



Annual Review of Cancer Biology
Small-Molecule Approaches to
Targeted Protein Degradation

Tyler B. Faust,^{1,2,*} Katherine A. Donovan,^{1,2,*}
Hong Yue,^{1,2} Philip P. Chamberlain,³
and Eric S. Fischer^{1,2}

¹Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, Massachusetts 02215, USA; email: Eric_Fischer@dfci.harvard.edu

²Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, USA

³Neomorph, Inc., San Diego, California 92121, USA; email: phil@neomorph.com

Annu. Rev. Cancer Biol. 2021. 5:9.1–9.21

The *Annual Review of Cancer Biology* is online at
cancerbio.annualreviews.org

<https://doi.org/10.1146/annurev-cancerbio-051420-114114>

Copyright © 2021 by Annual Reviews.
All rights reserved

*These authors contributed equally to this article

Keywords

ubiquitin, molecular glue, protein degradation, PROTAC

Abstract

Many essential biological processes are regulated through proximity, from membrane receptor signaling to transcriptional activity. The ubiquitin-proteasome system controls protein degradation, with ubiquitin ligases as the rate-limiting step. Ubiquitin ligases are commonly controlled at the level of substrate recruitment and, therefore, by proximity. There are natural and synthetic small molecules that also operate through induced proximity. For example, thalidomide is effective in treating multiple myeloma and functions as a molecular glue that stabilizes novel protein-protein interactions between a ubiquitin ligase and proteins not otherwise targeted by the ligase, leading to neo-substrate degradation. Emerging data on new degrader molecules have uncovered diverse mechanisms distinct from molecular glues, which often mirror the regulatory mechanisms that control substrate-ligase proximity in nature. In this review, we summarize our current understanding of biological and synthetic regulation of protein degradation and share our view on how these diverse mechanisms have inspired novel therapeutic directions.



1. INTRODUCTION

Targeted protein degradation (TPD) is a rapidly evolving concept in drug discovery. By using small molecules (commonly referred to as degraders) to redirect the cellular machinery to destroy specific proteins, researchers have the opportunity to discover powerful new therapeutics in multiple disease areas. Importantly, proteins that were considered undruggable to conventional therapeutic strategies can be targeted for degradation. Despite these bold claims, small-molecule protein degraders are far from being distant research goals, as there are several approved anticancer drugs that achieve clinical efficacy through protein degradation.

The fundamental approach in TPD is to discover synthetic low-molecular weight molecules that alter the specificity of the cellular machinery involved in protein degradation: the ubiquitin-proteasome system (UPS). In the UPS, ubiquitin is covalently attached to a target protein, a process referred to as ubiquitination. Ubiquitination is carried out by a three-enzyme cascade consisting of a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin ligase (E3), which act in sequence to attach ubiquitin to a lysine side chain on the substrate protein. While the major role of ubiquitination is to initiate protein degradation, multiple additional functions for this posttranslational modification (PTM) have been uncovered, including the regulation of transcription, localization, and signaling, which have been reviewed elsewhere (Dwane et al. 2017). After a substrate has been modified with a degradative ubiquitin signal, it is shuttled to the 26S proteasome for degradation.

Ubiquitin E3 ligases confer specificity in the UPS. Ubiquitination is a proximity-driven reaction such that recruitment to a ligase through a sequence or structural degron on a target is generally sufficient for modification (Zheng & Shabek 2017). Given the energetically expensive nature of ubiquitin-dependent degradation and protein resynthesis, many regulatory mechanisms have evolved to precisely control access of a substrate to its cognate ligase (**Figure 1**). These mechanisms include PTM, protein quality control, substrate oligomerization, and ligand binding. Considering the important role of regulating protein expression, it is not surprising that many

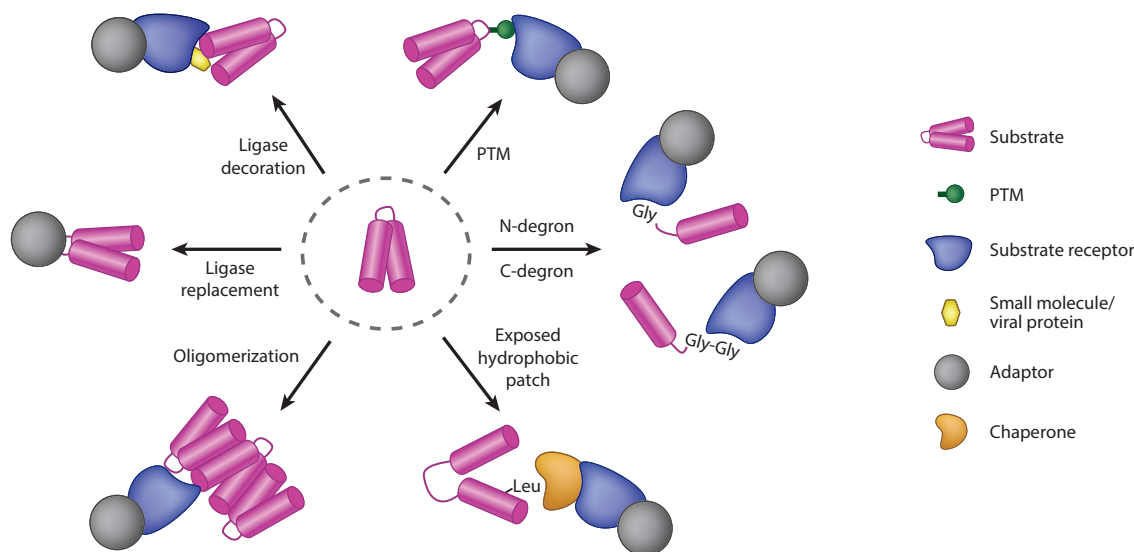


Figure 1

Regulated recruitment of a substrate to a ubiquitin ligase: schematic demonstrating the different ways in which substrate proteins are recruited to E3 ubiquitin ligases for ubiquitination. Abbreviation: PTM, posttranslational modification.

pathogens encode accessory proteins to hijack the UPS as part of their replication cycle. Altogether, the UPS is a vast signaling network to precisely tune cellular proteostasis.

The UPS offers tremendous and largely untapped opportunities for therapeutic intervention. There are already molecules in the clinic that target the UPS, including proteasome inhibitors and lenalidomide, both of which are effective treatments in hematologic malignancies (Manasanch & Orłowski 2017, Palumbo et al. 2012). Lenalidomide binds to the substrate receptor cereblon (CRBN) of the CRL4^{CRBN} E3 ubiquitin ligase and scaffolds a protein-protein interaction (PPI) to recruit multiple neo-substrates for ubiquitination and subsequent degradation (Chamberlain et al. 2014, Donovan et al. 2018, Fischer et al. 2014, Ito et al. 2010, Krönke et al. 2014, Lu et al. 2014). The ability to alter the specificity of E3 ubiquitin ligases with small molecules and redirect them to proteins linked to disease is an exciting area for therapeutic expansion. It is becoming increasingly clear that small-molecule ligands that lead to protein degradation are more prevalent than initially anticipated and that the mechanisms by which degradation can be achieved are diverse. While overwhelming at first, when classified alongside our understanding of how ligases are controlled by nature, it becomes clear that the diversity of mechanisms for small-molecule degraders is reflective of that observed in nature. In this review, we contrast new synthetic modalities of TPD with biological UPS regulatory mechanisms in health and disease, and we consider their future therapeutic potential in the context of past and current clinical development.

2. REGULATED RECRUITMENT OF SUBSTRATE TO LIGASE

The past decades of research have uncovered numerous mechanisms by which substrate-ligase interactions can be regulated. Here we broadly classify and summarize well-characterized substrate recruitment mechanisms. For each class, we describe biological examples of regulation and then compare these to synthetic examples where small molecules perturb these systems to achieve substrate degradation.

2.1. Substrate Posttranslational Modifications

PTMs are the covalent attachment of functional groups to proteins to broaden their functional diversity. Strong regulatory mechanisms exist to prevent unnecessary protein degradation. PTMs fill this role by acting as a trigger to facilitate recognition by ligase substrate receptors and thereby initiate subsequent degradation.

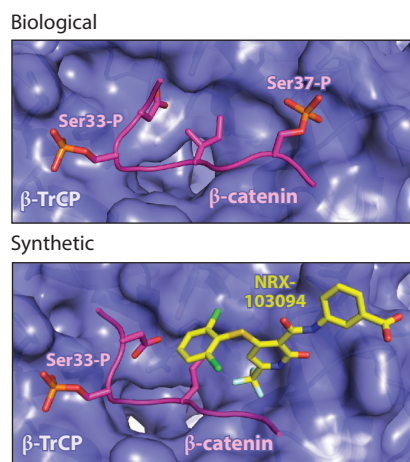
2.1.1. Biological. While certain degrons are recognized by their primary amino acid sequence, a common regulatory mechanism is for ligases to bind a PTM protein that often results from an upstream signaling input. One of the most well-studied examples of PTM-degrons are the phosphodegrons, where phosphorylation of key serine, threonine, or tyrosine residues is required for degron recognition. The cullin ligase SCF (Skp, cullin, F-box), composed of CUL1, Skp1, and Rbx1, contains numerous substrate receptors, termed F-box proteins, that specifically recognize phosphodegrons. These F-box proteins include Skp2, which recognizes p27^{Kip1} phosphorylated at Thr187 (Hao et al. 2005); β -TrCP, which binds doubly phosphorylated β -catenin (Wu et al. 2003); and Fbxw7, which binds doubly phosphorylated cyclin E (Hao et al. 2007). The degron motifs from the above examples are short amino acid sequences; for example, in β -catenin, the motif is DpSG Φ XpS, where Φ is a hydrophobic residue and X is any amino acid. Typically, upon binding to the E3 ligase, the phosphorylated residues on the substrate protein are buried in the F-box receptors and make extensive hydrogen bonds and charged interactions with the ligase (Figure 2a). The range of affinities among ligase-phosphodegron pairs is surprisingly large, with



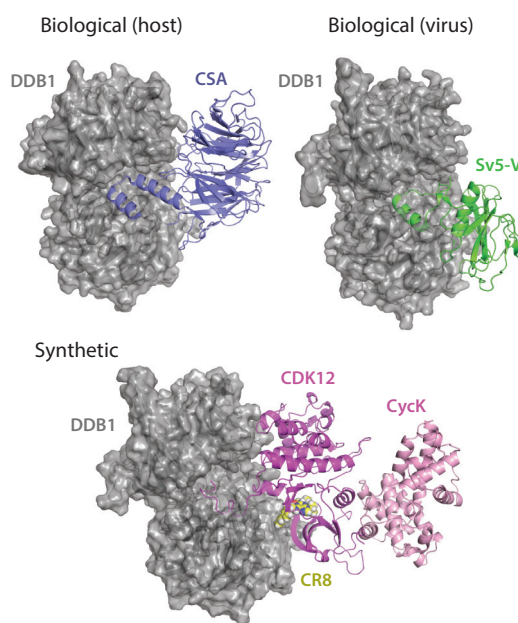
7 μM for Skp2-Cks1-p27^{Kip1}, 0.5 μM for β -TrCP- β -catenin, and 70 nM for Fbxw7-cyclin E, demonstrating that high-affinity interactions are not necessary to drive ligase-substrate binding.

