

# Recent advances in host–virus interactomics during entry and infection

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**Abstract:** Viral infections and pandemics result in millions of deaths worldwide each year. Viruses exploit host cellular processes, not only to gain entry and to deliver their genetic cargo, but also to counteract and use host immune defenses. To this end, a variety of ingenious strategies have evolved in viruses that involve fusion between virus and host membranes, channel formation through the host plasma membranes, disruption of the membrane vesicles, or a combination of these events. The entry and infection pathways of virus are thus largely defined by the interactions between virus particles and their cell surface and cytoplasmic receptors. A thorough analysis of virus–host interactomes may reveal novel mechanisms in virus entry, virus infection, and pathogenic strategies to modulate host metabolic pathways. The study of viral entry, infection, and pathogenesis has evolved over a long period. A host of next-generation technological advancements in this field has been discussed in this review.

**Keywords:** RNA interference, high-throughput, bioinformatics, viral entry, viral infection, virus–host interactions

## Introduction

Viruses, obligate intracellular parasites, are metastable molecular assemblages that should be unlocked systematically during cellular entry by specific molecular and/or cellular environmental cues, with minimal energetic input.<sup>1</sup> The transport of genetic material and other essential components through the host cell barriers requires, in most cases, precise attachment of the virions to the cell surface receptor(s) of the permissive host cells. Sometimes, viruses require additional co-receptor(s) on the host cell surface in order to complete successful entry within the cell. Therefore, the presence of cell receptors and co-receptors, where required, determines the fate of viral attachment and entry, especially in animal cells.

The diffusion rates of free virions toward the host cell, as well as the concentrations of virions and host cells, determine the probability and frequency of collision between them, and the initial interaction is guided by electrostatic forces.<sup>2,3</sup> The subsequent high-affinity binding depends on hydrophobic and other forces whose strength and specificity are governed primarily by the conformations of the interacting viral and cellular interfaces.<sup>2</sup> The avidity of virus binding to cells depends on the engagement of multiple receptor-binding sites on the virion and the fluid nature of the plasma membrane. Studies on the kinetics of virus spread in several viruses (human cytomegalovirus, vesicular stomatitis virus, and T7 bacteriophage) have been undertaken with the help of mathematical modeling.<sup>4–6</sup> Investigation on the parameters of viral infection and the patterns of infection spread in oncolytic viruses (eg, modified adenovirus

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particles or conditionally-replicating adenoviruses [CRAds]) revealed that the probability of an initially infected host cell forming a plaque was 28% and the spread of infection from initially infected cells to the secondarily infected cells was generally inefficient.<sup>7</sup>

Subsequent to successful binding to specific cell surface receptor(s), the entry and uncoating of virion nucleocapsids through the plasma membranes is mediated by transport pathways specific to each group of viruses. The mechanism of virus entry can be simple fusion of viral membranes with cellular plasma membranes via activation of viral fusion proteins, as in many enveloped viruses (members of family Paramyxoviridae), and via receptor-mediated endocytic pathways (influenza virus, family Orthomyxoviridae).<sup>8</sup> The entry of nonenveloped viruses, on the other hand, employs varied mechanisms: from disruption of endosome at low internal pH (adenovirus; family Adenoviridae), through pore formation at the plasma membrane or endosomes (poliovirus; family Picornaviridae), or via caveolae (SV40; family Polyomaviridae).<sup>1,9</sup> Recently, macropinocytosis or cellular drinking has come to the focus, as a means of unusual clathrin-independent endocytic pathway of cell entry in a growing list of viruses, which include Ebola virus (EBOV; family Filoviridae), adenovirus 35, influenza A virus, Kaposi's sarcoma-associated herpesvirus (family Herpesviridae), vaccinia virus (VV) extracellular virions (family Poxviridae), VV mature virions of the IHD-J strain, Nipah virus (family Paramyxoviridae), and Old World Arenaviruses (Lassa virus and Lymphocytic choriomeningitis mammarenavirus; family Arenaviridae).<sup>10,11</sup> Viruses that require replication of their genomes within the host nucleus employ a variety of strategies to transport their genomic cargo: disassembly at the nuclear pore complex, through nuclear envelope disruption or both.<sup>12</sup>

## Early studies on host–virus interactomics

Enzyme-based assays provided the initial clues concerning the chemical nature of cell surface components to which virions become attached. Early studies on identification of cell surface receptors depended on enzyme-based characterizations, such as in adenovirus and influenza virus.<sup>13,14</sup> The development of technologies such as monoclonal antibody-mediated immunoprecipitation or affinity chromatography, expression of cell surface receptors in non-susceptible cells through gene transformation, and subsequent molecular cloning of cellular receptors from the transformed non-susceptible cells paved the way toward identification of myriad of cell

surface receptors/co-receptors required for virus attachment (Table 1).<sup>15–17</sup> These discoveries largely depended on the cloning of the receptor genes that allowed detailed mutagenesis analyses and structural studies on host–virus interactions.

