Fine-Tuning Multiprotein Complexes Using Small Molecules

Andrea D. Thompson,[†,‡] Amanda Dugan,[†] Jason E. Gestwicki,*[,†,‡] and Anna K. Mapp*[,†,§]

[†]Program in Chemical Biology, [‡]Departments of Pathology and Biological Chemistry and the Life Sciences Institute, and [§]Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109, United States

ABSTRACT: Multiprotein complexes such as the transcriptional machinery, signaling hubs, and protein folding machines are typically composed of at least one enzyme combined with multiple non-enzymes. Often the components of these complexes are incorporated in a combinatorial manner, in which the ultimate composition of the system helps dictate the type, location, or duration of cellular activities. Although drugs and chemical probes have traditionally targeted the enzyme components, emerging strategies call for controlling the function of protein complexes by modulation of protein−protein interactions (PPIs). However, the challenges of targeting PPIs have been well documented, and the diversity of PPIs makes a "one-size-fits-all" solution highly unlikely. These hurdles are particularly daunting for PPIs that encompass large buried surface areas and those with weak affinities. In this Review, we discuss lessons from natural systems, in which allostery and other mechanisms are used to overcome the challenge of regulating the most difficult PPIs. These systems may provide a blueprint for identifying small molecules that target challenging PPIs and affecting molecular decision-making within multiprotein systems.

Natural Mechanisms for Tuning Complex Assembly and Function
(C) Dynamics
(B) PTMs
(A) Allostery
(D) Localization
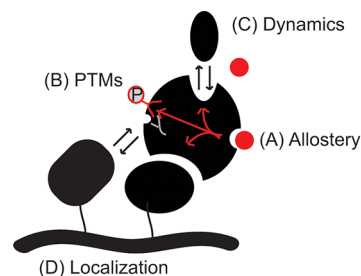
Protein−protein interactions (PPIs) form the backbone of nearly every facet of cellular function, as illustrated by proteome-wide maps composed of thousands of these contacts.[1] At the core of PPI networks are numerous multiprotein complexes dedicated to major cellular tasks, including systems involved in transcription, translation, trafficking, energy production, protein folding, cytokinesis, and signaling. These multiprotein complexes are typically composed of at least one enzyme, such as an ATPase, and a series of non-enzymatic factors, such as scaffolding proteins (Figure 1). These non-enzyme partners associate either stably or transiently with the complex and help fine-tune activity, subcellular location, and/or selectivity. One traditional goal of drug discovery and chemical biology has been to develop compounds that target the enzyme components of multiprotein complexes.[2] This approach has been fruitful, producing many of the most widely used chemical probes and drugs. Yet, PPIs within multiprotein complexes may provide an even greater number of opportunities, a prospect that is supported by a growing appreciation of the potential of PPIs as drug targets.[3,4]

Although the goal of modulating PPIs with small molecules has been recognized for some time, these interactions have been historically challenging to interrupt.[2,5,6] Indeed, the majority of PPIs have often been broadly classified as "undruggable" and among the estimated 650,000 PPIs, far less than 0.01% have been targeted with inhibitors.[2,6−8] However, a number of recent success stories, produced by both academic and industrial groups, have challenged the view that PPIs are uniformly insurmountable as targets.[9−11] Building on these past successes, we believe that by understanding the diverse strategies employed in nature to modulate PPIs, chemical biologists can further improve their ability to discover PPI modulators. To support this more optimistic view of PPI modulators, we highlight several representative examples of successful PPI modulator programs and discuss potential ways of accelerating their discovery in the future. In particular, we focus on the most challenging PPIs, the weak interactions that are central to many areas of biology yet remain the most difficult to target with small molecules.

ARCHITECTURE OF MULTIPROTEIN COMPLEXES

Given the central role of multiprotein complexes in biology, perturbing the assembly and/or disassembly of these structures has the potential to provide important insights. In this discussion, a multiprotein complex is defined as a system of proteins that assemble, either permanently or transiently, to perform specific tasks in the cell. Most multiprotein complexes contain at least one enzyme and typically have multiple non-enzyme components. The role of the non-enzymes is often to provide a scaffolding function, linking proteins together or controlling subcellular localization, as seen in GPCR recycling (Figure 1).[12] Also, the PPIs between the enzyme and non-enzyme can sometimes tune the biochemistry of the enzyme, enhancing K_m or k_{cat}, as seen in the Ras GTPase cycle or the Hsp70 ATPase system (Figure 1).[13,14] Finally, the protein surfaces used to form PPIs might be shared by multiple components, such that subunits compete for binding. In this way, complexes can be combinatorially assembled. This combinatorial assembly is present within the Hsp70 system to switch from a folding system to a degradation complex
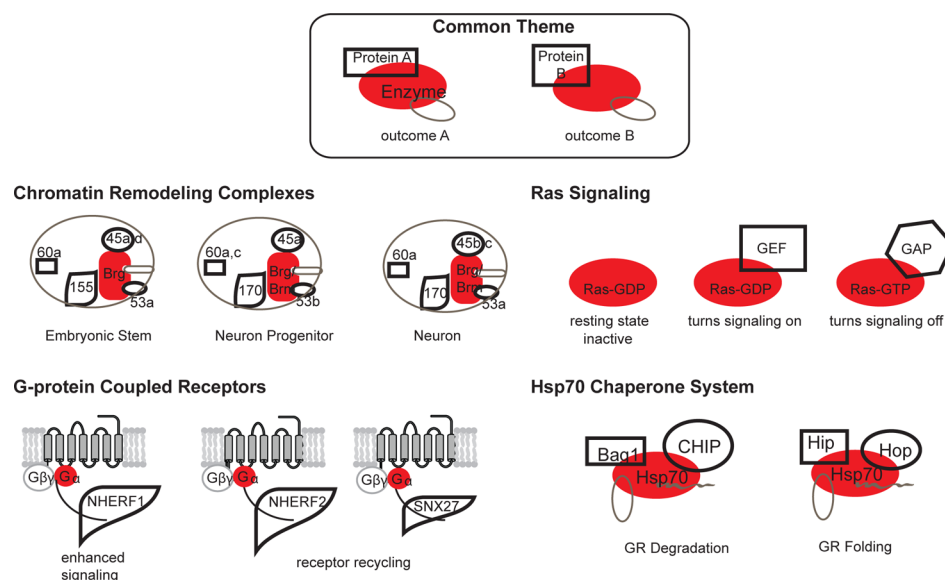
Figure 1. Multi-protein complexes are assembled from enzymes or receptors bound to multiple, non-enzyme partners through protein-protein interactions. The majority of protein complexes in biology share the common features of being assembled from protein-protein interactions (PPIs) between enzymes (red) and non-enzymes (white). These factors assemble into multi-protein systems that have emergent properties (*e.g.* biology not engendered by any individual component) and essential roles in molecular processes in the cell. To illustrate this idea, a few of the major protein complexes are shown. Switching components of these complexes can alter function; for example, the BAF45a component of the Brg/Brm complex defines neuronal progenitors, while BAF45b is only found in differentiated neurons.

(Figure 1). As another prime example, BAF-type chromatin remodeling complexes undergo subunit exchange during the transition from a pluripotent stem cell into a neuron progenitor cell and finally into a fully differentiated neuron (Figure 1).[15,16] In these systems, one appreciates why solely targeting the enzyme component might not be the most informative approach. Rather, inhibiting (or promoting) specific PPIs could be of great value for understanding a wide range of essential cellular processes.