As a further example, prolyl hydroxylation generates a PTM-degron that is sensed by the von Hippel-Lindau (VHL) substrate receptor of the CUL2 ligase. Hypoxia-inducible factor 1 (HIF-1) is a transcription complex that regulates the cellular response to oxygen availability. At low oxygen levels, HIF-1 activates a gene expression program to respond to hypoxia. Under normoxic conditions, Pro402 or Pro564 of the HIF-1 α subunit are hydroxylated and bound by VHL, leading to ubiquitination and degradation (Huang et al. 1998, Kaelin 2018, Pugh et al. 1997). Shape

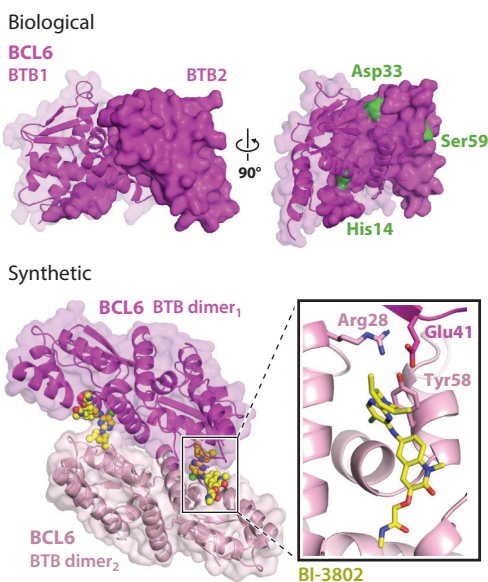
a PTM



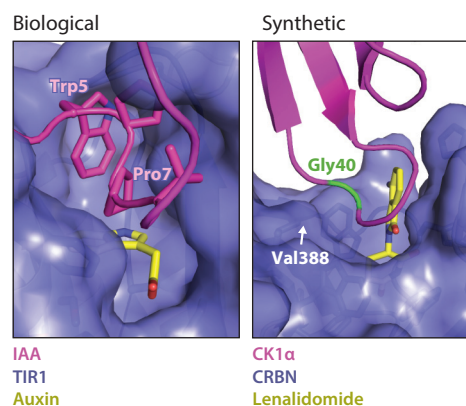
c Ligase replacement



b Oligomerization



d Molecular glues



(Caption appears on following page)

Figure 2 (Figure appears on preceding page)

Synthetic molecules repurpose biological regulatory mechanisms: paired examples of biological substrate-ligase recruitment mechanisms and synthetic drugs that hijack these processes for targeted protein degradation. (a) Biological PTM (*top*): Doubly phosphorylated β -catenin peptide binds the β -TrCP ligase (PDB ID 1p22). Synthetic PTM (*bottom*): NRX-103094 binds in the pocket left unoccupied by the β -catenin Ser37 mutation to stabilize substrate binding (PDB ID 6m91). (b) Biological oligomerization (*top*): homodimer of BCL6 BTB domains (PDB ID 3bin). The ligase CRL1^{FBXL17} recognizes nonfunctional BTB heterodimers through the degron residues highlighted in green (His14, Asp33, Ser59). Synthetic oligomerization (*bottom*): BI-3802 binds to the BTB domain of BCL6, inducing protein polymerization (PDB ID 6mxm; EMD-22265) and foci formation. The ligase SIAH1 colocalizes to the BCL6 foci through recognition of the VxP motif and induces degradation. (c) Biological ligase replacement (host; *top left*): The substrate receptor CSA (*blue*) binds to the adaptor protein DDB1 (*gray*) to assemble a functional CRL4 ligase (PDB ID 4a11). Biological ligase replacement (virus; *top right*): Sv5-V functions as a DCAF for the CRL4 ligase to recruit STAT proteins for degradation (PDB ID 2b51). Synthetic ligase replacement (*bottom*): CR8 binding to CDK12 induces the direct binding of a CDK12-CycK complex to DDB1, leading to degradation (PDB ID 6td3). (d) Biological molecular glue (*left*): The plant hormone auxin binds in a hydrophobic pocket of the TIR1 receptor, with IAA Trp5 and Pro7 stacking on top of the ligand (PDB ID 2p1q). Synthetic molecular glue (*right*): Lenalidomide binds the substrate receptor CRBN to recruit CK1 α through its β -hairpin loop (PDB ID 5fqd). CK1 α Gly40 packs between lenalidomide and CRBN Val388. Abbreviations: DCAF, DDB1- and CUL4-associated factor; EMD, Electron Microscopy Data Bank identifier; PDB ID, Protein Data Bank identifier; PTM, posttranslational modification; Sv5-V, simian virus 5 V protein.

complementarity and VHL residues positioned for optimal hydrogen bonding with the degron explain the exquisite sensitivity for a single hydroxyl group, with an affinity of 30 nM (Hon et al. 2002, Min et al. 2002). The affinity of VHL for a peptide containing unmodified Pro564 is 1,000-fold weaker than the hydroxylated peptide, again demonstrating that certain substrate receptors are highly sensitive to subtle chemical modifications of the degron motif. In addition to the PTMs described above, methylation, acetylation, and *N*-glycosylation also generate PTM-degrons (Lee & Zhou 2007, Leng et al. 2018, Van Nguyen et al. 2016, Yoshida et al. 2002). While there is great chemical diversity in PTM-degrons, they each enable tightly regulated access of a substrate to its ligase.

2.1.2. Synthetic. One liability of PTM-degrons is that they are disabled by missense mutations at acceptor sites, a recurrent feature in cancer (Mészáros et al. 2017). In particular, β -catenin is stabilized by mutations at Ser33 or Ser37 in its doubly phosphorylated degron, which prevents tight binding and ubiquitination by its substrate receptor, β -TrCP. A recent drug discovery effort identified small molecules that enhance the interaction between β -TrCP and a β -catenin peptide singly phosphorylated at Ser33 by binding in a small hydrophobic pocket that is available due to the loss of the phosphate on Ser37 (Simonetta et al. 2019). The molecules contain a 6-trifluoromethylpyridone core that binds in the vacant pocket on β -TrCP, making hydrophobic contacts with both the ligase and substrate (**Figure 2a**). The small molecule also functions as an anionic phosphate mimetic, hydrogen bonding with the backbone amide of β -TrCP Gly432. Potent derivatives of this core resulted in EC₅₀ (half-maximal effective concentration) values of 4 nM for the assembly of the ternary complex. This effort demonstrates the feasibility of using small molecules to glue together broken PTM-degron/ligase pairs.

PTM-binding ligases might be particularly amenable to drugging, and this concept is supported by the development of high-affinity ligands for the VHL receptor. VHL was one of the first ligases to be used in a proof-of-concept for proteolysis-targeting chimeras (PROTACs) (Lai & Crews 2017). The initial VHL binders were HIF-1 α peptide mimetics containing a hydroxylated proline. Subsequent efforts were dedicated to synthesizing a small-molecule VHL binder, ultimately resulting in a ligand with an affinity of 185 nM (Galdeano et al. 2014). The latest



generation of VHL ligands have been used in a variety of PROTACs to degrade BCR-ABL, $ERR\alpha$, RIPK2, and BET proteins (Lai & Crews 2017). With the success of degradation through VHL recruitment, it will be worth considering whether other PTM-binding ligases should be prioritized for small-molecule development.

2.2. Protein Quality Control

Cells have developed exquisite mechanisms of protein quality control to regulate proteostasis. Misfolded proteins can be detrimental to the cell and can be recognized by chaperones via their exposed hydrophobic patches and are either refolded or directed to cellular degradation machinery for elimination from the cell.

2.2.1. Biological. A class of substrate degrons are presented to ligases as a result of protein quality control. In this system, degron exposure can occur upon unfolding, proteolytic cleavage, or failure to assemble into a functional protein complex. The chaperones Hsp90, Hsp70, and the constitutively expressed Hsc70 assist in folding client proteins to their native state. Hsp70 recognizes exposed hydrophobic residues, which can result from stress-induced protein denaturation. The inability of a client to properly fold leads to a form of molecular triage in which the unfolded protein is targeted for degradation. The ubiquitin ligase CHIP (C terminus of Hsc70-interacting protein) binds to all three chaperones through a TPR (tetratricopeptide repeat) motif (Connell et al. 2001, Demand et al. 2001) and induces ubiquitination of client proteins, playing an essential role in the degradative triage of protein quality control.

There are several quality control pathways that recognize degrons exposed at either the N or C terminus. For N-terminal degrons, the classic regulatory mechanism is the N-end rule, which consists of an Arg/N-end and an Ac/N-end branch. In the Arg/N-end pathway, proteins with basic or large hydrophobic residues at the N-terminus are recognized by the N-recognin ligases UBR1, UBR2, and UBR4. In the case of UBR1, an acidic cleft in its UBR box binds the N-terminal positively charged amine, as well as the side chain of the first basic residue (Choi et al. 2010). The Ac/N-end rule is proposed to regulate protein complex stoichiometry, in which excess subunits are recruited to a ubiquitin ligase by their exposed N-terminal acetylation mark that would normally be buried by complex formation (Hwang et al. 2010). N-terminal glycine was recently shown to function as an N-degron targeted by $CUL2^{ZYG11B}$ and $CUL2^{ZER1}$ (Timms et al. 2019) in the clearance of protein fragments generated by caspase cleavage, or by the failure to undergo N-myristoylation.

Similarly, the DesCEND (destruction via C-end degrons) pathway degrades many proteins with C-terminal degron motifs. Multiple $CUL2$ (KLHDC2, KLHDC3, KLHDC10, APPBP2, FEM1B, and FEM1A-C) and $CUL4$ (DCAF12 and TRPC4AP) substrate receptors recognize a variety of one- to four-residue C-degrons (Koren et al. 2018, Rusnac et al. 2018). For both N- and C-degrons, a simple dipeptide motif is often sufficient for ligase recruitment, highlighting again that rather minimal structural requirements can lead to highly specific ligase interactions.