In contrast to studies on isolated viral proteins and their interactions with cellular targets, global analyses of virus–host interactions have been studied through a variety of genomics and proteomic tools.<sup>18</sup> Numerous proteomic studies have been carried out to study the effect of viral infections on human and other cells. 2D gel electrophoresis of whole-cell lysates taken before and after infection followed by mass spectrometry (MS) identified the proteins detected in a gel.<sup>19,20</sup> High-throughput yeast two-hybrid (HT-Y2H) assays have been useful in exploring protein–protein interactions (PPIs) in *Saccharomyces cerevisiae* using full-length predicted open reading frames.<sup>21</sup> The study of virus–host PPIs has been possible through binary Y2H assays or complex affinity purification followed by MS.<sup>22</sup> However, the Y2H system of detection of virus–host PPIs has few formidable limitations: frequent false positives; limitation of analysis of hydrophobic membrane proteins, owing to the expression of the reporter system within the nuclei; and lack of mammalian posttranslational modifications in yeast.<sup>20</sup> Several strategies have been developed in order to overcome these limitations, such as the use of novel N-terminal bait and C-terminal bait and prey fusion-protein vectors, validation of Y2H data through biochemical and/or cell-based assays, and co-immunoprecipitation studies. In HT-Y2H assays, the limitations discussed above have been addressed through several ingenious study designs, such as the Luminescence-based Mammalian IntERactome (LUMIER) mapping, and other variations of luminescence-based protein-fragment complementation assays, eg, split-yellow fluorescent protein (YFP) or split-luciferase-based methods.<sup>23–25</sup>

Virion purification and MS screens have identified several candidate human proteins that putatively interact with human immunodeficiency virus 1 (HIV-1; family Retroviridae).<sup>26</sup> Physiological implications of virus–virus protein interaction in VV, hepatitis C virus (HCV), potato virus A, pea seed-borne mosaic virus, and T7 phage have also been studied.<sup>27</sup> Systematic Y2H virus–host interaction screens, which are subsequently validated by a variety of methods, have been used to chart several virus–host PPIs, such as in HCV, Epstein–Barr virus (EBV), KSHV and varicella-zoster virus (VZV), dengue virus (DENV), and HIV-1.<sup>20,28–32</sup> Chikungunya virus (CHIKV; family Togaviridae)–host-protein interactions have been investigated through HT-Y2H assays that are validated by protein interaction mapping.<sup>33</sup> Out of

**Table 1** Known host cell receptors and co-receptors involved in attachment and entry of medically important viruses

Virus	Family	Receptor(s)	Co-receptor(s)
EBV	Herpesviridae	CD21, CD35	HLA-II, Integrin
HSV-1/2	Herpesviridae	Heparan sulfate	Integrin $\alpha\beta 6$ , TNFRSF14, Nectin-1, Nectin-2
HCMV	Herpesviridae	Heparan sulfate	Integrin $\alpha\beta 3$ , Integrin $\beta 1$ , EGFR
KSHV/HH-8	Herpesviridae	Heparan sulfate, EphA2	Integrin $\alpha 3\beta 1$ , $\alpha\beta 3$ , $\alpha\beta 5$
VZV	Herpesviridae	Heparan sulfate, M6P-R	IDE
EBOV	Filoviridae	TIM-1, NPC1	
Henipavirus (NiV)	Paramyxoviridae	EFNB2, EFNB3	
MeV	Paramyxoviridae	CD46, SLAM	
RABV	Rhabdoviridae	nAChR, NCAM, p75NTR	
SARS-CoV	Coronaviridae	ACE2 or L-SIGN	
HAV	Picornaviridae	TIM-1	
POV	Picornaviridae	CD155	
EV71	Picornaviridae	PSGL-1, SCARB2	
CVB	Picornaviridae	DAF, CAR (occludin)	
Rhinovirus (major group) (HRV14)	Picornaviridae	ICAM-1	
Rhinovirus (minor group) (HRV2)	Picornaviridae	VLDL-R	
Adenovirus (Ad2/Ad5) (Mastadenovirus)	Adenoviridae	CAR, $\alpha_M\beta_2$ , $\alpha L\beta 2$	Integrin $\alpha\beta 3$ , $\alpha\beta 5$ , $\alpha\beta 1$ , $\alpha 3\beta 1$ , $\alpha 5\beta 1$
Arenaviruses (LASV, LCMV) (Old world Complex)	Arenaviridae	$\alpha$ -Dystroglycan	
Arenaviruses (JUNV, SABV) (New world Complex)	Arenaviridae	Transferrin receptor I	
ANDV	Bunyaviridae	$\alpha_V\beta_3$ Integrin	
Phleboviruses (RVFV, CHFV)	Bunyaviridae	DC-SIGN (CD209)	
Norovirus	Caliciviridae	HBGA	
JEV	Flaviviridae	Laminin, CD4, Hsp70, Hsp90	
HCV	Flaviviridae	CD81, SR-B1	Claudin-1
DENV	Flaviviridae	DC-SIGN (CD209)/L-SIGN, heparan sulfate, mannose receptor	nLc4Cer
WNV	Flaviviridae	DC-SIGN, DC-SIGNR (CD209L)	
HBV	Hepadnaviridae	ASGPR, NTCP	
IAV	Orthomyxoviridae	Sialic acid, DC-SIGN (CD209)/L-SIGN	
Human papillomavirus (HPV16/HPV18)	Papillomaviridae	Syndecan-1	CD151, Integrin $\alpha 6\beta 4$
Polyomavirus (JC/SV40)	Polyomaviridae	LSTc/GM1	5-HT2
Rotavirus	Reoviridae	Sialic acid, integrins, Hsc70	JAM-A
HIV-1	Retroviridae	CD4	CCR5 or CXCR4
HTLV-1	Retroviridae	GLUT-1	NRP-1
CHIKV	Togaviridae	PHB	
SINV	Togaviridae	Laminin receptor	
SFV	Togaviridae	Sphingolipid	