In contrast to protein–ligand interfaces (PLIs) such as those between enzymes and their substrates,[5,17] PPIs are larger and flatter[18] (Figure 2A), with an average surface area of 1940 ± 760 Å^2. Sometimes, a disproportionate amount of the binding free energy (ΔG) is found within specific residues, termed "hotspots".[19] Other times, ΔG is distributed over a larger surface area or in a few regions separated by large distances. Consistent with their distribution of contact areas, PPIs also exhibit a wide range of affinity values, with examples of picomolar dissociation constants in more stable complexes and up to millimolar values in transient complexes,[20–22] Several examples of PPIs with a range of affinities and contact areas are highlighted in Figure 2.[23–36] The scope of this diversity is postulated to be even wider than currently appreciated, given the underrepresentation in the Protein Data Bank (PDB) of transient, low affinity PPIs.[22,37]

To date, the PPIs characterized by tight affinities and small surface areas (i.e., most similar to PLIs) have proven to be the most amenable to targeting with small molecules. Often, structure-based approaches have proven successful for this category of interactions. For example, pioneering work has been performed on SMAC mimetics (Figure 2A) and inhibitors of p53-MDM2.[10,11] These targets are characterized by binding sites composed of a short peptide interacting with a deep pocket, as in the SMAC interaction with IAPs (Figure 2A), or a single α helix interacting with a well-defined cleft, as in p53-MDM2. To explore how general these characteristics are for all

known PPI inhibitors, the 2P2IDB and TIMBAL databases were queried to sort known PPI inhibitors based on the surface areas and affinities of their targets. We found that PPIs with smaller surface areas (less than <1,800 Å^2) and relatively strong affinity (less than 1 μM) are targeted by 68% of reported small molecules (Figure 2D).[10,11] In contrast, only 10% bind to large surface area-strong binding PPIs (Figure 2D). One potential explanation for this preference is that concise, strong affinity PPIs rely on binding pockets that are best "fit" by low molecular mass (<500 Da) compounds, and these compounds are most common in drug discovery libraries.[2,5] Also, it is relatively straightforward to envision how competitive inhibitors could block these interactions because the ΔG is contributed by a handful (typically less than 5) of tightly clustered residues.[9,38]

What are the specific challenges to circumvent in successfully blocking the "difficult" PPIs, those with weak affinity and/or large surface area? Large binding surface areas have been particularly difficult to directly (*i.e.*, orthosterically) inhibit with small molecules, as the compound must compete with a much larger protein for binding. In some cases, "hotspots" can be used to generate potent inhibitors.[19] Still, accessing hotspots often requires extensive structural knowledge, and in many cases, hotspots are either far removed from each other or not present at all. Another challenge is that inhibitors of large surface areas tend to have high molecular weights, often not conforming to the standard Lipinski's Rule of 5 (Ro5).[39,40] In fact, many successful PPI inhibitors deviate from the Ro5,[11] potentially creating challenges with pharmacokinetics and oral bioavailability. Another significant problem is that many important PPIs are of weak affinity (Figure 1D). From a practical perspective, these weak systems are also challenging to study using traditional structural and biophysical methods, such as ITC.[22,37] These challenges have slowed discovery of successful inhibitors for the most challenging PPIs. In the
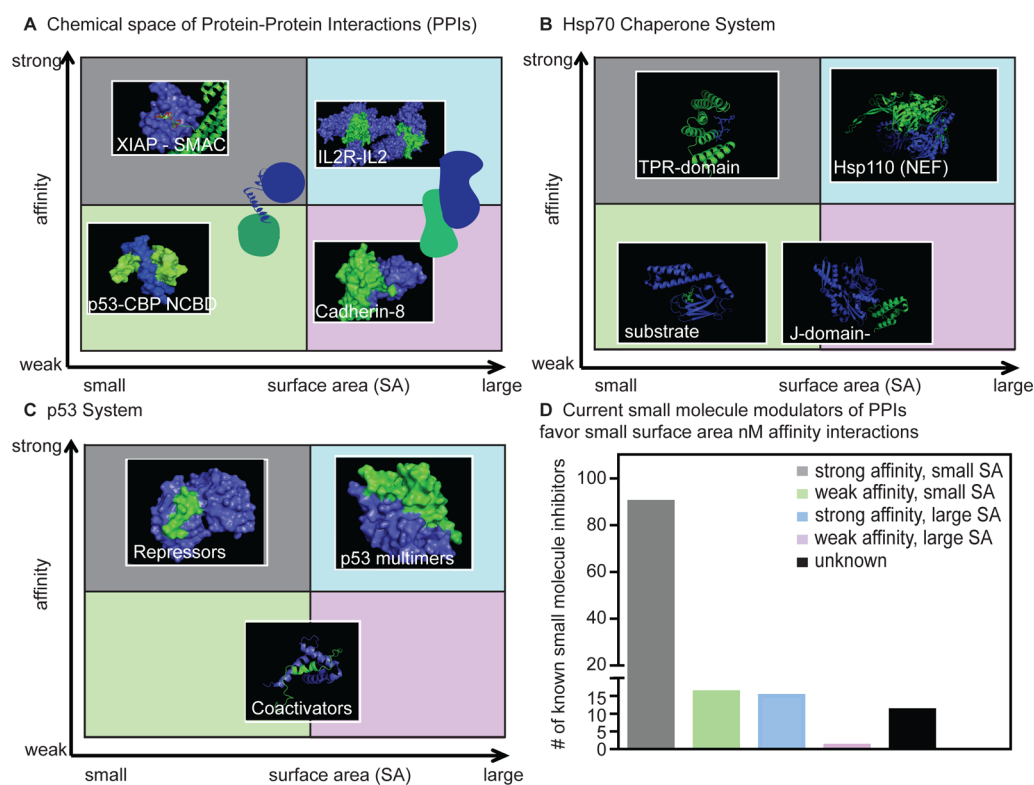
Figure 2. Not all protein-protein interactions are created equal: some PPIs are harder to inhibit than others. (A) PPIs can be roughly categorized by their apparent affinity and the buried surface area involved in the contact. This type of analysis creates four major quadrants of interactions: strong and concise, strong and broad, weak and concise, and weak and broad. Examples of each type of interaction from the Protein Data Bank (PDB) are shown. Strong and concise: XIAP-SMAC (2JK7, 1G73); strong and broad: IL-2-IL2R (2ERJ); weak and concise: p53-CBP NCBD complex (2L14); weak and broad: cadherin-8 homer dimer (1ZXK). (B) Multiple categories of PPIs are often found within a single complex, as illustrated by the Hsp70 system in which the four major types of PPIs are represented in interactions between Hsp70 and its associated partners: substrate (1DKY); TPR-domain protein (3Q49); nucleotide exchange factor (3C7N); J-domain (2QWN). (C) The p53 system is another example of a system in which multiple types of PPIs are found in the same complex. The repressor Mdmx (3DAC); p53 self-association (1PET); transcriptional coactivators (2K8F). (D) PPIs with known inhibitors were acquired from the 2P2IDB and TIMBAL databases and their binding affinities (as reported in the PDBbind[115]) and the surface are of the PPI (as measured by InterProSurf [116]) were determined. Placing these PPIs into categories revealed that small molecules which target PPIs with smaller concise surface ares (<1,800 Å) and relatively strong affinities (<1 mM) represent 68% of known PPI inhibitors.

next section, we describe how nature has evolved mechanisms to circumvent these issues.

◼ MODULATING PROTEIN−PROTEIN INTERACTIONS: LESSONS FROM NATURE

Allostery. Allostery is one of the most widely used mechanisms by which natural ligands control the assembly and disassembly of multiprotein complexes. Allostery is defined as binding at one site which regulates a function at a distant site[41] and classic examples include cooperative oxygen binding to hemoglobin or feedback inhibition within metabolic pathways. Allosteric mechanisms can also be used to control PPIs. For example, galactose promotes the formation of a stable complex between Gal3p and Gal80p, which subsequently activates the transcription of galactose catabolizing enzymes in yeast.[42] In these systems, even low molecular weight compounds are able to dramatically impact protein function by regulating PPIs at a distance.

Nature's use of allostery to control protein complex formation is not surprising given several advantages of this strategy. First, allosteric binding sites are often distinct from reactive centers in enzymes; thus, their topologies are not constrained by active site chemistry. This feature means that it is sometimes possible to discriminate between members of a

related enzyme family (*e.g.*, kinases) by taking advantage of their unique protein interactions rather than their highly conserved active sites. Another advantage of allostery is that small molecules can sometimes be used to reshape even the most challenging PPI surfaces by binding to sites far from the actual interface.[43] This feature is particularly advantageous when the PPI itself is very complex and shallow. In these cases, it might be preferable to seek out allosteric sites that are deeper and more amenable to binding. Finally, allosteric compounds can sometimes influence the decision to bind one protein partner over another at shared interface.[44] By subtly changing the topology of a surface, these molecules can favor binding to specific components of a complex, thereby influencing function. Such mechanisms are not possible with orthosteric compounds, which compete for binding at a given interface and, as such, cannot discriminate between two different proteins.

