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# New horizons for antiviral drug discovery from virus–host protein interaction networks

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Viruses are recurrent socio economical and health problems each year worldwide. Current drugs are mainly directed against viral components and select resistant strains that urge the need to develop new antiviral therapeutics. High-throughput screening technologies now allow to draw comprehensive genome-wide maps of physical and genetic virus–host interactions. This has been done recently for several viruses such as HIV, HCV, DENV and FLUAV and revealed a wealth of potential antiviral cellular targets. Systems-level analysis of virus–host protein networks and subnetworks begins to uncover several specific points of intervention for a human centered drug development. We present here this new paradigm in antiviral drug discovery together with the first promising antiviral molecules.

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## Introduction

Viruses are major threats for the populations. They kill millions of people worldwide and cause millions of work day lost each year. Preventive vaccines can provide life-long protection but concern only a limited number of viruses.

The current therapeutic arsenal against viral diseases is mainly focused on the targeting of viral components and more specifically of viral enzymes (Figure 1). An advantage of this approach is that it is only targeting viral components that are essential for the replication cycle so that the drugs are more likely to induce minimal side toxic effect. A disadvantage of targeting viral components is that the diversity of druggable viral targets is very limited owing to the small genome of most viruses of medical interest. In addition, these viruses often have the ability to mutate rapidly because of their low-fidelity replication process. This genetic diversity confers a robustness against viral targets oriented drugs and allows the emergence of strains resistant to the treatment. For example, treatment of the 2009 pandemic influenza A H1N1 virus with neuraminidase inhibitors led to the emergence of resistant viruses in the population [1]. New drugs allowing combination therapy to increase efficiency, avoid selection of resistant strains and enlarge the therapeutic arsenal are clearly needed for a wide diversity of viruses to provide a better protection to the populations. This mainly relies on alternative strategies for drug discovery.

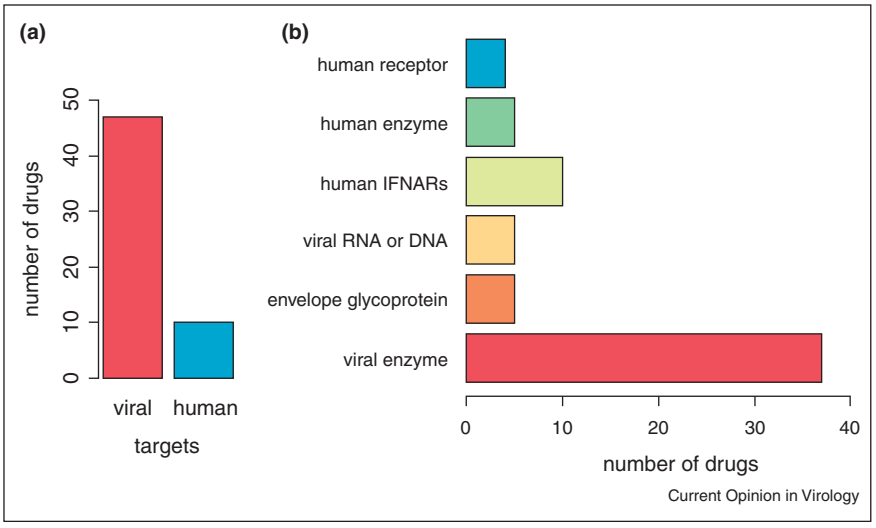
All viruses are obligate intracellular parasites. They can only replicate by entering a host cell and by using or taking the control of cellular functions that are essential for the production and assembly of their building blocks. The global understanding of a viral infection at the cell level therefore depends on the knowledge of all possible interactions between the viral and the host components. Most functions being supported by proteins interacting with other proteins, it is crucial to identify all cellular proteins interacting with viral proteins in order to identify the cellular functions that are mandatory for viral replication and to develop innovative therapeutic strategies targeting cellular proteins or functions.

