HHS Public Access

Author manuscript

Nat Chem Biol. Author manuscript; available in PMC 2021 April 01.

Published in final edited form as:

Nat Chem Biol. 2020 April ; 16(4): 369–378. doi:10.1038/s41589-020-0469-1.

Unifying principles of bifunctional, proximity-inducing small molecules

Christopher J. Gerry[1,2,3], **Stuart L. Schreiber**[1,2,*]

[1]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts, USA

[2]Chemical Biology and Therapeutics Science Program, Broad Institute, Cambridge, Massachusetts, USA

[3]Present address: Vertex Pharmaceuticals, Boston, Massachusetts, USA

Abstract

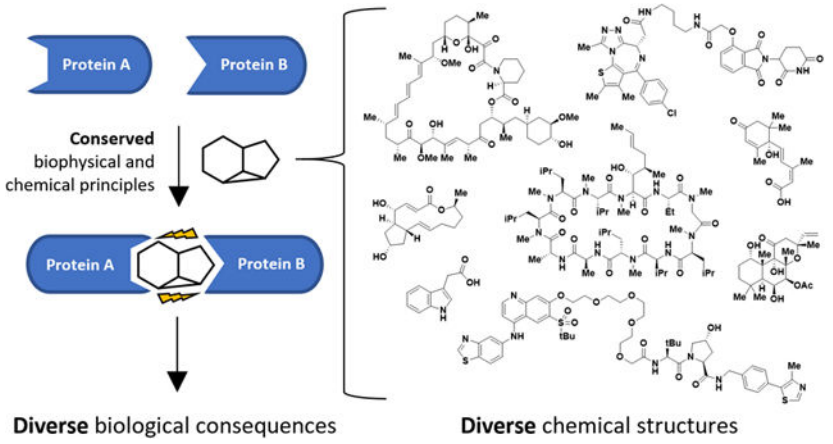
Nature uses a variety of tools to mediate the flow of information in cells, many of which control distances between key biomacromolecules. Researchers have thus generated compounds whose activities stem from interactions with two (or more) proteins simultaneously. In this Perspective, we describe how these “bifunctional” small molecules facilitate the study of an increasingly wide range of complex biological phenomena and enable the drugging of otherwise challenging therapeutic targets and processes. Despite their structural and functional differences, all bifunctional molecules employ Nature’s strategy of altering interactomes and inducing proximity to modulate biology. They therefore exhibit a shared set of chemical and biophysical principles that have not yet been appreciated fully. By highlighting these commonalities—and their wide-ranging consequences—we hope to chip away at the artificial barriers that threaten to constrain this interdisciplinary field. Doing so promises to yield remarkable benefits for biological research and therapeutics discovery.

Graphical Abstract

*To whom correspondence should be addressed: stuart_schreiber@harvard.edu.

Conflicts of Interest

S.L.S. serves on the Board of Directors of the Genomics Institute of the Novartis Research Foundation (“GNF”); is a shareholder and serves on the Board of Directors of Jnana Therapeutics; is a shareholder of Forma Therapeutics; is a shareholder and advises Decibel Therapeutics and Eikonizo Therapeutics; serves on the Scientific Advisory Boards of Eisai Co., Ltd., Ono Pharma Foundation, Exo Therapeutics, and F-Prime Capital Partners; and is a Novartis Faculty Scholar. C.J.G. is an employee of Vertex Pharmaceuticals.



Multiple classes of perturbagens are often needed to study biological systems thoroughly. Natural genetic or physiologic variations that affect outcomes of interest, for example, are a particularly powerful source of insight, especially into human physiology. Alternatively, experimental genetic or small-molecule perturbations can play complementary roles in dissecting biological phenomena with levels of temporal and spatial control not often afforded by natural variation.[1]

It is becoming increasingly clear that small molecules can achieve results not easily realized by their experimental genetics-based counterparts; instead, the effects of small molecules more closely resemble those of natural genetic alterations under positive selection. Small molecules are often thought to be synonymous with the term “inhibitors,” but many compounds either enhance the activities of their targets or impart novel functions. For example, diacylglycerols, phorbol esters, and bryostatins are activators of protein kinase C (PKC)—hydrophilic motifs in the small molecules bind PKC, and hydrophobic moieties recruit PKC to the plasma membrane.[2–4] This mechanism of action (MoA) reveals another underappreciated feature of small molecules: the ability to modulate biology by engaging multiple targets. We will refer to such compounds as “bifunctional,” though they have also been called “molecular glues”.[5]

Bifunctional small molecules come in many shapes and sizes (Fig. 1). Sometimes bifunctionality is apparent from analysis of chemical structure alone. Such compounds often comprise two small-molecule binders connected by a covalent linker, yielding “bivalent,” dumbbell-shaped molecules that can be synthesized using rational design.[6,7] In other cases, bifunctionality is less obvious. The macrocyclic natural products rapamycin, FK506, and cyclosporin A, for example, lack obvious linkers but are also bivalent as they each bind two protein targets.[8–10] These compounds engage their multiple binding partners in a defined order such that the first binding event generates a “composite” compound–protein interface that engages the second target. But because the compound alone often has low or no affinity for the second binding partner,[9] the identification and *de novo* design of these types of small molecules has been challenging. Both of these classes are distinct from “monovalent” allosteric modulators that promote new protein– protein interactions without contacting the second protein directly.[11]

In lieu of a comprehensive list, this Perspective offers vignettes that highlight the range of biology that can be studied with bifunctional compounds. The broad utility of molecular glues as chemical tools was first demonstrated effectively by inducing the proximity of designed, dominant drug-responsive fusion proteins.[12] Over time, this approach was adapted to native proteins, facilitating the study and treatment of disease. These experiments have often used linker-containing compounds to co-localize a specific pair of protein targets. But it also appears that bifunctionality in synthetic compounds *without* linkers is far more common than previously thought. Complex biological phenomena—from cellular signaling to the behavior of the human immune system—have been studied using these types of molecules.

Despite differences in structure, source, activity, and nomenclature (*e.g.* CIPs, CIDs, PROTACs, dimerizers, and molecular glues), many bifunctional small molecules operate *via* the same general mechanism: modulation of protein proximity. We believe that siloing these compounds into distinct categories has obscured this important commonality. Therefore, this Perspective will highlight a set of unifying chemical and biophysical principles that are shared by the ensemble of bifunctional small molecules: 1) binding and co-localization of more than one biomacromolecular binding partner to generate multimeric complexes, 2) arrangement of binding partners in biologically meaningful orientations, and 3) orchestration of extensive networks of intermolecular interactions. Recognizing that these outcomes can be achieved with a wide variety of chemotypes presents researchers with opportunities to modulate biological systems in novel ways.

Information transfer in biology

Biology operates in a crowded milieu. It is estimated that up to 40% of a cell's total volume is filled by proteins, nucleic acids, and other biomacromolecules.[13] Signals must traverse this congested landscape in a rapid—yet tightly regulated—manner, so Nature has several approaches to encourage meaningful biomolecular interactions. Many involve the induction of protein proximity to increase reaction rates. Co-localization allows reactants to interact (pseudo-)intramolecularly, causing dramatic rate acceleration due to a decreased loss of translational and rotational entropy. In some cases, the analogous intermolecular reaction would require impossibly high reactant concentrations (≥100 M) to achieve the same rate.[14] This effect is measured using a term called effective molarity, which is equal to the quotient of the intramolecular reaction's first-order rate constant and the intermolecular reaction's second-order rate constant.[15]

As noted decades ago by Albert Eschenmoser (among others), proximity alone is not always sufficient to achieve rate acceleration; the participating species must also be able to adopt a reactive orientation in three-dimensional space. In an instructive example, Eschenmoser observed that a system ostensibly primed for a rapid intramolecular reaction actually proceeds *via* a multi-step intermolecular process. Despite the proximity of the nucleophile and pendant electrophile—which would form a six-membered endocyclic transition state—the molecular geometry prevents the two species from achieving the requisite orientation for an S_N2 reaction to occur.[16] Jack Baldwin later described "rules" for intramolecular ring closure that extend this principle to a variety of ring sizes and electrophile geometries.[17]

Returning to the cell, scaffolding proteins can increase reaction rates by adhering to the physical organic chemistry principles outlined above. Scaffolding proteins both co-localize enzymes and their substrates and arrange them in reactive orientations that minimize entropic costs (Fig. 2).[18] In doing so, they can regulate signaling pathways in several ways: they can bind multiple signaling enzymes to increase the rates at which they interact, localize signaling components to specific organelles or cellular compartments, or modulate feedback mechanisms.[19] A well-studied example is Ste5, which binds enzymes in the mating mitogen-activated protein kinase (MAPK) signaling pathway in the yeast *Saccharomyces cerevisiae*.[20] Ste5 contains separate binding sites for the MAPK Fus3, the MAPK kinase (MAP2K) Ste7, and the MAP2K kinase (MAP3K) Ste11. It also facilitates binding of input proteins, such as the MAP3K kinase (MAP4K) Ste20.[18]

