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REVIEW

Rational design of protein-protein interaction inhibitors

Cite this: DOI: 10.1039/x0xx00000x

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Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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Protein-protein interactions are at the heart of most physiopathological processes. As such, they have attracted considerable attention for designing drugs of the future. Although initially considered as high-value but difficult to identify, low molecular weight compounds able to selectively and potently modulate protein-protein interactions have recently reached clinical trials. Along with high-throughput screening of compound libraries, combining structural and computational approaches has boosted this formerly minor area of research into a currently tremendous active field. This review highlights the very recent developments in the rational design of protein-protein interaction inhibitors.

Introduction

Drug discovery is a long, costly, multi-step endeavour which aims at reducing all possible risks to deliver a novel therapeutic solution to previously unmet clinical needs. To reduce chemical risks, empirical rules are used to filter the chemical space and retain drug-like low molecular weight compounds. Reduction of the biological risk is addressed by considering privileged target families (e.g., G protein-coupled receptors, kinases) whose activation/inhibition by drug-like compounds are likely to correct or reverse pathological states. Until recently, mostly single macromolecules (proteins, nucleic acids) have been considered as potential drug targets. Out of the 68,000 proteins currently annotated in UniProt for the human proteome,[1] only about 300 targets[2] have been addressed by current drugs, and the large majority of single targets are still awaiting first-in class drugs.

Beside single targets, large scale genomics and proteomics[3] have identified complex networks of targets and pathways regulating physiopathological processes in a coordinated manner. The current human protein-protein interactome has been estimated at between 130,000[4] and 650,000[5] complexes, out of which only a tiny amount is known, and only a very few[6-8] has been the object of a drug discovery initiative. Protein-protein interactions (PPI) therefore describe a totally new biological space that attracts more and more attention, with already 26 PPI inhibitors[9, 10] under clinical development, notably in the oncology field.[11] Despite PPIs may adopt quite different sizes, shapes and electrostatics,[12] identifying high-affinity PPI inhibitors is a considerable challenge for many reasons: (i) contrarily to conventional targets, a medicinal chemist cannot start inhibitor design from the structure of endogenous ligands, (i) PPIs often involve flat surfaces delocalized over multiple epitopes, usually lack well-defined buried cavities[13] typical of conventional targets, and are significantly larger (ca. 1000-3000 Å^2) than enzyme/receptor pockets (300-1000 Å^2), (iii) high-throughput screening of traditional compound libraries often return no viable hits[14] for the main reason that PPI inhibitor chemical space is quite different from that described by traditional drug-like compounds.[10] Nonetheless, thank to bioinformatics and proteomics-guided prioritization of therapeutically relevant protein-protein complexes, more and more PPI inhibitors are currently reported. Several excellent reviews[6, 7, 9, 11, 15-18] have already been published on experimental methods (high throughput screening, biochemical and cellular assays, fragment-based approaches) suitable to discover PPI inhibitors. The present report will only cover computer-aided approaches, with a major emphasis on structure-based methods and recent discoveries (2012-2014).

Databases

Preliminary access to experimentally validated data is key to launch a drug discovery program on PPI modulators. A multitude of databases storing genomics, proteomics and structural data are currently available to help the medicinal chemist. We will here briefly review these archives, focusing mostly on easily interpretable structural data.

PPI databases

Many experimental methods with different throughputs (from low to high) have been developed to characterize binary interactomes in various species, among which the most prominent have been the yeast two-hybrid (Y2H) assays, and mass-spectrometry (MS) coupled to co-immunoprecipitation or co-affinity purification.[19] These experimental data are stored in many primary databases (Table 1) that are difficult to mine due to their large heterogeneity. Metadatabases have been derived thereof to facilitate their analysis, among which the most popular are APID and PRIMOS (Table 1). These metadatabases covers a wide range of organisms and notably offer the possibility to mine experimental PPI data according to disease relevance or inter-organism crosstalk, and provide graphic tools to visualize complex networks of interacting proteins and identifying important protein nodes (hubs).

Table 1. Protein-protein interaction databases

Database	Interactions	website	Reference
BIND	32,211	http://bond.unleashedinformatics.com	20
DIP	78,191	http://dip.doe-mbi.ucla.edu/dip/Main.cgi	21
HPRD	41,327	http://www.hprd.org/	22
IntAct	448,986	http://www.ebi.ac.uk/intact/	17
MIPS	9,835	http://mips.helmholtz-muenchen.de/proj/ppi/	23
APID	196,700	http://bioinfow.dep.usal.es/apid/index.htm	24
PRIMOS	384,127	http://primos.fh-hagenberg.at/	19

It is however very difficult, from this huge amount of data, to clearly prioritize PPIs for a drug discovery program. Attempts to classify the PPIs by structural druggability[25] (although ligandibility[26] is probably a better term) are worth mentioning but should be taken with care due to the still insufficient number of existing PPI three-dimensional (3D) structures .

Ligand databases

Initially limited to a limited subset of inhibitors able to disrupt few PPIs (e.g. p53/MDM2, Bcl-Xl/Bak, IL-2/IL-2Rα),[7, 27] the repertoire of PPI inhibitors rises constantly thanks to exciting developments in biophysical fragment screening.[15, 28]
Three publicly-available databases storing information on PPIs and their inhibitors (Table 2) may be used to better describe the structural properties of druggable PPIs and the chemical space associated with their disruptors.