2.2.2. Synthetic. The estrogen receptor α ($ER\alpha$) is a ligand-activated transcription factor and an important drug target in breast cancer with multiple approved therapies. It was found that the potent antiestrogen compound ICI 164,384 induces the degradation of $ER\alpha$ (Dauvois et al. 1992). A close analog of ICI 164,384, fulvestrant (Faslodex[®], 2002), belongs to a family of selective ER downregulators (SERDs), many of which have been approved by the FDA (US Food and Drug Administration) for the treatment of metastatic breast cancers or have recently entered clinical trials for their ability to downregulate $ER\alpha$ (Osborne et al. 2004, Xiong et al. 2017). The



proposed mechanism for SERDs is that binding to ER α induces conformational changes that expose a hydrophobic patch on the surface of ER α , leading to degradation (Wittmann et al. 2007). The structure of ER α in complex with the modulator GW5638 reveals that the small molecule repositions helix 12 of ER α away from a buried core and exposes hydrophobic residues (Leu536, Leu539, Leu540, and Met543) to the protein exterior (Wu et al. 2005). Furthermore, recent work has shown that fulvestrant treatment leads to ER nuclear immobilization (Guan et al. 2019). Interestingly, creating hydrophilic or charged substitutions for the hydrophobic residues of helix 12 both blocked degradation and prevented immobilization. While the mechanism connecting exposed hydrophobicity to nuclear immobilization and protein turnover is unresolved, fulvestrant clearly takes advantage of protein quality control surveillance.

The induction of surface hydrophobicity, termed hydrophobic tagging (HyT), has also been utilized to develop degraders for the androgen receptor (AR). In this strategy, the attachment of a hydrophobic adamantyl group to an AR ligand (e.g., RU59063) mimics a partially unfolded state once the compound is bound to AR (Gustafson et al. 2015). Whether through forced surface hydrophobicity (SERDs) or imitated instability (HyT), eliciting the activity of the protein quality control machinery for target destruction is an effective strategy.

2.3. Substrate Oligomerization

To balance the targeting of unfolded proteins mentioned above, cells also contain quality control systems targeted towards dimerized or oligomerized proteins, enabling coordinated regulation of cellular proteostasis. These higher-order protein structures are recognized by specific ligases, resulting in ubiquitination and subsequent degradation.

2.3.1. Biological. Many proteins require dimerization or higher-order oligomerization for activity. Several protein domains serve as dimerization modules, including the BTB domain, the RING domain, and coiled coils. The BTB domain generally leads to homodimerization between identical proteins. It was recently shown that the ubiquitin ligase CRL1^{FBXL17} functions in BTB homodimerization quality control, in which the ligase binds and ubiquitinates nonfunctional BTB heterodimers (Mena et al. 2018). The heterodimer degron contains three residues (His15, Asp34, and Ala60) adjacent to the dimer interface, and the current model is that proper homodimerization buries these degron residues and prevents FBXL17 recognition (**Figure 2b**). Depletion of FBXL17 levels increased the formation of nonfunctional BTB heterodimers in cells, highlighting the extent of cellular surveillance mechanisms to maintain a functional signaling proteome (Mena et al. 2018).

2.3.2. Synthetic. B cell lymphoma 6 (BCL6) is a master transcriptional repressor and known driver of germinal center-derived lymphoma development. Drug discovery efforts have focused on the disruption of the PPI between BCL6 and its cofactors for the treatment of diffuse large B cell lymphomas. Recently, an effective BCL6 degrader, BI-3802, was discovered (Kerres et al. 2017). Investigation into the degradation mechanism revealed that BI-3802 binding to the BTB domain of BCL6 stimulates the formation of regular higher-order oligomers (**Figure 2b**). This polymerization of BCL6 alters its cellular localization and promotes the formation of BCL6 foci and degradation (Stabicki et al. 2020b). Since it has been speculated that many proteins evolve on the edge of self-assembly due to internal symmetries and many can self-assemble into higher-order structures in response to cellular stimuli (Garcia-Seisdedos et al. 2017, Li et al. 2012), this mechanism creates a clear therapeutic opportunity for using small molecules to trigger substrate oligomerization to enhance ligase binding.



2.4. Replacement of Ligase Components to Redirect Ubiquitination

Cullin ligases are modular assemblies that utilize a large pool of different substrate receptors to recruit a diverse array of substrates for ubiquitination. This modular nature of ligases can be hijacked through structural and functional mimicry to induce degradation of neo-substrates.

2.4.1. Biological. Viral and bacterial pathogens have evolved an impressive form of structural and functional mimicry in which they hijack the host UPS by circumventing substrate receptors to induce the degradation of host factors that interfere with viral replication. Both the paramyxovirus V protein and the hepatitis B virus X protein (HBx) bind the DDB1 adaptor of the CUL4 ligase to enable efficient viral replication. The V protein mimics a substrate receptor and recruits the STAT proteins, which are critical for interferon signaling, for degradation (**Figure 2c**). Both the V protein and HBx utilize a short helical motif to dock into the β -propeller C of DDB1 (Li et al. 2010). The substrate receptors for CUL4 also utilize a helix-loop-helix motif to bind DDB1 in an identical manner (**Figure 2c**) (Fischer et al. 2011, Li et al. 2010); therefore, the viruses have evolved precise structural mimicry that enables effective integration into the host cell UPS system.

2.4.2. Synthetic. A small-molecule cyclin-dependent kinase (CDK) inhibitor, CR8, has been shown to induce the degradation of cyclin K (Słabicki et al. 2020a). CR8-induced degradation of cyclin K bypasses the requirement of a canonical substrate receptor for the CUL4A ligase. Instead, CR8 acts as a molecular glue between the DDB1 adaptor protein and the CDK12-cyclin K complex. A C-terminal extension of CDK12 mimics DCAF binding by inserting itself into the cleft between β -propeller A and C (**Figure 2c**). CR8 binds the active site of CDK12 and makes contacts with both DDB1 and the kinase, while cyclin K is positioned away from DDB1 toward the E2-conjugating enzyme for proximity-mediated ubiquitination. This unique mode of degradation follows the commonly used principle of direct ligase adaptor binding to achieve ubiquitination. As several other kinase inhibitors, such as MELK-T1 (MELK inhibitor) and PF-06447475 (LRRK2 inhibitor), have also been implicated as inducers of degradation (Beke et al. 2015, Jones 2018, Lobbstaël et al. 2016), it will be interesting to identify the mechanism of action employed for their degradation.

2.5. Decoration of the E3 Ligase Machinery to Redirect Ubiquitination

As described above, it is possible to repurpose the E3 ligase machinery by replacing specific components with exogenous molecules to create a new enzyme. It is also possible to redirect E3 ligase enzymes by binding to the intact E3 complex and altering the substrate receptor surface to alter ligase specificity. Recently, surface decoration of E3 ligases has garnered considerable attention, and since there is a considerable body of literature available, here we separately describe heterobifunctional small molecules and molecular glues.

2.5.1. Heterobifunctional redirection of E3 ligases. Heterobifunctional binding factors exhibit dual specificity, which enables the simultaneous engagement of both target and ligase substrate receptors, leading to proximity-mediated ubiquitination of the target protein, followed by proteasomal degradation.

2.5.1.1. Biological. Viral mechanisms have been described where the DCAF is bound by a viral factor that then acts to recruit host proteins, thereby triggering their destruction. For example, the HIV-1 protein Vpr binds to DCAF1 and directs the CRL4^{DCAF1} E3 ubiquitin ligase to recruit

UNG2, MUS81, and SLX4 (Ahn et al. 2010, Bouhamdan et al. 1996, Laguette et al. 2014, Wu et al. 2016). The HIV-2 Vpx protein similarly binds to DCAF1 and has been reported to trigger the degradation of SAMHD1 (Ahn et al. 2012, Hrecka et al. 2011). Structural studies on the DDB1-DCAF1-Vpr-UNG2 complex reveal that Vpr may mimic both a natural substrate of DCAF1 and the endogenous partner of UNG2 in order to scaffold the complex leading to ubiquitination and degradation of UNG2.

2.5.1.2. Synthetic. Heterobifunctional molecules composed of E3-binding moieties linked to target-protein-binding moieties were first described at the beginning of the millennium (Deshaies et al. 2006, Sakamoto et al. 2001). The linked molecules must be able to simultaneously bind ligase and substrate to scaffold the ternary complex leading to ubiquitination and degradation. The first examples, PROTACs, were derived from peptides targeting VHL and demonstrated early proof-of-concept but were not suitable for in vivo use. Subsequent work was able to expand this concept to other ligases using nonpeptidic small-molecule moieties (Itoh et al. 2010, Okuhira et al. 2011, Schneekloth et al. 2008), and eventually in vivo protein knockdown was achieved (Bondeson et al. 2015, Winter et al. 2015). This heterobifunctional approach is conceptually appealing and can take advantage of known binding moieties, which has led to successful degradation of a vast array of target proteins (de Wispelaere et al. 2019, Jiang et al. 2019, Lai et al. 2016, Silva et al. 2019, Winter et al. 2015). Although a comprehensive survey is beyond the scope of this review, this topic has been extensively reviewed elsewhere (Bondeson & Crews 2017, Burslem & Crews 2020, Neklesa et al. 2017, Verma et al. 2020).

2.5.2. Ligase-substrate protein-protein stabilizers (molecular glues). Following a mechanism similar to heterobifunctional molecules, molecular glues also recruit target proteins to the E3 ligase substrate receptor for proximity-mediated ubiquitination. However, molecular glues are unidirectional molecules that act to bridge interactions between the target protein and the ligase substrate receptor.

2.5.2.1. Biological. Plant growth and development are controlled by structurally distinct phytohormones including auxins, jasmonates, gibberellins, strigolactones, and salicylic acid (Guilfoyle et al. 2015). These phytohormones act as molecular glues for stabilizing the interactions between SCF substrate receptors and various transcriptional repressors. Auxins bind to the substrate receptor TIR1, leading to the recruitment of the Aux/IAA (indole-3-acetic acid) repressors (Dharmasiri et al. 2005, Rogg & Bartel 2001), and to a hydrophobic surface pocket on TIR1, where an auxin carboxyl group anchors the small molecule to the floor of the pocket through a salt bridge and hydrogen bonding with the receptor (**Figure 2d**) (Tan et al. 2007). An analogous example is jasmonate, where the active form of the hormone is (3*R*,7*S*)-jasmonoyl-*L*-isoleucine (JA-Ile), which binds to the SCF substrate receptor COI1 (coronatine insensitive 1) to direct the ubiquitination of the jasmonate ZIM domain family of transcriptional repressors (Xu et al. 2002). Similar to auxin, (3*R*,7*S*)-JA-Ile is bound in a surface pocket of COI1, secured to the basic pocket floor via a network of salt bridges and hydrogen bonding (Sheard et al. 2010). Hydrophobic packing with the receptor further stabilizes the small molecule toward the top of the pocket. Given the number of plant hormone molecular glues and the structural parallels with mammalian hormones, it is possible that this molecular glue degradation mechanism is prevalent among higher-order species.