Functionally related biomolecules can also be co-localized by liquid–liquid phase separations. Just as lipid membranes define the boundaries of cellular compartments, membrane-less, phase-separated droplets can do the same.[21] This phenomenon helps to explain the behavior of super-enhancers (SEs), which regulate the transcription of genes critical for cell identity. SEs are clusters of enhancers that bind master transcription factors and the Mediator complex to boost the transcription of nearby genes to levels higher than traditional enhancers can achieve.[22] Many SE components have large intrinsically disordered domains that promote the formation of phase-separated “condensates” with high densities of transcription-promoting molecules.[23,24] By squeezing the transcriptional machinery into tiny volumes, SE phase-separated condensates maximize the rates of intermolecular interactions and bio-organic reactions required for transcriptional activity.[24]

As can be seen in X-ray co-crystal structures, bifunctional small molecules leverage the same set of physical organic chemistry principles. Rapamycin establishes high local molarity of FKBP12 at a portal to the active site of mTOR, thereby preventing kinase-mediated information transfer to mTOR substrates (Fig. 3A).[25] Changes in the location or orientation of FKBP12 relative to mTOR likely would result in a different effect on mTOR activity. Structural data can also provide insight into the mechanisms of targeted protein degraders. A crystal structure of the linker-containing compound MZ1 complexed with VHL (an E3 ubiquitin ligase) and BRD4 reveals an extensive network of protein–protein and compound–protein interactions (Fig. 3B).[26] These contacts increase the half-life of the complex—and thus the rate of BRD4 ubiquitination—by decreasing its dissociation rate.[27] The non-linker-containing degrader lenalidomide, meanwhile, “reprograms” the E3 ligase cereblon (CRBN) by generating a composite surface that recognizes a different set of protein substrates, such as the kinase CK1α (Fig. 3C).[28] These three structures highlight principles common to all bifunctional small molecules: multimeric complex formation, precise arrangement of binding partners, and the organization of intermolecular interactions.

Bifunctional natural products

Despite the conventional wisdom that small molecules must be large and complex to modulate protein–protein interactions (PPIs), natural selection has generated a set of bifunctional natural products that exhibit a variety of chemical structures (Fig. 4). On one end of the spectrum, the structurally unassuming phytohormones indole-3-acetic acid (IAA)

and methyl jasmonate promote the degradation of their cognate receptors by recruiting E3 ubiquitin ligases,[29,30] which mirrors the MoAs of synthetic linker-containing protein degraders. But unlike those dumbbell-shaped compounds, IAA and methyl jasmonate have structures that suggest neither their MoA nor their bifunctional nature. The comparably simple natural product abscisic acid binds pyrabactin resistance 1 (PYR1) in an internal cavity, prompting dramatic conformational changes that increase the protein's affinity for its type 2C protein phosphatase (PP2C) binding partners.[31] This activity is not contingent upon direct contact with proteins other than PYR1.

On the other end of the structural complexity scale are stereocenter-laden macrocycles that were early on noted for their ability to suppress the human immune system. FK506 and rapamycin both bind FKBP12, but the resultant compound–protein complexes inhibit different proteins to modulate intracellular signaling (calcineurin and mTOR, respectively).[8–10,32] These observations highlight that different small-molecule binders of the same protein may result in divergent neo-binding abilities. Along those lines, the cyclic peptide cyclosporin A is also a bifunctional calcineurin inhibitor like FK506,[33] but it first binds cyclophilin instead of FKBP12. Cyclophilin is also a target of sanglifehrin A, but the resulting complex engages IMP dehydrogenase 2 instead of calcineurin.[34] These discoveries provided the early hints that altering interactomes and modulating protein proximity may indeed be routine but previously cryptic features of small molecules.[35]

Inducing proximity of fusion proteins

The scope of biology that can be modulated with natural products—let alone the subset of bifunctional natural products—is limited. In response, researchers who were inspired by the mechanisms of bifunctional natural products wondered if proximity-based regulation of protein activity could be extended to synthetic compounds and non-natural PPIs. Starting in the early 1990s, they began to generate molecular glues known as "chemical inducers of proximity" (CIPs).[11,12,36] Rather than modulate protein function directly (*e.g.* by engaging in competitive enzyme inhibition), these tool compounds co-localize their targets *via* formation of a ternary complex. CIP-mediated increases in effective molarity were hypothesized to mimic the reaction-rate-enhancing effects of scaffolding proteins, but do so for pairs of proteins that natural selection had neglected.[36] Many of the first molecular glues were homo- or heterodimeric analogs of bifunctional natural products known to bind their targets with high affinity and selectivity. Protein engineering was then used to extend the utility of these molecules beyond their natural binding partners and signaling pathways. The breadth and depth of biology that can be studied using this approach have already been reviewed extensively,[12] so we will instead highlight a handful of illuminating case studies.

Proteins containing FKBP12 domains can be brought in proximity *via* treatment with the molecular glue FK1012: a cell-permeable FK506 dimer in which both ends retain their ability to bind FKBP12. As a proof of concept, FK1012—synthesized from FK506 using a single olefinmetathesis reaction[37]—was first shown to activate T-cell receptor signaling in cells containing an engineered version of the TCR ζ chain.[38] This strategy was then extended to heterodimeric bifunctional molecules. An early example, FKCsA was developed

to induce the proximity of FKBP12- and cyclophilin-containing fusion proteins, yielding control over different phases of cell-signaling cascades.[39]

Not all dimerizers of fusion proteins, however, contain linkers. Rapamycin has been used to assemble “split” versions of proteins whose halves have been fused to either FKBP12 or FRB (the domain of mTOR that binds FKBP12–rapamycin). One such example is the split-NIa protease from the tobacco etch virus (TEV). Originally described in 2006, split-TEV exhibits protease activity only in the presence of rapamycin.[40] This tool has been used to study many cellular phenomena, such as the consequences of caspase activation.[41] Similarly, an engineered split-Cas9 system has been developed in which rapamycin treatment induces genomic or transcriptional manipulations.[42] To avoid the mTOR-mediated independent actions of rapamycin in cells, a bump–hole system using methallyl-rapamycin and a mutant FRB having a compensatory methallyl pocket was developed.[43]

Induced proximity of fusion proteins has also been applied in the clinic. Cell-based therapies for cancer can be hindered by life-threatening toxicity, particularly *via* graft-versus-host disease (GVHD). One approach to limiting GVHD is the incorporation of a T-cell “safety switch” that induces apoptosis upon small-molecule treatment. An engineered version of caspase-9 that is fused to two FKBP12 domains was shown to be effective in patients who had received stem-cell transplants to treat relapsed leukemia.[44] This in-human experiment demonstrates that induced proximity is not only a useful tool for studying biology, but also a potential strategy for treating disease.

Compounds with linkers that engage native proteins

As discussed above, experiments throughout the 1990s showed that rationally designed bifunctional compounds could influence biology *via* the simple act of bringing two fusion proteins closer together in a cell. Having established this proof of concept with engineered proteins, researchers then shifted to the engagement of native proteins with “direct” binders that have minimal effects on intrinsic activity. Linker-containing molecular glues can be synthesized by tethering two compounds that bind the targets of interest. Ideally, chemists would use small-molecule binders that are already known to both 1) engage the desired targets without altering their activity substantially, and 2) contain a position at which a long, flexible linker can be attached without disrupting binding.[45] Such a modular, “plug and play” type of system can make the syntheses of these types of compounds more efficient.

Though easy to overlook, seemingly minor changes to the structure of the linker can have dramatic biological consequences. Even if appropriate binding elements are placed at the ends of the bifunctional molecule, they must be connected such that the bound proteins can interact in a meaningful way.[46] Linkers, therefore, should be sufficiently long and flexible to enable the proteins to adopt a reactive conformation, but short enough to minimize the entropic costs of complexation. As seen in Figure 3B, some of these entropic costs can be offset further *via* enthalpically favorable interactions between the linker and target proteins.[26] This phenomenon is reminiscent of the MoAs of non-linker “composite” binders, such as rapamycin and FK506. A key difference, however, is that linker-containing compounds typically engage their two binding partners independently. Without a defined binding order,

linker compounds can exhibit a “hook effect” in which ternary complex concentration paradoxically decreases at high ligand concentrations (due to the competing formation of binary complexes).[47] Complex7 stability is also contingent upon the complementarity of the protein surfaces being co-localized.[26] These factors all confound the prediction of optimal linker properties *a priori*, so chemists often test a suite of analogs with different linkers.[48]

Despite these challenges, the study of wild-type proteins has several benefits. Experiments with native proteins are more likely to be physiologically and therapeutically relevant than those using engineered proteins not found in humans. Also, these rationally designed, linker-containing compounds may be more easily adapted into therapeutic agents than ones acting on genetic fusion targets. And finally, the use of synthetic ligands removes the need for natural products and their derivatives, which can be challenging to synthesize. In recent years, this direct-binder-based approach has exploded in popularity, sophistication, ambition, and scope (Fig. 5).

Protein degradation.

