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Inhibitors of Protein-Protein Interactions (PPIs): An Analysis of Scaffold Choices and Buried Surface Area

Xu Ran and **Jason E. Gestwicki***

Institute for Neurodegenerative Diseases and Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA 94158

Abstract

Protein-protein interactions (PPI) were once considered "undruggable", but clinical successes, driven by advanced methods in drug discovery, have challenged that notion. Here, we review the last three years of literature on PPI inhibitors to understand what is working and why. From the 66 recently reported PPI inhibitors, we found that the average molecular weight was significantly greater than 500 Da, but that this trend was driven, in large part, by the contribution of peptide-based compounds. Despite differences in average molecular weight, we found that compounds based on small molecules or peptides were almost equally likely to be potent inhibitors (K_D < 1 μM). Finally, we found PPIs with buried surface area (BSA) less than 2000 $Å^2$ were more likely to be inhibited by small molecules, while PPIs with larger BSA values were typically inhibited by peptides. PPIs with BSA values over 4000 $Å^2$ seemed to create a particular challenge, especially for orthosteric small molecules. Thus, it seems important to choose the inhibitor scaffold based on the properties of the target interactions. Moreover, this survey suggests a (more nuanced) conclusion to the question of whether PPIs are good drug targets; namely, that some PPIs are readily "druggable" given the right choice of scaffold, while others still seem to deserve the "undruggable" moniker.

Introduction

Protein-protein interactions (PPIs) are potential drug targets for a broad range of therapeutic areas, such as oncology [1, 2*, 3, 4*, 5], immune-checkpoints for cancer immunotherapy [6], tropical infectious diseases [7*], neurological disorders [8], heart failure [9] and inflammation and oxidative stress [10]. Interest in these targets is further heightened by the fact that modern proteomics studies have shown that there are an estimated 650,000 PPIs, compared to only ~20,000 protein coding genes [11]. At the same time, gene-editing methods are making it possible to create point mutations within the genomes of mammalian cells [12*], allowing validation of individual PPIs as putative drug targets with unprecedented precision. Finally, because the interfaces are often less conserved than active

*Correspondence: University of California San Francisco, Sandler Center, 674 Nelson Rising Lane, San Francisco, CA 94158, (415) 502 7121, jason.gestwicki@ucsf.edu.

sites, PPI inhibitors are also commonly thought to have a greater opportunity for being selective [13].

This current enthusiasm is in contrast with the attitude twenty years ago, when PPIs were commonly regarded to as “undruggable”. This conclusion was based, in part, on a growing number of crystal structures that showed protein interfaces with daunting large (1,000–2,000 $Å^2$) and flat interfacial areas, when compared to traditional targets, such as enzyme active sites (~300–500 $Å^2$) [4]. However, even at that time, “undruggable” was an admittedly broad designation, because peptide hormones that act at PPIs had long been approved as clinical drugs [14]. The 1990s also saw the approval of small molecule PPI modulators (sometimes stabilizers instead of inhibitors), such as Tirofiban and various taxanes [15–17]. What were the common features of these early compounds? How were they able to escape the dogma? On commonality amongst the early PPI inhibitors is that they were based on natural products. While this feature likely helped the molecules overcome the challenges of inhibiting PPIs, it also gave them poor oral bioavailability and low cell-permeability. Thus, these successes did not clarify whether PPIs inhibitors could play an important role across the landscape of drug discovery. Fortunately, the number of PPI inhibitors that are either approved or under late-stage clinical investigation has expanded over the years. One milestone was the 2016 approval of the Bcl-2 inhibitor, Venclexta (ABT-199). This compound was derived from a fragment-based screen [18*] (rather than a natural product) and it is orally active for the treatment of chronic lymphocytic leukemia [19*]. Now, the possibilities of targeting a range of PPIs with compounds from different sources seems increasingly likely.

