

Targeted protein degradation: from mechanisms to clinic

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Abstract

Targeted protein degradation refers to the use of small molecules to induce the selective degradation of proteins. In its most common form, this degradation is achieved through ligand-mediated neo-interactions between ubiquitin E3 ligases – the principal waste disposal machines of a cell – and the protein targets of interest, resulting in ubiquitylation and subsequent proteasomal degradation. Notable advances have been made in biological and mechanistic understanding of serendipitously discovered degraders. This improved understanding and novel chemistry has not only provided clinical proof of concept for targeted protein degradation but has also led to rapid growth of the field, with dozens of investigational drugs in active clinical trials. Two distinct classes of protein degradation therapeutics are being widely explored: bifunctional PROTACs and molecular glue degraders, both of which have their unique advantages and challenges. Here, we review the current landscape of targeted protein degradation approaches and how they have parallels in biological processes. We also outline the ongoing clinical exploration of novel degraders and provide some perspectives on the directions the field might take.

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Introduction

Targeted protein degradation (TPD) generally refers to the use of a small molecule to induce degradation of a specific target protein. The concept of TPD was first conceived and demonstrated by recruiting a target protein to a ubiquitin ligase¹, but the idea has since been expanded to include many other cellular degradation pathways^{2–11}. For ubiquitin-mediated degradation, these molecules are commonly referred to as degraders and often categorized into molecular glue degraders and PROTACs (proteolysis targeting chimeras)¹. Although both intend to achieve the same outcome – recruitment of the target protein to a ubiquitin ligase for induced degradation, important differences exist (Box 1). Molecular glue degraders are monovalent small molecules that bind to either the ligase or target (without appreciable affinity for the other) and facilitate a neo-interface between the E3 ubiquitin ligase, the target and the molecular glue. PROTACs contain two distinct binding moieties (often referred to as warheads), one binding the E3 ligase and one binding the target, which are connected by a linker to induce proximity^{1,12}. In either scenario, an E3 ligase of choice is recruited to a target protein leading to its ubiquitylation and proteasomal degradation. TPD through the use of molecular glue degraders and PROTACs has become a widely explored therapeutic modality and powerful research tool¹³. The clinical uses of thalidomide, lenalidomide and pomalidomide (thalidomide derivatives), retrospectively found to function as molecular glues, have been established thus de-risking TPD as a therapeutic mechanism of action^{7,8,14,15}. Together with advances in the development of PROTACs, this has led to a wave of new degraders entering clinical development¹⁶. Although the concept of small molecule-induced proximity is currently undergoing rapid expansion, it is also becoming increasingly clear that many of these principles have long been exploited naturally and synthetically, providing mechanistic insights for the development of novel synthetic molecules¹⁷.

Maintaining protein homeostasis and modulating protein levels at precise times and locations are critical processes in the cell and for organismal physiology. Although protein synthesis is controlled by factors modulating transcription and translation, protein removal in mammals is largely controlled by two main pathways, the ubiquitin proteasome system (UPS) and the autophagy–lysosome pathway^{18,19}. The UPS is responsible for the majority of regulated degradation and the clearance of misfolded or damaged proteins, whereas the autophagy–lysosomal pathway is capable of degrading proteins and whole organelles as a response to stress such as nutrient starvation^{18,20}. In this Review, we focus on mechanisms of UPS-mediated degradation that rely on the activity of ubiquitin ligases as central players of the ubiquitylation cascade^{21–23} (Fig. 1a).

Selective degradation of disease-associated proteins is a powerful therapeutic tool, particularly for proteins that it has not been possible to target through other modalities. Although conceptualized two decades ago¹, excitement for the clinical applications of TPD has now accelerated with the discovery that the mode of action for the US Food and Drug Administration (FDA)-approved drugs thalidomide, lenalidomide and pomalidomide involves recruitment of the cullin-RING E3 ubiquitin ligase (CRL) CUL4A/B–RBX1–DDB1–CRBN (CRL4^{CRBN}) to the transcription factors IKZF1 (also known as Ikaros) and IKZF3 (Aiolos), leading to their subsequent ubiquitylation and degradation^{3,4,7,8,24}. More importantly, targeted degradation of IKZF1 and IKZF3 not only underlies the efficacy of these drugs in multiple myeloma but is also well tolerated.

Importantly, TPD has grown to encompass a range of other mechanisms, not necessarily mediated by E3 ligase, to degrade target

proteins. This Review serves to describe the growing number of mechanisms by which small molecules lead to proteasomal protein degradation, contrasts them with naturally occurring processes, and discuss insights and perspectives from their emerging clinical use.

Principles of targeted protein degradation

TPD commonly refers to degradation mediated by the ubiquitin proteasome system. Specificity in this pathway is conferred by recognition of target proteins by hundreds of distinct ubiquitin E3 ligases. Although many ligases will carry out non-proteolytic ubiquitylation, such as the monoubiquitylation of histone 2A at Lys119 (H2AK119ub)²⁵, a large number of ligases are responsible for protein degradation²⁰. Ligases commonly recognize substrates through short peptide motifs (referred to as degrons or linear degrons) or through small structural features (also often referred to as degrons or structural degrons) (Fig. 1b). Regulation of ubiquitin ligases often involves modulating the affinity between a ligase and its cognate degron, for example through post-translational modification of the degron^{26,27}. A classic example of a canonical ligase–degron pair is the CUL2–ELOB–ELOC–von Hippel–Lindau (VHL) ligase (CRL2^{VHL}), which recognizes a short peptide sequence in the transcription factor HIF-1 α only when a proline in the degron is hydroxylated²⁸ (Fig. 1c). Degrons are defined as being necessary and sufficient to trigger induced degradation and can still mediate E3 ligase recognition and subsequent degradation when fused to an arbitrary protein such as GFP²⁹, which suggests that most ligases are agnostic to the identity of the substrate – a pre-requisite for TPD approaches. This idea is further corroborated by evolution, as the substrate receptors of CRLs (the specificity conferring subunit of these multi-component ligase complexes; Fig. 1d) evolved independently of the remaining machinery^{30–32}. In addition, as detailed below, E3 ligases can be co-opted to ubiquitinate other substrates via small molecules or exogenous proteins, thus laying the foundation for TPD.

Additional components of the UPS have critical roles in ubiquitin-mediated protein degradation, such as the ATPase p97 (also known as VCP), which can extract or unfold poly-ubiquitylated proteins before proteasomal degradation, shuttling factors that bind to and deliver poly-ubiquitylated proteins to the proteasome, or deubiquitinating enzymes that can counteract E3 ligases. Additional factors such as sub-cellular localization and the nature of the protein of interest can influence the success of PROTAC-mediated degradation³³. Although the importance of these factors for TPD has been established^{34,35}, many mechanistic details of these additional steps and how they are differentially important for different protein families remain largely elusive and are outside the scope of this Review.

Naturally occurring molecular glue degraders

Endogenous and natural small molecules and peptides can induce protein–protein interactions, including facilitating ubiquitin ligase interactions with proteins that they would not naturally interact with, termed neo-substrates. Viruses hijack host E3 ligases, frequently CRLs, to degrade host target proteins, thereby augmenting infectivity and virion production³⁶ (Fig. 2a). Viruses use small peptides or proteins to alter the substrate specificity of ubiquitin ligases. A well-known example is the E7 protein of the human papillomavirus (HPV), which facilitates ubiquitylation and degradation of the host phosphorylated retinoblastoma protein (pRB) tumour suppressor through a direct interaction with CRL2, leading to oncogenic transformation³⁷. Other viruses use other CRLs for similar effects. The latency-associated nuclear antigen (LANA), encoded by Kaposi sarcoma-associated

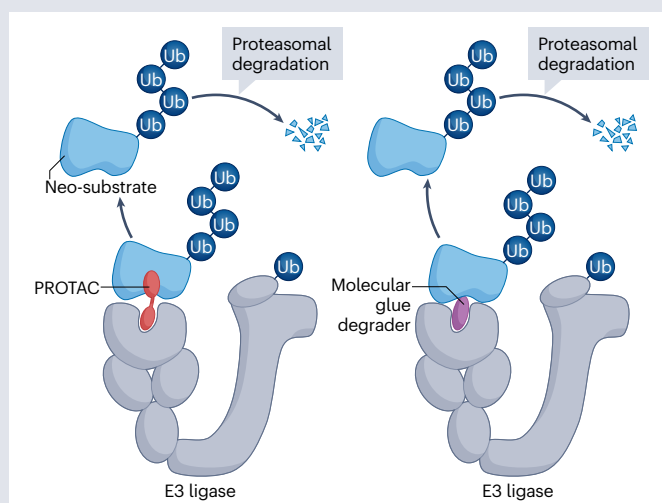
Box 1

PROTACs versus molecular glue degraders

PROTACs are modular molecules, wherein their initial design benefit derives from chemical matchmaking by linking a target binding ligand (target moiety) with the E3 ligase binding ligand (E3 moiety), resulting in a bifunctional molecule (see the figure). In theory, this design enables the creation of PROTACs for any protein of interest for which a ligand with suitable affinity exists; however, in reality considerable constraints exist. Target–PROTAC–ligase interactions form intricate composite small molecule–protein interaction surfaces and defined ternary complexes that only specific linker configurations can support^{64,212}. Hence, finding the correct linker for a target–ligase pair has become a priority in the early discovery of functional PROTACs. In addition to this empirical process, a major drawback of PROTACs is their actual size, often approaching or exceeding 1,000Da, which leads to more chemical liabilities such as poor solubility and absorption, distribution, metabolism and excretion properties²¹³. Chemical optimization based on medicinal chemistry principles therefore has had to focus on reducing molecular weights and finding a fine-tuned balance between activity and overall pharmacokinetic properties. The PROTAC linker is frequently rigidified and shortened, making the distinction between the E3 ligase and target binders less visible in the optimized PROTAC molecule. It is important to note that the prerequisite for a PROTAC is the presence of a small-molecule binder to the target protein, which therefore limits the PROTAC-targetable space to proteins with ligandable pockets. However, if the ligand used is an inhibitor, target inhibition is often retained in the final PROTAC, which can confound the pharmacology.

