Titolo rivista/libro: Critical reviews in microbiology (Online)

Titolo articolo/sezione: Bridging the past and the future of virology: Surface plasmon resonance as apowerful tool to investigate virus/host interactions

Autore/i: Rusnati M, Chiodelli P, Bugatti A, Urbinati C

ISSN: 1549-7828

DOI: 10.3109/1040841X.2013.826177

Anno: 2015

Volume: 41

Fascicolo: 2

Editore:

Pag. iniziale: 238

Pag. finale: 260

Critical Reviews in Microbiology

http://informahealthcare.com/mby
ISSN: 1040-841X (print), 1549-7828 (electronic)

Crit Rev Microbiol, 2015; 41(2): 238–260
 DOI: 10.3109/1040841X.2013.826177

informa
healthcare

REVIEW ARTICLE

Bridging the past and the future of virology: Surface plasmon resonance as a powerful tool to investigate virus/host interactions

Marco Rusnati, Paola Chiodelli, Antonella Bugatti, and Chiara Urbinati

Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

Abstract

Despite decades of antiviral drug research and development, viruses still remain a top global healthcare problem. Compared to eukaryotic cells, viruses are composed by a limited numbers of proteins that, nevertheless, set up multiple interactions with cellular components, allowing the virus to take control of the infected cell. Each virus/host interaction can be considered as a therapeutical target for new antiviral drugs but, unfortunately, the systematic study of a so huge number of interactions is time-consuming and expensive, calling for models overcoming these drawbacks. Surface plasmon resonance (SPR) is a label-free optical technique to study biomolecular interactions in real time by detecting reflected light from a prism-gold film interface. Launched 20 years ago, SPR has become a nearly irreplaceable technology for the study of biomolecular interactions. Accordingly, SPR is increasingly used in the field of virology, spanning from the study of biological interactions to the identification of putative antiviral drugs. From the literature available, SPR emerges as an ideal link between conventional biological experimentation and system biology studies functional to the identification of highly connected viral or host proteins that act as nodal points in virus life cycle and thus considerable as therapeutical targets for the development of innovative antiviral strategies.

Keywords

Protein–protein interactions, virus binding assays, virus receptors

History

Received 7 February 2013
Revised 12 July 2013
Accepted 15 July 2013
Published online 5 September 2013

Introduction

Viruses are the etiological cause of important human diseases worldwide. Despite decades of drug research and development, they are still a top global healthcare problem. As a consequence, virus detection, the study of their mechanism of action and the identification of new antiviral drugs remain extremely important for medical healthcare. Although very simple if compared to eukaryotic cells, viruses are very heterogeneous in their structures and mechanisms of action and are prone to a high mutation rate, making their study very diversified and difficult. Although a virus encodes only tens of proteins, it succeeds in taking control of a whole eukaryotic cell since its few proteins set up multiple interactions with cellular components, sometimes out-competing physiological ligands. In effect, every step of the virus life cycle depends on molecular interactions (Figure 1): during the early phases of infection, proteins of the viral envelope acting as determinant of infectivity bind to host cell surface receptors (Bowden et al., 2011). Once internalized, viral components bind to intracellular host cytoskeleton, second messengers, nucleic acids (Ou et al., 2010) and components of cellular secretory pathway (Hsieh et al., 2010), promoting virus replication and new virion assembly and egress. In the meantime, infected cells release virokines or viroceptors, a peculiar class of viral proteins that, acting in a cytokine-like manner, interfere with the host immune system, maintaining a suitable environment for viral infection and replication (Smith & Kotwal, 2001). Each virus/host interaction can be considered as a therapeutical target for the development of antiviral drugs (Brown et al., 2011), but their systematic study may be quite complex, time-consuming and expensive, calling for models overcoming these drawbacks. To this aim, several high-throughput methodologies have been developed in the last years, including proteomic, genomic and computational biology approaches. These methodologies have provided and are still providing an incessant torrent of ‘‘-omics’’ data, functional to the definition of the closely related viral ‘‘interactome’’, ‘‘infectome’’ and ‘‘diseasome’’ (Kadaveru et al., 2008; Viswanathan & Fruh, 2007).

Surface plasmon resonance (SPR) is a handy-user, reliable and high-throughput optical technique to evaluate biomolecular interactions. It has been launched less than 20 years ago and has been exploited in a variety of fields, including the study of the virus/host interactions. Here the contribution given by SPR to the advance of the knowledge in the field of virology will be reviewed, discussing its limits, advantages and future developments.

SPR spectroscopy: an introduction

A comprehensive description of SPR technology can be easily found elsewhere. However, to understand how SPR has

Address for correspondence: Prof. Marco Rusnati, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, Brescia 25123, Italy. E-mail: rusnati@med.unibs.it

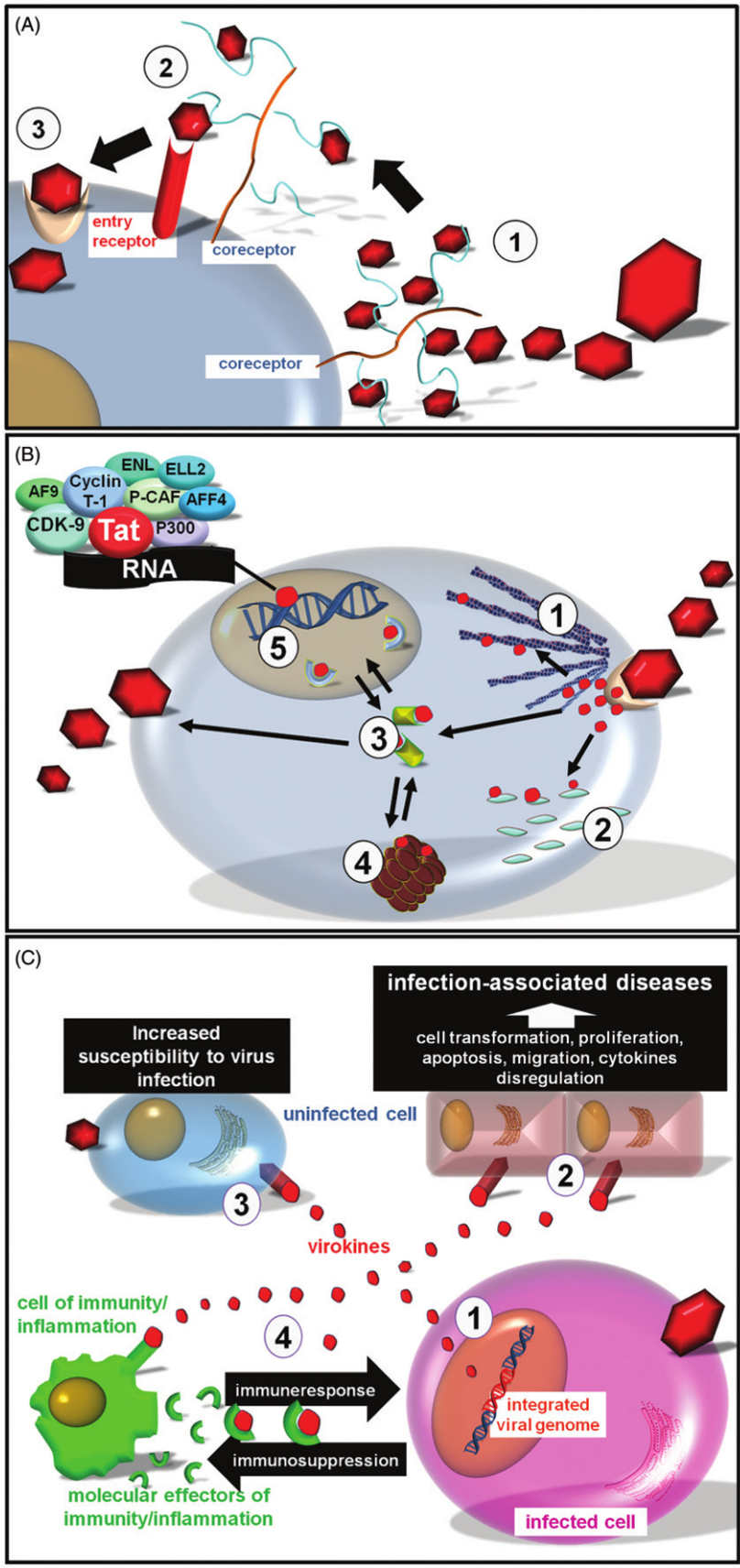

Figure 1. Schematic representations of the virus/host interactome. (A) In the early phases of infection, the virus binds to co-receptors of target cell, increasing its concentration at the cell surface and resulting protected from degradation (1). Then, co-receptors present the virus to entry-receptors (2) that mediate virus fusion and internalization (3). (B) Inside the infected cell, viral proteins can interact with cyto-skeleton's components (1), intracellular second messengers (2), cellular chaperones (3), components of the proteasome (4), transactivating factors and nucleic acids (5), often generating multimolecular complexes such that occurring among Tat, RNA and cofactors. (C) Infected cells release virokines (1) that hijack specific receptors on unin-fected cells inducing pathological effects that lead to infection-associated diseases (2) or increasing cell susceptibility to infection (3). Other extracellular interactions occur between viral proteins and effectors of inflammation and immunity that lead to virus neutralization or, alternatively, favor virus spread by inducing immunosuppression (4).

contributed (and may further contribute) to virology, a cursory appreciation of its basic fundamentals is needed.

A typical setup of a solid-phase bioassay based on SPR spectroscopy is sketched in Figure 2(A). A polarized beam of monochromatic visible light is passed through a prism fitted with a glass slide coated with about 50 nm of gold, from which it is reflected. An electric field intensity, known as evanescent wave, is generated when the light strikes the glass

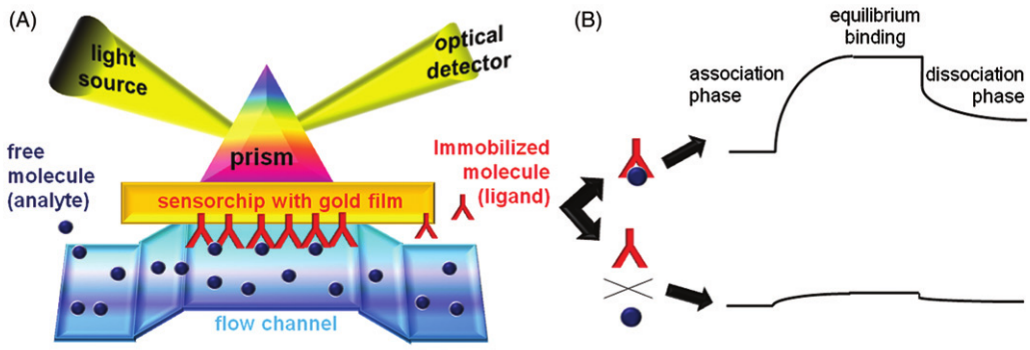

Figure 2. Schematic representation of SPR technology. (A) The molecule immobilized onto the sensorchip is named ligand whereas the putative partner injected into the microfluidic system is named analyte. (B) The real-time progress of the ligand/analyte interaction is monitored as a sensorgram. The analyte binds to surface-immobilized ligand during injection, resulting in an increase in the RU signal (association phase) and then in a transient equilibrium binding phase. At the end of the injection the analyte is replaced by a continuous flow of buffer, with the consequent decrease in the RU signal reflecting the dissociation of the analyte from the surface. In the presence of slow k_{off}, an additional regeneration step is required to remove the analyte bound to the immobilized ligand (not shown). This allows to perform several cycles of binding at different analyte concentrations, a procedure required to get accurate measurements.

in total internal reflection conditions. The evanescent wave is absorbed by the free electron clouds of the gold layer generating electron charge density waves called plasmons and causing a reduction in the intensity of the reflected light. The angle corresponding to the sharp intensity minimum that occurs at the SPR condition is called resonance angle. It depends on the refractive index of the material above (about 300 nm) the gold surface, and is monitored following the specularly reflected light intensity versus angle at fixed wavelengths or versus wavelength at fixed angle. In a SPR assay, the receptor specific for a particular analyte is chemically immobilized to the gold film. When the sensor is exposed to a sample containing that analyte, the analyte/receptor interaction causes an increase of the mass that, changing the refractive index at the gold surface, leads to the shift of the resonance angle that eventually provides label-free transduction of the binding reaction. The data are then presented as a real-time graph (sensorgram) of the response units (RU, directly proportional to the increase of the mass of the complex formed at the surface of the gold film) against time (Figure 2B). SPR allows the detection of analytes over a wide range of molecular weights and binding affinities, from weakly interacting small molecules (as antiviral prodrugs) to huge structures (as intact virions). In respect to conventional fluorescent-, enzyme- or radio-labeled assays, SPR adds to real-time, label-free molecular recognition other advantages, including the possibility to investigate and manipulate minute concentrations of molecules semi-automatically in a multiplexed way. Also, SPR gives access to:

(i) kinetics analyses that, by evaluating the association (k_{on}) and dissociation (k_{off}) rates, measure how fast a molecule binds to and detaches from another;
(ii) equilibrium analyses, that allow the determination of the stoichiometry of the interaction (further discussed in the Section ''What must be improved'') and of the equilibrium dissociation constant (K_D), inversely proportional to the binding affinity;
(iii) determination of thermodynamic parameters such as changes in enthalpy (ΔH) and entropy (ΔS) by measuring rate constants at different temperatures;
(iv) sequential injection of distinct analytes over an immobilized receptor, that can give insights on the formation of multi-molecular complexes.

Exploiting SPR in virology

The use of SPR has seen tremendous growth over the last two decades, and this trend is predicted to continue as the technology becomes more accessible and its applications more diverse. Accordingly, the number papers containing SPR analyses in the field of virology has steadily increased, with a total of about 1000 papers published to date (Figure 3A). About 37% of these papers deal with HIV, with the remaining papers scattered among the other viruses in percentages equal or lower than 10% (Figure 3B). For the majority of the viruses considered, the papers containing SPR-analyses impact the total scientific production with percentages equal to about 0.1%, with the only remarkable exceptions of SARS and ebola virus (0.5%).

As shown in Figure 3(C), few SPR analyses are dedicated to virus detection or genotyping, also if 2012 registered a remarkable increment of this kind of study. Rather, SPR has been mainly used to identify antiviral antibodies/drugs and to characterize viruses/host interactions. Due to the huge amount of literature available, in this review we will discuss only the latter type of study.

The various virus/host interactions can be ideally divided in five groups: viral proteins self interactions, viral envelope proteins interacting with host receptors, viral enzymes/transactivating factors interacting with host intracellular proteins or with host nucleic acids and virokines interacting with cellular or molecular host structures. Among these groups, SPR has been mainly exploited to study the interactions of viral determinants of infectivity with host receptors (Figure 3D). This is not surprising since the early stages of virus infection are widely recognized as promising targets for the development of vaccines or antiviral drugs.

SPR analyses of viral proteins self-interaction

The structural organization of viruses is based on different viral proteins that assemble, mature and bud into infective

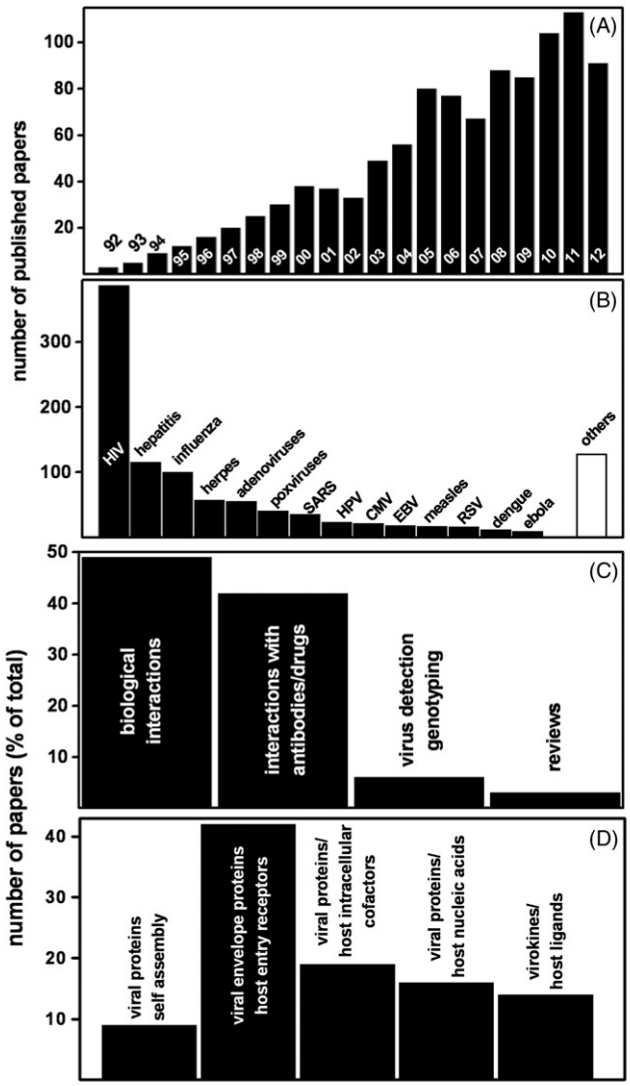

Figure 3. Statistic of virology-oriented papers containing SPR analyses. (A) SPR analyses published in the last 20 years in the field of virology. (B) Distribution of SPR analyses among the various viruses. (C) Distribution of SPR analyses among the various areas of virology. (D) Distribution of SPR analyses among the various categories of virus/host interactions.

virions. This process has been so far investigated mainly by high-resolution X-ray crystallography, cryo-electron microscopy and mathematical models (Twarock, 2006). These studies pointed out a common theme in virion assembly that is the conformational change of interacting viral proteins and the consequent allosteric regulation of their aggregation. In turn, this points to the prevention of viral proteins assembly by non-competitive small molecules as an attractive antiviral strategy (Zlotnick & Mukhopadhyay, 2011). Relevant to this point, dedicated SPR models have been successfully set up to characterize allosteric regulation of protein bindings and to identify second site non-competitive binders (Navratilova et al., 2012) that, if appropriately exploited, would positively impact this area of virology.