Table 2. Database of low molecular-weight PPI inhibitors

Database	Ligands	website	Reference
2P2I	71	http://2p2idb.cnrs-mrs.fr/	12
iPPI-DB	1,650	http://www.ippidb.cdithem.fr/	10
TIMBAL	6,896	http://mordred.bioc.cam.ac.uk/timbal	29

The 2P2Idb database[12] is a hand-curated repository of protein-protein complexes of known X-ray structure (X-ray diffraction, nuclear magnetic resonance spectroscopy) for which at least one low molecular weight orthosteric inhibitor has been co-crystallized with one of the two protein partners. It currently describes 71 inhibitors for 14 PPIs, clustered in two groups (Figure 1) with respect to the nature of the interface (protein-peptide, protein-protein). Companion tools (2P2I inspector,[30] 2P2I score,[30] 2P2I hunter[31]) are provided to analyse PPIs at a structural level, predict their structural druggability and design PPI focussed libraries, respectively.

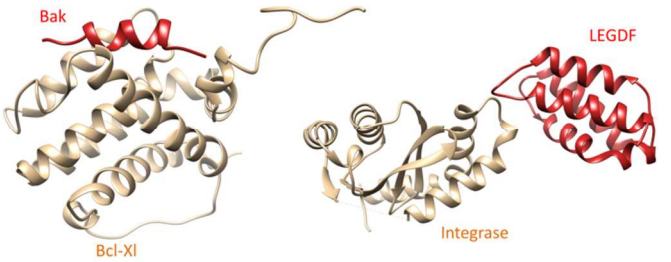

Figure 1. Prototypical examples of class I (left panel) and class II PPIs (right panel), exemplified by the Bcl-Xl/Bak (PDB id 1BXL) and Integrase/LEDGF (PDB id 2B4J) complexes, respectively. Class I PPIs involve the interaction of a globular protein with a peptide or a single secondary structure (α-helix, β-strand) of a second protein partner. Class II PPIs are characterized by the interaction of two globular proteins.

The iPPI-DB[10] is another manually curated database from world patents and medicinal chemistry literature, focussing on low molecular weight orthosteric inhibitors, disease-related protein-protein interfaces and a clear biochemical readout (e.g. fluorescence polarisation, enzyme-linked immunosorbent assay). The database archives 1,650 PPI inhibitors targeting 13 families of homologous PPI targets mainly involved in cancer, immune disorders and infectious diseases.
Last, the TIMBAL database[29] reports ca. 7,000 inhibitors for 50 known PPIs. Contrarily to the two other databases, TIMBAL is maintained through a predefined list of PPIs and automated searches in ChEMBL[32] and the Protein Data Bank.[33] Contrarily to the other databases, TIMBAL also registers short peptides with an upper molecular weight limit of 1,200 Da. It should be pointed that most of the 15,000 uncurated biological data present in TIMBAL arise from a single target family (Integrins) and should be considered with care.
Analysing the content of these databases enables a first comparison of PPI inhibitors versus drugs, as well as PPIs amenable to disruption versus standard heterodimers. PPI surfaces disrupted by inhibitors tend to be smaller, more hydrophobic and accessible than standard heterodimers.[12] As a consequence, low molecular weight PPI inhibitors tend to be larger, more hydrophobic and more aromatic-rich than standard drugs. Interestingly, many of them (ca. 60%) still comply with Lipinski's rule-of-five,[10] revealing some hopes in the developabililty of such compounds.
However, it should be stated that the set of empirical rules designed to discriminate druggable from non-druggable PPIs, as well as to distinguish PPI inhibitors from conventional drug-

like compounds still rely on a very limited set of highly homologous data (PPIs, inhibitors), and should therefore be regarded with caution. Increasing coverage of the PPI repertoire by future experimental screens will undoubtedly lead to a better definition of PPI biological and chemical spaces. We therefore expect in the future the above-mentioned rules to be refined and be more descriptive of the true world of PPI inhibitors, notably with respect to rational design of PPI focussed libraries.

Rational design of PPI modulators

Sequence-based approaches

Whatever the nature of the PPI (type I or type II, see definition above), PPI interfaces are often characterized by the presence of hotspots,[34] in other words anchor residues that contribute the most to the binding free energy of the protein-protein complex. The interaction of a single modified amino acid with a single anchor residue might be sufficient to disrupt a PPI as elegantly demonstrated by Lin et al. in a recent study.[35] Capitalizing on the presence of a reactive cysteine (C246) at the interface of the complex between caspase-7 (CASP7) and the X-linked inhibitor of apoptosis protein (XIAP), they designed the N-iodoacetyl-lysine amino acid derivative **1** (Figure 2) that covalently traps C246 and further disrupts the XIAP-CASP7 complex, therefore triggering CASP7-dependent apoptosis and killing MCF-7 breast cancer cells (EC_{50} = 0.64 μM) previously resistant to chemotherapy.