Molecular glue degraders bind to the target protein, or more frequently, the E3 ligase, imparting additional chemical properties to the protein surface and enhancing structural complementarity,

thereby leading to direct neo-interactions between the target and ligase (see the figure). The ability for molecular glue-bound proteins to recognize structural motifs, such as beta-hairpin loops (in the case of immunomodulatory imide drugs that bind CRBN)^{73,74}, allows them to engage targets based on structural complementarity rather than ligand binding, enabling degradation of previously unligandable proteins. Molecular glue degraders generally have a lower molecular weight and thereby usually better chemical properties, providing for an easier starting point for medicinal chemistry optimization than PROTACs. The critical challenge with molecular glue degraders is the discovery of initial molecules beyond the well-established CRBN system.



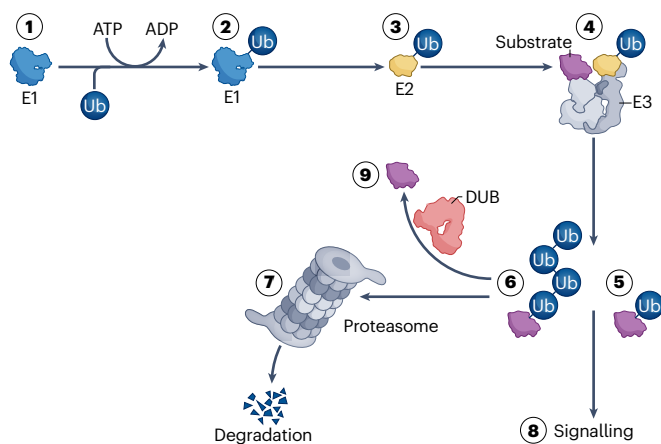
herpesvirus (KSHV), hijacks CLR5, interacting with, ubiquitylating, and degrading host p53 and VHL³⁸. The Vif and Vpu proteins of the human immunodeficiency virus (HIV) similarly interact with CRL5 and CRL1 to degrade A3 proteins and CD4, respectively^{39,40}. Notably, in the case of Vif, RNA serves as a molecular glue facilitating the interaction between the ubiquitin ligase, Vif and the A3 proteins^{41,42} (Fig. 2b). These mechanisms shed light on the breadth of the biochemical space available to engineer novel interactions between E3 ligases and proteins of interest.

In addition to protein-mediated or nucleic acid-mediated neo-substrate interactions that have been characterized in viruses, plants have adapted a similar mechanism through the use of auxins, a class of phytohormones used to regulate growth⁴³ (Fig. 2c). Auxins are soluble small molecules that control gene expression. The SCF^{TIR1} pathway is prototypic of auxin signalling; auxin response factors (ARFs) bind to auxin responsive elements (AREs) at auxin-responsive promoters leading to transcription whereas early auxin-response proteins bind to ARFs to inhibit transcription^{17,44,45}. Auxins such as indole-3-acetic acid (IAA) bind to the F-box containing SCF^{TIR1} complex, leading to the proteasomal degradation of the early auxin-response proteins⁴⁶. Importantly, IAA enhances and/or stabilizes the interaction

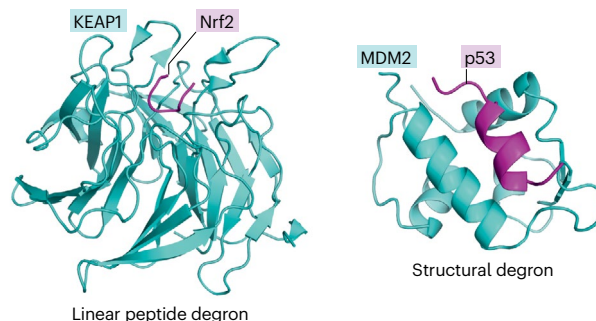
between TIR1, the substrate receptor of the SCF^{TIR1} complex and early auxin-response proteins and is a naturally occurring molecular glue that leads to degradation, which has led to its use as an inducible decon system⁴⁷. A myriad of developmental processes is controlled by auxins, including lateral root formation, new leaf formation and leaf vascular development, and main root growth, highlighting the physiological importance and intricacies of small molecule-induced degradation^{43,44}.

No direct mammalian counterpart to auxins had been identified until a study in 2021 demonstrated that zinc ions act as molecular glues in the reductive stress response⁴⁸. Degradation of the mitochondrial protein FNIP1 reduces reductive stress resulting from oxidative phosphorylation. As demonstrated by X-ray crystallography, two Zn²⁺ ions are needed to selectively recruit CUL2^{FEMIB} to reduced FNIP1 during reductive stress⁴⁸ (Fig. 2a). Interestingly, premature degradation of FNIP1 is prevented by the BEX family of proteins (BEX2 and BEX3), which act as pseudo-substrate inhibitors of CUL2^{FEMIB}. Indeed, BEX deletions lead to the accumulation of reactive oxygen species and developmental disorders^{48,49}. Zinc ions therefore represent an example of an endogenous molecular glue that tightly regulates a key biological process.

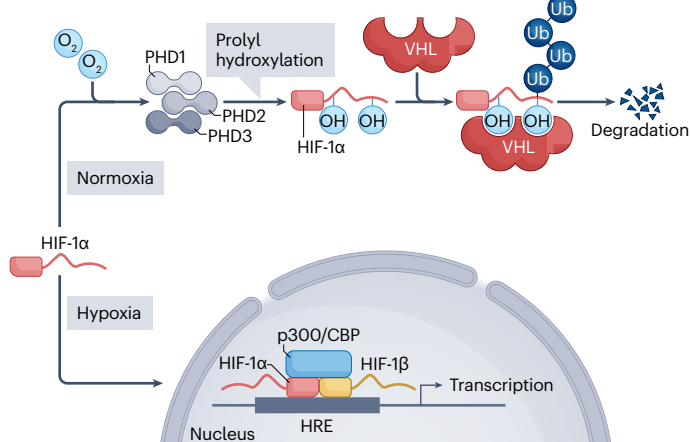
a Ubiquitin proteasome degradation



b Protein degron types



c Conditional protein degrons



d CRL architecture

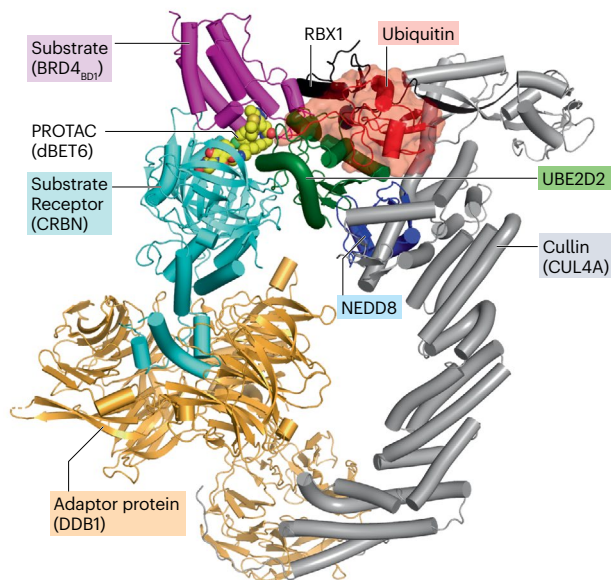


Fig. 1 | Ubiquitin-mediated degradation systems and degrons. **a**, The ubiquitin proteasome pathway. The ubiquitylation pathway is a multi-enzyme cascade that canonically utilizes one of two E1 activating enzymes, a range of E2 enzymes and an E3 ubiquitin ligase to achieve transfer of ubiquitin to the substrate. (1) The E1 adenylates the C-terminal carboxyl group of ubiquitin in an ATP-dependent process known as activation. (2) Ubiquitin is transferred to a cysteine residue on E1. (3) Ubiquitin is transferred to the active-site cysteine of an E2 enzyme. For most ligases, (4) E2-bound ubiquitin binds to an E3 ligase and (5) discharges the ubiquitin to a proximal substrate wherein ubiquitin forms an isopeptide bond, typically with a lysine of the substrate. Some ligases such as HECT or RBR involve an intermediate step of ubiquitin being transferred to an active-site cysteine in the ligase. (6) Ubiquitylation of the substrate can lead to (7) the protein being shuttled to proteasome and degraded or (8) various signalling events. (9) The process of ubiquitylation is countered by the removal of ubiquitin catalysed by deubiquitinase enzymes (DUBs). **b**, Two main types of degrons. Linear degron exemplified by peptide Nrf2, which is recognized by E3 ligase CRL3^{KEAP1} for degradation (Protein Data Bank (PDB) code 2DYH). Structural degron, the