Viral proteins' self-assembly occurs with a mean affinity (K_D in the micromolar range) that is significantly lower than those of viral proteins' interactions with host structures (Table 1 and Figure 5A). Since viral proteins self-assembly takes place in secluded environment such as specialized membrane microdomains (Bieniasz, 2009), it is possible that no selective pressure weights on this process, at variance with what occurs for envelope glycoproteins that, to successfully bind host receptors, must outcompete physiological ligands, thus requiring to develop high affinity interactions.

SPR analyses of viral envelope proteins interacting with cell surface receptors

Very frequently, a productive infection is the result of a multistep process during which the virus initially interacts with ''co-receptors'' [often represented by glycosphingolipids or proteoglycans (Urbinati et al., 2008)] that protect virion from degradation (Bobardt et al., 2003) and allow its concentration at the cell surface, compensating for the low expression of the ''actual'' entry receptors (often represented by glycoproteins) (Gallay, 2004) (Figure 1A). Then, co-receptors present virions to entry-receptors that mediate virus fusion and internalization (Nowak & Chou, 2009). Thus, the formation of multimeric complexes among viral envelope proteins and different host receptors represents a common theme in the process of virus infection that can be appropriately studied by SPR, as demonstrated by the successful characterization of the complexes formed by HIV-1 gp120 and CD4 with CCR5 (Lam et al., 2008), with CXCR4 (Chien et al., 2008), with heparan sulfate proteoglycans (HSPGs) (Crublet et al., 2008) and with Langerin and DC-SIGN (Hijazi et al., 2011).

SPR-calculations of K_D values for viral protein/host receptor interactions can be very heterogeneous, passing from low nanomolar to micromolar values [i.e. the interaction of HIV gp120 with CD4 and of adenovirus fiber knob protein with coxsackie B virus-adenovirus receptor (CAR) (Table 2)]. To this variability surely contributes the high rate of mutation of viral envelope proteins, but a significant burden is also brought by the heterogeneous procedures adopted for receptor immobilization that can span from the most simple amine-coupling (that yields a random orientation of the immobilized receptor) to the most complex incorporation of receptors in lipid layers (Figure 4A). The simpler immobilization procedures are expected to be the most artifactual, providing binding parameters hardly comparable to those calculated in living cells. At variance, the most complex and time-consuming procedures are likely expected to guarantee the proper orientation and stability of the immobilized receptor, thus providing binding conditions closer to the physiological settings. A good compromise is the use of N- or C-terminal tagged receptors [i.e. with Fc (Jennings et al., 2008), glutathione-S-transferase, maltose-BP (Zanier et al., 2005), Gal4 DNA-binding domain (Nedialkov & Triezenberg, 2004) and the FLAG epitope (Navaratnarajah et al., 2008)] to be coupled to specific ligands immobilized to the sensorchip (Figure 4A).

Among the various host structures that act as virus receptors, HSPGs are the most shared (Table 2). HSPGs are present on the surface of almost all eukaryotic cells and consist of a core protein with attached glycosaminoglycan (GAG) chains represented by unbranched anionic polysaccharides (Lindahl et al., 1994). The interactive capacity of HSPGs depends mainly on their GAG portion. Accordingly, heparin (structurally resembling the GAG chain of HSPGs) interacts with a wide array of viral proteins (Tables 2 and 6)

Table 1. SPR analysis of the interactions between viral proteins.

Virus	Interacting viral proteins	K_D (nM)	Reference
HPV	E6 oncoprotein/ubiquitin ligase E6AP	µM range	Zanier et al., 2005
		µM range	Liu et al., 2009b
		30 000	Zanier et al., 2009
	E7 protein/E2 protein	730	Saitoh et al., 2008
Hepatitis viruses	NS3 protease/NS4A cofactor	1900–5000	Gallo et al., 2010
	Core protein self-assembly	µM range	Kang et al., 2008
	Core protein/envelope protein	µM-mM range	Choi et al., 2004
	NS5A polymerase/NS3 helicase	nd	Jennings et al., 2008
HIV-1	Nucleocapsid protein/reverse transcriptase	1700	Ramboarina et al., 2004
	Integrase/reverse transcriptase	61	Wilkinson et al., 2009
		141	Herschhorn et al., 2008b
SARS-CoV	Nucleocapsid protein self assembly	nd	Luo et al., 2004b
	Nucleocapsid protein/membrane protein	550	Luo et al., 2006
Measles virus	Nucleoprotein self-assembly	81	Bourhis et al., 2005
Rotavirus	NSP4 enterotoxin/outer capsid VP4 protein	470	Hyser et al., 2008
Influenza virus	Polymerase subunits (PB1/PA)	1600	Wunderlich et al., 2011
	Polymerase subunits (PB2/PA)	nd	Ng et al., 2012
Chandipura virus	Nucleocapsid N protein/P phospoprotein	10 000	Majumdar et al., 2004
HSV	Type 1 primosome/single strand DNA binding protein (ICP8)	nd	Falkenberg et al., 1997
Dengue virus	Surface premembrane protein/envelope protein	290–730	Zhang et al., 2012

nd: not determined.

Table 2. SPR analysis of the interaction of intact viruses or of viral envelope proteins with host cell surface receptors functional to infection.

Viral protein	Host binder	K_D (nM)	Reference
HIV-1			
gp120	CD4	2.6	Ferrer et al., 1999
		30.1–1190	Ryzhova et al., 2002
		38–494	Martin-Garcia et al., 2005
		5.5–10.5	Dey et al., 2009
		5.6–7.7	Stricher et al., 2008
		86	Zhao et al., 2005
		9.2	Zhao et al., 2005
		22–220	Myszka et al., 2000
		0.9–8.9	VanCott et al., 1994
		5.3–97.2	Cocklin et al., 2007
		8	Crublet et al., 2008
		48.1	Chaudhari et al., 2006
		429	Biorn et al., 2004
		1.98	Frey et al., 2008
		1–7	Feng et al., 2011
	Heparin (HSPGs analog)	220	Moulard et al., 2000
		0.6	Bugatti et al., 2007
	CCR5	1900	Lam et al., 2008
	CXCR4	nd	Chien et al., 2008
gp140	CD4	2.43	Hijazi et al., 2011
	DC-SIGN	62–3270	
	Langerin	11–800	
gp41	Cellular receptor p45	nd	Xiao et al., 2000
Poxviruses			
Vaccinia virus envelope protein A27	Heparin (HSPGs analog)	12.5	Shih et al., 2009
		7.7	Ho et al., 2005
	Chondroitin sulfate	32.3	Shih et al., 2009

Table 2. Continued

Viral protein	Host binder	K_D (nM)	Reference
Adenoviruses			
Intact virion	Desmoglein-2	nd	Wang et al., 2010a
Virus-like particles	Desmoglein-2	2.5	
Fiber knob protein	CAR	7.3–6400	Kirby et al., 2001
		0.9–26.4	Lortat-Jacob et al., 2001
		14.8	Kirby et al., 2000
		20	Seiradake et al., 2006
	CD46	13–284	Cupelli et al., 2010
		13.7–15.5	Wang et al., 2007
		0.02–0.4	Trinh et al., 2012
	αvβ3 integrin	nd	Lord et al., 2006
Exon	Heparin (HSPGs analog)	nd	Corjon et al., 2011
HSV			
Glycoprotein B	Heparin (HSPGs analog)	770	Williams & Straus, 1997
	Heparan sulfate, chondroitin sulfate, dermatan sulfate	nd	
Glycoprotein D	Herpes virus entry mediator	1500–3200	Willis et al., 1998
	Heparin (HSPGs analog)	3200	Willis et al., 1998
Hepatitis viruses			
Envelope glycoprotein E2	CD81	10–92	Nakajima et al., 2005
	Heparin (HSPGs analog)	5.2	Barth et al., 2003; Barth et al., 2006
Envelope glycoprotein E1	Heparin (HSPGs analog)	53	Barth et al., 2006
Influenza viruses			
Intact virion	Fetuin	0.2–0.9	Meng et al., 2010
A hemagglutinin	Fetuin	100	Meng et al., 2010; Takemoto et al., 1996
	Sialic acid-bearing glycans	1.8	Suenaga et al., 2012
		2.5–2.9	Lewallen et al., 2009
EBV			
Surface glycoprotein gp350/220	CD21	4.6–45	Sarrias et al., 2001
	Chondoritin sulfate	nd	Birkmann et al., 2001
Glycoproteins gHgL complex	αvβ5 integrin	4.3	Chesnokova & Hutt-Fletcher, 2011
	αvβ6 integrin	2.4	
	αvβ8 integrin	6	
Measles virus			
Intact virion	Heparin, chondoritin sulfate	nd	Terao-Muto et al., 2008
Hemagglutinin	Signaling lymphocytic activation molecule	80	Navaratnarajah et al., 2008
		180–800	Santiago et al., 2002
	CD46	79	Navaratnarajah et al., 2008
		95–250	Santiago et al., 2002
HPV			
Virus-like particles	Tight junction MAGI-1	1100–3300	Fournane et al., 2010
	Heparin (HSPGs analog)	nd	Lembo et al., 2008
Reoviruses			
Intact virion	Glycophorin	4.8	Barton et al., 2001
Attachment protein σ1	Junctional adhesion molecule-A	2.4	Guglielmi et al., 2007
Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8)			
Envelope glycoprotein K8-1	Heparin (HSPGs analog)	48	Birkmann et al., 2001
Human rhinovirus			
Intact virion	ICAM-1	700	Casasnovas & Springer, 1995
		180–380	Xing et al., 2000
Echovirus			
Intact virion	CD55	3000	Lea et al., 1998
		700–4000	Pettigrew et al., 2006

(*continued*)

Table 2. Continued

Viral protein	Host binder	K_D (nM)	Reference
Dengue virus			
Envelope protein	Heparin (HSPGs analog)	56 31	Marks et al., 2001 Zhang et al., 2002
	Heparan sulfate, chondroitin sulfate, dermatan sulfate, hyaluronic acid	nd	Marks et al., 2001
Poliovirus			
Intact virion	Poliovirus receptor	170–430 670	Xing et al., 2000 McDermott et al., 2000
Nipah virus			
Attachment protein G	Ephrin B2 Ephrin B3	0.1 2.8	Negrete et al., 2006
Respiratory syncizial virus			
Attachment glycoprotein G	DC-SIGN L-SIGN	nd nd	Johnson et al., 2011

Fetuin and glycophorin were used as sialic acid-bearing receptor analogs. nd: not determined.

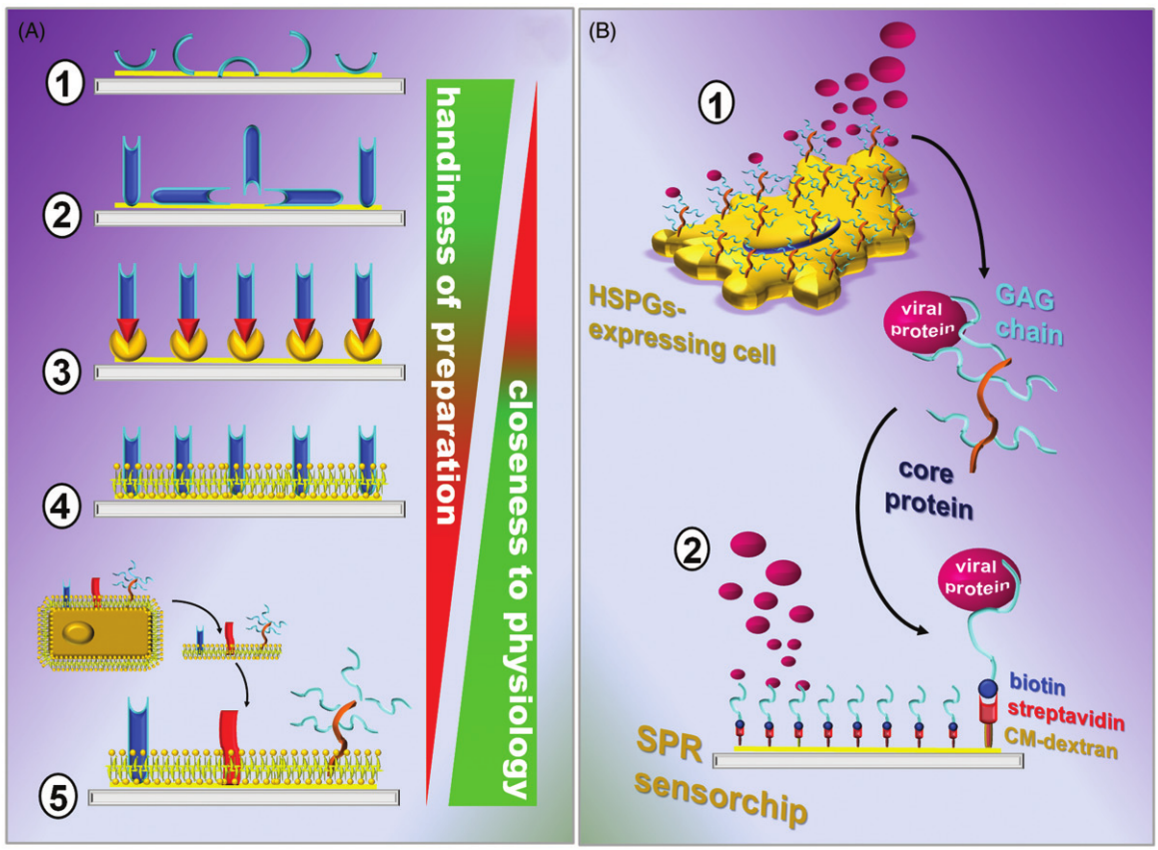

Figure 4. Procedures of immobilization of virus receptors to SPR surfaces. (A) The simplest amine-coupling leads to the immobilization of randomly oriented receptor-derived peptides (i.e. extracellular binding domains) (1) or intact receptors (2). Alternatively, virus receptors can be produced fused with a tag that is then exploited for its immobilization in a proper orientation (3). To increase the physiology of the SPR experimental conditions, lipid mixtures are deposited on the sensorchip, ''reproducing'' a membrane lipid bilayer that favors receptor stability and orientation (4). Finally, membranes isolated from living cells can be directly immobilized to the sensorchip, retaining the presence of possible co-receptors (5). (B) HSPGs are composed of a core protein attached to heparan sulfate chains that mediate the binding to viral proteins (1). Heparin is a structural analog of heparan sulfate that can be biotinylated at its reducing end and immobilized through streptavidin to the carboxymethyl (CM) dextran of the sensorchip (2), allowing SPR analyses predictive of the interactions occurring *in vivo* between viral proteins and cell surface-associated HSPGs.

and heparin immobilized to a SPR sensorchip resembles cell surface-associated HSPGs (Figure 4B), as demonstrated by the fact that the K_D values calculated with this model are comparable to those calculated for HSPGs associated to living cells (Rusnati et al., 2009). The biological importance of heparin/HSPG in virology luckily meets the easiness of heparin-based SPR analyses. This is mainly due to the fact that the immobilization of heparin evades those problems related to orientation and stability that instead affect those SPR analyses requiring the immobilization of proteins to the sensorchip (Figure 4B).

