyl-pyrophosphate and geranylgeranyl-pyrophosphate, which are inevitable for proper cell signaling. The chemical nature of individual statins is very diverse, which causes significant differences in their transport across cellular membranes, and thus in their antiproliferative efficiency. So far, the detailed mechanism of their action has not been fully explained.

The aim of the presented work was to study the antiproliferative potency of eight commercially available statins using three pancreatic cancer cell line models (MIA PaCa-2, CAPAN-2 and BxPC-3) and to compare their potency using cell models expressing organic anion-transporting polypeptides (OATPs), such as Hep G2 and HEK 293T cell lines. Further, we have studied the statin transport efficiency in MIA PaCa-2 and Hep G2 cells treated by individual statins in concentration and time dependent manner. For that purpose, ultrahigh pressure liquid chromatography together with tandem high resolution molecular spectroscopy (UHPLC-HRMS/MS) has been employed.

In summary, we found that the action of individual statins strongly differed. Among the most potent ones in terms of antiproliferative effects in pancreatic cancer model belong cerivastatin, simvastatin and pitavastatin in cell lines without the OATPs expression. Contrary to that, OATP positive cells were very sensitive also to pravastatin.

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LB-094
Molecular modeling of formate dehydrogenase modified forms: what modifications can block the substrate channel?

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NAD+-dependent formate dehydrogenases(FDH) catalyze the oxidation of formate to CO_2 with reduction of NAD+ to NADH. Eukaryotic FDHs catalyze this reaction by the ordered kinetic mechanism, whereas bacterial enzymes act by the random mechanism. The substrate channel in bacterial FDHs may be responsible for the random mechanism. Thus, search for substitutions blocking the substrate channel is helpful for better understanding of kinetic mechanisms at the molecular level. On the basis of a structural analysis and multiple alignment of FDHs representative sequences, we suggested following substitutions in FDH structure from Pseudomonas sp. (PDB ID 2NAC): F311W, Y102F and double F311W Y102F. All modifications were performed using the program UCSF Chimera. We used GROMACS software for their characterization. The F311W modification has demonstrated the minimum radius of the substrate channel (0,7 Å after 6 ns of MD simulation). The superposition of 2NAC structure and Y102F demonstrates that hydrophobic side chains around substrate channel came closer together (per ~0,4 Å) in Y102F after 30 ns of MD simulation. Resulting hydrophobic cavity hampers the charged substrate migration to active center, as it becomes energetically unfavorable. The structural analysis of double mutant F311W Y102F shows that the substrate channel is sterically blocked. Molecular modeling reveals the modifications blocking substrate channel of FDH (F311W Y102F, F311W, Y102F) which may be useful in following experimental investigation of FDHs kinetic mechanism. All simulations were performed using the facilities of the Supercomputer Centers "Lomonosov", "Chebushev" [Sadovnichy *et al.*, 2013] and "Arian Kuzmin".

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LB-095
Molecular modeling of Gmf to Arp2/3 complex binding: search amino acids important for interaction

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Arp2/3 complex plays a key role in driving cell motility, endocytosis, and intracellular transport. The yeast homolog of glia maturation factor (GMF) promotes debranching of actin networks by binding with Arp2/3. However, the molecular mechanism underlying actin debranching remains not fully understood. Thus, identifying of amino acid residues essential for interaction between Gmf and Arp2/3 in different sites of binding is important for better understanding of the molecular mechanisms of actin nucleation. Here we apply the molecular modeling, docking and molecular dynamics (MD) methods for screening interactions between Gmf and Arp2/3 complex. The docking and molecular models of Gmf to Arp2/3 complex were created using the UCSF Chimera software. GROMACS software was used for MD simulation and searching of amino acid residues important for interaction. The docking of Gmf to Arp2/3 complex suggests the formation of a salt bridge between LYS336 (Arp2 subunit) and ARG24 (Gmf). MD simulations of Arp2/3 complex with Gmf reveal an increase in the number of hydrogen bonds between Arp2 subunit and Gmf (from 1to4) and emergence the pairs within 0.35 nm between ArpC1/Arc40 subunit. Interactions between Arp2/3 complex with Gmf (LYS336-TYR84, LYS331-THR27 and double bond between LYS20 = ASP292) appear to be involved in the bond network. Resulting model may be useful in following experimental investigation using the method of electron microscopy for interpretation of the structure of Arp2/3 complex with Gmf.

This work was supported by RSF grant №14-14-00234. All simulations were performed using the facilities of the Supercomputer Centers "Lomonosov" [Sadovnichy *et al.*, 2013] and "Arian Kuzmin".

LB-096
Synaptic tenacity – beyond one molecule or another

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Activity-dependent modifications to synaptic connections – synaptic plasticity – is widely believed to represent a fundamental mechanism for altering network function, giving rise to emergent phenomena commonly referred to as learning and memory. This belief also implies, however, that synapses, when *not* driven to change their properties by physiologically relevant stimuli, should retain these properties over time. Otherwise, physiologically relevant modifications would be gradually lost amidst spurious changes and spontaneous drift. We refer to the expected tendency of synapses to hold onto their properties as "synaptic tenacity".

Over recent years, molecular imaging studies have changed our notion of the synapse, from that of a "structure" to that of a dynamic molecular assembly at steady state. These studies, com-

bined with proteomics and additional approaches, collectively indicate that synaptic molecular dynamics are dominated by the exchange and interchange of synaptic molecules, rather than protein synthesis and degradation, with the latter acting over longer time scales. Yet, regardless of their source and time scales, these continuous dynamics would seem to challenge the tenacity exhibited by individual synaptic sites. Indeed, recent studies from our lab and others indicate that the tenacity of individual synapses is inherently limited and that synaptic properties change spontaneously and extensively. We have also found, however, that these changes do seem to be governed by certain principles which become apparent when synapses are studied as individual entities on the one hand and populations on the other. This work, and the insights it has provided will be described.

LB-097
Role of flotillins in bacterial membrane organization

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Flotillins are associated with functional membrane domains (FMMs), or lipid rafts in pro- and eukaryotes. FMMs are physically and functionally separated parts of the plasma membrane. Due to the different lipid and protein composition of these domains they contribute in supplying a suitable environment for vital cellular processes including protein secretion, signal transduction, transport and cell wall metabolism.

Absence of flotillins *in vivo* leads to coalescence of distinct domains of high membrane order and, hence, loss of flotillins in the bacterial plasma-membrane reduces membrane heterogeneity. This loss of heterogeneity leads to impairment of vital cellular processes such as protein secretion. Therefore, it can be concluded that bacteria actively organize their membrane using specialized protein scaffolds. However, the molecular details of how flotillins interact with the membrane and maintain membrane organization are unknown.

In this study we focus on elucidating the molecular properties of prokaryotic flotillins. Therefore, we investigate two flotillins, FloT and FloA, from Bacillus subtilis. We have shown that FloT and FloA likely adhere to the membrane with an N-terminal hairpin loop and oligomerize through their prohibitin homology domain (PHB) *in vivo* and *in vitro*. One of the hypotheses is that oligomers form ring-like structures outlining the FMMs. In order to address this hypothesis full-lenght FloT was purified and reconstituted into liposomes. We apply various methods to characterize the organization of the flotillin oligomers in the reconstituted system and aim to understand its influence on membrane organization.

LB-098
Interaction and replication of ssDNA viruses and associated satellites

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Begomoiviruses infect a wide range of cultivated and the noncultivated plants worldwide causing a tremendous loss to yield directly or indirectly. *E. prostrata* is a weed found besides the water channels and agriculture fields in Pakistan, India, and China. A newly found virus *Altenethera yellow vein virus* (AlYVV) causing typical begomovirus like symptoms of vein yellowing and stunting is found to interact with satellite viral molecules. This project is designed for developing diagnostic tools, diversity studies and molecular characterization for identifying the viruses and their associated satellite molecules in the *E. prostrata* found in the different localities of the Punjab region of Pakistan. This project also includes the study of interaction of satellite molecules with the helper viral molecules for the onset of disease. Eclipta samples showing typical vein yellowing symptoms will be collected from the different districts of the Punjab, Pakistan. Amplification of the full-length viral molecules and their associated satellite molecules will be through PCR and RCA. Cloning of the full-length viral molecules, sequencing, recombination analysis and phylogenetic studies will be carried out to conclude how many different viruses and associated satellite molecules infect *E. Prostrata*. Infectious molecules of the full-length viruses and satellite molecules will be constructed for infectivity analysis to fulfil the Koch's postulate. Agroinfiltration method will be used to carry out infectivity studies on *Nicotiana benthamiana* and the *E. prostrata* plants. This study will contribute in understanding the diversity of satellite molecules interacting with AlYVV.

LB-099
Development of new antibacterial drugs based on inhibitors of histone-like HU-proteins

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Bacterial infections in susceptible of medical treatment with modern antibiotics are course of more than 50 000 deaths every year all over the world. Antibiotic resistance is recognized as a growing healthcare problem. Thus, development of new antibacterial substances is highly requested. HU proteins are the most abundant DNA-binding proteins in prokaryotic organisms. These small (about 90 amino acids per monomer) basic proteins occur as though homo- or hetero dimers, bind DNA in non-specific manner and play substantial role in processes of DNA repair, recombination and replication[1, 2]. Moreover, knockout of HU protein gene sufficiently represses bacteria growth and is lethal in many cases [3]. Thus Hu-proteins could be a good target for development of new antibacterial substances. Using molecular modeling with the structure of HU-protein from *Spiroplasmameliferum* (HUSpm) at highest resolution (1.36Å) [4] as a model, we identified a number of potential molecules, which bind to HUSpm in substrate cavity or in the interior of dimeric contact. Subsequent EMSA experiments demonstrated that three of the identified molecules inhibit DNA binding by HUSpm with IC_{50} in micromolar concentration. Future optimization of these molecules is in progress.

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LB-101
Identification of CDC-48 inhibitors in *C. elegans*

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The conserved AAA ATPase CDC-48 regulates a wide range of processes during the cell cycle. It generates mechanical force by the hydrolysis of ATP to promote the disassembly of protein complexes and it acts as a chaperone unfolding its substrates.

CDC-48 was first identified in a screen for mutants affecting cell division cycle (cdc) in yeast, but it has been mainly studied for its role in regulating cell proteostasis through the ubiquitin-proteasome system. It is also involved in several cell division processes, but the mechanisms are not well understood yet. The diverse roles of CDC-48 depend on its association with a large network of adaptors that confers substrate specificity.

Our aim is to understand the functions of CDC-48 in cell division in the *C. elegans* embryo. I will present a screening approach that we have designed to identify compounds that impair the association of CDC-48 with its adaptors. Such compounds will allow us to inhibit specific functions of CDC-48 and therefore dissect its diverse role in cell division.

LB-102
Involvement of long non coding RNAs in EGFR expression regulation in lung cancer

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Long non-coding RNAs (lncRNAs) represent one of the new frontiers in molecular biology. They compose a heterogeneous group of RNA molecules, over 200 bases long, which regulate a broad spectrum of molecular and cellular functions including transcription tune-up. Epidermal growth factor receptor (EGFR) is a major therapeutic target in lung cancer. Although EGFR mutations have been thoroughly studied and characterized, the link between abnormal EGFR expression and lung cancer pathogenesis is still not clear. Here we investigate EGFR mRNA expression in lung cancer and its potential regulation by the 2 long non-coding RNAs (LOC102723622 and EGFR-AS1) located within the EGFR locus.

The expression of EGFR, EGFR-AS1 and LOC102723622 was assessed by RT-qPCR in 80 lung tumor and adjacent normal tissue samples. In addition, we analyzed the methylation status of EGFR gene promoter by bisulfite pyrosequencing.

Only LOC102723622 showed significant elevated expression in tumors compared to normal lung tissue (median fold change = 3.5, $p = 0.019$). Expression of EGFR was correlated to EGFR-AS1 expression (Rho=0.43, $p = 0.005$). EGFR promoter was unmethylated in both tumor and normal tissues.

The significance of LOC102723622 overexpression must be further investigated to ascertain its functional importance in lung cancer development. In addition, this study demonstrates a unique potential epigenetic regulation of EGFR through an intragenic lncRNA.

LB-103
The influence of Ras/PKA signaling pathway on viability of *Saccharomyces cerevisiae* under calorie restriction and different stress conditions

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Ras/PKA pathway is an evolutionarily conserved signal transduction mechanism found in animal and fungi. It regulates cellular growth and aging in response to changing carbon level and other environmental conditions. In this study we analyzed *Saccharomyces cerevisiae* as a model organism to investigate the influence of various components of the Ras/PKA signal transduction pathway to cell viability and longevity under calorie restriction and various stress conditions. We used mutant strains with altered expression of Ras, PKA and PDE genes. These strains were grown in media containing different amounts of glucose and subjected to heat, osmotic or acidic stress conditions.

As expected, deletions of RAS1 and RAS2 genes and insertion of the human Ha-Ras led to the increased resistance to the stress conditions and higher cell viability while grown in the medium with different concentrations of glucose. Deletions of the PDE2 and PKA genes decreased cell viability and caused early entry to the death stage. However, PDE1 deletion and double deletion of both phosphodiesterase genes extended stationary stage and increased cell resistance to the stress conditions. Surprisingly, addition of the second copy of the RAS genes to the PKA deletions containing strains led to the increased cell viability and resistance. This could be due to feedback effect of the PKA to the cAMP synthesis rate by phosphorylation of the adenylatcyclase.

All these results contribute to the better understanding of the regulatory network associated with aging and influenced by calorie restriction combined with various stresses.

