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Defining Pharmacological Targets by Analysis of Virus–Host Protein Interactions

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Contents

1. Cellular Proteins Exist in Complexes	224
2. Discovering Virus–Host Protein Complexes	225
3. Different Pathogens Exploit Similar Cellular Processes	226
4. Viral Proteins Interact With Host Proteins in Complexes	228
5. Development of Small Molecules Disrupting PPIs	230
6. Targeting Virus–Host PPIs With Small Molecules	234
7. Small Molecules Disrupting Host Complexes Implicated in Viral Replication	235
8. Concluding Remarks	236
Acknowledgments	237
References	237

Abstract

Viruses are obligate parasites that depend on cellular factors for replication. Pharmacological inhibition of essential viral proteins, mostly enzymes, is an effective therapeutic alternative in the absence of effective vaccines. However, this strategy commonly encounters drug resistance mechanisms that allow these pathogens to evade control. Due to the dependency on host factors for viral replication, pharmacological disruption of the host–pathogen protein–protein interactions (PPIs) is an important therapeutic alternative to block viral replication. In this review we discuss salient aspects of PPIs implicated in viral replication and advances in the development of small molecules that inhibit viral replication through antagonism of these interactions.



1. CELLULAR PROTEINS EXIST IN COMPLEXES

Chromatographic resolution of human cell extracts subsequently analyzed by quantitative tandem mass spectrometry resulted in the identification of 13,993 physical interactions established by 3006 individual proteins. Computational analysis of the physical interactions led to the mapping of 622 complexes, suggesting an average of 4 proteins per complex (Havugimana et al., 2012). These estimates are also supported by other analysis indicating that most of the mammalian complexes are formed by the association of three or four different proteins. Importantly, some host proteins are found in more than one complex at a time (Wong et al., 2008). Similarly, a study in human cells combining large-scale immunoprecipitation of 331 bait proteins followed by high-throughput mass spectrometry analysis led to the discovery of 6463 interactions between 2235 unique proteins, suggesting an average of 3 proteins per complex (Ewing et al., 2007).

Protein complexes are stable assemblies that carry out most of the biochemical activities in the cell. CORUM, a public database of mammalian protein complexes, compiles complexes reported in nonhigh-throughput, physical interaction experiments. Of the proteins annotated in CORUM, 78% belong to one protein complex. Fig. 1 represents the frequency of annotated complexes in the CORUM database. Interestingly, protein complexes implicated in subcellular localization processes are notoriously overrepresented in this database, whereas others

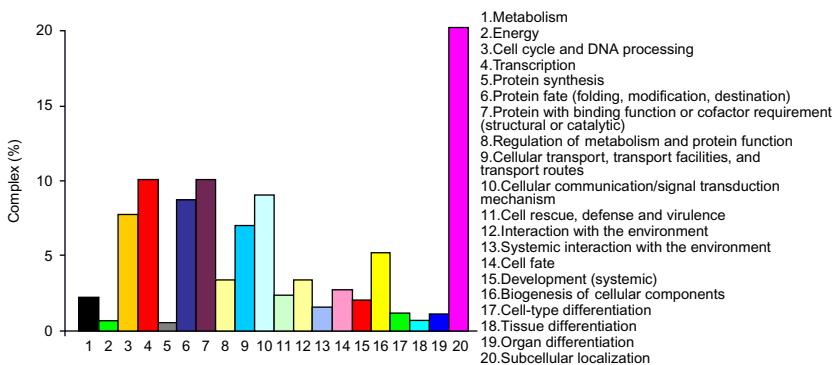


Fig. 1 Functional annotation of protein complexes from mammalian organisms reported in individual experiments compiled in CORUM (10/16/2017; <http://mips.helmholtz-muenchen.de/corum/#>).

implicated in energy production, protein synthesis, and tissue differentiation are underrepresented.

In these collection of experimentally characterized protein complexes, approximately 16% of all predicted human open reading frames are organized in 1815 protein complexes (Ruepp et al., 2010). However, this number is expected to be only a minor fraction of the human complexome considering that in yeast 45% of the encoded proteins reside in complexes (Ruepp et al., 2010).



2. DISCOVERING VIRUS–HOST PROTEIN COMPLEXES

Hypothesis-driven experiments conducted at small scale have notably contributed to our understanding of protein–protein interactions (PPIs) implicated in viral replication. In addition, high-throughput technologies analyzing PPIs, gene loss of function, or transcriptomic profiles have been utilized to define cellular complexes implicated in different steps of the viral life cycle. Large-scale physical analysis of PPI is conducted by yeast two-hybrid (Y2H) screens (Calderwood et al., 2007; de Chassey et al., 2008; Fossum et al., 2009; Lee et al., 2011; Rajagopala, Casjens, & Uetz, 2011; Shapira et al., 2009; Trigg et al., 2017; Uetz et al., 2006) and tandem affinity purification followed by mass spectrometry (TAP–MS) (Germain et al., 2014; Jager, Cimermancic, et al., 2011; Jager, Gulbahce, et al., 2011; Pichlmair et al., 2012; Ramage et al., 2015; Rozenblatt-Rosen et al., 2012). Y2H screens can analyze only binary interactions, whereas TAP–MS allows for the identification of more complex interactors. Then, the physical interactions identified with these methods are subjected to organization in functional networks by computational approaches. Despite of their rate of success in PPIs identification, both methodologies interrogating physical interactions of proteins fail to detect multiple types of PPIs. For example, interactions involving transmembrane proteins, or transient/weak interactions escape detection.

Similar to the physical methods, computational systems biology approaches successfully predict exploitation of biological processes by viruses (Kitano, 2002a, 2002b; Navratil, de Chassey, Combe, & Lotteau, 2011; Zak, Tam, & Aderem, 2014). These methods use gene expression and gene loss-of-function systemic analysis to identify potential host factor networks implicated in viral replication (Hirsch, 2010).

The main problem that the high-throughput functional approaches discussed earlier has is the poor data overlap resulting from these analyses.

This is even though these methods have a high degree of similarity or complementary. Therefore, validation of the discovered PPIs is required (Rozenblatt-Rosen et al., 2012; Shapira et al., 2009). This validation involves the demonstration of the interaction by coimmunoprecipitation analysis, particularly during infection. Prioritization analysis of PPIs can be implemented by combining the findings from the direct interaction approaches with comprehensive gene loss-of-function screenings.

An example of the effectiveness of this multifactorial approach for the identification of PPIs is a study aimed to identify host-influenza PPIs (Shapira et al., 2009). In this study, 35% of the 1745 genes initially found to be involved in viral protein interactions were demonstrated to influence viral replication by subsequent RNAi screening. This high rate of positive validation may have been the result of the selection of the final candidate genes by a combined analysis of the results obtained in Y2H screens using viral and host proteins (direct interactors), in transcriptional profiling experiments to define genes regulated by viral infection, and in *in silico* predictions of genes implicated in the protein networks found in the first two experimental approaches.