alpha helix of p53, is recognized by its cognate E3 ligase MDM2 (PDB: 1YCR). **c**, Conditional protein degrons. Post-translational protein modification can be a trigger for degradation. In normoxia or high oxygen concentrations, HIF-1 α is hydroxylated on proline residues by iron-dependent and oxygen-dependent prolyl hydroxylases (PHD1, PHD2 and PHD3) producing a degron recognized by an E3 ligase CRL2^{VHL}, resulting in its ubiquitination and degradation. By contrast, in hypoxia, the hydroxylation process is impaired and HIF-1 α forms a complex with HIF-1 β ; the transcriptional coactivator p300–CPB binds hypoxia response element (HRE) and activates transcription. **d**, General architecture of cullin-RING ligases (CRLs). CRL complexes are formed of one of the seven cullin proteins (CUL1, CUL2, CUL3, CUL4A, CUL4B, CUL5 or CUL7) bound to an adaptor protein (such as DDB1) and substrate receptor (such as CRBN). The example shows a model of a fully neddylated CRL^{CRBN} E3 ligase bound to dBET6 PROTAC in complex with BRD4_{BD1}, with the E3 ligase engaged with E2 UBE2D2 charged with ubiquitin. The model was composed by combining structures of parts of the complex and alignment over a common subunit of CRBN–dBET6–BRD4_{BD1} (PDB: 6BOY), DDB1 (PDB: 4AOK) and CRL4–RBX1–NEED8–UBE2D2–ubiquitin (PDB: 8B31).

First forays into targeted protein degradation by design

The concept of bifunctional molecules as a means to recruit a ubiquitin ligase to a target of interest for protein degradation (that is,

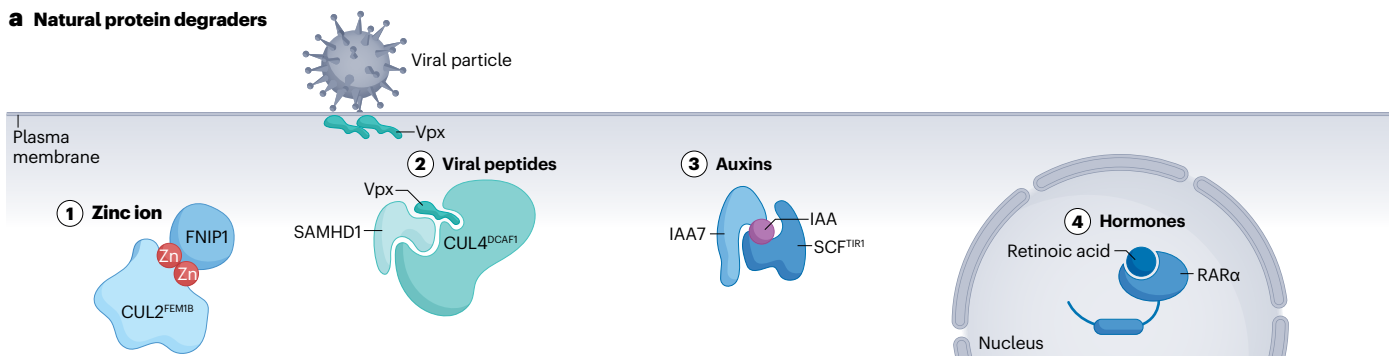
PROTACs) was first described in 2001 (refs. 1,50). These early studies have focused on recruiting target proteins to the SKP1–CUL1–F-box (SCF) complex¹ via a peptide-based small molecule (Protac-1), leading to Protac-1-dependent ubiquitylation and degradation of MetAP-2 in

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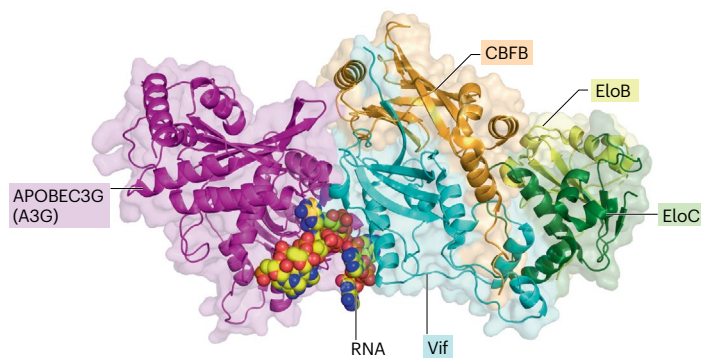
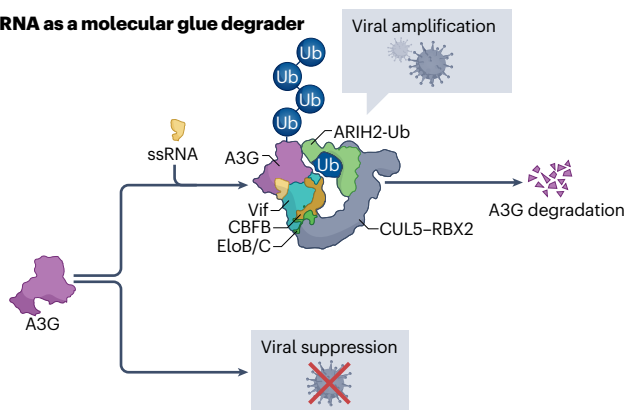
cell lysates¹. The first entirely small-molecule PROTAC was described in 2008, in which a small-molecule binder for the E3 ubiquitin ligase MDM2 was conjugated to the androgen receptor ligand with a linker,

resulting in the cellular degradation of androgen receptor in cells⁵¹. The modular design of PROTACs consisting of E3 moiety and target moiety and a linker remains the definition and distinction from molecular glue

a Natural protein degraders



b RNA as a molecular glue degrader



c Hormone as a molecular glue degrader

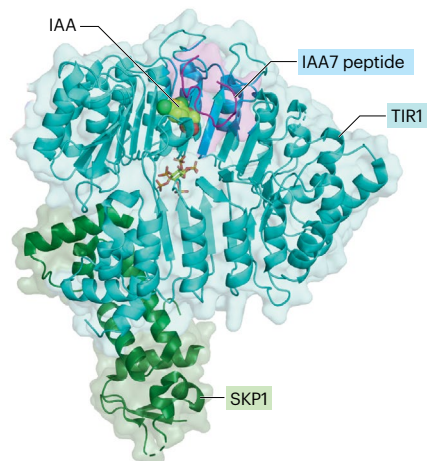
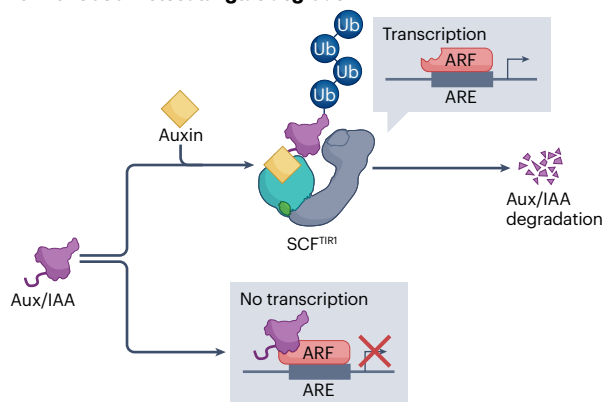
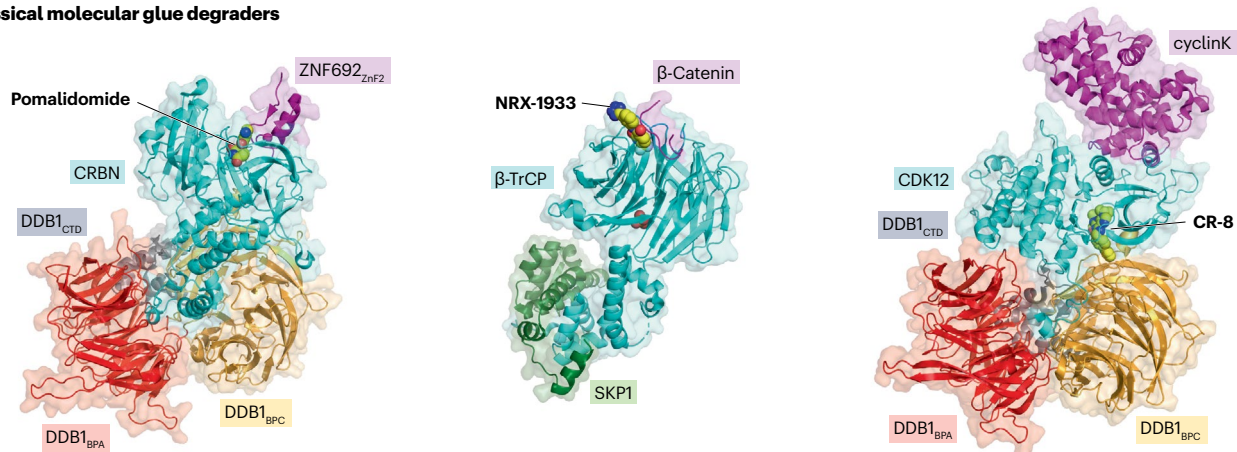


Fig. 2 | Examples of natural means of biomolecule-induced degradation.