Natural examples may point the way to synthetic strategies for controlling the assembly and disassembly of PPIs, especially in systems that are particularly challenging for direct inhibition.[41] As such, a very active area of research is currently focused on defining interactions between metabolites and/or secondary messengers and proteins.[45] These efforts have the potential to greatly improve our ability to rationally target PPIs. For example, porphobilinogen synthase (PBGS) regulates its

1313

dx.doi.org/10.1021/cb300255p | *ACS Chem. Biol.* 2012, 7, 1311−1320
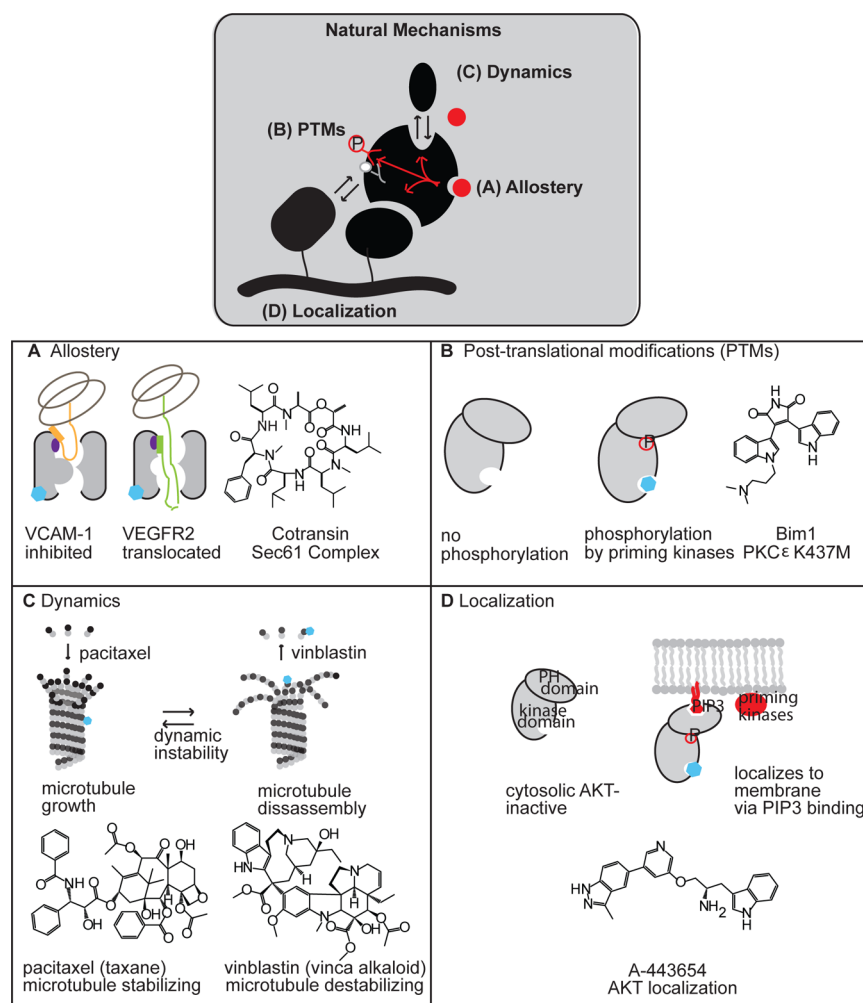
Figure 3. Nature-inspired strategies for modulating difficult PPIs. Natural multi-protein systems use mechanisms such as allostery, PTMs, dynamics, and subcellular localization to control their assembly and disassembly. Small molecules have been found to access similar mechanisms, such as; (A) allosteric regulation of the Sec61 translocon by cotransin, with blocks translocation of VCAM-1 without affecting VEGFR2 (shown in green). (B) conformational change in PKCε caused by Bim1, which allows binding to priming kinases, (C) altering microtubule dynamics with pacitaxel and vinblastin, and (D) membrane localization of Akt induced by the kinase inhibitor A-443654. In all figures the blue hexagon denotes the small molecules and red denotes phosphorylation or PIP3. Proteins and interacting proteins are shown in gray and black unless otherwise noted.

activity *via* a dynamic equilibrium between two alternative and functionally distinct conformations, an active octamer and a low activity hexamer. Only the active octamer binds to Mg^{2+}, which enforces the conformational change. Appreciating this natural conformation equilibrium and the ability of the magnesium ion to influence the system, Lawrence and colleagues identified a small molecule, Morphlock 1, which favors the low activity hexamer by binding to an interface only accessible in the conformation unique to hexamer assembly.[46,47] We anticipate that similar lessons from nature will be useful in providing paths toward small molecules that target the most challenging of PPIs.

Further illustrating the advantages of this approach, a number of synthetic molecules with allosteric mechanisms have been reported.[48−51] For example, a compound that binds the inducible nitric oxide synthase (iNOS) leads to disruption of a distant dimerization interface, inactivating the protein by blocking dimer formation.[52] In another example, Conn and colleagues have applied allosteric regulators to target G-protein coupled receptors (GPCRs), finding molecules that activate or inhibit discrete subsets of downstream pathways by changing

the PPIs that occur between the GPCRs and their effector proteins.[53] Another example is found in the allosteric modulation of the Sec61 translocon (Figure 3A). The Sec61 complex is responsible for transporting nascent polypeptides across the ER membrane.[54] As a nascent polypeptide is synthesized, a signal sequence in its N-terminus binds directly to the Sec61 complex,[55,56] which triggers an allosteric conformational change to open the channel to the ER. Recently both Besmer and Garrison identified small molecules that modulate the Sec61 translocon complex in an interesting way. These compounds, such as cotransin (CT), cause a selective decrease in translocation of vascular cell adhesion molecule 1 (VCAM1) without affecting the levels of other membrane proteins (Figure 3A).[57,58] CT appears to block the interaction between VCAM-1 and Sec61 *α* by favoring binding to Sec61*β*. These examples and others illustrate how allostery can be used to impact seemingly intractable PPIs.

Allostery, in concert with multivalency and other mechanisms, can also tightly regulate subcellular localization of key subunits. A classic example of the central role localization plays in cellular function is signaling in the T-cell receptor (TCR)

complex,[59] in which the antigen−MHC complex recruits TCRs at the membrane. As with other mechanisms, this type of regulation can be exploited by small molecules. For example, an Akt kinase inhibitor was shown to activate Akt signaling by inducing a conformational change, increasing affinity for phosphatidylinositol (3,4,5)-triphosphate (PIP3) in the membrane[60] (Figure 3D). In turn, localization to the membrane promotes the interaction of Akt with priming kinases. This example highlights how taking advantage of natural conformational changes can be used to alter localization and complex formation.

Post-translational Modifications. A distinct and widely used mechanism to regulate complex assembly is post-translational modifications (PTMs) including phosphorylation, glycosylation, acetylation, ubiquitination, neddylation, and sumolyation, among others.[61] PTMs occur at many PPI interfaces to promote or inhibit binding, as exemplified by SH2 domain binding to phosphorylated tyrosine residues.[61] However, PTMs can also occur far from PPI surfaces, changing protein stability, folding, or conformation and regulating complex formation.[61]

There are several ways small molecules could use PTM-like mechanisms to bring about changes in PPIs. One dramatic way is through formation of drug−protein adducts. Several examples of covalent drugs with low toxicity are known, including aspirin, proteasome inhibitors, and acetaminophen.[62] Although these classic examples are all enzyme inhibitors, similar mechanisms appear to operate at PPIs, such as those used by compounds that target the NRF2−KEAP1 complex.[63] Another inventive mechanism in which small molecules leverage PTMs is exemplified by the paradoxical activation observed with a PKCε kinase inhibitor.[64] A K437 M mutant of PKCε has been shown to be overcome by the binding of an ATP active site inhibitor, which promotes a conformational change that facilitates interaction with and phosphorylation by priming kinases, effectively activating this signaling pathway (Figure 3B). These studies exemplify how small molecules can alter function through tuning PTMs and regulating PPIs.