2.5.2.2. Synthetic. Thalidomide, lenalidomide, and pomalidomide are FDA-approved drugs and were utilized as therapeutics long before they were classified as molecular glues. These molecules create a structural bridge between the CRL4^{CRBN} ligase and several neo-substrates,



including IKZF1/3, SALL4, and CK1 α (Donovan et al. 2018; Gandhi et al. 2014; Ito et al. 2010; Krönke et al. 2014, 2015; Lu et al. 2014; Matyskiela et al. 2018a). Thalidomide analogs (sometimes referred to as CRBN modulators) bind in a hydrophobic pocket on CRBN, creating a favorable interaction surface for neo-substrates.

Similarly, aryl sulfonamides, including indisulam, tasisulam, and E7820, bind to the CRL4^{DCAF15} E3 ligase and create a structural bridge between the DCAF15 substrate receptor and the neo-substrates, RBM39 and RBM23 (Bussiere et al. 2020, Du et al. 2019, Faust et al. 2020, Han et al. 2017, Ting et al. 2019, Uehara et al. 2017). The sulfonamides bind in a shallow pocket between the N- and C-terminal domains of DCAF15 with relatively weak affinity (E7820 4 μ M, indisulam > 50 μ M) for DCAF15. Once the ligand is bound, ternary complex formation is driven by a large protein interaction surface between the RRM2 domain of RBM39 and DCAF15. The RRM2 primarily interacts with DCAF15 through its two α -helices, with the α 1 helix buried in the sulfonamide-binding cleft of DCAF15 contacting both the ligase and the ligand.

While the first PROTACs have entered clinical trials (NCT03888612 and NCT04072952, <https://clinicaltrials.gov>), neither the structures of these molecules nor their exact properties have been disclosed. Our current best understanding of properties necessary for successful clinical translation is thus limited to a rather small set of molecular glue drugs, yet they are highly informative for establishing a conceptual framework for TPD.

3. LESSONS FROM POSTCLINICAL DEVELOPMENT

Surprisingly, given their serendipitous discovery, clinical molecular glue-based degraders operate through the CUL4 substrate receptors CRBN or DCAF15 and therefore offer an interesting opportunity for comparison. Both classes of molecules were discovered and developed using empirical phenotypic approaches with limited knowledge of mechanism (Fukuoka et al. 2001, Owa et al. 1999). Furthermore, both were advanced into the clinic for the treatment of multiple myeloma and only subsequently were found to repurpose the CRL4 E3 ubiquitin ligase. Now that detailed structural and molecular information is available for both CRBN and DCAF15 systems, in addition to broader surveys of the neo-substrate proteins they target for degradation, we can attempt to use these two degradation systems to extract common principles and estimate the future potential for degradation therapeutics.

3.1. Cereblon Demonstrates Broad Substrate Accommodation

Structural studies on thalidomide analogs have elucidated the molecular basis for the recruitment of substrate proteins. CRBN modulators exhibit a wide range of neo-substrate proteins that they can recruit to CRL4^{CRBN} (An et al. 2017, Donovan et al. 2018, Krönke et al. 2015, Lu et al. 2014, Matyskiela et al. 2016, Petzold et al. 2016, Sievers et al. 2018). For example, the clinical activity in myeloma is mediated by recruitment of the zinc finger transcription factors IKZF1 and IKZF3 (Gandhi et al. 2014, Krönke et al. 2014, Lu et al. 2014), and the activity in del(5q) myelodysplastic syndrome is mediated by recruitment of the protein kinase CK1 α (Krönke et al. 2015). In addition, proteomic and library-based approaches have determined that considerably more substrates may be targetable, including many additional targets from the C₂H₂ zinc finger transcription factor family (Donovan et al. 2018, Sievers et al. 2018). All currently reported thalidomide analogs bind CRBN via the conserved glutarimide ring, with variable chemical groups decorating the phthalimide or isoindolinone ring displayed on the surface (Chamberlain et al. 2014, Fischer et al. 2014).

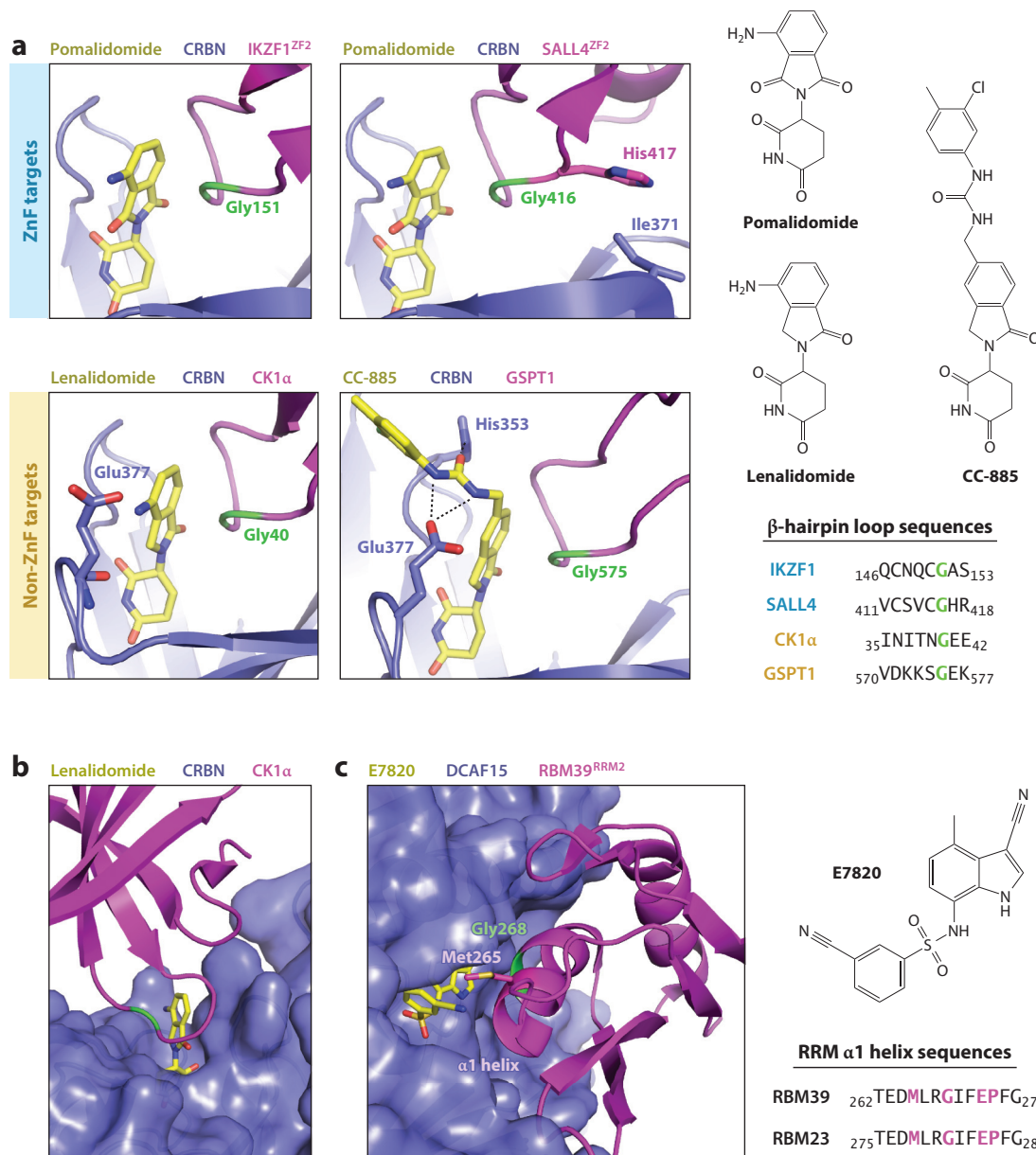
Despite exhibiting diverse protein folds and sequences, all currently described CRBN neo-substrates exhibit a shared structural degron composed of a β -hairpin loop containing a glycine

9.10 Faust et al.



residue in a specific position (**Figures 2d** and **3a**) (Donovan et al. 2018; Matyskiela et al. 2016, 2020; Petzold et al. 2016; Sievers et al. 2018). This degron mediates interactions both with the surface of CRBN and with the bound CRBN modulator.

Chemical modification of thalidomide analogs has been shown to alter the recruitment of neo-substrates. For example, extension on the isoindolinone ring found on lenalidomide results in iberdomide and CC-92480, molecules with enhanced CRBN binding and potent degradation of IKZF1/3 (Hansen et al. 2020, Matyskiela et al. 2018b). Iberdomide is in phase Ib clinical trials in



(Caption appears on following page)

Figure 3 (Figure appears on preceding page)

Principles of substrate recruitment for CRBN and DCAF15. (*a, left*) Cartoon representation of CRBN bound to neo-substrates. A β -hairpin loop with a positionally conserved glycine is the degron for CRBN neo-substrates, including the IKZF1 (PDB ID 6h0f) and SALL4 (PDB ID 6uml) ZnF transcription factors (*top*), and the non-ZnF targets CK1 α (PDB 5fqd) and GSPT1 (PDB ID 5hxb) (*bottom*). (*a, right*) The chemical structures of pomalidomide, lenalidomide, and CC-885, as well as the β -hairpin loop sequences. The conserved glycine residues in the degron are colored in green in both the cartoon structures and the sequences. (*b*) Lenalidomide bound to CRBN is exposed on the surface of the receptor, where it contacts the CK1 α degron. (*c, left*) A surface representation of DCAF15 demonstrates that the sulfonamide E7820 is buried in a groove, making it less accessible to neo-substrates compared to lenalidomide bound to CRBN. The second RRM of RBM39 displays high shape complementarity to the ligand-bound face of DCAF15, packing tightly against the receptor to contact E7820 through Met265. RBM39 Gly268 (*green*) is a critical position for indisulam resistance mutations. (*Right*) The chemical structure of E7820 and the sequences of the α 1 helix for the DCAF15 neo-substrates RBM39 and RBM23. Residues in magenta in the sequence are the positions of indisulam resistance mutations. Abbreviations: PDB ID, Protein Data Bank identifier; RRM, RNA recognition motif; ZnF, zinc finger.

lupus and in phase II for relapsed refractory myeloma; CC-92480 is in phase I clinical trials for relapsed refractory myeloma.

In addition to altering the potency, chemical modification can also enable novel neo-substrate recruitment. For example, of the approved thalidomide analogs (thalidomide, lenalidomide, and pomalidomide), lenalidomide is unique in degrading CK1 α (Krönke et al. 2015). Upon CK1 α binding to lenalidomide-CRBN, the 3 position of the lenalidomide phthalimide ring comes into proximity of the backbone carbonyl of CRBN Glu377 (**Figure 3a**). Thalidomide and pomalidomide contain a carbonyl at this phthalimide 3 position, which would clash sterically with CK1 α , providing a rationale for the differences in selectivity (Petzold et al. 2016).