In this review, we present a state of the art of virus–host protein interaction knowledge with a focus on works that aimed at comprehensively identify host factors relevant for viral replication. We discuss how this knowledge will rationally orientate the antiviral drug discovery towards critical cellular proteins to improve therapeutic intervention and reduce safety risks (Figure 2).

## The landscape of virus–host interactions

Until 2007, physical protein–protein interactions occurring during viral replication have essentially been identified by low-throughput experiments. Several databases have been developed to integrate all these data scattered in the literature. Among them, VirusMint [2] and VirHostNet [3] store respectively 1854 and 3113 virus–host protein–protein interactions (Table 1A). Although these databases contain the largest and the most confident datasets publicly available, thousands of interactions still remain to be extracted from the literature. By combining original data mining tools and a systematic effort of

**Figure 1**



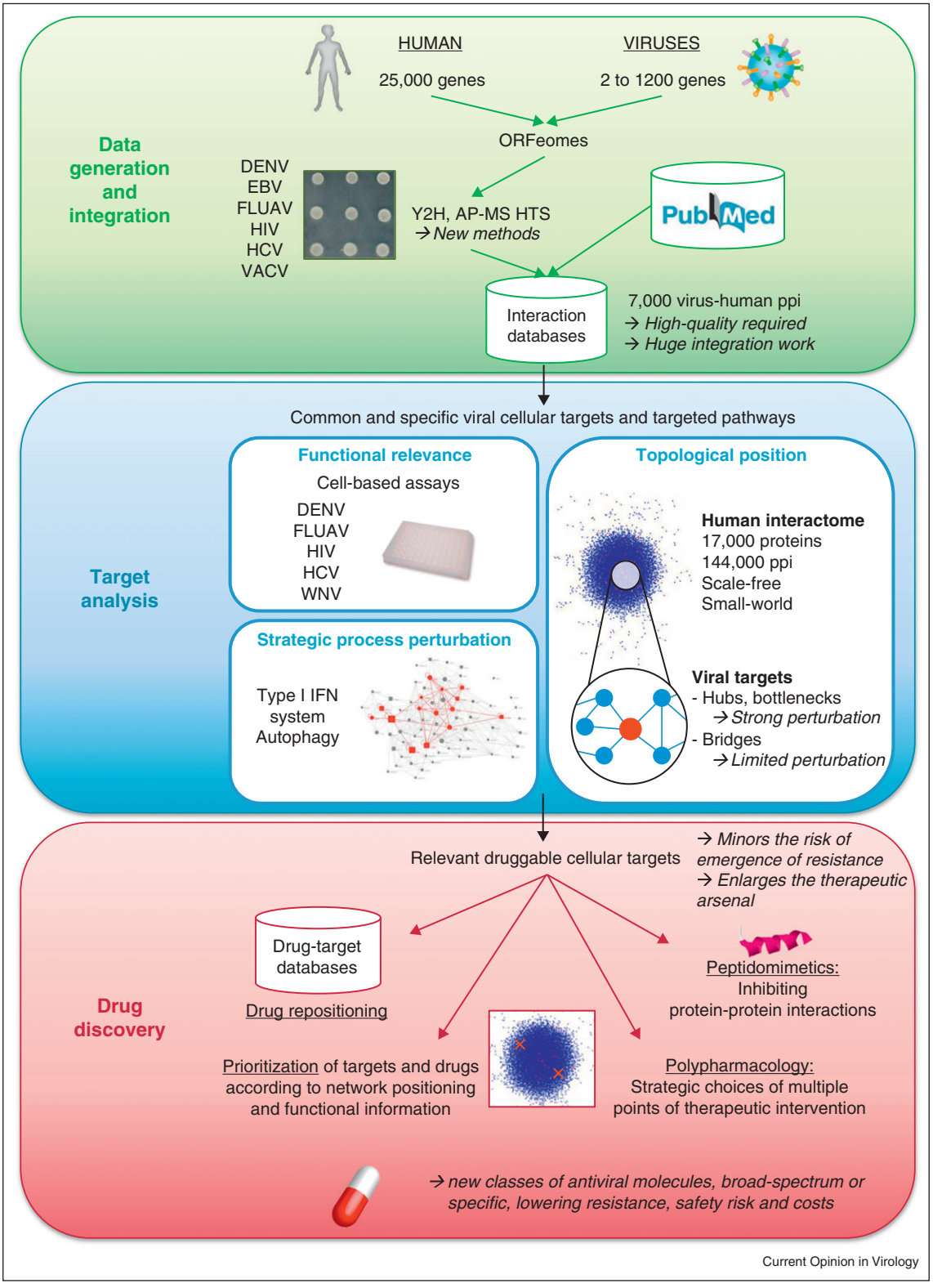

Current FDA-approved antiviral drugs and their targets. **(a)** Number of FDA-approved antiviral drugs targeting viral and human cellular elements. All interferon molecules have been gathered as a single molecule. **(b)** Distribution of FDA-approved antiviral drugs according to the nature of their target: human receptor, enzyme or IFNARs or viral RNA, DNA, envelope glycoprotein or enzyme. (Source DrugBank).

manual curation, an up to date dataset of nearly 7000 virus–host protein interactions will soon be provided to the scientific community (de Chassey, personal communication). However, for a comprehensive identification of cellular functions mandatory for viral replication, additional data generation and integration are still necessary. This is not an easy task considering that the human genome encodes more than 20 000 protein-coding genes [4]. The diversity of the virus realm, from the smallest virus (hepatitis delta virus, 2 mature proteins) to the largest DNA viruses (hundreds of proteins), illustrates the huge number of virus–host interactions remaining to be identified to obtain a complete picture of viral infections. The construction of the first comprehensive virus–host interactomes was rendered possible by several technological breakthroughs – the construction of physical viral and human ORFeomes [5,6] – and the development of high-throughput technologies such as yeast-two-hybrid (Y2H) or affinity