Often separated into a class of its own, targeted protein degradation *via* linker-containing bifunctional molecules is just one potential consequence of induced proximity, albeit the one with arguably the greatest immediate opportunity for translation to novel types of therapeutics. Though a detailed accounting of this technique lies beyond the scope of this Perspective, it has been documented elsewhere.[48,49]

As mentioned above, a well-studied means to achieve degradation is to co-localize the target protein with an E3 ubiquitin ligase, which results in polyubiquitination of the target and degradation by the 26S proteasome.[50] A well-known manifestation of this technique are proteolysis-targeting chimeras (PROTACs), which were first developed in 2001.[51] This and related strategies have yielded valuable chemical tools, such as a bifunctional degrader of pathogenic tau protein accumulations.[52] Researchers have also leveraged the linker’s influence on ubiquitination efficiency to generate “photoswitchable” degraders whose activities are controlled by light.[53,54] The effectiveness of these technologies underscore the importance of orientation when attempting to spark new biological interactions.

Bifunctional-compound-mediated degradation, however, need not involve binders of E3 ubiquitin ligases. Compounds containing solvent-exposed hydrophobic motifs can cause molecular chaperones to mistakenly identify the target as misfolded and promote its degradation.[55,56] Endosome targeting chimeras (ENDTACs) facilitate the degradation of extracellular protein targets by recruiting them to CXCR7, inducing endocytosis, and enabling degradation by the lysosome.[57] Lysosome targeting chimeras (LYTACs) comprise a multivalent mannose-6-phosphate (M6P)-containing glycopolypeptide conjugated to an antibody that binds an extracellular or membrane-bound target.[58] After binding, the cation-independent M6P receptor shuttles the complex to the lysosome for degradation. Irrespective of the mechanism by which it is achieved, targeted protein degradation is a flagship example of using bifunctional compounds to study biological phenomena.

Protein–protein interaction inhibition.

Bifunctional small molecules can inhibit protein activity by means other than degradation. Similar to how rapamycin must first bind FKBP12 before it can inhibit mTOR, a small molecule–protein complex may be more capable of binding proteins that are otherwise difficult to modulate with small molecules alone. Even if co-localization does not alter the target's activity directly, the newly recruited protein may hinder or block access to the target's active site (see Fig. 3A). This strategy can be applied to the inhibition of PPIs, which often involve large, shallow binding planes. An early instance of this approach sought to prevent the aggregation of β-amyloid (Aβ) as a proposed strategy for treating Alzheimer's disease.[59] The successful development of molecular glues that mitigate Aβ fibrillogenesis supported the notion that linker-containing bifunctional compounds can prevent protein aggregation.[60]

More recently, bifunctional inhibitors of K-Ras have been described.[61] Ras mutants are common drivers of cancer, but nearly all efforts to engage them with peptides or small molecules have failed. Compounds that bind covalently to cysteine-containing K-Ras mutants have been generated,[62] but they are ineffective against the most common oncogenic variants. Using these binders as a starting point, it was hypothesized that recruitment of immunophilin proteins (*e.g.* FKBP12 and cyclophilin) *via* molecular glues would inhibit K-Ras activity. Biochemical testing of compounds comprising K-Ras binders tethered to immunophilin ligands revealed an inhibitor of the interaction between K-Ras(M72C) and its effectors Raf and Sos.[61] This unconventional medicinal chemistry campaign—which focused primarily on linker chemistry—established that recruitment of a steric obstacle to the proper location can inhibit oncogenic K-Ras.

Immune system direction.

Immunotherapies, particularly antibody-based checkpoint inhibitors, have significantly advanced the treatment of cancer. Experiments in the early 1990s demonstrated that bifunctional small molecules could also direct proteins—including components of the immune system—to specific cell types. Though reminiscent of antibody–drug conjugates,[63] the fundamental chemical biology of this technique is reversed: a small molecule recruiting an antibody to achieve selective cell killing. In an early manifestation of this approach, conjugation of dinitrobenzene (DNP) to either biotin or CD4 recruited a monoclonal anti-DNP antibody to streptavidin or the HIV envelope protein gp120, respectively.[64] This *in vitro* proof of concept was extended to live cells shortly thereafter.[65]

Bifunctional compounds have more recently been used to recruit antibodies to cancer cells. Structural data informed the design of molecular glues containing a non-peptidic binder of the extracellular domain of $\alpha_v\beta_3$ integrin linked to the trisaccharide antigen galactosyl-α(1–3)galactose (α-Gal).[66] α-Gal-targeting antibodies bind much more tightly to multivalent displays of antigen, so the high abundance of $\alpha_v\beta_3$ integrin on the surfaces of tumors and tumoral endothelial cells present an opportunity for bifunctional compounds to direct antibodies towards those cell types.[67] This hypothesis proved correct: the bifunctional compounds only killed cell lines expressing high levels of $\alpha_v\beta_3$ integrin, whereas the chemotherapy doxorubicin tethered to the $\alpha_V\beta_3$ integrin binder killed cells

indiscriminately[68]. Other research groups have generated antibody-recruiting small molecules (ARMs) that target metastatic cancer cells and HIV particles, among others.[69,70]

This concept has been further extended to the recruitment of neutrophils to pathogenic fungi. Molecular glues comprising the *N*-formylated tripeptide fMLP (which binds FPR1 on neutrophils) covalently tethered to an antifungal drug successfully directed neutrophils to the fungus *Aspergillus fumigatus* in model systems, including a zebrafish infection model.[71] Compound treatment also enhanced the fungicidal activity of patient-derived neutrophils. These results, and others,[72] show that molecular glues can nudge the immune system towards a wide variety of targets.

Transcription elongation.

Bifunctional molecules can also induce transcription at specific parts of the genome. Proximity of RNA polymerase II (Pol II) is necessary but not sufficient for transcription at a given genetic locus to take place; the positive transcription elongation factor b (P-TEFb) must be present and active for Pol II to be in a productive elongation state.[73] An international collaboration of researchers generated bifunctional molecules that contain the small molecule JQ1 (a binder of BRD4, which associates with active P-TEFb) linked to polyamides that bind specific DNA sequences in a predictable manner. The molecular glue Syn-TEF1 was shown to activate transcription of the *FXN* gene—which is silenced in patients with the neurodegenerative disease Friedrich's ataxia—in various disease-relevant contexts.[74]

Compounds without linkers that engage native proteins

Increasingly sophisticated and thorough studies of small-molecule MoAs have revealed that bioactive compounds lacking canonical linkers often behave not as inhibitors of single enzymes, but as modulators of biologically meaningful PPIs—they alter the interactomes of their targets. Though they are more challenging to design "rationally," they act *via* the same biology described above: proximity as a regulator of activity.

Complex stabilization.

Small-molecule-induced stabilization of multimeric protein complexes can have pronounced biological consequences. For example, the natural products paclitaxel and discodermolide stabilize microtubule polymers in such a way that blocks mitosis and triggers apoptosis.[75] A synthetic small molecule with the same MoA is synstab A (a portmanteau from "synthetic" and "stabilizer"). Synstab A was identified from a commercially available compound collection *via* a phenotypic screen searching for small-molecule modulators of mitosis. Though many of the assay positives destabilized microtubules (52/139), synstab A was shown to be a stabilizer, promoting interactions between alpha and beta tubulin.[76] Notably, synstab A is significantly less complex than paclitaxel and discodermolide (Fig. 6A).

Complex stabilization can also result in enzyme inhibition. In one illustrative example, a small-molecule screen uncovered an inhibitor (CC0651) of the E2 ubiquitin ligase hCdc34, which was originally proposed to act *via* an allosteric mechanism.[77] Further examination of the CC0651–hCdc34A–ubiquitin ternary complex revealed that CC0651 stabilizes otherwise

weak interactions between hCdc34A and ubiquitin by binding at the interface of the two proteins.[78] Like synstab A—as well as the auxin and jasmonate natural products—CC0651 is a structurally simple compound with a low molecular weight (442 Da). Complex molecular features, therefore, are not a requirement for bifunctionality.

Counter-intuitively, small-molecule stabilizers can block PPIs indirectly. The transcription factor Myc, a regulator of cellular proliferation, is overexpressed in many cancers. To activate transcription, Myc must first form a heterodimeric complex with the transcription factor Max. Myc has been unreceptive to small-molecule engagement, so attention has shifted to disrupting the Myc–Max dimer. A recent small-molecule microarray screen identified binders of purified Max protein. An optimized analog of an assay positive, KI-MS2–008, was found to stabilize the Max homodimer and thus render Max less capable of binding Myc. KI-MS2–008 treatment prompted a decrease of both Myc protein levels and Myc-regulated transcripts in cells, and it exhibited significant efficacy against cellular and murine models of Myc-driven cancers.[79] Sequestering binding partners in stabilized protein complexes, therefore, may prove to be a useful general strategy for modulating pathogenic PPIs.

Advances in screening technology are needed to exploit more fully the biological and therapeutic potential of small molecules that alter interactomes. To address this need, an international collaboration between scientists from both academia and industry has recently developed a disulfide-trapping-based platform to identify fragments that stabilize PPIs.[80] The interaction between estrogen receptor α (ERα) and the regulatory protein 14-3-3σ was chosen as an initial test system. Screening a custom library of 1,600 disulfide-containing fragments revealed compounds that stabilize the interaction between 14-3-3σ and a 15-mer phosphopeptide derived from ERα in a dose-dependent manner. These impressive results establish a direct means to identify starting points for stabilizers of PPIs.

Protein destabilization.