The easiest way to inhibit a PPI is to start with the amino acid sequence of one interacting epitope, notably if the latter is part of a regular secondary structure (α-helix, β-strand, β-turn). For example, α-helical peptides mimicking the sequence of protein transmembrane domains may disrupt PPIs quite efficiently.[36, 37] Due to poor pharmacokinetic profiles, linear peptides are good *in vitro* tools but usually not efficient clinical candidates. Chemical modifications are required to stabilize their secondary structures in physiological media and prevent early degradation. Among the most exciting developments in this area[38, 39] is the design of stapled peptides.[40, 41] Stapled peptides are synthetic analogues of α-helical protein epitopes involved in a PPI, and in which a covalent hydrocarbon linkage connects adjacent turns of the helix. Stapling is known to significantly increase the *in vivo* half-life of the natural peptide (increasing proteolytic stability), decrease the entropic cost of binding, and even enable cellular uptake.[42] Many stapled peptides with potent in vivo activities have already been reported.[39] One of these stapled peptides (ATSP-7041, compound **2**, Figure 2) just entered clinical development as a dual nM MDM2/MDMX inhibitor for p53-dependent cancer therapy.[43]

Heterocyclic scaffolds mimicking secondary structures can also be obtained by solution-phase synthesis to afford peptidomemitic libraries amenable to PPI inhibition. Whitby et al. notably reported the design of 8000 member 4-acetamido-3-alkoxy-benzamide focused library featuring weak p53/MDM2 inhibitors and potent HIV-1/gp41 inhibition (compound **3**, Figure 2).[44] When the peptide epitope is not structured, developing macrocylic analogues is more difficult but still feasible as recently demonstrated by Glas et al.[38] who successfully improved 14-3-3 binding of a 11-mer peptide from a bacterial ExoS virulence factor by cross-linking binding amino acids with polymethylene linkers, up to an *in vitro* 40 nM disruptor of the ExoS/14-3-3 interaction (compound **4**, Figure 2). Interestingly, the cross-linker was not only chosen to rigidify the natural ExoS peptide structure but also to directly provide additional hydrophobic interactions to the 14-3-3 binding site.[38]

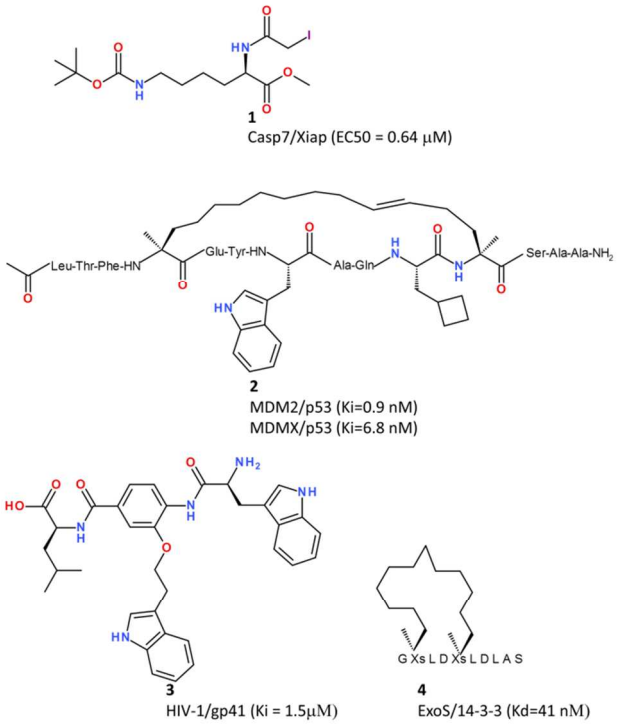

Figure 2. Peptidomimetics as PPI disruptors

Only in exceptional cases is the natural unmodified peptide directly usable as PPI inhibitor. One recent example is the 28 amino acid cell-penetrating peptide (p28) from a bacterial azurin redox protein, that binds to the DNA-binding domain of the p53 tumor suppressor and inhibit p53 degradation by interfering with the Cop1-mediated ubiquitination,[45] thereby enhancing p53 levels in cancer cells and exhibiting antitumoral efficacy in patients with advanced solid tumors.[46]

Pharmacophore-based approaches

As defined by the IUPAC,[47] a pharmacophore is "an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response."

Although pharmacophores are mainly used to align and compare ligands sharing the same target,[48] the same concept can be easily transferred to PPIs in which one partner is the "receptor" and the second one the "ligand". Pharmacophore features (hydrophobic, aromatic, H-bond donor and H-bond acceptor, positively and negatively ionisable) can therefore be manually or automatically mapped to atoms of the ligand in direct interaction with the receptor. The resulting pharmacophore can then be used to identify a compound library for hits fulfilling the defined query. Several tools (e.g. LigandScout,[49] DiscoveryStudio,[50] PocketQuery[51]) can be directly used to map PPI pharmacophores onto protein-protein X-ray structures (Figure 3).

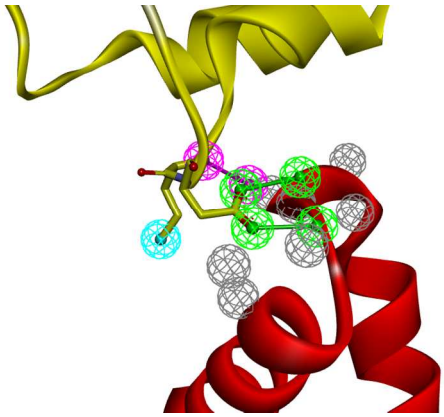

Figure 3. Example of a PPI pharmacophore mapped onto interacting atoms of human LEDGF (yellow ribbons) bound to HIV-1 integrase (red ribbons, PDB ID 2B4J). The PPI pharmacophore is composed of 2 h-bond donors (magenta balls), two H-bond acceptors (green balls), one hydrophobic feature (cyan ball) and 6 exclusion volumes (gray balls)