purification followed by mass spectrometry (AP-MS) [7]. The two technologies appear to cover different aspects of the interactome, binary interaction (Y2H) versus complex identification (AP-MS), and provide complementary information for a better picture of interaction networks [8]. The high throughput Y2H approach was used to construct the first two genome-wide virus–host interactomes for the Epstein-Barr virus (EBV) and hepatitis C (HCV) viruses [9,10]. Since then, with the same technology, genome-wide dengue virus (DENV)–host, influenza virus (FLUAV)–host and vaccinia virus (VACV)–host interactomes have been published [11,12••,13]. More recently, the first systematic AP-MS study has been carried out for human immunodeficiency virus (HIV) and identified 435 human protein targets [14••]. The weak but significant overlap with literature data strongly underlines the necessity for a diversification method and for iterative implementation of the same method in order to construct complete virus–host interactomes with highly confident interaction dataset [15]. Between 5 and 20% of the targeted cellular proteins and biological processes are shared by all the viruses studied so far [16•]. These common targets or pathways are good candidates to identify broad spectrum antiviral drugs.

## Searching for biologically relevant targets

A challenging goal is to identify among all these biophysical interactions those that are biologically relevant. High-throughput RNA interference technology contributes to provide such information. The development of genome-wide siRNA libraries and large-scale assays monitoring viral replication constituted major breakthroughs towards this goal. Since 2008, several laboratories performed large-scale siRNA screens to identify host factors that are essential for infection and replication of viruses causing public health problems (FLUAV [12,17–22], HCV [23,24], DENV [25], WNV [26]). As for the physical interaction screens, overlaps of datasets for a given virus are extremely low although significant [27•,14••,28]. Experimental conditions and statistical analysis dramatically impact the results of the screens. However, a strong overlap is observed at the level of biological processes. Analyzing the screens at this level allows discriminating virus species, supporting the biological relevance of these datasets [27•,29]. Interestingly,

**Figure 2**




Overview of the innovative strategy from virus–host interaction data generation to the rational discovery of new antiviral drug candidates.