Alternatively, some non-linker bifunctional compounds destabilize their protein targets and facilitate their degradation. Several estrogen receptor (ER) antagonists have been found retrospectively to be ER degraders. The synthetic steroid fulvestrant, for example, degrades ERα by enabling interactions with the intermediate filament proteins cytokeratins 8 and 18, which associate with proteasomes in the nuclear matrix.[81] Fulvestrant's long alkyl side chain inspired scientists at Genentech to develop GNE-0011, a JQ1-based BRD4 degrader that was not linked to an E3 ligase binder.[82] The only modification needed to convert JQ1 from an inhibitor to a degrader of BRD4 was the replacement of an aryl chloride with a propargylamine motif; no traditional linker was required (Fig. 6B). The ability of this minor structural change to alter compound MoA suggests that targeted degraders may be more common than previously realized.

Interactome modulation.

Just as linker-containing bifunctional small molecules can induce the proximity of proteins that otherwise have minimal affinity for one another, non-linker molecular glues can alter the interactomes of their targets. Immunomodulatory imide drugs (IMiDs) comprise the multiple

myeloma drug thalidomide and its derivatives. Phthalimides linked to small-molecule protein binders can effect targeted protein degradation,[6] but IMiD treatment alone is also sufficient to alter the target specificity of the E3 ligase CRBN (see Fig. 3C). Thalidomide blocks CRBN autoubiquitination,[83] inhibits the ubiquitination of native substrates (*e.g.* MEIS2), and promotes the ubiquitination of neo-substrates (*e.g.* IKZF1 and IKZF3).[84] Biophysical experiments and X-ray crystallography data suggest that these new interactions would not have taken place in the absence of IMiD.[28]

Similarly, researchers working independently at Eisai and the University of Texas Southwestern Medical Center discovered that aryl sulfonamides known as SPLAMs (*e.g.* indisulam and tasisulam) induce degradation of the RNA splicing factor RBM39 by promoting interactions with the CUL4–DCAF15 E3 ubiquitin ligase.[85,86] SPLAM-mediated degradation of RBM39 causes RNA splicing defects to accumulate in a manner that is harmful to cancer cell lines, and this sensitivity correlates with DCAF15 expression. Indisulam appears to have no affinity for either RBM39 or DCAF15 in isolation, which suggests that it binds both members of the complex simultaneously.[5,85,86]

Bifunctional interactome modulators can also induce the degradation of their target(s) in a proteasome-independent manner. A recent study describes molecular glues that reduce levels of pathogenic huntingtin protein (mHTT) by autophagic clearance in both cells and *in vivo* models of Huntington's disease.[87] A small-molecule microarray yielded compounds that bind mHTT and microtubule-associated protein 1A/1B light chain 3 (LC3), which is localized to the membranes of autophagosomes. While the structural bases of these compound–protein interactions remain unclear, the authors suggest that their molecular glues interact with the polyglutamine motif found in mHTT but not wild-type protein.[87]

Interactome modulation need not always result in protein degradation. The sensitivity of cancer cell lines to the synthetic compound DNMDP was shown to be correlated with *PDE3A* expression, which encodes phosphodiesterase 3A.[88] Further analysis revealed that DNMDP not only induces an interaction between PDE3A with the protein Schlafen 12 (SLFN12), but also exhibits its greatest toxicity in cells with high *PDE3A* and *SLFN12* expression; reduction of either *PDE3A* or *SLFN12* mRNA levels decreases sensitivity to DNMDP. It remains unknown if DNMDP stabilizes an endogenous interaction or induces a new interaction between PDE3A and SLFN12.

Transporter inhibition.

Natural products provided the original proof of concept that non-linker bifunctional small molecules can inhibit protein activity *via* induced proximity. Accordingly, a library of 45,000 rapamycin-inspired macrocycles termed rapafucins was recently synthesized.[89] Like rapamycin and FK506, rapafucins comprise two distinct "domains": an FKBP-binding domain that engages FKBP12, and a variable effector domain that promotes ternary complex formation with a second protein.[90]

Cell-based screening of the rapafucin library and subsequent optimization of assay hits yielded rapadocin, an equilibrative nucleoside transporter 1 (ENT1) inhibitor that blocks cellular nucleoside uptake.[89] Surprisingly, rapadocin alone has affinity for both FKBP12 and

ENT1, though the presence of FKBP12 enhances ENT1 inhibition significantly. These findings epitomize improved understandings of the design, synthesis, and potential biological consequences of molecular glues without linkers.

Protein–protein interaction disruption.

This Perspective focuses on the ability of bifunctional molecules to bring proteins closer together, but we also should note how they can obstruct PPIs. The small-molecule PPI inhibitor literature is vast,[91] so we will focus on ostensible enzyme inhibitors functioning as PPI disruptors.

Lysine-specific histone demethylase 1 (LSD1) is a candidate therapeutic target for acute myeloid leukemia (AML), prompting the development of LSD1 inhibitors that were thought to interfere with demethylase activity.[92] However, an LSD1 inhibitor from Takeda was determined to block the interaction between LSD1 and the transcription factor GFI1B,[93] and LSD1 catalytic activity was shown to be unnecessary to maintain AML proliferation or maturation arrest.[94] To study the MoA of LSD1 inhibitors in greater detail, a method called CRISPR-suppressor scanning was invented.[95] This technique yielded a more nuanced and comprehensive understanding of the MoA of LSD1 inhibitors, and it confirmed that disruption of the LSD1– GFI1B interaction is the clinically relevant mechanism.

Similarly, recent experiments with kinase inhibitors have revealed that our understanding of small-molecule-based kinase inhibition is incomplete. Several ATP-competitive kinase inhibitors, including clinically approved drugs, lower the levels of their targets in cells. A subset of these compounds appear to prevent their target kinases from interacting with Cdc37, which blocks recruitment to the chaperone Hsp90.[96] Other kinase inhibitors have been found to promote either proteasomal or lysosomal degradation of their targets, though in many cases the mechanistic details are unknown.[97]

Concluding thoughts and future outlook

Bifunctional small molecules are everywhere. Not only does Nature use them to regulate cellular information flow and homeostasis, but chemists also synthesize them—both knowingly and unknowingly—to populate screening libraries and analyze biological systems. Due to the difficultly of modeling the consequences of a compound–protein binding event, rationally designed molecular glues have largely comprised known “monovalent” binders that are stitched together with a covalent linker. The development of rapadocin,[89] however, suggests that detailed mechanistic information can aid the design of bifunctional compounds that engage in “composite” or allosteric binding. And as methods to study small-molecule MoAs continue to improve, researchers will be able to determine which compounds regulate protein proximity and how they do so. These efforts would benefit from the recognition that bifunctionality can have a broad range of chemical and biological manifestations (see Fig. 5).

Conventional wisdom says that bifunctional molecules and PPI modulators should be large and complex to engage multiple protein surfaces simultaneously. While this may be true in some cases, it should also be recognized that compounds like auxin (MW = 175 Da) and

thalidomide (MW = 258 Da) are bifunctional. Small, simple compounds like these can facilitate complex formation because proteins appear to be primed for multimeric assembly. In an illustrative study, the introduction of hydrophobicity-raising point mutations into protein complexes from *E. coli* prompted assembly into supramolecular fibrils or foci for each of the 12 complexes tested.[98] The ability of hydrophobic small molecules to engender similar changes in protein topography, therefore, should not be surprising, and the underlying mechanisms of these genetic- and binding-based perturbations are almost certainly related.

We will end this Perspective with some prospective thoughts. Bifunctional small molecules combined with protein engineering have enabled the detailed study of vast swaths of biology over the past 25 years: signal transduction, chromatin remodeling, protein transport, transcriptional regulation, and many other facets of cell behavior.[12] Perhaps even more importantly, **each achievement derived from an engineered system also provides evidence that the same result can be obtained *via* chemically induced proximity of the native system**. Different bifunctional compounds will be needed—a daunting hurdle, to be sure—but the same chemical and biophysical principles apply in both scenarios. Therefore, we find the future of bifunctional small molecules to be as exciting as its opportunities are numerous.

The identification and development of bifunctional compounds, both with and without linkers, would benefit from improvements in binding-based screening technology. Biochemical enzyme inhibition assays have proven amenable to high-throughput screening, but it remains challenging to detect small molecule–protein binding events with a similar level of efficiency. Even if a small-molecule binder does not alter the activity or interactome of its target directly, it could be tethered to another binder to realize new biology. Biophysical techniques and library synthesis strategies (*e.g.* DNA-encoded libraries and fragment-based screening in cells)[99,100] that enable affinity-based screening are promising developments.

Moving forward, we should continue to recognize the myriad ways in which small molecules can interact with proteins. Bifunctional compounds have proven to be a powerful but underappreciated modality. Be they synthesized in a lab or by Nature, bifunctional small molecules exhibit activities that can be difficult to reproduce with other methods. And for all their structural and functional differences, the principles undergirding their biological mechanisms are the same. Nature has given us the blueprints; chemical biologists are making them come to life.

Acknowledgments

The authors thank B. Melillo, J. Ostrem, and B. Wagner for their critical feedback on the manuscript. Research from the Schreiber laboratory in the area covered by this Perspective was generously supposed by the National Institute of General Medical Sciences.