Using a manual PPI pharmacophore defined from the X-ray structure of the Annexin A2/S100A10 complex, a pro-angiogenic complex), Reddy et al.[52] derived a simple pharmacophore (2 hydrophobes, 2 H-bond donors, 2 H-bond acceptors) using the Unity program,[53] and screened a library of 700,000 compounds to select 586 hits which were further docked to the Annexin A2 binding site to retain only 190 candidates with both a good docking and pharmacophore fitness score (Table 3). Out of the 190 tested compounds, 7 hits blocked the interaction between S100A10 and the annexin A2 N-terminus in a competitive fluorescent binding assay, with the most potent PPI inhibitor (compound **5**, Figure 4) exhibiting an IC_{50} of 24 μM.[52]

Geppert et al.[54] reported the rational discovery of a low molecular weight inhibitor of the complex between interferon-α (IFN-α) and its receptor (IFNAR2). Fortunately, the PPI interface was small enough (ca. 800 Å^2) to be targeted by a small heterocyclic compound. After identifying major hotspots at the IFN-α surface, a fuzzy receptor-based pharmacophore was determined using the VirtualLigand approach,[55] which assigns pharmacophoric features to Gaussian densities. Screening a collection of 556,000 commercially available compounds retained six virtual hits, out of which two were weak IFN-α inhibitors, but one (compound **6**, Figure 4) was confirmed by NMR and surface plasmon resonance (SPR) to bind to IFN-α with a dissociation constant (Kd) of 4 μM and to inhibit IFN-α responses in various cell assays. The novel inhibitor may be useful to reduce IFN-α titers in autoimmune disorders.

Table 3. Protein-protein pharmacophore searches to identify PPI inhibitors

Target	Library size	Tested	Hits	Ref.
Annexin A2/S100A10	700,000	190	7	52
INFAR2/IFN-α	556,000	6	3	54
p53/MDM2	21,287	15	6	56
Nrf2/Keap1	21,199	17	1	57
PKCε/RACK2	330,000	19	1	58

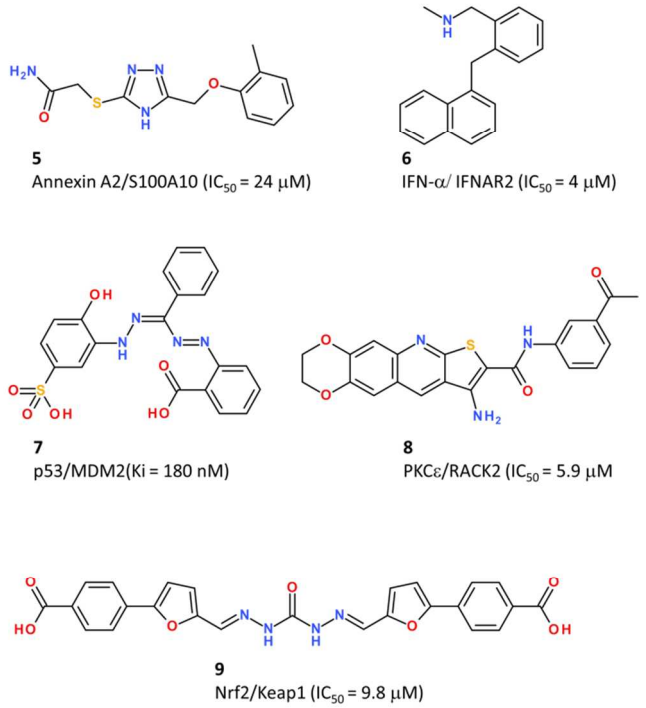

Figure 4. PPI inhibitors identified by pharmacophore-based virtual screening.

Due to the inherent complexity of PPI pharmacophores (many features covering a large surface), combining several pharmacophores into a consensus model may help to retrieve essential features and simplify pharmacophore queries. Xue et al. applied this approach to the identification of p53-MDM2 inhibitors.[56] The p53-MDM2 complex has become a prototypical PPI for its biological background (this interaction plays an important role in regulating the transcriptional activity of tumour cells) and the many high affinity low molecular-weight inhibitors of this PPI identified by various screening approaches.[59] Starting from a set of 15 MDM2-peptide X-ray

structures, a common features structure-based pharmacophore (2 H-bond donors, one H-bond acceptor, 2 aromatic rings, one hydrophobe) was first identified. In addition, a receptor-ligand pharmacophore (five hydrophobes, one aromatic, one H-bond donor) was generated from a separate set of 10 MDM2-non peptide complexes. Merging both pharmacophores and retaining the most common features led to an ensemble pharmacophore definition (two aromatic rings, two hydrophobes, one H-bond donor) talking in to account both peptide and non-peptide binding. This pharmacophore was used to screen a collection of 21,287 commercially available compounds, and led to a hit list of 15 compounds out of which 6 were confirmed as p53-MDM2 inhibitors using an *in vitro* fluorescence polarization assay.[56] The most potent inhibitor (compound **7**, Figure 4) is a 180 nM MDM2 inhibitor. Despite a good selectivity in a MTT tumour cell proliferation assay ($p53^{+/+}$ vs. $p53^{-/-}$ cells), compound **7** was a weak inhibitor (IC_{50} = 85 μM) of tumour cell growth, because of poor pharmacokinetic properties.