**Table 1**

**List of relevant interaction databases**

A. Databases providing lists of protein–protein interactions between viruses and human

| Database | Website | Manual curation | Last release date |
|---|---|---|---|
| VirHostNet | http://pbildb1.univ-lyon1.fr/virhostnet | x | 2010/11 |
| VirusMINT | http://mint.bio.uniroma2.it/virusmint | x | 2008/09 |
| HPIDB | http://agbase.msstate.edu/hpi/main.html | | 2012/04 |
| Phisto | http://www.phisto.boun.edu.tr | | 2012/03 |
| BIND | http://bind.ca | x | 2006/07 |
| IntAct | http://www.ebi.ac.uk/intact | x | 2012/06 |
| PIG | Discontinued | | – |

B. Databases providing lists of drug-target interactions

| Database | Website | Last release date |
|---|---|---|
| DrugBank | www.drugbank.ca | 2011/01 |
| Therapeutic Target DB | http://xin.cz3.nus.edu.sg/group/ttd/ttd.asp | 2011/08 |
| PharmGKB | www.pharmgkb.org | 2012/06 |
| STITCH | http://stitch.embl.de | 2012/04 |
| SuperTarget | http://insilico.charite.de/supertarget | 2011/11 |
| ChEMBL | www.ebi.ac.uk/chembldb | 2012/02 |

while 0.5–4% of genome-wide siRNA screen datasets are known interactors of viruses, 12% of cellular interactors appear to be essential host factors in the works of Shapira *et al.* [12••] on FLUAV and Jäger *et al.* on HIV [14••]. This observation justifies an orthogonal approach for the identification of cellular targets that are the most functionally relevant for the development of new antiviral drugs.

## Viral targets in the cellular protein network

The host protein interactome is an abstract representation of the cell where proteins are nodes and interactions between proteins are edges (Box 1). The human interactome stored in iRefweb database [30], which integrates several publicly available protein interaction databases, is composed of more than 17 000 proteins connected by more than 144 000 interactions. Most of the nodes make only few connections with other nodes while a few hub nodes are highly connected. The topological structure of this network is thus not random but is commonly believed to be scale-free as its degree distribution tends to follow a power law [31]. This network feature displays a high level of robustness against random node removal but is critically dependent on a limited number of highly connected proteins since targeted attacks of hubs dramatically change its structure [32]. A corollary to this architecture is that the distance between any couple of nodes is very short so that these networks are considered to be small-world [33]. Hence, the specific perturbation of a protein is susceptible to modify the behavior of many other proteins in the network.

Considering these features, one can ask whether viral targets are randomly distributed in the human protein network or occupy specific positions. The first clues came from analyzing the data from several virus–host protein interaction databases [34]. From these analyses, viruses appeared to significantly target hub and bottleneck proteins. This trend was later confirmed from unbiased large-scale virus–host protein interaction datasets also

**Box 1 Topological measures of interactomes. For topological analysis, protein interaction maps are converted into graphs with proteins as nodes and interactions as edges. A variety of graph-theoretical measures are thus used to describe these graphs.**

- The degree of a protein is a local centrality measure indicating its number of interactions with its direct neighbour proteins. Proteins with a high degree, that is, highly connected to other proteins, are called 'hubs'.
- The shortest path length between two proteins corresponds to the minimum number of interactions to go from one protein to the other. The average path length of a network summarizes the typical separation between two proteins.
- The betweenness of a protein is a global centrality measure that reflects the number of shortest paths that go through this protein. Proteins with a high betweenness, that is, highly central in the network, are called 'bottlenecks'.
- The bridging centrality of a protein reflects the significance of this protein in maintaining connectivity in the network. A high bridging centrality implies a high betweenness and a low degree relatively to the neighbourhood. Proteins with a high bridging centrality, that is, located between densely connected regions in the network, are called 'bridges'.
- The local clustering coefficient of a protein measures the tendency of its neighbour proteins to be linked. The global clustering coefficient of a network is the average local clustering coefficient of all proteins of the network. It reflects the tendency of the network to form highly interconnected, redundant and cohesive regions. High global clustering coefficient combined with small average path length is typical of small-world network architecture.

demonstrating that targeted cellular proteins are closely interconnected [16•]. Apart from these proteins that are Achille's heel of the human interactome, viruses also massively attack bridging proteins [35]. These proteins may be involved in cross-talks between cellular functions. Interestingly, their specific removal has a lower impact on network topology than hubs. In a drug discovery perspective it can be anticipated that targeting bridging proteins with small molecules would generate minimal toxicity or adverse side effect.