Chemical extension of the isoindolinone ring to produce the molecule CC-885 leads to the recruitment and degradation of GSPT1, a translation factor with a key role in protein synthesis and cellular proliferation (Matyskiela et al. 2016). While CC-885 retains the ability to degrade IKZF1, lenalidomide and pomalidomide do not induce GSPT1 degradation, highlighting the importance of the additional chemical extension for GSPT1 recruitment (**Figure 3a**). CC-885 exhibited broad antiproliferative activity against a panel of tumor cell lines, with subnanomolar potency against acute myeloid leukemia (AML) lines and ex vivo patient-derived cells. A GSPT1 degrader, CC-90009, has been reported to be in phase I clinical trials in AML (Surka et al. 2020). All of these examples clearly demonstrate that alterations to the surface-exposed features of the small molecule (**Figure 3b**) can dramatically impact the neo-substrate profile of CRBN, both for the C₂H₂ transcription factors and other targets, and they indicate that the rational design of molecular glues targeting selected proteins of interest is within reach.

The downside to the broad neo-substrate scope exhibited by the thalidomide analogs is the risk of toxicities such as the teratogenicity that occurred in the thalidomide birth defect tragedy in the 1960s. The embryonic transcription factor SALL4 is a C₂H₂ zinc finger transcription factor and a CRBN neo-substrate that is robustly degraded in the presence of thalidomide (Donovan et al. 2018, Matyskiela et al. 2018a). Haploinsufficiency in SALL4 causes a genetic syndrome so similar to thalidomide-induced teratogenicity that there are cases where SALL4 mutations were misdiagnosed as thalidomide embryopathy (Kohlhase et al. 2003, 2005).

While CRBN has shown broad neo-substrate accommodation, not all ligase systems can be expected to exhibit similar levels of substrate flexibility, and further examples have now been described with more narrow activity range.

3.2. DCAF15 and Shape Complementarity

Aryl sulfonamides were found to target the G1 phase of the cell cycle, resulting in cell cycle arrest and subsequent cell death (Owa et al. 1999) through an unknown mechanism. Further

investigation into the mode of action of these sulfonamides determined that the molecules bind to DCAF15 to induce the degradation of RBM39 (Han et al. 2017, Uehara et al. 2017). Structural studies into the binding mode of RBM39 with DCAF15 determined that, unlike CRBN, the interaction is primarily driven by protein-protein contacts between DCAF15 and the RNA recognition motif (RRM) domain, including multiple side chain interactions and buried hydrophobic residues (Bussiere et al. 2020, Du et al. 2019, Faust et al. 2020). While thalidomide analogs have their phthalimide ring protruding from the surface-exposed hydrophobic pocket on CRBN (**Figure 3b**), sulfonamides are buried in a DCAF15 cavity (**Figure 3c**). With high shape complementarity, the second RRM of RBM39 binds DCAF15 and interacts with the sulfonamide through Met265. Resistance mutations in RBM39 that prevent sulfonamide-dependent degradation cluster on the DCAF15-binding face of the $\alpha 1$ helix (Han et al. 2017, Uehara et al. 2017). In particular, RBM39 Gly268 cannot tolerate any sidechain-bearing residue, highlighting how tightly RBM39 packs into the DCAF15-sulfonamide interface.

Proteomic and bioinformatic approaches uncovered RBM23 as an additional neo-substrate for sulfonamide-bound DCAF15. RBM23 contains an RRM in which the $\alpha 1$ helix is perfectly conserved with the RBM39 sequence (**Figure 3c**), again demonstrating the higher constraints in neo-substrate recruitment for DCAF15 compared to CRBN. The high specificity for RBM23 and RBM39 likely limits the ability of DCAF15 to recruit diverse substrates for degradation. However, the shape complementarity between DCAF15 and the highly conserved RRM domain suggests that there is an opportunity to develop new chemical matter to recruit other RRM-containing proteins for degradation. The success of sulfonamides hijacking DCAF15 despite the lack of an obvious small-molecule binding cavity demonstrates that a broader range of ubiquitin ligases can likely be targeted by molecular glues. The differences in mechanism for immunomodulatory imide drugs (IMiDs) and sulfonamides emphasize that there are many ways to neofunctionalize the UPS for substrate degradation.

4. THE ROAD AND CHALLENGES AHEAD

While the positive impact of lenalidomide on patients and the propulsion of PROTACs to the forefront of chemical biology is exciting, we should not forget that TPD as a therapeutic modality is still in its infancy. We are just now witnessing the first-in-human dosing of PROTACs targeting the AR (ARV-110; NCT03888612) and ER (ARV-471; NCT04072952), as well as the clinical development of CRBN modulators that are not direct descendants of thalidomide and that were developed with molecular knowledge of the mechanism of action (CC-90009, CC-92480). While the understanding of small-molecule-mediated degradation may still be limited, the understanding of proximity-inducing therapeutics has come a long way from the discovery of rapamycin (Vezina et al. 1975) and the idea of rapalog (Bayle et al. 2006) to viral proteins (Ahn et al. 2010, Li et al. 2010), plant hormones (Kepinski & Leyser 2004, Rogg & Bartel 2001, Ueguchi-Tanaka et al. 2007, Xu et al. 2002), and now the widespread application of TPD in chemical biology (Bondeson & Crews 2017, Neklesa et al. 2017). The field of proximity-inducing therapeutics—with TPD as its spearhead—is at a tipping point for becoming a widely accepted drug discovery paradigm. To deliver on the promises that have been made on behalf of the modality, we have to continue to address key challenges in the discovery and development of these therapeutics, some of which we discuss here.

For TPD and proximity-inducing therapeutics more broadly, the fundamental promise is that the target space is no longer limited to enzymes, receptors, and other activities that can be directly agonized or antagonized. Instead, the field has progressed to include several methods of protein inactivation that are more reminiscent of genetic loss, including the recruitment of E3 ubiquitin

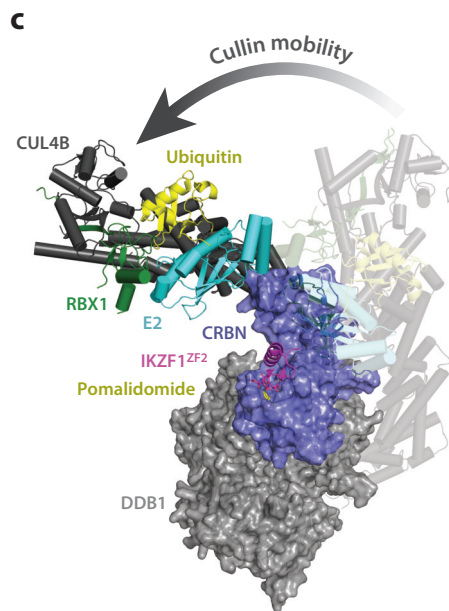
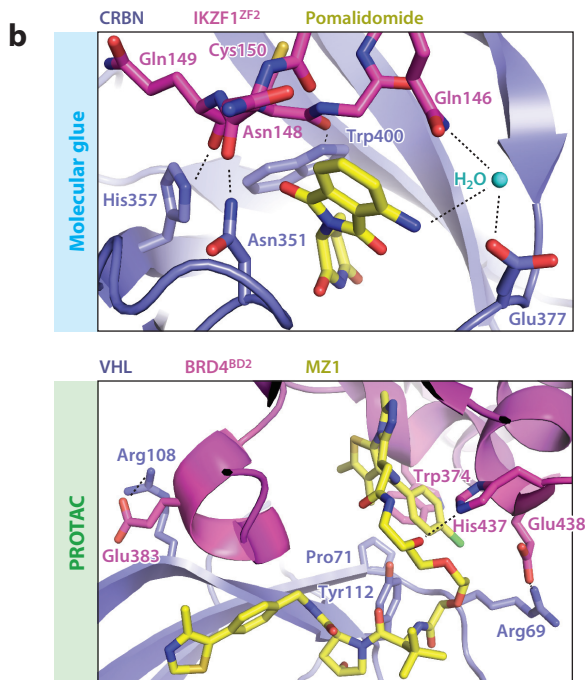
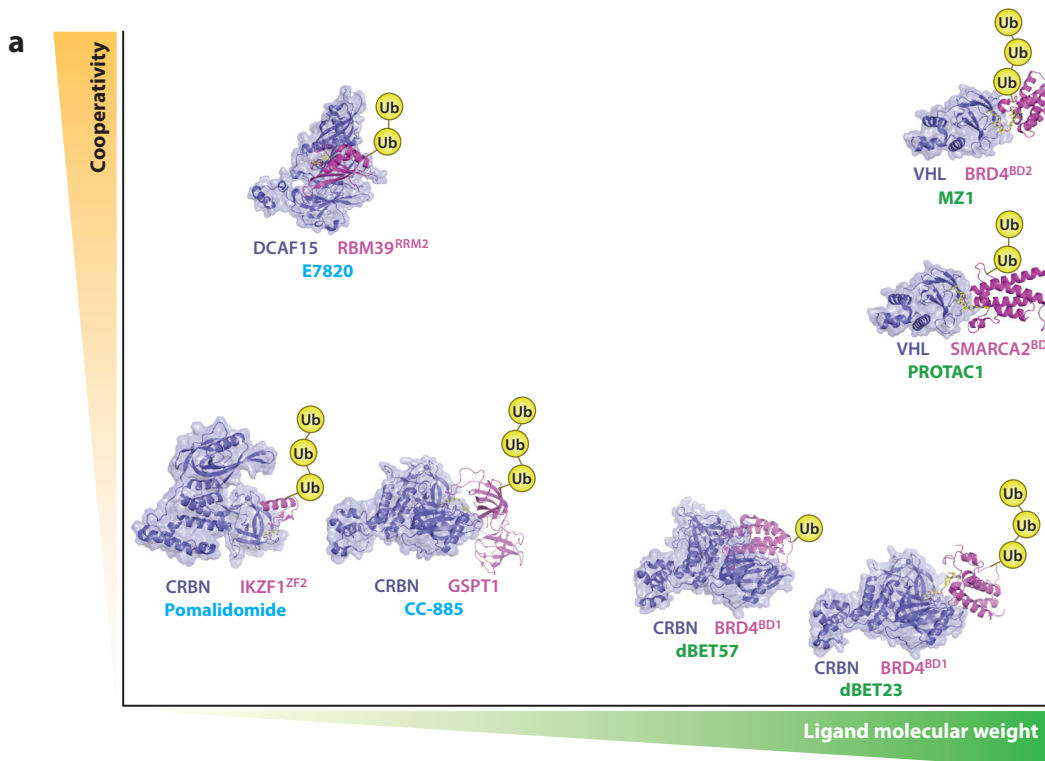


ligases to targets for degradation, induced oligomerization to block activity, or sequestration into nonfunctional compartments. However, delivering on this promise relies heavily on the ability to first identify the targets that can be dimerized with small molecules and to subsequently devise and optimize such molecules while retaining necessary drug-like properties to support human use. To date, the majority of described TPD examples were discovered through a brute force approach using existing ligands (PROTACs), were serendipitously discovered (IMiDs, indisulam, fulvestrant), or have been developed out of a narrow rationale approach (CRBN modulators). Chemoproteomic methods have now further added DCAF16 (Zhang et al. 2019), RNF4 (Ward et al. 2019), and RNF114 (Spradlin et al. 2019) as ligases compatible with TPD. Conceptually, the process of dimerizing two proteins can be broken down into how compatible the proteins are with respect to surface complementarity and surface chemistry and how a small molecule can add the necessary binding energy for productive binding. Similar to classical inhibitor development, in which hit finding, lead optimization, and development are aided by structural understanding, high-throughput screening, and modeling, we must identify and develop the critical tools needed to support the discovery pipeline for dimerizing molecules.