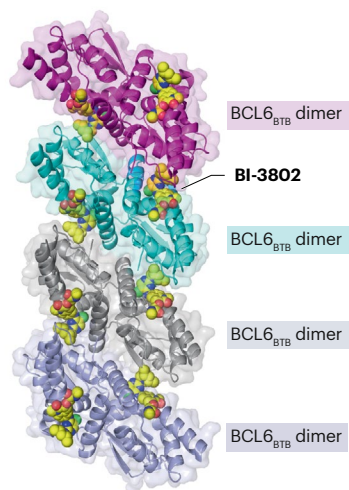
a, Naturally occurring protein degraders. (1) Two Zn²⁺ ions are required to recruit E3 ubiquitin ligase CUL2^{FEM1B} to reduced FNIPI during reductive stress. (2) Viral peptides hijack native E3 ligases to redirect them for degradation of host proteins. Viral peptide Vpx1 binds CUL4^{DCAF1} E3 ubiquitin ligase and induces degradation of host restriction factor protein SAMHD1. (3) Plant hormone auxin binds to an E3 ubiquitin ligase SCF^{TIR1} and leads to degradation of auxin response proteins modulating plant growth. (4) Nuclear receptors undergo proteasomal degradation following ligand binding. **b**, RNA as a molecular glue degrader. Ternary complex of the RNA mediating interaction between human E3 ligase adaptor Vif and viral factor

APOBEX3G (A3G) (PDB: 8CX0). The native function of host deaminase A3G (which is packaged into a viral capsid) is to block viral replication. Degradation of A3G is mediated by the recognition of the unpaired single-stranded RNA (ssRNA) by viral factor Vif, which hijacks CUL5 E3 ubiquitin ligase (the CUL5-RBX2-EloB-EloC-CBFB-ARIH2 complex shown in the figure is loaded with ubiquitin) and allows the virus to replicate. **c**, Hormone as a molecular glue degrader. Plant hormone auxin mediates an interaction between plant E3 ubiquitin ligase SCF^{TIR1} and IAA7 peptide (PDB: 2PIN). Auxin results in degradation of Aux/IAA, preventing it from binding auxin response factors (ARFs) and auxin response elements (AREs), thus releasing suppression of downstream genes, resulting in cell division, stem and root growth.

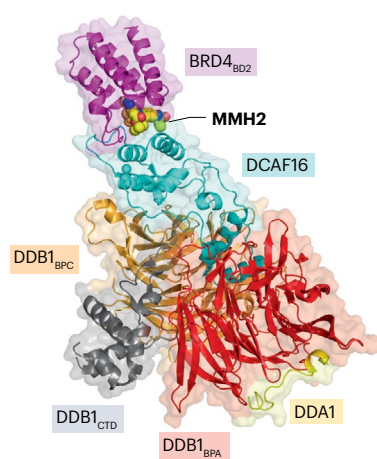
a Classical molecular glue degraders



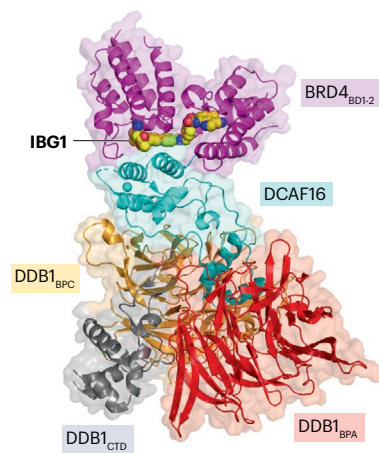
b Protein polymerization



Template-assisted covalent labelling



Intramolecular bivalent degrader glues



c PROTACs

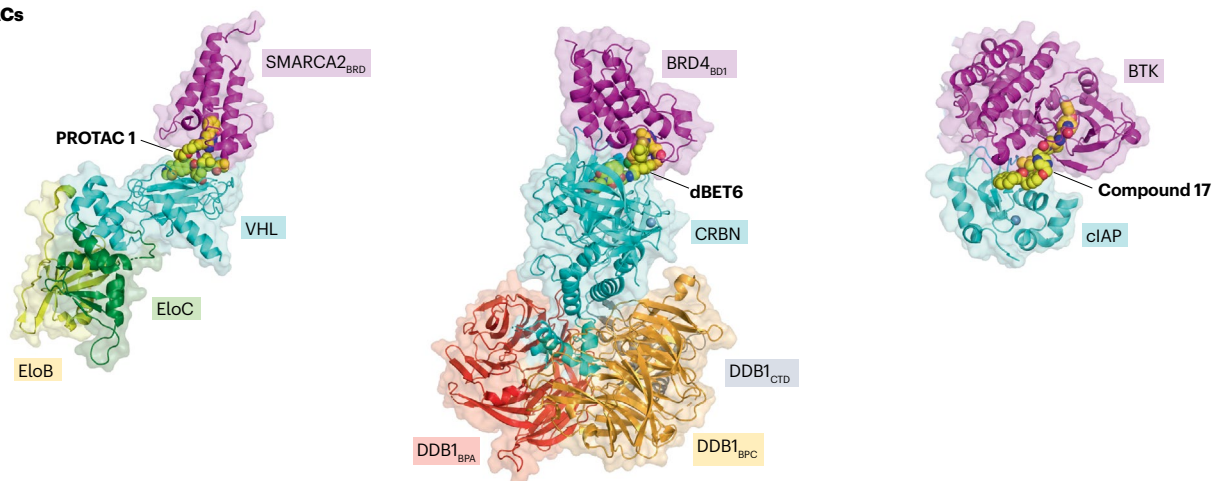


Fig. 3 | Diverse molecular mechanisms can lead to substrate recruitment for targeted protein degradation. Representative examples of structurally characterized molecular recognition events are shown. **a**, Classical molecular glue interactions exemplified by pomalidomide (PDB: 6H0G), NRX-1933 (PDB: 6M93) and CR-8 (PDB: 6TD3). **b**, Non-canonical or more recent discovery mechanisms such as polymerization (PDB: 6XMX), *trans*-covalent glues

(PDB: 8G46) or intramolecular glues (PDB: 8OV6). **c**, PROTACs that have been demonstrated to form intricate ternary complexes (VHL PDB: 6HAY; CRBN PDB: 6BOY; cIAP PDB: 6W7O). Models built into cryo-EM or X-ray crystallography maps were obtained from the protein databank and rendered in PyMOL. For chemical structures, see Supplementary Fig. 1.

degraders, which are monovalent. The modular design of PROTACs makes their discovery more rational in principle as one can modularly use binders for ligase and target, but it also dictates some challenging chemical properties for drug development. Specifically, PROTAC design usually leads to large molecules (≥ 700 Da) that require extensive and often empirical optimization to achieve pharmacokinetic properties sufficient for clinical use.

Although the early studies regarding PROTAC development were known to a small expert community, the true potential of PROTACs became apparent after the discovery of potent small-molecule binders such as thalidomide and a ligand for the ligase CRL2^{VHL} inspired by the HIF-1 α peptide^{6,52}. Although thalidomide was serendipitously uncovered to be a potent ligand for CRL4^{CRBN} exhibiting chemical properties favourable for PROTACs^{3,4,6,53}, discovery of binders to VHL, the substrate receptor of the CRL2^{VHL} E3 ligase, required extensive chemistry efforts using the hydroxyproline on HIF-1 α (Hyp564) as a starting point. Hydroxyproline analogues were designed in silico and later with structure-guided medicinal chemistry and optimized for VHL binding. The use of these potent and relatively small E3 ligase ligands was demonstrated to be highly effective in proof-of-principle PROTAC molecules targeting bromodomain-containing protein 4 (BRD4) and the peptidyl-prolyl *cis*-*trans*-isomerase FKBP12 for degradation^{54–56}.