## Networking the innate immune processes

Facing the complexity of an interactome at the cellular level, it may also be worth considering working at the level of intracellular biological processes that are highly relevant to viral infection. With a systems biology approach it becomes possible to build a detailed interactome map delineating subnetworks of proteins involved in these strategic processes, to explore the theoretical robustness of such subnetworks and to study their perturbation. One expectation of this approach is the identification of multiple cellular therapeutic targets from one or several subnetworks for the development of polypharmacology as well as of specific and large spectrum anti-viral drugs.

Hosts display several innate defense mechanisms allowing a rapid response to an infection. Pathogens have evolved the ability to escape this response or to exploit it to their own benefit. Several attempts have been developed to collect and integrate interaction data relative to this system. For example, InnateDB is a gateway to biomolecules and their interactions that govern the innate immune response [36]. It integrates manually curated data and data from public databases, providing an invaluable resource to facilitate the exploration of innate immunity in a systems-oriented manner. Oda and Kitano proposed a comprehensive map of the toll-like receptor (TLR) signaling network [37]. TLRs are sensors of pathogens whose activation turns on a series of anti-infection mechanisms including the type I interferon pathway. The TLR signaling network has a bow-tie architecture where the protein MYD88 is a non-redundant core and therefore a major weakness of the system. Interestingly, A46R from VACV and NS5A from HCV target MYD88 and severely impair TLRs signaling [38,39]. Globally, the type I interferon system is massively attacked by viruses with a broad spectrum of targeting strategies and outcomes, from different levels of inhibition (HCV) to diversion (HIV) [40].

Autophagy is another ancestral innate cell defense mechanism that can drive pathogens to degradative lysosomes but that can also be exploited by pathogens to their own benefit [41]. A subnetwork of proteins involved in this process has been recently established and extended to new cellular protein partners [42,43]. Its targeting by viruses is significant especially by RNA viruses [42].

## Antiviral drugs targeting host factors

The targeting of host factors can dramatically enlarge the pool of druggable targets. This change in the paradigm of drug discovery for viral diseases is recent and is already awarded with several promising molecules. The Maraviroc (Celsentri) developed by Pfizer has been FDA-approved in 2007 as a CCR5 co-receptor antagonist for the treatment of HIV [44]. The targeting of cellular host receptors is also a strategy proposed by NexBio, a start-up biopharmaceutical company. Its molecule DAS181, currently in phase II clinical trial, is a recombinant sialidase fusion protein administered by oral inhalation route and that inactivates influenza virus receptors in patient's respiratory tract [45]. Functional Genetics develop a human monoclonal antibody against TSG101, a protein [46] exposed on the surface of infected cells by a variety of viruses. This antibody is currently undergoing phase I clinical trial for an influenza indication.

One way to speed up drug discovery at a low financial risk is to find new indications for existing drugs. This process is called drug repositioning or repurposing [47]. Several libraries of small molecules are composed of known biologically active compounds. Some are even dedicated to FDA-approved drugs, like the Prestwick Chemical Library. Cell-based high-throughput screens (HTS) have been performed to identify regulators of replication for viruses like influenza virus or hepatitis C virus. Molecules have been identified that target intracellular host proteins, with either antiviral or proviral activities [48,49]. Other HTS focused on innate immune processes, for instance to identify approved molecules enhancing the interferon signaling pathway. In a secondary screen selected drugs are tested for their antiviral activity [50].