**Note:** Adapted with permission from Flint et al,<sup>15</sup> Grove and Marsh,<sup>16</sup> and ViralZone ([viralzone.expasy.org](http://viralzone.expasy.org)), SIB Swiss Institute of Bioinformatics, PMID:20947564.<sup>17</sup>

**Abbreviations:** EBV, Epstein–Barr virus; HSV-1/2, Herpes simplex virus 1/2; HCMV, human cytomegalovirus; KSHV, Kaposi's sarcoma-associated herpesvirus; HH-8, human herpesvirus 8; VZV, varicella-zoster virus; EBOV, Ebola virus; NiV, Nipah virus; MeV, measles virus; RABV, rabies virus; SARS-CoV, severe acute respiratory syndrome-coronavirus; HAV, Hepatitis A virus; POV, poliovirus; EV71, enterovirus 71; CVB, coxsackievirus B; HRV14, human rhinovirus 14; HRV2, human rhinovirus 2; LASV, Lassa virus; LCMV, lymphocytic choriomeningitis mammarenavirus; JUNV, Junin mammarenavirus; SABV, Sabia mammarenavirus; ANDV, Andes virus; RVFV, Rift Valley fever virus; CHFV, Crimean-Congo hemorrhagic fever virus; JEV, Japanese encephalitis virus; HCV, hepatitis C virus; DENV, dengue virus; WNV, west Nile virus; HBV, hepatitis B virus; IAV, influenza A virus; HPV, human papillomavirus; JC/SV40, John Cunningham virus; SV40, simian vacuolating virus 40; HIV-1, human immunodeficiency virus 1; HTLV-1, human T-cell leukemia virus 1; CHIKV, chikungunya virus; SINV, sindbis virus; SFV, Semliki Forest virus; CD4/21/35/46/81/155, clusters of differentiation 4/21/35/46/81/155; HLA-II, human leucocyte antigen-II; TNFRSF14, Tumor necrosis factor receptor superfamily, member 14; EphA2, ephrin receptor tyrosine kinase A2; M6P-R, mannose-6-phosphate receptor; IDE, insulin degrading enzyme; TIM-1, T-cell immunoglobulin and mucin domain 1; NPC-1, Niemann-Pick C 1; EFNB2/B3, ephrin B2/3; SLAM, signaling lymphocyte-activation molecule; nAChR, nicotinic acetylcholine receptor; NCAM, neural cell adhesion molecule; p75NTR, p75 neurotrophin receptor; ACE2, angiotensin 1 converting enzyme 2; DC-SIGN, dendritic cell-specific ICAM-3 grabbing non-integrin; DC-SIGNR, dendritic cell-specific ICAM-3 grabbing non-integrin receptor; L-SIGN, liver/lymph node-specific intracellular adhesion molecule-3 grabbing non-integrin; PSGL-1, P-selectin glycoprotein ligand 1; SCARB2, scavenger receptor class B, member 2; DAF, decay accelerating factor; CAR, coxsackievirus and adenovirus receptor; ICAM-1, intercellular adhesion molecule-1; VLDL-R, very low density lipoprotein receptor; HBGA, histo-blood group antigen; SRB-1, scavenger receptor class B, member 1; ASGPR, asialoglycoprotein receptor; NTCP, sodium taurocholate receptor; LSTc, sialylacto-N-tetraose c; GM1, monosialotetrahexosylganglioside 1; Hsc70, heat shock cognate protein 70; GLUT-1, glucose transporter-1; PHB, prohibitin; nLc4Cer, neolactotetraosylceramide; 5-HT2, 5-hydroxytryptamine receptor 2; JAM-A, junctional adhesion molecule-A; CCR5, C-C chemokine receptor type 5; CXCR4, C-X-C chemokine receptor type 4; NRP-1, neuropilin-1.