References

1. Rakhit R, Navarro R & Wandless TJ Chemical biology strategies for posttranslational control of protein function. Chem. Biol 21, 1238–1252 (2014). [PubMed: 25237866]

2. Kraft AS & Anderson WB Phorbol esters increase the amount of Ca2+, phospholipid-dependent protein kinase associated with plasma membrane. Nature 301, 621–623 (1983). [PubMed: 6828143]
3. Rando RR & Kishi Y Structural basis of protein kinase C activation by diacylglycerols and tumor promoters. Biochemistry 31, 2211–2218 (1992). [PubMed: 1540576]
4. Wender PA et al. Modeling of the bryostatins to the phorbol ester pharmacophore on protein kinase C. Proc. Natl. Acad. Sci 85, 7197–7201 (1988). [PubMed: 3174627]
5. Che Y, Gilbert AM, Shanmugasundaram V & Noe MC Inducing protein-protein interactions with molecular glues. Bioorg. Med. Chem. Lett 28, 2585–2592 (2018). [PubMed: 29980357]
6. Winter GE et al. Phthalimide conjugation as a strategy for in vivo target protein degradation. Science. 348, 1376–1381 (2015). [PubMed: 25999370]
7. Bondeson DP et al. Catalytic in vivo protein knockdown by small-molecule PROTACs. Nat. Chem. Biol 11, 611–617 (2015). [PubMed: 26075522]
8. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P & Snyder SH RAFT1: A mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. Cell 78, 35–43 (1994). [PubMed: 7518356]
9. Schreiber SL & Crabtree GR The mechanism of action of cyclosporin A and FK506. Immunol. Today 13, 136–142 (1992). [PubMed: 1374612]
10. Sabers CJ et al. Isolation of a Protein Target of the FKBP12-Rapamycin Complex in Mammalian Cells. J. Biol. Chem 270, 815–822 (1995). [PubMed: 7822316]
11. Austin DJ, Schreiber SL & Crabtree GR Proximity versus allostery: the role of regulated protein dimerization in biology. Chem. Biol 1, 131–136 (1994). [PubMed: 9383382] This paper contrasts the concept of induced proximity to that of the general phenomenon of allosteric regulation. The authors propose that induced co-localization of proteins via synthetic “dimerizers” may be sufficient to study a variety of biological systems.
12. Stanton BZ, Chory EJ & Crabtree GR Chemically induced proximity in biology and medicine. Science. 359, eaao5902 (2018). [PubMed: 29590011] This review discusses the breadth of biological systems that have been studied with molecular glues known as chemical inducers of proximity (CIPs). It primarily focuses on cases involving protein engineering.
13. Ellis RJ & Minton AP Cell Biology - Join the crowd. Nature 425, 27–28 (2003). [PubMed: 12955122]
14. Page MI & Jencks WP Entropic Contributions to Rate Accelerations in Enzymic and Intramolecular Reactions and the Chelate Effect. Proc. Natl. Acad. Sci 68, 1678–1683 (1971). [PubMed: 5288752]
15. Kirby AJ Effective Molarities for Intramolecular Reactions in Advances in Physical Organic Chemistry (eds. Gold V & Bethell D) 17, 183–278 (Academic Press, 1980).
16. Tenud L, Farooq S, Seibl J & Eschenmoser A Endocyclische S N -Reaktionen am gesättigten Kohlenstoff? Vorläufige Mitteilung. Helv. Chim. Acta 53, 2059–2069 (1970).This paper describes a nucleophilic substitution reaction in which the intramolecular pathway that proceeds via an endocyclic transition state is disfavored. In doing so, it reveals that increasing the proximity of reactive species is not always sufficient to achieve rate acceleration.
17. Baldwin JE Rules for ring closure. J. Chem. Soc. Chem. Commun 734–736 (1976). doi:10.1039/c39760000734
18. Good MC, Zalatan JG & Lim WA Scaffold Proteins: Hubs for Controlling the Flow of Cellular Information. Science. 332, 680–686 (2011). [PubMed: 21551057]
19. Shaw AS & Filbert EL Scaffold proteins and immune-cell signalling. Nat. Rev. Immunol 9, 47–56 (2009). [PubMed: 19104498]
20. Chol K-Y, Satterberg B, Lyons DM & Elion EA Ste5 tethers multiple protein kinases in the MAP kinase cascade required for mating in S. cerevisiae. Cell 78, 499–512 (1994). [PubMed: 8062390]
21. Alberti S Phase separation in biology. Curr. Biol 27, R1097–R1102 (2017). [PubMed: 29065286]
22. Whyte WA et al. Master Transcription Factors and Mediator Establish Super-Enhancers at Key Cell Identity Genes. Cell 153, 307–319 (2013). [PubMed: 23582322]
23. Hnisz D, Shrinivas K, Young RA, Chakraborty AK & Sharp PA A Phase Separation Model for Transcriptional Control. Cell 169, 13–23 (2017). [PubMed: 28340338]

24. Sabari BR et al. Coactivator condensation at super-enhancers links phase separation and gene control. Science. 361, eaar3958 (2018). [PubMed: 29930091]
25. Yang H et al. mTOR kinase structure, mechanism and regulation. Nature 497, 217–223 (2013). [PubMed: 23636326] This paper describes several X-ray crystal structures of mTOR complexed with various regulatory small molecules and protein binding partners. Analysis of these structures reveals that rapamycin–FKBP12 inhibits mTOR by physically blocking substrates from accessing its active site.
26. Gadd MS et al. Structural basis of PROTAC cooperative recognition for selective protein degradation. Nat. Chem. Biol 13, 514–521 (2017). [PubMed: 28288108]
27. Kopytek SJ, Standaert RF, Dyer JC & Hu JC Chemically induced dimerization of dihydrofolate reductase by a homobifunctional dimer of methotrexate. Chem. Biol 7, 313–321 (2000). [PubMed: 10801470]
28. Petzold G, Fischer ES & Thomä NH Structural basis of lenalidomide-induced CK1α degradation by the CRL4CRBN ubiquitin ligase. Nature 532, 127–130 (2016). [PubMed: 26909574] This paper examines the structural underpinnings of lenalidomide's ability to alter the substrate specificity of the E3 ubiquitin ligase cereblon (CRBN).
29. Woodward AW & Bartel B Auxin: Regulation, Action, and Interaction. Ann. Bot 95, 707–735 (2005). [PubMed: 15749753]
30. Chini A et al. The JAZ family of repressors is the missing link in jasmonate signalling. Nature 448, 666–671 (2007). [PubMed: 17637675]
31. Nishimura N et al. Structural Mechanism of Abscisic Acid Binding and Signaling by Dimeric PYR1. Science. 326, 1373–1379 (2009). [PubMed: 19933100]
32. Brown EJ et al. A mammalian protein targeted by G1-arresting rapamycin–receptor complex. Nature 369, 756–758 (1994). [PubMed: 8008069]
33. Liu J et al. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. Cell 66, 807–815 (1991). [PubMed: 1715244] This paper identifies calcineurin as the shared target of cyclosporin A (complexed with cyclophilin) and FK506 (complexed with FKBP12). It also shows that the rapamycin–FKBP12 complex, cyclophilin alone, and FKBP12 alone do not inhibit calcineurin.
34. Pua KH, Stiles DT, Sowa ME & Verdine GL IMPDH2 Is an Intracellular Target of the Cyclophilin A and Sanglifehrin A Complex. Cell Rep. 18, 432–442 (2017). [PubMed: 28076787]
35. Schreiber SL A Chemical Biology View of Bioactive Small Molecules and a Binder-Based Approach to Connect Biology to Precision Medicines. Isr. J. Chem 59, 52–59 (2019). [PubMed: 31123369]
36. Crabtree GR & Schreiber SL Three-part inventions: intracellular signaling and induced proximity. Trends Biochem. Sci 21, 418–422 (1996). [PubMed: 8987395]
37. Diver ST & Schreiber SL Single-Step Synthesis of Cell-Permeable Protein Dimerizers That Activate Signal Transduction and Gene Expression. J. Am. Chem. Soc 119, 5106–5109 (1997).
38. Pruschy MN et al. Mechanistic studies of a signaling pathway activated by the organic dimerizer FK1012. Chem. Biol 1, 163–172 (1994). [PubMed: 9383386]
39. Belshaw PJ, Ho SN, Crabtree GR & Schreiber SL Controlling protein association and subcellular localization with a synthetic ligand that induces heterodimerization of proteins. Proc. Natl. Acad. Sci 93, 4604–4607 (1996). [PubMed: 8643450]
40. Wehr MC et al. Monitoring regulated protein-protein interactions using split TEV. Nat. Methods 3, 985–993 (2006). [PubMed: 17072307]
41. Gray DC, Mahrus S & Wells JA Activation of specific apoptotic caspases with an engineered small-molecule-activated protease. Cell 142, 637–646 (2010). [PubMed: 20723762]
42. Zetsche B, Volz SE & Zhang F A split-Cas9 architecture for inducible genome editing and transcription modulation. Nat. Biotechnol 33, 139–142 (2015). [PubMed: 25643054]
43. Liberles SD, Diver ST, Austin DJ & Schreiber SL Inducible gene expression and protein translocation using nontoxic ligands identified by a mammalian three-hybrid screen. Proc. Natl. Acad. Sci 94, 7825–7830 (1997). [PubMed: 9223271]
44. Di Stasi A et al. Inducible Apoptosis as a Safety Switch for Adoptive Cell Therapy. N. Engl. J. Med 365, 1673–1683 (2011). [PubMed: 22047558] This paper demonstrates the ability of a

molecular glue to induce the activity of engineered caspase 9 in stem-cell transplant patients. In doing so, it establishes that bifunctional-compound-induced proximity of fusion proteins can have therapeutic benefits in the clinic.