Along the same lines, two X-ray structures were used to derive inhibitors of the PPI between Keap1 and Nrf2, a complex involved in the response to oxidative stress.[57] The two PPI pharmacophores were merged into a single query consisting of one H-bond donor, two H-bond acceptors and three negative ionisable centers. To afford some fuzziness in the search, up to two features were allowed to be missed by virtual hits. Since the Keap1-binding epitope of Nrf2 is composed of several acidic residues, only compounds bearing a negative charge were searched among a full commercial library of 251,774 compounds. The remaining 21,199 hit list was matched to the pharmacophore, and led after confirmation with docking and MM-PBSA scoring, to a list of 17 potential hits which were tested for Keap1-Nrf2 inhibition using an *in vitro* fluorescence polarization assay. A single compound (compound **9**, Figure 3) was confirmed *in vitro* as a moderately potent Keap1-Nrf2 inhibitor with an EC_{50} if 9.8 μM.[57] Interestingly, the inhibitor activated Nrf2 transcriptional activity.

When both protein partners involved in the PPI have not been co-crystallized, it is still possible to rationally discover PPI inhibitors, starting from the sole X-ray structure of one of the two proteins. This approach was followed by Rechfeld et al. in the discovery of PKCε-RACK2 inhibitors.[58] Starting from the X-ray structure of the PKCε octameric epitope binding to RACK2 (a receptor for activated protein kinase C), a simple peptide-based pharmacophore model (3 H-bond donor/acceptor, one hydrophobe) was defined and used to screen a collection of 330,000 compounds. Out of 19 virtual hits, a thienoquinoline was found to disrupt the PPI *in vitro* and served as a query for a secondary screen for chemically similar analogues, to led to compound **8** (Figure 4) as a micromolar potent PKCε-RACK2 inhibitor (IC_{50}=5.9μM) which also inhibited PKCε downstream signalling, HeLa cancer cell migration and invasion.[58]

Last, pharmacophore searches may be used to prioritize privileged scaffolds for synthesizing PPI-focused libraries. For example, Fry et al. reported a rational approach to PPI library design targeting α-helical binding epitopes.[60] Starting from the known X-ray structure of a α-helical p53 epitope binding to MDM2, a three point pharmacophore, featuring the three important hydrophobic side chains (Phe19, Trp23, Leu26) of the p53 peptide, was designed and used to find heterocylic scaffolds among the CSD database[61] of small molecule X-ray structures. Several small-sized libraries (ca. 100 members) were synthetized from each hit and tested for general inhibition of PPIs involving a α-helical epitope (e.g. MDM2, BCL2, BCL-XL, MCL1). Although no potent hit could be discovered, the average hit rate was far superior (4%) to what should be expected from a random screen. Moreover, many starting hits exhibited good ligand efficiencies,[60] and are therefore interesting starting points for hit to lead optimization.

Despite its apparent simplicity, PPI-based pharmacophore search is a fast, cost-effective and simple *in silico* approach to discover the very first inhibitors of a particular PPI. Of course, all successful examples mentioned above imply that the PPI is of manageable size and does not involve a too large and complex binding epitope. Beside the existence of a X-ray or NMR structure of the protein-protein(peptide) complex, it is therefore equally important to properly select PPIs amenable to pharmacophore-based searches, notably with respect to the complexity of the query (5-6 features) and its hydrophobic/hydrophilic balance.

Docking-based approaches

At first sight, protein-ligand docking should be considered as the most intuitive and logical computational tool to predict likely ligands of any target of known 3D structure.[62] Unfortunately, severe drawbacks associated with the scoring of protein-ligand interactions render that tool usually suitable for positioning a ligand into a binding site, but rarely to predict binding free energies or to precisely rank ligands by decreasing affinity.[63] Moreover, the ability of docking algorithms to anchor ligands to flat PPI surfaces has long remained elusive. In a benchmark study, Krüger et al. used two popular docking tools (AutoDock, Glide) to reproduce the known X-ray structure of PPI inhibitors to their target.[64] Surprisingly, the performance of these standard docking programs with respect to the positioning of the ligand (rmsd to the X-ray structure) was only moderately affected by switching from conventional targets to PPIs. Although PPI inhibitors with more than 10 rotatable bonds were found more difficult to properly dock, a good pose was generated in ca. 54% of the 80 PPI inhibitors considered. Docking to PPIs providing at least one charge residue was favoured over those purely hydrophobic.[64] There are therefore no particular reasons to discard docking-based approaches from rational PPI inhibitor discovery scenarios. Many of the following success stories support this assumption.

We will not here review the many recent reports describing docking as a mean to predict the binding mode of a PPI inhibitor discovered by an experimental screening method.[59, 65-68] The next section will only focus on inhibitors discovered by a docking-based virtual screening campaign (Table 4).

Table 4. Protein-protein inhibitors discovered by docking-based screening

Target	Library size	Tested	Hits	Ref.
TLR4/MD-2	50,000	14	3	[69]
uPA-uPAR	5,000,000	50	3	[70]
IL-6/gp130	9	2	2	[71]
Keap1-Nrf2	153,611	65	9	[72]
CRYAB/VEGF	139,735	40	4	[73]
NRP-1/VEGF-	429,623	1,317	56	[74]
PPxY/Nedd4	4,800,000	20	1	[75]
p53/MDM2	87,430	295	1	[76]

Despite an apparent unsuitable large and concave cavity, the MD-2-binding site at the surface of the Toll-like receptor 4 (TLR4) was selected for pharmacophore-constrained FlexX[77] docking of a library of 49,600 compounds pre-filtered for 3D shape similarity to an existing TLR4 antagonist.[69] 40 virtual hits were selected for *in vitro* TLR4 binding and functional antagonism, and 3 of them could be confirmed experimentally. The most potent antagonist (compound **10**, Figure 5) blocked TLR4 in a gene receptor assay with an IC_{50} of 16.6 µM and inhibited pro-inflammatory cytokine release (e.g. TNF-α) from human peripheral blood mononuclear cells upon LPS activation. Due to unfavourable aqueous solubility, the compound could not be tested in vivo but represent a good starting hit for developing small molecule TLR4 antagonists for the treatment of neuropathic pain and sepsis.