What drove this evolution from “undruggable” to increasingly common? One significant development was greater basic knowledge of PPI structure and energetics. Early observations by Arkin, Wells and others suggested that the interaction energy (ΔG_{bind}) of PPIs was often not evenly distributed across the entire buried surface area (BSA). Rather, mutagenesis showed that there are “hot spots” that confer a disproportionate amount of the ΔG_{bind} [17,20]. By placing molecules at these sites, orthosteric (*i.e.* competitive) inhibitors with relatively low molecular mass values could be created, even if the PPI itself had a comparatively large BSA (Fig 1A). Another major advance was the realization of the plasticity of many protein interaction surfaces [4]. Indeed, molecules that take advantage of conformational changes are sometimes able to inhibit seemingly intractable PPIs [21,22]. In some cases, these molecules bind at the interface itself and remodel the topology of the contact surface. However, other compounds bind to distal sites to influence the PPI. For example, JG-98 binds with a sub-micromolar affinity to an allosteric site on Hsp70, disrupting an interaction with BAG3 that is >20 Å away. This interaction has a BSA of 4,473 $Å^2$ and it involves two different subdomains [23*], so it is difficult to imagine how an orthosteric inhibitor might be capable of doing that job. Other technological advances have focused on creating inhibitors from non-traditional scaffolds, often inspired by natural products. For example, new methods for creating cell-permeable peptides, such as helical regions that are covalently “stapled” along the backbone to improve their cLogP and proteolytic stability [11,24*,25] or polycationic “tags” to improve passive permeability [26], have seen significant progress in the last decade. Other advances have included semi-synthetic [27] and synthetic methods [28,29] for creating a greater range of biologically

active macrocycles [30] and advanced methods for creating peptide-inspired foldamers [31]. Now, an entire zoo of scaffolds - small molecules, peptides and others - are available for targeting PPIs. These approaches are further supplemented by the emergence of fundamentally new techniques, such as proteolysis targeting chimera (PROTAC) molecules, in which the target protein is degraded instead of trying to physically block its interactions [32*]. Given the rise of so many new approaches for inhibiting PPIs, it seems worth performing a retrospective analysis to find out what is working.

Here, we present a survey of inhibitors targeting PPIs, with a special focus on those described since 2015. Our hypothesis is that the field of PPI inhibitors may be mature enough that "rules" could emerge from an overview of the types of strategies, targets and molecules being deployed. Indeed, in retrospective studies performed in 2012 and 2015 [13,33,34], putative PPI targets were divided into four categories based on their affinity (K_D) and BSA values (Fig 1B). When known inhibitors were placed into these quadrants, it became clear that some PPIs were easier to inhibit than others. For example, the majority (~80%) of reported inhibitors (at that time) were found to target PPIs that have a small, concise surface area (<2000 $Å^2$) and a strong interaction ΔG_{bind} (K_D < 200 nM). These compounds were also the most potent, suggesting that there was simply more binding energy available. In contrast, the other categories of PPIs seemed to be less amenable to inhibitors (~20% of the total), including those with: (a) weak affinity and small, concise BSA values or (b) those with a large BSA and tight affinity. At the extreme end, there were no reported inhibitors of PPIs within the category characterized as having weak interactions over a large surface area. Together, this analysis seems to roughly match biophysical intuition. For example, PPIs with a concise surface area and tight affinity would be likely to have closely spaced "hot spots" and be most amenable to orthosteric inhibition by low molecular mass, high ligand efficiency (LE) compounds. Conversely, large surface areas or weak affinities both create theoretical challenges in the pursuit of inhibitors, requiring either larger molecules (> 500 Da) or possibly allosteric mechanisms (see Fig 1A).

In this review, we were particularly interested in learning whether PPI inhibitors derived from small molecules were being used to target different types of PPIs than those derived from peptides or other scaffolds. In other words, with the rise in new technologies for making and discovering PPI inhibitors, we wondered whether any of them had become more/less dominant in recent years. We were also interested in whether recent examples might continue the early trends about non-equivalent "druggability" of PPIs or whether the difficult categories were becoming more amenable to inhibition as technology develops. More broadly, it seemed important to periodically re-evaluate these questions (since 2015) in an effort to understand what is working and what gaps remain. Before continuing with this task, we first provide brief vignettes of some recent case studies, sequestered into sections based on whether the inhibitor was derived from a small molecule, peptide or alternative scaffold.