Additional E3 ligases that have been enabled so far include HDM2 (also known as MDM2)^{57,58} and members of the inhibitor of apoptosis (IAP) protein family^{59,60}, which were enabled by the discovery or repurposing of small-molecule ligands. Each of these ligases and discovered ligands carries its own, at times undesirable characteristics, such as the auto-degradation of IAP caused by some IAP ligands, or inhibition of the function of HDM2 owing to the high affinity of HDM2 binders leading to p53 dependent toxicity. A stated goal of expanding the ligase repertoire has been to achieve tissue-restricted degradation of targets whose systematic depletion would not be tolerated. Although this goal has so far not been fully realized yet, it could offer a path to highly differentiated therapeutics and for mitigating on-target, off-tissue toxicities⁶¹. Efforts in academia and industry have led to the discovery of hundreds of PROTACs for targets including kinases, hormone receptors, and proteases¹², with a number of these now in clinical development. Large scale efforts have been conducted to map degradable targets, and methods developed such as dedicated chemo-proteomics approaches^{34,62}. These studies (extensively reviewed elsewhere^{63,64}) have demonstrated the ability of PROTACs to remove proteins rapidly and selectively from the cell and have accelerated the ability to design and synthesize these molecules⁵⁶.

Serendipitous discovery of new protein degraders

Although there is now tremendous excitement in the field of TPD with a growing number of molecular glues and PROTACs identified and in development, the clinical use of molecular glues has been slower.

An unexpected clinical proof of concept

A catalysing event for TPD was undoubtedly the clinical proof of concept for small molecule-mediated TPD through synthetic degraders,

which arose from an unexpected line of research: the search for the mechanism of clinical activity of the thalidomide derivative lenalidomide in multiple myeloma^{65,66}. Thalidomide was discovered and marketed as non-barbiturate sedative and anti-emetic in the 1950s and 1960s (ref. 67). Its popularity led to widespread use, particularly during pregnancy, which resulted in tragic consequences: many birth defects were discovered to be caused by thalidomide teratogenicity^{68,69}. Decades after the drug was withdrawn from markets, it was found to be effective for the treatment of leprosy-related erythema nodosum and for multiple myeloma and other haematological malignancies, but the mechanism of action remained elusive^{70,71}. In 2010, thalidomide was reported to directly bind to – and hypothesized to inhibit – cereblon (CRBN)⁶, the substrate receptor protein of the CRL4^{CRBN} E3 ubiquitin ligase. In an unexpected twist, studies investigating the clinical activity of thalidomide and lenalidomide in multiple myeloma found that these drugs act as molecular glue degraders, resulting in the degradation of the transcription factors IKZF1 and IKZF3^{3,4,6–8,24}. Lenalidomide now forms the backbone of treatments for multiple myeloma and subsets of myelodysplastic syndrome (5q-MDS)⁷². Structural biology studies have revealed that thalidomide and derivatives reshape the surface of CRBN, allowing it to bind to a beta-hairpin in the zinc-finger domain, forming the structural degron with a glycine residue at a key position. This degron was quickly realized to be not only present in many C₂H₂ zinc-finger (ZnF) transcription factors, but also in other proteins^{73,74}. This discovery had a profound impact on the rise of TPD as it provided clinical proof of concept and established the efficacy and safety of molecular glue degraders in the clinic^{12,75}. Moreover, the 260 Da thalidomide possesses highly favourable chemical properties to serve as an E3 ligase binder for the development of PROTACs, and is, often with some minor modifications, therefore widely used in PROTACs including dominantly in clinical stage drugs^{54,55} (Fig. 3).

In addition to catalysing widespread interest and investments into drug discovery for TPD, CRBN binders related to thalidomide have proved to be unexpectedly versatile in targeting a wide variety of previously undruggable targets. Structural studies^{73,74,76} and a systematic search for neo-substrates⁷⁶ have quickly revealed that immunomodulatory imide drugs (IMiDs; including thalidomide, lenalidomide and pomalidomide) target zinc-finger transcription factors and other proteins beyond IKZF1 and IKZF3, including the probable culprit of the embryotoxicity of thalidomide, SALL4 (refs. 77,78). These findings led academic and biopharmaceutical groups to develop libraries in search of new molecular glues repurposing CRBN and resulted in successful demonstration of TPD for novel targets such as GSPT1 (ref. 79), IKZF2 (also known as Helios)^{80,81}, PATZ1 (ref. 82) or CDK2 (ref. 83).

The discovery that IMiDs, including the widely used drug lenalidomide, work by inducing neo-substrate interactions, also provided important answers to concerns about risks or class toxicities associated with TPD drugs. Clinical data beyond IMiDs and later-stage PROTACs (such as ARV-471) are still very limited; however, a few early observations include the following: first, the induced degradation of proteins such as IKZF1, IKZF3 or oestrogen receptor (ER) itself does not seem

to present degradation-based toxicities such as the overloading of the UPS. Second, embryotoxicity is probably not limited to thalidomide as many molecular glue degraders or PROTACs based on CRBN ligands can also exert neo-substrate activity, including degradation of SALL4^{84,85}. This finding suggests that the CRBN warhead alone can cause off-target effects and might be an additional reason for the pursuit of alternative E3 ligases for use in TPD. Third, these human toxicities do not tend to be observed in species commonly used for toxicity studies such as rodents or canines, which have species-specific polymorphisms rendering them inert to molecular glue-mediated neo-substrate activity^{85,86}. Specifically, CRBN mutations in these species disrupt G-loop-mediated recruitment of neo-substrates subsequent to IMiD binding, preventing their induced degradation⁸⁷. Accordingly, toxicity studies need to be in the appropriate CRBN knock-in models⁸⁷ or non-human primates. Fourth, given the documented off-target effects and their specificity to humans, target and molecule-specific toxicities must be carefully controlled for and considered. Finally, although CRBN has been extensively utilized in the TPD space, knowledge about its biological function or native substrates remains limited. CRBN has been proposed to recognize C-terminally cyclized glutamine or asparagine residues^{88,89}, but the biological function remains to be fully elucidated^{90,91}. Not unexpected given that they represent isosteres of IMiDs, these degron-inspired CRBN binders can be derivatized to potent CRBN ligands for TPD application⁹².

Molecular glue degraders beyond thalidomide derivatives

In 2017, the aryl sulfonamide indisulam (E7070) was identified as a molecular glue that facilitates a neo-interaction between the DDB1 and cullin-associated factor DCAF15 as part of the CUL4–DDB1–DDA1–DCAF15 (CRL4^{DCAF15}) ligase complex and the splicing factor RBM39, leading to RBM39 degradation^{93,94}. Indisulam leads to G1 cell cycle arrest and cell death. Indisulam-mediated toxicity requires RBM39 degradation, which can be abrogated by mutations to the degron in RBM39 in cell lines and xenograft models^{95,96}. Recombinant studies have demonstrated indisulam-dependent ternary complex formation, involving RBM39 and DCAF15, which was similarly abrogated through the same degron mutants. Subsequent structural studies have elucidated the mechanism by which DCAF15 interacts with the alpha helical degron in RBM39 through nonpolar interactions in an indisulam-dependent manner^{97–99}. This phenomenon was found to also underlie the activity of other sulfonamides, namely tasisulam and chloroquinoxaline. Both IMiDs and aryl sulfonamide small molecules establish neo-interactions between substrate adaptors and targets but differ at the molecular level in how they engage ligases. Aryl sulfonamides bind to DCAF15 with weak affinity (apparent dissociation constant ($K_{d(\text{app})}$) \approx 3 μM for indisulam or $K_{d(\text{app})}$ $>$ 50 μM for tasisulam) and yet potently stabilize the interaction with the alpha-helical degron in RBM39, whereas IMiDs bind more strongly ($K_{d(\text{app})}$ $<$ 1 μM) and recruit beta-hairpin based degrons. Owing to the largely side chain-mediated interaction of the DCAF15–aryl sulfonamide–RBM39 complex, the interaction is also more specific^{97–99}.

Since the initial characterization of synthetic molecular glues, numerous examples of neo-interactions leading to target degradation have been identified. The majority of these glues remodel the interface between the protein target and the E3 ligase substrate receptor, but interesting variations of this concept have been recognized. The integration of multiple unbiased genetic and chemical screening approaches led to the identification of a small molecule, CR8 (a known CDK inhibitor) that results in the selective degradation of cyclin K¹⁰⁰ (Fig. 3a). What was surprising, however, was that although genetic

screens highlighted the role of CUL4 and DDB1 in mediating the degradation, no protein serving as the substrate receptor (such as a DCAF) was identified. Instead, *in vitro* co-immunoprecipitation experiments and structural analyses demonstrated that CR8 instead binds CDK12 and creates a novel interface that glues the CDK12–cyclinK complex to DDB1, placing CDK12 in the position wherein a substrate receptor would normally bind, thereby inducing cyclin K degradation. This example, and the expansion of CR8 derivatives, highlights an underappreciated flexibility in recruiting CRLs by molecular glues and suggests that multiple interfaces – not necessarily involving the target itself – can be remodelled to induce TPD^{101–103}.