In effect, several proteins acting as virus receptors present structural features that make their immobilization very difficult. Paradigmatic are seven transmembrane-spanning chemokine receptors, deeply involved in HIV biology but basically neglected in SPR analyses. This mainly depends on their structural complexity that, in turn, makes their sensorchip-immobilization a hard challenge. In the past, this has been tentatively overcome by amine-coupling of linear peptides representing their extracellular domains (Baritaki et al., 2002), a simple model that, however, did not resemble physiological interactions. More recently, procedures have been set up to incorporate on the sensorchip lipid mixtures that, resembling plasma membrane, allow a stable and properly oriented immobilization of intact receptors (Navratilova et al., 2005). These procedures have been successfully used to characterize the interaction of HIV gp120 with CCR5 (Lam et al., 2008) and of HIV p17 with CXCR1 (Giagulli et al., 2012) and CXCR2 (Caccuri et al., 2012). Very promising in this context is also the possibility to transfect cells to overexpress specific viral receptors and then to capture to the sensorchip the membranes isolated from transfected cells (Zhu et al., 2009). In effect, it is well known that host plasma membranes play essential roles in virus infection (Ghanam et al., 2012; Moriishi & Matsuura, 2012) pointing to lipid layers as particularly important in SPR analyses of virus/host receptors interaction. In particular, gangliosides are sialic acid-containing glycosphingolipids largely represented in eukaryotic cell membrane and involved in virus attachment (Taube et al., 2010). Although a significant amount of SPR analyses has been done to study the interactions of viral proteins with gangliosides and other lipids, affinity values have been seldom calculated in these analyses (Table 3). This is likely due to the complexity of proteins/lipids interactions, often characterized by multiphasic kinetics and/or cooperativity. Briefly, cooperativity is a case of allostery in which a macromolecule (i.e. a lipid) has more than one binding site and the interaction of the ligand (i.e. a viral protein) at one site increases its affinity at the contiguous site. Multiphasic or cooperative interactions are difficult to study with classical binding assays, while suitable SPR model have been developed to this aim (Galdiero et al., 2010; Nilsson et al., 2010).

Although within the limit of the high variability of the K_D values calculated, it seems that viral proteins bind host receptors with affinities that are lower than those of physiological ligands (Figure 5B). On the other hand, the overall affinity of the interaction of viral proteins with HSPGs seems higher than those with chemokine receptors or with integrins (Figure 5B), suggesting that HSPGs are ''more hijackable'' by viruses, thus explaining their diffusion as viral co-receptors. Finally, the interaction of viral determinant of infectivity with host receptors seems to occur with an overall affinity (K_D in the high nanomolar range) that is lower than that of the other viral proteins with nucleic acids and of virokines with their receptors (K_D in the low nanomolar) (Figure 5A). Relevant to this point, *in vivo,* viral determinants of infectivity often interact with their specific entry receptors only after a proper presentation by co-receptors while, in SPR analyses, they are analyzed in the presence of isolated receptors, likely underestimating their affinity.

SPR analyses of viral proteins interacting with intracellular host factors

After virus internalization or following *de novo* synthesis from integrated viral genome, viral proteins enter the crowded cytoplasmic compartment, where they set up the various

Table 3. SPR analysis of the interaction of viruses or of viral envelope proteins with plasma membrane lipids.

Viral protein	Host binder	K_D (nM)	Reference
HIV-1			
gp41	Lipid monolayer and bilayer	nd	Galdiero et al., 2010
p6	Lipid bilayer	μM range	Solbak et al., 2012
Adenoviruses			
Fiber knob protein	Ganglioside GD_{1A}	19 000–265 000	Nilsson et al., 2010
HSV			
Glycoprotein B	Lipid monolayer and bilayer	nd	Galdiero et al., 2010
Glycoprotein H	Lipid monolayer and bilayer	nd	
Influenza viruses			
Intact virion	Sialoglycolipid neomembrane	nd	Critchley & Dimmock, 2004
	Gangliosides Neu5Acα2-3nLc4Cer, Neu5Acα2-6nLc4Cer and GM3	nd	Hidari et al., 2007
Parvovirus			
Virus-like particles	Globotetraosylceramide	nd	Kaufmann et al., 2005

nd: not determined.

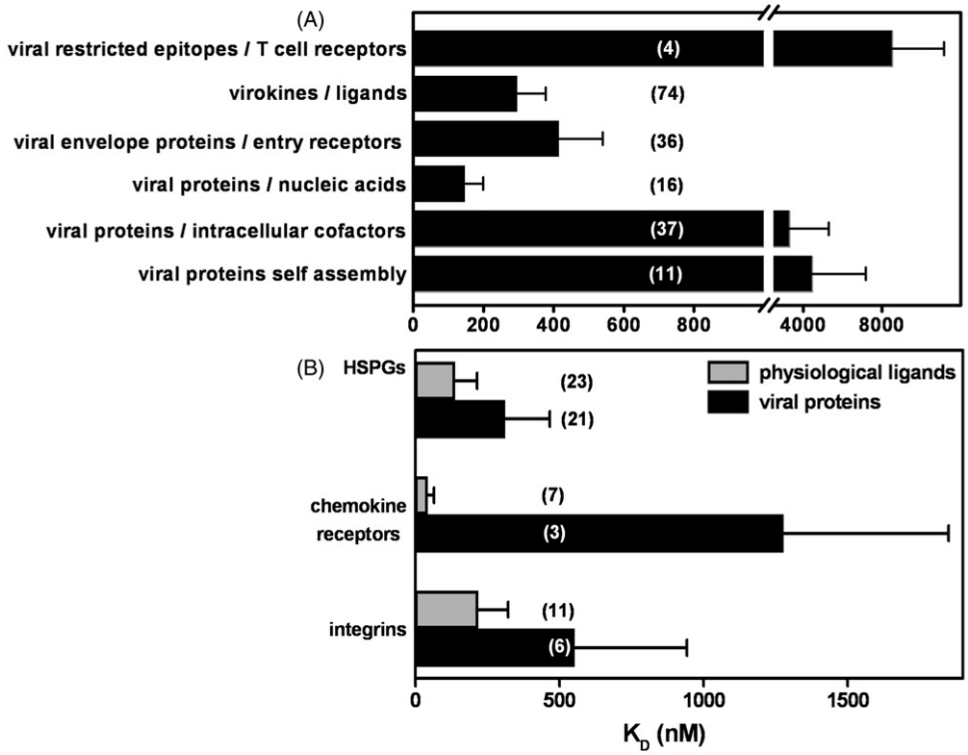

Figure 5. (A) Affinity of the various categories of virus/host interactions. The mean value of the dissociation constant (K_D) of the interactions of viral proteins with different host structures are reported. (B) Affinity of the interactions of host structures with physiological ligand or with viral proteins. The mean value of the K_D of the interactions of viral determinant of infectivity, virokines or physiological ligands with selected receptors are shown. The number of measurements taken in consideration are reported in brackets.

interactions required to virus replication, assembly and egress. Different types of host intracellular proteins are involved in these interactions (Table 4 and Figure 1B): cytoskeleton components, mainly implicated in virus endocytosis and new virions egress (Taylor et al., 2011); second messengers and enzymes, exploited by the virus to maintain a cell environment favorable to its replication (Greco-Stewart & Pelchat, 2010); cellular chaperones, that marshal viral components from and to the various cellular compartments (Stamminger, 2008); proteasome components, that modulate the stability of viral proteins (Choi et al., 2012); transactivating factors and DNA-associated proteins, that regulate replication and transcription of viral nucleic acids (Engelhardt & Fodor, 2006).

Very frequently these interactions lead to the formation of large multimeric complexes, as exemplified by the main transactivator of HIV-1 Tat that, to exert its full transactivating activity, set up a complex with RNA and nine different cofactors (He et al., 2010) (Figure 1B). These multimeric complexes are often characterized by allosteric regulation (Leavitt et al., 2004) that can be properly dissected and characterized by SPR (Navratilova et al., 2012) as successfully performed for the study of the interaction of the HIV-1 Gag protein with Tsg101 and ubiquitin (Garrus et al., 2001) and of the Epstein-Barr virus (HBV) EBNA-5 protein with MDM2 and p53 (Kashuba et al., 2010).

Interestingly, intracellular host proteins can also interact with viral proteins within mature virions. Although originally considered purification contaminants, it is now accepted that host components are present in virions, although their roles remain unknown. HSV is particularly prone to incorporate host proteins, but also vaccinia virus (VV), influenza virus and HIV share this capacity. The host proteins more often found in virions are actin, annexins, cofilin, translation factors, GAPDH, heat shock proteins, pyruvate kinase M2 and Rab GTPases (Lippe, 2012). The exploiting of SPR in a systematic research and characterization of their interactions inside the virion may contribute to the better comprehension of this otherwise obscure field of virology.

SPR analyses of viral proteins interacting with host nucleic acids

Historically, the interaction of viral enzymes/transactivators with host nucleic acids received great attention and several biochemical models (including SPR) have been developed (Majka & Speck, 2007). An important issue in protein/DNA interaction is the discrimination of specific (productive) bindings with an actual biological meaning from aspecific bindings that often occur with highly negatively charged nucleic acids. This discrimination can be usually achieved only by time consuming binding assays at different times and temperatures hardly practicable with classical biochemical models. At variance, due to its quick and high-throughput features, SPR has proven to be appropriate for this type of studies (Oda & Nakamura, 2000). Another important technical issue that makes SPR analysis a first choice for the analysis of viral proteins interaction with nucleic acids is the easiness of the surface-immobilization of DNA or RNA, usually achieved through their biotinylation and binding to streptavidin sensorchips. Once surface-immobilized, nucleic acids do not pose problems of orientation or of masking of functional domains (Majka & Speck, 2007), resulting in

Table 4. SPR analysis of the interaction of viral proteins with intracellular host co-factors.

Viral protein	Host binder	K_D (nM)	Reference
HIV-1			
gp120	Intracellular mannan-binding protein	1.7–5.0	Nonaka et al., 2007
	H1 histone	34.3	Mamikonyan et al., 2008
integrase	H1 histone	38.3	
gp41	Cellular transportin 3	261	Krishnan et al., 2010
Gag	Phosphoinositide phosphates	μM range	Anraku et al., 2010
	Endosomal sorting complexes required for transport-associated protein Alix	μM range	Munshi et al., 2007
	Tsg101	μM range	Garrus et al., 2001
Nef	Calmodulin	94	Matsubara et al., 2005
	Src kinase Hck	250	Manninen et al., 1998
Vpr	Cyclophilin A	μM range	Solbak et al., 2010
		280	Solbak et al., 2011
	Adenine nucleotide translocator	10–100	Sabbah et al., 2006
		9.7	Jacotot et al., 2001
	Importin-α	4300–8900	Takeda et al., 2011
Tat	IκB-α	178	Vitagliano et al. 2011
	Dopamine transporter	nd	Zhu et al., 2009
Reverse transcriptase	Topoisomerase I	nd	Takahashi et al., 2004
Adenoviruses			
Early trascription unit E3	AP-1	480–520	Hilgendorf et al., 2003
	AP-2	300–400	
Virus-like particles	Ubiquitin-protein ligase WWP1	65	Galinier et al., 2002
Rep52 protein	Protein kinase PrKX	385	Chiorini et al., 1998
	Protein kinase A	320	
Rep68 protein	Protein kinase PrKX	2100	
Rep78 protein	Protein kinase PrKX	680	
Co-activator protein E1A 13S	NF-kB p65	26	Paal et al., 1997
HSV			
Co-activator protein VP16	TATA-binding protein	35	Nedialkov et al., 2003
		16	Bamdad, 1998
		44	Nedialkov & Triezenberg, 2004
	TATA-binding protein -associated factor 9	73 000	Nedialkov & Triezenberg, 2004
	Transcription factor TFIIA	nd	
	Transcription factor TFIIB	3000	
	Swi1and Snf5 subunits of the chromatin remodeling complex	nd	Ferreira et al., 2005
Exonuclease UL12	DNA double-strand break-sensing complex	31.1	Balasubramanian et al., 2010
Hepatitis viruses			
Core protein	Microtubulin	75–100	Roohvand et al., 2009
	Nucleophosmin-1	2510	Lee et al., 2009
	Aminoacylase 3	10 100	Tsirulnikov et al., 2012
	Apolipoprotein B mRNA-editing enzyme catalytic polypeptide 1-like	nd	Zhao et al., 2010
NS5A polymerase	Fyn tyrosine kinase	556–629	Shelton & Harris, 2008
	FK506-binding protein 8	82	Okamoto et al., 2008
HBx co-activator	Glioma-associated oncogene homologue 1	μM range	Jo et al., 2011
Influenza viruses			
Polymerase PB2	Human importin-α1,3,5,7	1.3–8.5	Boivin & Hart, 2011
Measles virus			
Nucleocapsid protein	Heat shock protein 72	1000	Zhang & Oglesbee, 2003
Helical ribonucleoprotein	Heat shock protein 72	16	Zhang & Oglesbee, 2003

(*continued*)

Table 4. Continued

Viral protein	Host binder	K_D (nM)	Reference
HPV			
E6 oncoprotein	Human homolog of the *Drosophila* discs large tumor suppressor protein/synapse associated protein	μM range	Liu et al., 2009b
	E6-binding protein	nd	Beerheide et al., 1999
E7 protein	Retinoblastoma tumor suppressor Rb	5000	Jung et al., 2005
SARS-CoV			
Nucleocapsid protein	Heterogeneous nuclear ribonucleoprotein A1	350	Luo et al., 2005
	Cyclophilin A	6.1–159	Luo et al., 2004a
		40	Chen et al., 2005c
	Proteasome subunit p42	nd	Wang et al., 2010b
Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8)			
Viral K15 protein	Intersectin 2	13 500	Lim et al., 2007
Viral G protein-coupled receptor	Protein tyrosine phosphatase Shp2	6.1	Philpott et al., 2011
Ebola virus			
Matrix protein VP40	Human ubiquitin ligase Nedd4	800	Timmins et al., 2003
Poxviruses			
V39 subunit of VV poly(A) polymerase	S-adenosyl-L-methionine	nd	Shi et al., 1996
A46 protein of VV	MyD88-adaptor like protein	1.7	Oda et al., 2011

SPR was used to assess the kinetics of interaction between the indicated viral proteins and their cognate/putative viral or host binder. nd: not determined.

highly accessible DNA or RNA surface, the only drawback being possible non-specific binding of positively charged proteins, a problem that can be solved by immobilizing the protein and injecting the nucleic acids.

Collectively, the affinity of the interaction of viral proteins with nucleic acids (K_D in the low nanomolar range) seems the highest among all the other interactions considered (Figure 5A). Relevant to this point, although nucleic acids are repetitive macromolecules devoid of the structural complexity that usually confer to an interaction high affinity and specificity, they can set up multimeric interaction with viral proteins (Figure 1B) that are very often cooperative (Majka & Speck, 2007), two features that may increase the length of the nucleic acid recognized by the transcription factors and the specificity and affinity of the binding. Also, the stacking interactions between the nucleotide bases and aromatic residues of the protein and hydrogen bonds between the protein and the nucleic acids, allow an exquisite tuning of the interaction. Finally, nucleic acids are endowed with intrinsic flexibility, which, through their looping, allows long-range interactions of viral proteins with distal DNA/RNA elements, again increasing complexity, affinity and specificity. Appropriate SPR analyses with DNA or RNA have been optimized to unravel the intricacy of their interaction with protein (Okumoto et al., 2002; Smith et al., 2002). Accordingly, SPR allowed the successful characterization of complex interactions between DNA and proteins from the most important human viruses (Table 5).

SPR analyses of interactions of viral proteins released by infected cells

In the extracellular environment, an intricate network of interactions occurs between viral proteins released by infected cells and the surrounding components of the host (Table 6 and Figure 1C). Some of these interactions are functional to the protection of the host from virus infection, as typically exemplified by the binding of T cell receptors or to defensins to viral peptides. Interestingly, T cell receptors bind viral restricted peptides with a mean affinity (K_D in the micromolar range) that is the lowest among those here considered (Table 6 and Figure 5A). This may be due to two concurrent reasons: the interactions involving short linear peptides devoid of structural complexity (as processed viral peptides) are usually characterized by low affinity. On the other hand, T cell receptors maintain the capacity to recognize a broad range of restricted peptides, lowering their specificity and affinity for their ligands.