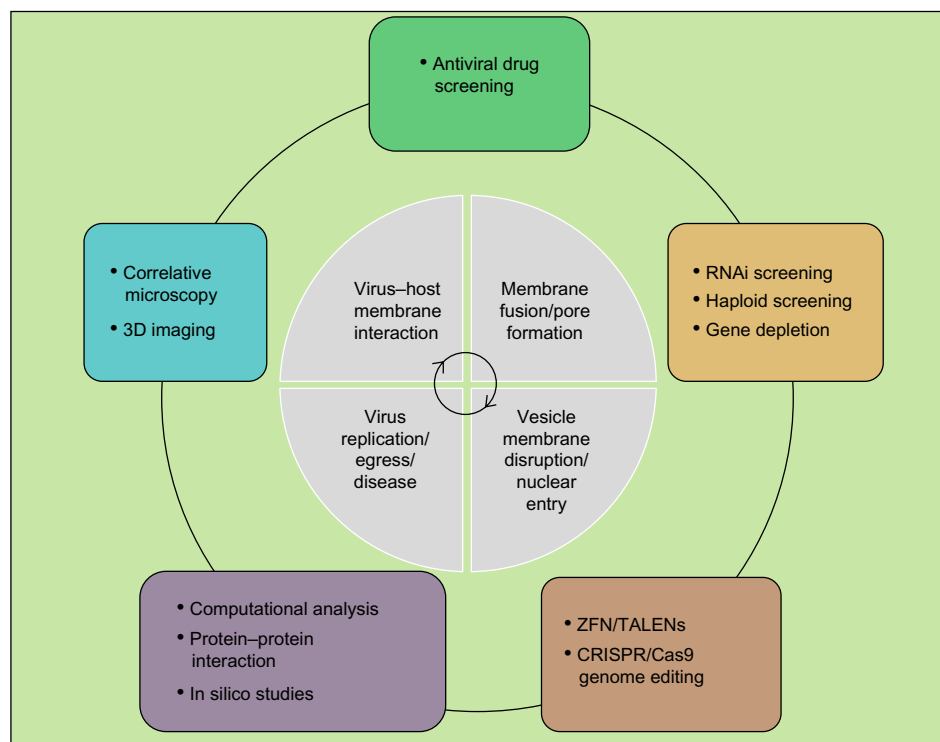
total 30 distinct interactions, 22 high-confidence interaction data sets were generated that contained 21 interactions supported by nsP2 and one by nsP4. The results were further validated in protein complementation assay. In order to identify interacting host proteins, a CHIKV–host-protein interaction map was built up, which indicated several candidate proteins of translational machinery (hnRNP-K), RNA splicing factors (SRSF3), and cytoskeletal proteins (VIM, TACC3, CEP55, and KLC4), that are targeted by CHIKV nsP2. Other cellular proteins identified with nsP2 as bait are regulators of gene transcription and the proteins involved in protein degradation and/or autophagy. A similar study explored putative interacting host factors in severe acute respiratory syndrome-coronavirus (SARS-CoV; family Coronaviridae) replication.<sup>34</sup>

The Y2H and small interfering RNA (siRNA) screens have been useful in selecting host cofactors interacting with influenza virus, which Shaw classified into several gene clusters, namely, ribosome, coat protein complex I (COPI) vesicle, proton-transporting V-type ATPase complex, spliceosome, nuclear pore/envelope, and kinase/signaling.<sup>35</sup> The involvement of ribosome in host mRNA degradation (influenza virus and SARS-CoV), vATPase in pH-dependent

endosomal release (SARS-CoV, Semliki Forest virus; family Togaviridae), and nuclear pore-associated protein such as human KPNA1 (Karyopherin alpha 1 or importin alpha 5) and MAPK-signaling pathway in infections of SARS-CoV and HCV has been elucidated through these studies.<sup>20</sup>

The study of HIV–human protein interaction by Jäger et al employed affinity tagging and purification followed by MS that revealed several interactions of HIV-1 proteins in two human cell lines (HEK293 and Jurkat).<sup>36</sup> The authors scored the PPIs using an improvised scoring system (MS interaction statistics) and plotted 497 HIV–human PPIs involving 435 individual human proteins in an interaction map. In addition to several known interactions, the authors discovered novel interactions, such as the interactions of HIV Pol and/or protease (PR) with that of host eIF3 complex subunits, thus indicating a role of this host-protein complex in inhibition of HIV-1 reverse transcriptase.<sup>36</sup>

In order to model virus infection at the cellular level, we need exhaustive PPI data not only on intravirus–host interactomes but also on virus–host interactomes. Although far from complete, the studies discussed above have generated a huge primary database of intraviral and virus–host PPIs. These results are now being validated by detailed computational and



**Figure 1** Advanced tools in the study of host–virus interaction dynamics.

**Abbreviations:** RNAi, RNA interference; ZFN, zinc-finger nuclease; TALENs, transcription activator-like effector nucleases; CRISPR, clustered regularly interspaced short palindromic repeat; Cas 9, CRISPR-associated 9.

bioinformatic analyses. Such PPI networks and their analyses may prove to be a powerful resource for the development of antiviral therapeutic strategies.