Discovery of novel molecular glues

Thalidomide derivatives and indisulam have all been found to be molecular glue degraders retrospectively; efforts to discover novel molecular glues are required to expand the scope of potential targets. Perhaps one of the first published examples of a rational approach to the discovery of molecular glue degraders was the identification of small molecules that enhance the interaction between β -catenin (an oncogenic transcription factor) and its natural E3 ligase, SCF ^{β -TrCP}¹⁰⁴. β -Catenin is a Wnt pathway signalling protein, often dysregulated and stabilized in cancers¹⁰⁵. It is phosphorylated by glycogen synthase kinase 3 (GSK3) and casein kinase 1 (CK1), creating a phospho-degron that is recognized by β -TrCP, a substrate adaptor of the SCF ^{β -TrCP} CRL²⁶. Hypothesizing that small molecules could be found that overcome the phosphorylation dependence, the authors screened for and optimized small molecules and identified NRX-1933 as a molecular glue for the phospho- β -catenin– β -TrCP interaction (Fig. 3a). Further chemical optimization led to NRX-252114, which showed improved recruitment and ubiquitylation of a specific phosphorylated form of β -catenin¹⁰⁴.

An alternative approach is the chemical diversification of ligands for ligases predated to exhibit molecular glue activity, most prominently CRBN. Two published studies have taken advantage of this approach to target the IKZF2 zinc-finger transcription factor^{80,81}, a close relative of IKZF1 and IKZF3. By chemically diversifying glutarimide-based ligands of CRBN, initial hits were obtained that led to the development of NVP-DKY709 (ref. 80) and ALV1 and ALV2 (ref. 81), which all mediated recruitment of IKZF2 to CRBN. Structure-guided optimization ultimately led to highly potent and selective molecular glue degraders of IKZF2. This finding demonstrates that highly selective degradation is attainable even amongst closely related proteins using the same ligase. The first IKZF2 degraders are now in clinical development¹⁰⁶.

Another example is the discovery of thalidomide derivatives that target the translation termination factor GSPT1, which was found through phenotypic screening⁷⁴. Extended efforts have also been made to improve on the efficacy and tolerability of lenalidomide, for example optimizing the potency of IKZF1 and IKZF3 degradation and selectivity over other neo-substrates, leading to molecules such as CC-92480 (ref. 107). Pipeline programs disclosed by biotechnology and pharmaceutical companies, together with the growing number of targets identified for thalidomide derivatives in unbiased screens⁷⁶ suggests that there is significant room for expansion via this approach to enable degradation of other targets beyond closely related zinc-finger containing proteins. The discovery of novel molecular glue degraders is a fast-moving area of high interest in the field and as such is subject to extensive research. A number of innovative screening approaches have been devised and tested on established systems such as the use of isogenic cell lines lacking a key component of the UPS together with phenotypic screening^{103,108}, using large-scale, small-molecule and genetic

dependency data¹⁰⁰, or DNA-encoded libraries¹⁰⁹. A more complete account of this rapid development is summarized elsewhere^{90,110,111}.

Variations on targeted degradation

Although the majority of mechanisms being pursued in the field of TPD primarily focus on the induction of neo-interactions, it would be incorrect to consider them as a single mechanism. In fact, a number of variations on this concept exist that lead to different biological and clinical effects.

Endogenous small molecule-induced neo-interactions in nuclear receptors

Nuclear receptors are a family of structurally related transcription factors that are transcriptionally activated by ligands. A number of nuclear receptors undergo proteasomal degradation following ligand binding, including the retinoic acid receptor alpha (RARA), glucocorticoid receptor, and oestrogen receptor (ER)^{112–114}. Although not molecular glues in the traditional sense, nuclear receptor ligands (such as hormones) induce stereotypic structural changes following ligand binding that reveals a conserved, hydrophobic cleft that is recognized by nuclear co-activators (NCOAs). Thus, hormone binding leads to transcriptional activation of downstream nuclear receptor targets through NCOA binding. This hydrophobic cleft represents a shielded degron that is exposed following ligand binding¹¹⁵. The HECT E3 ubiquitin ligase UBR5 recognizes this shielded site and competes with NCOAs for nuclear receptor binding, leading to degradation. In this model, the nuclear receptor degron is twice-shielded: first, while in the inactive state through steric hindrance by helix 12 of the nuclear receptor and, second, through direct engagement with NCOAs.

A class of nuclear receptor ligands that act through a parallel, though disparate, mechanism are selective oestrogen receptor degraders (SERDs). These molecules target and degrade the ER for treatment of HER2 negative and hormone positive breast cancers^{116–118}. Fulvestrant, a prototypic SERD, includes a bulky hydrophobic tail attached to an oestradiol scaffold, and is a product of a systematic chemical effort aimed at modification of long-chain alkyl substitutes in the 7a-position of oestradiol^{117,119}. Although many additional SERDs have been developed, none had received FDA clearance until the approval of elacestrant in January 2023. The reasons for the relatively poor success rates of SERDs are unclear, but their mechanisms of ER antagonism and degradation are clearly not straightforward. Fulvestrant has been extensively investigated; studies at Genentech suggest that its primary anti-oestrogen effect comes not from ER elimination, but from markedly slowing the intra-nuclear motility of ER¹²⁰. Further studies have investigated numerous SERDs and found that acrylic side-chained SERDs engage the SUMO pathway and RNF111, whereas basic side-chained SERDs use UBR5 and a degradation pathway similar to that of oestradiol^{115,121}. Sumoylation involves a family of small proteins similar to ubiquitin in that they are attached to specific lysine residues leading to different aspects of protein regulation and function. In this case, RNF111 probably only recognizes sumoylated ER, allowing it to subsequently be ubiquitinated. Further studies are needed to understand the clinical consequences of engagement of either degradation pathway.

Small-molecule covalent degraders

Enabling additional ligases for TPD could expand the scope of targets^{34,122}, which has led investigators to explore alternative routes, such as covalent ligands^{123–125}. These molecules can directly modify components of the UPS through covalent or reversible covalent reactions,

changing the landscape of interacting partners, or can be used as handles for the development of PROTACs. For example, activity-based protein profiling demonstrated that nimbolide interacts with the E3 ligase RNF114 through a previously uncharacterized cysteine residue (C8) in an intrinsically disordered region needed for substrate recognition¹²⁶. Nimbolide normally functions by covalently modifying C8, which disrupts the ability of RNF114 to ubiquitylate and degrade p21 and other tumour suppressors (leading to the stabilization of these tumour suppressors and thus disruption of cancer cell proliferation). Nimbolide was then used as an E3 moiety in a PROTAC that recruits RNF114 to target the transcriptional and epigenetic regulator, BRD4 – which was subsequently successfully targeted for degradation¹²⁶.

Analogous studies have instituted covalent fragment-based ligand discovery (FBLD) screening using covalent fragments with broad cysteine reactivity^{123–125}. FBLD is useful in the identification of binding hot spots and to obtain starting points for iterative chemistry towards chemical probes. Fragments found to react with ubiquitin ligases are coupled to reversible inhibitors, including those against BRD4 and FKBP12, forming PROTACs that target E3 ligases including DCAF16 (ref. 127). Covalent modification of E3 ligases has potential benefits over reversible PROTACs¹²⁸; covalency, or directly linking the molecule to the E3 ligase, couples the residence time of the ligand on the ligase to the natural turnover time of the ligase¹²⁹, which could result in a more durable activity; however, whether this is advantageous has yet to be demonstrated. As a consequence, several other covalent degraders using ligases such as DCAF1, DCAF11, DCAF15, DCAF16, RNF116, FEM1B or even E2-conjugating enzymes have been characterized¹³⁰.

Finally, during screens to identify novel molecular glues that target BRD4, it was noted that a novel series of covalent molecules, based on a well-characterized BRD4 binder, a BET bromodomain inhibitor JQ1 (ref. 131), recruit the CUL4^{DCAF16} ligase to the second bromodomain of BRD4 (BRD4_{BD2}). Interestingly, BRD4_{BD2} binds the JQ1-based molecular glue MMH2 to form a complex that acts as a template to covalently modify DCAF16, which in turn stabilizes the entire BRD4_{BD2}–CUL4^{DCAF16} complex, leading to BRD4 degradation. Cryo-electron microscopy structural studies and subsequent genetic validation have identified a cysteine at position 58 of DCAF16 as the recipient of covalent modification (Fig. 3b). These findings demonstrate a covalent labelling in trans, referred to as ‘template-assisted covalent modification’, in which a small molecule reversibly binds to one protein and is then covalently attached to a recruited protein^{132,133}.

A parallel study has identified an intra-molecular bifunctional small molecule, IBG1, which engages two bromodomains of BRD4 and thereby positions them in a manner to form a neo-substrate for the CUL4^{DCAF16} ligase¹³⁴. Interestingly, the initial set of molecules was identified in a screen for BRD4 targeting PROTACs based on aryl sulfonamide-based DCAF15 ligands, and was subsequently identified in a CRISPR screen to act via DCAF16 substrate receptor¹³⁴. Structural studies have shown that the degrader molecules engage both BRD4 bromodomains leading to a large conformational change and inducing contacts with DCAF16 (Fig. 3b). This finding demonstrates how subtle differences can lead to complete rewiring of protein–protein interactions, highlighting the importance of proper experimental controls when determining the mechanisms of action of degraders.

Degradation by small molecule-induced polymerization

Another mechanism of small molecule-induced degradation is drug-induced polymerization of the target protein that triggers subsequent ubiquitylation and proteasomal degradation. The small molecule

Glossary

Acetylation tagging

(AceTAG). Acetylation tagging system in which heterobifunctional molecules composed of ligands to acetyltransferase p300/CBP are linked by binder to FKBP12^{F36V} and can acetylate FKBP12^{F36V}-tagged target proteins.