3. DIFFERENT PATHOGENS EXPLOIT SIMILAR CELLULAR PROCESSES

Comparison of the cellular complexes exploited by different pathogens indicates a significant overlap, leading to the definition of cellular processes implicated in infection (Brander & Walker, 2000; Davis et al., 2015; Dyer, Murali, & Sobral, 2008; Hirsch, 2010; Hiscott, Nguyen, Arguello, Nakhaei, & Paz, 2006; Pichlmair et al., 2012; Rozenblatt-Rosen et al., 2012; Shapira et al., 2009). Therefore, shared cellular complexes could constitute broad-spectrum therapeutic targets potentially impairing more than one pathogen. An example of this strategy is discussed later when we consider the implication of protein translation in the mechanism of replication of multiple viruses.

The overlap in the utilization of cellular complexes between different pathogens also brings support to the hypothesis that the innate immune system has evolved the ability of detecting patterns of pathogenicity. These patterns are generated by the activation of similar biological processes by different pathogens, indicating the existence of pathogen-induced processes (Dyer et al., 2008; Pichlmair et al., 2012; Vance, Isberg, & Portnoy, 2009).

The viral manipulation of the activity of type I interferon (IFN)-stimulated genes (ISG) illustrates this concept. Protein Kinase, RNA-activated (PKR) is an ISG that upon activation phosphorylates the α -subunit of the translation initiation factor eIF-2, inhibiting protein synthesis. Viral dsRNA is the main PKR activator in virus-infected cells, whereas the cellular protein PACT (Protein activator of PKR), that heterodimerizes with PKR, is an important activator of the kinase in stressed, noninfected cells in the absence of dsRNA (Li et al., 2006; Patel & Sen, 1998). HIV-1 evades the robust type I IFN response mounted by plasmacytoid dendritic cells in infected individuals by rewiring the PACT/PKR complex. In infected cells, HIV-1 TAR nucleates a complex with the viral protein Tat and the cellular proteins PACT and ADAR1 (Adenosine Deaminase Acting on RNA 1). In this complex, ADAR1 inhibits PACT PKR activatory function by direct interaction with PACT (Clerzius et al., 2013). Another example of pathogen-induced host complex rewiring will be discussed later in relation to the role of HIV-1 accessory proteins in the targeting of the ubiquitin/proteasome system to degrade different restriction factors.

Importantly, multiple viruses including Middle East Respiratory Syndrome Coronavirus, Herpes Simplex Virus, Ebola Virus, Influenza Virus, and Orf Virus also inactivate PACT, inhibiting PKR activation. Similar to HIV-1, ADAR1 influences replication of Measles Virus, Vesicular Stomatitis Virus, Polyoma DNA Virus, West Nile Virus, Yellow Fever Virus, Chikungunya Virus, Venezuelan Equine Encephalitis Virus, and viruses of the Paramyxoviridae and the Rhabdoviridae families (Chukwurah, Handy, & Patel, 2017). Therefore, the PACT-ADAR1-PKR axis is a cellular pathway commonly manipulated by different viruses that could constitute a therapeutic target with broad antiviral activity or pathogen-induced processes detected by the innate immune system. The hijacking of similar cellular pathways by different viruses also allows for the identification of broader biomarkers of viral infection.

Generally viral pathogens target cell cycle regulation, nuclear transport, and immune response processes (Dyer et al., 2008; Shapira et al., 2009). ssRNA(–) viral proteins tend to interact with processes implicated in the protection of RNA from degradation and RNA processing, whereas dsRNA viruses are more connected to protein degradation processes. DNA viruses, however, target proteins connecting the cell cycle with other processes such as chromosomal and transcriptional homeostasis (Pichlmair et al., 2012; Shapira et al., 2009).



4. VIRAL PROTEINS INTERACT WITH HOST PROTEINS IN COMPLEXES

RNAi-based screens have also been useful in identifying host factors implicated in viral replication. Interestingly, the number of host factors identified for the different viruses analyzed in these screens outnumber by a factor of 10 the proteins encoded by these viruses (Hirsch, 2010). This disproportion could illustrate the multifunctional character of viral proteins. In this case viral proteins are expected to establish multiple independent binary interactions with independent host proteins. Alternatively, viral proteins could interact with host proteins organized in complexes. Therefore, RNAi-mediated downregulation of the subunits of these protein complexes will produce similar phenotypes.

Correspondingly, Y2H screenings have found that viral proteins establish a disproportionate number of binary interactions with the human proteome. These numbers of interactions registered for viral proteins exceed the predicted number of interactions estimated from the analysis of the human interaction network (Shapira et al., 2009). For example, assessment of the interaction of each of the 10 viral proteins encoded by influenza with 12,000 human ORF through Y2H screening found 135 interactions with 87 human proteins (Shapira et al., 2009). These evidences support a model indicating that viral proteins evolve multiple direct interactions. Furthermore, computational analysis of host factors implicated in these pairwise interactions indicated that the host proteins occupy central positions, hub proteins, within the cellular interactome (Shapira et al., 2009). This higher than expected connectivity suggests that the direct interactions of viral proteins with host factors allow the access of the virus to cellular complexes.

Computational analysis of the interaction of human proteins binding to different viral proteins encoded by 35 different viruses showed that approximately $97\% \pm 9.1\%$ of the 1396 unique targeted human proteins interact at least with one other human protein (Dyer et al., 2008). These data indicate that the majority of the host factors implicated in viral replication are in complexes. These conclusions are further supported by TAP-MS and functional genomic studies. TAP-MS studies of the virus-host interactome indicate that viral proteins tend to interact with proteins that have multiple interacting partners and participate in more cellular pathways (protein hubs), and also with proteins that are central to many pathways and, therefore, occupy a more central position within these networks (protein bottlenecks)

(Dyer et al., 2008; Pichlmair et al., 2012; Shapira et al., 2009). For example, in a TAP–MS experiment were mapped 3787 complex associations between 54 viral proteins from different viruses and 1079 host proteins (Rozenblatt-Rosen et al., 2012), highlighting the high degree of connectivity of the interacting proteins.

As discussed above, some of the host factors predicted, by the combined transcriptional profiling and *in silico* analyses, to interact with host proteins implicated in direct binary contacts with influenza proteins (Y2H interactors) were demonstrated to influence viral replication in functional screenings (Shapira et al., 2009). These findings demonstrate that influenza, and potentially other viruses, hijacks cellular networks by combining physical and regulatory (transcriptional level) interactions with these pathways. This multilayer regulation offers further alternatives of blocking physical or regulatory interactions aiming to impair viral replication. Interestingly some of the host factors found in the networks of protein interacting with viral proteins are induced at the transcriptional level by infection in a type I IFN-independent manner (Shapira et al., 2009). This indicates that viruses modulate, at the transcriptional levels, the abundance of proteins that will engage in physical binary or multiprotein complex interactions. Therefore, computational analysis of the pathways discovered by functional genomics could lead to the identification of protein networks implicated in physical interactions with viral proteins.

Protein complexes established by viruses seem to determine the disease associated to the infection. For example, analysis of the interactome of E6 proteins from HPV types differing in their oncogenic potential associates with different subset of host proteins (Rozenblatt-Rosen et al., 2012). Therefore, disruption of these complexes could also prevent viral pathogenesis.