■ STRATEGIES FOR IDENTIFYING COMPOUNDS THAT CAN INHIBIT OR PROMOTE COMPLEX FORMATION

How can we take these lessons from nature and develop ways to identify compounds that target PPIs? Toward these goals, a variety of screening platforms and assays can be utilized. For example, many of the compounds discussed earlier act allosterically. Thus, techniques that identify small molecule allosteric binding sites could have broad utility in the search for PPI modulators. One of the first major techniques used for finding PPI inhibitors, disulfide tethering, focused PPI inhibitor discovery on hotspot residues using covalent coupling.[65] Disulfide tethering has also been utilized to identify novel allosteric inhibitors. For example, an allosteric inhibitor of caspase 1/7 that traps it in an inactive conformation was identified using this technique.[66] Another related approach to this problem is fragment-based screening (FBS).[67] FBS purposefully employs weak binding (mM) small molecule "fragments" of less than ∼200 Da. These chemical libraries are screened using techniques sensitive enough to measure weak affinity interactions, such as Thermofluor, ITC, NMR, surface plasmon resonance, and X-ray crystallography.[68] These initial hits are developed further by either linking or growing fragments to improve affinity and activity. These methods

have proven to be particularly powerful for PPIs because they can identify unanticipated allosteric sites.[69] Further, this technique has been applied to find competitive inhibitors of larger PPIs, such as the Bcl-XL/Bcl-2-BAD/BAX interaction.[70]

Computational approaches offer a complementary method to predict allosteric binding sites suitable for regulating PPIs.[5] Some techniques leverage "statistical coupling" of amino acid residues throughout evolution.[71,72] The theory behind these techniques is that if two positions are functionally coupled, their amino acid identities should be constrained through evolution. Other computational methods simulate mutations and map structural perturbations or perform computational alanine scanning.[73,74] Another approach, called anisotropic thermal diffusion, tracks the propagation of kinetic energy emanating from a heated target location.[75] Finally, the protein structure can be searched using van der Waals probes to identify potential ligand sites in allosteric locations.[76−78] This list is not meant to be inclusive but rather to illustrate that computational efforts toward this goal have been extensive, suggesting the possibility of rationally designing allosteric PPI regulators. For example, the previously mentioned, Morphlock-1, was identified through *in silico* screening of an allosteric site.[46]

Another "nature-inspired" way to develop PPI modulators may be to alter protein dynamics. Like many other cellular complexes, both the ribosome and microtubules rely on the dynamic interchange of subunits to function.[79,80] Upon looking at the complicated and dynamic protein complexes that make up microtubules and the ribosome, one might conclude that these complexes would be very difficult drug targets. Yet, widely used therapeutics, such as antibiotic and taxanes, demonstrate that disrupting dynamics can enable small molecule modulation of even the most challenging drug targets (Figure 3C). In nature, the affinities of the PPIs in these systems are tightly controlled to ensure that they are strong enough to form complexes yet weak enough to allow exchange.[81,82] Thus, small molecules need only to perturb this narrow affinity window to disrupt dynamic exchange. One technique which may allow one to identify such small molecules is Forster resonance energy transfer (FRET), which remains a workhorse method to monitor protein dynamics. This approach has been successfully used to identify small molecules that alter protein conformation and PPI dynamics both *in vitro* and *in vivo*.[83−86] Another approach is to employ isotopically labeled unnatural amino acids and NMR spectroscopy to measure conformational changes in the presence of small molecules. An advantage of this approach is that it simplifies the NMR spectra, making it suitable for larger proteins. This approach was used to study the binding of a small molecule to the thioesterase domain of fatty acid synthase (FAS-TE), a protein of therapeutic interest in the treatment of cancer and obesity.[87] Finally, selective labeling of side chain methyl groups, combined with NMR, has proven to be sensitive to structure, dynamics, and conformational changes.[88] These spectroscopic methods provide valuable information regarding conformational transitions and are particularly well suited for discovery of PPI inhibitors that alter dynamics. The goal in many of these discovery efforts is to screen for compounds that shift an ensemble and stabilize specific, active, or inactive conformers.[89]

Despite the challenges outlined above, a surprising number of PPI inhibitors have been identified by HTS methods. For example, ubistatins, which stabilize cyclin B by blocking ubiquitin PPIs, were identified using a screen in *Xenopus*

extracts.[90] Biochemical methods for screening difficult PPIs include FRET, fluorescence polarization (FP), capillary electrophoresis, and flow cytometry, and several groups have explored ways of making these platforms favor the discovery of allosteric molecules.[91,92] For example, high concentrations of an interacting partner can be used to disfavor binding to orthosteric ligands and enrich for allosteric compounds. Other HTS methods have also been specially designed to favor the identification of PPI inhibitors or activators. For example, chemoproteomic profiling allows measurements of drug-induced changes in protein complex formation.[93,94] Another promising approach is "grey-box" screening, in which multiprotein complexes are reconstituted *in vitro*, and then an HTS method is used to find molecules that impact the biochemical properties of the complex. For example, in a screen for inhibitors of the p21-activated kinase (Pak1), Deacon and colleagues chose to use the full-length protein, including its non-catalytic domain, in complex with an activator protein, Cdc42, and a substrate, maltose binding protein.[95] By including this full complex, they identified a noncompetitive inhibitor IPA-3 that binds the autoregulatory non-catalytic domain and blocks activation by Cdc42. In another example, the prokaryotic Hsp70 system, composed of DnaK and DnaJ, was reconstituted and screened.[51] DnaK is an ATPase that is stimulated by the non-enzyme DnaJ. An HTS method using a DnaK-DnaJ mixture identified molecules that blocked ATPase activity by selectively disrupting the weak interactions between these two proteins.[51,96,97] These examples suggest that "grey-box" screening has the potential to identify small molecules enriched in their ability to "fine-tune" complex formation.

One powerful way to uncover inhibitors of PPIs is to directly measure the binding between protein partners and screen for compounds that disrupt this contact. However, one of the challenges imposed by difficult PPIs is that most HTS platforms for measuring binding affinities are less suited for characterization of transient, lower affinity interactions.[98,99] Toward that goal, several methods have proven useful in identifying transiently interacting protein partners in cells including yeast-2-hybrid (Y2H) systems, bimolecular fluorescence complementation (BiFC), and *in vivo* cross-linking strategies.[100−103] Although there are caveats to each of these methods, their proper implementation provides the ability to measure transient, moderate affinity PPIs in cells. *In vivo* cross-linking using unnatural amino acid mutagenesis has also recently been identified as a powerful tool for covalently capturing both high affinity and transient, lower affinity protein−protein interactions in yeast.[101,104] Combined with mass spectrometric methods, this technique creates a powerful platform for characterizing weak PPIs and developing inhibitors of these contacts.

■ TOWARD COMPOUND LIBRARIES ENRICHED FOR PPI INHIBITORS

Compounds that target PPIs tend to be higher molecular mass, which is expected on the basis of the more complex topology of these interactions.[11] In addition, many successful modulators of PPIs are natural products, which are typically larger and more topologically complex than average synthetic compounds. These observations have led many groups to consider that typical commercial chemical libraries may not be the most appropriate for finding PPI inhibitors, owing to the tendency of these collections to be composed of low mass and low complexity molecules. Accordingly, many new methods such as

DNA encoded combinatorial libraries (DELs) and improved diversity-oriented synthesis (DOS) strategies have been developed to produce more complex compounds.[105−108] Similarly, chemical-protein hybrids,[109] secondary structure mimetics,[110] aptamers,[111] and antibody-like molecules[112] have been developed in an attempt to better match the topology of PPIs. Finally, natural products, metabolites, and natural product-like collections are finding renewed use as sources of PPI modulators.[113,114] Unfortunately, these concepts have still not been widely adapted by public screening facilities; as of 2010, only 1% of the NIH Molecular Libraries Small Molecule Repository was natural product-like.[106] However, a greater focus on the biology of PPIs, especially the most difficult PPIs, may drive the development of additional commercial collections that cater to the particular needs of these systems.