45. Cyrus K et al. Jostling for Position: Optimizing Linker Location in the Design of Estrogen Receptor-Targeting PROTACs. ChemMedChem 5, 979–985 (2010). [PubMed: 20512796]
46. Krishnamurthy VM, Semetey V, Bracher PJ, Shen N & Whitesides GM Dependence of Effective Molarity on Linker Length for an Intramolecular Protein–Ligand System. J. Am. Chem. Soc 129, 1312–1320 (2007). [PubMed: 17263415]
47. Douglass EF, Miller CJ, Sparer G, Shapiro H & Spiegel DA A Comprehensive Mathematical Model for Three-Body Binding Equilibria. J. Am. Chem. Soc 135, 6092–6099 (2013). [PubMed: 23544844]
48. Lai AC & Crews CM Induced protein degradation: an emerging drug discovery paradigm. Nat. Rev. Drug Discov 16, 101–114 (2017). [PubMed: 27885283] This review summarizes several small-molecule-based approaches to targeted protein degradation, such as PROTACs, hydrophobic tagging (HyT), and selective hormone receptor degraders (*e.g.* SERDs).
49. Chamberlain PP & Hamann LG Development of targeted protein degradation therapeutics. Nat. Chem. Biol 15, 937–944 (2019). [PubMed: 31527835]
50. Dikic I Proteasomal and Autophagic Degradation Systems. Annu. Rev. Biochem 86, 193–224 (2017). [PubMed: 28460188]
51. Sakamoto KM et al. Protacs: Chimeric molecules that target proteins to the Skp1–Cullin–F box complex for ubiquitination and degradation. Proc. Natl. Acad. Sci 98, 8554–8559 (2001). [PubMed: 11438690]
52. Silva MC et al. Targeted degradation of aberrant tau in frontotemporal dementia patient-derived neuronal cell models. Elife 8, (2019).
53. Pfaff P, Samarasinghe KTG, Crews CM & Carreira EM Reversible Spatiotemporal Control of Induced Protein Degradation by Bistable PhotoPROTACs. ACS Cent. Sci 5, 1682–1690 (2019). [PubMed: 31660436]
54. Reynders M et al. PHOTACs Enable Optical Control of Protein Degradation. ChemRxiv (2019). doi:10.26434/chemrxiv.8206688.v2
55. Neklesa TK et al. Small-molecule hydrophobic tagging–induced degradation of HaloTag fusion proteins. Nat. Chem. Biol 7, 538–543 (2011). [PubMed: 21725302]
56. Long MJC, Gollapalli DR & Hedstrom L Inhibitor Mediated Protein Degradation. Chem. Biol 19, 629–637 (2012). [PubMed: 22633414]
57. Nalawansha DA et al. Targeted Protein Internalization and Degradation by ENDosome TArgeting Chimeras (ENDTACs). ACS Cent. Sci 5, 1079–1084 (2019). [PubMed: 31263767]
58. Banik S, Pedram K, Wisnovsky S, Riley N & Bertozzi C Lysosome Targeting Chimeras (LYTACs) for the Degradation of Secreted and Membrane Proteins. ChemRxiv (2019). doi:10.26434/chemrxiv.7927061
59. Schellenberg GD & Montine TJ The genetics and neuropathology of Alzheimer's disease. Acta Neuropathol. 124, 305–323 (2012). [PubMed: 22618995]
60. Gestwicki JE, Crabtree GR & Graef IA Harnessing Chaperones to Generate Small-Molecule Inhibitors of Amyloid Aggregation. Science. 306, 865–869 (2004). [PubMed: 15514157]
61. Zhang Z & Shokat KM Bifunctional Small-Molecule Ligands of K-Ras Induce Its Association with Immunophilin Proteins. Angew. Chemie Int. Ed 58, 16314–16319 (2019).
62. Ostrem JM, Peters U, Sos ML, Wells JA & Shokat KM K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. Nature 503, 548–551 (2013). [PubMed: 24256730]
63. Koppel GA Recent advances with monoclonal antibody drug targeting for the treatment of human cancer. Bioconjug. Chem 1, 13–23 (1990). [PubMed: 2095201]
64. Shokat KM & Schultz PG Redirecting the immune response: ligand-mediated immunogenicity. J. Am. Chem. Soc 113, 1861–1862 (1991).
65. Bertozzi C & Bednarski M C-glycosyl compounds bind to receptors on the surface of Escherichia coli and can target proteins to the organism. Carbohydr. Res 223, 243–253 (1992). [PubMed: 1596922]

66. Owen RM et al. Bifunctional Ligands that Target Cells Displaying the αvβ3 Integrin. ChemBioChem 8, 68–82 (2007). [PubMed: 17154219]
67. Danhier F, Le Breton A & Préat V RGD-Based Strategies To Target Alpha(v) Beta(3) Integrin in Cancer Therapy and Diagnosis. Mol. Pharm 9, 2961–2973 (2012). [PubMed: 22967287]
68. Carlson CB, Mowery P, Owen RM, Dykhuizen EC & Kiessling LL Selective Tumor Cell Targeting Using Low-Affinity, Multivalent Interactions. ACS Chem. Biol 2, 119–127 (2007). [PubMed: 17291050]
69. Parker CG, Domaoal RA, Anderson KS & Spiegel DA An Antibody-Recruiting Small Molecule That Targets HIV gp120. J. Am. Chem. Soc 131, 16392–16394 (2009). [PubMed: 19839582]
70. Rullo AF et al. Re-engineering the Immune Response to Metastatic Cancer: Antibody-Recruiting Small Molecules Targeting the Urokinase Receptor. Angew. Chemie Int. Ed 55, 3642–3646 (2016).
71. Jones CN et al. Bifunctional Small Molecules Enhance Neutrophil Activities Against Aspergillus fumigatus in vivo and in vitro. Front. Immunol 10, 1–13 (2019). [PubMed: 30723466]
72. McEnaney PJ, Parker CG, Zhang AX & Spiegel DA Antibody-Recruiting Molecules: An Emerging Paradigm for Engaging Immune Function in Treating Human Disease. ACS Chem. Biol 7, 1139–1151 (2012). [PubMed: 22758917]
73. Adelman K & Lis JT Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. Nat. Rev. Genet 13, 720–731 (2012). [PubMed: 22986266]
74. Erwin GS et al. Synthetic transcription elongation factors license transcription across repressive chromatin. Science. 358, 1617–1622 (2017). [PubMed: 29192133]
75. Jordan MA & Wilson L Microtubules and actin filaments: dynamic targets for cancer chemotherapy. Curr. Opin. Cell Biol 10, 123–130 (1998). [PubMed: 9484604]
76. Haggarty SJ et al. Dissecting cellular processes using small molecules: identification of colchicine-like, taxol-like and other small molecules that perturb mitosis. Chem. Biol 7, 275–286 (2000). [PubMed: 10780927]
77. Ceccarelli DF et al. An Allosteric Inhibitor of the Human Cdc34 Ubiquitin-Conjugating Enzyme. Cell 145, 1075–1087 (2011). [PubMed: 21683433]
78. Huang H et al. E2 enzyme inhibition by stabilization of a low-affinity interface with ubiquitin. Nat. Chem. Biol 10, 156–163 (2014). [PubMed: 24316736]
79. Struntz NB et al. Stabilization of the Max Homodimer with a Small Molecule Attenuates Myc-Driven Transcription. Cell Chem. Biol 26, 711–723.e14 (2019). [PubMed: 30880155]
80. Sijbesma E et al. Site-Directed Fragment-Based Screening for the Discovery of Protein–Protein Interaction Stabilizers. J. Am. Chem. Soc 141, 3524–3531 (2019). [PubMed: 30707565]
81. Long X & Nephew KP Fulvestrant (ICI 182,780)-dependent Interacting Proteins Mediate Immobilization and Degradation of Estrogen Receptor-α. J. Biol. Chem 281, 9607–9615 (2006). [PubMed: 16459337]
82. Blake RA Abstract 4452: GNE-0011, a novel monovalent BRD4 degrader in Cancer Research 79, 4452 (American Association for Cancer Research, 2019).
83. Ito T et al. Identification of a Primary Target of Thalidomide Teratogenicity. Science. 327, 1345–1350 (2010). [PubMed: 20223979]
84. Fischer ES et al. Structure of the DDB1–CRBN E3 ubiquitin ligase in complex with thalidomide. Nature 512, 49–53 (2014). [PubMed: 25043012]
85. Han T et al. Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15. Science. 356, eaal3755 (2017). [PubMed: 28302793]
86. Uehara T et al. Selective degradation of splicing factor CAPERα by anticancer sulfonamides. Nat. Chem. Biol 13, 675–680 (2017). [PubMed: 28437394]
87. Li Z et al. Allele-selective lowering of mutant HTT protein by HTT–LC3 linker compounds. Nature 575, 203–209 (2019). [PubMed: 31666698]
88. de Waal L et al. Identification of cancer-cytotoxic modulators of PDE3A by predictive chemogenomics. Nat. Chem. Biol 12, 102–108 (2016). [PubMed: 26656089]
89. Guo Z et al. Rapamycin-inspired macrocycles with new target specificity. Nat. Chem 11, 254–263 (2019). [PubMed: 30532015]