Activity-based protein profiling

(ABPP). A proteomics-based technology that uses chemical probes, usually consisting of a reactive group (warhead), a chemical linker and a reporter group including a tag. Probes whose reactive groups react with target proteins are isolated and identified via proteomics.

Antibody targeting chimaeras

(AbTACs). Recombinant bispecific antibodies that recruit E3 ligases bound to the membrane for degradation of cell-surface proteins.

Cullin-RING E3 ubiquitin ligase

(CRL). Family of E3 ubiquitin ligases that contain a cullin protein (CUL1, CUL2, CUL3, CUL4A, CUL4B, CUL5 or CUL7). These multicomponent complexes frequently include adaptor proteins in between the substrate receptors.

DDB1 and cullin-associated factor

(DCAF). Proteins associated with cullin proteins, such as substrate receptors or substrate adaptors, in CRLs.

Dephosphorylation-targeting chimaeras

(DEPTACs). Heterobifunctional peptides that recruit a phosphatase to a target protein. The approach was demonstrated to dephosphorylate tau with chimaeric peptides composed of tau binding motif, linker, PP2A phosphatase binding motif and cell-penetrating sequence.

Deubiquitinase-targeting chimaeras

(DUBTACs). Heterobifunctional small molecules consisting of a deubiquitinase recruiter and a target binder. A DUBTAC has been demonstrated to deubiquitinate Δ F508-CFTR by using a covalent allosteric OTUB1 recruiter linked to lumacaftor, a Δ F508-CFTR binding small molecule.

Fragment-based ligand discovery

(FBLD). A ligand discovery approach that uses small chemical fragments instead of elaborated structures in an attempt to cover wider chemical spaces with fewer molecules. After initial low-affinity binding, fragments are discovered — often using chemoproteomics approaches; these fragments are elaborated (such as the addition of functional groups, atoms or other scaffolds) into larger molecules with improved binding affinity.

Immunomodulatory imide drugs

(IMiDs). A group of drugs that include the small molecules thalidomide, lenalidomide and pomalidomide, known for their immunomodulatory functions. IMiDs bind CRBN and cause degradation of transcription factors including IKZF1/3.

Lysosome-targeting chimaeras

(LYTACs). Antibodies modified with a lysosome-targeting ligand, such as the tri-GalNAc substrate of asialoglycoprotein receptor (ASGPR), thus enabling the internalization of the antibody bound with its protein target. LYTACs target cell surface proteins for lysosomal degradation.

mRNA display technology

A display-based technique that evolves peptides or proteins that bind to a specific target. A DNA library is first synthesized with sites for T7 RNA polymerase transcription and ribosomal binding. DNA libraries are transcribed in vitro to an mRNA library and ligated to a DNA spacer attached to puromycin. The resulting library is translated in vitro to a peptide library that is covalently linked to the mRNA and selected for binding to an immobilized target. The mRNA-DNA duplex resulting from the bound peptides is sequenced and amplified with error-prone PCR to increase diversity of the mRNA library for the next iterative cycle.

Nuclear co-activators

(NCOAs). A family of proteins that bind to nuclear hormone receptors to facilitate transcriptional activation. NCOAs often bind a conserved hydrophobic cleft within nuclear receptors, which allows them to engage a broad number of targets.

Phosphate-recruiting chimaeras

(PhoRCs). Heterobifunctional small molecules that recruit phosphatase to a target protein. The PhoRC system was demonstrated with DDO3711, which is a bifunctional small molecule that binds phosphatase PP5 and recruits it to ASK1 via the active site inhibitor of ASK1.

Phosphorylation inducing chimaeras

(PHICs). Heterobifunctional small molecules that recruit a kinase to a target protein. PHICs were demonstrated to phosphorylate BRD4 by recruitment of AMPK or PKC kinases.

Phosphorylation-targeting chimaeras

(PhosTACs). Heterobifunctional small molecules that recruit phosphatase to a target protein. The PhosTAC approach was demonstrated using FKBP12^{F36V}-tagged phosphatase and HaloTagged-target protein with a PhosTAC composed of FKBP12^{F36V} small-molecule binder and a halo ligand.

Regulated induced proximity targeting chimaeras

(RIPTACs). Bifunctional small molecules that bring into proximity two proteins, a disease-specific protein target with a pan-essential effector protein, resulting in context-specific toxicity.

Sumoylation

Sumoylation is a post-translational modification that involves the attachment of SUMO proteins (SUMO1, SUMO2 or SUMO3), small ubiquitin-like peptides, to lysine residues that lead to different aspects of protein regulation and/or function. SUMO proteins are attached to targets in an analogous process to ubiquitin, via an E1, SUMO E2 and SUMO E3 ligases.

BI-3802 was previously known to degrade B cell lymphoma 6 (BCL6), albeit by an unknown mechanism¹³⁵. Time-course immunofluorescence studies have demonstrated that green fluorescence protein (GFP)-tagged BCL6 formed fluorescent foci after BI-3802 treatment before vanishing, suggesting that BCL6 was aggregating before degradation¹³⁶. Intriguingly, negative-stain electron microscopy studies have demonstrated BCL6 filaments, which indicates polymerization

instead of aggregation. More detailed structural studies, coupled with genetic validation, have shown that BI-3802 forms a symmetric neo-interaction interface between two homodimers of the BCL6 Bric-à-brac (BTB) domain, facilitating self-polymerization, which in turn leads to enhanced recruitment of the SIAH1/2 ligase¹³⁶ (Fig. 3b).

A similar mechanism was also hinted at during efforts to research acute promyelocytic leukaemia. Acute promyelocytic leukaemia is a

Table 1 | Clinical trials utilizing degrader molecules

Degrader	Company	Phase	Target	E3	Indication
Molecular glue degraders					
CC-220	BMS	III	IKZF1/3	CRBN	Multiple myeloma (lymphoma, phase I)
CC-92480	BMS	III	IKZF1/3	CRBN	Multiple myeloma
CC-99282	BMS	II	IKZF1/3	CRBN	Lymphoma
E7820	Eisai	II	RBM39	DCAF15	Myeloid cancers
CC-122	BMS	I	IKZF1/3	CRBN	Lymphoma
CFT-7455	C4	I	IKZF1/3	CRBN	Multiple myeloma and lymphoma
KPG-818	Kangpu	I	IKZF1/3	CRBN	Systemic lupus erythematosus, multiple myeloma and lymphoma
ICP-490	Innocare	I	–	CRBN	Multiple myeloma and diffuse large B cell lymphoma
KPG-121	Kangpu	I	IKZF1/3, CK1 α	CRBN	Prostate cancer
BTX-1188	BioTheryX	I	GSPT1, IKZF1/3	CRBN	Haematological and solid malignancies
CC-90009	BMS	I	GSPT1	CRBN	Acute myeloid leukaemia and myelodysplastic syndrome
MRT-2359	Monte Rosa	I/II	GSPT1	CRBN	MYC-driven solid tumours
NVP-DKY709	Novartis	I	IKZF2/4	CRBN	Solid tumours
TQB3820	Sino	I	IKZF1/3	CRBN	Multiple myeloma
Lenalidomide analogue	Starton	I	IKZF1/3	CRBN	Multiple myeloma
PROTACS					
ARV-471	Arvinas	III	Oestrogen receptor	CRBN	Breast cancer
ARV-110	Arvinas	II	Androgen receptor	CRBN	Prostate cancer
ARV-766	Arvinas	I	Androgen receptor	CRBN	Prostate cancer
CC-94676	BMS	I	Androgen receptor	CRBN	Prostate cancer
AC-0176	Accutar	I	Androgen receptor	CRBN	Prostate cancer
HP518	Hinova	I	Androgen receptor	NA	Prostate cancer
GT20029	Kintor	I	Androgen receptor	CRBN	Androgenetic alopecia and acne vulgaris
KT-474	Kymera	I	IRAK4	CRBN	Immuno-inflammatory skin diseases
KT-413	Kymera	I	IRAK4, IKZF1/3	CRBN	MYD88-mutant tumours
KT-333	Kymera	I	STAT3	NA	Liquid and solid tumours, T cell lymphomas
NX-2127	Nurix	I	BTK, IKZF1/3	CRBN	B cell malignancy
NX-5948	Nurix	I	BTK	CRBN	B cell malignancies and autoimmune diseases
HSK29116	Haisco	I	BTK	NA	B cell malignancy
BGB-16673	BeiGene	I	BTK	NA	B cell malignancy
DT-2216	Dialectic	I	BCL-X _L	VHL	T cell lymphomas
CFT-8634	C4	I	BRD9	CRBN	Synovial sarcoma, SMARCB1-null solid tumours
FHD-609	Foghorn	I	BRD9	NA	Synovial sarcoma, SMARCB1-del tumours
CFT-1946	C4	I	BRAF V600	CRBN	BRAF V600 mutant solid tumours, non-small-cell lung cancer, colorectal cancer and melanoma
CG001419	Cullgen	I	NTRK	CRBN	NA
SERDs					
Elacestrant	Radius Health	III	ER	–	Breast cancer
Amcenestrant	Sanofi	III	ER	–	Breast cancer
Giredestrant	Roche	III	ER	–	Breast cancer
Imlunestrant	Eli Lilly	III	ER	–	Breast cancer