■ SUMMARY

PPIs are emerging as promising drug targets, and reports of PPI inhibitors have become increasing widespread. The next frontier in PPI research is to go beyond the concise, tight affinity PPIs that have constituted a majority of the published success stories thus far. Rather, the next phase is to understand how to target the PPIs with large and complex surface topologies and those with weak, transient contacts. Because these "challenging" PPIs are the least amenable to classic orthosteric inhibitors, it seems likely that new strategies will be needed. Herein we have discussed the varied ways in which the cell naturally regulates and modulates PPIs. From these observations, the themes of allosteric inhibition and PTMs become readily apparent, and a few synthetic small molecules have already accessed these natural regulatory mechanisms to fine-tune protein complex formation. Deployment of new HTS methodologies and carefully designed chemical libraries may further accelerate discovery of molecules with activity on these difficult systems. By continuing to look to nature for inspiration, chemical biologists have the potential to expand the number of "druggable" PPIs.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: gestwick@umich.edu; amapp@umich.edu.

Notes

The authors declare no competing financial interest.

Keywords

Multi-protein complexes: structures formed by non-covalent interactions between two or more proteins

Protein-protein interaction (PPI): occurs when proteins bind to one another *via* a direct physical interaction surface

Protein-ligand interface (PLI): the binding surface between a protein and its cognate ligand; typically smaller and more topologically defined than a PPI

PPI modulator: small "drug-like" molecule capable of either promoting or inhibiting the binding interaction between two proteins

Orthosteric inhibitor: a PPI modulator that inhibits protein interactions through direct binding competition

Allosteric binding: binding at one site that regulates function or binding at a distant site

Post-translational modifications (PTMs): covalent modifications of individual amino acid side chains that regulate the structure; localization and function of proteins including phosphorylation, glycosylation, acylation and ubiquitylation

Chemical probes: small molecules or chemical matter that can be utilized as research tools to study biology

High-throughput screening (HTS): a discovery experiment in which a rapid and efficient biological or chemical assay is employed to evaluate large numbers of chemical or biologicall agents for a particular activity

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Rual, J. F., Venkatesan, K., Hao, T., Hirozane-Kishikawa, T., Dricot, A., Li, N., Berriz, G. F., Gibbons, F. D., Dreze, M., Ayivi-Guedehoussou, N., Klitgord, N., Simon, C., Boxem, M., Milstein, S., Rosenberg, J., Goldberg, D. S., Zhang, L. V., Wong, S. L., Franklin, G., Li, S., Albala, J. S., Lim, J., Fraughton, C., Llamosas, E., Cevik, S., Bex, C., Lamesch, P., Sikorski, R. S., Vandenhaute, J., Zoghbi, H. Y., Smolyar, A., Bosak, S., Sequerra, R., Doucette-Stamm, L., Cusick, M. E., Hill, D. E., Roth, F. P., and Vidal, M. (2005) Towards a proteome-scale map of the human protein-protein interaction network. *Nature 437*, 1173−1178.

(2) Hopkins, A. L., and Groom, C. R. (2002) The druggable genome. *Nat. Rev. Drug Discovery 1*, 727−730.

(3) Cravatt, B. F., Simon, G. M., and Yates, J. R., 3rd (2007) The biological impact of mass-spectrometry-based proteomics. *Nature 450*, 991−1000.

(4) Powers, E. T., Morimoto, R. I., Dillin, A., Kelly, J. W., and Balch, W. E. (2009) Biological and chemical approaches to diseases of proteostasis deficiency. *Annu. Rev. Biochem. 78*, 959−991.

(5) Surade, S., and Blundell, T. L. (2012) Structural biology and drug discovery of difficult targets: the limits of ligandability. *Chem. Biol. 19*, 42−50.

(6) Overington, J. P., Al-Lazikani, B., and Hopkins, A. L. (2006) How many drug targets are there? *Nat. Rev. Drug Discovery 5*, 993−996.

(7) Drews, J. (2000) Drug discovery: a historical perspective. *Science 287*, 1960−1964.

(8) Stumpf, M. P., Thorne, T., de Silva, E., Stewart, R., An, H. J., Lappe, M., and Wiuf, C. (2008) Estimating the size of the human interactome. *Proc. Natl. Acad. Sci. U.S.A. 105*, 6959−6964.

(9) Wells, J. A., and McClendon, C. L. (2007) Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. *Nature 450*, 1001−1009.

(10) Bourgeas, R., Basse, M. J., Morelli, X., and Roche, P. (2010) Atomic analysis of protein-protein interfaces with known inhibitors: the 2P2I database. *PLoS One 5*, e9598.

(11) Higueruelo, A. P., Schreyer, A., Bickerton, G. R., Pitt, W. R., Groom, C. R., and Blundell, T. L. (2009) Atomic interactions and profile of small molecules disrupting protein-protein interfaces: the TIMBAL database. *Chem. Biol. Drug Des. 74*, 457−467.

(12) Romero, G., von Zastrow, M., and Friedman, P. A. (2011) Role of PDZ proteins in regulating trafficking, signaling, and function of GPCRs: means, motif, and opportunity. *Adv. Pharmacol. 62*, 279−314.

(13) Hohfeld, J., Cyr, D. M., and Patterson, C. (2001) From the cradle to the grave: molecular chaperones that may choose between folding and degradation. *EMBO Rep. 2*, 885−890.

(14) Young, A., Lyons, J., Miller, A. L., Phan, V. T., Alarcon, I. R., and McCormick, F. (2009) Ras signaling and therapies. *Adv. Cancer Res. 102*, 1−17.

(15) Lessard, J., Wu, J. I., Ranish, J. A., Wan, M., Winslow, M. M., Staahl, B. T., Wu, H., Aebersold, R., Graef, I. A., and Crabtree, G. R. (2007) An essential switch in subunit composition of a chromatin remodeling complex during neural development. *Neuron 55*, 201−215.

(16) Yoo, A. S., and Crabtree, G. R. (2009) ATP-dependent chromatin remodeling in neural development. *Curr. Opin. Neurobiol. 19*, 120−126.

(17) Perot, S., Sperandio, O., Miteva, M. A., Camproux, A. C., and Villoutreix, B. O. (2010) Druggable pockets and binding site centric chemical space: a paradigm shift in drug discovery. *Drug Discovery Today 15*, 656−667.

(18) Fuller, J. C., Burgoyne, N. J., and Jackson, R. M. (2009) Predicting druggable binding sites at the protein-protein interface. *Drug Discovery Today 14*, 155−161.

(19) DeLano, W. L. (2002) Unraveling hot spots in binding interfaces: progress and challenges. *Curr. Opin. Struct. Biol. 12*, 14−20.

(20) Lo Conte, L., Chothia, C., and Janin, J. (1999) The atomic structure of protein-protein recognition sites. *J. Mol. Biol. 285*, 2177−2198.

(21) Nooren, I. M., and Thornton, J. M. (2003) Diversity of protein-protein interactions. *EMBO J. 22*, 3486−3492.

(22) Nooren, I. M., and Thornton, J. M. (2003) Structural characterisation and functional significance of transient protein-protein interactions. *J. Mol. Biol. 325*, 991−1018.

(23) Wang, L., Liu, Y. T., Hao, R., Chen, L., Chang, Z., Wang, H. R., Wang, Z. X., and Wu, J. W. (2011)) Molecular mechanism of the negative regulation of Smad1/5 protein by carboxyl terminus of Hsc70-interacting protein (CHIP). *J. Biol. Chem. 286*, 15883−15894.

(24) Jiang, J., Maes, E. G., Taylor, A. B., Wang, L., Hinck, A. P., Lafer, E. M., and Sousa, R. (2007) Structural basis of J cochaperone binding and regulation of Hsp70. *Mol. Cell 28*, 422−433.

(25) Schuermann, J. P., Jiang, J., Cuellar, J., Llorca, O., Wang, L., Gimenez, L. E., Jin, S., Taylor, A. B., Demeler, B., Morano, K. A., Hart, P. J., Valpuesta, J. M., Lafer, E. M., and Sousa, R. (2008) Structure of the Hsp110:Hsc70 nucleotide exchange machine. *Mol. Cell 31*, 232−243.

(26) Ahmad, A., Bhattacharya, A., McDonald, R. A., Cordes, M., Ellington, B., Bertelsen, E. B., and Zuiderweg, E. R. (2011) Heat shock protein 70 kDa chaperone/DnaJ cochaperone complex employs an unusual dynamic interface. *Proc. Natl. Acad. Sci. U.S.A. 108*, 18966−18971.

(27) Zhu, X., Zhao, X., Burkholder, W. F., Gragerov, A., Ogata, C. M., Gottesman, M. E., and Hendrickson, W. A. (1996) Structural analysis of substrate binding by the molecular chaperone DnaK. *Science 272*, 1606−1614.