Other interactions instead lead to immunosuppression: virokines (also termed viroceptors) are viral proteins actively released by infected cells that bind and inactivate inflammatory cytokines or component of the complement cascade, creating a suitable habitat for viral replication and spreading (Smith & Kotwal, 2001). Also, some proteins of HIV (the gp120 envelope protein, the p17 matrix component and the transactivating factor Tat) are released by infected cells and, once in the extracellular environment, engage receptors of surrounding cells causing a variety of effects that concur to increase cell susceptibility to HIV infection and to promote the arise of AIDS-associated diseases (Bugatti et al., 2007; Fiorentini et al., 2006). Virokines bind to their receptors with affinities that are higher than those of the other virus/host interactions considered here (Figure 5A). Two lines of reasons may explain this feature: virokines are usually encoded by genes that viruses have acquired by eukaryotic cells and, as already mentioned, the interaction of eukaryotic proteins are usually characterized by affinities that are higher than those of viral proteins (Figure 5B). Also, in the course of virus evolution, virokines may mutate freely to increase their affinity for targets, being not burdened by

Table 5. SPR analysis of the interaction of viral proteins with viral or host nucleic acids.

Viral protein	Host binder	K_D (nM)	Reference
HIV-1			
Integrase	DNA	2.2–14 2.9–31.3 1.3–53	Ramcharan et al., 2006 Ramcharan et al., 2006 Yi et al., 1999
Reverse trascriptase	DNA	31 170	Herschhorn et al., 2008a Lin et al., 2000
	RNA–DNA hybrids	2.7–33.3	Gorshkova et al., 2001
Nucleocapsid protein	DNA	13.7–277	Ramboarina et al., 2004
	RNA	2.9–9 0.7 2.4	Ramboarina et al., 2004 Kim et al., 2002 Kim & Jeong, 2003
Rev	RNA	0.04–90 2.7 2000	Van Ryk & Venkatesan, 1999 West & Ramsdale, 1996 Gallego et al., 2003
Tat	RNA	1.8–7.8 46	Chaloin et al., 2005 Partidos et al., 2005
Gag	DNA	1.1–166.5	Stephen et al., 2007
SARS-CoV			
Nucleocapsid protein	RNA	0.7–15.1 11.9 4.6 2–1430	Chen et al., 2005a Huang et al., 2009 Yang et al., 2008 Spencer & Hiscox, 2006
	DNA	nd	Huang et al., 2009
Hepatitis viruses			
Proteinase 3C	RNA	μM-mM range	Peters et al., 2005
Core protein	DNA, RNA	nd	Tanaka et al., 2000
NS5B polymerase	RNA	nd	Nyanguile et al., 2010
Poxviruses			
E3L zα domain polymerase	DNA	57–177 7–25	Quyen et al., 2007 Hamilton et al., 2007
Influenza viruses			
Nucleoprotein	RNA	23.1 14–47	Ng et al., 2008 Tarus et al., 2012
	DNA	105	
Adenoviruses			
ss DNA binding protein	DNA	6	Dekker et al., 1998
Rep40 protein	DNA	nd	Dignam et al., 2007
HSV			
Uracil-DNA glycosylase helicase-primase	Uracil-DNA	4.6–1493	Panayotou et al., 1998
DNA helicase-primase	DNA	38–88	Chen et al., 2012
HPV			
E7 protein	DNA	180	Chinami et al., 1996

SPR was used to assess the kinetics of interaction between the indicated viral proteins and their target DNA/RNA. nd: not determined.

those constrains that weight instead on envelope proteins, whose mutation is limited by their structural function inside the virion.

As apparent by Table 6, virokines often exploit heparin/HSPGs as receptors. This, together with the fact that HSPGs act as co-receptors for many viruses (Table 2), point to these molecules as key actors in both early and late stages of the virus life cycle, including virus infection and the maintaining of a favorable environment for virus replication and spreading.

Concluding remarks

From the data summarized above, it emerges that SPR has already brought a reasonable contribution to the knowledge in the field of virology. However, a lot more can be done, provided a mandatory improvement of the reproducibility of the kinetic and affinity data generated that, in turn, would allow the scaling up of SPR from scattered analyses to a systematic study functional to the characterization of the virus/host interactome.

Table 6. SPR analysis of the interaction of intact viruses or released viral proteins (i.e. virokines) with host cell surface receptors or extracellular molecules.

Viral protein	Binder	K_D (nM)	Reference
HIV-1			
gp120	Heparin (HSPGs analog)	220	Moulard et al., 2000
		0.6	Bugatti et al., 2007
	τ-defensin retrocyclin-1	33	Gallo et al., 2006
		35.4	Wang et al., 2003
	α-defensin HD5	24.5	Lehrer et al., 2009
Tat	Heparin (HSPGs analog)	20	Urbinati et al., 2004
		64	Rusnati et al., 2001
	VEGFR2/KDR	0.07	Bugatti et al., 2010
	$\alpha_v\beta_3$ integrin	32	Urbinati et al., 2005
		19.9–40.3	Chiodelli et al., 2012
p17	Heparin (HSPGs analog)	190	Bugatti et al., 2013
	Interferon-γ	27.8	Flamminio et al., 1995
	CXCR1	1800	Giagulli et al., 2012
	CXCR2	130	Caccuri et al., 2012
gp41	τ-defensin retrocyclin-1	67.6	Gallo et al., 2006
Poxviruses			
Interferon-α/β-binding protein	Interferon α	0.1–0.6	Fernandez de Marco Mdel et al., 2009
	Interferon β	1.2–5.3	
	IL28A	14.9	
	IL29	13.5	
	Heparin (HSPGs analog)	3.8	Montanuy et al., 2011
Chemokines-binding protein	CCL2	0.4	Lateef et al., 2009
		0.3–1.3	Seet et al., 2001b
		0.3	Beck et al., 2001
	CCL3	0.1	Lateef et al., 2009
	CCL5	0.03	
	CXCL4	2058	Bahar et al., 2008
	CCL25	7.95–24.7	
	CCL26	71–87.2	
	CCL28	35.5–59.5	
	CCL21	116	
	IL-18	3	Meng et al., 2007
	Heparin (HSPGs analog)	446	Seet et al., 2001a
	CCL4,7,8,11,16,17,18	nd	Ng et al., 2001
VV complement control protein	C3b	μM range	Bernet et al., 2004
	C4b	μM range	
	Heparin (HSPGs analog)	230.0	Smith et al., 2003
	C3b, C4b	nd	Ahmad et al., 2010
IL18-binding protein	IL18	5.1	Esteban & Buller, 2004
		2.6	Esteban et al., 2004
		0.4–9.2	Calderara et al., 2001
	Heparin (HSPGs analog)	0.5	Xiang & Moss, 2003
Interferon-γ-binding protein	Interferon-γ	0.09	Symons et al., 2002
Cytokine response modifier B	Tumor necrosis factor	0.3	Alejo et al., 2006
	Lymphotoxin-α	7.5	
	CCL28	0.3	
	CCL25	0.5	
	CXCL12β	4.3	
	CXCL13	5.9	
	CXCL14	6.3	
	CXCL1	28.8	
	CXCL20	29.2	
Variola virus complement inhibitor	C3b, C4b	nd	Yadav et al., 2008

(*continued*)

Table 6. Continued

Viral protein	Binder	K_D (nM)	Reference
HSV			
Glycoprotein G1	CCL18	90.2	Viejo-Borbolla et al., 2012
	CCL22	nd	
	CCL25	4.7	
	CCL26	55	
	CCL28	68	
	CXCL9	38	
	CXCL10	457	
	CXCL11	10.9	
	CXCL12α	31.5	
	CXCL12β	7.7	
	CXCL13	13	
	CXCL14	420	
Glycoprotein G2	CCL18	28	
	CCL22	5.2	
	CCL25	1.6	
	CCL26	1.7	
	CCL28	3.2	
	CXCL9	12.3	
	CXCL10	5.5	
	CXCL11	6	
	CXCL12α	6.5	
	CXCL12β	2.2	
	CXCL13	4.3	
	CXCL14	4.3	
Glycoprotein B	A-defensins	30.3–2880	Yasin et al., 2004
	T-defensin	13.3–295	
Glycoprotein D1	α-defensin	23.5	Lehrer et al., 2009
Cytomegalovirus			
Immunoevasin UL16	MHCI related molecule B	66–68	Muller et al., 2010
Viral Fc receptor gp68	Non-immune IgG (*via* Fc)	60–1600	Sprague et al., 2008
Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8)			
Complement control protein	C3b	120	Mark et al., 2004
		470–10 000	Spiller et al., 2003
	C4b	54.0	Mark et al., 2004
		25–6000	Spiller et al., 2003
viral IL6	gp130	2200	Aoki et al., 2001
Adenoviruses			
Intact virion	Coagulation factor X	1.83	Waddington et al., 2008
Hexon	Coagulation factor IX	3.3–16.7	Johnson et al., 2011
	Coagulation factor X	1.9–19.4	Waddington et al., 2008
		2.7–54	Greig et al., 2009
Hepatitis viruses			
Core protein	Non-immune IgG (*via* Fc)	nd	Maillard et al., 2004
	Apolipoprotein AII	nd	Sabile et al., 1999
Influenza viruses			
A hemagglutinin	Human 1-acid glycoprotein	nd	Mandenius et al., 2008
Rotavirus			
Enterotoxin NSP4	α_1 integrin	1100	Seo et al., 2008
	α_2 integrin	2700	
EBV			
EBV-restricted epitops	T cell receptors	15 000	Gras et al., 2009
		8900	
		8100	Ely et al., 2006
		μM range	Miles et al., 2010
		2200	Gras et al., 2010
EBV-encoded Il-10	IL-10 receptor	121–232	Yoon et al., 2012

HIV gp120/heparin interaction has been here included due to the ''cytokine-like'' activity of the free monomeric gp120. nd: not determined.

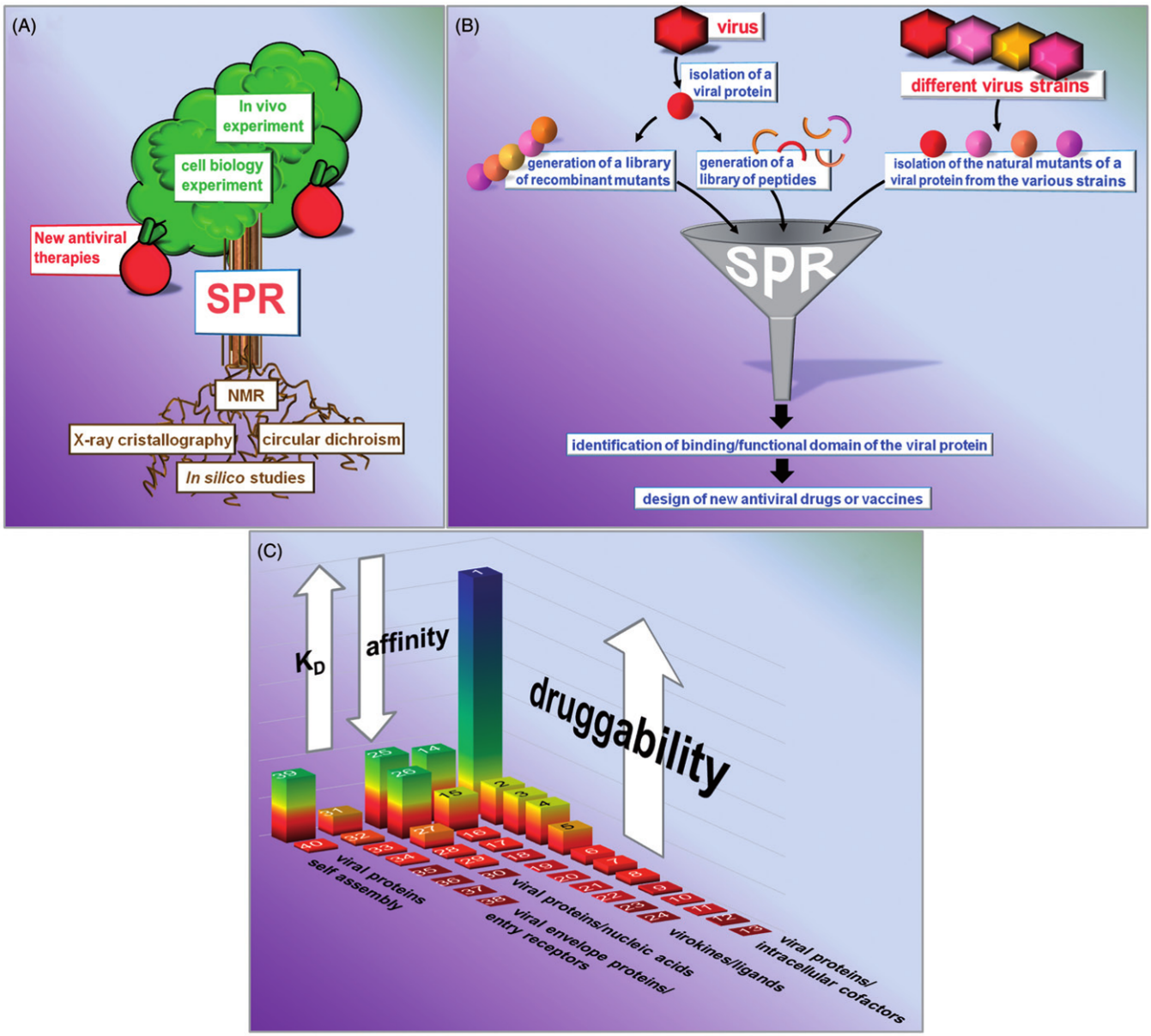

Figure 6. Contribution given by SPR to virology: (A) SPR can be considered as a bridge ideally connecting computational predictions and biochemical studies to *in vitro* and *in vivo* experimentation. (B) SPR can be ideally exploited in the process of identification of bioactive domains of viral proteins, functional to the design of antiviral drugs and vaccines. (C) SPR can be used to rank the interactions occurring between viral and host structures with the aim to identify the most druggable ones: in the representative plot shown here each numbered histogram corresponds to one of the available SPR-generated K_D values of HIV/host interactions grouped in the various categories already used above. A higher K_D value (blue) means a lower affinity interaction and thus a possible higher druggability.

What has been done

SPR has widely been exploited for the characterization of many virus/host interactions and for the identification/development of new antiviral drugs or vaccines (not discussed in this review), providing a steadily increasing flow of data otherwise hardly achievable with classical binding assays based on protein labeling. Other free-label methodologies to study macromolecular interaction are available among which isothermal titration calorimetry (ITC) that, however, in respect to SPR, still presents some disadvantages, including high sample consumption and difficulties in performing kinetic and multiplex analyses. Accordingly, ITC has been exploited in no more than 170 papers dedicated to the characterization of virus/host interactions.

Thus, SPR emerges as an ideal bridge between the structural studies of viruses typically performed *in silico*, by X-ray crystallography, NMR or circular dichroism spectroscopy and the biological studies performed with cell cultures or *in vivo* (Figure 6A). This bridging is well illustrated by the studies aimed at the identification of critical amino acids implicated in the interaction of viral proteins with host receptors, that are almost mandatory to the design of antiviral drugs/vaccines and that usually require the screening of large library of peptides or of recombinant mutants (Figure 6B). Remarkable are the SPR analyses of the interaction of synthetic peptides representing fragments of the HCV p68 protein with the Fc portion of human IgG (Sprague et al., 2008) and of synthetic peptides from the transcription factor VP16 of HSV with the TATA-box binding protein (Nedialkov

& Triezenberg, 2004). Also, a large array of recombinant mutants of HIV integrase have been assayed by SPR for their interaction with DNA (Ramcharan et al., 2006) and the same has been performed for the interaction of HIV Nef mutants with Hck-SH3 (Manninen et al., 1998). Regarding adenoviruses, worth to note is the paper by Alba et al. (2009) in which the adenovirus 5 hexon was point mutated, expressed in intact virions and then assayed for its binding to coagulation factor X. Also, SPR has been instrumental in evaluating the interaction of fiber knob protein mutants with CAR and in the reverse approach, namely the screening of peptides representing the immunoglobulin domain of CAR for their capacity to bind to the fiber knob protein (Kirby et al., 2000).

SPR turned out to be useful also in the study of natural mutants of viral proteins, also burdened by the use of wide arrays of molecules (Figure 6B). Exemplificative in this case is the evaluation of the CD4-binding capacity of gp120 mutants from different HIV strains, that demonstrated how these interactions can occur with variable affinities with important implications in the design of vaccines targeting the gp120/CD4 interaction (Cocklin et al., 2007; Martin-Garcia et al., 2005; Owen et al., 2004; Pashov et al., 2005).