Recent Examples of Small-Molecules as Inhibitors of Protein-Protein Interactions

Small-molecules clearly have a number of advantages compared to other potential therapeutic modalities, including metabolic stability, bio-distribution, shelf life, manufacturing cost, pharmacokinetics and permeability [35,36]. In addition, there are well-established tools available to support chemical discovery and optimization, including computer-assisted drug design (CADD) and high-throughput screening infrastructure. However, it seems possible that small molecules might have some disadvantages when PPIs are the target, because of their relatively limited surface area. Here, we briefly summarize small molecule-based inhibitors that have been described in the last few years. We don't intend this discussion to be inclusive of the targets or the approaches, but; rather, to give a sense of the scope of the discovery efforts (Fig 2) and illustrate some themes.

The concept of hotspots at orthosteric sites is continuing to influence modern PPI inhibitor design. For example, Liu *et al.* developed a series of pyrrolinones to target a hotspot in the uPAR-uPA interaction [37]. Interestingly, they found that large jumps in potency were gained when the series made an unexpected cation-pi interaction with an arginine in uPAR, which was outside the anticipated hotspot region but helped favorably position the scaffold. Structure-based approaches to target hotspots have also been used to create low nanomolar inhibitors of menin-MLL interactions [38,39*], which were potent in mouse models of leukemias caused by MLL translocations. In another compelling study in an entirely distinct biological system, Dawidowski *et al.* targeted the PPI between PEX14 and PEX5 that is required for peroxisome maturation in *Trypanosoma* species [7*]. In this study, they performed two, sequential screening campaigns: firstly, an *in silico* 3D pharmacophore-based search followed by docking; secondly, an NMR-based fragment screening from their in-house 1500 compound library to further exclude off-target effects and reduce toxicity of the initial hit. This effort produced sub-micromolar inhibitors (~ 0.2 to 0.5 μM) of the PPI that mimicked the natural hotspot in the contact region and validated PEX14-PEX5 as a new target for the treatment of parasitic infections by *Trypanosomes*. It also highlights the interdisciplinary nature of modern PPI inhibitor programs, incorporating biophysical methods, disease models and CBDD. For some types of PPIs, especially those with concise BSA values, this combination has created a well-worn pathway to small molecule-based inhibitors.

Transcription factor PPIs were also popular, and very challenging, targets in the last few years. For example, Illendula *et. al.* developed the first small-molecule targeting the transcription factor fusion CBFβ-SMMHC [40*]. They used a fluorescence resonance energy transfer (FRET) assay to screen the National Cancer Institute's Diversity Set; revealing the most potent molecule, AI-10-49, as a bivalent small-molecule that can restore transcriptional activity of RUNX1 in acute myeloid leukemia (AML), and selectively induce cancer cell death *in vivo*. In some ways, the PPIs of transcription factors, such as CBFβ-SMMHC, are prime examples of the most challenging targets. They are dynamic, polar and often lack detailed structural information [41]. In the CBFβ-SMMHC example, the active compound acts at an allosteric pocket, perhaps creating a template for future studies.

Covalent inhibitors of other transcription factors have also been reported [42,43], providing a potentially complementary strategy.

Another theme in the recent literature is a continued development of chemical screening libraries that are enriched for PPI inhibitors. Essentially, these collections tend to be more "natural product-like", having higher average molecular mass and more stereocenters. For example, Vincendeau *et al.* screened a natural product library to identify an anthraquinone derivative that inhibits binding of NEMO-Ubiquitin, a large interface area of 4520 $Å^2$ [44]. Similarly, inhibitors inspired by natural products also led to the discovery of the first non-azaphilone containing chlorofusin as an inhibitor of MDM2/p53 [45]. Another approach is to assemble subsets of compounds that are cherry-picked from traditional screening decks. Venkitaraman *et al.* took this strategy to build 17000 rationally-selected compounds, yielding an inhibitor of the PPI between AURKA and TPX2 [46]. These molecules were found to act at an allosteric site instead of directly binding to the PPI interface, a growing trend in kinase inhibitor programs that is often used to generate more selectivity [47].