(28) Scheufler, C., Brinker, A., Bourenkov, G., Pegoraro, S., Moroder, L., Bartunik, H., Hartl, F. U., and Moarefi, I. (2000) Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. *Cell 101*, 199−210.

(29) Feng, H., Jenkins, L. M., Durell, S. R., Hayashi, R., Mazur, S. J., Cherry, S., Tropea, J. E., Miller, M., Wlodawer, A., Appella, E., and Bai, Y. (2009) Structural basis for p300 Taz2-p53 TAD1 binding and modulation by phosphorylation. *Structure 17*, 202−210.

(30) Lee, W., Harvey, T. S., Yin, Y., Yau, P., Litchfield, D., and Arrowsmith, C. H. (1994) Solution structure of the tetrameric minimum transforming domain of p53. *Nat. Struct. Biol. 1*, 877−890.

(31) Popowicz, G. M., Czarna, A., and Holak, T. A. (2008) Structure of the human Mdmx protein bound to the p53 tumor suppressor transactivation domain. *Cell Cycle 7*, 2441−2443.

(32) Lee, C. W., Martinez-Yamout, M. A., Dyson, H. J., and Wright, P. E. (2010) Structure of the p53 transactivation domain in complex with the nuclear receptor coactivator binding domain of CREB binding protein. *Biochemistry 49*, 9964−9971.

(33) Patel, S. D., Ciatto, C., Chen, C. P., Bahna, F., Rajebhosale, M., Arkus, N., Schieren, I., Jessell, T. M., Honig, B., Price, S. R., and Shapiro, L. (2006) Type II cadherin ectodomain structures: implications for classical cadherin specificity. *Cell 124*, 1255−1268.

(34) Stauber, D. J., Debler, E. W., Horton, P. A., Smith, K. A., and Wilson, I. A. (2006) Crystal structure of the IL-2 signaling complex: paradigm for a heterotrimeric cytokine receptor. *Proc. Natl. Acad. Sci. U.S.A. 103*, 2788−2793.

(35) Sun, H., Stuckey, J. A., Nikolovska-Coleska, Z., Qin, D., Meagher, J. L., Qiu, S., Lu, J., Yang, C. Y., Saito, N. G., and Wang, S. (2008)) Structure-based design, synthesis, evaluation, and crystallographic studies of conformationally constrained Smac mimetics as

inhibitors of the X-linked inhibitor of apoptosis protein (XIAP). *J. Med. Chem. 51*, 7169−7180.

(36) Wu, G., Chai, J., Suber, T. L., Wu, J. W., Du, C., Wang, X., and Shi, Y. (2000) Structural basis of IAP recognition by Smac/DIABLO. *Nature 408*, 1008−1012.

(37) Vaynberg, J., Fukuda, T., Chen, K., Vinogradova, O., Velyvis, A., Tu, Y., Ng, L., Wu, C., and Qin, J. (2005) Structure of an ultraweak protein-protein complex and its crucial role in regulation of cell morphology and motility. *Mol. Cell 17*, 513−523.

(38) Arkin, M. R., and Wells, J. A. (2004) Small-molecule inhibitors of protein-protein interactions: progressing towards the dream. *Nat. Rev. Drug Discovery 3*, 301−317.

(39) Lipinski, C., and Hopkins, A. (2004) Navigating chemical space for biology and medicine. *Nature 432*, 855−861.

(40) Lipinski, C. A. (2000) Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods 44*, 235−249.

(41) Lindsley, J. E., and Rutter, J. (2006) Whence cometh the allosterome? *Proc. Natl. Acad. Sci. U.S.A. 103*, 10533−10535.

(42) Jiang, F., Frey, B. R., Evans, M. L., Friel, J. C., and Hopper, J. E. (2009) Gene activation by dissociation of an inhibitor from a transcriptional activation domain. *Mol. Cell. Biol. 29*, 5604−5610.

(43) Weinkam, P., Pons, J., and Sali, A. (2012) Structure-based model of allostery predicts coupling between distant sites. *Proc. Natl. Acad. Sci. U.S.A. 109*, 4875−5880.

(44) Kenakin, T. P. (2010) Ligand detection in the allosteric world. *J. Biomol. Screening 15*, 119−130.

(45) Li, X., Gianoulis, T. A., Yip, K. Y., Gerstein, M., and Snyder, M. (2010) Extensive in vivo metabolite-protein interactions revealed by large-scale systematic analyses. *Cell 143*, 639−650.

(46) Lawrence, S. H., Ramirez, U. D., Tang, L., Fazliyez, F., Kundrat, L., Markham, G. D., and Jaffe, E. K. (2008) Shape shifting leads to small-molecule allosteric drug discovery. *Chem. Biol. 15*, 586−596.

(47) Lawrence, S. H., Ramirez, U. D., Selwood, T., Stith, L., and Jaffe, E. K. (2009) Allosteric inhibition of human porphobilinogen synthase. *J. Biol. Chem. 284*, 35807−35817.

(48) Roman, D. L., Blazer, L. L., Monroy, C. A., and Neubig, R. R. (2010) Allosteric inhibition of the regulator of G protein signaling-Galpha protein-protein interaction by CCG-4986. *Mol. Pharmacol. 78*, 360−365.

(49) Mallya, M., Phillips, R. L., Saldanha, S. A., Gooptu, B., Brown, S. C., Termine, D. J., Shirvani, A. M., Wu, Y., Sifers, R. N., Abagyan, R., and Lomas, D. A. (2007) Small molecules block the polymerization of Z alpha1-antitrypsin and increase the clearance of intracellular aggregates. *J. Med. Chem. 50*, 5357−5363.

(50) Blazer, L. L., Roman, D. L., Chung, A., Larsen, M. J., Greedy, B. M., Husbands, S. M., and Neubig, R. R. (2010) Reversible, allosteric small-molecule inhibitors of regulator of G protein signaling proteins. *Mol. Pharmacol. 78*, 524−533.

(51) Chang, L., Miyata, Y., Ung, P. M., Bertelsen, E. B., McQuade, T. J., Carlson, H. A., Zuiderweg, E. R., and Gestwicki, J. E. (2011) Chemical screens against a reconstituted multiprotein complex: myricetin blocks DnaJ regulation of DnaK through an allosteric mechanism. *Chem. Biol. 18*, 210−221.

(52) McMillan, K., Adler, M., Auld, D. S., Baldwin, J. J., Blasko, E., Browne, L. J., Chelsky, D., Davey, D., Dolle, R. E., Eagen, K. A., Erickson, S., Feldman, R. I., Glaser, C. B., Mallari, C., Morrissey, M. M., Ohlmeyer, M. H., Pan, G., Parkinson, J. F., Phillips, G. B., Polokoff, M. A., Sigal, N. H., Vergona, R., Whitlow, M., Young, T. A., and Devlin, J. J. (2000) Allosteric inhibitors of inducible nitric oxide synthase dimerization discovered via combinatorial chemistry. *Proc. Natl. Acad. Sci. U.S.A. 97*, 1506−1511.

(53) Conn, P. J., Christopoulos, A., and Lindsley, C. W. (2009) Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat. Rev. Drug Discovery 8*, 41−54.

(54) Osborne, A. R., Rapoport, T. A., and van den Berg, B. (2005) Protein translocation by the Sec61/SecY channel. *Annu. Rev. Cell. Dev. Biol. 21*, 529−550.

(55) Kim, S. J., Mitra, D., Salerno, J. R., and Hegde, R. S. (2002) Signal sequences control gating of the protein translocation channel in a substrate-specific manner. *Dev. Cell. 2*, 207−217.

(56) Rutkowski, D. T., Lingappa, V. R., and Hegde, R. S. (2001) Substrate-specific regulation of the ribosome- translocon junction by N-terminal signal sequences. *Proc. Natl. Acad. Sci. U.S.A. 98*, 7823−7828.

(57) Garrison, J. L., Kunkel, E. J., Hegde, R. S., and Taunton, J. (2005) A substrate-specific inhibitor of protein translocation into the endoplasmic reticulum. *Nature 436*, 285−289.