To account for the conformational flexibility of proteins, Khanna at al. reported a cascade docking-based virtual screening for discovering inhibitors of the interaction between urokinase-type plasminogen activator (uPA) and the urokinase receptor (uPAR).[70] Two X-ray structure of the uPAR were first used to docking a collection of 5 million commercially available compounds using AutoDock4.[78] 10,000 top-ranked virtual hits were further docked, still with AutoDock, to 50 molecular dynamics snapshots of the uPAR structure, leading to 500 top-ranked compounds which, in a third step, were docked using a different program (Glide) on the 50 receptor conformers. After clustering the top 250 compounds by chemical similarity, the highest scoring compounds from each of the top 50 clusters were finally selected, purchased and evaluated *in vitro* in a fluorescence polarization assay. Among the three validated hits, the most potent inhibitor (Compound **11**, Figure 5) binds to uPRA with a submicromolar affinity (K_d=310 nM) and inhibits the uPA-uPAR interaction with an IC_{50} of 10 µM.[70] The hit blocked invasion of breast cancer cells but not their migration or adhesion. A close analogue of compound **11** was recently shown to be efficient in an *in vivo* breast cancer metastasis assay.[79]

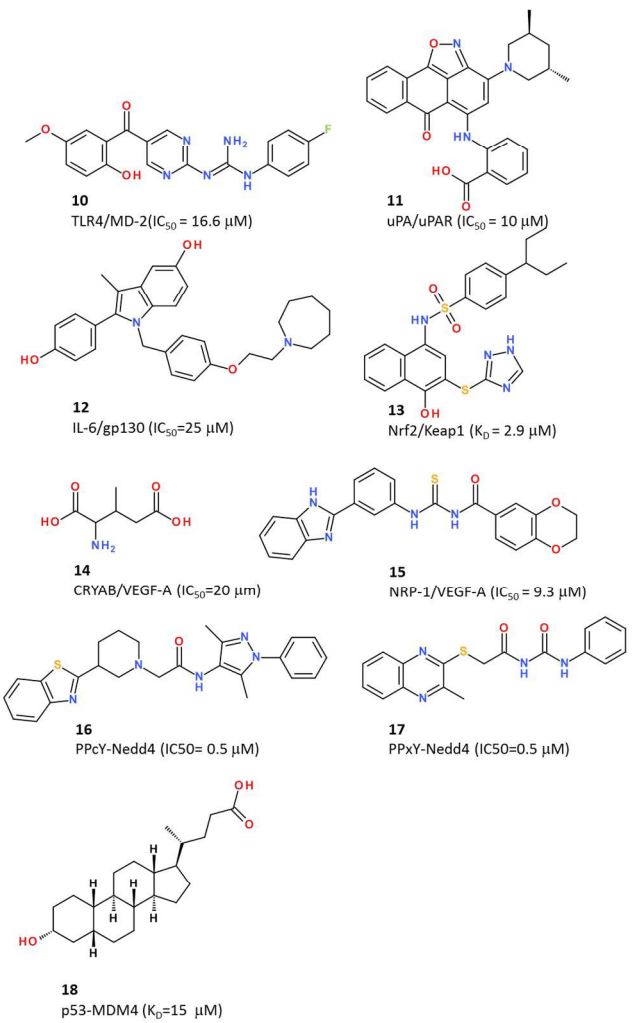

Figure 5. PPI inhibitors identified by docking-based virtual screening.

Docking is not limited to the study of single protein-ligand interactions. In an elegant study, Li et al. reports a computational method enabling the simultaneous docking of multiple fragments to a single binding site, by analogy to experimental fragment screening.[71] When applied to the PPI between IL-6 and gp130, simultaneous docking of two fragment pools (6 and 3 fragments, respectively) targeting two different hotspots at the PPI, two theoretical ligands could be reconstructed after tethering the best fragments at each hotspot. Searching for known drugs[80] which are chemically similar to the two virtual hits suggested than two estrogen receptor modulators (raloxifene, bazedoxifene) may bind to the gp130/IL-6 PPI. Effective binding of both drugs to gp130 was confirmed experimentally, as well as inhibition of IL-6 induced STAT3 phosphorylation in various cancer cell lines defective in estrogen receptor expression. Bazedoxifene (compound **12**, Figure 5) was the most efficient (IC_{50}= 25 µM) in inhibiting the ER-independent IL6-induced breast cancer cell proliferation, thereby offering some repositioning potential in the treatment of IL-6/gp130/STAT3 dependent tumours.[71]

The Nrf2-Keap1complex, previously investigated using a pharmacophore-based approach (see previous section) was also used for docking 300,000 commercially available compounds with the program Glide. Among the chemically diverse 65 top-ranking hits, 9 compounds were confirmed to be PPI inhibitors, the most potent disruptor (compound **13**, Figure 5) exhibiting a K_D of 2.9 μM in a fluorescence anisotropy-based assay.

