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Bifunctional chemical probes inducing protein–protein interactions

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Inducing biomolecular interactions with synthetic molecules to impact biological function is a concept of enormous appeal. Recent years have seen a resurgence of interest in designing bispecific molecules that serve as bridging agents to bring proteins together. Pioneering structural and biophysical investigation of ternary complexes formed by mono-functional and bifunctional ligands highlights that proximity-induced stabilization or *de novo* formation of protein–protein interactions is a common feature of their molecular recognition. In this review, we illustrate these concepts and advances with representative case studies, and highlight progress over the past three years, with particular focus on recruitment to E3 ubiquitin ligases by 'molecular glues' and chimeric dimerizers (PROTACs) for targeted protein degradation. This approach promises to significantly expand the range of tractable targets for chemical biology and therapeutic intervention.

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Introduction

Protein–protein interactions (PPIs) mediate most intracellular processes, so it is not surprising that modulation of PPIs using small molecules is one of the 'holy grails' of pharmacology and chemical biology [1]. Despite the often large and flat interfaces of protein complexes, recent years have witnessed progress in developing small-molecule probes and drugs that disrupt PPIs with high binding affinity, selectivity, and suitable pharmacokinetic properties, reviewed elsewhere [2,3•]. An opposite strategy has fascinated scientists for decades: stabilizing or forming *de novo* PPIs with interfacial molecules, also called 'molecular glues' [4,5]. By bringing together two proteins that would not normally interact, control can be gained in principle over protein fate, localization and function, impacting cellular signalling [6]. Bifunctional chimeric molecules composed of two binding units, often referred to as chemical inducers of dimerization (CIDs) or chemical inducers of proximity (CIPs), enable recruitment of two targets simultaneously [7,8••]. Dimerization can be for molecules of the same protein (homo-dimerizer) or different proteins (hetero-dimerizer). Formation of the desired ternary complex is more productive when the dimerizer binds one protein more tightly in the presence of the other protein rather than its absence — a thermodynamic characteristics of ternary equilibria known as cooperativity [9,10,11•,12•].