Almost 78% of the human–pathogen PPIs reported belong to HIV-1 (Dyer et al., 2008; Fahey et al., 2011; Ruepp et al., 2010). Generally host factors interacting with viral proteins tend to preserve the host protein interactions mapped in noninfected cells (Rozenblatt-Rosen et al., 2012; Yu et al., 2011), indicating that the normal host protein complexes rather than new virus-induced protein complexes are implicated in viral replication. This opens the opportunity to utilize the current body of knowledge on the human interactome to define the host complex hijacked by viruses. However, examples of virus-specific complex variants also occur. Among them, one of the best characterized is the complex between HIV-1 Vif and the transcriptional and protein degradation cellular machineries.

Vif removes APOBEC3 family restriction factors by targeting the ubiquitin ligase complex CUL5 to this restriction factor, and inducing its proteasome-mediated degradation. Other HIV-1 proteins, Vpu and Vpr, also exploit cullin-RING E3 ligase to degrade other restriction factors. Vif directly interacts with the substrate, the transcription factor CBF- β , the N-terminus of CUL5 (cullin-RING E3 ligase), and the heterodimeric substrate adaptor EloB/EloC. In turn, the C-terminus of CUL5 binds Rbx2 and recruits an E2 ubiquitin conjugation enzyme that mediates the transfer of poly-ubiquitin to the substrate proteins (Jager, Kim, et al., 2011; Zhang, Du, Evans, Yu, & Yu, 2011). CBF- β is required in this complex for the assembly of the Vif-CUL5 E3-ubiquitin-ligase complex, but not for the binding of Vif to the substrate (Zhang et al., 2011). CBF- β normally heterodimerizes with the RUNX family of transcription factors preventing its degradation and Vif competes with RUNX1 for binding to CBF- β (Guo et al., 2014).

The surface area buried at the interface of the interaction of Vif and CBF- β is large ($\sim 4800 \text{ \AA}^2$) suggesting that is undruggable (Salter, Morales, & Smith, 2014), as we will discuss later for other surfaces of PPIs. However, alanine scanning indicated that aa 5–11 of Vif are a hot spot (defined in a later section of this review) in the interaction with CBF- β . Furthermore, residues Phe68 and Ile55 of CBF- β establish important interactions with Trp5 in Vif through hydrophobic interactions (Desimmie, Smith, Matsuo, Hu, & Pathak, 2017), highlighting further opportunities for disruption of this complex with small molecules. Therefore, these structural characteristics of the Vif-CBF- β surface of interaction do not exclude its amenability to small-molecule interference, as we will show later for similar PPIs.



5. DEVELOPMENT OF SMALL MOLECULES DISRUPTING PPIs

Interfaces of PPI were considered undruggable for sometime, mainly because these surfaces of interactions are bigger than classical druggable protein surfaces, such as allosteric and catalytic sites in enzymes. The total area buried by the interactors in the binding site in PPIs is in average $1600 (\pm 400) \text{ \AA}^2$, with some proteins extending to $2000\text{--}4660 \text{ \AA}^2$. In contrast, surfaces of interaction between proteins and small molecules are approximately from 300 to 1000 \AA^2 (Arkin, Tang, & Wells, 2014; Lo Conte, Chothia, & Janin, 1999). In addition, the surface of protein-protein interaction,

although very variable in characteristics, was generally considered large patches of segmented complementary surfaces lacking small grooves or pockets (Hopkins & Groom, 2002; Jones & Thornton, 1996, 1997; Lo Conte et al., 1999).

Furthermore, attempts to find small molecules interfering with PPIs showed a very high failure rate (~60%) (Brown & Superti-Furga, 2003), therefore reinforcing the concept that surface of PPIs is not druggable. In correlation with this, estimates using very stringent concepts for drugs indicate that only approximately 10% of the human proteome could be targeted by oral, drug-like small molecule. These predictions also indicate that of the druggable gene products only between 5% and 50% were implicated in diseases (Hopkins & Groom, 2002).

Our understanding of the rules driving the PPIs importantly changed with the discovery that the different residues buried in the large surfaces of interactions contribute differentially to the binding strength of the interacting proteins (Clackson & Wells, 1995). This characteristic reduced considerably the area required to be targeted by small molecules. Interrogation of the surfaces of binding in PPIs by mutagenesis of individual residues (alanine scanning) indicated that mutation of a few residues implicated in these binding surfaces was sufficient to disrupt the PPIs. Residues importantly contributing to the binding free energy were defined as hot spots (Clackson & Wells, 1995). These residues tend to cluster in tightly packed regions in the center of the surfaces of interactions and are called hot regions (Clackson & Wells, 1995; Keskin, Ma, & Nussinov, 2005). Hot spots can be amenable to drug targeting due to their small surface area and important contribution to the total binding energy of the complex. However, not always hot spots are vulnerable to drugs, as additional topological constraints for drug binding limit the number of druggable hot spots (Zerbe, Hall, Vajda, Whitty, & Kozakov, 2012). In addition, *in silico* analysis indicated that interfaces could contain more than one hot region, and in some PPIs the establishment of the complex depends on the cooperative interaction of different hot spots within the region (Keskin et al., 2005).

By 2011, more than 40 PPIs have been reported to be targeted by small molecules (Morelli, Bourgeas, & Roche, 2011). Twenty-seven protein–protein complexes were included in the 2016 release of the small-molecule orthosteric modulators of PPIs database 2P2Idb. In this site are listed only those PPIs in which there is structural information of the protein–protein and protein–inhibitor complexes (Basse, Betzi, Morelli, & Roche, 2016).

The majority of the most successful PPIs inhibitors target hot-spot residues that cluster in small binding pockets (250–900 Å²), and the surface of interaction of the binding proteins has short primary sequences (Arkin et al., 2014).

Structural analysis of protein–inhibitor complexes indicated that in some cases small molecules interrupting PPIs bind to cavities not present in the surface of interaction of the protein–protein complex or in the interactor proteins when they are in isolation. These findings indicate some degree of adaptability in the surfaces of interaction initially considered lacking of druggable groves or pockets. According to *in silico* simulations the opening of some of these binding pockets is transient (Wells & McClendon, 2007).

Most of the small molecules interfering with PPIs bind directly to the implicated surfaces of interactions (orthosteric modulators) by targeting hot spot residues or by molecular mimicry of elements of secondary structures (Arkin et al., 2014; Basse et al., 2016; Fry, 2006; Wells & McClendon, 2007; Yin & Hamilton, 2005). However, several successful examples of small molecules disrupting PPIs act through their binding to allosteric sites (Crump et al., 2004; McMillan et al., 2000; Oswald et al., 2016; Roche et al., 2016; Szilagyi, Nussinov, & Csermely, 2013).

A well-characterized model of small-molecule disruption of a host PPI, deposited in the 2P2Idb database, is the MDM2/p53 interaction. MDM2 binds to p53, impairing its transcriptional activity and stability. The interaction surfaces are formed by a hydrophobic pocket (aa 25–109) in the N-terminus of MDM2 that is occupied by the hydrophobic side of an amphipathic α -helix in p53 (aa 19–26). Mutations at any of the four residues within the MDM2-binding pocket or of three residues within the p53 α -helix block this PPI (Moll & Petrenko, 2003). Small molecules interrupting this interaction bind to the p53-binding pocket in MDM2 (Vassilev et al., 2004).