In TPD, a critical step is the identification of a ligase suitable for recruitment. So far, empirical data suggest the following. (a) Not every ligase is suitable for TPD. This includes sulfonamide-bound DCAF15, which relies heavily on favorable PPIs between DCAF15 and targets for productive complex formation, thus limiting the range of potential targets that can be recruited by the currently available chemical matter. (b) Not every ligase/target pair is compatible. This has been demonstrated with matched pairs of CRBN and VHL-recruiting kinase PROTACs. Although both ligase-recruiting molecules can degrade c-Abl, only CRBN can induce degradation of BCR-Abl due to unfavorable PPI between BCR-Abl and VHL (Smith et al. 2019). (c) Productive ternary complex formation drives TPD, but recent work has shown that the requirement for productivity varies across ligase/target pairs, with some pairs driven through affinity and others driven by cooperative PPIs (Donovan et al. 2020). With the growing number of detailed studies, it is clear that the development of a molecule to catalyze target ubiquitination by a ubiquitin ligase is a process that requires optimization of many parameters. As an example of this larger parameter space, there is a clear correlation of cooperativity with efficacy in the case of the VHL-recruiting SMARCA PROTACs (Farnaby et al. 2019), while there is no cooperativity correlation in the case of CRBN-recruiting BRD4 PROTACs (**Figure 4a**) (Nowak et al. 2018). These differences can be explained by the complex nature of the molecular recognition event and the fact that ligases are different from each other. The formation of a productive ternary complex is the unifying theme across PROTACs and molecular glue degraders (**Figure 4b,c**), and optimization should focus on the process to be catalyzed.

In an attempt to support the development of degraders, many groups have identified an urgent need for computational approaches, and this has led to the establishment of a variety of advances to molecular modeling of ternary complex formation (Drummond & Williams 2019, Nowak et al. 2018). While these have successfully supported the discovery of individual PROTACs, they are unable to address the major challenges in the field, including accurate predictions of the tractability of a target/ligase pair, and optimization of the productive transition state of ubiquitin transfer. Molecular modeling is further limited by the lack of high-quality structural information on many targets and ligases, which must be complemented by improved homology modeling and, hopefully in the near future, structure prediction methods (Senior et al. 2020, Yang et al. 2020). In the case of molecular glues, we must find ways to better predict the strength of interactions derived from *in silico* docking experiments to aid off-target identification and better de-risk discovery efforts. While these are considerable challenges, the rapidly evolving computational structural





(Caption appears on following page)



Figure 4 (Figure appears on preceding page)

Features of small-molecule degraders. (a) Plot of the cooperativity of ternary complex formation versus ligand molecular weight. The structure of each ternary complex is placed to qualitatively reflect its relative values for cooperativity and molecular weight (PDB IDs: IKZF1-pomalidomide, 6h0f; GSPT1-CC-885, 5hxb; BRD4-dBET23, 6bn7; BRD4-dBET57, 6bnb; BRD4-MZ1, 5t35; SMARCA2-PROTAC1, 6hay; RBM39-E7820, 6q04). Molecular glue molecule names are colored in cyan and PROTAC molecule names are colored in green. The degradation efficiency for each complex is represented by yellow ubiquitins (3 ubiquitins = $DC_{50} < 50$ nM, 2 ubiquitins = $DC_{50} < 500$ nM, 1 ubiquitin = $DC_{50} > 500$ nM). (b) Comparison of the substrate-ligase interfaces between a molecular glue (pomalidomide; *top*) and a PROTAC (MZ1; *bottom*). Both ternary complexes utilize protein-ligand and protein-protein interactions to stabilize the complex. (c) Mobility of an assembled CRL4^{CRBN} ligase bound to a charged E2~Ub (UBE2D). The bound substrate is presented to the E2~Ub in multiple conformations to increase the likelihood of efficient ubiquitin transfer (PDB IDs: IKZF1, 6h0f; DDB1-CUL4B, 4a0l; RBX1-UBE2D, 6ttu). Abbreviations: DC_{50} , half-maximal degradation concentration; PDB ID, Protein Data Bank identifier; PROTAC, proteolysis-targeting chimera.

biology toolbox will productively converge with our understanding of the molecular workings of E3 ubiquitin ligases.

5. CONCLUSIONS AND PERSPECTIVES

Progress in the field over recent years has brought us to an exciting inflection point in the discovery of degradation therapeutics: Progress has brought PROTAC approaches to the point of clinical development, and at the same time, molecular glue molecules have been discovered in approved drugs, providing clinical validation for the approach and enabling additional molecules to be advanced. Structural studies on ternary complexes for both PROTACs and molecular glue scaffolded complexes have revealed that the distinction between glues and PROTACs may exist on a spectrum, with varying dependence on direct small-molecule affinity and PPIs (**Figure 4**). The research community has in rapid succession discovered novel examples of small-molecule catalyzed protein degradation, which, together with detailed mechanistic studies, will soon put us in position to support rational, target-based drug discovery with impactful structure-based drug design. Finally, the progress in TPD has sparked interest in proximity-inducing therapeutics beyond degradation systems to systems in which the induced scaffolding of macromolecular interactions can provide benefits to human health.

SUMMARY POINTS

1. Many cellular mechanisms exist to regulate the recruitment of a substrate protein to a ubiquitin ligase.
2. Synthetic degrader molecules leverage diverse substrate-ligase recruitment mechanisms to achieve targeted protein degradation.
3. The most advanced clinical degrader molecules—thalidomide, its analogs, and sulfonamides—operate through a ligase-substrate protein-protein stabilizer (molecular glue) mechanism.
4. Molecular glues can operate through considerably different principles. In the CRBN-IMiD (immunomodulatory imide drug) system, the small molecule binds to a pocket in the receptor to recruit neo-substrates through a simple structural degron. In the DCAF15-sulfonamide system, the small molecule binds to an open groove in the receptor buried away from potential binding partners and is only accessible to neo-substrates with strict shape (RNA recognition motif fold) and sequence requirements.

DISCLOSURE STATEMENT

E.S.F. is a founder, scientific advisory board (SAB) member, and equity holder of Civetta Therapeutics, Jengu Therapeutics (board member), and Neomorph, Inc.; is an equity holder of C4 Therapeutics; and is or has consulted for Novartis, AbbVie, Astellas, Deerfield, and EcoR1. The Fischer lab receives or has received research funding from Novartis, Deerfield, and Astellas. P.P.C. is an employee and equity holder of Neomorph, Inc. and an equity holder of BMS.

ACKNOWLEDGMENTS

This work was supported by NIH (National Institutes of Health)/NCI (National Cancer Institute) grants R01CA214608 and R01CA218278 (to E.S.F.) and a Mark Foundation Emerging Leader Award (to E.S.F.). E.S.F. is a Damon Runyon-Rachleff Innovator supported in part by the Damon Runyon Cancer Research Foundation (DRR-50-18). T.B.F. is supported by an NCI F32 fellowship (1F32CA232772-01).

LITERATURE CITED

- Ahn J, Hao C, Yan J, DeLucia M, Meherns J, et al. 2012. HIV/SIV accessory virulence factor Vpx loads the host cell restriction factor SAMHD1 onto the E3 ubiquitin ligase complex CRL4DCAF1. *J. Biol. Chem.* 287:12550–58
- Ahn J, Vu T, Novince Z, Guerrero-Santoro J, Rapic-Otrin V, Gronenborn AM. 2010. HIV-1 Vpr loads uracil DNA glycosylase-2 onto DCAF1, a substrate recognition subunit of a cullin 4A-RING E3 ubiquitin ligase for proteasome-dependent degradation. *J. Biol. Chem.* 285:37333–41
- An J, Ponthier CM, Sack R, Seebacher J, Stadler MB, et al. 2017. pSILAC mass spectrometry reveals ZFP91 as IMiD-dependent substrate of the CRL4^{CRBN} ubiquitin ligase. *Nat. Commun.* 8:15398
- Bayle JH, Grimley JS, Stankunas K, Gestwicki JE, Wandless TJ, Crabtree GR. 2006. Rapamycin analogs with differential binding specificity permit orthogonal control of protein activity. *Chem. Biol.* 13:99–107
- Beke L, Kig C, Linders J, Boens S, Boeckx A, et al. 2015. MELK-T1, a small-molecule inhibitor of protein kinase MELK, decreases DNA-damage tolerance in proliferating cancer cells. *Biosci. Rep.* 35(6):e00267
- Bondeson DP, Crews CM. 2017. Targeted protein degradation by small molecules. *Annu. Rev. Pharmacol. Toxicol.* 57:107–23
- Bondeson DP, Mares A, Smith IE, Ko E, Campos S, et al. 2015. Catalytic *in vivo* protein knockdown by small-molecule PROTACs. *Nat. Chem. Biol.* 11:611–17
- Bouhamdan M, Benichou S, Rey F, Navarro J-M, Agostini I, et al. 1996. Human immunodeficiency virus type 1 Vpr protein binds to the uracil DNA glycosylase DNA repair enzyme. *J. Virol.* 70:697–704
- Burslem GM, Crews CM. 2020. Proteolysis-targeting chimeras as therapeutics and tools for biological discovery. *Cell* 181:102–14
- Bussiere DE, Xie L, Srinivas H, Shu W, Burke A, et al. 2020. Structural basis of indisulam-mediated RBM39 recruitment to DCAF15 E3 ligase complex. *Nat. Chem. Biol.* 16:15–23
- Chamberlain PP, Lopez-Girona A, Miller K, Carmel G, Pagarigan B, et al. 2014. Structure of the human Cereblon–DDB1–lenalidomide complex reveals basis for responsiveness to thalidomide analogs. *Nat. Struct. Mol. Biol.* 21:803–9
- Choi WS, Jeong B-C, Joo YJ, Lee M-R, Kim J, et al. 2010. Structural basis for the recognition of N-end rule substrates by the UBR box of ubiquitin ligases. *Nat. Struct. Mol. Biol.* 17:1175–81
- Connell P, Ballinger CA, Jiang J, Wu Y, Thompson LJ, et al. 2001. The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. *Nat. Cell Biol.* 3:93–96
- Dauvois S, Daniellian PS, White R, Parker MG. 1992. Antiestrogen ICI 164,384 reduces cellular estrogen receptor content by increasing its turnover. *PNAS* 89:4037–41
- de Wispelaere M, Du G, Donovan KA, Zhang T, Eleuteri NA, et al. 2019. Small molecule degraders of the hepatitis C virus protease reduce susceptibility to resistance mutations. *Nat. Commun.* 10:3468