(58) Besemer, J., Harant, H., Wang, S., Oberhauser, B., Marquardt, K., Foster, C. A., Schreiner, E. P., de Vries, J. E., Dascher-Nadel, C., and Lindley, I. J. (2005) Selective inhibition of cotranslational translocation of vascular cell adhesion molecule 1. *Nature 436*, 290−293.

(59) Klemm, J. D., Schreiber, S. L., and Crabtree, G. R. (1998) Dimerization as a regulatory mechanism in signal transduction. *Annu. Rev. Immunol. 16*, 569−592.

(60) Okuzumi, T., Fiedler, D., Zhang, C., Gray, D. C., Aizenstein, B., Hoffman, R., and Shokat, K. M. (2009) Inhibitor hijacking of Akt activation. *Nat. Chem. Biol. 5*, 484−493.

(61) Deribe, Y. L., Pawson, T., and Dikic, I. (2010) Post-translational modifications in signal integration. *Nat. Struct. Mol. Biol. 17*, 666−672.

(62) Potashman, M. H., and Duggan, M. E. (2009) Covalent modifiers: an orthogonal approach to drug design. *J. Med. Chem. 52*, 1231−1246.

(63) Ahn, Y. H., Hwang, Y., Liu, H., Wang, X. J., Zhang, Y., Stephenson, K. K., Boronina, T. N., Cole, R. N., Dinkova-Kostova, A. T., Talalay, P., and Cole, P. A. (2010) Electrophilic tuning of the chemoprotective natural product sulforaphane. *Proc. Natl. Acad. Sci. U.S.A. 107*, 9590−9595.

(64) Cameron, A. J., Escribano, C., Saurin, A. T., Kostelecky, B., and Parker, P. J. (2009) PKC maturation is promoted by nucleotide pocket occupation independently of intrinsic kinase activity. *Nat. Struct. Mol. Biol. 16*, 624−630.

(65) Erlanson, D. A., Braisted, A. C., Raphael, D. R., Randal, M., Stroud, R. M., Gordon, E. M., and Wells, J. A. (2000) Site-directed ligand discovery. *Proc. Natl. Acad. Sci. U.S.A. 97*, 9367−9372.

(66) Scheer, J. M., Romanowski, M. J., and Wells, J. A. (2006) A common allosteric site and mechanism in caspases. *Proc. Natl. Acad. Sci. U.S.A. 103*, 7595−7600.

(67) Coyne, A. G., Scott, D. E., and Abell, C. (2010) Drugging challenging targets using fragment-based approaches. *Curr. Opin. Chem. Biol. 14*, 299−307.

(68) Murray, C. W., and Rees, D. C. (2009) The rise of fragment-based drug discovery. *Nat. Chem. 1*, 187−192.

(69) Jahnke, W., Rondeau, J. M., Cotesta, S., Marzinzik, A., Pelle, X., Geiser, M., Strauss, A., Gotte, M., Bitsch, F., Hemmig, R., Henry, C., Lehmann, S., Glickman, J. F., Roddy, T. P., Stout, S. J., and Green, J. R. (2010) Allosteric non-bisphosphonate FPPS inhibitors identified by fragment-based discovery. *Nat. Chem. Biol. 6*, 660−666.

(70) Oltersdorf, T., Elmore, S. W., Shoemaker, A. R., Armstrong, R. C., Augeri, D. J., Belli, B. A., Bruncko, M., Deckwerth, T. L., Dinges, J., Hajduk, P. J., Joseph, M. K., Kitada, S., Korsmeyer, S. J., Kunzer, A. R., Letai, A., Li, C., Mitten, M. J., Nettesheim, D. G., Ng, S., Nimmer, P. M., O'Connor, J. M., Oleksijew, A., Petros, A. M., Reed, J. C., Shen, W., Tahir, S. K., Thompson, C. B., Tomaselli, K. J., Wang, B., Wendt, M. D., Zhang, H., Fesik, S. W., and Rosenberg, S. H. (2005) An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature 435*, 677−681.

(71) Lockless, S. W., and Ranganathan, R. (1999) Evolutionarily conserved pathways of energetic connectivity in protein families. *Science 286*, 295−299.

(72) Suel, G. M., Lockless, S. W., Wall, M. A., and Ranganathan, R. (2003) Evolutionarily conserved networks of residues mediate allosteric communication in proteins. *Nat. Struct. Biol. 10*, 59−69.

(73) Massova, I., and Mobashery, S. (1999) Structural and mechanistic aspects of evolution of beta-lactamases and penicillin-binding proteins. *Curr. Pharm. Des. 5*, 929−937.

(74) Schuyler, A. D., Carlson, H. A., and Feldman, E. L. (2010) Computational methods for identifying a layered allosteric regulatory mechanism for ALS-causing mutations of Cu-Zn superoxide dismutase 1. *Proteins 79*, 417−427.

(75) Ota, N., and Agard, D. A. (2005) Intramolecular signaling pathways revealed by modeling anisotropic thermal diffusion. *J. Mol. Biol. 351*, 345−354.

(76) Hendlich, M., Rippmann, F., and Barnickel, G. (1997) LIGSITE: automatic and efficient detection of potential small molecule-binding sites in proteins. *J. Mol. Graph. Model. 15*, 359−363 , 389.

(77) Kalidas, Y., and Chandra, N. (2008) PocketDepth: a new depth based algorithm for identification of ligand binding sites in proteins. *J. Struct. Biol. 161*, 31−42.

(78) Lexa, K. W., and Carlson, H. A. (2010) Full protein flexibility is essential for proper hot-spot mapping. *J. Am. Chem. Soc. 133*, 200−202.

(79) Desai, A., and Mitchison, T. J. (1997) Microtubule polymerization dynamics. *Annu. Rev. Cell. Dev. Biol. 13*, 83−117.

(80) Steitz, T. A. (2008) A structural understanding of the dynamic ribosome machine. *Nat. Rev. Mol. Cell. Biol. 9*, 242−253.

(81) LaRiviere, F. J., Wolfson, A. D., and Uhlenbeck, O. C. (2001) Uniform binding of aminoacyl-tRNAs to elongation factor Tu by thermodynamic compensation. *Science 294*, 165−168.

(82) Schrader, J. M., Chapman, S. J., and Uhlenbeck, O. C. (2011) Tuning the affinity of aminoacyl-tRNA to elongation factor Tu for optimal decoding. *Proc. Natl. Acad. Sci. U.S.A. 108*, 5215−5220.

(83) Davydov, D. R., Rumfeldt, J. A., Sineva, E. V., Fernando, H., Davydova, N. Y., and Halpert, J. R. (2012)) Peripheral ligand-binding site in cytochrome P450 3A4 located with fluorescence resonance energy transfer (FRET). *J. Biol. Chem. 287*, 6797−6809.

(84) Bill, A., Blockus, H., Stumpfe, D., Bajorath, J., Schmitz, A., and Famulok, M. (2011) A homogeneous fluorescence resonance energy transfer system for monitoring the activation of a protein switch in real time. *J. Am. Chem. Soc. 133*, 8372−8379.

(85) Coward, P., Conn, M., Tang, J., Xiong, F., Menjares, A., and Reagan, J. D. (2009) Application of an allosteric model to describe the interactions among retinol binding protein 4, transthyretin, and small molecule retinol binding protein 4 ligands. *Anal. Biochem. 384*, 312−320.

(86) Soderholm, J. F., Bird, S. L., Kalab, P., Sampathkumar, Y., Hasegawa, K., Uehara-Bingen, M., Weis, K., and Heald, R. (2011) Importazole, a small molecule inhibitor of the transport receptor importin-beta. *ACS Chem. Biol. 6*, 700−708.

(87) Cellitti, S. E., Jones, D. H., Lagpacan, L., Hao, X., Zhang, Q., Hu, H., Brittain, S. M., Brinker, A., Caldwell, J., Bursulaya, B., Spraggon, G., Brock, A., Ryu, Y., Uno, T., Schultz, P. G., and Geierstanger, B. H. (2008) In vivo incorporation of unnatural amino acids to probe structure, dynamics, and ligand binding in a large protein by nuclear magnetic resonance spectroscopy. *J. Am. Chem. Soc. 130*, 9268−9281.

(88) Tugarinov, V., and Kay, L. E. (2005) Methyl groups as probes of structure and dynamics in NMR studies of high-molecular-weight proteins. *ChemBioChem 6*, 1567−1577.