Most of the data on small molecules targeting PPIs in the 2P2Idb database correspond to host–host PPIs and only two are relevant to viruses. That is the case of the interaction between the HPV proteins E2 and E1, and of allosteric inhibitors of HIV-1 integrase activity (ALLINIs) (Engelman, Kessl, & Kvaratskhelia, 2013). HPV E2 and E1 bind cooperatively to the viral origin of DNA replication and are required for initiation of DNA replication (Berg & Stenlund, 1997). Indandione derivatives that bind to the E2 transactivation domain block this interaction. One of these small molecules was shown to contact 7 of the 20 residues implicated in this interaction.

ALLINIs bind to the dimer interface of integrase to the same residues of the host protein LEDGF/p75 (Engelman et al., 2013). LEDGF/p75 is a chromatin-bound protein required for efficient HIV-1 cDNA integration (Llano et al., 2006; Shun et al., 2007), an essential step in the viral life cycle. LEDGF/p75 binds to the dimer interface of the catalytic core domain of integrase. Two residues in the integrase-binding domain of LEDGF/p75 are essential in this interaction. Asp366 in an interhelical loop of the host protein contacts, via hydrogen bonds, with residues Glu170 and His171 in one integrase monomer, whereas LEDGF/p75 residue Ile365 interacts with a hydrophobic pocket (Leu102, Ala128, Trp132) in the other integrase subunit (Cherepanov, Ambrosio, Rahman, Ellenberger, & Engelman, 2005; Cherepanov, Sun, et al., 2005). ALLINIs bind to the viral integrase at the LEDGF/p75-binding site, preventing the binding of LEDGF/p75 and affecting integrase catalytic core domain dimerization. This disrupts integrase assembly with viral DNA and allosterically inhibits its activity (Engelman et al., 2013). 2-(Quinolin-3-yl) acetic acid derivatives (LEDGINs), and in particular compound 6, showed potent inhibitory activity of HIV-1 replication (Christ et al., 2010). This compound establishes hydrogen bonds with integrase residues Glu170, His171, and Thr173 that are hot spots in the LEDGF/p75-binding site in integrase (Christ et al., 2010). As ALLINIs were more potent against HIV-1 in cells lacking LEDGF/p75, the contribution of LEDGF/p75-binding inhibition to their mechanism of action is not clear (Wang et al., 2012).

Another successful strategy in the design of small molecules interfering viral replication has been the targeting of multimeric viral RNA polymerases. Influenza Virus and Vaccinia Virus encode RNA polymerases formed by the essential interaction of several subunits. In Influenza Virus each of the three viral subunits of the polymerase, PB1, PB2, and PA, is required to assemble in a complex for the polymerase activity. Therefore, small molecules that interrupt the binding of PB1 into a hydrophobic groove in the C-terminus of PA (He et al., 2008) block replication of influenza A and B viruses (Muratore et al., 2012).

Similarly, Vaccinia Virus depends for replication on the formation of a trimeric complex between the DNA polymerase E9, the uracil DNA glycosylase D4, and the viral protein A20 (Stanitsa, Arps, & Traktman, 2006). Small molecules disrupting this complex by impairing the interaction D4–A20 inhibit replication of Vaccinia Virus and Cowpox Virus (Schormann et al., 2011).



6. TARGETING VIRUS–HOST PPIs WITH SMALL MOLECULES

There are two types of physical interactions that can be targeted to interrupt the utilization of cellular complexes by the virus: the virus–host interface or the host–host interface of complexes hijacked by the virus. Targeting protein interaction surfaces containing viral proteins is always associated with the selection of mutant viruses that escape control of the inhibitor (De Clercq, 2007; Strasfeld & Chou, 2010). This is the result of the highly mutagenic rate of viruses. However, targeting host complexes utilized by viruses at the host–host interface is expected to do not exhibit mechanisms of resistance, since host proteins are genetically more stable than their viral counterparts. Therefore, disruption of the host complexes exploited by viruses rather than interference of the virus–host protein interface is an attractive alternative to avoid the selection of escape mutants resistant to the inhibitors.

A major disadvantage of this strategy is the potential toxicity associated to the disruption of cellular complexes. Potentially, this could be minimized if the interruption of the complex is transient. Viral replication is a fast process that occurs in a few hours, and disruption of these complexes for a few hours could greatly affect the ability of the virus to replicate without affecting cellular viability. We will later discuss experimental findings supporting this view.

In addition, the high degree of functional overlap in the human proteome, that is absent in viruses because of their relative smaller genomes, could also ameliorate the toxicity caused by the disruption of host–host PPIs implicated in viral replication. The differences in functional redundancy suggest that the disruption of cellular complex will be more tolerable for the host than the pathogen. In support of this view, shRNA-based studies have showed that many host factors required for viral replication do not carry essential cellular functions. For example, out of 54,509 transcripts permanently targeted with lentivirus-encoded shRNAs, 17% were dispensable for the cell and deficient stable cell lines were developed but a fraction of them were required for efficient HIV-1 replication (Yeung, Houzet, Yedavalli, & Jeang, 2009).

Finally, targeting host complexes relevant for different viruses could allow the development of broad-spectrum antivirals.



7. SMALL MOLECULES DISRUPTING HOST COMPLEXES IMPLICATED IN VIRAL REPLICATION

Protein translation is essential for the virus and the cell. However, transient interference with protein synthesis at the initiation step has been demonstrated to be more detrimental for the pathogen than the host at a cellular level.

Both cellular mRNA and mRNA from different viruses carry a 7-methylguanosine cap (cap) on their 5' terminal nucleotide. Therefore, cap-dependent cellular translation is exploited by many different viruses. Critical to this translation mechanism is the eukaryotic initiation factor 4F complex (eIF4F). This complex is required for the efficient recruitment of ribosomes to capped mRNAs. The eIF4F complex includes a cap-binding protein (eIF4E), an RNA helicase (eIF4A), and a large scaffolding subunit (eIF4G). eIF4E protein binds to cap-mRNA and then recruits eIF4G that binds eIF4A. Therefore, inhibitors of eIF4E–eIF4G interaction (i.e., 4E2RCat (Cencic, Hall, et al., 2011)) or the helicase activity (hippuristanol) drastically impair translation.

4E2RCat completely blocks the replication of the human Alphacoronavirus 229E (Cencic, Desforges, et al., 2011) or Murine Norovirus 1 (Royall et al., 2015) at doses that impair approximately 40% of the cellular protein synthesis (Cencic, Desforges, et al., 2011) or do not alter at all cell viability. At these doses 4E2RCat affects eIF4E–eIF4G interaction as determined by coimmunoprecipitation (Royall et al., 2015). These results indicate that Coronaviruses are more dependable on eIF4F for ribosome recruitment mRNAs than the host.