LB-104
Mic10 oligomerizes to bend mitochondrial inner membranes at cristae junctions

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The highly folded mitochondrial inner membrane is functionally compartmentalized into domains defined by different degrees of membrane curvature. Cristae membranes are invaginations towards the matrix from the boundary membrane. They are physically separated from the remainder part of the inner membrane by cristae junctions. These highly curved, narrow tubular openings are believed to be essential for the distribution of ions, metabolites and proteins along the inner membrane and they play a central role in apoptosis. Despite their physiological importance the molecular nature of these structures remains elusive.

Here we show that Mic10, a core subunit of the recently discovered MICOS complex changes membrane morphology *in vitro* and *in vivo*. We demonstrate that Mic10 spans the inner mito-

chondrial membrane in a hairpin topology and membrane shaping relies on the proteins ability to oligomerize through a glycine-rich motif. Oligomerization mutants lost their ability to directly induce membrane curvature and mitochondria carrying these mutants display highly decreased numbers of cristae junctions. Thus, we demonstrate that membrane sculpting by Mic10 is essential for cristae junction formation.

LB-105
Noninvasive biomarkers of stress: salivary alpha amylase, myeloperoxidase and total antioxidant capacity

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The objective of present study is to investigate the effect of stress on salivary parameters in young medical students.

Material and methods: The study groups consist of 30 medical students. Saliva samples were collected between 10.00 and 11.00 am on the day of their Biochemistry practical examination (stress condition). And next samples were collected between 10.00 and 11.00 am, 15 days after completion of the examination (relax condition). Salivary alpha amylase and myeloperoxidase activities were estimated by modified method of Huggins et al and of Andrew J et al respectively. Total antioxidant capacity in saliva were estimated by method of Koracevic D *et al*. Statistical analysis was done by OpenEpi software.

Result: Student paired t test was applied. Normality of distribution was checked by shapiro- wilk test. The salivary amylase level was significantly increased on the day of examination (Mean ± SD= 84448.76 ± 9046.13) than relaxed condition (Mean ± SD = 47,075.9 ± 2433.17) ($p < 0.05$). Myeloperoxidase activity was also significantly increased on the day of examination (Mean ± SD= 0.0563 ± 0.038) compared to that on relaxed condition (Mean ± SD = 0.0352 ± 0.0265, ($p < 0.05$). There was no significant difference in salivary protein levels . Total antioxidant capacity was significantly low in stress (Mean ± SD= 0.487 ± 0.092, $p < 0.05$) than relaxed condition (Mean ± SD = 0.730 ± 0.233) ($p < 0.05$).

Conclusion: Salivary alpha amylase and myeloperoxidase were increased in stress. TAC were significantly decreased in stress. These can be used as stress markers noninvasively.

LB-106
Apolipoprotein b deficient serum fails to prevent oxidation of placental villous membrane in diabetics.

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This study is done to find the ability of apolipoprotein B deficient serum to protect placental villous membrane from oxidation and to correlate it with paraoxonase activity in normal healthy and diabetic females.

Apolipoprotein B deficient serum of 30 diabetic and 30 age matched healthy females was used to evaluate lipid hydroperoxides and HDL-PON activity. Placental villous membrane was separated and homogenized with PBS and was oxidized with Cu^{2+} for 20 h at 37°C. The lipid hydroperoxides were estimated in this membrane and also after 3-hrs incubation with apolipoprotein B deficient serum from both groups. Lipid hydroperoxides and PON1 arylesterase were measured by methods described earlier (Ferretti *et al*., Eckerson *et al*.)

PON 1 arylesterase activities of apolipoprotein B deficient serum of diabetics (mean ± SD = 36.684 ± 5.63, CI= 35.664 – 37.704) was found significantly lower than healthy females (mean±SD = 56.162 ± 6.79, CI= 54.922 – 57.402) ($p < 0.001$). Apolipoprotein B deficient serum from diabetic patients shows significantly higher levels (mean ± SD = 1.171 ± 0.099, CI = 1.135–1.207) of lipid hydroperoxides ($p < 0.001$) than healthy counterparts (mean ± SD = 1.042 ± 0.101, CI = 1.006–1.078) showing that Apolipoprotein B deficient serum of diabetics protects the placental villous membrane from oxidation less efficiently than its healthy counterparts which shows different distribution curves. Correlational statistics using Pearsons correlation coefficient indicates that as paraoxonase levels increases, lipid hydroperoxides levels decreases. ($r = -0.188$).

Decrease in paraoxonase activity in diabetics may lead to decreased protection against peroxidation of placental villous membranes leading to complications in diabetic pregnancy.

LB-107
microRNA-155 impairs autophagy in chondrocytes by targeting autophagy genes

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Defective autophagy has recently emerged as a feature of aging-related pathologies, including osteoarthritis (OA) and is associated with reduced activity or expression of autophagic proteins and abnormal signaling events, including mTOR hyperactivation. Mechanisms responsible for defective autophagy in OA remain to be elucidated.

Here we have evaluated the role of miR-155, which is overexpressed in OA, in suppression of autophagy in the T/C28a2 human chondrocyte cell line and in human primary chondrocytes.

Rapamycin and 2-deoxyglucose (2-DG) were used to stimulate autophagy in primary human articular chondrocytes and the T/C28a2 cells and cells were transfected with GAPMER, antisense oligonucleotides, and MIMIC, double-stranded RNAs, specific for miR-155.

We observed that autophagy flux induced by rapamycin and 2-DG was significantly increased after downregulation of miR-155 with GAPMER, and significantly decreased after miR-155 MIMIC transfection in T/C28a2 cells. Furthermore, miR-155 negatively modulated the basal autophagy flux in human primary chondrocytes following GAPMER or MIMIC transfection.

These effects of miR-155 on autophagy activation were related to suppression of gene expression of some key autophagy regulators factors predicted previously by employing miRWalk and TargetScan databases. Surprisingly, we also observed that miR-155 decreased mTOR pathway activation.

These findings demonstrate that mIR-155 is a potent regulator of autophagy in chondrocytes by suppressing the levels of autophagy proteins and mTOR signalling.

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LB-108
The changes in the experimental course of molecular biology for medical graduates in Peking University Health Science Centre

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Peking University Health Science Centre was the first Medical Institution of Western Medicine founded by Chinese Government in 1912 and now is one of the most outstanding medical institutions in China with an enrolment of about 1500 students (including 800 graduate students) each year. The experimental course of molecular biology was first set up in 1992 and since then, the contents of the course have been modified several times. The aims of the course changes are not only to help students to acquire the basic technical skills, but also to keep up with the development of the subject in the world. The contents of the course were selected according to the demand of most researchers in China during the corresponding periods. The history of course change may divide into 4 stages and is expected to provide a chance to review the insight on the development of molecular biology education in China.

In the first two stages (1992–1996, and 1996–2000) since the course was set up, we focused on introducing individual experiments. These experiments were essential in most biomedical research institutes, but it couldn't reflect an entire research approach. In the third stage (2000–2010), the course was organised as one "recombinant DNA construction project", which was aimed to help students to know the whole process for an entire experiment. In the fourth stage (2010–), some new techniques were introduced as an advanced teaching block. The feedback from students proved the course to be an effective and useful training program.

LB-109
microRNA-9 mediates oxidative stress-induced cytotoxicity in chondrocytes by targeting Sirt-1

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Increasing evidence suggests that oxidative stress may be a key pathogenic factor in age-related disorders, including osteoarthritis (OA). A useful alternative to current, unsatisfactory drug treatment of OA may be represented by food-derived molecules, such as hydroxytyrosol (HT), able to interfere with the processes involved in OA development and progression. In addition, several microRNAs (miRs) have been reported to be responsive to oxidative stress and to play a key role in the modulation of different signalling pathways dysregulated in degenerative diseases.

The object of this study has been to investigate the involvement of miRs to address the cytotoxicity of hydrogen peroxide (H_2O_2) and the mechanistic aspects of protective action of HT in human cell line C-28/I2.

We observed that HT is able to reduce cell death and prevent the decrease of protein levels of Sirt-1 caused by H_2O_2. Among different candidate miRs targeting Sirt-1, we verified that HT inhibits the increase of miR-9 levels following treatment with H_2O_2.

We found out that miR-9 silencing led to a complete loss of the H_2O_2-induced cytotoxicity and rescues protein levels of Sirt-1 reduced after H_2O_2 treatment. Indeed, we observed that pre-miR-9 transfection interfered with the pro-survival action of HT and decreased protein expression of Sirt-1.

We speculate that miR-9 is a potent mediator of cytotoxicity mediated by H_2O_2 and may be a key target of the cytoprotective activity exerted by HT in C-28/I2.

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LB-110
Structural basis of vitamin B3 transport

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Recently we determined the first crystal structure of vitamin B3 transporter (PnuC) [1] from the family of bacterial Pnu transporters, which is distantly related to the family of sugar SWEET transporters. PnuC catalyzes cellular uptake of the NAD+ precursor nicotinamide riboside (NR) via facilitated-diffusion mechanism linked to metabolic trapping in the cytoplasm by the specific kinase NadR, which converts NR into nicotinamide mononucleotide (NMN) and NAD+. We have also determined the crystal structure of this kinase and performed thorough biochemical characterisations of PnuC transporter. Obtained results allowed us proposing the mechanism of vitamin B3 transport across the biological membrane.

[1] Jaehme M, Guskov A, Slotboom DJ (2014) *Nat Struct Mol Biol*. 21(11):1013–5.

LB-111
How to avoid the most frequent mistakes in Western blot analysis – FTO protein as general example

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The Western blot is a widely used analytical technique used to detect specific proteins in a sample of tissue homogenate or cell extract. To obtain reliable results it is necessary to exclude sources of potential false positive results, such as: (I) primary antibodies recognizing very similar or identical sequence of examined protein but also presented in other proteins, (II) secondary antibodies bounding not only primary antibodies but also immunoglobulins occurring in a sample. To avoid these false positive results we can use series of approach. First of all, verification of sequence recognized by primary antibody in bioinformatic databases to check if the sequence is present yet in other proteins than our protein of interest. Second, using two different primary antibodies recognizing two different sequences of examined protein. Third, to avoid ghost bands it is necessary to use proper concentration of antibodies in an experiment and do not choose too long time of exposure. In the case when secondary antibody attached to immunoglobulin present in a sample overlaps a signal of the protein of interest, to remove this antibody it is necessary to use protein G or A immobilized on agarose. Last but not least, performed siRNA transfection gives us the greatest certainty that our antibodies recognize the examined protein. In our study with the use of Western Blot analysis, we examine connected with obesity development FTO protein, one of the member of AlkB dioxygenase superfamily.

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LB-112
Molecular characterisation of the cross-talk between bone morphogenetic protein and LIM domain kinase 1 signalling

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Bone or body morphogenetic proteins (BMPs) are secreted growth factors of the transforming growth factor-β superfamily. They play important roles in embryonic development and tissue regeneration, not only in bone. In addition to the classical phosphorylation of Smad transcription factors, it is now clear that there is extensive cross-talk of BMP signalling with other pathways. A well-established example is the activation of LIM domain kinase 1 (LIMK1) by BMP7 in neuronal cells, resulting in dendrite outgrowth. LIMK1 promotes reorganisation of the actin cytoskeleton by phosphorylation and inactivation of the actin regulator cofilin. This is mediated by the BMP type II receptor (BMPRII), which interacts with LIMK1 through its unique C-terminal cytoplasmic tail region.

In this study we aimed to investigate further the molecular mechanism of LIMK1 activation by BMPRII. LIMK1 overexpression had differential effects on early (Id1 gene expression) and late (alkaline phosphatase) BMP signalling outcomes in C2C12 cells, a model for BMP-induced osteogenic differentiation. These effects were attributed to the inhibition of BMPRII internalisation by LIMK1. Furthermore, we demonstrated that BMPRII phosphorylates LIMK1 in the linker between the PDZ and kinase domains. We also narrowed down and validated the LIMK1 binding site in BMPRII using a combination of SPOT peptide, isothermal titration calorimetry and co-immunoprecipitation assays. LIMK1 binding-deficient BMPRII was generated to assess effects on BMP signalling. Using information about the binding site will help us to design strategies for blocking the interaction in cells. Moreover, it lays the groundwork for structural studies on LIMK1 and the BMPRII tail.

LB-113
Translation in the presence of macrolide antibiotics

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Macrolides are clinically important antibiotics that inhibit translation. They bind to the ribosome near the entrance of the nascent peptide exit tunnel, adjacent to the peptidyl transferase center. Because macrolides partially obstruct the tunnel, they inhibit translation by hindering the passage of the nascent polypeptide and by destabilizing the association of peptidyl-tRNA with the ribosome. However, some short peptides are able to displace macrolides from the actively translating ribosomes during translation termination.

By modifying the nascent chain amino acid sequence, we identified peptides which are able to displace macrolides during translation elongation. By analyzing ribosome profiling data we further showed a larger set of natural proteins, which were able to bypass the antibiotic in the tunnel without displacing it.

In addition, we were able to show that some polypeptides that span the entire length of the exit tunnel do not preclude (re-) binding of telithromycin, a ketolide antibiotic. Therefore, our findings may clarify the general understanding of macrolide action.

LB-114
Mechanisms of FGF1 membrane translocation

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Fibroblast growth factors (FGFs) and their receptors (FGFRs) regulate cell survival, proliferation, differentiation and migration, and overactivation of FGFRs has been found to contribute to development of various breast, bladder, prostate, endometrial, lung and haematological cancers. In addition to the canonical role of the receptor tyrosine kinase activity of FGFRs in signaling, our group has previously revealed another and less well characterized mechanism that involves a direct action of FGF1 in the cell nucleus. In order to reach the nucleus, FGF1 first has to bind its receptor, become endocytosed together with its receptor, translocate from an endocytic compartment to the cytosol, and shuttle into the nucleus via an importin-dependent process. It is still not known which endosomal compartment membrane translocation occurs from and how FGF1 is translocated across the endosome membrane. In the present project we propose to use an engineered peroxidase (APEX) for electron microscopy of FGF1-containing endosomes and a proximity biotinylation approach to identify protein partners that interact with FGF1 and its receptors in endosomes in order to identify components of the endosomal FGF1 translocon.