Recent Examples of Peptides as Inhibitors of Protein-Protein Interactions

It is logical to consider peptides as potential inhibitors of PPIs, as they can be mimics of the natural interaction. Indeed, many groups have designed peptide-based inhibitors (Fig 3) using information gleaned from co-crystal structures of the protein targets [48,49]. However, poor membrane permeability and rapid metabolic instability are often major limitations to their clinical application. Accordingly, advances that tackle these pharmacological problems, such as macrocycles [50], short peptide mimetics [51], introduction of non-natural amino acids [52,53], conformational restricted cyclized peptides [24,54,55], and non-peptide mimetics of α-helical peptides [56] are important milestones.

Recent years have seen peptide-based inhibitors used in many different indications and often as first-in-class inhibitors. For example, Milroy *et al.* were inspired by co-crystal structure of the PPI stabilizer fusicoccin A to develop an inhibitor of tau binding to 14-3-3 for the potential treatment of Alzheimer's disease [56]. Ran *et al.* employed triazole-stapling strategy to make a 300-times more potent TRF2-based peptide, which blocked a previously under-explored RAP1-TRF2 interaction in the shelterin complex [57]. Ran *et al.* rationally designed and screened a peptide library to identify a molecule that inhibits the transcription factor: heat shock factor 1 (HSF1) [58]. Although HSF1 is a large, topologically complex protein, the authors found that optimized peptides derived from the natural inhibitory region, HR-C, limit DNA binding. Zhang *et al.* rationally designed peptidomimetics that potently bind to APC at its interface with Asef. They further optimized several peptides by attaching a transcription trans-activating (TAT) sequence to increase cell permeability and used a cellular thermal-shift assay (CETSA) to show that they disrupt APC-Asef interactions in colorectal cancer cells [59]. Jendrny and Beck-Sickinger took a different approach to modify their peptide-based inhibitor of serpin protease PPIs, by grafting it into a loop of sunflower trypsin inhibitor (SFTI-1) [60]. This chimeric SFTI-1 stabilized the active conformer of the inhibitor and increased its stability. In contrast, an interesting discovery from Giralt *et al.* indicated that a stable secondary structure might not be required in all cases, as they found that flexible structures had greater potency compared to those in helical conformations [61].

Finally, it is worth noting that peptide-based discovery programs have the advantage of established screening technologies, such as phage display and SICLOPPS, that continue to evolve. Phage display was used by Bertoldo *et al.* to identify macrocyclic, rather than linear, peptides that bound to a previous difficult region of beta-catenin [62]. Male *et al.* screened a 3.2 million member SICLOPPS library to identify a peptide that binds to the CMG2 receptor required for uptake of bacterial-derived toxins [63]. Interestingly, the top hits from that screen were linear peptides, rather than the macrocycles that are typically expected from SICLOPPS screens [64*]. New methods for chemically modifying peptides on phage are also emerging [65], promising to diversify the functionality of these materials.

PPI Inhibitors from Other Miscellaneous Classes

One of the other recent trends in PPI inhibitor discovery is the use of molecules that don't readily fit into the designation of small molecules or peptides. For example, organometallic complexes [66–69], foldamer helix mimetics [70,71], peptoids [72] and monobodies [73] have provided supplementary approaches to target PPIs. Several bifunctional molecules of high molecular mass were also reported [74,75], and we have placed them into this category because of their non-tradition size. Whether any of these molecules can be further optimized into drug candidates is still in question, given the limited *in vivo* studies thus far; however, such strategies may be particularly effective as research tools. One counter-intuitive example is worth pointing out: In the process of studying the mechanism of a false positive screening hit, Lumb *et. al.* solved the X-ray crystal structure of an ordered conglomerate of an aggregated small-molecule, bound to TNF-α [76*]. Such a compound would normally be termed a pan-assay interference (PAINS) concern, so one wonders how many other false positives have this surprisingly specific interface with their protein target. Although the molecule is likely not a lead candidate, its contacts with TNF- might help inform future studies.