Hippuristanol is a natural product that inhibits the activity of eIF4A by binding to its C-terminal domain. This compound impairs eIF4A RNA-binding, ATPase, and helicase activities. Hippuristanol specifically impaired replication of poliovirus (Bordeleau et al., 2006), Caliciviruses (Chaudhry et al., 2006), Human Cytomegalovirus (Lenarcic, Ziehr, De Leon, Mitchell, & Moorman, 2014), and Junin Virus (Linerio, Thomas, Boccaccio, & Scolaro, 2011). Despite the cell toxicity of hippuristanol (Bordeleau et al., 2006) at the doses and length of the treatment used in these reports, viral replication was affected specifically since this compound did not alter cellular viability. For example, 6-h treatment of RAW 264.7 cells with hippuristanol reduced cell viability by 10%, but Murine Norovirus production was reduced by over 3000-fold (Chaudhry et al., 2006).

As described earlier, eIF4E interacts with eIF4G and assembles into the eIF4F complex together with eIF4A on the capped mRNA. eIF4F complex formation leads to eIF4E phosphorylation at Ser209 by the eIF4G-associated kinase Mnk1. Inhibition of Mnk1 by CGP57380 specifically impairs protein synthesis in a variety of large DNA viruses (Walsh, Mathews, & Mohr, 2013). For example, this compound reduced by 10^2 -fold the replication of HSV-1 in quiescent primary fibroblasts without affecting cell viability (Walsh & Mohr, 2004). In addition, CGP57380 inhibits reactivation of Kaposi's sarcoma-associated Herpesvirus (Walsh et al., 2013). Because Mnk1 is not essential for cell growth or development, it is a potential therapeutic target (Ueda, Watanabe-Fukunaga, Fukuyama, Nagata, & Fukunaga, 2004).

There are other examples of small molecules inhibiting PPIs in host complex that are not essential for the cell. Some compounds targeting host-virus PPIs implicated in the earlier steps of the HIV-1 life cycle are in this category. For example, the HIV-1 entry inhibitor Maraviroc, that reached clinical use, acts through an allosteric mechanism. This drug binds to a hydrophobic pocket located in the transmembrane domain of CCR5, altering the conformation of the extracellular domain of CCR5 and preventing in this manner the binding of Env (Roche et al., 2016).

In addition, the small molecule PF74, that inhibits HIV-1 replication by triggering early uncoating (Shi, Zhou, Shah, Aiken, & Whitby, 2011), binds to a pocket encompassing the N-terminal domain-C-terminal domain interface of CA in the assembled capsid (Bhattacharya et al., 2014). Cleavage and polyadenylation-specific factor 6 (CPSF6) also binds to the same pocket in the capsid during HIV trafficking to the nucleus. This PPI delays uncoating, allowing HIV-1 to evade the innate immune activation through cytoplasmic nucleic acid sensor signaling (Rasaiyaah et al., 2013).



8. CONCLUDING REMARKS

We have presented literature evidences indicating that viruses interact for replication with cellular complexes. Within these complexes, viral proteins bind to cellular proteins that are central (bottleneck proteins) in the interacting networks and establish multiple interactions in the human interactome (hub proteins). These PPIs offer different opportunities for the development of small molecules to block viral replication. Virus-virus, virus-host, and host-host PPIs are amenable for pharmacological disruption. Different viruses utilize similar host protein complexes during

infection, showing overlap in the cellular processes hijacked. Targeting these shared cellular processes opens the possibility to generate broad-spectrum antivirals and general biomarkers of viral infection. Potentially, the innate immune system recognizes these shared pathogen-induced processes to counteract infection. A better understanding of this mechanism could greatly impact the development of stronger vaccine adjuvants.

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REFERENCES

- Arkin, M. R., Tang, Y., & Wells, J. A. (2014). Small-molecule inhibitors of protein-protein interactions: Progressing toward the reality. *Chemistry & Biology*, *21*(9), 1102–1114. <https://doi.org/10.1016/j.chembiol.2014.09.001>.
- Basse, M. J., Betzi, S., Morelli, X., & Roche, P. (2016). 2P2Idb v2: Update of a structural database dedicated to orthosteric modulation of protein-protein interactions. *Database (Oxford)*, *2016*. <https://doi.org/10.1093/database/baw007>.
- Berg, M., & Stenlund, A. (1997). Functional interactions between papillomavirus E1 and E2 proteins. *Journal of Virology*, *71*(5), 3853–3863.
- Bhattacharya, A., Alam, S. L., Fricke, T., Zadrozny, K., Sedzicki, J., Taylor, A. B., et al. (2014). Structural basis of HIV-1 capsid recognition by PF74 and CPSF6. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(52), 18625–18630. <https://doi.org/10.1073/pnas.1419945112>.
- Bordeleau, M. E., Mori, A., Oberer, M., Lindqvist, L., Chard, L. S., Higa, T., et al. (2006). Functional characterization of IREs by an inhibitor of the RNA helicase eIF4A. *Nature Chemical Biology*, *2*(4), 213–220. <https://doi.org/10.1038/nchembio776>.
- Brander, C., & Walker, B. D. (2000). Modulation of host immune responses by clinically relevant human DNA and RNA viruses. *Current Opinion in Microbiology*, *3*(4), 379–386.
- Brown, D., & Superti-Furga, G. (2003). Rediscovering the sweet spot in drug discovery. *Drug Discovery Today*, *8*(23), 1067–1077.
- Calderwood, M. A., Venkatesan, K., Xing, L., Chase, M. R., Vazquez, A., Holthaus, A. M., et al. (2007). Epstein-Barr virus and virus human protein interaction maps. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(18), 7606–7611. <https://doi.org/10.1073/pnas.0702332104>.
- Cencic, R., Desforges, M., Hall, D. R., Kozakov, D., Du, Y., Min, J., et al. (2011). Blocking eIF4E-eIF4G interaction as a strategy to impair coronavirus replication. *Journal of Virology*, *85*(13), 6381–6389. <https://doi.org/10.1128/JVI.00078-11>.
- Cencic, R., Hall, D. R., Robert, F., Du, Y., Min, J., Li, L., et al. (2011). Reversing chemoresistance by small molecule inhibition of the translation initiation complex eIF4F. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(3), 1046–1051. <https://doi.org/10.1073/pnas.1011477108>.
- Chaudhry, Y., Nayak, A., Bordeleau, M. E., Tanaka, J., Pelletier, J., Belsham, G. J., et al. (2006). Caliciviruses differ in their functional requirements for eIF4F components.