LB-115
Spermidine protects human chondrocytes from oxidative stress and reduces cell death by autophagy induction

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A major research challenge is the development of strategies for the prevention/disease modifying treatment of Osteoarthritis (OA), the degenerative disease of articular cartilage. At present, there are no therapies which prevent or arrest OA progression. In this scenario an alternative and safe opportunity is represented by nutraceuticals. Spermidine (SPD), a natural dietary compound, belongs to the class of polyamines, naturally occurring polycations. It is known that SPD extends lifespan in yeast and flies by an autophagy-dependent mechanism.

We focused on the ability of SPD to attenuate oxidative stress after H_2O_2 treatment and to induce autophagic mechanisms in OA articular chondrocytes.

Chondrocytes from knee cartilage of adult OA patients were pre-incubated with SPD and then exposed to H_2O_2. H_2O_2 markedly increased cell death was measured by Sytox Green, a probe that penetrates cells with compromised plasma membranes. SPD pre-treatment strongly reduced H_2O_2 dependent cell death and the extent of γH2AX-foci, markers of DNA damage that form

when both DNA strands are broken closely. Moreover, SPD induced autophagy was measured by the increased signal of microtubule-associated protein 1 light chain 3 II (LC3 II), a marker of autophagosomes. Our findings therefore indicate an antioxidant/chondroprotective activity of SPD after an oxidative stimulus mimicking the environment of OA cartilage. The combined reduction of γH2AX-foci and increased LC3II signal suggest that SPD promotes an efficient autophagic flux. Therefore, we propose that SPD can be considered an interesting tool for OA prevention and treatment.

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LB-116
Roles for free iron in AlkB-dependent and independent mechanisms in alkylated DNA repair

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Escherichia coli hemH accumulates protoporphyrin IX photosensitizing cells to visible light. We have shown that intracellular free iron in *hemH* mutants is double that observed in $hemH^+$ strain. The aim of this study was to recognize the influence of free iron on AlkB-directed repair of alkylated DNA analyzing survival and mutation induction after visible light irradiation and MMS-treatment of *E. coli* AB1157 *hemH* and *alkB* mutants. *E.coli* AlkB dioxygenase constitutes repair system using Fe(II) and 2-oxoglutarate to initiate oxidative dealkylation of DNA/RNA bases. We have established that the frequency of MMS-induced Arg^+ revertants in AB1157 $alkB^+hemH^-$/pMW1 was 40 and 26% reduced comparing to the $alkB^+$ $hemH^-$ and $alkB^+$ $hemH^+$/pMW1 strains. The effect was observed only in bacteria irradiated with λ>320 nm light prior MMS-treatment. This finding indicates efficient repair of alkylated DNA in photosensibilized cells in the presence of higher free iron pool and AlkB concentrations. Interestingly, 31% decrease in the level of Arg^+ reversion was observed in irradiated and MMS-treated $hemH^-$ $alkB^-$ comparing to the $hemH^+$ $alkB^-$ strain. Also, the level of Arg^+ revertants in the irradiated/MMS treated $hemH^-$ $alkB^-$ mutant was significantly lower (34%) in comparison to the same strain but MMS-treated only. These indicate AlkB-independent repair involving Fe ions and reactive oxygen species and may be caused by non-enzymatic dealkylation of alkylated dNTPs in *E. coli* cells. In *in vitro* studies, the absence of AlkB protein in the presence of iron ions allowed etheno(ε)dATP and εdCTP to spontaneously convert to dAMP and dCMP.

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LB-117
Production of the plant triterpene friedelin in *Saccharomyces cerevisiae*

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Friedelin is an interesting cetonic pentacyclic triterpene isolated from the leaves of *Maytenus ilicifolia*. It is precursor of the quinonemethide triterpenoids maytenin and pristimerin, important anti-inflammatory agents. Triterpenes are formed by the cyclization of oxidosqualene catalyzed by oxidosqualene cyclases (OSC). In *Saccharomyces cerevisiae*, the sole OSC is a lanosterol synthase (encoded by *ERG7*), which takes part in the biosynthesis pathway for ergosterol, an essential component of the plasma membrane. The presence of the same precursor, oxidosqualene, in yeast and in plants allows the production of triterpenes in *S. cerevisiae* and enables the functional characterization of plant OSC genes, which was the aim of the present study. We used a *S. cerevisiae* strain generated by crossing CEN.PK2 strain and the *ERG7* Decreased Abundance by mRNA Perturbation (DAmP) strain. After induction of the expression of the plant OSC gene in yeast, the triterpene fraction was extracted and analyzed by GC-MS. Chromatographic analysis coupled with mass spectrometry from the extract of recombinant yeast showed the presence of friedelin as the sole pentacyclic triterpene. Therefore, functional characterization in yeast was able to confirm the identity of the coding sequence cloned from the leaves of *M. ilicifolia* as friedelin synthase. This is the second plant friedelin synthase gene identified so far and the comparison with these and other triterpene and primary metabolism OSC genes will contribute to the understanding of their enzymatic metabolism. Moreover, our work demonstrated that *S. cerevisiae* is a suitable model for heterologous friedelin production.

LB-118
microRNA-499 protects the cardiomyocytes from LPS-induced apoptosis

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Lipopolysaccharide (LPS) is a major mediator in sepsis-induced cardiac apoptosis. However, its mechanism is unclear. In our previous study, we demonstrated that microRNA-499 (miR-499) inhibited apoptosis during cardiac differentiation of P19CL6 cells. It is unknown whether miR-499 is involved in LPS-induced cardiac apoptosis.

We treated neonatal rat ventricular myocytes with LPS and then examined the expression of miR-499 and its several potential target genes. The results showed miR-499 level was reduced in dose- and time-dependent manners with LPS. The expression of *Sox6* and *Pdcd4* was significantly upregulated. The results of annexin V/PI- or TUNEL-staining demonstrated that LPS-induced apoptosis rate was reduced in the cardiomyocytes treated with miR-499 mimic, while it was potentiated in Inhibitor-treated cardiomyocytes. In contrast to miR-499, *Sox6* or *Pdcd4* overexpression enhanced the apoptosis rate from 50% to almost 80%, while knockdown of either *Sox6* or *Pdcd4* decreased the apoptosis rate from approximately 50% to 25%. We further demonstrated that miR-499 mimic attenuated *Sox6* or *Pdcd4* expression, whereas miR-499 inhibitor elevated its expression; the miR-499-mediated protective effects were blocked in the presence of *Sox6* or *Pdcd4*-encoding exogenous constructs that did not contain the 3′-UTR in which contains the miR-499 binding sites. These results suggest that *Sox6* and *Pdcd4* both are direct miR-499 targets. We also found miR-499 overexpression inhibited *Bad*, *Bax* and *Bid* expression, and promoted *Bcl-xL* expression; cotransfection of *Sox6* or *Pdcd4* with miR-499 reversed the miR-499′s effects on *Bad*, *Bax*, *Bid* and *Bcl-xL* expression, indicating the existence of a LPS-miR-499-*Sox6*/*Pdcd4*-apoptosis pathway.

LB-119
Second step of genome-wide association study: Validation of 50 single nucleotit polymorphisms associated with sporadic colorectal cancer in Turkish population

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Colorectal cancer (CRC) is the second most seen neoplasia in developed countries and over 75% of the CRC cases are sporadic. Although it is one of the most well studied cancer type, mechanisms that trigger sporadic CRC together with its predisposing genes have not been entirely exhausted. In this study, our aim is to identify gene loci responsible for sporadic CRC susceptibility in Turkish population and define new genes which predispose CRC in our population.

Our study is consisted of a family based genomewide association study (GWAS) results and their validation by using KASP (Kompetitive allele spesific amplification) technology. In the first step, we identified 75 CRC associated SNPs by 250K Affymetrix chips in 51 trio. In the second step we used KASP technology to investigate 75 SNPs in 1000 cases and 1000 healthy controls. So far, we studied 50 SNPs in 913 patientand 607 controls. Statistical analysis was carried out by Cochran-Armitagechi-square test.

In this study 5 out of 50 SNPs were found to be associated with sporadic CRC in Turkish population. With regards to our study preliminary results cancer associated SNPs localized in 1q43, 4p15.2, 5q11.2, 6p25.2 and 10p14.

This study is the very first CRC GWAS in Turkish population and it includes preliminary results of the validation studies. With this study, we seek to explain the molecular background of sporadic CRC by investigating and subsequently revealing the related genes in our population of interest.

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LB-120
N-myc downstream-regulated gene 2 (NDRG2) suppresses the epithelial.mesenchymal transition (EMT) in breast cancer cells via STAT3/Snail signaling

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Although NDRG2 has recently been found to be a candidate tumor suppressor, its precise role in the epithelial.mesenchymal transition (EMT) is not well understood. In the present study, we demonstrated that NDRG2 overexpression in MDA-MB-231 cells down-regulated the expression of Snail, a transcriptional repressor of E-cadherin and a key regulator of EMT, as well as the phosphorylation of signal transducer and activator of transcription 3 (STAT3), an oncogenic transcription factor that is activated in many human malignancies including breast cancer. In addition, we confirmed that the expression of Snail and phospho-STAT3 was recovered when NDRG2 was knocked down by siRNA in MCF7 cells in which NDRG2 is endogenously expressed. Interestingly, MDA-MB-231-NDRG2 cells showed remarkably decreased Snail expression after treatment with JSI-124 (also known as cucurbitacin I) or Stattic, STAT3 inhibitors, compared to MDA-MB-231-mock cells. Moreover, STAT3 activation by EGF treatment induced higher Snail expression, and NDRG2 overexpression resulted in the inhibition of Snail expression in MDAMB-231 cells stimulated by EGF in the absence or presence of STAT3 inhibitor. Treatment of MDA-MB-231 cells with STAT3 inhibitor led to a moderate decrease inwound healing and migration capacity, whereas STAT3 inhibitor treatment of MDA-MB-231-NDRG2 cells resulted in a significant attenuation of migration in both resting and EGF-stimulated cells. Collectively, our data demonstrate that the inhibition of STAT3 signaling by NDRG2 suppresses EMT progression of EMT via the down-regulation of Snail expression.

LB-121
"Quorum sensing in times of cholera" – targeting bacterial virulence regulators in *V. cholerae* with electrophilic probes

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Antibiotic-resistant pathogens have become a rising and troubling phenomenon in recent years. A potential solution may come through fighting infections by targeting virulence mechanisms as opposed to killing the bacteria, thereby avoiding the strong selective pressures that encourage antibiotic-resistant strains to develop. One strategy for doing so is to disrupt quorum sensing (QS), the mechanism by which bacteria communicate with one another via small signaling molecules in order to coordinate their behavior, often including pathogenic behavior. For example, in *Vibrio cholerae*, a Gram-negative pathogen estimated to cause 100,000-120,000 deaths each year, QS mediates biofilm formation and the production of virulence factors through CAI-1, an α-hydroxyl ketone signaling molecule.

We have synthesized CAI-1 analogues containing electrophilic moieties with the aim of covalently binding them to a specific cysteine residue in the CAI-1 receptor, CqsS. Once the probe is covalently bound, it will be possible to selectively label the modified CAI-1 with a fluorescent aminooxy-tag as previously reported by our group. The advantage of this method is that the binding of the probe to the receptor and the labeling are two separate reactions, thereby avoiding a decrease in affinity of the probe to the receptor due to a bulky tag. Moreover, covalently binding a CAI-1 analogue to its receptor can be later used for live cell imaging and further investigation of the QS system in *V. cholerae*, opening the way for innovative, new ways to treat the disease.

LB-122
Structural elucidation of bi-specificity of A-domains as a basis to activate non-natural amino acids

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Many biologically active peptide secondary metabolites of bacteria are produced by modular enzyme complexes, the non-ribosomal peptide synthetases. Substrate selection occurs through an adenylation (A) domain, which activates the cognate amino acid with high fidelity. The recently discovered A domain of an Anabaenopeptin synthetase from *Planktothrix agardhii* (ApnA A_1) is

capable of activating two chemically distinct amino acids (Arg and Tyr).

We present crystal structures of the A domain that reveal how the enzyme can adapt both substrates. Analysis of the binding pocket led to the identification of three residues that are critical for substrate recognition. Systematic mutagenesis of these residues created A domains that became mono-specific, or changed substrate specificity to tryptophan. The non-natural amino acid substrate 4-azidophenylalanine is also efficiently activated by a mutant A domain, enabling the production of diversified non-ribosomal peptides for bioorthogonal labeling.

LB-123
Introduction of a salt bridge increases the thermal stability of a polyester hydrolase from *Thermobifida fusca*

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Thermobifida fusca is a thermophilic actinomycete isolated from compost. The strain *T. fusca* KW3 contains the genes *tfcut1* and *tfcut2* encoding for two polyester hydrolases capable of hydrolyzing polyethylene terephthalate (PET). TfCut2 contains a Ca^{2+} binding site and displays a higher thermostability and activity compared to TfCut1. Ca^{2+} bound to this site was shown to increase the melting point of TfCut2 by 14°C.