Analysis of Recent PPI Inhibitors: Molecular Mass, Potency and BSA

From these case studies, it seems that different types of scaffolds (*i.e.* small molecules, peptides, other) are all being generated and that they are being directed against a wide range of PPI targets and indications. To more quantitatively address this question, we performed a PubMed search for PPI inhibitors in the period from Jan 1st 2015 to March 1st 2018. From this list of ~140 examples, we excluded molecules that only had reported cellular activity (*e.g.* EC_{50}) and only used those clear biophysical K_D or K_i values. This search criteria yielded 66 compounds [6,7,23,35–38,40,44–46,55,57,59,60,62,63,70–75,77–131]. Each of these inhibitors was then manually categorized as being either a small molecule, peptide or belonging to neither group. Overall, the average molecular mass of these compounds was significantly larger (~800 Da) than the typical Lipinski range (Fig 4A). However, the small molecule subset largely conformed to the 500 Da cutoff, while the peptides and other categories were almost entirely above this value (see Fig 4A). Next, we prepared a pie chart to analyze which types of scaffolds were being explored (Fig 4B). From the pool of 66 inhibitors, it was clear that small-molecules are the dominant strategy, with over 50% of total cases. Peptides were also quite common, with just under 40%, while only ~10% were in the miscellaneous category. Then, the compounds with the most promising potency values

(K_D < 1 μM) were extracted, giving a pool of the 40 best inhibitors. Interestingly, these compounds had approximately the same distribution as the total pool (Fig 4C), suggesting that sub-micromolar inhibitors can be identified using any of the approaches.

We were also interested in whether the type of scaffold (*e.g.* small molecule, peptide) impacted the type of PPI that was targeted. To approach this question, we calculated the BSA values for 50 of the 66 PPI interfaces that had sufficient structural information, using the reported PDB structures and UCSF Chimera [132]. When we plotted the molecular weight of the inhibitor against the calculated BSA values, we found that more than 80% of the small molecule were inhibiting PPIs with interfacial area below 2000 $Å^2$ (Fig 5). Conversely, ~50% of the peptides were directed against PPIs with BSA > 2000 $Å^2$, suggesting that they tend to be better for inhibiting large interactions.

Finally, we wanted to understand the relationships between inhibitor potency and the BSA of the target PPI. A plot shows that the majority of potent inhibitors (K_D < 1 μM) seemed to be clustered against targets with low BSA (Fig 6), consistent with pre-2015 studies [13]. Thus, concise PPIs seemed to be the most amenable to the discovery of inhibitors. However, it is interesting to note that sub-micromolar inhibitors of large interfaces were also identified. For targets in the range between 2000 $Å^2$ and 4000 $Å^2$, there were 5 potent, reported inhibitors and there were even three inhibitors of targets in the BSA range >4000 $Å^2$. Interestingly, inhibitors of these large targets tended to be peptide-based. For example, two of the inhibitors of large (>4000 $Å^2$ BSA) interfaces were cyclic peptides targeting DOCK2-Rac1 (6160 $Å^2$) and Shh-HHIP (5048 $Å^2$), respectively [92,95]. Overall, 6/8 inhibitors of large PPIs were peptides. In addition, one of the small molecules is known to have an allosteric mechanism (Hsp70-BAG3; 4473 $Å^2$) [23]). Together, these collective findings suggest that orthosteric small molecules might be more challenging to develop against these types of PPIs.

Summary and Prospectus

The last few years have continued to remove the undeserved stigma of PPIs being “undruggable”. At the same time, it is becoming increasingly clear that some PPIs are more difficult to tackle than others and that the original concerns may sometimes be justified. Thus, it seems that a more nuanced and sophisticated answer to the question of whether PPIs can be inhibited is that all targets are not equal. For each individual system, its own idiosyncratic features (*i.e.* are high resolution crystal structures available? do conformational changes accompany binding? is there a natural ligand to start with? what is the BSA?) will likely dictate whether a small molecule, peptide or other approach might be best and whether the search will ultimately be successful in producing a sub-micromolar inhibitor. For example, from our analysis here, we would argue that peptide-based compounds might be preferred for PPIs with large BSA values (>2000 $Å^2$). We would also conclude that small molecules are still the go-to method for amenable PPIs, such as those with low BSA.