The study of virus attachment and entry entered a new era in the last decade with the development of a host of gene-disruption technologies.<sup>37</sup> These new technologies include multiplex screenings of small molecule inhibitors, RNA interference (RNAi) screening, haploid screening and genome editing, host–virus interactomics, 3D imaging, and in silico approach (Figure 1). This review explores recent advances and discusses the potential novel applications in the study of virus entry and infection.

## New approach on virus attachment and infection

### Genetic approach to identify host factors involved in viral infection

In an effort to understand the genetic basis of host susceptibility to viral infection/disease, researchers have identified genes that determine the outcome of virus–host interactions. Host proteins involved in susceptibility to viral infections have been explored through genetic mapping and positional cloning techniques in experimental animals. Although limited by the availability of inbred mouse strains and their inherent genetic diversities, these approaches are based on the identification of individuals who differ in susceptibilities to viral infections. Mice that differ in their inherent susceptibilities can be bred and the progenies can be subjected to linkage analyses to identify chromosomal location of candidate genes. Studies in this line have created low-resolution mapping of several mouse genes responsible for susceptibility/resistance to mouse cytomegalovirus (MCMV; reviewed in Webb et al<sup>38</sup>), West Nile virus<sup>39</sup> and mouse adenovirus type 1 (MAV-1<sup>40</sup>).<sup>38–40</sup> The pattern of susceptibility/resistance to MCMV infection has identified the *Cmv1* locus, a stimulatory member (Ly49H) of Ly49 receptor gene family (homolog of human KIR genes). The importance of *Cmv1/Ly49H* in modulating MCMV infection in mouse has underlined the role of stimulatory KIRs in controlling viral infections through their interactions with major histocompatibility complex-I (MHC-I) molecules.<sup>38</sup> Interferon (IFN)-inducible proteins are important in determining the outcome of many host–virus interactions. Genetics and biochemical studies have established the inhibitory roles of IFN-induced 2',5'-oligoadenylate synthetase (OAS) family of enzymes and RNase L in infections with Picornaviruses and with Flaviviruses (West Nile virus) in mouse models. Systematic

genetic crosses in inbred mice initially identified a resistance/susceptibility locus (*Flv/Wmv*) with a chromosomal region. Subsequent positional cloning technique identified the gene cluster encoding the IFN-inducible OAS family of proteins.<sup>39</sup> Welton et al used a genome-wide search strategy, quantitative trait locus (QTL) detection, to identify host factors associated with susceptibility of congenic mouse to MAV-1.<sup>40</sup> The *H-2<sup>s</sup>* haplotype of SJL/J mice was shown not to be associated with susceptibility to MAV-1, and the susceptibility of MAV-1-infected (BALB/cJ × SJL/J) F1 mice was intermediate between that of SJL/J and BALB/cJ mice, indicating that susceptibility is a genetically controlled quantitative trait. Through the analyses of 192 backcross progeny in a genome scan with polymorphic markers, a major QTL was detected on chromosome 15 (logarithm of odds [LOD] score of 21) and MAV-1 susceptibility was shown to be unlinked to the *H-2* major histocompatibility locus. Through the development of high-resolution genetic map, a diverse panel of inbred mouse strains was used to identify QTLs associated with regulation of host responses to influenza infection.<sup>41</sup> A novel *N*-ethyl-*N*-nitrosourea-mediated germline mutagenesis in MCMV-resistant mice and backcrossing led to the identification and positional cloning of host susceptibility genes.<sup>42</sup> A similar approach was used to identify a mouse gene, *Eif2ak4* (encoding GCN2), involved in susceptibility to MCMV and human adenovirus.<sup>43</sup>