A major hurdle in PPI inhibitor development is the frequently objected high molecular weight and unflavoured pharmacokinetic properties. Chen et al. strikingly contradicted this dogma by reporting a very low molecular weight inhibitor of the αB-crystallin (CRYAB)/$VEGF_{-A}$ interaction.[73] CRYAB is a protein overexpressed in triple-negative breast cancer cells that acts as a chaperone to several proteins including the pro-angiogenic vascular endothelial growth factor (VEGF). Disrupting the interaction between CRYAB and VEGF-A is therefore a potential approach to cancer cell proliferation and invasion. The VEGF-binding site on the surface of the CRYAB X-ray structure was therefore targeted by docking 140,000 compounds from the NCI database using the Dock6.5 program (UCSF). Despite a very modest molecular weight (161.16 Da), one compound (compound **14**, Figure 5) was identified as an *in vitro* disruptor of the CRYAB/VEGF-A interface with an IC_{50} of ca. 20μM. Intraperitoneal injection of compound **14** (200 mg/kg) remarkably supresses tumour growth *in vivo* in human breast cancer xenograft models. VEGF-A is an important angiogenic factor that interacts with many other partners, notably the family of neuropilin receptors (NRP-1, NRP-2) whose inhibition leads to cancer cell apoptosis. The PPI between the C-terminal end of $VEGF\text{-}A_{165}$ and the tandem b1 and b2 domains of NRP-1 was targeted for docking 430,000 molecules with a consensus docking approach relying on two docking programs (Surflex-Dock[81] and ICM[82]). A consensus list of 1,317 top-scoring compounds was retained for their *in vitro* anti-proliferative activity and binding to NRP-1 using a chemiluminescent assay.[74] 56 molecules (hit rate of 4.2 %) antagonized the NRP-1/VEGF-A interaction by at least 30% at the concentration of 10 μM. The best hit (compound **15**, Figure 5) is the first non-peptide NRP-1/VEGF-A antagonist (IC_{50} = 34 μM) and displays remarkable anti-proliferative effects (IC_{50} = 0.2 μM) on breast cancer cells. Administered at the dose of 50 mg/kg in NOG-xenografted mice, compound **15** strongly inhibit tumour growth inhibition by inducing cell apoptosis, without any effect on pro-angiogenic kinases.

Although most of the above reported therapeutical indications remain in the oncology field, PPI inhibitors have clear potential in other areas, notably infectious diseases as recently demonstrated by Han et al.[75] who reported the structure-based discovery of antiviral compounds inhibiting viral-host interactions. The PPI target is the complex between the conserved L-domain PPxY sequence of several viral matrix proteins (e.g. Ebola, Marburg, Lassa fever, VSV) and the ubiquitin ligase Nedd4 protein. Docking ca. 5 million compounds (ZINC database)[83] on the Nedd4 X-ray structure with the AutoDock4 program, yielded to the evaluation of 20 compounds, out of which one molecule was confimed as a true inhibitor of the PPI in a cellular assay. Acquiring close analogs of the initial hit led to two more potent inhibitors (compounds **16** and **17**, Figure 5) as submicromolar inhibitors of the PPxY-Nedd4 interaction *in vitro*.[75] Both compounds exhibit antibudding activity against Ebola, Lassa fever, Marburg and VSV viruses, thereby decreasing viral titers, without apparent cytotoxicity on HEK293T cells.

Natural compounds are also a major source of potentially interesting PPI inhibitors. By docking a library of commercially available compounds to the p53 binding site, Vogel et al. recently reported lithocholic acid (compound **18**, Figure 5), a secondary bile acid, as a weak binder (K_D of 15 μM) to MDM4 and MDM2 proteins with a slight preference for MDM4.[76] The natural compound was further shown to inhibit p53-MDM4 interactions and promote apoptosis in a p53-dependent manner by inducting caspase3/7.

Conclusions

We should acknowledge that peptides usually remain a good starting point to derive PPI inhibitors. Given the increasing number of high resolution X-ray structures of biologically relevant protein-protein complexes, the number of potentially increasing PPIs is likely to significantly rise in the next years. Provided that molecular rules exist to prioritize the most interesting anchoring residues at the interface, continuous protein epitopes can be easily converted into linear peptides for quick experimental validation. Recent progress in peptide stabilisation by chemical stapling next opens an immense field for deriving either pharmacological tools or drug candidates. Numerous successes in identifying non-peptide PPI inhibitors also exist. The present review has only considered inhibitors mostly discovered by a rational structure-based virtual screening approach. Despite the few cases described herein (15 in total), examples are pretty much indicative of results than can be reasonably achieved. Comparing properties of PPIs (Figure 6A, B) and their inhibitors (Figure 6C) with previously reported larger PPI data,[64] some trends could be verified. Considering success as the availability of low micromolar non-peptide inhibitors, successfully targeted PPIs present a higher proportion of charged residues with respect to conventional targets (sc-PDB data).[84] Unsurprisingly, PPI inhibitors bind to smaller cavities (200-350 $Å^3$) than that presented by conventional targets (450-800 $Å^3$ range). Consequently, PPI inhibitors present a high proportion of aromatic rings, amide moieties and charged groups (Figures 4, 5) that hampers their druggability potential, as estimated here by the QED metric[85] (Figure 6C). We notice a significant proportion of negatively charged compounds, suggesting that a strong electrostatic interaction with the target is often mandatory to reach detectable affinity to PPI-participating cavities.