SPR has been instrumental in the identification of highly connected, central host structures that act as ''nodal points'' in the biology of certain viruses, as for HSPGs/heparin that were demonstrated to bind simultaneously different HIV proteins, being thus involved in different phase of the virus life cycle. In effect, SPR helped in demonstrating that HSPGs bind to gp120 both when embedded in the virus envelope (thus mediating virus attachment) and when released in the extracellular environment (thus exerting a cytokine-like activity) (Bugatti et al., 2007). Also, SPR was used to demonstrate that HSPGs/heparin act as receptors for the free form of Tat, mediating several pathological effects in different HIV-non-permissive cells that contribute to AIDS progression and related diseases (Rusnati & Presta, 2002). More recently, SPR was used to characterize the binding of heparin to p17 matrix protein that, once released by HIV-infected cells, binds to HSPGs of different leukocytes inducing their functional subversion (Bugatti et al., 2013). Thus, SPR has been instrumental in the identification of HSPGs as an ideal target to design novel anti-HIV drugs endowed with multi-target activity. Accordingly, SPR contributed to the identification of heparin-like compounds able to effectively inhibit Tat, gp120 and p17 (Bugatti et al., 2007, 2013). A systematic and standardized use of SPR may lead to the identification of additional ''nodal points'' for other viruses, with clear benefits for the comprehension of virus biology and for the therapy of viral diseases.

What must be improved

Looking at the tables, it is apparent that the K_D values calculated by SPR for a given interaction can be very different. Two sets of reasons contribute to this high variability, the first of which is basically technical:

(i) The temperature (Boulter et al., 2007; Ely et al., 2006; Gakamsky et al., 2007; Miles et al., 2010), the pH (Khurana et al., 2010; Pan et al., 2010), the flow rate, the composition of the binding medium (Lortat-Jacob et al., 2001) and the amount of ligand immobilized onto the sensorchip (Dimmock & Hardy, 2004; Zhang & Oglesbee, 2003).

(ii) The alternative use of the two binders as immobilized ligand or as free analyte, that can generate contrasting results: a few papers reported similar binding parameters independent of the binder chosen for immobilization (Chaloin et al., 2005), while usually binding parameters are reported that can be of magnitude of one order different depending on the alternative immobilization of one of the two binders (Bernet et al., 2004; Waddington et al., 2008; Zhao et al., 2005).

(iii) The procedure of immobilization. Remarkable in this case are the works by Khilko (Khilko et al., 1993) and Vaisocherova (Vaisocherova et al., 2007) where peptides from cytomegalovirus or EBV were purposefully immobilized with different procedures and assayed for their capacity to bind specific ligands. Protein immobilization to the sensorchip is often achieved by simple amine-coupling of one of the two binders to the carboxymethyl dextran of the sensorchip. However, this procedure yields a ''random'' protein immobilization, with undesired effects such as the masking of binding sites or the loss of protein folding (Figure 4A). As a consequence, the amount of ligand effectively available for the binding may be quite different from the total amount of RU of protein immobilized onto the surface, hampering an accurate calculation of the stoichiometry of the interaction. As an example, it was demonstrated that only 40% of randomly immobilized VV polymerase VP55 was available for binding to the VP39 regulatory subunit while up to 99.6% of the His-tagged VP55 retained its binding capacity when immobilized by chelation (Gershon & Khilko, 1995). The choice of a proper immobilization is particularly important for host receptors such as seven transmembrane-spanning chemokine receptors (see above) and for viral proteins such as HIV gp41 (Kim et al., 2007; Sun et al., 2008; Veiga et al., 2009), whose stability *in vivo* is ensured by the presence of lipid membranes.

(iv) The fitting model used. All SPR analyzers are provided with software that calculate kinetic parameters on the basis of a default 1:1 model but alternative models fitting multivalent bindings are available that must be taken in consideration when dealing with viral proteins that exist as multimers (i.e. HIV gp120 or adenovirus 2 fiber protein) or with host structures that can accommodate more than one viral protein simultaneously (i.e. proteoglycans) (Greig et al., 2009; Lewallen et al., 2009; Nakajima et al., 2005; Sprague et al., 2008; Yu et al., 2006).

(v) The procedure of calculation of the K_D value. All SPR analyzers are provided with software that directly extrapolates K_D from the k_{off}/k_{on} ratio. Alternatively, K_D can be calculated by the Scatchard plot analysis of equilibrium binding data. When in agreement, the two calculations indicate the good quality of the SPR analysis, but this kind of comparison is seldom used (Liu et al., 2007; Zanier et al., 2005). It must be pointed

out, however, that in some cases this comparison is not possible. If a plateau cannot be reached for each analyte injection, a proper Scatchard plot analysis of equilibrium binding data cannot be performed. Conversely, if binding rates are too fast, only the analysis at the equilibrium can be performed.

The second line of reasons that contribute to the variability of SPR-generated data depends on intrinsic structural and biological features of the viral proteins or of their ligands, and thus it can be a source of knowledge instead that an artifact to be avoided:

(vi) The use of proteins derived from different virus strains, that can be very different at a structural level, thus displaying different binding capacity for their ligands (Chen et al., 2005b; Cocklin et al., 2007; Cupelli et al., 2010; Fu et al., 2011; Greig et al., 2009; Meng et al., 2010; Nyanguile et al., 2010; ter Meulen et al., 2006; Zhou et al., 2008).

(vii) The use of proteins in their glycosilated or deglycosilated forms (i.e. recombinant proteins produced in mammalian cells or bacteria) (Bahar et al., 2008; Dey et al., 2005). In this case, SPR can help in understanding the contribution of sugar chains to interactions.

(viii) The use of viral proteins in monomeric or multimeric forms. This is of importance for those proteins that oligomerize, such as the HIV-1 proteins gp120 (Frey et al., 2008) or gp41 (Liu et al., 2009a), adenovirus 2 fiber protein (Lortat-Jacob et al., 2001), S glycoprotein of coronaviruses (CoV) (Tripet et al., 2006) and the E6 protein of HPV (Zanier et al., 2009).

All the criticalities listed above call for a standardization of the experimental conditions, of the calculation procedures and (whenever possible) of the interactants used. Accordingly, several SPR benchmark studies have been launched with the aim to generate more comparable results (Navratilova et al., 2007). This goal is mandatory for the scaling up of SPR from scattered analyses of virus/host interactions to an ''interactomic'' level, functional to system biology studies.

What can be done

The concept of druggability has been recently arrived to limelight as a parameter to lead drug discovery in the field of virology. Briefly, it consists in the prediction of a putative inhibitor to bind a viral protein or its host ligand preventing their interaction, hence interfering with the virus life cycle. This is classically carried out by screening large library of putative antiviral molecules identifying those endowed with the maximal affinity for the target protein and then by validating this value to predict the actual antiviral efficacy of the drug (Cheng et al., 2007). However, a preliminary screening can be carried out to identify those virus/host interactions that occur with the lowest affinity, thus likely corresponding to those more easily displaceable with specific inhibitors (Seco et al., 2009). Actually, a possible limit of this approach consists of the fact that a low affinity binding may corresponds to a low specific interaction. This calls again for a cautious judgment of SPR data that must be critically pondered together with other parameters such as the abundance, accessibility and biological importance of the viral or host proteins, leading to the identification of suitable therapeutical targets. SPR may be functional to a systematic ranking of the virus/host interactions on the basis of their affinity (Kastritis & Bonvin, 2010) (Figure 6C). Unfortunately, to date such prediction remains outside our reach due to the large variability of the data generated by SPR. However, some guidelines can be drawn from dedicated benchmark studies to improve SPR analysis, making it exploitable for a systematic approach in antiviral drug research.

Another hot topic in antiviral drug discovery is that of ''multitarget'' drugs that, interfering with different viral proteins simultaneously, may limit the rise of drug resistant viral strains that represents to date the major burden of common antiviral mono-therapies (Jenwitheesuk et al., 2008). The development of multitarget drugs requires the identification of hub-proteins playing multiple important roles in virus life cycle, a research appropriately approached by system biology studies. Briefly, system biology is an interdisciplinary experimental and computational field of study that focuses on complex interactions within biological systems with aim to identify novel key features of cell signaling networks. In the last years, it has been widely used in a variety of biomedical contexts, including the deciphering of the network of virus/host interactions (the so call ''interactome'') (Neveu et al., 2012; Sorathiya et al., 2010). The rationale of system biology is that multiple regulatory cascades can be converged into hub-proteins/interactions whose inhibition can affect multiple signaling pathways, commensurate with the administration of multiple drugs that would hopefully cause an overall failure of the ''disease system''. The expectation from such ''connectivity maps'' is to provide better tools for drug discovery, avoiding the low yield, elevated costs, and high risk of failures of traditional, ''monotarget'' drug screening. Although within the limit of the variability of the binding data generated, from what discussed here SPR clearly demonstrated to be a first-choice technology to validate the identification of viral proteins or host receptors that play nodal roles in virus life cycle as well as in the identification of multitarget drugs. Consequently, SPR emerges as a promising tool to efficiently connect system biology studies of virus/host interactome to the discovery of new multitarget antiviral drugs.

Acknowledgements

Largeness of the topic precluded a complete citation of the literature. We apologize with those whose work is not mentioned herein.

Declaration of interest

No financial or personal interest to be disclosed. This work was supported by Istituto Superiore di Sanita' (AIDS Grants # 40H.51) and by the Italian Ministry of University and Scientific Research (MIUR) to M.R.

References

Ahmad M, Raut S, Pyaram K, et al. (2010). Domain swapping reveals complement control protein modules critical for imparting cofactor

and decay-accelerating activities in vaccinia virus complement control protein. J Immunol 185:6128–37.
Alba R, Bradshaw AC, Parker AL, et al. (2009). Identification of coagulation factor (F)X binding sites on the adenovirus serotype 5 hexon: effect of mutagenesis on FX interactions and gene transfer. Blood 114:965–71.
Alejo A, Ruiz-Arguello MB, Ho Y, et al. (2006). A chemokine-binding domain in the tumor necrosis factor receptor from variola (smallpox) virus. Proc Natl Acad Sci U S A 103:5995–6000.
Anraku K, Fukuda R, Takamune N, et al. (2010). Highly sensitive analysis of the interaction between HIV-1 Gag and phosphoinositide derivatives based on surface plasmon resonance. Biochemistry 49: 5109–16.
Aoki Y, Narazaki M, Kishimoto T, Tosato G. (2001). Receptor engagement by viral interleukin-6 encoded by Kaposi sarcoma-associated herpesvirus. Blood 98:3042–9.
Bahar MW, Kenyon JC, Putz MM, et al. (2008). Structure and function of A41, a vaccinia virus chemokine binding protein. PLoS Pathog 4:e5.
Balasubramanian N, Bai P, Buchek G, et al. (2010). Physical interaction between the herpes simplex virus type 1 exonuclease, UL12, and the DNA double-strand break-sensing MRN complex. J Virol 84: 12504–14.
Bamdad C. (1998). The use of variable density self-assembled monolayers to probe the structure of a target molecule. Biophys J 75: 1989–96.
Baritaki S, Zafiropoulos A, Sioumpara M, et al. (2002). Ionic interaction of the HIV-1 V3 domain with CCR5 and deregulation of T lymphocyte function. Biochem Biophys Res Commun 298:574–80.
Barth H, Schafer C, Adah MI, et al. (2003). Cellular binding of hepatitis C virus envelope glycoprotein E2 requires cell surface heparan sulfate. J Biol Chem 278:41003–12.
Barth H, Schnober EK, Zhang F, et al. (2006). Viral and cellular determinants of the hepatitis C virus envelope-heparan sulfate interaction. J Virol 80:10579–90.
Barton ES, Connolly JL, Forrest JC, et al. (2001). Utilization of sialic acid as a coreceptor enhances reovirus attachment by multistep adhesion strengthening. J Biol Chem 276:2200–11.
Beck CG, Studer C, Zuber JF, et al. (2001). The viral CC chemokine-binding protein vCCI inhibits monocyte chemoattractant protein-1 activity by masking its CCR2B-binding site. J Biol Chem 276: 43270–6.
Beerheide W, Bernard HU, Tan YJ, et al. (1999). Potential drugs against cervical cancer: zinc-ejecting inhibitors of the human papillomavirus type 16 E6 oncoprotein. J Natl Cancer Inst 91:1211–20.
Bernet J, Mullick J, Panse Y, et al. (2004). Kinetic analysis of the interactions between vaccinia virus complement control protein and human complement proteins C3b and C4b. J Virol 78:9446–57.
Bieniasz PD. (2009). The cell biology of HIV-1 virion genesis. Cell Host Microbe 5:550–8.
Biorn AC, Cocklin S, Madani N, et al. (2004). Mode of action for linear peptide inhibitors of HIV-1 gp120 interactions. Biochemistry 43: 1928–38.
Birkmann A, Mahr K, Ensser A, et al. (2001). Cell surface heparan sulfate is a receptor for human herpesvirus 8 and interacts with envelope glycoprotein K8.1. J Virol 75:11583–93.
Bobardt MD, Saphire AC, Hung HC, et al. (2003). Syndecan captures, protects, and transmits HIV to T lymphocytes. Immunity 18:27–39.
Boivin S, Hart DJ. (2011). Interaction of the influenza A virus polymerase PB2 C-terminal region with importin {alpha} isoforms provides insights into host adaptation and polymerase assembly. J Biol Chem 286:10439–48.
Boulter JM, Schmitz N, Sewell AK, et al. (2007). Potent T cell agonism mediated by a very rapid TCR/pMHC interaction. Eur J Immunol 37: 798–806.
Bourhis JM, Receveur-Brechot V, Oglesbee M, et al. (2005). The intrinsically disordered C-terminal domain of the measles virus nucleoprotein interacts with the C-terminal domain of the phosphoprotein via two distinct sites and remains predominantly unfolded. Protein Sci 14:1975–92.
Bowden TA, Yvonne Jones E, Stuart DI. (2011). Cells under siege: viral glycoprotein interactions at the cell surface. J Struct Biol 175:120–6.
Brown JR, Magid-Slav M, Sanseau P, Rajpal DK. (2011). Computational biology approaches for selecting host-pathogen drug targets. Drug Discov Today 16:229–36.
Bugatti A, Chiodelli P, Rosenbluh J, et al. (2010). BSA conjugates bearing multiple copies of the basic domain of HIV-1 Tat: prototype for the development of multitarget inhibitors of extracellular Tat. Antiviral Res 87:30–9.
Bugatti A, Giagulli C, Urbinati C, et al. (2013). Molecular interaction studies of HIV-1 matrix protein p17 and heparin: identification of the heparin-binding motif of p17 as a target for the development of multitarget antagonists. J Biol Chem 288:1150–61.
Bugatti A, Urbinati C, Ravelli C, et al. (2007). Heparin-mimicking sulfonic acid polymers as multitarget inhibitors of human immunodeficiency virus type 1 Tat and gp120 proteins. Antimicrob Agents Chemother 51:2337–45.
Caccuri F, Giagulli C, Bugatti A, et al. (2012). HIV-1 matrix protein p17 promotes angiogenesis via chemokine receptors CXCR1 and CXCR2. Proc Natl Acad Sci U S A 109: 14580–5.
Calderara S, Xiang Y, Moss B. (2001). Orthopoxvirus IL-18 binding proteins: affinities and antagonist activities. Virology 279:22–6.
Casasnovas JM, Springer TA. (1995). Kinetics and thermodynamics of virus binding to receptor. Studies with rhinovirus, intercellular adhesion molecule-1 (ICAM-1), and surface plasmon resonance. J Biol Chem 270:13216–24.
Chaloin O, Peter JC, Briand JP, et al. (2005). The N-terminus of HIV-1 Tat protein is essential for Tat-TAR RNA interaction. Cell Mol Life Sci 62:355–61.
Chaudhari A, Fialho AM, Ratner D, et al. (2006). Azurin, Plasmodium falciparum malaria and HIV/AIDS: inhibition of parasitic and viral growth by Azurin. Cell Cycle 5:1642–8.
Chen H, Gill A, Dove BK, et al. (2005a). Mass spectroscopic characterization of the coronavirus infectious bronchitis virus nucleoprotein and elucidation of the role of phosphorylation in RNA binding by using surface plasmon resonance. J Virol 79:1164–79.
Chen L, Gui C, Luo X, et al. (2005b). Cinanserin is an inhibitor of the 3C-like proteinase of severe acute respiratory syndrome coronavirus and strongly reduces virus replication in vitro. J Virol 79:7095–103.
Chen Y, Bai P, Mackay S, et al. (2012). Herpes simplex virus type 1 helicase-primase: DNA binding and consequent protein oligomerization and primase activation. J Virol 85:968–78.
Chen Z, Mi L, Xu J, et al. (2005c). Function of HAb18G/CD147 in invasion of host cells by severe acute respiratory syndrome coronavirus. J Infect Dis 191:755–60.
Cheng AC, Coleman RG, Smyth KT, et al. (2007). Structure-based maximal affinity model predicts small-molecule druggability. Nat Biotechnol 25:71–5.
Chesnokova LS, Hutt-Fletcher LM. (2011). Fusion of Epstein-Barr virus with epithelial cells can be triggered by alphavbeta5 in addition to alphavbeta6 and alphavbeta8, and integrin binding triggers a conformational change in glycoproteins gHgL. J Virol 85:13214–23.
Chien MP, Jiang S, Chang DK. (2008). The function of coreceptor as a basis for the kinetic dissection of HIV type 1 envelope protein-mediated cell fusion. FASEB J 22:1179–92.
Chinami M, Inoue M, Masunaga K, et al. (1996). Nucleic acid binding by zinc finger-like motif of human papillomavirus type 16 E7 oncoprotein. J Virol Methods 59:173–6.
Chiodelli P, Urbinati C, Mitola S, et al. (2012). Sialic acid associated with alphavbeta3 integrin mediates HIV-1 Tat protein interaction and endothelial cell proangiogenic activation. J Biol Chem 287:20456–66.
Chiorini JA, Zimmermann B, Yang L, et al. (1998). Inhibition of PrKX, a novel protein kinase, and the cyclic AMP-dependent protein kinase PKA by the regulatory proteins of adeno-associated virus type 2. Mol Cell Biol 18:5921–9.
Choi AG, Wong J, Marchant D, Luo H. (2012). The ubiquitin-proteasome system in positive-strand RNA virus infection. Rev Med Virol 23:85–96.
Choi KJ, Lim CW, Yoon MY, et al. (2004). Quantitative analysis of the interaction between the envelope protein domains and the core protein of human hepatitis B virus. Biochem Biophys Res Commun 319: 959–66.
Cocklin S, Gopi H, Querido B, et al. (2007). Broad-spectrum anti-human immunodeficiency virus (HIV) potential of a peptide HIV type 1 entry inhibitor. J Virol 81:3645–8.
Corjon S, Gonzalez G, Henning P, et al. (2011). Cell entry and trafficking of human adenovirus bound to blood factor X is determined by the fiber serotype and not hexon:heparan sulfate interaction. PLoS One 6:e18205.