(89) Al-Hashimi, H. M. (2010) Biochemistry. Exciting structures. *Science 329*, 1295−1296.

(90) Verma, R., Peters, N. R., D'Onofrio, M., Tochtrop, G. P., Sakamoto, K. M., Varadan, R., Zhang, M., Coffino, P., Fushman, D., Deshaies, R. J., and King, R. W. (2004) Ubistatins inhibit proteasome-dependent degradation by binding the ubiquitin chain. *Science 306*, 117−120.

(91) Bobkova, E. V., Weber, M. J., Xu, Z., Zhang, Y. L., Jung, J., Blume-Jensen, P., Northrup, A., Kunapuli, P., Andersen, J. N., and Kariv, I. (2010) Discovery of PDK1 kinase inhibitors with a novel mechanism of action by ultrahigh throughput screening. *J. Biol. Chem. 285*, 18838−18846.

(92) Converso, A., Hartingh, T., Garbaccio, R. M., Tasber, E., Rickert, K., Fraley, M. E., Yan, Y., Kreatsoulas, C., Stirdivant, S., Drakas, B., Walsh, E. S., Hamilton, K., Buser, C. A., Mao, X., Abrams, M. T., Beck, S. C., Tao, W., Lobell, R., Sepp-Lorenzino, L., Zugay-Murphy, J., Sardana, V., Munshi, S. K., Jezequel-Sur, S. M., Zuck, P. D., and Hartman, G. D. (2009) Development of thioquinazolinones, allosteric Chk1 kinase inhibitors. *Bioorg. Med. Chem. Lett. 19*, 1240−1244.

(93) Erlbruch, A., Hung, C. W., Seidler, J., Borrmann, K., Gesellchen, F., Konig, N., Kubler, D., Herberg, F. W., Lehmann, W. D., and Bossemeyer, D. (2010) Uncoupling of bait-protein expression from the prey protein environment adds versatility for cell and tissue interaction proteomics and reveals a complex of CARP-1 and the PKA Cbeta1 subunit. *Proteomics 10*, 2890−2900.

(94) Moulick, K., Ahn, J. H., Zong, H., Rodina, A., Cerchietti, L., Gomes DaGama, E. M., Caldas-Lopes, E., Beebe, K., Perna, F., Hatzi, K., Vu, L. P., Zhao, X., Zatorska, D., Taldone, T., Smith-Jones, P., Alpaugh, M., Gross, S. S., Pillarsetty, N., Ku, T., Lewis, J. S., Larson, S. M., Levine, R., Erdjument-Bromage, H., Guzman, M. L., Nimer, S. D., Melnick, A., Neckers, L., and Chiosis, G. (2011) Affinity-based proteomics reveal cancer-specific networks coordinated by Hsp90. *Nat. Chem. Biol. 7*, 818−826.

(95) Deacon, S. W., Beeser, A., Fukui, J. A., Rennefahrt, U. E., Myers, C., Chernoff, J., and Peterson, J. R. (2008) An isoform-selective, small-molecule inhibitor targets the autoregulatory mechanism of p21-activated kinase. *Chem. Biol. 15*, 322−331.

(96) Miyata, Y., Chang, L., Bainor, A., McQuade, T. J., Walczak, C. P., Zhang, Y., Larsen, M. J., Kirchhoff, P., and Gestwicki, J. E. (2010) High-throughput screen for Escherichia coli heat shock protein 70 (Hsp70/DnaK): ATPase assay in low volume by exploiting energy transfer. *J. Biomol. Screening 15*, 1211−1219.

(97) Wisen, S., Bertelsen, E. B., Thompson, A. D., Patury, S., Ung, P., Chang, L., Evans, C. G., Walter, G. M., Wipf, P., Carlson, H. A., Brodsky, J. L., Zuiderweg, E. R., and Gestwicki, J. E. (2010) Binding of a small molecule at a protein-protein interface regulates the chaperone activity of hsp70-hsp40. *ACS Chem. Biol. 5*, 611−622.

(98) Berggard, T., Linse, S., and James, P. (2007) Methods for the detection and analysis of protein-protein interactions. *Proteomics 7*, 2833−2842.

(99) Perkins, J. R., Diboun, I., Dessailly, B. H., Lees, J. G., and Orengo, C. (2010) Transient protein-protein interactions: structural, functional, and network properties. *Structure 18*, 1233−1243.

(100) Kerppola, T. K. (2008) Bimolecular fluorescence complementation (BiFC) analysis as a probe of protein interactions in living cells. *Annu. Rev. Biophys. 37*, 465−487.

(101) Krishnamurthy, M., Dugan, A., Nwokoye, A., Fung, Y. H., Lancia, J. K., Majmudar, C. Y., and Mapp, A. K. (2011) Caught in the act: covalent cross-linking captures activator-coactivator interactions in vivo. *ACS Chem. Biol. 6*, 1321−1326.

(102) Pellegrini, M. (2003) Defining interacting partners for drug discovery. *Expert Opin. Ther. Targets 7*, 287−297.

(103) Tanaka, Y., Bond, M. R., and Kohler, J. J. (2008) Photocrosslinkers illuminate interactions in living cells. *Mol. Biosyst. 4*, 473−480.

(104) Majmudar, C. Y., Lee, L. W., Lancia, J. K., Nwokoye, A., Wang, Q., Wands, A. M., Wang, L., and Mapp, A. K. (2009) Impact of nonnatural amino acid mutagenesis on the in vivo function and binding modes of a transcriptional activator. *J. Am. Chem. Soc. 131*, 14240−14242.

(105) Clark, M. A. (2010) Selecting chemicals: the emerging utility of DNA-encoded libraries. *Curr. Opin. Chem. Biol. 14*, 396−403.

(106) Dandapani, S., and Marcaurelle, L. A. (2010) Grand challenge commentary: Accessing new chemical space for 'undruggable' targets. *Nat. Chem. Biol. 6*, 861−863.

(107) Dandapani, S., and Marcaurelle, L. A. (2010) Current strategies for diversity-oriented synthesis. *Curr. Opin. Chem. Biol. 14*, 362−370.

(108) Goldman, S. (2010) Genetic Chemistry: Production of non-native compounds in yeast. *Curr. Opin. Chem. Biol. 14*, 390−395.

(109) Gestwicki, J. E., Crabtree, G. R., and Graef, I. A. (2004) Harnessing chaperones to generate small-molecule inhibitors of amyloid beta aggregation. *Science 306*, 865−869.

(110) Fletcher, S., and Hamilton, A. D. (2005) Protein surface recognition and proteomimetics: mimics of protein surface structure and function. *Curr. Opin. Chem. Biol. 9*, 632−638.

(111) Buerger, C., and Groner, B. (2003) Bifunctional recombinant proteins in cancer therapy: cell penetrating peptide aptamers as inhibitors of growth factor signaling. *J. Cancer Res. Clin. Oncol. 129*, 669−675.

(112) Traczewski, P., and Rudnicka, L. (2011) Treatment of systemic lupus erythematosus with epratuzumab. *Br. J. Clin. Pharmacol. 71*, 175−182.

(113) Drewry, D. H., and Macarron, R. (2010) Enhancements of screening collections to address areas of unmet medical need: an industry perspective. *Curr. Opin. Chem. Biol. 14*, 289−298.

(114) Renner, S., Popov, M., Schuffenhauer, A., Roth, H. J., Breitenstein, W., Marzinzik, A., Lewis, I., Krastel, P., Nigsch, F., Jenkins, J., and Jacoby, E. (2011) Recent trends and observations in the design of high-quality screening collections. *Future Med. Chem. 3*, 751−766.

(115) Wang, R., Fang, X., Lu, Y., and Wang, S. (2004) The PDBbind database: collection of binding affinities for protein-ligand complexes with known three-dimensional structures. *J. Med. Chem. 47*, 2977−2980.

(116) Negi, S. S., Schein, C. H., Oezguen, N., Power, T. D., and Braun, W. (2007) InterProSurf: a web server for predicting interacting sites on protein surfaces. *Bioinformatics 23*, 3397−3399.

1320

dx.doi.org/10.1021/cb300255p | *ACS Chem. Biol.* 2012, 7, 1311−1320