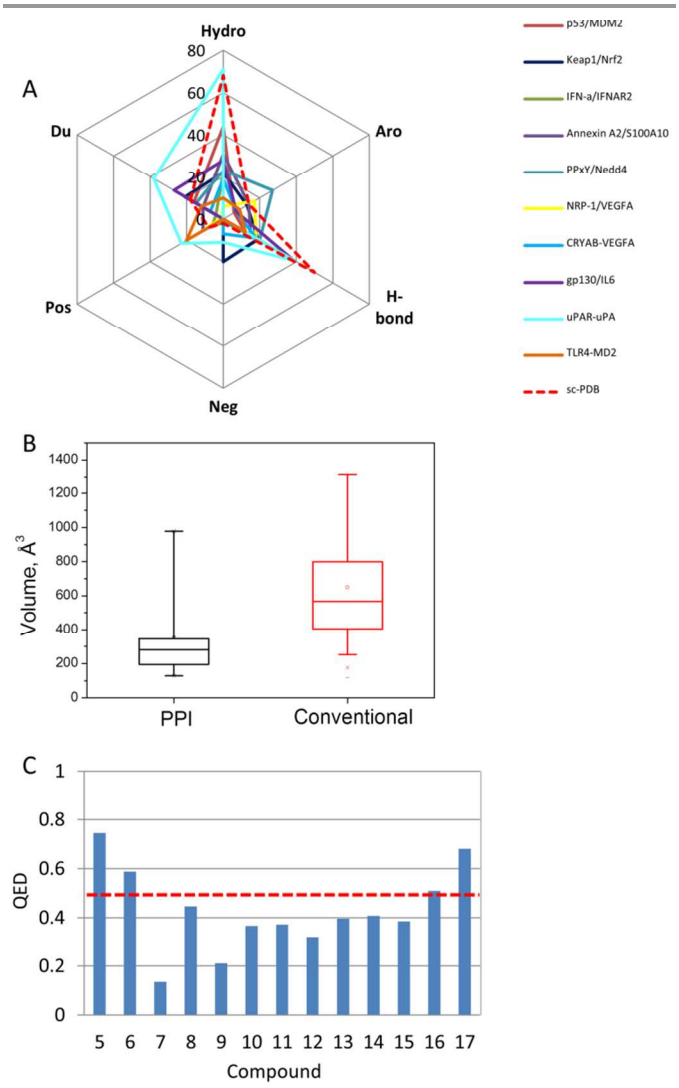

Figure 6. Properties of PPIs and their inhibitors: A) Cavity properties expressed in percentage according to the cavity detection VolSite program[86] (Hydro, hydrophobic; Aro, aromatic; H-bond, H-bond accepting/donating properties; Neg: Negatively charged; Pos, positively charged, Du: fully accessible; B) Cavity volumes targeted by PPI inhibitors (this review) and conventional ligands (sc-PDB data[84]). The box delimits the 25th and 75th percentiles, the whiskers delimit the 5th and 95th percentiles. The median and mean values are indicated by a horizontal line and an empty square in the box.; C) Quantitative estimate of Drugagbility (QED)[85] of the inhibitors. QED values for true drug-like compounds should be over 0.5 (red broken line)

However, the current survey also indicates that there is no absolute dogma with respect to PPI inhibitor identification. Very low molecular weight compounds (compounds **1**, **6** and **14**) have been successfully identified as PPI disruptors.

Beside interfacial inhibitors, there exist promising alternative ways of inhibiting PPIs. For example, PPI stabilizers[87, 88] (e.g. paclitaxel, rapmycine, forskolin) bind to rim exposed pockets at or very close to the interface, and also lead to the functional inactivation of the protein-protein complex. Such stabilizers are frequent in the nature, and this area still has not been fully exploited up to now. Likewise, the allosteric inhibition of PPIs, at pockets remote from the interface, clearly deserves some consideration. Such pockets have been shown to be frequent at the close vicinity of two proteins chains in close interaction,[89] and represent, at least for some of them, more ligandable pockets than those presented by PPIs.

Although dominated by a continent of flat and featureless interfaces, the PPI world is also populated by very different islands in terms of shape and electrostatics that should not been discarded. Many factors are likely to increase our knowledge of PPIs and their inhibitors among which: (i) the increasing number of biologically relevant and crystallized protein-protein complexes, (ii) the development of label-free experimental screening techniques, (iii) the significant contribution of molecular simulations to detect transient interfaces. Medicinal chemistry will be key actors to transform moderately potent PPI inhibitor hits into clinical candidates with desired pharmacokinetic properties.

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Rational design of protein-protein interaction inhibitors

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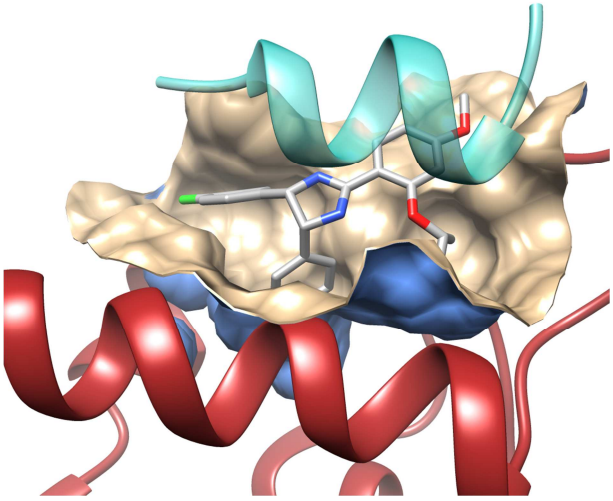

Low molecular weight compound competing for the binding of the p53 tumor suppressor to the MDM2 oncoprotein.