What is next for PPI inhibitor discovery? How do we improve discovery, especially for the PPIs with large BSA values? One exciting speculation is that cryo-EM methods may allow atomistic resolution of previously inaccessible protein complexes. For example, Merk *et al.*

have been able to identify the binding site of ligands in isocitrate dehydrogenase, lactate dehydrogenase and glutamate dehydrogenase at < 4 Å resolution [133*], potentially opening the way to CBDD or other strategies for structure-guided design. Recent advances in the computational identification of cryptic binding sites may also create opportunities for the rational development of allosteric inhibitors [134,135]. Finally, it seems that PROTACS molecules or covalent inhibitors [136] might be fundamentally new ways target the currently "undruggable" systems. More broadly, retrospective analyses, such as this one, might reveal the gaps: the problems that lack a reliable, current solution. These are likely the topics that need the most innovation.

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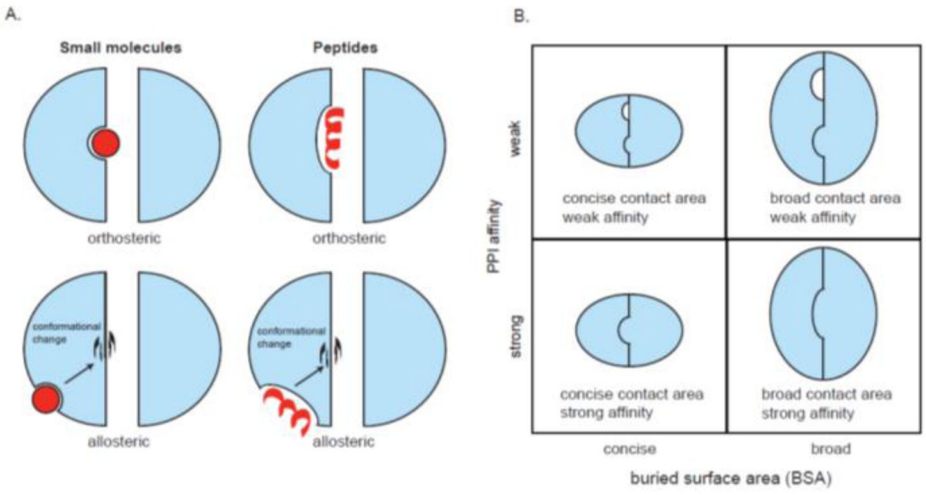
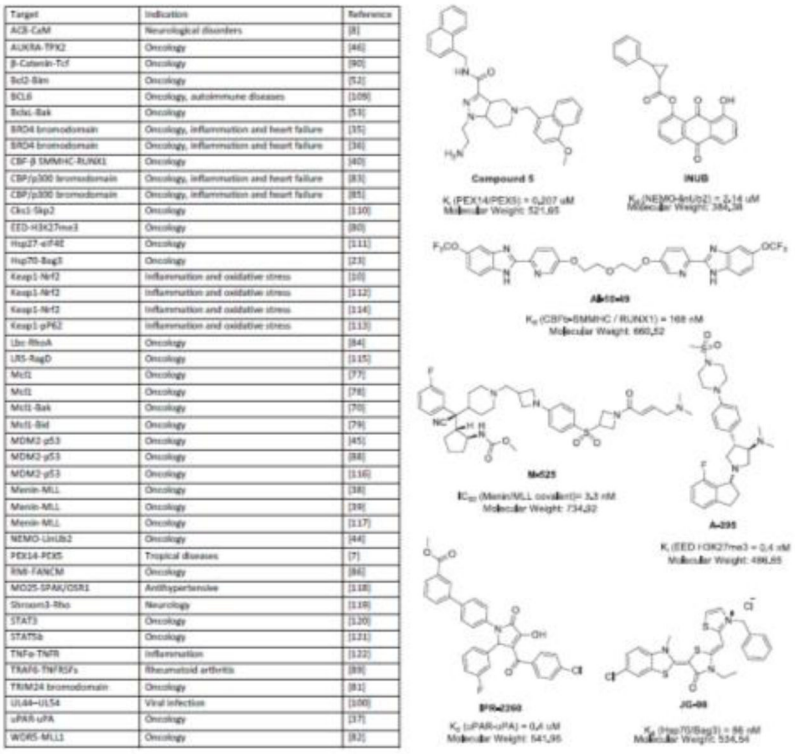


Figure 1.