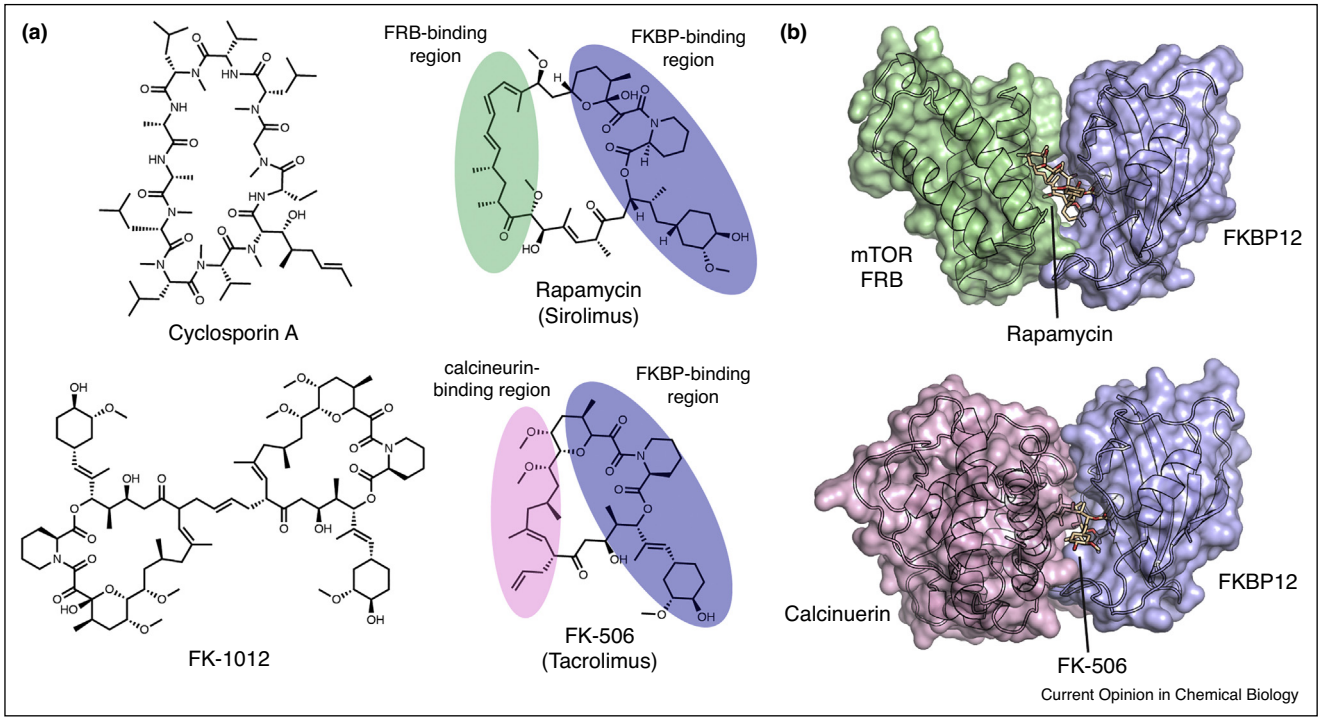
Chemical inducers of dimerization

Molecules that function as CIDs are found in Nature. Amongst natural CIDs are complex natural products that have macrocyclic structures, such as rapamycin, cyclosporine A, and the immunosuppressive drug FK506 (tacrolimus) (Figure 1a). These molecules exert their immunosuppressant activity by recruiting immunophilins, such as the FK506 binding protein FKBP12, to form a ternary complex with a second target protein. In the case of rapamycin and cyclosporine/FK506 the recruited proteins are mTOR and calcineurin, respectively. As a result of the induced dimerization, the activity of the target is inhibited [13]. Crystal structures of ternary complexes FKBP12:rapamycin:mTOR (FRB domain) [14], and FKBP12:FK506:calcineurin [15] elucidated the structural basis for the molecular recognition (Figure 1b). Biophysical investigation of the thermodynamics of the ternary equilibria revealed that rapamycin binds to mTOR with 2000-fold enhanced binding affinity when pre-bound to FKBP12, hence exhibits high cooperativity [16]. These discoveries were taken a step further by developing synthetic bifunctional ligands that can be used to control the intracellular oligomerization of specific proteins. Early work on a dimer of FK506, called FK1012 (Figure 1a), artificially promoted FKBP12 homodimerization [17]. More recently, Zhang and Shokat developed bifunctional molecules that recruit the cancer target Ras in a tripartite complex with FKBP12 and cyclophilin A as a strategy to interfere with Ras activity and block its downstream signaling pathway [18].

Monovalent PPI stabilizers

Nature also exploits endogenous ligands and cofactors to stabilize native interactions with physiological consequences. Serendipitous discoveries have unveiled surprisingly simple

Figure 1

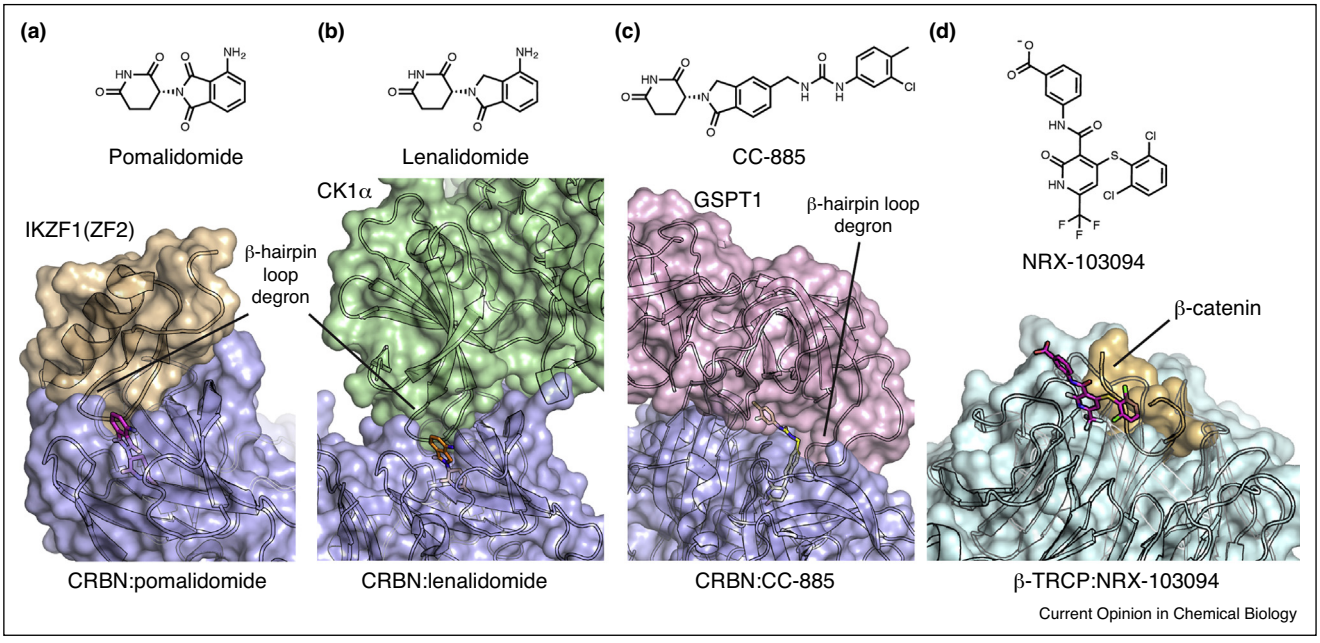


Chemical Inducers of Dimerization (CIDs). **(a)** Chemical structures of natural and synthetic CIDs. **(b)** Crystal structures of cooperative ternary complexes formed by rapamycin (top, PDB code 1FAP [14]) and FK-506 (bottom, PDB code 1TCO [15]).

natural compounds, conceptually monovalent, that form *de novo* protein–protein complexes. Plant hormones auxin and jasmonate bind to specific E3 Cullin RING ligases (CRLs) and promote recruitment of neo-substrates via a ligand-dependent degron mechanism [19,20]. The PPI-stabilizing feature of small molecules is however not limited to natural ones: it is critical to the pharmacological activity of synthetic molecules too. For example, the immunomodulatory drugs (ImIDs) thalidomide and its analogues pomalidomide, lenalidomide and CC-885 (Figure 2a–c) exert their anti-cancer and immunosuppressant activity by binding to cereblon (CRBN), the substrate-recognition subunit of the $CRL4A^{CRBN}$ E3 ligase complex [21,22,23•]. ImIDs act as CRBN modulators to aid recruitment of 'neo-substrate' proteins including the transcription factors Ikaros (IKZF1) and Aiolos (IKZF3), CK1α, and GSPT1. These neo-substrates form tight complexes with varying specificies for different CRBN:ligand complexes, ultimately leading to neo-substrate degradation [21,22,24,25]. More recently, neo-substrate SALL4 has been identified as the target responsible for the infamous teratogenicity of thalidomide [26,27]. Pioneering structural studies by the groups of Thoma and Chamberlain have elucidated the structural basis of the ImID-induced recruitment of neo-substrates to CRBN [28,29]. The ImID-dependent degron was revealed to be a tight hairpin loop present in many zinc finger proteins, that has exquisite structurally conserved features despite low-sequence conservation across the neo-substrates [30••] (Figure 2a–c).