- Demand J, Alberti S, Patterson C, Höhfeld J. 2001. Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. *Curr. Biol.* 11:1569–77
- Deshaies RJ, Crews C, Sakamoto KM. 2006. *Proteolysis targeting chimeric pharmaceutical*. Eur. Patent EP1322750A4
- Dharmasiri N, Dharmasiri S, Estelle M. 2005. The F-box protein TIR1 is an auxin receptor. *Nature* 435:441–45
- Donovan KA, An J, Nowak RP, Yuan JC, Fink EC, et al. 2018. Thalidomide promotes degradation of SALL4, a transcription factor implicated in Duane Radial Ray syndrome. *eLife* 7:e38430
- Donovan KA, Ferguson FM, Bushman JW, Eleuteri NA, Bhunia D, et al. 2020. Mapping the degradable kinome provides a resource for expedited degrader development. *Cell*. In press
- Drummond ML, Williams CI. 2019. *In silico* modeling of PROTAC-mediated ternary complexes: validation and application. *J. Chem. Inf. Model.* 59:1634–44
- Du X, Volkov OA, Czerwinski RM, Tan H, Huerta C, et al. 2019. Structural basis and kinetic pathway of RBM39 recruitment to DCAF15 by a sulfonamide molecular glue E7820. *Structure* 27:1625–33.e3
- Dwane L, Gallagher WM, Chonghaile TN, O'Connor DP. 2017. The emerging role of non-traditional ubiquitination in oncogenic pathways. *J. Biol. Chem.* 292:3543–51
- Farnaby W, Koegl M, Roy MJ, Whitworth C, Diers E, et al. 2019. BAF complex vulnerabilities in cancer demonstrated via structure-based PROTAC design. *Nat. Chem. Biol.* 15:672–80
- Faust TB, Yoon H, Nowak RP, Donovan KA, Li Z, et al. 2020. Structural complementarity facilitates E7820-mediated degradation of RBM39 by DCAF15. *Nat. Chem. Biol.* 16:7–14
- Fischer ES, Böhm K, Lydeard JR, Yang H, Stadler MB, et al. 2014. Structure of the DDB1–CRBN E3 ubiquitin ligase in complex with thalidomide. *Nature* 512:49–53
- Fischer ES, Scrima A, Böhm K, Matsumoto S, Lingaraju GM, et al. 2011. The molecular basis of CRL4^{DDB2/CSA} ubiquitin ligase architecture, targeting, and activation. *Cell* 147:1024–39
- Fukuoka K, Usuda J, Iwamoto Y, Fukumoto H, Nakamura T, et al. 2001. Mechanisms of action of the novel sulfonamide anticancer agent E7070 on cell cycle progression in human non-small cell lung cancer cells. *Investig. New Drugs* 19:219–27
- Galdeano C, Gadd MS, Soares P, Scaffidi S, Van Molle I, et al. 2014. Structure-guided design and optimization of small molecules targeting the protein–protein interaction between the von Hippel–Lindau (VHL) E3 ubiquitin ligase and the hypoxia inducible factor (HIF) alpha subunit with in vitro nanomolar affinities. *J. Med. Chem.* 57:8657–63
- Gandhi AK, Kang J, Havens CG, Conklin T, Ning Y, et al. 2014. Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex CRL4^{CRBN}. *Br. J. Haematol.* 164:811–21
- Garcia-Seisdedos H, Empereur-Mot C, Elad N, Levy ED. 2017. Proteins evolve on the edge of supramolecular self-assembly. *Nature* 548(7666):244–47
- Guan J, Zhou W, Hafner M, Blake RA, Chalouni C, et al. 2019. Therapeutic ligands antagonize estrogen receptor function by impairing its mobility. *Cell* 178:949–63.e18
- Guilfoyle T, Hagen G, Larrieu A, Vernoux T. 2015. Comparison of plant hormone signalling systems. *Essays Biochem.* 58:165–81
- Gustafson JL, Neklesa TK, Cox CS, Roth AG, Buckley DL, et al. 2015. Small-molecule-mediated degradation of the androgen receptor through hydrophobic tagging. *Angew. Chem. Int. Ed.* 54:9659–62
- Han T, Goralski M, Gaskill N, Capota E, Kim J, et al. 2017. Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15. *Science* 356:eaal3755
- Hansen JD, Correa M, Nagy MA, Alexander M, Plantevin V, et al. 2020. Discovery of CRBN E3 ligase modulator CC-92480 for the treatment of relapsed and refractory multiple myeloma. *J. Med. Chem.* 63(13):6648–76
- Hao B, Oehlmann S, Sowa ME, Harper JW, Pavletich NP. 2007. Structure of a Fbw7–Skp1–cyclin E complex: multisite-phosphorylated substrate recognition by SCF ubiquitin ligases. *Mol. Cell* 26:131–43
- Hao B, Zheng N, Schulman BA, Wu G, Miller JJ, et al. 2005. Structural basis of the Cks1-dependent recognition of p27^{Kip1} by the SCF^{Skp2} ubiquitin ligase. *Mol. Cell* 20:9–19
- Hon W-C, Wilson MI, Harlos K, Claridge TD, Schofield CJ, et al. 2002. Structural basis for the recognition of hydroxyproline in HIF-1 α by pVHL. *Nature* 417:975–78



- Hrecka K, Hao C, Gierszewska M, Swanson SK, Kesik-Brodacka M, et al. 2011. Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. *Nature* 474:658–61
- Huang LE, Gu J, Schau M, Bunn HF. 1998. Regulation of hypoxia-inducible factor 1 α is mediated by an O₂-dependent degradation domain via the ubiquitin-proteasome pathway. *PNAS* 95:7987–92
- Hwang C-S, Shemorry A, Varshavsky A. 2010. N-terminal acetylation of cellular proteins creates specific degradation signals. *Science* 327:973–77
- Ito T, Ando H, Suzuki T, Ogura T, Hotta K, et al. 2010. Identification of a primary target of thalidomide teratogenicity. *Science* 327:1345–50
- Itoh Y, Ishikawa M, Naito M, Hashimoto Y. 2010. Protein knockdown using methyl bestatin– ligand hybrid molecules: design and synthesis of inducers of ubiquitination-mediated degradation of cellular retinoic acid-binding proteins. *J. Am. Chem. Soc.* 132:5820–26
- Jiang B, Wang ES, Donovan KA, Liang Y, Fischer ES, et al. 2019. Development of dual and selective degraders of cyclin-dependent kinases 4 and 6. *Angew. Chem. Int. Ed.* 58:6321–26
- Jones LH. 2018. Small-molecule kinase downregulators. *Cell Chem. Biol.* 25:30–35
- Kaelin WG. 2018. The von Hippel–Lindau tumor suppressor protein. *Annu. Rev. Cancer Biol.* 2:91–109
- Kepinski S, Leyser O. 2004. Auxin-induced SCF^{TIR1}–Aux/IAA interaction involves stable modification of the SCF^{TIR1} complex. *PNAS* 101:12381–86
- Kerres N, Steurer S, Schlager S, Bader G, Berger H, et al. 2017. Chemically induced degradation of the oncogenic transcription factor BCL6. *Cell Rep.* 20:2860–75
- Kohlhase J, Chitayat D, Kotzot D, Ceylaner S, Froster UG, et al. 2005. *SALL4* mutations in Okhiro syndrome (Duane–radial ray syndrome), acro–renal–ocular syndrome, and related disorders. *Hum. Mutat.* 26:176–83
- Kohlhase J, Schubert L, Liebers M, Rauch A, Becker K, et al. 2003. Mutations at the *SALL4* locus on chromosome 20 result in a range of clinically overlapping phenotypes, including Okhiro syndrome, Holt–Oram syndrome, acro–renal–ocular syndrome, and patients previously reported to represent thalidomide embryopathy. *J. Med. Genet.* 40:473–78
- Koren I, Timms RT, Kula T, Xu Q, Li MZ, Elledge SJ. 2018. The eukaryotic proteome is shaped by E3 ubiquitin ligases targeting C-terminal degrons. *Cell* 173:1622–35.e14
- Krönke J, Fink EC, Hollenbach PW, MacBeth KJ, Hurst SN, et al. 2015. Lenalidomide induces ubiquitination and degradation of CK1 α in del(5q) MDS. *Nature* 523:183–88
- Krönke J, Udeshi ND, Narla A, Grauman P, Hurst SN, et al. 2014. Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. *Science* 343:301–5
- Laguette N, Brégnard C, Hue P, Basbous J, Yatim A, et al. 2014. Premature activation of the SLX4 complex by Vpr promotes G2/M arrest and escape from innate immune sensing. *Cell* 156:134–45
- Lai AC, Crews CM. 2017. Induced protein degradation: an emerging drug discovery paradigm. *Nat. Rev. Drug Discov.* 16:101–14
- Lai AC, Toure M, Hellerschmied D, Salami J, Jaime-Figueroa S, et al. 2016. Modular PROTAC design for the degradation of oncogenic BCR–ABL. *Angew. Chem. Int. Ed. Engl.* 55:807–10
- Lee J, Zhou P. 2007. DCAF5, the missing link of the CUL4–DDB1 ubiquitin ligase. *Mol. Cell* 26:775–80
- Leng F, Yu J, Zhang C, Alejo S, Hoang N, et al. 2018. Methylated DNMT1 and E2F1 are targeted for proteolysis by L3MBTL3 and CRL4^{DCAF5} ubiquitin ligase. *Nat. Commun.* 9:1641
- Li P, Banjade S, Cheng H-C, Kim S, Chen B, et al. 2012. Phase transitions in the assembly of multivalent signalling proteins. *Nature* 483:336–40
- Li T, Robert EI, Van Breugel PC, Strubin M, Zheng N. 2010. A promiscuous α -helical motif anchors viral hijackers and substrate receptors to the CUL4–DDB1 ubiquitin ligase machinery. *Nat. Struct. Mol. Biol.* 17:105–11
- Lobbstaal E, Civiero L, De Wit T, Taymans J-M, Greggio E, Baekelandt V. 2016. Pharmacological LRRK2 kinase inhibition induces LRRK2 protein destabilization and proteasomal degradation. *Sci. Rep.* 6:33897
- Lu G, Middleton RE, Sun H, Naniang M, Ott CJ, et al. 2014. The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. *Science* 343:305–9
- Manasanch EE, Orlowski RZ. 2017. Proteasome inhibitors in cancer therapy. *Nat. Rev. Clin. Oncol.* 14:417–33
- Matyskiela ME, Clayton T, Zheng X, Mayne C, Tran E, et al. 2020. Crystal structure of the SALL4–pomalidomide–cereblon–DDB1 complex. *Nat. Struct. Mol. Biol.* 27:319–22