- The Journal of Biological Chemistry*, 281(35), 25315–25325. <https://doi.org/10.1074/jbc.M602230200>.
- Cherepanov, P., Ambrosio, A. L., Rahman, S., Ellenberger, T., & Engelman, A. (2005). Structural basis for the recognition between HIV-1 integrase and transcriptional coactivator p75. *Proceedings of the National Academy of Sciences of the United States of America*, 102(48), 17308–17313.
- Cherepanov, P., Sun, Z. Y., Rahman, S., Maertens, G., Wagner, G., & Engelman, A. (2005). Solution structure of the HIV-1 integrase-binding domain in LEDGF/p75. *Nature Structural & Molecular Biology*, 12(6), 526–532.
- Christ, F., Voet, A., Marchand, A., Nicolet, S., Desimmié, B. A., Marchand, D., et al. (2010). Rational design of small-molecule inhibitors of the LEDGF/p75-integrase interaction and HIV replication. *Nature Chemical Biology*, 6(6), 442–448. <https://doi.org/10.1038/nchembio.370>.
- Chukwurah, E., Handy, I., & Patel, R. C. (2017). ADAR1 and PACT contribute to efficient translation of transcripts containing HIV-1 trans-activating response (TAR) element. *The Biochemical Journal*, 474(7), 1241–1257. <https://doi.org/10.1042/BCJ20160964>.
- Clackson, T., & Wells, J. A. (1995). A hot spot of binding energy in a hormone-receptor interface. *Science*, 267(5196), 383–386.
- Clerzius, G., Shaw, E., Daher, A., Burugu, S., Gelinás, J. F., Ear, T., et al. (2013). The PKR activator, PACT, becomes a PKR inhibitor during HIV-1 replication. *Retrovirology*, 10, 96. <https://doi.org/10.1186/1742-4690-10-96>.
- Crump, M. P., Ceska, T. A., Spyropoulos, L., Henry, A., Archibald, S. C., Alexander, R., et al. (2004). Structure of an allosteric inhibitor of LFA-1 bound to the I-domain studied by crystallography, NMR, and calorimetry. *Biochemistry*, 43(9), 2394–2404. <https://doi.org/10.1021/bi035422a>.
- Davis, Z. H., Verschuere, E., Jang, G. M., Kleffman, K., Johnson, J. R., Park, J., et al. (2015). Global mapping of herpesvirus-host protein complexes reveals a transcription strategy for late genes. *Molecular Cell*, 57(2), 349–360. <https://doi.org/10.1016/j.molcel.2014.11.026>.
- de Chasse, B., Navratil, V., Tafforeau, L., Hiet, M. S., Aublin-Gex, A., Agaoglu, S., et al. (2008). Hepatitis C virus infection protein network. *Molecular Systems Biology*, 4, 230. <https://doi.org/10.1038/msb.2008.66>.
- De Clercq, E. (2007). The design of drugs for HIV and HCV. *Nature Reviews. Drug Discovery*, 6(12), 1001–1018. <https://doi.org/10.1038/nrd2424>.
- Desimmié, B. A., Smith, J. L., Matsuo, H., Hu, W. S., & Pathak, V. K. (2017). Identification of a tripartite interaction between the N-terminus of HIV-1 Vif and CBFbeta that is critical for Vif function. *Retrovirology*, 14(1), 19. <https://doi.org/10.1186/s12977-017-0346-5>.
- Dyer, M. D., Murali, T. M., & Sobral, B. W. (2008). The landscape of human proteins interacting with viruses and other pathogens. *PLoS Pathogens*, 4(2), e32. <https://doi.org/10.1371/journal.ppat.0040032>.
- Engelman, A., Kessl, J. J., & Kvaratskhelia, M. (2013). Allosteric inhibition of HIV-1 integrase activity. *Current Opinion in Chemical Biology*, 17(3), 339–345. <https://doi.org/10.1016/j.cbpa.2013.04.010>.
- Ewing, R. M., Chu, P., Elisma, F., Li, H., Taylor, P., Climie, S., et al. (2007). Large-scale mapping of human protein-protein interactions by mass spectrometry. *Molecular Systems Biology*, 3, 89. <https://doi.org/10.1038/msb4100134>.
- Fahey, M. E., Bennett, M. J., Mahon, C., Jager, S., Pache, L., Kumar, D., et al. (2011). GPS-Prot: A web-based visualization platform for integrating host-pathogen interaction data. *BMC Bioinformatics*, 12, 298. <https://doi.org/10.1186/1471-2105-12-298>.
- Fossum, E., Friedel, C. C., Rajagopala, S. V., Titz, B., Baiker, A., Schmidt, T., et al. (2009). Evolutionarily conserved herpesviral protein interaction networks. *PLoS Pathogens*, 5(9), e1000570. <https://doi.org/10.1371/journal.ppat.1000570>.

- Fry, D. C. (2006). Protein–protein interactions as targets for small molecule drug discovery. *Biopolymers*, 84(6), 535–552. <https://doi.org/10.1002/bip.20608>.
- Germain, M. A., Chatel–Chaix, L., Gagne, B., Bonneil, E., Thibault, P., Pradezynski, F., et al. (2014). Elucidating novel hepatitis C virus–host interactions using combined mass spectrometry and functional genomics approaches. *Molecular & Cellular Proteomics*, 13(1), 184–203. <https://doi.org/10.1074/mcp.M113.030155>.
- Guo, Y., Dong, L., Qiu, X., Wang, Y., Zhang, B., Liu, H., et al. (2014). Structural basis for hijacking CBF-beta and CUL5 E3 ligase complex by HIV-1 Vif. *Nature*, 505(7482), 229–233. <https://doi.org/10.1038/nature12884>.
- Havugimana, P. C., Hart, G. T., Nepusz, T., Yang, H., Turinsky, A. L., Li, Z., et al. (2012). A census of human soluble protein complexes. *Cell*, 150(5), 1068–1081. <https://doi.org/10.1016/j.cell.2012.08.011>.
- He, X., Zhou, J., Bartlam, M., Zhang, R., Ma, J., Lou, Z., et al. (2008). Crystal structure of the polymerase PA(C)–PB1(N) complex from an avian influenza H5N1 virus. *Nature*, 454(7208), 1123–1126. <https://doi.org/10.1038/nature07120>.
- Hirsch, A. J. (2010). The use of RNAi-based screens to identify host proteins involved in viral replication. *Future Microbiology*, 5(2), 303–311. <https://doi.org/10.2217/fmb.09.121>.
- Hiscott, J., Nguyen, T. L., Arguello, M., Nakhaei, P., & Paz, S. (2006). Manipulation of the nuclear factor-kappaB pathway and the innate immune response by viruses. *Oncogene*, 25(51), 6844–6867. <https://doi.org/10.1038/sj.onc.1209941>.
- Hopkins, A. L., & Groom, C. R. (2002). The druggable genome. *Nature Reviews. Drug Discovery*, 1(9), 727–730. <https://doi.org/10.1038/nrd892>.
- Jager, S., Cimermancic, P., Gulbahce, N., Johnson, J. R., McGovern, K. E., Clarke, S. C., et al. (2011). Global landscape of HIV–human protein complexes. *Nature*, 481(7381), 365–370. <https://doi.org/10.1038/nature10719>.
- Jager, S., Gulbahce, N., Cimermancic, P., Kane, J., He, N., Chou, S., et al. (2011). Purification and characterization of HIV–human protein complexes. *Methods*, 53(1), 13–19. <https://doi.org/10.1016/j.ymeth.2010.08.007>.
- Jager, S., Kim, D. Y., Hultquist, J. F., Shindo, K., LaRue, R. S., Kwon, E., et al. (2011). Vif hijacks CBF-beta to degrade APOBEC3G and promote HIV-1 infection. *Nature*, 481(7381), 371–375. <https://doi.org/10.1038/nature10693>.
- Jones, S., & Thornton, J. M. (1996). Principles of protein–protein interactions. *Proceedings of the National Academy of Sciences of the United States of America*, 93(1), 13–20.
- Jones, S., & Thornton, J. M. (1997). Analysis of protein–protein interaction sites using surface patches. *Journal of Molecular Biology*, 272(1), 121–132. <https://doi.org/10.1006/jmbi.1997.1234>.
- Keskin, O., Ma, B., & Nussinov, R. (2005). Hot regions in protein–protein interactions: The organization and contribution of structurally conserved hot spot residues. *Journal of Molecular Biology*, 345(5), 1281–1294. <https://doi.org/10.1016/j.jmb.2004.10.077>.
- Kitano, H. (2002a). Computational systems biology. *Nature*, 420(6912), 206–210. <https://doi.org/10.1038/nature01254>.
- Kitano, H. (2002b). Systems biology: A brief overview. *Science*, 295(5560), 1662–1664. <https://doi.org/10.1126/science.1069492>.
- Lee, S., Salwinski, L., Zhang, C., Chu, D., Sampankanpanich, C., Reyes, N. A., et al. (2011). An integrated approach to elucidate the intra-viral and viral–cellular protein interaction networks of a gamma-herpesvirus. *PLoS Pathogens*, 7(10), e1002297. <https://doi.org/10.1371/journal.ppat.1002297>.
- Lenarcic, E. M., Ziehr, B., De Leon, G., Mitchell, D., & Moorman, N. J. (2014). Differential role for host translation factors in host and viral protein synthesis during human cytomegalovirus infection. *Journal of Virology*, 88(3), 1473–1483. <https://doi.org/10.1128/JVI.02321-13>.
- Li, S., Peters, G. A., Ding, K., Zhang, X., Qin, J., & Sen, G. C. (2006). Molecular basis for PKR activation by PACT or dsRNA. *Proceedings of the National Academy of Sciences of*