Plant hormones and ImiDs are notable examples of small molecules that bind to an E3 ligase and 'hijack' its activity toward a new protein. However, a small molecule may also increase ligase:substrate binding affinity that may have been weakened, for example as a result of mutations, by stabilizing PPIs between the E3 and its natural substrate. If these mutations are involved in disease, rescuing weakened interactions with such 'molecular glues' could open up new therapeutic opportunities. This strategy was recently applied by a team at Nurix Inc. to stabilize the interaction between the E3 ligase SCF^{β}-TRCP and its natural substrate β-catenin [31••]. This PPI is weakened as a result of cancer-driving mutations on the phospho-degron of β-catenin, that prevent phosphorylation events crucial to formation of the native PPI. Crystal structure of β-TRCP in complex with NRX-103094 and a β-catenin-degron mutant peptide revealed the compound bound snugly at the protein–peptide interface, with its trifluoromethylpyridone group occupying a pocket revealed by the β-catenin mutation and forming tight hydrophobic interactions with both proteins to stabilize the ternary complex [31••] (Figure 2d).

Figure 2



Molecular glues of E3 ubiquitin ligases. **(a–c)** Co-crystal structures of ternary complexes CRBN:pomalidomide:Ikaros (a), PDB code 6H0F [30••]), CRBN:lenalidomide:CK1α (b), PDB code 5FQD [28]), and CRBN:CC-885:GSPT1 (c), PDB code 5HXB [29]) illustrate the range of binding modes for neo-substrate recruitment accommodated by the different ImID compounds. The structurally conserved β-hairpin loop that function as ligand-dependent neo-substrate degron is highlighted. **(d)** Co-crystal structure of ternary complex β-TRCP:NRX-103094:β-catenin (PDB code 6M91 [31••]).

Other notable examples of PPI stabilizers, beyond E3 ligases, are small molecules that strengthen the interaction between 14-3-3 proteins and their cognate substrate partners, reviewed elsewhere [5,32]. In a recent study, Sijbesma *et al.* applied cysteine-targeting site-directed fragment screening to discover orthosteric stabilizers of the 14-3-3:ERα interaction [33].

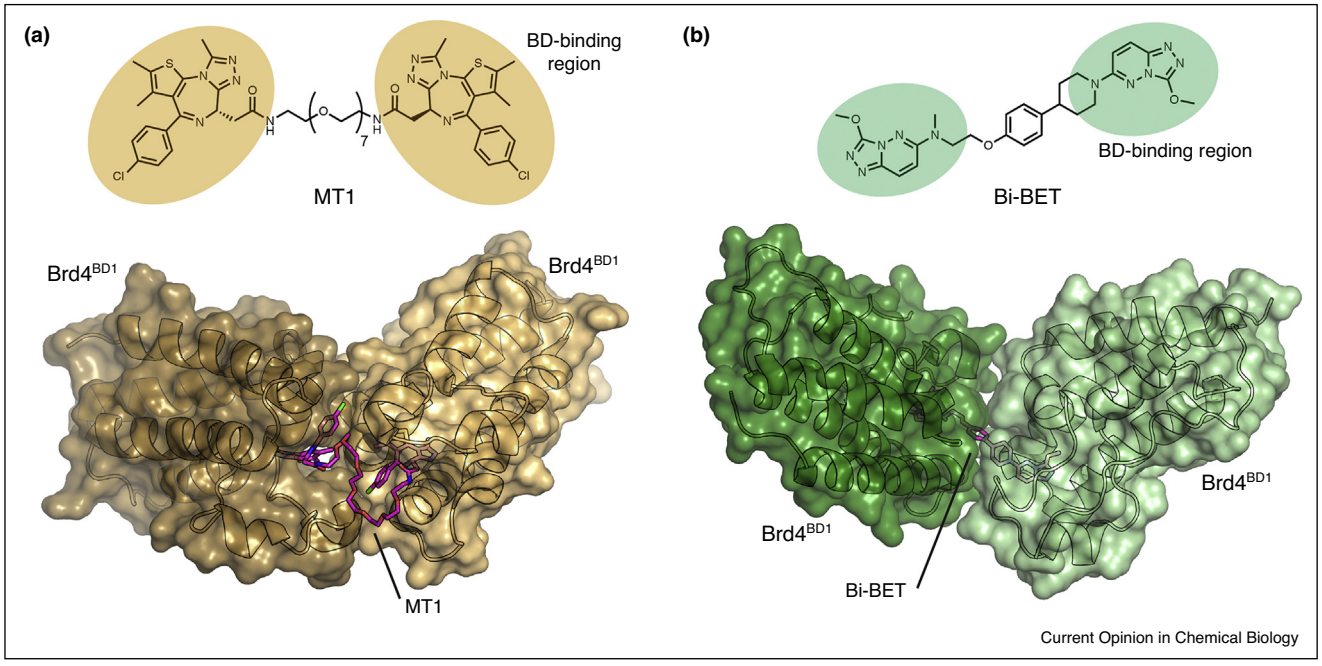
While prominent examples, the compounds described so far either bind preferentially to only one of the two proteins in the complex, or bind weakly to the individual components, and show measurable binding affinity only as part of a fully formed complex. This means that even when a first protein–ligand pair is identified, there is little control on which second target can be recruited. The target scope can be expanded conceptually by designing bifunctional molecules.