Critchley P, Dimmock NJ. (2004). Binding of an influenza A virus to a neomembrane measured by surface plasmon resonance. Bioorg Med Chem 12:2773–80.
Crublet E, Andrieu JP, Vives RR, Lortat-Jacob H. (2008). The HIV-1 envelope glycoprotein gp120 features four heparan sulfate binding domains, including the co-receptor binding site. J Biol Chem 283: 15193–200.
Cupelli K, Muller S, Persson BD, et al. (2010). Structure of adenovirus type 21 knob in complex with CD46 reveals key differences in receptor contacts among species B adenoviruses. J Virol 84: 3189–200.
Dekker J, Kanellopoulos PN, van Oosterhout JA, et al. (1998). ATP-independent DNA unwinding by the adenovirus single-stranded DNA binding protein requires a flexible DNA binding loop. J Mol Biol 277: 825–38.
Dey AK, Khati M, Tang M, et al. (2005). An aptamer that neutralizes R5 strains of human immunodeficiency virus type 1 blocks gp120-CCR5 interaction. J Virol 79:13806–10.
Dey B, Svehla K, Xu L, et al. (2009). Structure-based stabilization of HIV-1 gp120 enhances humoral immune responses to the induced co-receptor binding site. PLoS Pathog 5:e1000445.
Dignam SS, Collaco RF, Bieszczad J, et al. (2007). Coupled ATP and DNA binding of adeno-associated virus Rep40 helicase. Biochemistry 46:568–76.
Dimmock NJ, Hardy SA. (2004). Valency of antibody binding to virions and its determination by surface plasmon resonance. Rev Med Virol 14:123–35.
Ely LK, Beddoe T, Clements CS, et al. (2006). Disparate thermodynamics governing T cell receptor-MHC-I interactions implicate extrinsic factors in guiding MHC restriction. Proc Natl Acad Sci U S A 103:6641–6.
Engelhardt OG, Fodor E. (2006). Functional association between viral and cellular transcription during influenza virus infection. Rev Med Virol 16:329–45.
Esteban DJ, Buller RM. (2004). Identification of residues in an orthopoxvirus interleukin-18 binding protein involved in ligand binding and species specificity. Virology 323:197–207.
Esteban DJ, Nuara AA, Buller RM. (2004). Interleukin-18 and glycosaminoglycan binding by a protein encoded by Variola virus. J Gen Virol 85:1291–9.
Falkenberg M, Bushnell DA, Elias P, Lehman IR. (1997). The UL8 subunit of the heterotrimeric herpes simplex virus type 1 helicase-primase is required for the unwinding of single strand DNA-binding protein (ICP8)-coated DNA substrates. J Biol Chem 272: 22766–70.
Feng Y, McKee K, Tran K, et al. (2011). Biochemically defined HIV-1 envelope glycoprotein variant immunogens display differential binding and neutralizing specificities to the CD4-binding site. J Biol Chem 287:5673–86.
Fernandez de Marco Mdel M, Alejo A, Hudson P, et al. (2009). The highly virulent variola and monkeypox viruses express secreted inhibitors of type I interferon. FASEB J 24:1479–88.
Ferreira ME, Hermann S, Prochasson P, et al. (2005). Mechanism of transcription factor recruitment by acidic activators. J Biol Chem 280: 21779–84.
Ferrer M, Sullivan BJ, Godbout KL, et al. (1999). Structural and functional characterization of an epitope in the conserved C-terminal region of HIV-1 gp120. J Pept Res 54:32–42.
Fiorentini S, Marini E, Caracciolo S, Caruso A. (2006). Functions of the HIV-1 matrix protein p17. New Microbiol 29:1–10.
Flamminio G, Caruso A, Poiesi C, et al. (1995). Aspects of molecular interaction between HIV p17 and human gamma interferon. AIDS Res Hum Retroviruses 11:1441–7.
Fournane S, Charbonnier S, Chapelle A, et al. (2010). Surface plasmon resonance analysis of the binding of high-risk mucosal HPV E6 oncoproteins to the PDZ1 domain of the tight junction protein MAGI-1. J Mol Recognit 24:511–23.
Frey G, Peng H, Rits-Volloch S, et al. (2008). A fusion-intermediate state of HIV-1 gp41 targeted by broadly neutralizing antibodies. Proc Natl Acad Sci U S A 105:3739–44.
Fu J, Li L, Bouvier M. (2011). Adenovirus E3–19K proteins of different serotypes and subgroups have similar, yet distinct, immunomodulatory functions toward major histocompatibility class I molecules. J Biol Chem 286:17631–9.
Gakamsky DM, Lewitzki E, Grell E, et al. (2007). Kinetic evidence for a ligand-binding-induced conformational transition in the T cell receptor. Proc Natl Acad Sci U S A 104:16639–44.
Galdiero S, Falanga A, Vitiello G, et al. (2010). Role of membranotropic sequences from herpes simplex virus type I glycoproteins B and H in the fusion process. Biochim Biophys Acta 1798:579–91.
Galinier R, Gout E, Lortat-Jacob H, et al. (2002). Adenovirus protein involved in virus internalization recruits ubiquitin-protein ligases. Biochemistry 41:14299–305.
Gallay P. (2004). Syndecans and HIV-1 pathogenesis. Microbes Infect 6: 617–22.
Gallego J, Greatorex J, Zhang H, et al. (2003). Rev binds specifically to a purine loop in the SL1 region of the HIV-1 leader RNA. J Biol Chem 278:40385–91.
Gallo M, Bottomley MJ, Pennestri M, et al. (2010). Structural characterization of the Hepatitis C Virus NS3 protease from genotype 3a: the basis of the genotype 1b vs. 3a inhibitor potency shift. Virology 405:424–38.
Gallo SA, Wang W, Rawat SS, et al. (2006). Theta-defensins prevent HIV-1 Env-mediated fusion by binding gp41 and blocking 6-helix bundle formation. J Biol Chem 281:18787–92.
Garrus JE, von Schwedler UK, Pornillos OW, et al. (2001). Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. Cell 107:55–65.
Gershon PD, Khilko S. (1995). Stable chelating linkage for reversible immobilization of oligohistidine tagged proteins in the BIAcore surface plasmon resonance detector. J Immunol Methods 183: 65–76.
Ghanam RH, Samal AB, Fernandez TF, Saad JS. (2012). Role of the HIV-1 matrix protein in Gag intracellular trafficking and targeting to the plasma membrane for virus assembly. Front Microbiol 3:55.
Giagulli C, Magiera AK, Bugatti A, et al. (2012). HIV-1 matrix protein p17 binds to the IL-8 receptor CXCR1 and shows IL-8-like chemokine activity on monocytes through Rho/ROCK activation. Blood 119: 2274–83.
Gorshkova, II, Rausch JW, Le Grice SF, Crouch RJ. (2001). HIV-1 reverse transcriptase interaction with model RNA-DNA duplexes. Anal Biochem 291:198–206.
Gras S, Burrows SR, Kjer-Nielsen L, et al. (2009). The shaping of T cell receptor recognition by self-tolerance. Immunity 30:193–203.
Gras S, Chen Z, Miles JJ, et al. (2010). Allelic polymorphism in the T cell receptor and its impact on immune responses. J Exp Med 207: 1555–67.
Greco-Stewart V, Pelchat M. (2010). Interaction of host cellular proteins with components of the hepatitis delta virus. Viruses 2:189–212.
Greig JA, Buckley SM, Waddington SN, et al. (2009). Influence of coagulation factor x on in vitro and in vivo gene delivery by adenovirus (Ad) 5, Ad35, and chimeric Ad5/Ad35 vectors. Mol Ther 17:1683–91.
Guglielmi KM, Kirchner E, Holm GH, et al. (2007). Reovirus binding determinants in junctional adhesion molecule-A. J Biol Chem 282: 17930–40.
Hamilton MD, Nuara AA, Gammon DB, et al. (2007). Duplex strand joining reactions catalyzed by vaccinia virus DNA polymerase. Nucleic Acids Res 35:143–51.
He N, Liu M, Hsu J, et al. (2010). HIV-1 Tat and host AFF4 recruit two transcription elongation factors into a bifunctional complex for coordinated activation of HIV-1 transcription. Mol Cell 38:428–38.
Herschhorn A, Oz-Gleenberg I, Hizi A. (2008a). Mechanism of inhibition of HIV-1 reverse transcriptase by the novel broad-range DNA polymerase inhibitor N-{2-[4-(aminosulfonyl)phenyl]ethyl}-2-(2-thienyl)acetamide. Biochemistry 47:490–502.
Herschhorn A, Oz-Gleenberg I, Hizi A. (2008b). Quantitative analysis of the interactions between HIV-1 integrase and retroviral reverse transcriptases. Biochem J 412: 163–70.
Hidari KI, Shimada S, Suzuki Y, Suzuki T. (2007). Binding kinetics of influenza viruses to sialic acid-containing carbohydrates. Glycoconj J 24:583–90.
Hijazi K, Wang Y, Scala C, et al. (2011). DC-SIGN increases the affinity of HIV-1 envelope glycoprotein interaction with CD4. PLoS One 6: e28307.
Hilgendorf A, Lindberg J, Ruzsics Z, et al. (2003). Two distinct transport motifs in the adenovirus E3/10.4–14.5 proteins act in concert to down-modulate apoptosis receptors and the epidermal growth factor receptor. J Biol Chem 278:51872–84.

Ho Y, Hsiao JC, Yang MH, et al. (2005). The oligomeric structure of vaccinia viral envelope protein A27L is essential for binding to heparin and heparan sulfates on cell surfaces: a structural and functional approach using site-specific mutagenesis. J Mol Biol 349:1060–71.

Hsieh MJ, White PJ, Pouton CW. (2010). Interaction of viruses with host cell molecular motors. Curr Opin Biotechnol 21:633–9.

Huang CY, Hsu YL, Chiang WL, Hou MH. (2009). Elucidation of the stability and functional regions of the human coronavirus OC43 nucleocapsid protein. Protein Sci 18:2209–18.

Hyser JM, Zeng CQ, Beharry Z, et al. (2008). Epitope mapping and use of epitope-specific antisera to characterize the VP5* binding site in rotavirus SA11 NSP4. Virology 373:211–28.

Jacotot E, Ferri KF, El Hamel C, et al. (2001). Control of mitochondrial membrane permeabilization by adenine nucleotide translocator interacting with HIV-1 viral protein rR and Bcl-2. J Exp Med 193: 509–19.

Jennings TA, Chen Y, Sikora D, et al. (2008). RNA unwinding activity of the hepatitis C virus NS3 helicase is modulated by the NS5B polymerase. Biochemistry 47:1126–35.

Jenwitheesuk E, Horst JA, Rivas KL, et al. (2008). Novel paradigms for drug discovery: computational multitarget screening. Trends Pharmacol Sci 29:62–71.

Jo BB, Jeong MS, Park SY, et al. (2011). The binding of hepatitis B virus X protein to glioma-associated oncogene homologue 1 and its biological characterization in vitro. Appl Biochem Biotechnol 165: 109–22.

Johnson TR, McLellan JS, Graham BS. (2011). Respiratory syncytial virus glycoprotein G interacts with DC-SIGN and L-SIGN to activate ERK1 and ERK2. J Virol 86:1339–47.

Jung SO, Ro HS, Kho BH, et al. (2005). Surface plasmon resonance imaging-based protein arrays for high-throughput screening of protein-protein interaction inhibitors. Proteomics 5:4427–31.

Kadaveru K, Vyas J, Schiller MR. (2008). Viral infection and human disease–insights from minimotifs. Front Biosci 13:6455–71.

Kang H, Yu J, Jung G. (2008). Phosphorylation of hepatitis B virus core C-terminally truncated protein (Cp149) by PKC increases capsid assembly and stability. Biochem J 416:47–54.

Kashuba E, Yurchenko M, Yenamandra SP, et al. (2010). Epstein-Barr virus-encoded EBNA-5 forms trimolecular protein complexes with MDM2 and p53 and inhibits the transactivating function of p53. Int J Cancer 128:817–25.

Kastritis PL, Bonvin AM. (2010). Are scoring functions in protein-protein docking ready to predict interactomes? Clues from a novel binding affinity benchmark. J Proteome Res 9:2216–25.

Kaufmann B, Baxa U, Chipman PR, et al. (2005). Parvovirus B19 does not bind to membrane-associated globoside in vitro. Virology 332: 189–98.

Khilko SN, Corr M, Boyd LF, et al. (1993). Direct detection of major histocompatibility complex class I binding to antigenic peptides using surface plasmon resonance. Peptide immobilization and characterization of binding specificity. J Biol Chem 268: 15425–34.

Khurana S, Chearwae W, Castellino F, et al. (2010). Vaccines with MF59 adjuvant expand the antibody repertoire to target protective sites of pandemic avian H5N1 influenza virus. Sci Transl Med 2: 15ra5.

Kim M, Qiao Z, Yu J, et al. (2007). Immunogenicity of recombinant human immunodeficiency virus type 1-like particles expressing gp41 derivatives in a pre-fusion state. Vaccine 25:5102–14.

Kim MY, Jeong S. (2003). RNA aptamers that bind the nucleocapsid protein contain pseudoknots. Mol Cells 16:413–17.

Kim SJ, Kim MY, Lee JH, et al. (2002). Selection and stabilization of the RNA aptamers against the human immunodeficiency virus type-1 nucleocapsid protein. Biochem Biophys Res Commun 291: 925–31.

Kirby I, Davison E, Beavil AJ, et al. (2000). Identification of contact residues and definition of the CAR-binding site of adenovirus type 5 fiber protein. J Virol 74:2804–13.

Kirby I, Lord R, Davison E, et al. (2001). Adenovirus type 9 fiber knob binds to the coxsackie B virus-adenovirus receptor (CAR) with lower affinity than fiber knobs of other CAR-binding adenovirus serotypes. J Virol 75:7210–14.