## Antiviral drug screening

Conventional therapy against viral infections has specifically targeted specific viral proteins and enzymes, but this therapy regime is fraught with danger of severe cellular toxicity or the chance of viral targets evolving to resistant varieties, owing to viruses' intrinsic capacity for rapid genetic changes and evolution of their fitness levels. Therefore, the current need is to develop a combination therapy that increases specificity and efficiency, avoids selection of resistant strains, and enlarges the therapeutic arsenal. This requires alternative therapeutic interventions, such as targeting critical host proteins required for virus entry and replication. Documenting and understanding virus–host PPIs is a prerequisite to identify cellular “druggable” targets. Few promising host-protein antagonistic drugs are ready to be launched commercially, such as Maraviroc (a CCR5 co-receptor antagonist) for the HIV treatment, DAS181 (a recombinant sialidase fusion protein) for influenza virus infection, and TSG101 (human monoclonal antibody against a surface receptor expressed in infected cell) for a variety of viruses.<sup>32</sup> Through a process of drug repurposing, the available libraries of small drug

molecules can be rescreened for novel interactions with host cell proteins. Cell-based high-throughput screenings of drugs with known biological functions have been useful in identifying host proteins that enhance IFN signaling pathways or regulate replication of viruses, namely, HCV and influenza virus.<sup>32</sup>

In vitro cytopathic effects-based high-throughput screening assays to identify novel antiviral drugs against bluetongue virus,<sup>44</sup> influenza virus,<sup>45–47</sup> SARS-CoV,<sup>48</sup> yellow fever virus,<sup>49</sup> and Crimean-Congo hemorrhagic fever virus<sup>50</sup> have identified unique antivirals. In a novel modification of studying virus–host interactions, a systematic large number of pair-wise drug interactions were explored.<sup>51</sup> This multiplex screening method (multiplex screen for interacting compounds) employed a combination of cell culture, immunostaining, fluorescence imaging, and bioinformatics tools and tested approximately 500,000 drug pairs that synergistically inhibited HIV replication in HeLa-based MAGI cells expressing CD4 receptor. The multiplex screen for interacting compounds screening methodology not only identified promising drug pairs but also identified novel targets for HIV therapies that inhibit viral assembly and release.

## RNAi screening

RNAi involves genetic modulation of virtually any gene by reducing its mRNA levels and, therefore, subsequent protein expression.<sup>52,53</sup> The availability of libraries of siRNAs directed toward almost every human gene enabled several genome-wide high-throughput RNAi (HT-RNAi) screens to identify key host proteins involved in viral infections, such as in influenza virus,<sup>47,54</sup> HIV-1,<sup>55–57</sup> and HCV.<sup>58,59</sup> Although these initial studies generated a wealth of promising leads for drug designing, the outcome of HT-RNAi screens is fraught with many pitfalls: errors in RNAi reagent design, inhomogeneous staining, differences in cell growing properties, transfection/infection efficiencies, signal-detection methodologies, data variations from plate to plate, as well as reliability and reproducibility of readouts. Therefore, bioinformatics and robust statistical approaches may increase the sensitivity of HT-RNAi technology in identifying bonafide hits.<sup>60</sup> Integration of HT-RNAi screens with PPI databases may be approached to strengthen the significance and reliability of identification of host dependency factors, reduce false positives and false negatives, and identify novel cellular targets.<sup>60</sup>

## Gene depletion

High-throughput genetic screens can identify not only specific virus–host interactions but also components of cellular

pathways that are needed for virus replication. This could lead to the development of multiple drug targets. Novel haploid host–gene disruption through non-lethal antiviral insertional mutagenesis and gene trapping methods have led to the identification of host cell receptors for influenza A and EBOV infections.<sup>61,62</sup> Haploid genetic approach has identified host factors required for entry of Lassa virus mediated by the Lassa glycoprotein, in addition to known entry receptor (glycosylated  $\alpha$ -dystroglycan), receptor modifiers, and additional genes.<sup>63</sup> However, haploid screens may miss biologically significant targets due to induced lethality by mutagenesis.<sup>37</sup> More recently, Petersen et al employed insertionally mutagenized haploid human cell line, transcription activator-like effector nuclease-driven gene disruption, and a large-scale siRNA screen to identify members of the major cellular cholesterol regulatory pathway as important targets in entry of Andes virus (family Bunyaviridae).<sup>64</sup>

## CRISPR/Cas9 genome editing

New genetic engineering technologies that specifically target genome loci through DNA double-strand breaks (DSBs) can be classified into three novel platforms: zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the most powerful clustered regularly interspaced short palindromic repeat (CRISPR)-associated 9 (Cas9) guided silencing.<sup>65–67</sup> These new gene-depletion strategies can be used to target putative host cell proteins suspected to be involved in virus interactions.

CRISPR loci evolved in bacteria and archaea as a part of adaptive immune system, which acquire foreign DNA resulting in the production of surveillance complexes consisting of short complementary guide RNAs (gRNAs) and an endonuclease (Cas9). When coexpressed in cells, gRNA and Cas9 can target DNA, namely, putative host-protein gene, for modification/silencing. The gRNAs contain a 20 base-pair guide sequence, which recruits the gRNA/Cas9 complex to its target sequence by base pairing. Successful binding of Cas9 to the target and subsequent endonuclease disruption requires the correct protospacer adjacent motif trinucleotide sequence immediately following the target sequence. Endonuclease Cas9 cuts both strands of DNA causing a DSB, which lies three to four nucleotides upstream of the protospacer adjacent motif sequence. The DSBs may be repaired by an error-prone non-homologous end joining (NHEJ) DNA repair mechanism, which often results in the generation of inserts/deletions at the DSB site. This may lead to disruption of open reading frames in the target genes.