Bivalent inhibitors

Bivalent inhibitors can engage two molecules of target protein simultaneously, thereby potentially aiding pharmacological potency due to an avidity effect. Illendula *et al.* developed compound AI-10-49, a bivalent inhibitor of the PPI between transcription factors CBFβ-SMMHC (fusion of core binding factor b and smooth-muscle myosin heavy chain) and RUNX1, which sustains acute myeloid leukemia (AML) growth. AI-10-49 was significantly more potent that the parent monovalent compound, displayed favorable pharmacokinetics, and delayed leukemia progression in mice [34].

Two groups at Dana-Farber [35••] and AstraZeneca [36••] reported bivalent inhibitors of the bromo and extraterminal domain (BET) proteins Brd2, Brd3, and Brd4 (Figure 3). Most BET inhibitors such as the archetypical JQ1 and IBET-762, bind to BET proteins monovalently by targeting their bromodomain. A bivalent approach to small-molecule inhibition stood as an attractive opportunity for this target class, because all BET proteins contain two distinct bromodomains at their N-terminal region, termed BD1 and BD2 [37,38]. Both MT1 [35••] and biBETs [36••,39] were found to bind intramolecularly two domains of a single BET protein. Co-crystal structures and allied biophysical studies evidenced the inhibitors bridging across two molecules of bromodomains and inducing extensive intermolecular PPIs [35••,36••] (Figure 3). In MT1, two molecules of JQ1 are joined together using a linker length of seven ethylene glycol units (PEG-7 linker) (Figure 3a). MT1 was found to be 100-fold more potent than the corresponding monovalent inhibitor JQ1 at blocking Brd4 from binding to chromatin in cells [35••]. BiBET, initially developed to downregulate androgen receptor signalling, was revealed to engage simultaneously two bromodomains of a BET protein [36••] (Figure 3b). Cells treated with these bivalent compounds showed inhibition of cell

Figure 3



Bivalent BET inhibitors. Crystal structures of avid ternary complexes formed by MT1 **(a)**, PDB code 5JWM [35••]) and Bi-BET **(b)**, PDB code 5AD3 [36••]) with two protomers of the first bromodomain of Brd4 ($Brd4^{BD1}$).

growth in a manner consistent with sensitivity to BET inhibition, and with a remarkable enhancement in potency, consistent with strong avidity effects [35••,36••].

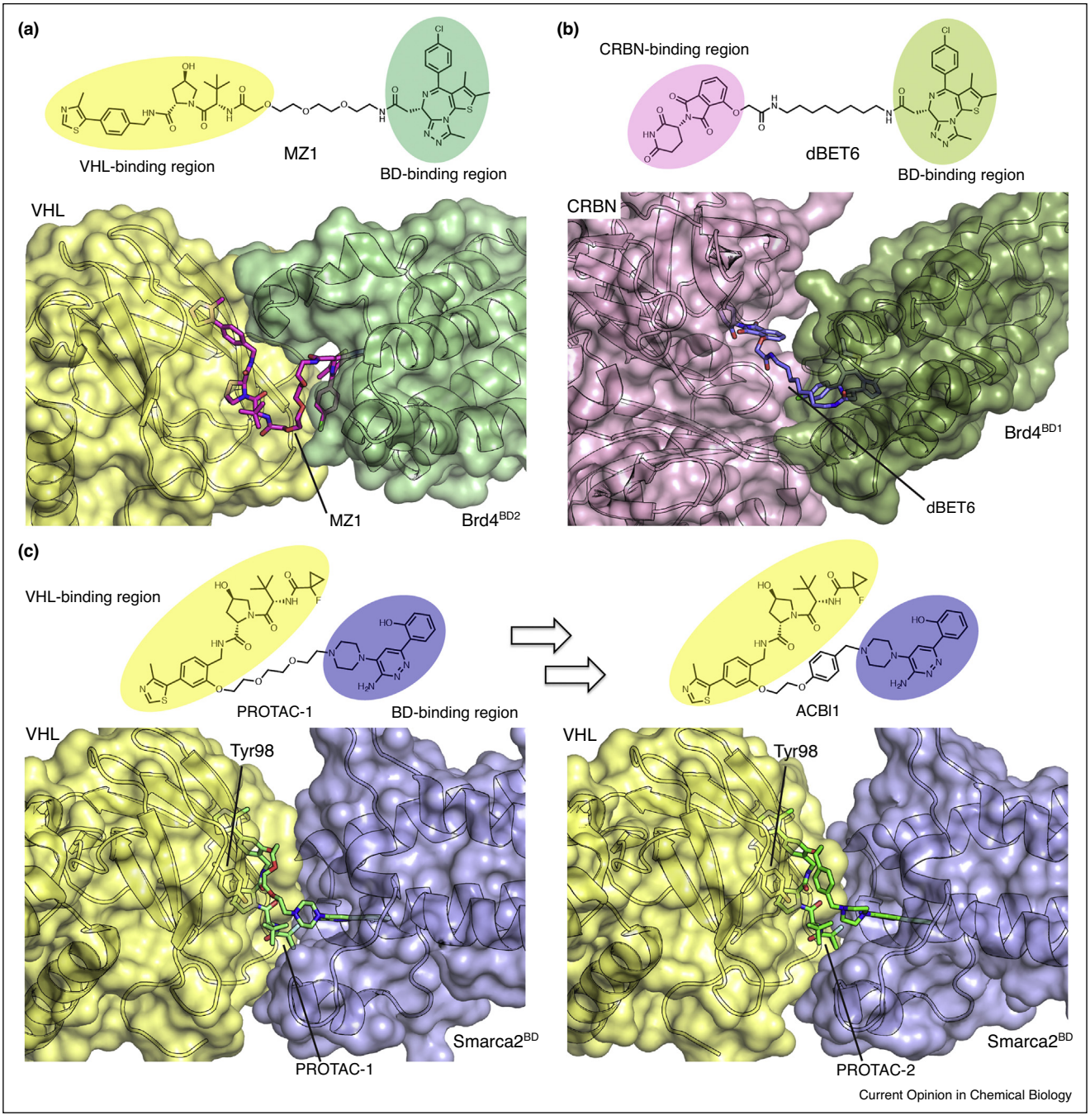
Bifunctional degraders: proteolysis-targeting chimeras (PROTACs)