A promising refinement of this approach is the rational pre-selection of drugs using the physical and genetic virus–host interaction data. Several databases are interesting resources that combine drug data with drug target information (Table 1B). These databases show partially overlapping targets (254 in common for DrugBank and Therapeutic Target Database for instance) indicating that the number of drug targets is still an opened question [51]. Interestingly, according to our own virus–host interaction dataset, numerous drug targets from these databases are also targeted by a viral protein (267 targets from DrugBank, resampling test, $p$-value $< 10^{-4}$, de Chassey, unpublished data).

The drug repositioning strategy is an evident consequence of the genome-wide exploration of genetic and physical virus–host interactions. For example, Karlas *et al.* identified CLK1 as an essential host factor for the influenza virus and showed that the chemical inhibitor of

this protein, TG003, inhibited the viral replication. In agreement with the known regulation of alternative splicing by CLK1, TG003 reduced the level of spliced viral M2 RNA [19]. Similarly, König *et al.* showed that KN-93, a specific inhibitor of CAMK2B, induced a strong inhibition of influenza viral replication at 100 μM with no obvious side toxic effect *in vitro* [18]. Combining physical and genetic virus–host interaction data, it is also possible to draw a hierarchy of targets and drugs with a higher potential of modulating viral replication. This led to the identification of HSP90AA1 which appeared particularly critical in FLUAV replication. Drugs targeting this protein, like Geldanamycin, are indeed highly efficient in inhibiting influenza virus replication [27].

## Perspectives

Viral infections are major public health problem with emerging, re-emerging diseases and appearance of resistance to conventional drug therapies. The recent shift in drug discovery, from a virus-centered view to host-oriented targets, unveils a wealth of potential antiviral drugs essentially through a drug repositioning strategy. High-throughput screens for virus–host interactions considerably enriched our knowledge of viral infections and reinforced the rational for this drug discovery process. Nevertheless, the systems based approach to drug discovery against viral infections is still in its infancy as the first HTS genome-wide screens were performed in the late years of the previous decade. New screens are clearly needed as the picture of interactions is far from being complete and concerns only a small subset of viruses. Various HTS methods have to be used or developed and efforts for a large-scale application of the protein complementation assay technology (PCA) [52], the luminescence-based mammalian interactome mapping (LUMIER) [53] or the mammalian protein–protein interaction trap (MAPPIT) [54] will undoubtedly be fruitful.

The integration of multiple orthogonal datasets coming from other '-omics' approaches (metabolomics, transcriptomics…) will be decisive to further strengthen the rational identification of relevant targets. With these integrative approaches, the drug discovery process will move from a protein centric view toward a network centric view. For this, a crucial issue will be the consideration of the position of the therapeutic targets within the network. For example, one would be cautious with drugs targeting proteins whose localization is susceptible to induce undesired side network pertubation potentially responsible for side toxic effect. The potential of this new vision will also be illustrated by the development of a polypharmacology strategy using compounds with multiple targets or combination of compounds acting on different targets rationally selected in the network.

Finally, network biology approaches are concomitant to the emerging field of protein–protein interaction inhibitors [55••]. Drug developers are today essentially focusing on the oncology area but the accumulation of data on virus–host protein–protein interactions offers opportunities for new classes of antivirals. Mapping technologies, structural and computational tools are being developed to delineate protein complex interfaces as well as short peptide sequences involved in the interactions [56]. This approach provides an invaluable information to enlarge the chemical space spanned by libraries and should motivate innovative strategies for identifying these peptides and developing peptidomimetics.

## Conclusion

Recent efforts to integrate protein interaction data from the literature and to develop technologies for the screening of genome-wide protein interaction between a virus and its host now offer intriguing opportunities for antiviral drug discovery. Therapeutic relevance of cellular targets can be explored by placing these proteins in the context of the human protein interaction network and by the use of computational and network-based tools. In addition, cell-based assays can provide orthogonal information for target validation before initiation of a drug discovery process. Although these approaches are still in their infancy, a wide diversity of new classes of antiviral molecules with reduced safety risk and cost and low resistance induction is already arising through drug repositioning strategies, peptidomimetics or polypharmacology.

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- •• of outstanding interest

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