Krishnan L, Matreyek KA, Oztop I, et al. (2010). The requirement for cellular transportin 3 (TNPO3 or TRN-SR2) during infection maps to human immunodeficiency virus type 1 capsid and not integrase. J Virol 84:397–406.

Lam SN, Acharya P, Wyatt R, et al. (2008). Tyrosine-sulfate isosteres of CCR5 N-terminus as tools for studying HIV-1 entry. Bioorg Med Chem 16:10113–20.

Lateef Z, Baird MA, Wise LM, et al. (2009). Orf virus-encoded chemokine-binding protein is a potent inhibitor of inflammatory monocyte recruitment in a mouse skin model. J Gen Virol 90:1477–82.

Lea SM, Powell RM, McKee T, et al. (1998). Determination of the affinity and kinetic constants for the interaction between the human virus echovirus 11 and its cellular receptor, CD55. J Biol Chem 273: 30443–7.

Leavitt SA, Schon A, Klein JC, et al. (2004). Interactions of HIV-1 proteins gp120 and Nef with cellular partners define a novel allosteric paradigm. Curr Protein Pept Sci 5:1–8.

Lee SJ, Shim HY, Hsieh A, et al. (2009). Hepatitis B virus core interacts with the host cell nucleolar protein, nucleophosmin 1. J Microbiol 47: 746–52.

Lehrer RI, Jung G, Ruchala P, et al. (2009). Multivalent binding of carbohydrates by the human alpha-defensin, HD5. J Immunol 183: 480–90.

Lembo D, Donalisio M, Rusnati M, et al. (2008). Sulfated K5 *Escherichia coli* polysaccharide derivatives as wide-range inhibitors of genital types of human papillomavirus. Antimicrob Agents Chemother 52:1374–81.

Lewallen DM, Siler D, Iyer SS. (2009). Factors affecting protein-glycan specificity: effect of spacers and incubation time. Chembiochem 10: 1486–9.

Lim CS, Seet BT, Ingham RJ, et al. (2007). The K15 protein of Kaposi's sarcoma-associated herpesvirus recruits the endocytic regulator intersectin 2 through a selective SH3 domain interaction. Biochemistry 46:9874–85.

Lin S, Long S, Ramirez SM, et al. (2000). Characterization of the ''helix clamp'' motif of HIV-1 reverse transcriptase using MALDI-TOF MS and surface plasmon resonance. Anal Chem 72:2635–40.

Lindahl U, Lidholt K, Spillmann D, Kjellen L. (1994). More to ''heparin'' than anticoagulation. Thromb Res 75:1–32.

Lippe R. (2012). Deciphering novel host-herpesvirus interactions by virion proteomics. Front Microbiol 3:181.

Liu H, Fu J, Bouvier M. (2007). Allele- and locus-specific recognition of class I MHC molecules by the immunomodulatory E3–19K protein from adenovirus. J Immunol 178:4567–75.

Liu J, Deng Y, Dey AK, et al. (2009a). Structure of the HIV-1 gp41 membrane-proximal ectodomain region in a putative prefusion conformation. Biochemistry 48:2915–23.

Liu Y, Cherry JJ, Dineen JV, et al. (2009b). Determinants of stability for the E6 protein of papillomavirus type 16. J Mol Biol 386:1123–37.

Lord R, Parsons M, Kirby I, et al. (2006). Analysis of the interaction between RGD-expressing adenovirus type 5 fiber knob domains and alphavbeta3 integrin reveals distinct binding profiles and intracellular trafficking. J Gen Virol 87:2497–505.

Lortat-Jacob H, Chouin E, Cusack S, van Raaij MJ. (2001). Kinetic analysis of adenovirus fiber binding to its receptor reveals an avidity mechanism for trimeric receptor-ligand interactions. J Biol Chem 276: 9009–15.

Luo C, Luo H, Zheng S, et al. (2004a). Nucleocapsid protein of SARS coronavirus tightly binds to human cyclophilin A. Biochem Biophys Res Commun 321:557–65.

Luo H, Chen Q, Chen J, et al. (2005). The nucleocapsid protein of SARS coronavirus has a high binding affinity to the human cellular heterogeneous nuclear ribonucleoprotein A1. FEBS Lett 579:2623–8.

Luo H, Wu D, Shen C, et al. (2006). Severe acute respiratory syndrome coronavirus membrane protein interacts with nucleocapsid protein mostly through their carboxyl termini by electrostatic attraction. Int J Biochem Cell Biol 38:589–99.

Luo H, Ye F, Sun T, et al. (2004b). In vitro biochemical and thermodynamic characterization of nucleocapsid protein of SARS. Biophys Chem 112:15–25.

Maillard P, Lavergne JP, Siberil S, et al. (2004). Fcgamma receptor-like activity of hepatitis C virus core protein. J Biol Chem 279:2430–7.

Majka J, Speck C. (2007). Analysis of protein-DNA interactions using surface plasmon resonance. Adv Biochem Eng Biotechnol 104:13–36.

Majumdar A, Bhattacharya R, Basak S, et al. (2004). P-protein of Chandipura virus is an N-protein-specific chaperone that acts at the nucleation stage. Biochemistry 43:2863–70.

Mamikonyan G, Kiyatkin A, Movsesyan N, et al. (2008). Detection of the active components of calf thymus nuclear proteins (TNP), histones that are binding with high affinity to HIV-1 envelope proteins and CD4 molecules. Curr HIV Res 6:318–26.
Mandenius CF, Wang R, Alden A, et al. (2008). Monitoring of influenza virus hemagglutinin in process samples using weak affinity ligands and surface plasmon resonance. Anal Chim Acta 623:66–75.
Manninen A, Hiipakka M, Vihinen M, et al. (1998). SH3-Domain binding function of HIV-1 Nef is required for association with a PAK-related kinase. Virology 250:273–82.
Mark L, Lee WH, Spiller OB, et al. (2004). The Kaposi's sarcoma-associated herpesvirus complement control protein mimics human molecular mechanisms for inhibition of the complement system. J Biol Chem 279:45093–101.
Marks RM, Lu H, Sundaresan R, et al. (2001). Probing the interaction of dengue virus envelope protein with heparin: assessment of glycosaminoglycan-derived inhibitors. J Med Chem 44:2178–87.
Martin-Garcia J, Cocklin S, Chaiken IM, Gonzalez-Scarano F. (2005). Interaction with CD4 and antibodies to CD4-induced epitopes of the envelope gp120 from a microglial cell-adapted human immunodeficiency virus type 1 isolate. J Virol 79:6703–13.
Matsubara M, Jing T, Kawamura K, et al. (2005). Myristoyl moiety of HIV Nef is involved in regulation of the interaction with calmodulin in vivo. Protein Sci 14:494–503.
McDermott Jr BM, Rux AH, Eisenberg RJ, et al. (2000). Two distinct binding affinities of poliovirus for its cellular receptor. J Biol Chem 275:23089–96.
Meng B, Marriott AC, Dimmock NJ. (2010). The receptor preference of influenza viruses. Influenza Other Respi Viruses 4:147–53.
Meng X, Leman M, Xiang Y. (2007). Variola virus IL-18 binding protein interacts with three human IL-18 residues that are part of a binding site for human IL-18 receptor alpha subunit. Virology 358:211–20.
Miles JJ, Bulek AM, Cole DK, et al. (2010). Genetic and structural basis for selection of a ubiquitous T cell receptor deployed in Epstein-Barr virus infection. PLoS Pathog 6:e1001198.
Montanuy I, Alejo A, Alcami A. (2011). Glycosaminoglycans mediate retention of the poxvirus type I interferon binding protein at the cell surface to locally block interferon antiviral responses. FASEB J 26: 1960–71.
Moriishi K, Matsuura Y. (2012). Exploitation of lipid components by viral and host proteins for hepatitis C virus infection. Front Microbiol 3:54.
Moulard M, Lortat-Jacob H, Mondor I, et al. (2000). Selective interactions of polyanions with basic surfaces on human immunodeficiency virus type 1 gp120. J Virol 74:1948–60.
Muller S, Zocher G, Steinle A, Stehle T. (2010). Structure of the HCMV UL16-MICB complex elucidates select binding of a viral immunoevasin to diverse NKG2D ligands. PLoS Pathog 6:e1000723.
Munshi UM, Kim J, Nagashima K, et al. (2007). An Alix fragment potently inhibits HIV-1 budding: characterization of binding to retroviral YPXL late domains. J Biol Chem 282:3847–55.
Myszka DG, Sweet RW, Hensley P, et al. (2000). Energetics of the HIV gp120-CD4 binding reaction. Proc Natl Acad Sci U S A 97:9026–31.
Nakajima H, Cocquerel L, Kiyokawa N, et al. (2005). Kinetics of HCV envelope proteins' interaction with CD81 large extracellular loop. Biochem Biophys Res Commun 328:1091–100.
Navaratnarajah CK, Vongpunsawad S, Oezguen N, et al. (2008). Dynamic interaction of the measles virus hemagglutinin with its receptor signaling lymphocytic activation molecule (SLAM, CD150). J Biol Chem 283:11763–71.
Navratilova I, Macdonald G, Robinson C, et al. (2012). Biosensor-based approach to the identification of protein kinase ligands with dual-site modes of action. J Biomol Screen 17:183–93.
Navratilova I, Papalia GA, Rich RL, et al. (2007). Thermodynamic benchmark study using Biacore technology. Anal Biochem 364: 67–77.
Navratilova I, Sodroski J, Myszka DG. (2005). Solubilization, stabilization, and purification of chemokine receptors using biosensor technology. Anal Biochem 339:271–81.
Nedialkov YA, Shooltz DD, Triezenberg SJ. (2003). Purification and protein interaction assays of the VP16C transcription activation domain. Methods Enzymol 370:522–35.
Nedialkov YA, Triezenberg SJ. (2004). Quantitative assessment of in vitro interactions implicates TATA-binding protein as a target of the VP16C transcriptional activation region. Arch Biochem Biophys 425: 77–86.
Negrete OA, Wolf MC, Aguilar HC, et al. (2006). Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. PLoS Pathog 2:e7.
Neveu G, Cassonnet P, Vidalain PO, et al. (2012). Comparative analysis of virus-host interactomes with a mammalian high-throughput protein complementation assay based on *Gaussia princeps* luciferase. Methods 58:349–59.
Ng A, Tscharke DC, Reading PC, Smith GL. (2001). The vaccinia virus A41L protein is a soluble 30 kDa glycoprotein that affects virus virulence. J Gen Virol 82:2095–105.
Ng AK, Chan WH, Choi ST, et al. (2012). Influenza polymerase activity correlates with the strength of interaction between nucleoprotein and PB2 through the host-specific residue K/E627. PLoS One 7: e36415.
Ng AK, Zhang H, Tan K, et al. (2008). Structure of the influenza virus A H5N1 nucleoprotein: implications for RNA binding, oligomerization, and vaccine design. FASEB J 22:3638–47.
Nilsson EC, Storm RJ, Bauer J, et al. (2010). The GD1a glycan is a cellular receptor for adenoviruses causing epidemic keratoconjunctivitis. Nat Med 17:105–9.
Nonaka M, Ma BY, Ohtani M, et al. (2007). Subcellular localization and physiological significance of intracellular mannan-binding protein. J Biol Chem 282:17908–20.
Nowak SA, Chou T. (2009). Mechanisms of receptor/coreceptor-mediated entry of enveloped viruses. Biophys J 96:2624–36.
Nyanguile O, Devogelaere B, Vijgen L, et al. (2010). 1a/1b subtype profiling of nonnucleoside polymerase inhibitors of hepatitis C virus. J Virol 84:2923–34.
Oda M, Nakamura H. (2000). Thermodynamic and kinetic analyses for understanding sequence-specific DNA recognition. Genes Cells 5: 319–26.
Oda S, Franklin E, Khan AR. (2011). Poxvirus A46 protein binds to TIR domain-containing Mal/TIRAP via an alpha-helical sub-domain. Mol Immunol 48:2144–50.
Okamoto T, Omori H, Kaname Y, et al. (2008). A single-amino-acid mutation in hepatitis C virus NS5A disrupting FKBP8 interaction impairs viral replication. J Virol 82:3480–9.
Okumoto Y, Ohmichi T, Sugimoto N. (2002). Immobilized small deoxyribozyme to distinguish RNA secondary structures. Biochemistry 41:2769–73.
Ou HD, May AP, O'Shea CC. (2010). The critical protein interactions and structures that elicit growth deregulation in cancer and viral replication. Wiley Interdiscip Rev Syst Biol Med 3:48–73.
Owen SM, Rudolph DL, Wang W, et al. (2004). RC-101, a retrocyclin-1 analogue with enhanced activity against primary HIV type 1 isolates. AIDS Res Hum Retroviruses 20:1157–65.
Paal K, Baeuerle PA, Schmitz ML. (1997). Basal transcription factors TBP and TFIIB and the viral coactivator E1A 13S bind with distinct affinities and kinetics to the transactivation domain of NF-kappaB p65. Nucleic Acids Res 25:1050–5.
Pan YS, Wei HJ, Chang CC, et al. (2010). Construction and characterization of insect cell-derived influenza VLP: cell binding, fusion, and EGFP incorporation. J Biomed Biotechnol 2010:506363.
Panayotou G, Brown T, Barlow T, et al. (1998). Direct measurement of the substrate preference of uracil-DNA glycosylase. J Biol Chem 273: 45–50.
Partidos CD, Hoebeke J, Moreau E, et al. (2005). The binding affinity of double-stranded RNA motifs to HIV-1 Tat protein affects transactivation and the neutralizing capacity of anti-Tat antibodies elicited after intranasal immunization. Eur J Immunol 35:1521–9.
Pashov A, MacLeod S, Saha R, et al. (2005). Concanavalin A binding to HIV envelope protein is less sensitive to mutations in glycosylation sites than monoclonal antibody 2G12. Glycobiology 15:994–1001.
Peters H, Kusov YY, Meyer S, et al. (2005). Hepatitis A virus proteinase 3C binding to viral RNA: correlation with substrate binding and enzyme dimerization. Biochem J 385:363–70.
Pettigrew DM, Williams DT, Kerrigan D, et al. (2006). Structural and functional insights into the interaction of echoviruses and decay-accelerating factor. J Biol Chem 281:5169–77.
Philpott N, Bakken T, Pennell C, et al. (2011). The Kaposi's sarcoma-associated herpes virus G protein-coupled receptor contains an immunoreceptor tyrosine-based inhibitory motif that activates Shp2. J Virol 85:1140–4.

Quyen DV, Ha SC, Lowenhaupt K, et al. (2007). Characterization of DNA-binding activity of Z alpha domains from poxviruses and the importance of the beta-wing regions in converting B-DNA to Z-DNA. Nucleic Acids Res 35:7714–20.

Ramboarina S, Druillennec S, Morellet N, et al. (2004). Target specificity of human immunodeficiency virus type 1 NCp7 requires an intact conformation of its CCHC N-terminal zinc finger. J Virol 78: 6682–7.

Ramcharan J, Colleluori DM, Merkel G, et al. (2006). Mode of inhibition of HIV-1 Integrase by a C-terminal domain-specific monoclonal antibody. Retrovirology 3:34.

Roohvand F, Maillard P, Lavergne JP, et al. (2009). Initiation of hepatitis C virus infection requires the dynamic microtubule network: role of the viral nucleocapsid protein. J Biol Chem 284:13778–91.

Rusnati M, Bugatti A, Mitola S, et al. (2009). Exploiting Surface Plasmon Resonance (SPR) technology for the identification of fibroblast growth factor-2 (FGF2) antagonists endowed with anti-angiogenic activity. Sensors (Basel) 9:6471–503.

Rusnati M, Presta M. (2002). HIV-1 Tat protein: a target for the development of anti-AIDS therapies. Drug Fut 27:481–493.

Rusnati M, Urbinati C, Caputo A, et al. (2001). Pentosan polysulfate as an inhibitor of extracellular HIV-1 Tat. J Biol Chem 276: 22420–5.

Ryzhova E, Whitbeck JC, Canziani G, et al. (2002). Rapid progression to simian AIDS can be accompanied by selection of CD4-independent gp120 variants with impaired ability to bind CD4. J Virol 76:7903–9.

Sabbah EN, Delaunay T, Varin A, et al. (2006). Development and characterization of ten monoclonal anti-Vpr antibodies. AIDS Res Hum Retroviruses 22:630–9.

Sabile A, Perlemuter G, Bono F, et al. (1999). Hepatitis C virus core protein binds to apolipoprotein AII and its secretion is modulated by fibrates. Hepatology 30:1064–76.