Conventional genetic techniques to knockdown or knockout the expression level of proteins have been based on nucleic acids, for example antisense oligonucleotides, RNAi and siRNA, and more recently CRISPR–Cas9 [40]. A small-molecule approach to induce intracellular protein degradation combines the desired output of knockdown techniques with favorable pharmacological properties of small molecules as well as acute, fast, selective and reversible effects. Such 'chemical degraders' recruit target proteins to an E3 ubiquitin ligase, thereby inducing selective target ubiquitination and degradation [41,42]. One prominent class of bifunctional chemical degraders are known as proteolysis-targeting chimeras (PROTACs) [43•]. PROTACs contain two ligands, one for a target protein of interest and one for an E3 ligase, connected via a linker. The historical backdrop and latest developments of PROTACs have been extensively reviewed elsewhere [43•,44–46]. Here we briefly outline prominent and well-characterized PROTAC molecules described in 2015 [47] and review recent structural work that have evidenced how PROTACs induce protein–protein interactions within their ternary complexes.

The development of non-peptidic PROTACs with high cellular efficacies and specificities was ushered by the discovery of two low-molecular weight, specific, high-affinity E3 ligands, the VHL ligand VH032 [48–50] and the cereblon ligands (described above). These new E3 ligands enabled the assembly of potent and selective PROTACs against targeted proteins of interest [51••,52••,53••,54••]. The first examples of PROTACs recruiting $CRL2^{VHL}$ to induce degradation of a target were reported in mid-2015 by our laboratory [51••] and the Crews/GSK laboratories [52••]. Both studies used a VHL recruiting moiety of relatively small size (MW = 472 Da) and high binding affinity (K_d = 180 nM) for a compound targeting a PPI site [49]. Zengerle *et al.* linked the pan-selective BET inhibitor JQ1 to the VHL ligand via solvent-exposed regions using an optimized PEG-3 linker to afford MZ1 [51••] (Figure 4a). MZ1 qualified as a potent, fast and well-characterized BET PROTAC exerting preferential degradation of Brd4 [51••,55••,56•]. The same VHL ligand was linked to inhibitors for the serine/threonine kinase RIPK2 via a PEG-4 linker to yield PROTAC_RIPK2 [52••]. VHL-based PROTACs have since been deployed successfully against a wide array of target proteins [44,45,57].

The first example of PROTACs recruiting $CRL4^{CRBN}$ were reported around the same time as the VHL recruiting PROTACs by the Bradner laboratory (dBET1 [53••]) and Arvinas (ARV-825 [54••]). Winter *et al.* coupled JQ1 to pomalidomide to obtain dBET1 inducing potent

Figure 4



Bifunctional degraders (PROTACs). **(a)** Crystal structure of the cooperative ternary complex formed by MZ1 with VHL and the second bromodomain of Brd4 ($Brd4^{BD2}$) (PDB code 5T35 [55••]). **(b)** Crystal structure of non-cooperative ternary complex CRBN:dBET6:$Brd4^{BD1}$ (PDB code 6BOY [67•]). **(c)** Crystal structures of the cooperative ternary complexes formed by PROTAC-1 (left) and PROTAC-2 (right) with VHL and the bromodomain of Smarca2 ($Smarca2^{BD}$) (PDB codes 6HAY and 6HAX, respectively [68••]), which guided the design of potent PROTAC degrader ACBI1 via optimization of the linker conformation and introduction and stabilization of a π–π stack with VHL residue Tyr98. Note: ACBI-1 and PROTAC-2 differ by a single oxygen atom in the linker.

degradation of BET proteins [53••]. The compounds were later optimized to more potent BET degrader dBET6 [58] (Figure 4b). In a similar fashion, Lu *et al.* developed ARV825 by coupling BET inhibitor OTX015 (a close analogue of JQ1) to pomalidomide via a short alkyl linker [54••]. Both VHL-based and CRBN-based BET degraders potently and rapidly induce selective, reversible, and long-lasting degradation of BET proteins

in a proteasome-dependent and E3 ligase-dependent fashion in multiple cancer cell lines and are active in *in vivo* mouse models [58–61].