- Matyskiela ME, Couto S, Zheng X, Lu G, Hui J, et al. 2018a. SALL4 mediates teratogenicity as a thalidomide-dependent cereblon substrate. *Nat. Chem. Biol.* 14:981–87
- Matyskiela ME, Lu G, Ito T, Pagarigan B, Lu C-C, et al. 2016. A novel cereblon modulator recruits GSPT1 to the CRL4^{CRBN} ubiquitin ligase. *Nature* 535:252–57
- Matyskiela ME, Zhang W, Man H-W, Muller G, Khambatta G, et al. 2018b. A cereblon modulator (CC-220) with improved degradation of Ikaros and Aiolos. *J. Med. Chem.* 61:535–42
- Mena EL, Kjolby RA, Saxton RA, Werner A, Lew BG, et al. 2018. Dimerization quality control ensures neuronal development and survival. *Science* 362:eaap8236
- Mészáros B, Kumar M, Gibson TJ, Uyar B, Dosztányi Z. 2017. Degrons in cancer. *Sci. Signal.* 10:eaak9982
- Min J-H, Yang H, Ivan M, Gertler F, Kaelin WG, Pavletich NP. 2002. Structure of an HIF-1 α -pVHL complex: hydroxyproline recognition in signaling. *Science* 296:1886–89
- Neklesa TK, Winkler JD, Crews CM. 2017. Targeted protein degradation by PROTACs. *Pharmacol. Ther.* 174:138–44
- Nowak RP, DeAngelo SL, Buckley D, He Z, Donovan KA, et al. 2018. Plasticity in binding confers selectivity in ligand-induced protein degradation. *Nat. Chem. Biol.* 14:706–14
- Okuhira K, Ohoka N, Sai K, Nishimaki-Mogami T, Itoh Y, et al. 2011. Specific degradation of CRABP-II via cIAP1-mediated ubiquitylation induced by hybrid molecules that crosslink cIAP1 and the target protein. *FEBS Lett.* 585:1147–52
- Osborne C, Wakeling A, Nicholson R. 2004. Fulvestrant: an oestrogen receptor antagonist with a novel mechanism of action. *Br. J. Cancer* 90:S2–6
- Owa T, Yoshino H, Okauchi T, Yoshimatsu K, Ozawa Y, et al. 1999. Discovery of novel antitumor sulfonamides targeting G1 phase of the cell cycle. *J. Med. Chem.* 42:3789–99
- Palumbo A, Hajek R, Delforge M, Kropff M, Petrucci MT, et al. 2012. Continuous lenalidomide treatment for newly diagnosed multiple myeloma. *New Engl. J. Med.* 366:1759–69
- Petzold G, Fischer ES, Thomä NH. 2016. Structural basis of lenalidomide-induced CK1 α degradation by the CRL4^{CRBN} ubiquitin ligase. *Nature* 532:127–30
- Pugh CW, O'Rourke JF, Nagao M, Gleadow JM, Ratcliffe PJ. 1997. Activation of hypoxia-inducible factor-1; definition of regulatory domains within the α subunit. *J. Biol. Chem.* 272:11205–14
- Rogg LE, Bartel B. 2001. Auxin signaling: derepression through regulated proteolysis. *Dev. Cell* 1:595–604
- Rusnac D-V, Lin H-C, Canzani D, Tien KX, Hinds TR, et al. 2018. Recognition of the diglycine C-end degron by CRL2^{KLHDC2} ubiquitin ligase. *Mol. Cell* 72:813–22. e4
- Sakamoto KM, Kim KB, Kumagai A, Mercurio F, Crews CM, Deshaies RJ. 2001. Protacs: chimeric molecules that target proteins to the Skp1–Cullin–F box complex for ubiquitination and degradation. *PNAS* 98:8554–59
- Schneekloth AR, Pucheault M, Tae HS, Crews CM. 2008. Targeted intracellular protein degradation induced by a small molecule: en route to chemical proteomics. *Bioorg. Med. Chem. Lett.* 18:5904–8
- Senior AW, Evans R, Jumper J, Kirkpatrick J, Sifre L, et al. 2020. Improved protein structure prediction using potentials from deep learning. *Nature* 577:706–10
- Sheard LB, Tan X, Mao H, Withers J, Ben-Nissan G, et al. 2010. Jasmonate perception by inositol-phosphate-potentiated COI1–JAZ co-receptor. *Nature* 468:400–5
- Sievers QL, Petzold G, Bunker RD, Renneville A, Slabicki M, et al. 2018. Defining the human C2H2 zinc finger degrome targeted by thalidomide analogs through CRBN. *Science* 362:eaat0572
- Silva MC, Ferguson FM, Cai Q, Donovan KA, Nandi G, et al. 2019. Targeted degradation of aberrant tau in frontotemporal dementia patient-derived neuronal cell models. *eLife* 8:e45457
- Simonetta KR, Taygerly J, Boyle K, Basham SE, Padovani C, et al. 2019. Prospective discovery of small molecule enhancers of an E3 ligase-substrate interaction. *Nat. Commun.* 10:1402
- Slabicki M, Kozicka Z, Petzold G, Li Y-D, Manojkumar M, et al. 2020a. The CDK inhibitor CR8 acts as a molecular glue degrader that depletes cyclin K. *Nature* 585:293–97
- Slabicki M, Yoon H, Koeppl J, Nitsch L, Burman SSR, et al. 2020b. Small-molecule-induced polymerization triggers degradation of BCL6. *Nature*. In press
- Smith BE, Wang SL, Jaime-Figueroa S, Harbin A, Wang J, et al. 2019. Differential PROTAC substrate specificity dictated by orientation of recruited E3 ligase. *Nat. Commun.* 10:131

9.20 Faust et al.



- Spradlin JN, Hu X, Ward CC, Brittain SM, Jones MD, et al. 2019. Harnessing the anti-cancer natural product nimbolide for targeted protein degradation. *Nat. Chem. Biol.* 15:747–55
- Surka C, Jin L, Mbong N, Lu C-C, Jang IS, et al. 2020. CC-90009, a novel cereblon E3 ligase modulator, targets acute myeloid leukemia blasts and leukemia stem cells. *Blood*. In press
- Tan X, Calderon-Villalobos LIA, Sharon M, Zheng C, Robinson CV, et al. 2007. Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature* 446:640–45
- Timms RT, Zhang Z, Rhee DY, Harper JW, Koren I, Elledge SJ. 2019. A glycine-specific N-degron pathway mediates the quality control of protein N-myristoylation. *Science* 365:eaaw4912
- Ting TC, Goralski M, Klein K, Wang B, Kim J, et al. 2019. Aryl sulfonamides degrade RBM39 and RBM23 by recruitment to CRL4-DCAF15. *Cell Rep.* 29:1499–510.e6
- Ueguchi-Tanaka M, Nakajima M, Motoyuki A, Matsuoka M. 2007. Gibberellin receptor and its role in gibberellin signaling in plants. *Annu. Rev. Plant Biol.* 58:183–98
- Uehara T, Minoshima Y, Sagane K, Sugi NH, Mitsuhashi KO, et al. 2017. Selective degradation of splicing factor CAPER α by anticancer sulfonamides. *Nat. Chem. Biol.* 13:675–80
- Van Nguyen T, Lee JE, Sweredoski MJ, Yang S-J, Jeon S-J, et al. 2016. Glutamine triggers acetylation-dependent degradation of glutamine synthetase via the thalidomide receptor cereblon. *Mol. Cell* 61:809–20
- Verma R, Mohl D, Deshaies RJ. 2020. Harnessing the power of proteolysis for targeted protein inactivation. *Mol. Cell* 77(3):446–60
- Veziņa C, Kudelski A, Sehgal S. 1975. Rapamycin (AY-22, 989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J. Antibiot.* 28:721–26
- Ward CC, Kleinman JI, Brittain SM, Lee PS, Chung CYS, et al. 2019. Covalent ligand screening uncovers a RNF4 E3 ligase recruiter for targeted protein degradation applications. *ACS Chem. Biol.* 14:2430–40
- Winter GE, Buckley DL, Paulk J, Roberts JM, Souza A, et al. 2015. Phthalimide conjugation as a strategy for in vivo target protein degradation. *Science* 348:1376–81
- Wittmann BM, Sherk A, McDonnell DP. 2007. Definition of functionally important mechanistic differences among selective estrogen receptor down-regulators. *Cancer Res.* 67:9549–60
- Wu G, Xu G, Schulman BA, Jeffrey PD, Harper JW, Pavletich NP. 2003. Structure of a β -TrCP1-Skp1- β -catenin complex: destruction motif binding and lysine specificity of the SCF $^{\beta}$ -TrCP1 ubiquitin ligase. *Mol. Cell* 11:1445–56
- Wu Y, Zhou X, Barnes CO, DeLucia M, Cohen AE, et al. 2016. The DDB1-DCAF1-Vpr-UNG2 crystal structure reveals how HIV-1 Vpr steers human UNG2 toward destruction. *Nat. Struct. Mol. Biol.* 23:933–40
- Wu Y-L, Yang X, Ren Z, McDonnell DP, Norris JD, et al. 2005. Structural basis for an unexpected mode of SERM-mediated ER antagonism. *Mol. Cell* 18:413–24
- Xiong R, Zhao J, Gutgesell LM, Wang Y, Lee S, et al. 2017. Novel selective estrogen receptor downregulators (SERDs) developed against treatment-resistant breast cancer. *J. Med. Chem.* 60:1325–42
- Xu L, Liu F, Lechner E, Genschik P, Crosby WL, et al. 2002. The SCF $^{\text{COI1}}$ ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis. *Plant Cell* 14:1919–35
- Yang J, Anishchenko I, Park H, Peng Z, Ovchinnikov S, Baker D. 2020. Improved protein structure prediction using predicted interresidue orientations. *PNAS* 117(3):1496–503
- Yoshida Y, Chiba T, Tokunaga F, Kawasaki H, Iwai K, et al. 2002. E3 ubiquitin ligase that recognizes sugar chains. *Nature* 418:438–42
- Zhang X, Crowley VM, Wucherpfennig TG, Dix MM, Cravatt BF. 2019. Electrophilic PROTACs that degrade nuclear proteins by engaging DCAF16. *Nat. Chem. Biol.* 15:737–46
- Zheng N, Shabek N. 2017. Ubiquitin ligases: structure, function, and regulation. *Annu. Rev. Biochem.* 86:129–57