Saitoh T, Kuramochi K, Imai T, et al. (2008). Podophyllotoxin directly binds a hinge domain in E2 of HPV and inhibits an E2/E7 interaction in vitro. Bioorg Med Chem 16:5815–25.

Santiago C, Bjorling E, Stehle T, Casasnovas JM. (2002). Distinct kinetics for binding of the CD46 and SLAM receptors to overlapping sites in the measles virus hemagglutinin protein. J Biol Chem 277: 32294–301.

Sarrias MR, Franchini S, Canziani G, et al. (2001). Kinetic analysis of the interactions of complement receptor 2 (CR2, CD21) with its ligands C3d, iC3b, and the EBV glycoprotein gp350/220. J Immunol 167:1490–9.

Seco J, Luque FJ, Barril X. (2009). Binding site detection and druggability index from first principles. J Med Chem 52:2363–71.

Seet BT, Barrett J, Robichaud J, et al. (2001a). Glycosaminoglycan binding properties of the myxoma virus CC-chemokine inhibitor, M-T1. J Biol Chem 276:30504–13.

Seet BT, Singh R, Paavola C, et al. (2001b). Molecular determinants for CC-chemokine recognition by a poxvirus CC-chemokine inhibitor. Proc Natl Acad Sci U S A 98:9008–13.

Seiradake E, Lortat-Jacob H, Billet O, et al. (2006). Structural and mutational analysis of human Ad37 and canine adenovirus 2 fiber heads in complex with the D1 domain of coxsackie and adenovirus receptor. J Biol Chem 281:33704–16.

Seo NS, Zeng CQ, Hyser JM, et al. (2008). Integrins alpha1beta1 and alpha2beta1 are receptors for the rotavirus enterotoxin. Proc Natl Acad Sci U S A 105:8811–18.

Shelton H, Harris M. (2008). Hepatitis C virus NS5A protein binds the SH3 domain of the Fyn tyrosine kinase with high affinity: mutagenic analysis of residues within the SH3 domain that contribute to the interaction. Virol J 5:24.

Shi J, Gonzales RA, Bhattacharyya MK. (1996). Identification and characterization of an S-adenosyl-L-methionine: delta 24-sterol-C-methyltransferase cDNA from soybean. J Biol Chem 271:9384–9.

Shih PC, Yang MS, Lin SC, et al. (2009). A turn-like structure ''KKPE'' segment mediates the specific binding of viral protein A27 to heparin and heparan sulfate on cell surfaces. J Biol Chem 284: 36535–46.

Smith EA, Kyo M, Kumasawa H, et al. (2002). Chemically induced hairpin formation in DNA monolayers. J Am Chem Soc 124: 6810–11.

Smith SA, Kotwal GJ. (2001). Virokines: novel immunomodulatory agents. Expert Opin Biol Ther 1:343–57.

Smith SA, Sreenivasan R, Krishnasamy G, et al. (2003). Mapping of regions within the vaccinia virus complement control protein involved in dose-dependent binding to key complement components and heparin using surface plasmon resonance. Biochim Biophys Acta 1650:30–9.

Solbak SM, Reksten TR, Hahn F, et al. (2012). HIV-1 p6 – a structured to flexible multifunctional membrane-interacting protein. Biochim Biophys Acta 1828:816–23.

Solbak SM, Reksten TR, Wray V, et al. (2010). The intriguing cyclophilin A-HIV-1 Vpr interaction: prolyl cis/trans isomerisation catalysis and specific binding. BMC Struct Biol 10:31.

Solbak SM, Wray V, Horvli O, et al. (2011). The host-pathogen interaction of human cyclophilin A and HIV-1 Vpr requires specific N-terminal and novel C-terminal domains. BMC Struct Biol 11:49.

Sorathiya A, Bracciali A, Lio P. (2010). Formal reasoning on qualitative models of coinfection of HIV and Tuberculosis and HAART therapy. BMC Bioinformatics 11:S67.

Spencer KA, Hiscox JA. (2006). Characterisation of the RNA binding properties of the coronavirus infectious bronchitis virus nucleocapsid protein amino-terminal region. FEBS Lett 580:5993–8.

Spiller OB, Blackbourn DJ, Mark L, et al. (2003). Functional activity of the complement regulator encoded by Kaposi's sarcoma-associated herpesvirus. J Biol Chem 278:9283–9.

Sprague ER, Reinhard H, Cheung EJ, et al. (2008). The human cytomegalovirus Fc receptor gp68 binds the Fc CH2-CH3 interface of immunoglobulin G. J Virol 82:3490–9.

Stamminger T. (2008). Interactions of human cytomegalovirus proteins with the nuclear transport machinery. Curr Top Microbiol Immunol 325:167–85.

Stephen AG, Datta SA, Worthy KM, et al. (2007). Measuring the binding stoichiometry of HIV-1 Gag to very-low-density oligonucleotide surfaces using surface plasmon resonance spectroscopy. J Biomol Tech 18:259–66.

Stricher F, Huang CC, Descours A, et al. (2008). Combinatorial optimization of a CD4-mimetic miniprotein and cocrystal structures with HIV-1 gp120 envelope glycoprotein. J Mol Biol 382:510–24.

Suenaga E, Mizuno H, Penmetcha KK. (2012). Monitoring influenza hemagglutinin and glycan interactions using surface plasmon resonance. Biosens Bioelectron 32:195–201.

Sun ZY, Oh KJ, Kim M, et al. (2008). HIV-1 broadly neutralizing antibody extracts its epitope from a kinked gp41 ectodomain region on the viral membrane. Immunity 28:52–63.

Symons JA, Tscharke DC, Price N, Smith GL. (2002). A study of the vaccinia virus interferon-gamma receptor and its contribution to virus virulence. J Gen Virol 83:1953–64.

Takahashi H, Sawa H, Hasegawa H, et al. (2004). Topoisomerase I dissociates human immunodeficiency virus type 1 reverse transcriptase from genomic RNAs. Biochem Biophys Res Commun 313: 1073–8.

Takeda E, Murakami T, Matsuda G, et al. (2011). Nuclear exportin receptor CAS regulates the NPI-1-mediated nuclear import of HIV-1 Vpr. PLoS One 6:e27815.

Takemoto DK, Skehel JJ, Wiley DC. (1996). A surface plasmon resonance assay for the binding of influenza virus hemagglutinin to its sialic acid receptor. Virology 217:452–8.

Tanaka Y, Shimoike T, Ishii K, et al. (2000). Selective binding of hepatitis C virus core protein to synthetic oligonucleotides corresponding to the 5' untranslated region of the viral genome. Virology 270:229–36.

Tarus B, Bakowiez O, Chenavas S, et al. (2012). Oligomerization paths of the nucleoprotein of influenza A virus. Biochimie 94:776–85.

Taube S, Jiang M, Wobus CE. (2010). Glycosphingolipids as receptors for non-enveloped viruses. Viruses 2:1011–49.

Taylor MP, Koyuncu OO, Enquist LW. (2011). Subversion of the actin cytoskeleton during viral infection. Nat Rev Microbiol 9:427–39.

ter Meulen J, van den Brink EN, Poon LL, et al. (2006). Human monoclonal antibody combination against SARS coronavirus: synergy and coverage of escape mutants. PLoS Med 3:e237.

Terao-Muto Y, Yoneda M, Seki T, et al. (2008). Heparin-like glycosaminoglycans prevent the infection of measles virus in SLAM-negative cell lines. Antiviral Res 80:370–6.

Timmins J, Schoehn G, Ricard-Blum S, et al. (2003). Ebola virus matrix protein VP40 interaction with human cellular factors Tsg101 and Nedd4. J Mol Biol 326:493–502.
Trinh HV, Lesage G, Chennamparampil V, et al. (2012). Avidity binding of human adenovirus serotypes 3 and 7 to the membrane cofactor CD46 triggers infection. J Virol 86:1623–37.
Tripet B, Kao DJ, Jeffers SA, et al. (2006). Template-based coiled-coil antigens elicit neutralizing antibodies to the SARS-coronavirus. J Struct Biol 155:176–94.
Tsirulnikov K, Abuladze N, Vahi R, et al. (2012). Aminoacylase 3 binds to and cleaves the N-terminus of the hepatitis C virus core protein. FEBS Lett 586:3799–804.
Twarock R. (2006). Mathematical virology: a novel approach to the structure and assembly of viruses. Philos Transact A Math Phys Eng Sci 364:3357–73.
Urbinati C, Bugatti A, Giacca M, et al. (2005). alpha(v)beta3-integrin-dependent activation of focal adhesion kinase mediates NF-kappaB activation and motogenic activity by HIV-1 Tat in endothelial cells. J Cell Sci 118:3949–58.
Urbinati C, Bugatti A, Oreste P, et al. (2004). Chemically sulfated *Escherichia coli* K5 polysaccharide derivatives as extracellular HIV-1 Tat protein antagonists. FEBS Lett 568:171–7.
Urbinati C, Chiodelli P, Rusnati M. (2008). Polyanionic drugs and viral oncogenesis: a novel approach to control infection, tumor-associated inflammation and angiogenesis. Molecules 13:2758–85.
Vaisocherova H, Mrkvova K, Piliarik M, et al. (2007). Surface plasmon resonance biosensor for direct detection of antibody against Epstein-Barr virus. Biosens Bioelectron 22:1020–6.
Van Ryk DI, Venkatesan S. (1999). Real-time kinetics of HIV-1 Rev-Rev response element interactions. Definition of minimal binding sites on RNA and protein and stoichiometric analysis. J Biol Chem 274: 17452–63.
VanCott TC, Bethke FR, Kalyanaraman V, et al. (1994). Preferential antibody recognition of structurally distinct HIV-1 gp120 molecules. J Acquir Immune Defic Syndr 7:1103–15.
Veiga AS, Pattenden LK, Fletcher JM, et al. (2009). Interactions of HIV-1 antibodies 2F5 and 4E10 with a gp41 epitope prebound to host and viral membrane model systems. Chembiochem 10:1032–44.
Viejo-Borbolla A, Martinez-Martin N, Nel HJ, et al. (2012). Enhancement of chemokine function as an immunomodulatory strategy employed by human herpesviruses. PLoS Pathog 8: e1002497.
Viswanathan K, Fruh K. (2007). Viral proteomics: global evaluation of viruses and their interaction with the host. Expert Rev Proteomics 4: 815–29.
Vitagliano L, Fiume G, Scognamiglio PL, et al. (2011). Structural and functional insights into IkappaB-alpha/HIV-1 Tat interaction. Biochimie 93:1592–600.
Waddington SN, McVey JH, Bhella D, et al. (2008). Adenovirus serotype 5 hexon mediates liver gene transfer. Cell 132:397–409.
Wang H, Li ZY, Liu Y, et al. (2010a). Desmoglein 2 is a receptor for adenovirus serotypes 3, 7, 11 and 14. Nat Med 17:96–104.
Wang H, Liaw YC, Stone D, et al. (2007). Identification of CD46 binding sites within the adenovirus serotype 35 fiber knob. J Virol 81: 12785–92.
Wang Q, Li C, Zhang Q, et al. (2010b). Interactions of SARS coronavirus nucleocapsid protein with the host cell proteasome subunit p42. Virol J 7:99.
Wang W, Cole AM, Hong T, et al. (2003). Retrocyclin, an antiretroviral theta-defensin, is a lectin. J Immunol 170:4708–16.
West ML, Ramsdale TE. (1996). Binding kinetics and bioassay of RRE mRNA fragments to a peptide containing the recognition domain of HIV-1 Rev. Biomed Pept Proteins Nucleic Acids 2:85–8.
Wilkinson TA, Januszyk K, Phillips ML, et al. (2009). Identifying and characterizing a functional HIV-1 reverse transcriptase-binding site on integrase. J Biol Chem 284:7931–9.
Williams RK, Straus SE. (1997). Specificity and affinity of binding of herpes simplex virus type 2 glycoprotein B to glycosaminoglycans. J Virol 71:1375–80.
Willis SH, Rux AH, Peng C, et al. (1998). Examination of the kinetics of herpes simplex virus glycoprotein D binding to the herpesvirus entry mediator, using surface plasmon resonance. J Virol 72:5937–47.
Wunderlich K, Juozapaitis M, Ranadheera C, et al. (2011). Identification of high-affinity PB1-derived peptides with enhanced affinity to the PA protein of influenza A virus polymerase. Antimicrob Agents Chemother 55:696–702.
Xiang Y, Moss B. (2003). Molluscum contagiosum virus interleukin-18 (IL-18) binding protein is secreted as a full-length form that binds cell surface glycosaminoglycans through the C-terminal tail and a furin-cleaved form with only the IL-18 binding domain. J Virol 77:2623–30.
Xiao Y, Wu W, Dierich MP, Chen Y. (2000). HIV-1 gp41 by N-domain binds the potential receptor protein P45. Int Arch Allergy Immunol 121:253–7.
Xing L, Tjarnlund K, Lindqvist B, et al. (2000). Distinct cellular receptor interactions in poliovirus and rhinoviruses. EMBO J 19:1207–16.
Yadav VN, Pyaram K, Mullick J, Sahu A. (2008). Identification of hot spots in the variola virus complement inhibitor (SPICE) for human complement regulation. J Virol 82:3283–94.
Yang Y, Wang Q, Guo D. (2008). A novel strategy for analyzing RNA-protein interactions by surface plasmon resonance biosensor. Mol Biotechnol 40:87–93.
Yasin B, Wang W, Pang M, et al. (2004). Theta defensins protect cells from infection by herpes simplex virus by inhibiting viral adhesion and entry. J Virol 78:5147–56.
Yi J, Asante-Appiah E, Skalka AM. (1999). Divalent cations stimulate preferential recognition of a viral DNA end by HIV-1 integrase. Biochemistry 38:8458–68.
Yoon SI, Jones BC, Logsdon NJ, et al. (2012). Epstein-Barr virus IL-10 engages IL-10R1 by a two-step mechanism leading to altered signaling properties. J Biol Chem 287:26586–95.
Yu JS, Liao HX, Gerdon AE, et al. (2006). Detection of Ebola virus envelope using monoclonal and polyclonal antibodies in ELISA, surface plasmon resonance and a quartz crystal microbalance immunosensor. J Virol Methods 137:219–28.
Zanier K, Charbonnier S, Baltzinger M, et al. (2005). Kinetic analysis of the interactions of human papillomavirus E6 oncoproteins with the ubiquitin ligase E6AP using surface plasmon resonance. J Mol Biol 349:401–12.
Zanier K, Ruhlmann C, Melin F, et al. (2009). E6 proteins from diverse papillomaviruses self-associate both in vitro and in vivo. J Mol Biol 396:90–104.
Zhang F, Fath M, Marks R, Linhardt RJ. (2002). A highly stable covalent conjugated heparin biochip for heparin-protein interaction studies. Anal Biochem 304:271–3.
Zhang Q, Hunke C, Yau YH, et al. (2012). The stem region of premembrane protein plays an important role in the virus surface protein rearrangement during dengue maturation. J Biol Chem 287: 40525–34.
Zhang X, Oglesbee M. (2003). Use of surface plasmon resonance for the measurement of low affinity binding interactions between HSP72 and measles virus nucleocapsid protein. Biol Proced Online 5:170–81.
Zhao D, Wang X, Lou G, et al. (2010). APOBEC3G directly binds Hepatitis B virus core protein in cell and cell free systems. Virus Res 151:213–19.
Zhao Q, Ma L, Jiang S, et al. (2005). Identification of N-phenyl-N′-(2,2,6,6-tetramethyl-piperidin-4-yl)-oxalamides as a new class of HIV-1 entry inhibitors that prevent gp120 binding to CD4. Virology 339:213–25.
Zhou B, Liu J, Wang Q, et al. (2008). The nucleocapsid protein of severe acute respiratory syndrome coronavirus inhibits cell cytokinesis and proliferation by interacting with translation elongation factor 1alpha. J Virol 82:6962–71.
Zhu J, Mactutus CF, Wallace DR, Booze RM. (2009). HIV-1 Tat protein-induced rapid and reversible decrease in [3H]dopamine uptake: dissociation of [3H]dopamine uptake and [3H]2beta-carbomethoxy-3-beta-(4-fluorophenyl)tropane (WIN 35 428) binding in rat striatal synaptosomes. J Pharmacol Exp Ther 329:1071–83.
Zlotnick A, Mukhopadhyay S. (2011). Virus assembly, allostery and antivirals. Trends Microbiol 19:14–23.