VHL and CRBN ligands have also been conjugated to elements recruiting specific tags that can be fused to proteins of interest, leading to degradation of the tagged protein. Buckley *et al.* developed conjugates of VHL ligands to chloro-alkane moieties (HaloPROTACs) and studied their ability to induce the degradation of transiently expressed proteins fused to HaloTag7 [62•]. More recently, Tovell *et al.* qualified HaloPROTAC-E, a conjugate of potent VHL inhibitor VH298 [50], as their most potent degrader of representative endosomal proteins endogenously Halo-tagged using CRISPR–Cas9 [63•]. They showed that HaloPROTAC-E induced rapid (50% protein depletion in 30 min) and profound depletion (>95%) of target protein, retained after 48 hours [63•]. In a similar fashion, Nabet *et al.* developed the CRBN-based system dTAG, by conjugating a CRBN ligand to a bumped ligand for a mutant $FKBP12^{F36V}$ variant [64•]. Unlike RNAi knockdowns, these inducible-degron technologies are highly specific at post-translational level, can be developed to have little to no off-target effects, and allow for the rapid and reversible knockdown of any gene of interest modified with suitable fusion tags [65].

Structural and biophysical insights on PROTACs ternary complexes

An important step forward in the PROTAC field came in 2017 with the first description of a crystal structure revealing how a PROTAC binds in a ternary complex [55••]. Using X-ray crystallography, Gadd *et al.* showed that MZ1, when anchoring the ternary complex VHL:MZ1:Brd4, induces the formation of new interactions between VHL and the Brd4 bromodomain (Figure 4a). These two proteins do not interact with any measurable binding affinity in the absence of the compound. The structure revealed that MZ1 is 'sandwiched' between the two proteins, inducing extensive *de novo* protein–protein and protein–ligand contacts of both hydrophobic and electrostatic nature, which together bury >2600 $Å^2$ of protein surface area, of which ~700 $Å^2$ contributed by the induced PPI [55••]. Allied biophysics and mutagenesis swap experiments revealed that the PPI gained in the process contribute extra stabilization energy to the complex (>2 kcal/mol), beyond the sum of the binary binding energies, corresponding to >20-fold increase in binding affinity. This cooperativity greatly increases the potency of the PROTAC, enhancing the stability and population of the ternary complex, driving the preferential degradation of Brd4 [55••]. Guided by the crystal structure, degrader AT1 with enhanced selectivity for Brd4 was designed via a novel conjugation vector out of the VHL ligand [55••]. More recently, Roy *et al.* extended biophysical investigation of PROTAC ternary complexes to studying the kinetics of their formation and dissociation using surface plasmon resonance (SPR). By immobilizing biotinylated E3 ligase on a chip and flowing over PROTAC in the absence or presence of target protein, they were able to quantify the overall kinetics of the ternary complexes formed and show that the comparatively more stable and long-lived ternary complex formed by MZ1 with VHL and Brd4-BD2 drive faster rates of protein degradation in cell [66••].

Unlike in the case of monovalent molecular glues, formation of positively cooperative ternary complexes is not a strict requirement for productive targeted protein degradation. Already in 2017, Chan *et al.* reported another series of VHL-based BET degraders that were designed using IBET-726, a more potent BET inhibitor than JQ1, via a different conjugation vector than JQ1 [69]. This strategy led to negatively cooperative degraders, that form ternary complexes that dissociate comparatively much more rapidly than MZ1 [66••]. In spite of these unfavorable thermodynamics and kinetics features of ternary complex formation, Chan *et al.* showed that PROTACs based on IBET-726 were nevertheless active as BET degraders [69]. However, interestingly, they exhibited different BET isoform degradation profiles, and were found to behave more as inhibitors than degraders at higher concentrations, consistent with their greater binary binding affinities, negative cooperativities and more pronounced hook effects [69]. Similarly, active PROTAC degraders for the protein kinase BTK were developed that were also shown to exhibit negative cooperativity [70]. In 2018, Nowak *et al.* described the co-crystal structure of CRBN-based BET degrader dBET6 in complex with CRBN and Brd4-BD1 [67•] (Figure 4b). In contrast to the structure by Gadd *et al.*, in the ternary complex structure of CRBN:dBET6:Brd4-BD1 no obvious additional hydrogen bonds were observed from the PROTAC, and a lesser buried surface area was observed [71,72]. The structural features were consistent with negative cooperativity for dBET6 and related analogues observed in biophysical assays [67•]. Despite the negative cooperativity of these systems, favorable PPI were induced by the PROTAC. This suggests other unfavorable energetic contributions, likely strain of the PROTAC linker conformation forced in the ternary complex, must compensate and overcome these PPIs to result in an overall negative cooperativity. More recently, a team at the University and Dundee and Boehringer Ingelheim developed potent degraders for the BAF complex ATPase subunits SMARCA2 and SMARCA4 using structure-based PROTAC design [68••]. Farnaby *et al.* used a high-resolution crystal structure of a weak and partial, yet cooperative, PROTAC bound to VHL and the SMARCA2 bromodomain to guide further stabilization of the complex, achieving PROTAC AC-BI1 which potently impacted cancer cells rendered vulnerable to SMARCA2 knockdown [68••].