90. Bierer B, Somers P, Wandless T, Burakoff S & Schreiber S Probing immunosuppressant action with a nonnatural immunophilin ligand. Science. 250, 556–559 (1990). [PubMed: 1700475]

91. Scott DE, Bayly AR, Abell C & Skidmore J Small molecules, big targets: drug discovery faces the protein–protein interaction challenge. Nat. Rev. Drug Discov 15, 533–550 (2016). [PubMed: 27050677]

92. McGrath JP et al. Pharmacological Inhibition of the Histone Lysine Demethylase KDM1A Suppresses the Growth of Multiple Acute Myeloid Leukemia Subtypes. Cancer Res. 76, 1975–1988 (2016). [PubMed: 26837761]

93. Ishikawa Y et al. A Novel LSD1 Inhibitor T-3775440 Disrupts GFI1B-Containing Complex Leading to Transdifferentiation and Impaired Growth of AML Cells. Mol. Cancer Ther 16, 273–284 (2017). [PubMed: 27903753]

94. Maiques-Diaz A et al. Enhancer Activation by Pharmacologic Displacement of LSD1 from GFI1 Induces Differentiation in Acute Myeloid Leukemia. Cell Rep. 22, 3641–3659 (2018). [PubMed: 29590629]

95. Vinyard ME et al. CRISPR-suppressor scanning reveals a nonenzymatic role of LSD1 in AML. Nat. Chem. Biol 15, 529–539 (2019). [PubMed: 30992567]

96. Polier S et al. ATP-competitive inhibitors block protein kinase recruitment to the Hsp90-Cdc37 system. Nat. Chem. Biol 9, 307–312 (2013). [PubMed: 23502424]

97. Jones LH Small-Molecule Kinase Downregulators. Cell Chem. Biol 25, 30–35 (2018). [PubMed: 29174540] This review covers small-molecule-based methods to decrease levels of kinases in cells, particularly in the context of cancer. Both linker-containing molecules (*e.g.* PROTACs) and non-linker compounds are discussed, including several FDA-approved, ATP-competitive compounds (*e.g.* erlotinib and vemurafenib).

98. Garcia-Seisdedos H, Empereur-Mot C, Elad N & Levy ED Proteins evolve on the edge of supramolecular self-assembly. Nature 548, 244–247 (2017). [PubMed: 28783726] This paper studies the phenomenon by which proteins self-assemble into symmetric multimeric complexes. The authors often observed that a single point mutation was sufficient to catalyze polymerization, which suggests that proteins are primed for self-assembly.

99. Zimmermann G & Neri D DNA-encoded chemical libraries: foundations and applications in lead discovery. Drug Discov. Today 21, 1828–1834 (2016). [PubMed: 27477486]

100. Parker CG et al. Ligand and Target Discovery by Fragment-Based Screening in Human Cells. Cell 168, 527–541.e29 (2017). [PubMed: 28111073]

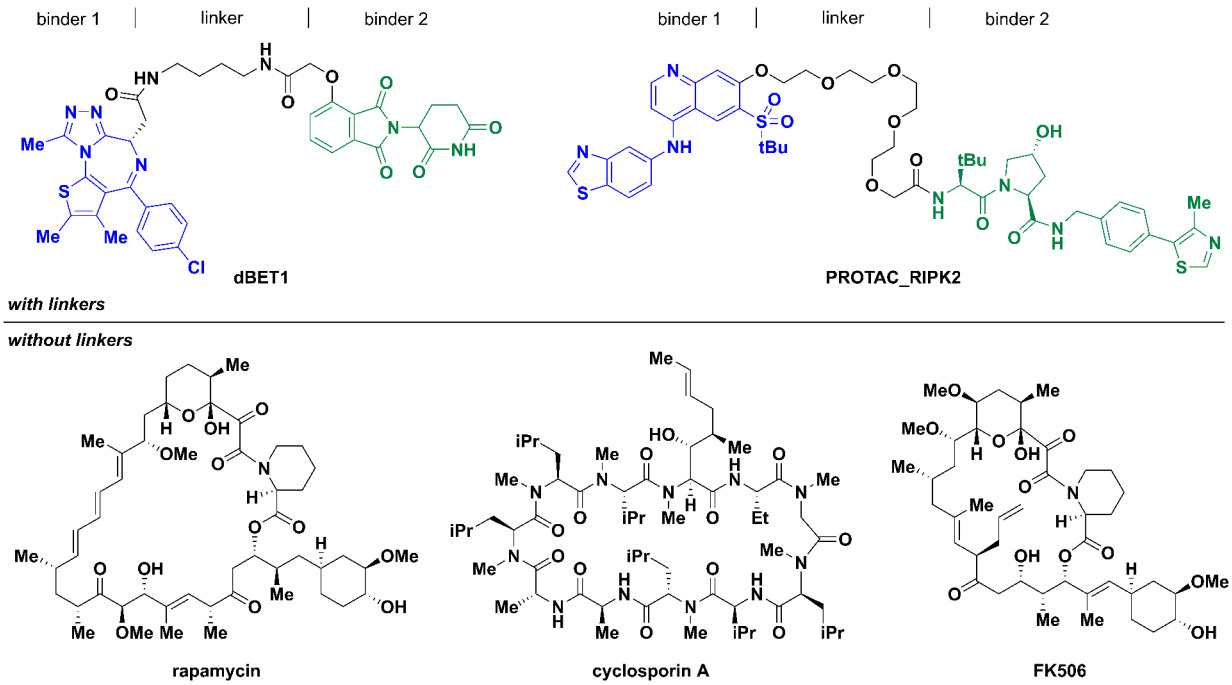


Fig. 1 |. Bifunctional compounds can be grouped into two classes: with and without linkers. Some bifunctional small molecules have dumbbell-like chemical structures that telegraph their bifunctionality (top); the target-binding motifs are highlighted in blue, and the ubiquitin-ligase-binding motifs are highlighted in green. Others have chemical structures that are less suggestive (bottom). We refer to these sets as having or lacking linkers, respectively.

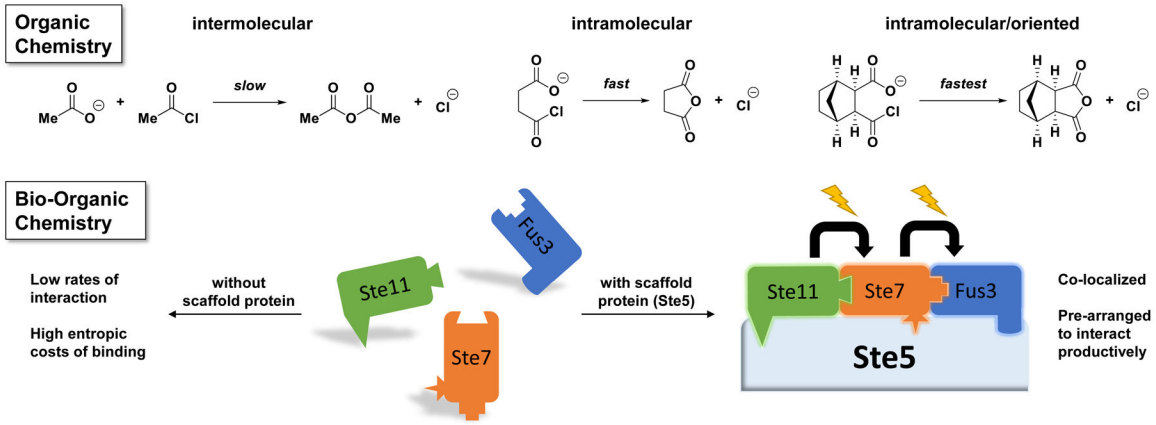


Fig. 2 |. Scaffold proteins leverage the physical organic chemistry principle of effective molarity to accelerate enzymatic reactions.

The co-localization and strategic orientation of reaction partners—functional groups (top) or enzymes (bottom)—can raise reaction rates substantially.