The influence of Ca^{2+} on the thermal stability of the enzyme was studied using molecular dynamics simulation experiments. The results indicated that an identical thermal stability of the enzyme can be obtained by replacing the Ca^{2+} binding site with the salt bridges D204R or E253R. Variants containing the salt bridge were shown to be able to hydrolyze PET films at 65°C in the absence of Ca^{2+}.

LB-124
Use of biomolecules delivery vector, adenovirus dodecahedron, for targeted cancer therapy

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Adenoviral dodecahedron (Dd) is a non-enveloped symmetrical virus-like particle (VLP). VLPs are multimeric stable proteinaceous nanostructures devoid of any genetic material, formed from functional viral proteins responsible for cell penetration, which ensures VLPs efficient cell entry. Dd, composed of twelve copies of the pentameric penton base (Pb) protein, is spontaneously generated in the baculovirus system upon the expression of the Pb gene of adenovirus serotype 3 (Ad3). The particle shows remarkable cell penetration ability with 200,000–300,000 Dd internalized into one cell in culture, conceivably delivering several millions of foreign cargo molecules to the target cell. For this reason, they are of great interest as a delivery vectors. Stability studies show that Dds can be conveniently stored and transported, and can potentially be used for therapeutic purposes under various climates. We have used Dd for delivery of small drugs for targeted cancer therapy. A cell-impermeant oncogene inhibitor or anti-cancer antibiotics doxorubicin and bleomycin were delivered as Dd conjugates, demonstrating significantly improved drug bioavailability. Recently we undertook some improvements in the protocols of Dd expression and purification, leading to considerable savings in time, improved yield and allowing the scaling-up of the protein production.

LB-125
Genetic analysis of the glycopeptidolipids (GPLs) synthetic pathway in *Mycobacterium intracellulare*

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Mycobacterium avium-intracellulare complex (MAC) has been classified into 31 serotypes based on the difference of oligosaccharide structure in glycopeptidolipids (GPLs) presented on the bacterial cell surface. However, biosynthesis pathways and responsible genes of serotype-specific GPLs have not been clear for most of the serotypes. We determined the chemical structure of GPL derived from *Mycobacterium intracellulare* serotype 16, and analyzed DNA sequence of the serotype 16-specific gene cluster for GPL biosynthesis. Structure of the serotype 16 GPL was similar to that of serotype 17, except for acylation. In this study, we determined DNA sequence of the serotype 17-specific gene cluster for GPL biosynthesis, and analyzed open reading frames (ORFs) found in the cluster. The cosmid libraries were constructed using the genomic DNA from *M. intracellulare* ATCC35763 (serotype 17). Functions of the ORF found in the cluster were examined by transformation of *M. avium* and *M. intracellulare*, and structures of GPLs produced in the transformants were analyzed. Fifteen ORFs were found in the serotype 17-specific gene cluster. The genetic organization between the *gtfB* and *drrC* of *M. intracellulare* ATCC35763 was closely related to that of *M. intracellulare* 13950 (serotype 16). Thirteen ORFs out of 15 were in common with those in serotype 16.

The relation between function of genes in GPL biosynthesis cluster and structure of GPL was also examined in other serotypes. As for the different genes between different serotypes, the functions were analyzed by over-expressing gene in MAC.

LB-126
Structural dynamics and ion channel activities of CyaA-hemolysin pore from *Bordetella pertussis* revealed how it may conduct cations

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Adenylate cyclase hemolysin (CyaA) is a toxin secreted by *Bordetella pertussis* that causes whooping cough. It uses its hemolysin domain (Hly) to cooperatively form a transmembrane oligomeric pore that conducts ions and subsequently lyses susceptible sheep erythrocytes. In this study, we incorporated CyaA-Hly into planar lipid bilayer (PLB) to investigate its ion channel activities and found that (1) CyaA-Hly was a cation-selective ion channel with conductance of ~35 and ~250 pS under symmetric and asymmetric conditions, (2) the potassium to chloride ion perme-

ability ratio was ~8, (3) CyaA-Hly conducted cations only in one direction and opened its channel only when negative voltages were applied, and (4) it had multiple open and closed time constants ranging from 20 ms to 5 s. Our previous works suggested that α2-loop-α3 hairpin of CyaA-Hly could be a potential pore-lining constituent that contained conserved Glu^{570} and Glu^{581} residues and were important for the hemolytic activity. Moreover, work done by others on osmotic protection assay of CyaA-toxin indicated that its pore was rather small with a diameter of ~0.8 nm. This led us to build a pore model of CyaA-Hly as a trimer consisting of three α2-loop-α3 hairpins. Then, we used it to study structural and dynamical properties via molecular dynamics (MD) method. Our pore model revealed two cation binding sites wihin the pore lumen formed by Glu^{570} and Glu^{581}, and helix packing between the hairpins. The dynamics of the CyaA-trimeric pore in DMPC bilayer during 50 ns MD simulations revealed how CyaA-Hly may conduct cations.

LB-127
Introducing positive charges to the pore interior of CyaA-hemolysin from *Bordetella pertussis* increased its hemolytic activity

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Adenylate cyclase-hemolysin (CyaA) is one of the toxins secreted by the bacterium *Bordetella pertussis* that causes whooping cough. It induces lysis of susceptible erythrocytes by forming ion channels *via* its hemolysin (Hly) domain. Our previous works suggested that amphipathic helix 3 in this domain formed the pore-lining surface of CyaA-Hly channel and the conserved Gln^{574} and Glu^{581} residues on this helix were important for its ion channel and hemolytic activities. Interestingly, in other highly-active hemolysins, the amino acids corresponding to these two positions are found to be both lysines hinting that positive charges are preferred in the pore interior for CyaA to be more hemolytically active. Moreover, mutagenic work done by other showed that replacing Glu^{581} with a lysine made CyaA four times more hemolytically active. In this study, we therefore decided to investigate this idea in more details and tried to understand what really happened at molecular levels. So we performed single-mutagenic substitutions at both positions by replacing each of them with either a positively-charged (K/R) or a polar- or a negatively-charged residue (Q/E). We could make CyaA-Hly up to 3.5 times more hemolytically active by replacing each of them with a positively charged or polar residue while introducing more negative charges abolished hemolytic and ion channel activities. In addition, we incorporated these mutants into planar lipid bilayer (PLB) and measured their ion channel activities under symmetric and asymmetric conditions and then performed molecular dynamics (MD) simulations of these mutants to investigate their ion channel activities and mechanisms.

LB-128
Thioredoxin reductase from *s. Coelicolor* as a drug target

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One of the best possibilities how to create or design the effective inhibitors seem to be compounds, which are designed after determination of protein structure with revealed other biochemical properties.

Thioredoxin reductase (TrxR) is part of thioredoxin system. TrxRs are homodimeric enzymes belonging to the flavin-pyridine-nucleotide-disulfid oxidoreductase family, which catalyze the transfer of two electrons from NADPH via FAD and N-terminal disulfide active site to the thioredoxin or other substrate. Bacterial TrxRs differ from mammalian in many aspects. They have different sizes, 3D structures, mechanism of catalysis and also position of active site. They are very specific and except for thioredoxin, they accept only a small amount of other substances.

Particularly, the absence of cooperating antioxidation system – glutathione-glutaredoxin system in some pathogenic bacteria (such as *Helicobacter pylori*, *Mycobacterium tuberculosis* and *Staphylococcus aureus*), makes the bacterial system essential for survival under oxidative stress. This provides an opportunity to kill these bacteria by targeting the thioredoxin system. Differences between mammalian and bacterial TrxR open up a new potential for designing new drugs or inhibitors.

TrxR from *S. coelicolor* is homodimeric low-molecular-weight protein (each monomer is 34,9 kDa). It shows 62% sequence identity to homologous enzyme *M. tuberculosis*, which was used for determination the structure of TrxR *S. coelicolor* by molecular replacement using available structures.

LB-129
GSK3β: a key regulator of oxidative stress in 3D-cultures of osteoarthritic chondrocytes

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Glycogen synthase kinase-3β (GSK3β) is a key regulator of chondrocyte signaling. When GSK3β is inhibited by phosphorylation, β-catenin translocates in the nucleus and activates pathways that promote chondrocyte hypertrophy and terminal differentiation, mimicking what happens in Osteoarthritis (OA). We previously demonstrated that GSK3β inhibition in chondrocyte monolayer cultures induces ROS generation and DNA damage. Here we focused on the oxidative response of chondrocytes in 3D-cultures downstream GSK3β inactivation, by evaluating reactive oxygen species (ROS) production, DNA damage and cell death.

Chondrocytes from knee cartilage of adult OA patients were cultured in micromasses and treated with GSK3β inhibitors (LiCl, SB216763 or insulin) during micromass maturation or for 16 h thereafter.

Following the three GSK3β inactivating stimuli left for 16 h, we observed increased ROS production as measured by Light-Sheet Microscope Fluorescence analysis of the ROS-specific probe dichlorofluorescein diacetate as well as increased signal of the mitochondrial probe Mitotracker Orange CMTMRos. In keeping with the findings of increased oxidative stress, GSK3β inactivation led to accumulation of higher levels of 8-oxo-dG DNA adducts with a mitochondrial pattern, in treated compared to unstimulated chondrocytes. Moreover, GSK3β inhibition increased cell death in 3D cell cultures after 5 days of treatment, as assessed by Live&Dead staining.

Our findings indicate that GSK3β plays a key role in maintaining oxidative stability in chondrocytes cultured within their extracellular matrix. GSK3β inactivation induces ROS production that leads to mitochondrial DNA damage and cell death.

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LB-130
Formulated diet as a supplement in the management of streptozotocin-induced diabetes mellitus in rats

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Diabetes mellitus is a metabolic disorder constituting a major health concern today whose prevalence has continuously increase worldwide over the past few decades. In the present study, diet was formulated, the glycaemic index of the formulated diet determined and its effectiveness tested in the management of streptozotocin-induced diabetic rats. Diabetes was induced by single intraperitoneal injection of streptozotocin 150 mg/Kg Bwt to the albino rats. The glycaemic index (GI) of the formulated diet was determined to be 57% (medium glycaemic index). Non diabetic controls and diabetic controls received balanced normal nutritive diet while the experimental animals were treated with 30%, 70%, and 100% of the formulated diet for a period of 28 days. There was significant decrease ($p < 0.05$) in blood glucose level of the diet treated groups when compared with the diabetic control group. There was a significant increase at ($p < 0.05$) in high density lipoprotein HDL value, significant decrease ($p < 0.05$) in triglyrceride, cholesterol and low density lipoprotein (LDL) values of the formulated diet treated groups when compared to the diabetic control group. A relative increase in body weight of the diet treated group was observed as against the diabetic control group; however, the effect was prominent in the group given 100% of the formulated diet. Thus, the present animal study evidenced the hypoglycaemic, hypocholesterolemic properties of the formulated diet suggesting that it could be used in management of diabetes.

Key words: Streptozotocin, diabetes mellitus, formulated diet, hypoglycaemic

LB-131
New structural insights into PARP3 function

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PARP3 is a member of the poly(ADP-ribose) polymerase superfamily that localises to sites of DNA damage – promoting canonical Non-Homologous End-Joining (cNHEJ) of DNA double-strand breaks, via its downstream recruitment of APLF.

PARP3, like the structurally related PARP1 and PARP2 enzymes, contains two highly conserved domains: WGR (Trp-Gly-Arg) and Catalytic, connected together by a short 15 amino acid linker region. In addition it also contains a largely uncharacterised 48 amino acid region at its N-terminus.

We have demonstrated that PARP3 binds preferentially to DNA duplexes containing single-strand breaks – with particularly high affinity for nicks containing a 5' phosphate – which stimulates the enzyme's ADP-ribosyl-transferase activity to a much greater extent than simple blunt-ended DSBs. We confirm that the mechanism of the ADP-ribosyl-transferase occurs in *cis* (as opposed to *trans*). We also define the residues of PARP3 involved and required for DNA-binding, and additionally reveal that the N-terminal region remains unfolded, even when bound to DNA.

LB-132
Contribution of electrostatic interactions to the binding of halogenated benzotriazoles by protein kinase CK2α confirmed by the single point mutation

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CK2 is a ubiquitous serine/threonine protein kinase, being one of the most pleiotropic of all protein kinases. It plays a key role in cell growth, differentiation, cell death and survival, and becomes the therapeutic target in cancer treatment.

One of the first potent and selective inhibitor of CK2α, directed towards the conserved ATP binding site, was 4,5,6,7-tetrabromobenzoteriazole (TBBt). We have recently shown that a balance of hydrophobic and electrostatic interactions contribute predominantly in binding of halogenated benzotriazoles to the ATP-binding site of hCK2α[1–3].

To assess the contribution of electrostatic interactions independently, we have used *in-silico* modeling to design the single-point mutation that should substantially decrease electrostatic interaction between a ligand and the protein. Thus, Asp175 is known for its function in coordination of a Mg^{2+} ion, which is required for ATP binding. It is a negatively charged residue closest to TBBt and its homologues.

Variation in the strength of ligand binding at the ATP-binding site were measured by DSF and MST techniques, both for the wild type protein and its D175N mutant. The thermodynamic data obtained clearly confirmed that ligand binding is driven by electrostatic interactions.

1. Wasik *et al. PLoS ONE* 7 (2012): e48898.
2. Winiewska *et al.* BBRC, 456 (2015) 282.
3. Winiewska *et al.* BBA (2015) doi:10.1016/j.bbapap.2015.04.004.

LB-133
Hyperpolarization-activated and cyclic nucleotide-gated channels in hippocampal neurons

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The hippocampal formation is considered a relay center for novel information in the vertebrate brain, participating in learning and memory functions of organisms. In general terms, initiation and coordination of signals in neuronal networks relies on a complex

interplay of ion channels. Amongst the different ion channel families, hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels play an essential role. In the murine CNS, HCN channels are expressed in a distinct and differential manner. In contrast to typical voltage-dependent channels, they are activated at negative membrane potentials. Furthermore, HCN channel activation can be modulated via direct binding of cAMP.