Together, these pioneering structural and biophysical studies highlight the importance of the ternary complex and its relevance as key intermediate species in the PROTAC mechanism of action [55••,66••,67•,68••]. They also underscore the advantage of the catalytic, substoichiometric mode of action of PROTAC degraders, that can achieve efficient target ubiquitination and proteasomal degradation even in cases where suboptimal ternary complex formation equilibria are involved [56•,67•,69,70,73]. Increasing evidence suggests that improving ternary complex formation parameters is an important strategy for optimizing the cellular activity and desired properties of PROTACs. This can contribute to achieving single-target selectivity beyond binary target engagement, and is likely to be required in cases where ligand binary binding affinities are relatively weak [55••,69,74–77]. Similarly, reliance on favorable ternary complex equilibria is expected to be important in designing occupancy-based bifunctional probes that, unlike PROTACs, must rely on stoichiometric formation of ternary complexes to trigger a cellular response.

Homo-PROTACs: dimerizers of E3 ubiquitin ligases

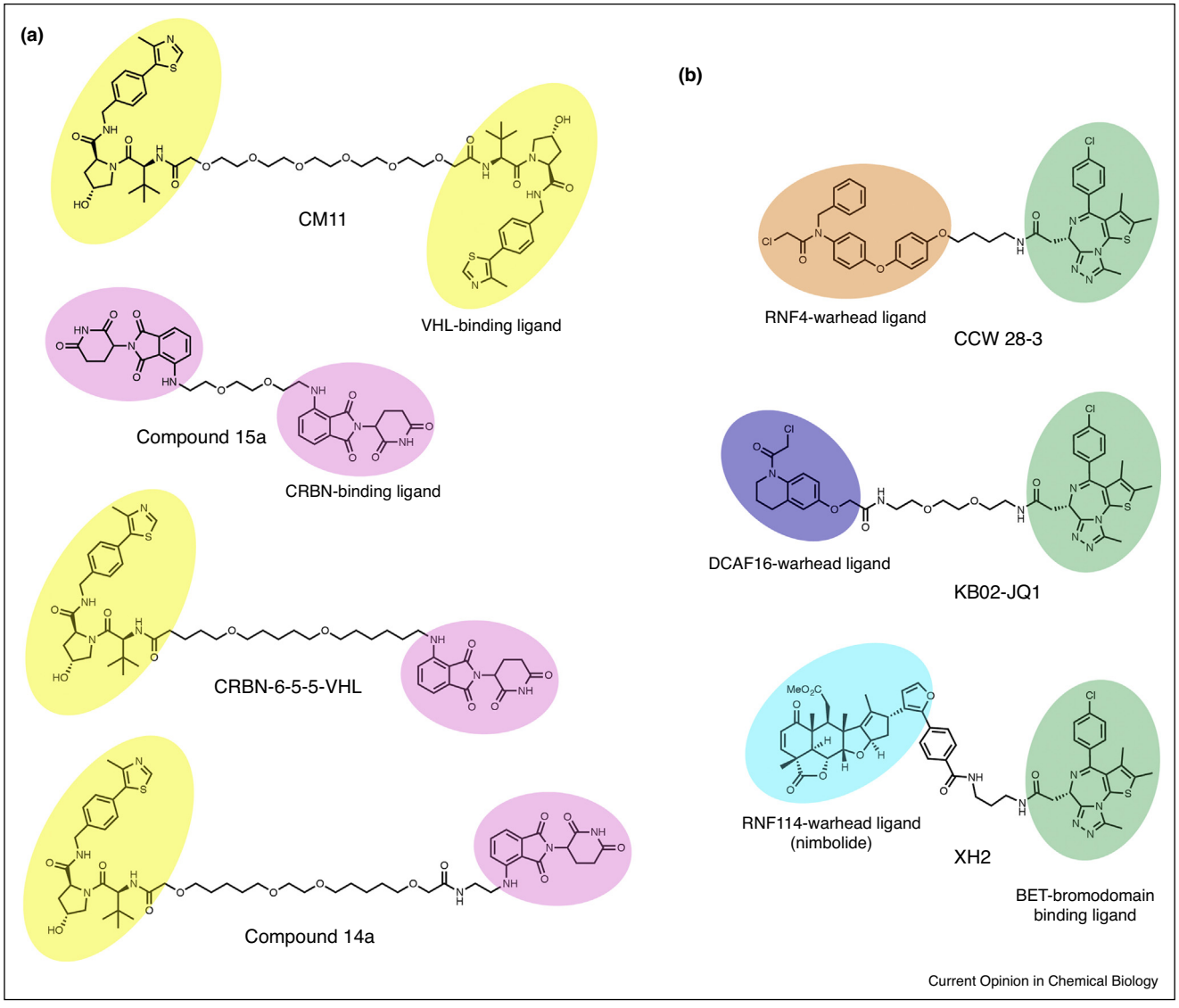
Merging the concepts of CIDs and PROTACs, in 2017 Maniaci *et al.* first showed that it is possible to dimerize an E3 ubiquitin ligase using double-headed molecules 'homo-PROTACs' as a strategy to induce E3 ligase degradation in cells [78•]. Bifunctional molecules were constructed of the same VHL ligand (VH032 or VH298), connected via a PEG linker in both symmetric and asymmetric fashions, to induce $CRL2^{VHL}$ dimerization. The best degrader identified, symmetric homo-PROTAC CM11 (Figure 5a), exhibited high cooperativity (~20) for VHL dimerization *in vitro*, and induced VHL degradation at concentrations >100-fold lower than the binary K_d, consistent with the substoichiometric mode of action. This work qualified CM11 as a fast, potent PROTAC inducing profound and prolonged degradation of VHL in different cell lines [78•]. Subsequently, the same concept was applied by Steinebach *et al.* to develop homo-PROTACs for the $CRL4^{CRBN}$ ligase, leading to compound 15a as the most active CRBN degrader [79•] (Figure 5a). Interestingly, VHL and CRBN could also be productively hijacked against one another. In two parallel studies, Steinebach *et al.* [80•] and Girardini *et al.* [81•] designed and characterized VHL-CRBN hetero-dimerizers CRBN-6-5-5-VHL and 14a, respectively (Figure 5a). Both PROTACs were found to preferentially induce CRBN degradation in a concentration and time-dependent fashion [80•,81•].