- the United States of America*, 103(26), 10005–10010. <https://doi.org/10.1073/pnas.0602317103>.
- Linero, F. N., Thomas, M. G., Boccaccio, G. L., & Scolaro, L. A. (2011). Junin virus infection impairs stress-granule formation in Vero cells treated with arsenite via inhibition of eIF2alpha phosphorylation. *The Journal of General Virology*, 92(Pt. 12), 2889–2899. <https://doi.org/10.1099/vir.0.033407-0>.
- Llano, M., Saenz, D. T., Meehan, A., Wongthida, P., Peretz, M., Walker, W. H., et al. (2006). An essential role for LEDGF/p75 in HIV integration. *Science*, 314(5798), 461–464.
- Lo Conte, L., Chothia, C., & Janin, J. (1999). The atomic structure of protein–protein recognition sites. *Journal of Molecular Biology*, 285(5), 2177–2198.
- McMillan, K., Adler, M., Auld, D. S., Baldwin, J. J., Blasko, E., Browne, L. J., et al. (2000). Allosteric inhibitors of inducible nitric oxide synthase dimerization discovered via combinatorial chemistry. *Proceedings of the National Academy of Sciences of the United States of America*, 97(4), 1506–1511.
- Moll, U. M., & Petrenko, O. (2003). The MDM2–p53 interaction. *Molecular Cancer Research*, 1(14), 1001–1008.
- Morelli, X., Bourgeas, R., & Roche, P. (2011). Chemical and structural lessons from recent successes in protein–protein interaction inhibition (2P2I). *Current Opinion in Chemical Biology*, 15(4), 475–481. <https://doi.org/10.1016/j.cbpa.2011.05.024>.
- Muratore, G., Goracci, L., Mercorelli, B., Foeglein, A., Digard, P., Cruciani, G., et al. (2012). Small molecule inhibitors of influenza A and B viruses that act by disrupting subunit interactions of the viral polymerase. *Proceedings of the National Academy of Sciences of the United States of America*, 109(16), 6247–6252. <https://doi.org/10.1073/pnas.1119817109>.
- Navratil, V., de Chassey, B., Combe, C. R., & Lotteau, V. (2011). When the human viral infectome and disease networks collide: Towards a systems biology platform for the aetiology of human diseases. *BMC Systems Biology*, 5, 13. <https://doi.org/10.1186/1752-0509-5-13>.
- Oswald, C., Rappas, M., Kean, J., Dore, A. S., Errey, J. C., Bennett, K., et al. (2016). Intracellular allosteric antagonism of the CCR9 receptor. *Nature*, 540(7633), 462–465. <https://doi.org/10.1038/nature20606>.
- Patel, R. C., & Sen, G. C. (1998). PACT, a protein activator of the interferon-induced protein kinase, PKR. *The EMBO Journal*, 17(15), 4379–4390. <https://doi.org/10.1093/emboj/17.15.4379>.
- Pichlmair, A., Kandasamy, K., Alvisi, G., Mulhern, O., Sacco, R., Habjan, M., et al. (2012). Viral immune modulators perturb the human molecular network by common and unique strategies. *Nature*, 487(7408), 486–490. <https://doi.org/10.1038/nature11289>.
- Rajagopala, S. V., Casjens, S., & Uetz, P. (2011). The protein interaction map of bacteriophage lambda. *BMC Microbiology*, 11, 213. <https://doi.org/10.1186/1471-2180-11-213>.
- Ramage, H. R., Kumar, G. R., Verschueren, E., Johnson, J. R., Von Dollen, J., Johnson, T., et al. (2015). A combined proteomics/genomics approach links hepatitis C virus infection with nonsense-mediated mRNA decay. *Molecular Cell*, 57(2), 329–340. <https://doi.org/10.1016/j.molcel.2014.12.028>.
- Rasaiyaah, J., Tan, C. P., Fletcher, A. J., Price, A. J., Blondeau, C., Hilditch, L., et al. (2013). HIV-1 evades innate immune recognition through specific cofactor recruitment. *Nature*, 503(7476), 402–405. <https://doi.org/10.1038/nature12769>.
- Roche, M., Borm, K., Flynn, J. K., Lewin, S. R., Churchill, M. J., & Gorry, P. R. (2016). Molecular gymnastics: Mechanisms of HIV-1 resistance to CCR5 antagonists and impact on virus phenotypes. *Current Topics in Medicinal Chemistry*, 16(10), 1091–1106.
- Royall, E., Doyle, N., Abdul-Wahab, A., Emmott, E., Morley, S. J., Goodfellow, I., et al. (2015). Murine norovirus 1 (MNV1) replication induces translational control of the host