Here, we investigated the expression profiles of HCN channels in hippocampal neurons. We used independent methods, *e. g.* immunohistochemistry, immunocytochemistry, and quantitative PCR, to investigate the expression pattern of individual HCN isoforms. We found that HCN 1, 2, and 4 are expressed differentially in the murine hippocampus. Thus, we sought an approach for further study of these proteins in hippocampal cells. We used cultures of murine primary hippocampal neurons (PHNs) which maintain many biochemical and electrophysiological properties that have been identified in the intact hippocampus. We found that HCN channels exhibit distinct expression patterns in cultivated PHNs. Most notably, subcellular distribution patterns of HCN isoforms in PHNs resembled that found in intact hippocampal tissue.

Our findings suggest that properties of individual signaling molecules can be studied in cultivated PHNs as a model. Finally, we established recombinant adeno-associated viral vectors (rAAVs) as an efficient tool to genetically modify hippocampal cell functions in forthcoming studies.

LB-134
Dynamics of cyclic adenosine monophosphate signaling in cell lines and primary hippocampal neurons

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With more than 800 members, G protein-coupled receptors (GPCRs) constitute the largest gene family encoding cell-surface receptors. They are targets for a wide range of drugs such as β-blockers, antihistamines and opiates. Once activated, GPCRs can control and modulate the concentration of the intracellular second messenger cyclic adenosine monophosphate (cAMP). The molecular components contributing to cAMP signaling have been studied extensively, yet little is known about the time constants of individual steps during the signal transduction process. Notably, cAMP signaling is highly compartmentalized within cells. Therefore, studying the spatial and temporal distribution of cAMP signals as well as investigating the kinetics of GPCR-mediated signal transduction is an important issue in current research. Genetically encoded sensors have been developed for monitoring intracellular dynamics of cAMP.

Epac1-camps, one of the first optogenetic sensors, is a versatile tool to detect intracellular changes in cAMP. However, expression of Epac1-camps in cells leads to a homogeneous distribution in the cytosol. For monitoring of local cAMP signals, we genetically modified the sensor to target the protein to distinct cellular compartments such as the plasma membrane and the nucleus. Based on the purified Epac1-camps proteins we examined biophysically whether the targeting sequences had any effect on the sensor properties. Further characterization of the different Epac1-camps versions was performed in HEK293 cells. Since cAMP signals play an important role in neuronal function recombinant adeno-associated viruses were generated allowing expression of the different Epac1-camps variants in primary hippocampal neurons to register and examine GPCR-mediated cAMP signals in these cells.

LB-135
The role of glycoprotein IIIa P1A1/A2 genotype in Turkish stroke patients

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Aspirin plays a crucial physiological and pathophysiological role in cardiovascular diseases and cerebrovascular diseases by irreversibly inhibiting thromboxane A2. Aspirin resistance occurs in 5–45% of high-risk patients, with various mechanisms proposed for its development. The resistance has close association with adverse cardiovascular outcomes and increased mortality. The relationships among aspirin resistance, aspirin dosage, type of aspirin, and glycoproteinIIIaP1A1/A2 genotype in patients with vascular risk factors were investigated in this study.

Seventy-five symptomatic, 133 asymptomatic patients with vascular risk factors who were using aspirin for primary or secondary prevention wereinvestigated. The symptomatic group was further classified into according to aspirin usage at the time of stroke. Aspirin resistance was measured by PFA-100 system (collagen/epinephrine cartridge) and glycoproteinIIIaP1A1/A2 genotype was determined.

The overall prevalence of aspirin resistance was 32.2%. The mean age of patients with aspirin resistance was significantly higher than that in those who didn't have resistance ($p = 0.009$). The prevalence of aspirin resistance was similar for the symptomatic and asymptomatic under aspirin therapy groups. The resistance rate was found to be highest with 100 mg enteric-coated preparation use (39.3%). Increasing the aspirin dosage and/or shifting to uncoated preparations caused a change in aspirin sensitivity of 36–60%. Repeated measurements showed development of aspirin resistance in 14% of patients who were sensitive to aspirin in previous measurements. GlycoproteinIIIaP1A1/A2 genotype, aspirin resistance, and development of atherothrombotic stroke were not significantly related.

The effect of aspirin can change by time, dosage, and type of preparation used but no relation with GlycoproteinIIIaP1A1/A2 genotype.

LB-136
A non-essential tryptophan residue reports structural changes of the protein backbone in a blue light photoreceptor

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Blue light sensing domains that use Flavin adenine dinucleotide as a chromophore (BLUF) show a unique photochemistry among the so far described photoreceptor families. The non-isomerizable flavin does not undergo any chemical reaction and only a subtle rearrangement in the hydrogen bond network around the flavin seems to discriminate between resting and signaling state. Nevertheless, the signaling state conformation is stable for seconds up to several minutes. The hydrogen bond rearrangement is driven by the light-induced formation and subsequent recombination of short-lived flavin radical species through proton coupled electron transfer between the flavin and a conserved tyrosine. Beyond that, little is known about the molecular details of the light state, how signal propagation to the protein surface is facilitated or

how communication with the biological effector is realized. Based on experimental results and theoretical calculations several models for light-activation have been proposed and are controversially discussed. Particularly, the relevance of a specific tryptophan residue for signal transduction remains unclear as direct experimental evidence is scarce. Here, we present an FT-IR spectroscopic study on the semi-conserved tryptophan residue in PixD using selective isotope labeling and selected mutations. Our results indicate that the side-chain of this amino acid does not experience drastic changes in its environment upon light activation, but is a marker for the conformational rearrangement at the b5-sheet. Moreover, replacement of tryptophan strongly affects the stability of the signaling state that is related to a discrete upshift of a protein side chain vibration that does not originate from the tryptophan.

LB-137
The role of *β2-syntrophin* in cell migration and proliferation

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The accelerated cell proliferation and the initiation of cell migration are two key components of malignant transformation. Cells have to first disrupt the integrity of the epithelial monolayer and then start migrating. Cell–cell adhesion and apicobasal polarity are essential for proper epithelial function and consist of two aspects regulated by the adaptor protein β2-syntrophin. Specifically, β2-syntrophin is required for optimal cell-cell adhesion and along with Par3 it regulates apicobasal polarity. Polarity proteins, in general, can either promote or impede cell migration leading to the malignant progression or inhibition of tumour metastasis, respectively. They are also implicated in the modulation of cell proliferation that is deregulated in cancer cells. In this study, it is shown that depletion of β2-syntrophin leads to a reduction of cell migration. In wound healing assays, wild type cells display a complete closure of the wound, whereas cells with β2-syntrophin knock-down do not reach that point within 24 h. It is also shown that knocking-down β2-syntrophin results in decreased cell proliferation that is observed 24 h post-plating and thereafter. We are currently investigating the mechanism by which β2-syntrophin regulates cell migration.

LB-138
Profiling of arsenite-induced lung toxicity in mice – a combined proteomic and transcriptomic approach

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Arsenite is a compound of special interest as it is an environmental pollutant and a highly potent carcinogen, but has also been shown to exhibit anticancer activity in various systems and is used as a compound to treat acute promyelocytic leukaemia. The aim of this study was to investigate changes in mRNA and protein levels in mouse tissues after exposure to arsenite. 8–12 week C57BL6/J mice were subcutaneously injected with arsenite (13 μg/gr body weight) or saline and sacrificed after 3 h. Lung tissue was homogenized and mRNA and proteins were extracted.

RNA sequencing and proteomic analysis were performed using an ion semiconductor sequencer and a high resolution and high accuracy Orbitrap mass spectrometer. RNA sequencing identified 1321 up-regulated and 1073 down-regulated genes. This result indicates that acute exposure to arsenite initiates very extensive changes in gene expression. More than 2200 proteins were identified and quantified and among them we found significant up-regulation for a number of proteins upon arsenite treatment. Many of these are known to be associated with acute inflammatory responses, while others are protease inhibitors, proteins with a defense function, or proteins known to increase in response to toxic stress. We will present a bioinformatic integration of both *omics* datasets, evaluating the changes associated with the pathogenesis of arsenite-induced toxicity.

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LB-139
Molecular dissection of ceramide-induced apoptosis using bifunctional lipid analogs

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Cells routinely synthesize ceramides in the endoplasmic reticulum (ER) as precursors for sphingolipids to form an impermeable plasma membrane. In addition to their role as central intermediates of sphingolipid biosynthesis, ceramides have been implicated as signaling molecules in cellular stress responses and apoptosis. Consequently, cells must regulate ceramide levels closely to meet metabolic demands without compromising their viability. We recently identified a candidate protein sensor for ceramides and found that cells lacking a functional sensor commit suicide by mistargeting ER ceramides to mitochondria (Tafesse *et al.*, 2014). How ER ceramides can reach mitochondria to trigger apoptosis is not known.

The aim of this project is to unravel the molecular principles that govern ceramide trafficking at the ER-mitochondrial interface and identify down-stream effectors responsible for mediating ceramide-induced apoptosis. For this purpose, we are using bifunctional ceramide analogues to trace ceramide-binding proteins (CBPs) at ER-mitochondrial junctions and mitochondrial membranes. With this screen, we found the mitochondria-localized phosphatidycholine transfer protein StarD7 to be also a CBP. Additional functional analyses support the idea, that StarD7 is involved in the process of mitochondrial apoptosis.

LB-140
A bioinformatic method to identify potential SNARE proteins

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Tail anchored proteins are a topologically distinct class of intracellular proteins defined by their single carboxy-terminal transmembrane domain with a cytosolic facing amino-terminus. Tail anchored proteins are involved in a range of key cellular functions including protein translocation and apoptosis. Additionally, within the tail anchored class of proteins are a set of vesicle fusion proteins called SNARE proteins. There is biomedical interest in SNARE drug delivery mechanisms. SNAREs can fuse liposomes containing various drug payloads into the membrane. This study aims to identify SNARE proteins in eukaryotic proteomes by filtering through large datasets using automatically pre-

dicted TrEMBL consensus, and manually annotated SWISS-PROT transmembrane regions. The pipeline generates a list of singlepass proteins with a transmembrane domain close to the C terminal, that are not splice isoforms. A previous study by Kalbfleisch *et al*. published in Traffic 2007 (8: *1687–1694*) predicted 411 tail anchor proteins. This study uses more stringent filtering methods, and a larger dataset, to identify 351 novel predicted tail anchored proteins from a comprehensive human dataset. The tools developed herein are openly available for re-application to other datasets. Notably, known SNARE transmembrane helices are highly hydrophobic even compared to other tail anchored transmembrane helices. We compare Kyte and Doolittle hydrophobicity profiles of our filtered human protein list against the profiles of previously known SNARE and tail anchored proteins. This provided a list of potential SNARE proteins in addition to potential spontaneously inserting tail anchored proteins similar to cytochrome b5 which have the least hydrophobic transmembrane helices.

LB-141
Biosynthetic and functional relationship between two sRNAs encoded on opposite DNA strands in *Escherichia coli*

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About a hundred species of small RNAs (sRNAs) has been identified in *Escherichia coli*. Many sRNAs function as central regulators in response to diverse environmental growth conditions. Two sRNAs, RyeA and RyeB, are encoded on opposite DNA strands at the same locus, sharing an overlapping transcribed region. Since they can base-pair through long complementary sequences, their biogenesis could affect each other, leading to changes in their cellular levels. However, their biosynthetic pathway remains unclear. In the current study, we defined transcription units of each sRNA and examined how one could affect the other for regulation of their biosyntheses. We found that RyeB biosynthesis is repressed by RyeA and vice versa, suggesting that their biosyntheses are reciprocally regulated. Furthermore, we demonstrated that RyeA regulates expression of *pphA*, a downsteam gene of *ryeA*.

LB-142
Influence of reactive oxygen species on HIF-1α and its crosstalk with notch signaling as a defensive mechanism against oxidative stress induced cell death

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There is evidence that Reactive oxygen species (ROS)-induced oxidative stress plays a key role in the etiology and/or progression of a number of human diseases including neurodegenerative diseases. Hence, it is urgent to discover effective therapeutic strategies for the treatment of these diseases. Thus, Study of antioxidants and signaling pathways involved in these diseases is effective. In the current study, hydrogen peroxide was used to evaluate the effects of oxidative stress on SK-N-MC cells death with focus on Notch-1, Foxo3a and HIF-1α signaling factors that regulate cellular responses to oxidative stress. Furthermore, we scrutinized the cross talk between HIF-1 and two other pathways. Our results revealed that H_2O_2 up-regulates HIF-1α, Notch-1 intracellular domain (NICD) and Foxo3a pathways. Moreover, we found that the suppression of HIF-1α expression in SK-N-MC cells enhanced vulnerability to oxidative stress-induced cell death. Additionally, our data showed that, under this circumstance, HIF-1α down-regulation also reduced cell content of Foxo3a and NICD. Our cumulative data indicated that HIF-1α has a neuroprotective role against oxidative stress which may be due to activation of downstream pathways including Foxo3a and Notch-1. Collectively, HIF-1-mediated neuroprotection could be important for the development of effective therapies to mitigate or prevent neurodegenerative diseases.