A review on the applications of CRISPR/Cas9 technology in human papillomaviruses (HPV16, HPV18; family Papillomaviridae), hepatitis B virus (family Hepadnaviridae), EBV (family Herpesviridae), and HIV-1 has recently been published.<sup>68</sup> These powerful genome editing technologies are sure to delve into many host–virus interactome studies in future.

## Novel applications Protein–protein interactomics and systems biology

Computational prediction of host–pathogen interactions has been developed that infers interactions among domains based on their occurrence in Y2H data. Sprinzak and Margalit demonstrated in *S. cerevisiae* that characteristic pairs of sequence-signatures can be learned from a database of experimentally determined interacting proteins in which one protein contains one sequence-signature and its interacting partner contains the other sequence-signature.<sup>69</sup> The sequence-signatures that appear together in interacting protein pairs more often than expected at random are termed correlated sequence-signatures. Dyer et al combined this domain-based approach with Bayesian statistics to predict interactions among human and *Plasmodium falciparum*.<sup>70</sup> Here, the researchers integrated a number of public intra-species PPI datasets with protein-domain profiles to develop a novel framework for predicting and studying host–pathogen PPI networks. This theme was translated into virus–human interactions where 182 unique human proteins with more than one viral interacting partner were identified.<sup>71</sup> Tastan et al used a multitude of information in a learning framework, namely, co-occurrence of functional motifs and their interaction domains and protein classes, gene ontology annotations, posttranslational modifications, tissue distributions and gene expression profiles, and topological properties of the human proteins in the prediction of PPIs between HIV-1 and human proteins.<sup>72</sup> The study predicted, among others, interactions of HIV-1 protein Tat and human vitamin D receptor, validating an earlier report that Tat acts with vitamin D receptor in a synergistic manner as a stimulator for HIV-1 LTR activity.<sup>72</sup>

While protein–protein-interaction databases provide a global view of cellular processes controlled by viruses, analyses of the structural details of individual proteins and their interaction interfaces would provide a dynamics of such interactions. Topological and functional analysis of virus–host interactome has been attempted through domain–domain interactions (DDIs).<sup>73</sup> Search for DDIs from PPI dataset

by Pfam scan and HMMER 3.0 against Pfam-A models identified 9,598 intrahost DDIs among 2,084 domains, 1,851 intravirus DDIs among 839 domains, and 269 virus–host DDIs between 87 viral domains from 49 viruses and 144 host domains after filtration. Based on this outcome, network distribution, network topology analysis, gene ontology enrichment analysis, and viral disease network were built up. Virus–host interactomes revealed that viruses use unique domains to interact with the same host partners with fundamental functions and conserved DDIs occurring in host interactomes to mediate the interspecies interaction. Thus, viruses seem to perturb the host cellular network by both common and unique strategies.<sup>73</sup>

## In silico studies

Viruses have a strong tendency to directly and indirectly target host proteins that are central to viral infectivity and replication. These proteins are characterized by high values of connectivity and centrality in the cellular network. The study of viral infection at the systems level is now possible through the analyses of virus–host PPIs. These technically challenging, time-consuming, and expensive experiments can be replaced by in silico studies through algorithm-based modeling of “viral infectomes”. Viral proteins seem to interact with highly connected and central proteins within the host infectome network in order to control essential functions of the host cell. In silico simulation of these topological perturbations associated with viral infections can help to identify suitable targets for drug design.<sup>74</sup>

Posttranslational modifications of multifunctional cholesterol transporter protein Niemann–Pick C1 (NPC1) have been studied in relation to EBOV binding and cellular entry in human.<sup>75</sup> NPC1 of nine species, including human, was subjected to multiple sequence analyses, using appropriate similarity matrix in ClustalW2. NetPhos 2.0 program was employed for phosphorylation site assessment of each Thr, Ser, and Tyr residue of the NPC1 protein using artificial neural network framework. Kinase specific phosphorylation sites in NPC1 were then predicted by NetPhosK 1.0 and KinasePhos 2.0. The O-β-GlcNAc modification potential sites in NPC1 were predicted by YinOYang 1.2 and ISO-GlyP, which identified potential o-glycosylation sites (Yin Yang sites) and enhancement value product values as an indication of glycosylation rates, respectively. Analyses of solvent accessibility of predicted Ser and Thr residues were done with NetSurfP v1.1, and secondary structure analysis was conducted using POLYVIEW server. Docking of spike glycoprotein of Zaire EBOV was then carried out using

structures of the receptor (NPC1), EBOV glycoprotein as ligand, and other active interacting residues for receptor and ligand positions as input with the help of HADDOCK server that used experimental knowledge-driven docking method. A host of revelations emerged from this study, highlighted by the prediction that NPC1-Yin Yang sites may not be important for virus attachment, whereas phosphosite 473 may be important for binding and entry of EBOV.<sup>75</sup> In another study, EBOV–human PPIs were investigated through in silico virtual spectroscopy (informational spectrum method) method to decipher key interactions of EBOV glycoprotein and host endothelial extracellular matrix.<sup>76</sup>