Summary and prospectus

The past few years have witnessed a renaissance in the enticing concept of bringing proteins together with small molecules, and growing efforts to apply it as a strategy to modulate protein's intracellular function, stability and levels. In large part fueled by the meteoric resurgence of PROTACs, designing bifunctional molecules to form ternary complexes is now establishing itself as a field on its own. Fundamental structural and biophysical investigation of recent years have revealed that the molecular recognition process is much more sophisticated than previously assumed [9]. In all systems structurally defined so far, highlighted herein, these chimeric molecules do much more than simply bringing two proteins into proximity with each other: they induce extensive protein–protein contacts of varying degrees of buried surface area. In hindsight, this is perhaps not surprising because once two proteins have been brought into proximity there exists significant inherent potential to aid interactions, as the entropic barrier to complex formation has already been largely overcome. This feature of molecular recognition is therefore likely to be much more fundamental and general than was previously anticipated for this class of molecule. It is also becoming increasingly evident, particularly for PROTACs, how nuances in cooperativity, stability, half-lives and perhaps geometry of ternary complexes can play important roles in defining the concentration-spectrum, target selectivity and time-dependency of compounds' activities in cells and *in vivo*. For example, high cooperativity alleviates the hook effect, and can render compound mode of action less dependent on cellular permeability/uptake. We therefore believe that these emerging principles of ternary complex recognition are ripe for guiding rational structure-based design of bifunctional chemical probe and therapeutics.

What is next for the design of molecular dimerizers? Clearly, bi-functional or multi-functional ligands will inspire creativity for tackling challenging and relevant biological systems that can be bridged via synthetic molecules. Notable are systems where monovalent interactions can be weak on their own, for example, carbohydrate binding elements and cell surface receptors [82], and where multiple domains are available for multivalent recognition of post-translational modifications, such as chromatin binding proteins [83]. Finding compounds that induce cellular PPIs with functional consequences remains a historic challenge. To tackle this challenge, it will be important to develop relevant screening approaches, and expand the chemical space covered by current compound libraries. For PROTACs and molecular glues, expanding to new E3 ubiquitin ligases beyond VHL and CRBN is an obvious next step. Recent efforts are directed towards identifying and characterizing more E3 CRLs that can be chemically tractable for targeted protein degradation. Complementary approaches involve de-orphanizing E3 ligases with new non-covalent ligands and degron peptides [31••,84–86], and modifying covalently nucleophilic residues for example, cysteines on the E3 protein using electrophilic warheads [87••,88••,89••] (Figure 5b). Beyond PROTACs and the targeting to E3 ligases, it will be interesting to watch the range of

Figure 5



Unconventional targeting of E3 ubiquitin ligases with bifunctional molecules. **(a)** Chemical structures of homo-dimerizers (homo-PROTACs) and hetero-dimerizers of the E3 ubiquitin ligases VHL and CRBN. **(b)** Chemical structures of covalent PROTACs recruiting novel E3 ligases RNF4, RNF114, and DCAF16.

combinations being exploited to recruit proteins on-demand for purposefully modulating cellular signaling. Recent approaches include designing conjugates that recruit nuclease enzymes to RNA (Ribotac) [90] and that bring extracellular proteins to cell surface lysosome targeting receptors to induce the degradation of secreted and membrane proteins [91,92]. As medicinal chemistry navigates chemical space beyond the Rule-of-five, and accepts and addresses the associated challenges, developing bifunctional molecules of suitable PK properties is proving more feasible than previously thought. The advances and exciting recent developments outlined in this review together suggest generality and outline fresh opportunities that can be achieved by bringing proteins together as powerful means to control or endow new protein function with small molecules.

Conflict of interest statement

The A.C. laboratory receives or has received sponsored research support from Boehringer Ingelheim, Nurix, Inc. and Ono Pharmaceuticals. A.C. is a scientific founder, director and shareholder of Amphista Therapeutics, a company that is developing targeted protein degradation therapeutic platforms. The remaining author reports no competing interests.

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- •• of outstanding interest

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