LB-143
The interplay between Notch1 and pin1 in sensitizing trastuzumab-resistant SKBR3 cells

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HER2 overexpression is estimated to be about 25–30% of invasive breast cancer. Trastuzumab (Herceptin) as a recombinant humanized monoclonal antibody directed against HER2 receptor, has been administered as treatment for metastatic HER2 positive breast cancer. The problematic issue in treatment of HER2 positive breast cancer cells is resistance to trastuzumab. One of the mechanisms of refractoriness to trastuzumab is activation of other signaling factors such as Notch1. In this study, we aimed to investigate whether the cross talk between pin1 and Notch1has the role in resistant cells. Our results indicated that the expression level of pin1 in resistant cells increased about 2 fold relative to sensitive SKBR3 cells. Besides, pin1 inhibition reduced the level of proliferation, colony formation and migration capacity of resistant SKBR3 cells. In addition, we found that pin1 is a downstream signal for Notch1 signaling pathway and Notch1 is activated by pin1 through a feed forward loop in sensitive cells. However, the inhibition of Notch1 cleavage in resistant cells did not affect pin1 level whereas pin1 inhibition reduced the level of Hes1 and increased the cellular content of Numb. Therefore, we concluded that Numb inhibition by pin1 appears to be considered as the function of pin1 in resistance to trastuzumab.

LB-144
Differential expression of glypican 3 and insulin-like growth factor-II mRNAs in liver tissues of hepatocellular carcinoma on top of HCV cirrhosis

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Background: Specific diagnosis of hepatocellular carcinoma (HCC) at early stage is of utmost importance. Increasing evidence indicates that abnormal hepatocellular carcinoma geens, glypican 3 (GPC-3) and insulin-like growth factor-II (IGF-II) expression were associated with the occurrence and progression of HCC. GPC3 confers oncogenecity through the interaction between IGF-II and its receptor, and the subsequent activation of the IGF-signaling pathway.
Aim of Work: Evaluation of GPC3 and IGF-II mRNAs expression differentially in HCC tissues.

Material and methods: Twenty one patients with HCC who had undergone hepatectomy were included in our study after obtaining informed consent. Total RNA was extracted from tissue and GPC3 mRNA and IGF-II mRNA in addition to Beta-actin mRNA as internal control were evaluated by Real time PCR in all samples.
Results: The 21 cases were distributed into 63% males and 37% females with median of age 55 years. The expression of GPC3 mRNA was positive in all HCC malignant tissue, and it was over expressed in 17/21 (81.8%); in respect to the grade of the tumor (1–3 grades), while in nonmalignant tissue it was over expressed only in 4/21 (18%). The IGF-II mRNA was over expressed only in two samples (9.5%) in both malignant and nonmalignant tissues.
Conclusion: The GPC3 and IGF II mRNA are good molecular markers for HCC, especially on top of cirrhosis due to HCV, and the use of Real time PCR is a sensitive method for relatrelative expression for genes in HCC malignant and nonmalignant tissues.

LB-145
Transcription factor co-occupied genomic regions constitute T helper cell subtype-specific enhancers

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Transcription factors (TFs) regulate cell type-specific gene expression programs by combinatorial binding to *cis*-genomic elements, particularly enhancers, subsequently leading to the recruitment of cofactors and the general transcriptional machinery to target genes. Using data integration of genome wide TF binding profiles we defined regions with combinatorial binding of lineage-specific master TFs (T-BET, GATA3, and ROR-γt) and STATs (STAT1 and STAT4, STAT6, and STAT3) in T helper (Th) 1, Th2, and Th17 cells, respectively. Stringently excluding promoter regions, we revealed precise genomic elements which were preferentially associated with the enhancer marks p300 and H3K4me1. Furthermore, closely adjacent TF co-occupied regions constituted larger enhancer domains in the respective Th cell subset with characteristics of so-called super-enhancers. Importantly, 89% of these super-enhancer regions were Th cell subtype-specific. The majority of genes associated with super-enhancers, including cytokine genes (such as *Ifng* in Th1, *Il13* in Th2, and *Il17a* in Th17 cells), showed strong transcriptional activity. Currently, we are performing TF binding site analyses within the genomic super-enhancer regions to identify further important TFs regulating the activity of these enhancers.

Altogether, the discovered catalogue of Th cell enhancers provides information about crucial Th cell subtype-specific regulatory hubs. Their detailed functional characterization and investigation will expand our understanding of distinct gene regulation processes in different Th cell subtypes as well as during physiological and pathophysiological Th cell fate decisions.

LB-146
Regulation of the fungal transcriptome in response to light

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Transcriptomic plasticity enhances organismal fitness and complexity. Light exposure regulates transcription of large sets of genes in eukaryotes across different kingdoms of life. Alternative splicing is another source of diversity in the transcriptome, but it is unclear to what extent light influences this process. To better understand how light influences the transcriptome of the filamentous fungus *Neurospora crassa*, we performed next-generation sequencing of RNA from dark-grown and light-pulsed cultures. Previous reports indicate that ~5% of *N. crassa* genes are regulated by light over time courses up to 4 h. Combining data from our 15 min and 4 h time points, we found ~13% of genes are regulated by light ($q < 0.05$). We attribute the increased number of light-responsive genes to differences in the light source used in our experiments. Despite high prevalence of alternative splicing in *N. crassa*, light has little influence on intron retention, the most common mode of alternative splicing in fungi. Instead, we detected putative alternative transcription start sites that are regulated by light. Our data suggest that in contrast to the strong transcriptional response to light, alternative splicing does not significantly contribute to light-driven changes in the *N. crassa* transcriptome. Current work includes generation of a debranchase deletion strain in order to stabilise lariat-introns, to aid in future splicing studies.

LB-147
The relation between lipid metabolism and autophagy in HepG2 cell line

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Autophagy is a mechanism involved in cellular homeostasis under basal and stressed conditions delivering cytoplasmic content to lysosomes. The potential role of autophagy is reported as an important factor in the lipid metabolism. Evident molecular data suggests relation of autophagy and lipid biosynthesis. Sterol-regulatory-element-binding protein-1c (SREBP-1c) and peroxisome-proliferator-activated receptor γ (PPARγ) are among two transcription factors that regulate hepatic lipogenesis and fatty acid oxidation. Therefore we aimed to investigate the effect of 3-Methyladenine (3MA) and rapamycin on SREBP-1c, PPARγ and LC3-I gene expression in HEPG2 cell line.

HepG2 cells were treated with 3-Methyladenine (3MA) and rapamycin as autophagy inhibitor and inducer respectively. MTT assay was used for cell viability analysis. At 6^{th}, 9^{th}, 12^{th}, 24^{th} hours SREBP-1c, PPARγ and LC3-I expression were analyzed. The duration of application at 3MA and rapamycin in HEPG2 cells were statistically significant ($p < 0.01$). PPARγ expression was doubled in 12^{th} hour of rapamycin induced cells with respect to 3MA and control. SREBP-1c expression was doubled in 3MA induced cell with respect to rapamycin and control. Although the cell vitality was lowest at the 9^{th} hour in 3MA induced cells, the increase in cell vitality at 12^{th} hour may suggest that neither the activators nor inhibitors of autophagy have an impact in cell survival.

Here we report the preliminary data of our study. Both the lack of the immunostaining and western blotting were the limitations of this report. Also other genes enrolling in lipid metabolism is under investigation.

LB-148

Monomeric proteins are the preferential substrates of the peroxisomal protein import machinery

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Peroxisomal matrix proteins are synthetized on cytosolic ribosomes and targeted to the peroxisomal membrane by the shuttling receptor PEX5. At the peroxisomal membrane, the PEX5-cargo complex gets inserted into the docking/translocation machinery with the concomitant release of the cargo protein into the peroxisomal matrix. It is widely accepted that peroxisomes have, unlike other organelles, the remarkable capacity to import already oligomerized proteins. However, it remains unknown whether or not these are the most frequent and preferred clients of the protein import machinery. In this work, we provide data suggesting that 1) PEX5 binds newly synthesized acyl-CoA oxidase 1 (ACOX1) and urate oxidase (UOX), inhibiting their oligomerization; 2) ACOX1 and UOX are much better imported in their oligomeric forms than after oligomerization; and 3) an ACOX1 lacking the peroxisomal targeting information can be piggybacked to peroxisomes with a PTS1 containing ACOX1 *in vivo*, but this process is very inefficient. These data support a model in which many of the protein translocation events occurring at the peroxisomal DTM involve monomeric cargoes.

LB-149

Mechanistic basis for site-specific functions of focal adhesion kinase

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Focal adhesion kinase (FAK) controls adhesion-dependent cell motility, survival, and proliferation, and plays a major role in development and cancer. Interestingly, FAK has different functions in different cellular compartments. For example at focal adhesions, FAK regulates integrin signalling in a kinase-dependent manner, whereas in the nucleus it exerts kinase-independent anti-apoptotic effects. We have combined SAXS with data from x-ray crystallography, NMR, bioinformatics, biochemical and functional analyses, to provide first structural insights into full-length FAK. Through specifically affecting the structural dynamics of FAK, we show that low-probability conformational transitions are of biological importance. Collectively, our data show how the dynamics and allosteric interplay between ligands and FAK's several domains controls site-specific activity of FAK. Our results reveal how FAK detects the coincidence of multiple signals to generate an environment-specific outcome.

LB-150

MD simulation of dynamics and transport in 5-HT3 receptor

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The 5-HT3 receptor is a member of cation selective ligand-gated ion channels. It plays an important role in functioning of central and peripheral nervous systems. There are currently no high resolution structures of the 5-HT3 receptor in open state, but crystal structure of 5HT-3 in complex with stabilizing nanobodies, as well as crystal structures of closely related proteins are available.

In this work molecular dynamics simulations of transmembrane domain of mouse serotonin 5HT-3 receptor was used to study dynamics and ion permeation. Transmembrane part of the receptor (X-ray structure PDB 4PIR) was used to set up the calculations. The structure was prepared in dimyristoyl-phosphatidylcholine lipid bilayer with TIP4P water model. GROMACS software was used for MD simulations. Position restraints were used on extracellular amino-acid residues of the ion channel. RMSD data after 15 ns of MD calculations indicates stability of the system. Minimum pore diameter remained at around 3,5 Å. Umbrella sampling method was used to estimate the potential of mean force for Na+ ions along the protein pore. All simulations were performed using "Arian Kuzmin" supercomputer center of NEFU, the work was supported by the Ministry of Education and Science of Russian Federation.

LB-151

Molecular and computational analysis of temperature compensation of the *Neurospora* circadian clock

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Circadian clocks are molecular oscillators that drive rhythms in gene expression, physiology and behaviour that ultimately allow organisms to anticipate and exploit predictable daily changes in their environment. A hallmark of circadian clocks is their ability to maintain a constant period in a range of temperatures. In the filamentous fungus *Neurospora crassa*, the circadian clock imparts rhythmicity on asexual spore development (conidiation) and both the circadian oscillator and its output are temperature compensated between 18°C and 32°C. Temperature compensation of circadian systems is thought to be encoded within the core circadian oscillator itself, but the underlying molecular mechanisms are not well understood. We have highlighted previously that in poikilothermic organisms such as *Neurospora,* temperature compensation must also occur downstream of the oscillator if a stable phase relationship with the environment is to be achieved, and identified the *Neurospora* blue-light photoreceptor VIVID (VVD) as important for this process. To gain further insight into how VVD exerts control on temperature compensation of the phase of circadian outputs we have performed next-generation sequencing of RNA from both wild type and *vvd* knockout cultures grown at different temperatures. This has allowed us to examine the effect VVD has on gene expression, relating to conidiation genes as well as the wider transcriptome. Using a dynamic model of the circadian clock, we present data that explore how temperature compensation functions within the

central clock machinery as well as in clock-controlled output pathways controlled by VVD.

LB-152
The effect of TFAM overexpression on mitochondrial gene expression

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Mitochondrial transcription factor A (TFAM) has emerged as an essential regulator of mitochondrial function in mammals due to its dual role as a core component of the mitochondrial transcription machinery and a key packaging factor of the mitochondrial genome. Previously, our group characterized TFAM as a key regulator of mtDNA copy number: Transgenic mice overexpressing human TFAM displayed a general increase in mtDNA copy number, but not an increase in respiratory chain capacity or mitochondrial mass. To decipher the effect of increased TFAM levels on mitochondrial gene expression itself, we generated transgenic mice expressing murine TFAM. Differences in the levels of mtDNA, *de novo* mitochondrial transcription, mitochondrial transcripts and respiratory chain components were assessed in relation to net TFAM overexpression under steady-state conditions. Furthermore, the effect of TFAM overexpression on mitochondrial replication was investigated.

LB-153
Development of new chemical probes for investigation of the CB1-D2 receptor complex

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G-Protein coupled receptors (GPCRs) are transmembrane proteins that initiate intracellular signalling cascades upon binding of a ligand. Formation of homodimeric GPCR complexes has been well established,[1] but it is now recognised that some GPCRs may form heterodimeric complexes composed of two different receptors.[2] Hence only a few studies on this highly interesting field have been published.[3]

The cannabinoid CB1/dopamine D2 heteromeric GPCR complex is proposed to form in highly localised areas of the brain where the two receptors are co-expressed in the cell membrane.[4] Importantly, evidence suggests that these heterodimers exhibit different downstream signalling behaviour to the corresponding monomers and homodimers. The CB1/D2 heterodimer is therefore a promising target for highly selective modulation of CNS signalling pathways that are important in a variety of clinical conditions.[5]

Current work is focussed on the development of divalent ligands, selective for the CB1/D2 heterodimer, to enable complete characterisation of the GPCR complex. Based upon this work, more advanced chemical probes are under investigation, to both facilitate *in situ* pharmacological evaluation of heterodimers and provide the foundation for controlled modulation of cannabinoid and dopamine-mediated CNS signalling pathways.