Schematic properties of PPI inhibitors. (A) Small molecules or peptides can inhibit PPIs by binding to the interface (orthosteric) or a distal site (allosteric). Peptides are often derived from natural protein partners and can be either structured (e.g. helical) or disordered (e.g. random coil). (B) Categorization of PPIs based on buried surface area (BSA) and the affinity of the PPI creates four types of interactions. In the last 20+ years of PPI inhibitor discovery, the majority of reported inhibitors have been directed at the concise/strong qudrant (bottom left). These compounds also tend to have the best potency values.See the text for references.



Target	Indication	Reference
ACB-CaM	Neurological disorders	[8]
AUKRA-TPX2	Oncology	[46]
β-Catenin-Tcf	Oncology	[90]
Bcl2-Bim	Oncology	[52]
BCL6	Oncology, autoimmune diseases	[109]
Bclxl-Bak	Oncology	[53]
BRD4 bromodomain	Oncology, inflammation and heart failure	[35]
BRD4 bromodomain	Oncology, inflammation and heart failure	[36]
CBF-β SMMHC-RUNX1	Oncology	[40]
CBP/p300 bromodomain	Oncology, inflammation and heart failure	[83]
CBP/p300 bromodomain	Oncology, inflammation and heart failure	[85]
Cks1-Skp2	Oncology	[110]
EED-H3K27me3	Oncology	[80]
Hsp27-eIF4E	Oncology	[111]
Hsp70-Bag3	Oncology	[23]
Keap1-Nrf2	Inflammation and oxidative stress	[10]
Keap1-Nrf2	Inflammation and oxidative stress	[112]
Keap1-Nrf2	Inflammation and oxidative stress	[114]
Keap1-p62	Inflammation and oxidative stress	[113]
Lbc-RhoA	Oncology	[84]
LRS-RagD	Oncology	[115]
Mcl1	Oncology	[77]
Mcl1	Oncology	[78]
Mcl1-Bak	Oncology	[70]
Mcl1-Bid	Oncology	[79]
MDM2-p53	Oncology	[45]
MDM2-p53	Oncology	[88]
MDM2-p53	Oncology	[116]
Menin-MLL	Oncology	[38]
Menin-MLL	Oncology	[39]
Menin-MLL	Oncology	[117]
NEMO-LinUb2	Oncology	[44]
PEX14-PEX5	Tropical diseases	[7]
RMI-FANCM	Oncology	[86]
MO25-SPAK/OSR1	Antihypertensive	[118]
Shroom3-Rho	Neurology	[119]
STAT3	Oncology	[120]
STAT5a	Oncology	[121]
TNFα-TNFR	Inflammation	[122]
TRAF6-TNFRSFs	Rheumatoid arthritis	[89]
TRIM24 bromodomain	Oncology	[81]
UL44–UL54	Viral infection	[100]
uPAR-uPA	Oncology	[37]
WDR5-MLL1	Oncology	[82]

Figure 2.
Targets, disease indications and chemical structures of select small molecule PPI inhibitors

Target	Indication	Reference
14-3-3/Tau	Alzheimer's disease	[74]
APC-Asef	Oncology	[59]
βArrestin-βAdapin2	Oncology	[61]
βCatenin-αCatenin	Oncology	[98]
βCatenin-ICAT	Oncology	[62]
βCatenin-TCF LEF	Oncology	[104]
Bcl2 A1	Oncology	[94]
BRCA1	Oncology	[96]
CD2-CD58	Oncology, immunology	[123]
CMG2-PA	Bacteria infection	[63]
DOCK2-Rac1	Oncology	[95]
GluK2-PSD-95	Ischemic Stroke	[124]
HDM2-p53	Oncology	[67]
HER2-EGFR	Oncology	[102]
HIF1a-HIF1b	Oncology	[105]
IGFBP3-Importin β	Oncology	[125]
Keap1-Nrf2	Inflammation and oxidative stress	[10]
KLK7-Serpin	Oncology	[60]
LeETR1-LeEIN2	Fruit ripening control	[126]
LeETR4-NR	Fruit ripening control	[103]
MDM2-p53	Oncology	[91]
mTOR- Rictor	Oncology	[127]
NCS1-Ric8a	Neurology	[128]
PARP1	Oncology	[129]
PD1-PD1L	Oncology, immunology	[6]
PDGFRβ-PDGFB	Liver fibrosis	[106]
PDK1	Oncology	[130]
PLK1	Oncology	[131]
RAP1-TRF2	Oncology	[57]
Shh	Oncology	[92]
TLE1	Oncology	[107]
VHL-HIFa	Oncology, immunology, inflammation	[97]
WDR5-MLL1	Oncology	[55]