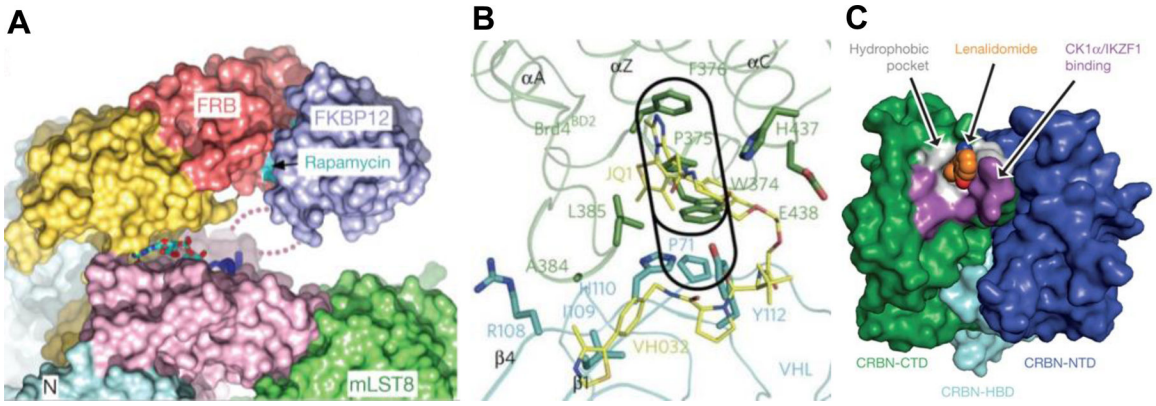


Fig. 3 |. X-ray crystallography data reveal common mechanistic principles of molecular glues. **A,** Rapamycin (cyan) inhibits the kinase activity of mTOR by promoting an interaction between FKBP12 (purple) and FRB (red) that occludes the mTOR catalytic site; reproduced and adapted from REF. 25. **B,** The ternary complex of MZ1 (yellow), VHL (cyan), and BRD4 (green), is characterized by an intricate web of intermolecular interactions that are required to maintain proximity; reproduced and adapted from REF. 26. **C,** The binding of lenalidomide (orange) to CRBN generates a surface (purple) that can engage neo-targets without disrupting ubiquitin ligase activity; reproduced and adapted from REF. 28.

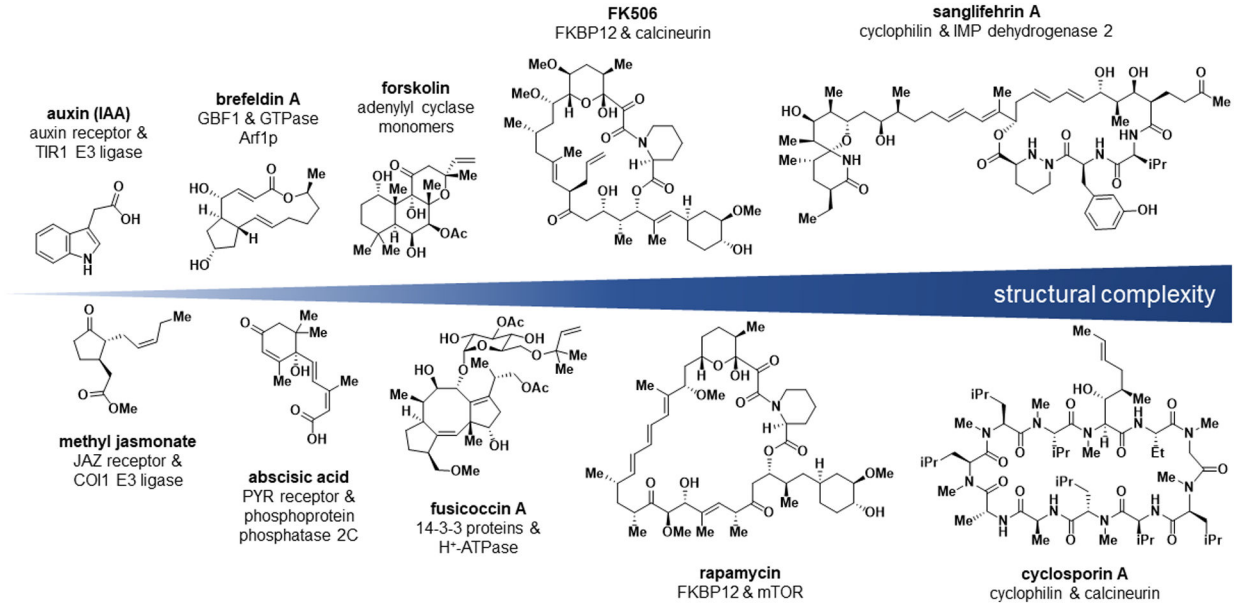


Fig. 4 |. Bifunctional natural products exhibit a wide range of structural complexity.

Ten bifunctional natural products are arranged in order of increasing structural complexity, and their protein targets are listed below the bolded compound names. This subset highlights the structural diversity contained within the ensemble of bifunctional natural products.

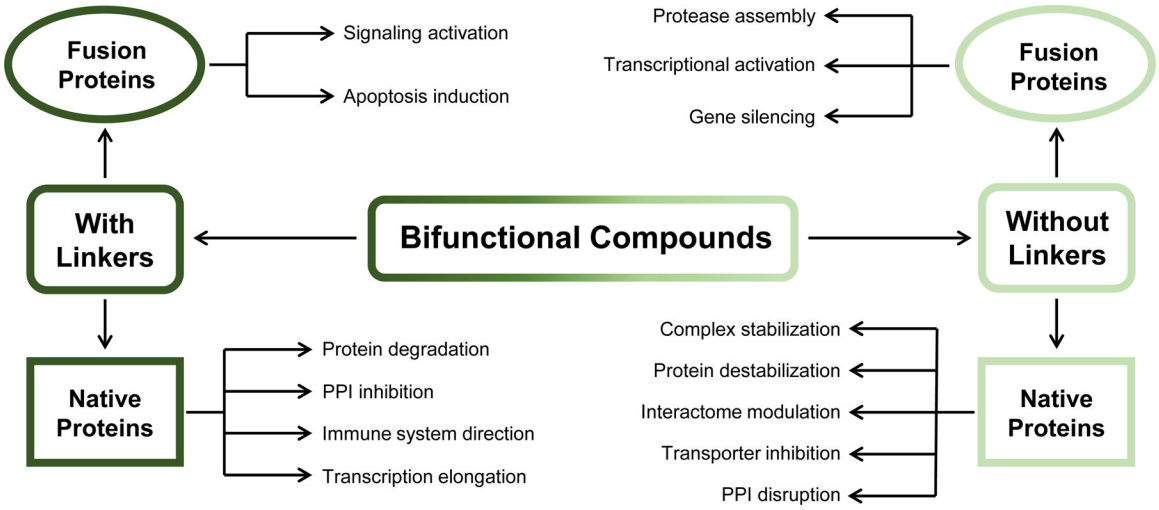


Fig. 5 |. Bifunctional compounds exhibit a variety of biological activities.

Though not an exhaustive list, the biological activities described in this manuscript highlight the breadth of biology that can be modulated and studied with bifunctional small molecules.

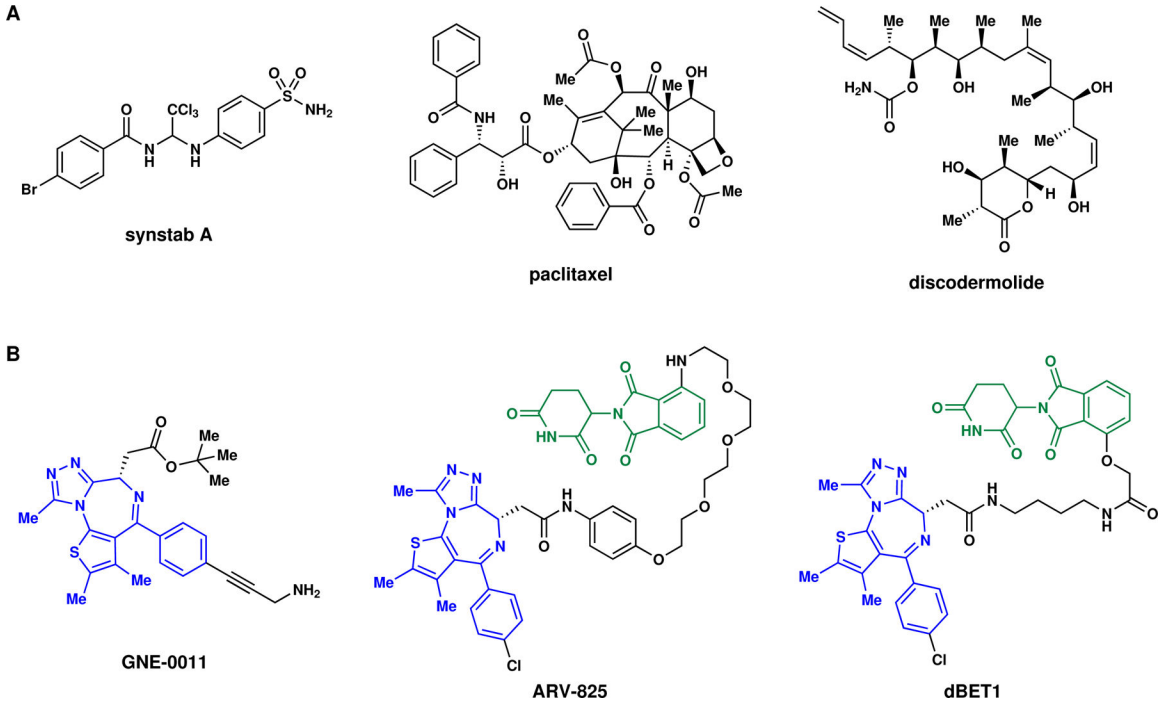


Fig. 6 |. Triads of bifunctional molecules with differing sets of structural features but shared biological activities.

A, Microtubule stabilizers. **B,** BRD4 degraders. The BRD4-binding motif is highlighted in blue, and the CRBN-binding motif is highlighted in green.