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LB-154
Specific gene silencing in *Escherichia coli* using artificial small RNA

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Knockdown or silencing of a specific gene presents a powerful strategy for elucidating gene function in a variety of organisms. To date, efficient silencing methods have been established in eukaryotes, but not bacteria. In this study, we developed an efficient and versatile gene silencing method using artificial small RNA (afsRNA) in *Escherichia coli*. For this purpose, target-recognizing sequences were introduced in specially designed RNA scaffolds to exist as single-stranded stretches in afsRNA. The translation initiation region of target genes was used as the sequence for afsRNA recognition, based on the theory that this site is usually highly accessible to ribosomes, and therefore, possibly, afsRNA. Genes tested with our protocol were effectively silenced by their cognate afsRNAs, clearly indicating that our protocol can be employed as a general laboratory method to silence specific mRNAs.

LB-155
Structural insight of T4 dCMP hydroxymethylase in ternary complex with dCMP and tetrahydrofolate

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dCMP hydroxymethylase (CH) is T-even phage specific viral enzyme that was firstly identified and its reaction is also the first recognized DNA modification for protecting the viral DNA from restriction-modification system of host bacteria. CH catalyzes hydroxymethylation of deoxycytidylate (dCMP) to 5-hydroxymethyl-dCMP (Hm5dCMP) using 5-methyltetrahydrofolate (CH2THF) as a methyl donor. We previously reported binary complex structure of T4CH with dCMP, but how this enzyme utilizes THF as a cofactor is largely unknown due to lacking of ternary complex structure. Here, we report crystal structure of T4CH in tertiary complex with dCMP and THF at 1.9 Å resolution. Space group belongs to an I 2 2 2 with unit cell parameters of a = 52.625, b = 75.226, and c = 154.087 Å, which differs from the crystal of binary complex. Interestingly, an iodide ion is essential for new crystal packing and also used for in-house phase determination. The structure was refined to free R factor of 18.83% an excellent stereochemistry. The bound dCMP and THF molecules were clearly observed in the electron density map. This ternary complex structure provides an insight into the enzymatic mechanism of dCMP hyroxymethylation catalyzed by T4 CH.

LB-156
Molecular aspects of the Q-cycle: Quinol binding and proton-coupled electron transfer

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The bc_1 complex is a central player in the conversion of energy into ATP synthesis in photosynthesis and respiration, and its overall mechanism, the Q-cycle, is well known. However, the quinol-protein interaction that initiates Q-cycle at the Q_o-binding site has not yet been described. Furthermore, the consequent reaction mechanism, namely a proton-coupled electron transfer between quinol and the bc_1 complex, lacks of a molecular description. Employing classical MD simulations in tandem with DFT calculations, the quinol binding motifs to the Q_o-site of bc_1 complex as well as reaction mechanism of charge transfer are investigated for a range of Q_o-site protonation states. The computations revealed a novel configuration of the key side groups at the Q_o-site site, such as H156, Y147 and E295, that stabilize the reaction complex and provide an optimal configuration prior to the charge transfer reactions between quinol and iron-sulfur cluster of the Rieske protein. Re-arrangements in the E295 and Y147 side chains were observed in all our simulations, showing intermediate bridging hydrogen bonding between quinol and E295, not observed before. Quantum chemistry calculations revealed a coupled nature of the charge transfer reactions and a well characterized reaction pathway was obtained.

LB-157
Interaction between inclusion complexes of halogenated benzotriazoles with cyclodextrins and protein kinase CK2

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CK2 is an ubiquitous, highly pleiotropic and constitutively active Ser/Thr protein kinase. Halogenated benzotriazoles are important group of kasein kinase 2 (CK2) inhibitors. One of the first potent and selective CK2 inhibitor was 4,5,6,7-tetrabromobenzotriazole (TBBT).

The interaction of the catalytic domain of human protein kinase CK2 with a series of brominated ligands, which represent all possible patterns of halogen substitutions to the benzene ring of benzotriazole, was previously studied by microscale thermophoresis (MST). This method allowed determination of binding affinities for seven ligands, with the exception of 4-BrBt and 4,7-Br_2Bt, which were found consistent with the values determine by isothermal titration calorimetry (ITC). However, a limited aqueous solubility of brominated benzotriazoles decreases their bioavability, and thus may affect their apparent activity.

To overcome this limitation, the solubility of halogenated ligands in presence of several cyclodextrins has been tested. The formation of inclusion complexes with β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD) and γ-cyclodextrin (γ-CD) in aqueous solutions, followed by UV-Vis spectroscopy, substantially improved the solubility of TBBt and its derivatives. The interaction between protein kinase CK2 and cyclodextrins, and their inclusion complexes with halogenated ligands as well, was further followed with the aid of the microscale thermophoresis. The results obtained clearly show that cyclodextrins only moderately affect binding of halogenated benzoitriazoles by CK2.

LB-158
Nuclear FGFR2 participates in the MLL-AF4 protein network that activates leukemia pathways

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The t(4;11)(q21;q23) chromosomal translocation causes a form of infant acute lymphoblastic leukemia (ALL) that has a very poor prognosis. It fuses *in frame* the *MLL* and *AF4* genes thereby resulting in a fusion gene that encodes the chimeric oncoprotein MLL-AF4. The MLL-AF4 chimera transactivates the *HOXA9* and *MEIS1* genes, which play a crucial role in leukemogenesis. We previously found that AF4, the most common MLL protein fusion partner, interacts with fibroblast growth factor receptor 2 (FGFR2), a member of the receptor tyrosine kinase superfamily. We have analyzed the role of this interaction in the context of t (4;11) ALL. *In vitro* binding and co-immunoprecipitation (ChIp) assays showed that FGFR2 directly interacts with the MLL-AF4 chimera in the nucleus. Inhibition of FGFR kinase activity, induced with the ATP-mimetic inhibitor PD173074, correlates with reduced transcription of *HOXA9* and *MEIS1* in the MLL/AF4-positive cell line RS4;11. siRNA knockdown of endogenous FGFR2 also results in reduced expression of *HOXA9* and *MEIS1*. We also found that PD173074 decreases the nuclear amount of FGFR2 in RS4;11 cells. However, ChIp assay did not reveal FGFR2 binding to the *HOXA9* promoter region that recruits the MLL-AF4 chimera. Taken together, these data indicate that FGFR2 is a new promising therapeutic target in t(4;11) (q21;q23) ALL.

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LB-159
Atg11 and Atg13 bind to the distinct regions in the MIT domain of Atg1

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Autophagy is an intracellular degradation pathway which categorizes into two groups, non-specific and specific pathway. In contrast to non-specific and bulk autophagy, selective autophagy requires the targeted recognition and removal of various cellular components including protein aggregates, mitochondria, peroxisomes, ribosomes and intracellular pathogens. In yeast, Atg11, which is scaffold protein, is not only related to cvt (cytoplasm-to-vacuole) pathway but also selective autophagy pathway. It has been reported that this protein interacts with some of autophagy proteins; Atg1, Atg11 itself, Atg17, Atg19, and Atg20. Here, we present that Atg11 interacts with the MIT (microtubule-interacting and transport) domain of Atg1 using *in vitro* pull-down assay. It has been known that the MIT domain of Atg1 binds to

the MIM domain of Atg13. Unexpectedly, the Atg11 also forms a ternary complex among MIT domain of Atg1, MIM domain of Atg13, and Atg11. This finding suggests that the Atg11 has a binding site on the MIT domain of Atg1, which is not interfered by the binding of Atg13.

LB-160
Anti-inflammatory effect of glycosylation product derived from high hydrostatic pressure enzymatic hydrolysate of flatfish byproduct and its mechanism

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In this study, flatfish byproducts were hydrolyzed by Protamex at high hydrostatic pressure and glycosylated with ribose to utilize protein of flatfish byproducts as a nutraceutical. We investigated anti-inflammatory effects of glycosylated fish byproduct protein hydrolysate (GFPH) and their anti-inflammatory mechanisms were elucidated in lipopolysaccharide (LPS)-stimulated RAW264.7 mouse macrophage. The results showed that GFPH suppress LPS-induced production of nitric oxide (NO) and prostaglandin E_2 (PGE_2) and expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) dose-dependently. Enzyme-linked immunosorbent assay (ELISA) kit clearly demonstrated that GFPH significantly reduced the productions of pro-inflammatory cytokines such as, interleukin (IL)-6, interleukin (IL)-1β and tumor necrosis factor (TNF)-α, and monocyte chemoattractant protein (MCP)-1. Moreover, GFPH reduced nuclear factor κB (NF-κB) and mitogen-activated protein kinases (MAPKs) activation. These results indicate that the inhibitory effects of GFPH on LPS-induced NO and PGE_2 production might be due to the suppression of NF-κB and MAPKs signaling pathway. Therefore, these results suggest that flatfish byproducts are latent bioactive resources and GFPH may have potential as a therapeutic agent in the treatment of various inflammatory diseases.

LB-161
Expression and characterization of *Pseudomonas aeruginosa* lipoxygenase

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Lipoxygenases (LOX) have been implicated in the biosynthesis of inflammatory mediators and in the pathogenesis of various diseases. Functional LOX have been detected in two of the three domains of terrestrial life (bacteria, eucarya) and the genome of *Pseudomonas aeruginosa* (PA) contains several LOX- sequences. To explore its structure function relation we recombinantly expressed it in *E. coli* and purified it to an apparent electrophoretic homogeneity. The enzyme migrated at 70 kDa in SDS PAGE and exhibited a catalytic turnover rate of 162 s^{-1} for linoleic acid oxygenation. Its iron content was 106 mol%. It's Km- and Vmax values for 10 different polyenoic fatty acids indicated that 8,11,14-eicosatrienoic acid and 4,7,10,13,16,19-docosahexaenoic acids are the most efficient substrates. Reaction products analyzed by GC/MS and chiral phase HPLC indicated a preferential S-lipoxygenation at the n-6 oxygen carbon atom. Since the X-ray structure of PA-LOX indicated the presence of a phospholipid molecule at the active site, we tested the possible membrane oxygenase activity of PA-LOX with mitochondrial membranes where they showed capability to oxidize membrane bound phospholipids with a molecular turnover rate of 0.3 s^{-1} indicating that free PUFAs are better substrates. Multiple enzyme mutants were generated to explore the structural basis of reaction specificity. An Ala420Gly exchange induced significant alterations in the reaction specificity of the enzyme, which was mirrored by a significant increase in 11R-oxygenation. The X-ray data from the crystallized wildtype (1.85 Å) PA-LOX and its mutant A420G (2.3 Å) variant suggested subtle structural differences behind the observed functional alterations.

LB-162
Comparison of antigenic proteins of hydatid cysts from sheep and mice by immunoblotting

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Hydatidosis (Echinococcosis) is a widespread zoonotic parasitic disease which has major medical and socio-economic costs for humans and also threatens livestock productivity caused by the cestode helminthes *Echinococcus granulosus*. The aim of this study was to analyse and compare the antigenic proteins of hydatid cysts of experimentally infected mice and naturally infected sheep by immunoblotting. For this reason 20 each infected sheep and infected mice, and 10 each healthy sheep and mice as control were used in the study. Cyst fluid and cyst membrane proteins which were derived from hydatidosis infected sheeps and mice were separated by SDS-PAGE. Antigenic proteins were determined by immunoblotting technique using positive serum samples obtained from infected sheep and mice. Moleculer weights of antigenic proteins from cyst fluid and cyst membrane of sheep were determined in sizes between 116 kDa and 26 kDa in size by SDS-PAGE. Molecular weights of antigenic proteins in cyst fluid and cyst membrane samples of mice were found between 106 kDa and 30 kDa. Common antigenic bands of hydatid fluid from infected sheep and mice were found to be 40 kDa and 26 kDa. Common antigenic bands of hydatid membrane from infected sheep and mice were found to be 48 kDa and 40 kDa in sizes. These identified antigenic proteins may have a high diagnostic value in the diagnosis of hydatidosis.

Key words: Antigenic protein, hydatid cyst, mice, sheep.

LB-163
The rhodopsin-guanylyl cyclase of the aquatic fungus *Blastocladiella emersonii* enables fast optical control of cGMP signaling

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Blastocladiomycota fungi form motile zoospores that are guided by sensory photoreceptors to optimal light conditions. Here we show that the microbial rhodopsin of *Blastocladiella emersonii* is a rhodopsin-guanylyl cyclase (RhGC). RhGC is the first member of a new rhodopsin class of light-activated enzymes. Upon light absorption, RhGC (D525) converts in 8 ms after a light flash into a blue-shifted signaling state P380 and recovers within 100 ms. RhGC was well expressed and produced cGMP in response to green light in *Xenopus* oocytes, CHO cells, and mammalian neurons. Cyclic GMP production was light dose-dependent, rapid and reproducible. Thus, RhGC is a versatile tool for optogenetic analysis of cGMP-dependent signaling processes in cell biology and the neurosciences.

LB-164

ERCC1-XPF complex stability and consequences of a COFS syndrome mutation F231L in ERCC1

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XPF forms a heterodimeric complex with ERCC1 that is functional in nucleotide excision repair (NER). *In vitro* XPF can also form homodimers. The homodimeric state is more stable than heterodimeric state at elevated temperatures and in denaturants, however, this conformation is not observed *in vivo*. We now find that the ERCC1-XPF heterodimer can rapidly dissociate. Whereas association of ERCC1-XPF is preferred *in vivo* and *in vitro*, we show that despite this rapid dissociation XPF preferentially re-associates with ERCC1, and that XPF only forms homodimers in the absence of ERCC1. Since the structures of XPF in the homodimer and in the heterodimer differ, our data suggest that XPF dissociated from ERCC1 has the proper conformation to re-associate with ERCC1 but that a structural transition is required for homodimerization. A point mutation in ERCC1, F231L, located at the hydrophobic interaction interface of ERCC1 and XPF, leads to severe NER pathway deficiencies. We analyze biophysical properties and report the NMR structure of the complex of the C-terminal $(HhH)_2$ domains of ERCC1-XPF that contains this mutation. The F231L mutation results in a small disturbance of the ERCC1-XPF interface where, in contrast to F231, L231 lacks interactions stabilizing the ERCC1-XPF complex. This results in a more dynamic complex causing reduced stability and increased dissociation of the mutant complex as compared to wildtype. Our data provides a biophysical explanation for the severe NER deficiencies caused by this mutation.