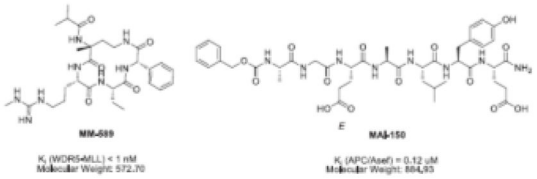


Figure 3.
Targets, disease indications and chemical structures of select peptide-based PPI inhibitors

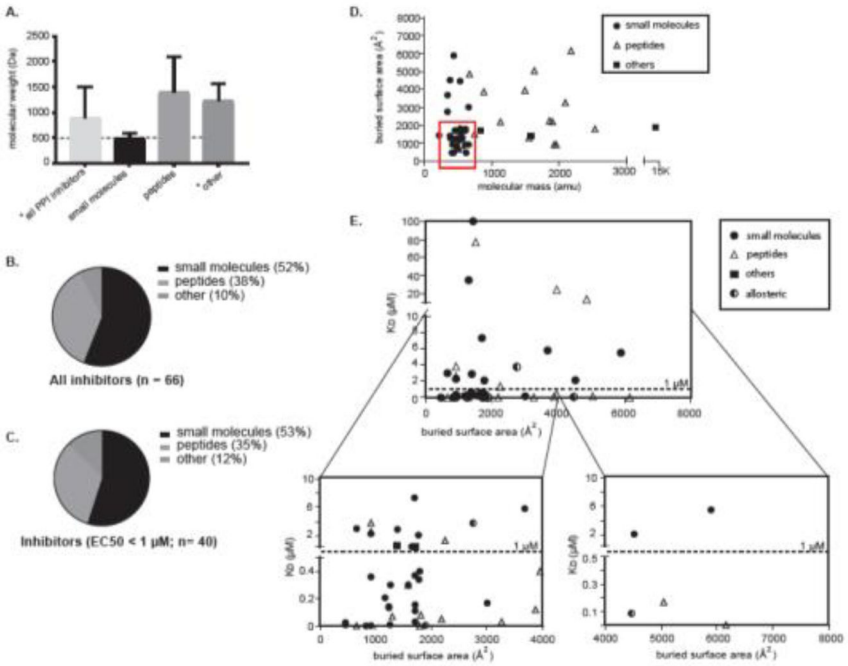


Figure 4.

Relative properties and composition of the technologies used to target PPIs (reported 1/15 to 3/18). (A)The average molecular mass of the three categories of PPI inhibitors. Error bars represent the standard error of the mean (SEM). The dotted line is set to 500 Da. * monobody excluded (B) The relative contriibution of the three inhibitor categories, calculated from the 66 literature examples with reported Kd/Ki value. (B) An analysis of the subset of 40 cases that have reported Kd/Ki values < 1 μM. (D) Distribution of PPI inhibitors based on their molecular weight and BSA of the target interface. The red box signifies the most drug-like inhibitors (e.g. those with the lowest mass and best potency).

(E) Distribution of recent PPI inhibitors (1/15 to 3/18). Each compound was manually designated as either a small molecule, peptide or miscellaneous. In addition, the mechanism-of-action was designated as orthosteric (solid color) or allosteric (split color). For each compound, its potency (Kd or Ki) was plotted against buried surface area (BSA) of the target PPI. The bottom two quadrants (the most potent molecules) are shown as close-ups for clarity.