- by regulating eIF4E activity during infection. *The Journal of Biological Chemistry*, 290(8), 4748–4758. <https://doi.org/10.1074/jbc.M114.602649>.
- Rozenblatt-Rosen, O., Deo, R. C., Padi, M., Adelmant, G., Calderwood, M. A., Rolland, T., et al. (2012). Interpreting cancer genomes using systematic host network perturbations by tumour virus proteins. *Nature*, 487(7408), 491–495. <https://doi.org/10.1038/nature11288>.
- Ruepp, A., Waegelé, B., Lechner, M., Brauner, B., Dunger-Kaltenbach, I., Fobo, G., et al. (2010). CORUM: The comprehensive resource of mammalian protein complexes—2009. *Nucleic Acids Research*, 38(Database issue), D497–501. <https://doi.org/10.1093/nar/gkp914>.
- Salter, J. D., Morales, G. A., & Smith, H. C. (2014). Structural insights for HIV-1 therapeutic strategies targeting Vif. *Trends in Biochemical Sciences*, 39(9), 373–380. <https://doi.org/10.1016/j.tibs.2014.07.001>.
- Schormann, N., Sommers, C. I., Prichard, M. N., Keith, K. A., Noah, J. W., Nuth, M., et al. (2011). Identification of protein–protein interaction inhibitors targeting vaccinia virus processivity factor for development of antiviral agents. *Antimicrobial Agents and Chemotherapy*, 55(11), 5054–5062. <https://doi.org/10.1128/AAC.00278-11>.
- Shapira, S. D., Gat-Viks, I., Shum, B. O., Dricot, A., de Grace, M. M., Wu, L., et al. (2009). A physical and regulatory map of host–influenza interactions reveals pathways in H1N1 infection. *Cell*, 139(7), 1255–1267. <https://doi.org/10.1016/j.cell.2009.12.018>.
- Shi, J., Zhou, J., Shah, V. B., Aiken, C., & Whitby, K. (2011). Small-molecule inhibition of human immunodeficiency virus type 1 infection by virus capsid destabilization. *Journal of Virology*, 85(1), 542–549. <https://doi.org/10.1128/JVI.01406-10>.
- Shun, M. C., Raghavendra, N. K., Vandegraaff, N., Daigle, J. E., Hughes, S., Kellam, P., et al. (2007). LEDGF/p75 functions downstream from preintegration complex formation to effect gene-specific HIV-1 integration. *Genes & Development*, 21(14), 1767–1778.
- Stanitsa, E. S., Arps, L., & Traktman, P. (2006). Vaccinia virus uracil DNA glycosylase interacts with the A20 protein to form a heterodimeric processivity factor for the viral DNA polymerase. *The Journal of Biological Chemistry*, 281(6), 3439–3451. <https://doi.org/10.1074/jbc.M511239200>.
- Strasfeld, L., & Chou, S. (2010). Antiviral drug resistance: Mechanisms and clinical implications. *Infectious Disease Clinics of North America*, 24(3), 809–833. <https://doi.org/10.1016/j.idc.2010.07.001>.
- Szilagyi, A., Nussinov, R., & Csermely, P. (2013). Allo-network drugs: Extension of the allosteric drug concept to protein–protein interaction and signaling networks. *Current Topics in Medicinal Chemistry*, 13(1), 64–77.
- Trigg, S. A., Garza, R. M., MacWilliams, A., Nery, J. R., Bartlett, A., Castanon, R., et al. (2017). CrY2H-seq: A massively multiplexed assay for deep-coverage interactome mapping. *Nature Methods*, 14(8), 819–825. <https://doi.org/10.1038/nmeth.4343>.
- Ueda, T., Watanabe-Fukunaga, R., Fukuyama, H., Nagata, S., & Fukunaga, R. (2004). Mnk2 and Mnk1 are essential for constitutive and inducible phosphorylation of eukaryotic initiation factor 4E but not for cell growth or development. *Molecular and Cellular Biology*, 24(15), 6539–6549. <https://doi.org/10.1128/MCB.24.15.6539-6549.2004>.
- Uetz, P., Dong, Y. A., Zeretzke, C., Atzler, C., Baiker, A., Berger, B., et al. (2006). Herpesviral protein networks and their interaction with the human proteome. *Science*, 311(5758), 239–242. <https://doi.org/10.1126/science.1116804>.
- Vance, R. E., Isberg, R. R., & Portnoy, D. A. (2009). Patterns of pathogenesis: Discrimination of pathogenic and nonpathogenic microbes by the innate immune system. *Cell Host & Microbe*, 6(1), 10–21. <https://doi.org/10.1016/j.chom.2009.06.007>.
- Vassilev, L. T., Vu, B. T., Graves, B., Carvajal, D., Podlaski, F., Filipovic, Z., et al. (2004). In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science*, 303(5659), 844–848. <https://doi.org/10.1126/science.1092472>.

- Walsh, D., Mathews, M. B., & Mohr, I. (2013). Tinkering with translation: Protein synthesis in virus-infected cells. *Cold Spring Harbor Perspectives in Biology*, 5(1), a012351. <https://doi.org/10.1101/cshperspect.a012351>.
- Walsh, D., & Mohr, I. (2004). Phosphorylation of eIF4E by Mnk-1 enhances HSV-1 translation and replication in quiescent cells. *Genes & Development*, 18(6), 660–672. <https://doi.org/10.1101/gad.1185304>.
- Wang, H., Jurado, K. A., Wu, X., Shun, M. C., Li, X., Ferris, A. L., et al. (2012). HRP2 determines the efficiency and specificity of HIV-1 integration in LEDGF/p75 knockout cells but does not contribute to the antiviral activity of a potent LEDGF/p75-binding site integrase inhibitor. *Nucleic Acids Research*, 40(22), 11518–11530. <https://doi.org/10.1093/nar/gks913>.
- Wells, J. A., & McClendon, C. L. (2007). Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. *Nature*, 450(7172), 1001–1009. <https://doi.org/10.1038/nature06526>.
- Wong, P., Althammer, S., Hildebrand, A., Kirschner, A., Pagel, P., Geissler, B., et al. (2008). An evolutionary and structural characterization of mammalian protein complex organization. *BMC Genomics*, 9, 629. <https://doi.org/10.1186/1471-2164-9-629>.
- Yeung, M. L., Houzet, L., Yedavalli, V. S., & Jeang, K. T. (2009). A genome-wide short hairpin RNA screening of jurkat T-cells for human proteins contributing to productive HIV-1 replication. *The Journal of Biological Chemistry*, 284(29), 19463–19473. <https://doi.org/10.1074/jbc.M109.010033>.
- Yin, H., & Hamilton, A. D. (2005). Strategies for targeting protein-protein interactions with synthetic agents. *Angewandte Chemie (International Edition in English)*, 44(27), 4130–4163. <https://doi.org/10.1002/anie.200461786>.
- Yu, H., Tardivo, L., Tam, S., Weiner, E., Gebreab, F., Fan, C., et al. (2011). Next-generation sequencing to generate interactome datasets. *Nature Methods*, 8(6), 478–480. <https://doi.org/10.1038/nmeth.1597>.
- Zak, D. E., Tam, V. C., & Aderem, A. (2014). Systems-level analysis of innate immunity. *Annual Review of Immunology*, 32, 547–577. <https://doi.org/10.1146/annurev-immunol-032713-120254>.
- Zerbe, B. S., Hall, D. R., Vajda, S., Whitty, A., & Kozakov, D. (2012). Relationship between hot spot residues and ligand binding hot spots in protein-protein interfaces. *Journal of Chemical Information and Modeling*, 52(8), 2236–2244. <https://doi.org/10.1021/ci300175u>.
- Zhang, W., Du, J., Evans, S. L., Yu, Y., & Yu, X. F. (2011). T-cell differentiation factor CBF-beta regulates HIV-1 Vif-mediated evasion of host restriction. *Nature*, 481(7381), 376–379. <https://doi.org/10.1038/nature10718>.