LB-165

Effect of azamethiphos and emamectin benzoate on transcriptional levels of detoxification proteins in *Caligus rogercresseyi*

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Caligus rogercresseyi, is the sea lice that severely affects the Chilean salmon farming industry Currently, decreased efficiencies of the drugs used against *C. rogercresseyi* have been reported. We determine the effect of emamectin benzoate and azamethiphos, on transcriptional levels of detoxification proteins in adult *Caligus rogercresseyi*.

RT-qPCR was used to quantify the mRNA expression levels of detoxification proteins (CYP3A, FMO-1 and GST) in the *C. rogercresseyi* treated *in vitro* with the antiparasitics.

C. rogercresseyi treated with emamectin benzoate showed a marked increase in CYP3A mRNA expression levels, unlike the observed level in samples treated with azamethiphos, which showed a slight decrease or no change. Also, we observed an increase in the expression levels of GST in specimens treated with emamectin benzoate and azamethiphos. Finally, the FMO levels tend to increase in all specimens treated, particularly at the highest concentrations used in each of the antiparasitics.

These results suggest that the reduced effectiveness of different treatments against *C. rogercresseyi* could be the result of the induction of enzymes involved in the metabolism and elimination of xenobiotics, thus affecting the antiparasitic pharmacokinetics. The knowledge about the mechanism involved in antiparasitic resistance observed in *C. rogercresseyi* will allow us to develop effective alternative treatments against this parasite.

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LB-166

Histone H3 lysine-to-methionine mutants as a paradigm to study chromatin signaling

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Histone H3 lysine(27)-to-methionine (H3K27M) gain-of-function mutations occur in highly aggressive pediatric gliomas. We established a *Drosophila* animal model for the pathogenic histone H3K27M mutation and show that its overexpression resembles polycomb repressive complex 2 (PRC2) loss-of-function phenotypes, causing derepression of PRC2 target genes and developmental perturbations. Similarly, an H3K9M mutant depletes H3K9 methylation levels and suppresses position-effect variegation in various *Drosophila* tissues. The histone H3K9 demethylase KDM3B/JHDM2 associates with H3K9M-containing nucleosomes, and its misregulation in *Drosophila* results in changes of H3K9 methylation levels and heterochromatic silencing defects. We have established histone lysine-to-methionine mutants as robust *in vivo* tools for inhibiting methylation pathways that also function as biochemical reagents for capturing site-specific histone-modifying enzymes, thus providing molecular insight into chromatin signaling pathways.

LB-167

Targeted deletion of rhodopsin photoreceptors in *Chlamydomonas reinhardtii* using zinc-finger nucleases and CRISPR-Cas9

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Chlamydomonas reinhardtii is a versatile model organism in many aspects of plant science because – in comparison to higher plants – it shows a fast rate of cell divison and thus allows speeding up experimental approaches. Our lab is interested in photoreceptors, primarily focusing on rhodopsins and their characterization.

The use of *Chlamydomonas* as a model organism has always been hindered by the inability to perform targeted genetics. *Chlamydomonas* has proven to be quite resistant to targeted gene modifications in the past, although it only comprises a haploid genome.

Thus, we are working to gene targeting in that organism enabeling us to characterize unknown photoreceptors.

Firstly, we created target specific zinc-finger nuclease and deleted the Channelrhodopsine-1(ChR1) gene encoding one of the primary photoreceptors mediating phototaxis. Now we are

studying effects of different light qualities on cell behavior in ChR1-depleted strains.

Secondly, we adapted the recently published gene targeting system CRISPR-Cas9 to *C. reinhardtii*. We identified an active RNAPIII promotor to drive sgRNA transcription. Next, we managed to create Cas9 expressing strains in different genetic backgrounds. *Sp*Cas9 localization to the nucleus could be shown by fluorescence microscopy. As a proof of principle we deleted the selectable gene FKB12.

This work was supported by the German Research Foundation, (FOR1261, to P.H.) and Russian Foundation for Basic Research (RFFI 14-04-92694, to I.S.)

LB-168
Identification of multiple target genes under RyhB small RNA regulation in *Vibrio parahaemolyticus*

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Vibrio parahaemolyticus is a halophilic Gram-negative bacterium that thrives in warm climates within marine or estuarine environments. Virulent strains can cause diarrhea and gastroenteritis upon the consumption of raw or undercooked seafood contaminated with the bacterium. The Fur-regulated small RNA, RyhB, was previously identified in *V. parahaemolyticus* as regulatory sRNA, and its modulation of siderophore biosynthesis was mediated base pairing between RyhB with the target mRNAs. In this study, we explored the other possible target genes under RyhB regulation, including the genes involved in iron metabolism, chemotaxis, motility and virulence of *Vibrio parahaemolyticus*. A *ryhB* deletion mutant and its complement strain were constructed; target genes expression among the described strains was detected and confirmed using qRT-PCR and Nothern blot hybridization. The results indicated that RyhB positively regulates *iscS* and negatively regulates *fhuE, cheY, motA, motB, lafK, flaD, flak, fliS, mam7, toxS, and toxR*. However, *ropS* is not regulated by RyhB. These data provide to our knowledge that RyhB represses expression of multiple mRNA targets in *Vibrio parahaemolyticus,* a food-borne human pathogen.

LB-169
The respiratory supercomplex III/IV from *Corynebacterium glutamicum*

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In the aerobic respiratory chain of the Gram-positive Actinobacterium *Corynebacterium glutamicum*, a supramolecular association of cytochrome bc_1 complex (complex III) and cytochrome aa_3 oxidase (complex IV) couples menaquinol oxidation to reduction of dioxygen. Here we isolated supercomplex III/IV to high homogeneity and purity enabling its biophysical and structural characterization which will be presented. The supercomplex was stable in size-exclusion chromatography and contained all subunits and cofactors as analyzed by SDS-PAGE, BN-PAGE, redox difference spectroscopy and EPR spectroscopy. Determination of redox midpoint potentials revealed how complexes are finedtuned for an integrated quinol oxidase activity. The study provides experimental evidence for the molecular co-evolution hypothesis of Q_o motif and quinone species (Kao and Hunte, *Genome Biol. Evol.* (2014) 6: 1894–1910). Comprehensive sequence and phylogenetic analysis support the supermolecular association of complex III and IV in Actinobacteria. A homology model of supercomplex III/IV was constructed, which is matching projection maps of highly uniform particles resolved by electron microscopy. The data further the understanding of the mechanism of the important bioenergetics complexes and could be of interest for optimizing efficiency of *C. glutamicum* in industrial production. Homologous complexes are evaluated as targets to combat drug-resistant forms of actinobacterial human pathogens such as *Corynebacterium diphtheriae* and *Mycobacterium tuberculosis*.

LB-170
Studies on nucleotide composition of viral genomes

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In 1968 Chargaff and his colleagues discovered a second parity rule: in a single DNA strand A≈T and C≈G. This rule has been extended to the longer reverse complement oligonucleotides. In 2006 Mitchell and Bridge showed that the original version of this rule holds for all types of dsDNA genomes, but not for ssDNA and RNA genomes.

To check whether the rule applies to the oligonucleotides in viral genomes, the viruses were divided into several groups (single-stranded, double-stranded DNA or RNA, retroviruses) and the groups were checked for correlations between absolute and relative reverse complement dinucleotide frequencies. Strong positive correlations between ApC/GpT, ApG/CpT, CpA/TpG and GpA/TpC contents in dsDNA viruses could be observed only between their relative frequencies. In the other groups of viruses some dinucleotide pairs shows even stronger negative correlation. Retroviruses, but not other groups of viruses, exhibit strong positive correlation between ApG/GpA. Some interesting relationships were also observed between relative trinucleotide frequencies. For example, relative TAA frequency is negatively correlated with TAC in dsDNA viruses and similar relationship is observed between TAG and TAC in retroviruses.

Lack of strong positive correlation between absolute frequencies of reverse complement nucleotides in dsDNA viruses contradicts the previous suggestion, that the parity at the level of single bases is a consequence of evolutionary forces acting on the oligonucleotide level. Negative correlation observed between trinucleotide pairs TAA/TAC and TAG/TAC may be caused by elimination of STOP codons, but some of the observed relationships between oligonucleotide frequencies still need to be explained.

LB-171
Cytotoxicity of new polyfluorinated 1,4-naphtoquinones containing aminoacid substituents and benzene fluorinated 2,2-Dimethyl-2,3-dihydro-1H-quinoline-4-ones

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Fluorinated derivatives of 1,4-naphthoquinones were shown to be highly potent inhibitors of Cdc25A and Cdc25B phosphatases and growth of tumor cells. Cdc25A and Cdc25B are overexpressed in various human tumors, which make them attractive drug targets for anticancer therapy. New conjugates of polyfluorinated 1,4-naphthoquinone core with amino acid fragments and benzene-fluorinated 2,2- dimethyl-2,3-dihydro-1H-quinolone-4-ones were synthesized.

The cytotoxicity of new quinones was tested in human myeloma, human mammary adenocarcinoma, human hepatocellular carcinoma HepG2, mouse fibroblasts and Chinese hamster fibroblast cells.

All the compounds suppressed the growth of three tumor lines – IC50 values ranging from 1,5-mkM for compounds **13, 15, 16**, 3–4mkM for **2, 14,18** and 20–40 mkM for **3–7** quinones). It could be observed that esterification of carboxyl group leads to the decrease of the IC50 value of derivatives.

Mutagenic and antioxidant properties were evaluated in Ames test. The data indicate that quinones are not mutagenic themselves and decrease efficiently the level of spontaneous muthagenesis and the mutagenic effect of H_2O_2. These data together with the better cytotoxic effect against cancer cells compared to normal mammalian cells, reveal quinones 2, 13, 15, 16, and 18 and quinolones f and g as best inhibitors of tumor cells growth among the substances tested.

LB-172
Elucidating the *in vivo* function of POLRMT in mouse models

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Mitochondria are involved in a variety of metabolic processes, which depend on a coordinated expression of the nuclear and the mitochondrial genomes (mtDNA). One of these processes is oxidative phosphorylation and disturbances in it have been implicated in several inherited and age-related diseases.

Transcription initiation of mammalian mtDNA *in vitro* requires mitochondrial RNA polymerase (POLRMT) and the mitochondrial transcription factor B2 that are recruited at the heavy- and light-strand promoters (HSP and LSP) when the mitochondrial transcription factor A is bound. Recent studies show that the mitochondrial transcription elongation factor enhances the processivity of POLRMT and abolishes premature transcription termination. Even though the components of this basal transcription machinery are known, the regulation of mtDNA expression still remains one of the most significant gaps in our knowledge of mitochondrial function.

Using a knockout mouse model, we could show that POLRMT has an exclusive mitochondrial role and that it is absolutely required for expression of mtDNA in mammals (Kühl *et al. Nature* 2014). Here, we present the characterization of this conditional *Polrmt* knockout mouse. POLMRT is essential for embryonic development and its tissue-specific disruption in heart leads to a very drastic mitochondrial phenotype with impaired mtDNA replication. Interestingly, at low POLRMT levels, we find a significant discrepancy in the initiation of mitochondrial transcription between HSP and LSP. Further, we present some preliminary data of our work aiming to elucidate the mechanisms that dynamically regulate gene expression by varying the *in vivo* expression of mitochondrial RNA polymerase in mouse.

LB-173
In-vitro cytotoxic and apoptotic activity of edible mushroom *Tricholoma anatolicum* H.H.Doğan & Intini extracts against HEPG-2 cells

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Edible mushrooms are a valuable source of nutritional ingredients and biologically active compounds and they have many medicinal properties, including the enhancement of antitumor activity. *Tricholoma anatolicum* is an endemic species of Turkey which only grows in the cedar forests of the Taurus Mountains in the Mediterranean region of our country. In this study, for the first time in the literature, *in vitro* cytotoxic and apoptotic activity of aqueous extracts of edible *Tricholoma anatolicum* (TA) mushroom against human liver carcinoma (HEPG-2) was aimed to determine. Dried mushroom samples were extracted by water at a ratio of 1:25 (w/v) at RT for 2 h. HEPG-2 cells were cultured at six different concentrations (1, 10, 50, 100, 250 and 500 μg/ml) of TA for 24 h. The percentage of cell viability was determined by trypan blue and XTT assays. TA inhibited the survival of HEPG-2 cells in a concentration-dependent manner. EC_{50} value of TA was calculated as 152.15 ± 0.54 and 328.15 ± 0.29 μg/ml for trypan blue and XTT assay, respectively. Furthermore, *Tricholoma anatolicum* caused HEPG-2 cell shrinkage and formation of apoptotic body, which are typical characteristics of apoptotic cell death. In addition, TA caused HEPG-2 cells apoptosis in a concentration dependent manner via formation of phosphatidylserine externalization, as evidenced by flow cytometry. Exposure of HEPG-2 cells to 500 μg/ml TA for 24 h resulted in a shift of 75% of the cell population from normal to the early/late apoptotic/necrotic stage. These findings suggest that *Tricholoma anatolicum* exhibits potential anticancer properties.

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