Although far from complete and still in a developmental flux, huge primary databases of intraviral and virus–host PPIs have been built so far, such as in VirHostNet and VirusMINT.<sup>77,78</sup> In combination with literature-derived low-throughput interaction data, Meyniel-Schicklin et al have used these high-quality curated PPI datasets in an integrative and comparative computational analysis of all intraviral and virus–human interactomes through the implementation of graph theory and other computational modelings.<sup>79</sup> Their study tried to delineate the intraviral and virus–human PPI network architectures and systems behavior of several human virus networks (DENV, HCV, EBV, herpes simplex virus 1 [HSV-1], SARS-CoV, VV, and VZV). The analyses of virus–human interactomes revealed that viral proteins that target a high number of host proteins are predicted to be more disordered than viral proteins targeting a single host protein. Analysis of viruses, according to the human proteins they target, has identified common and specific viral strategies at the topological as well as the functional levels.<sup>79</sup>

### 3D imaging

Visualization of viruses in host cells can reveal finer details of the structures and interactions in virus entry, viral genome replication, assembly, maturation, escape, and further infection. Traditional electron microscopy (EM) methods, complemented by fluorescence microscopy (FM) techniques, now allow us to follow the dynamics of virus–cell interactions. Multistep events of influenza virus entry have been visualized using real-time microscopy, which provided new insights into cellular endocytic pathways.<sup>80</sup> Two different host proteins that interact with the Sindbis virus at different stages of infection were identified using a green fluorescent protein-tagged viral protein.<sup>81</sup> The interactions between Sindbis virus and host proteins were explored by using a mutant virus expressing the viral nsP3 protein tagged with green fluorescent protein.<sup>81</sup> Direct observation of nsP3 localization and isolated

nsP3-interacting proteins at various times after infection was recorded via FM. Their results revealed that host factor recruitment to nsP3-containing complexes was time dependent, with a specific early and persistent recruitment of G3BP and a later recruitment of 14-3-3 proteins.<sup>81</sup>

High structural, as well as temporal, resolution can be obtained through sub-diffraction FM and correlative EM/FM approaches.<sup>82</sup> 3D reconstruction by transmission electron microscopy serial sections, electron tomography, and focused ion beam scanning electron microscopy plays significant roles. Focused ion beam scanning electron microscopy uses scanning electron beam to generate 3D images from serial section of cells of a resin bed, which captures virus entry in susceptible host cells.<sup>83</sup> Dramatic morphological changes in the plasma membrane at the site of entry for VV as well as changes in the vaccinia virion itself have been visualized in several studies.<sup>83</sup>

### Conclusion

Organisms impose multiple barriers to virus entry. However, viruses exploit fundamental cellular structures and processes to gain entry to cells, unpack their genetic cargo, and initiate productive replication. Viruses seem to target common and central host proteins for remodeling the cellular signaling pathways and machinery. Therefore, gaining knowledge on the functions of the individual viral proteins in the host cell and that on their interactions with cellular signaling pathways is of paramount importance in understanding the pathogenesis. Systems virology, a highly integrated interdisciplinary systems biology approach in virology, has been instrumental in the global understanding of hitherto unknown virus–host interactions and especially of the dynamic nature of host responses against viruses. The integration of high-throughput array-based genetic screening, computational systems biology, and in silico strategies has substantiated known interactions and unraveled new interactions between the virus and host proteins. Systems biology approaches may help to identify the impact of virulence factors on the host system based on computational models of signal transduction and pathway analysis. Advances in computational biology in molecular dynamics simulations are required to understand unique features of protein structures and the chain of events that ensue with the initial attachment and entry of virus. The completeness of virus–host PPIs will be cornerstone in the structure-based predictions of virus–host DDIs and virus-induced network perturbations in viral infections. The virus-centric approach in developing antiviral drugs might fail to produce desired effects due to the potential genetic

plasticity of the viruses, whereas a host-protein-based approach may identify a broad-range of antiviral drug target candidates. There are already a number of approved drugs designed against human proteins; however, considering the multidimensional nature of PPIs, there is a long way to go before the development of low-risk and low-cost antivirals.

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## Disclosure

The author declares no conflicts of interest in this work.

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