Table 1 (continued) | Clinical trials utilizing degrader molecules

Degrader	Company	Phase	Target	E3	Indication
SERDs (continued)					
Camizestrant	AstraZeneca	III	ER	–	Breast cancer
EA 114	Eagle	II	ER	–	Breast cancer
ZB-716	EnhancedBio	II	ER	–	Breast cancer
ZN-c5	Zentaris	II	ER	–	Breast cancer
AC-682	Accutar	I	ER	–	Breast cancer
D-0502	InventisBio	I	ER	–	Breast cancer
SCO-120	Sun Pharma	I	ER	–	Breast cancer
SIM-270	Simcere	I	ER	–	Breast cancer

BTK, Bruton's tyrosine kinase; CRBN, cereblon; ER, oestrogen receptor; NA, not available; SERD, selective oestrogen receptor degrader.

haematological malignancy driven by the t(15;17) PML–RARA fusion, which is unique in that it is degraded by two small molecules: arsenic trioxide causes oligomerization of PML and PML–RARA, leading to an increased interaction with UBC9 and ultimately degradation¹³⁷; and all-trans retinoic acid activates and subsequently degrades RARA (as discussed in the previous section regarding degradation of hormone receptors)^{114,137–140}.

Direct recruitment to the proteasome

The plethora of mechanisms that lead to ubiquitin-dependent TPD has led people to ask whether ubiquitination is necessary or could be bypassed. Further extending the breadth of approaches, a study has demonstrated direct 26S proteasomal recruitment in a PROTAC-like

approach¹⁴¹. mRNA display technology was leveraged to identify high-affinity peptides that associated with recombinant PSMD2, a proteasome subunit, with nanomolar affinity¹⁴¹. Cryo-electron microscopy studies have illustrated that the identified macrocyclic peptide, MC1, binds to a distinct region of PSMD2 between its C-terminal domain and helical solenoid, near the 26S proteasome pore. MC1 conjugates to high-affinity BRD4 sulfone ligands (BETi) led to the successful recruitment of BRD4 to the proteasome, resulting in BRD4 degradation.

Exploring the repertoire of E3 ligases in targeted protein degradation

CRBN and VHL are the two chief ligases engaged by PROTACs such as dBET6 or PROTAC-1 respectively; together with IAP (that is, compound 17)

Box 2

Ubiquitylation and/or proteasome-independent strategies of targeted protein degradation

Progress in small molecule-based hijacking of the ubiquitin proteasome system with PROTACs or molecular glues has inspired the development of many novel strategies that leverage proximity-based approaches to degrade proteins or organelles not easily tractable for the ubiquitin system. This area of research is rapidly evolving and the examples listed here should be seen as a snapshot and not an exhaustive list of possibilities.

LYTACs (lysosome targeting chimaeras) are antibodies modified by N-glycans capped with a ligand to a lysosomal trafficking receptor, such as manose-6-phosphate, a ligand to the cation-independent mannose-6-phosphate receptor (CI-MPR)⁵. LYTACs enable the targeting of extracellular or surface proteins for degradation by directing these to the internalizing CI-MPR. Although CI-MPR is ubiquitously expressed, an alternative receptor, ASGPR, is liver specific and enables restricted degradation of target proteins selectively within hepatocytes²¹⁴.

Another approach is technologies that rely on the use of autophagy (AUTACs, autophagy targeting chimeras) and autophagosome

(ATTECs, autophagosome targeting chimeras). AUTACs were developed following the observation that protein S-guanlylation induces autophagic clearance. AUTACs are bifunctional small molecules with one side consisting of a guanine mimetic *p*-fluorobenzylguanine (FBnG) moiety, linked via a linker to a ligand of a target of interest. AUTACs are capable of degrading intracellular proteins and fragmented mitochondria. ATTECs are bifunctional small molecules that use a ligand to the phagophore (autophagosome precursor) protein LC3, and a binder to the target of interest. ATTECs can induce degradation of intracellular proteins and lipids.

Finally, interest is growing in modalities that modulate protein stability in a ubiquitin dependent fashion. Small-molecule inhibitors to deubiquitinating enzymes (DUBs) or ubiquitin E3 ligases, although already known and not proximity-based in mechanism, are receiving increasing attention for their orthogonal ability to regulate protein stability. Furthermore, bifunctional molecules have been designed to target DUBs to proteins of interest leading to de-ubiquitylation and thereby stabilization²¹¹.

they form the relative rarity of small molecules that engage directly with E3 ligases, which has limited PROTAC expansion into new ligases^{84,142,143} (Fig. 3c). Although CRBN and VHL are, in general, highly effective ligases in terms of the degree of and rate of degradation, engagement of other ligases might have other benefits¹⁴⁴. The use of tissue-specific ligases in PROTAC design might be a method for providing further drug specificity and restricting unwanted off-target effects; engagement of ligases found only in the nervous or haematopoietic systems would enable PROTACs that bind to those ligases only to function in those sites, simplifying the necessity for restricting chemical distribution¹⁴⁴. Likewise, ligases that are predominantly nuclear or chromatin bound could be recruited by PROTACs for transcription factor targeting. Indeed, studies have begun to characterize tissue-specific E3 ligases, as well as their ligands and substrates, which might subsequently lead the expansion of PROTACs towards further targeted applications^{145,146}. Regardless, the limitation currently lies in the difficulty of identifying small molecules that engage specific ligases; a platform that robustly identifies molecule–ligase pairs would be highly valuable for PROTAC clinical development.

Selectivity and specificity of targeted protein degradation

Small-molecule protein degraders derive their selectivity from multiple factors, such as the engagement of an E3 ligase and/or the target proteins, protein–protein interactions, efficiency of ubiquitination expression levels of the target–E3 ligase pair, and degradation kinetics. The requirement for ternary complex formation and efficient target ubiquitination is the key characteristic driving degradation selectivity. Early studies with PROTACs derived from promiscuous kinase ligands have shown that ligase choice (CRBN or VHL) affects which kinases are degraded, and that PROTAC–target affinity can be decoupled from degradation efficacy^{62,147}. Although PROTACs derived from parental ligands maintain the inhibition effect of the parental molecule, molecular glue degraders binding to the E3 ligase (such as IMiDs) do not. The selectivity of degradation is also derived from the tissue expression of the target–ligase pair. Notably, low expression of VHL in platelets led to reduced platelet toxicity caused by a PROTAC targeting and degrading BCL-X_L⁶¹. The development of isoform-specific PROTACs for targets with nearly identical ligand binding sites (such as CDK4 and CDK6 (refs. 148,149), BRG and BRM¹⁵⁰, p38 α and p38 δ (ref. 151)) will further enhance the selectivity of TPD selectivity^{150,152–155}.

Molecular glue degraders and PROTACs are generally thought of as specific drugs; however, we and others have found off-target effects with a number of CRBN-based PROTACs, with engagement and degradation of targets such as IKZF1 or SALL4 (refs. 82,84), underlining the need to thoroughly characterize off-target degradation using targeted and unbiased approaches, before clinical investigation. Lastly, given the catalytic process involved in the action of small-molecule degraders, the degradation selectivity is also dependent on time, degradation kinetics and substrate competition¹⁵⁶.

Clinical perspectives

Despite the diversity of mechanisms of small molecule-induced protein degradation outlined in the previous section, relatively few molecules are FDA approved for active clinical use¹³⁷. The landscape of molecules in clinical development is much more diverse than our current clinical repertoire, with multiple PROTACs and molecular glue degraders in various stages of clinical trials. Many molecular glue degraders have entered phase I and II clinical trials, including a number targeting

IKZF1 and IKZF3 (CC-92480, CC220, ICP-490, CC-99282, CFT-7455, GT-919 and BTX-1188). As molecular glue targets are generally discovered rather than designed, clinical indications for molecular glues were initially restricted for use in haematological malignancies (for example, relapsed refractory multiple myeloma and acute myeloid leukaemia), although some are now being tested in solid tumours including lung cancers^{157–161} (Table 1). Other molecular glue degraders in clinical trials include E7820, which targets RBM39 in acute myeloid leukaemia¹⁶², and MRT-2359, which targets GSPT1 in solid tumours.

Given their relatively more straightforward design, more PROTACs are in clinical development, with a larger variety of target proteins. These targets include the androgen receptor (ARV-110, ARV-766 and CC-94676), ER (ARV-471 and AC682), BRD9 (FHD-609 and CFT8634), BCL-x_L (DT2216), IRAK4 (KT-474 and KT-413), STAT3 (KT-333), BTK (NX-2127 and NX-5948), TRK (CG001419) and EGFR-L858R (CFT8919) in a range of indications including haematological malignancies, solid tumours (including prostate, breast and lung cancers), synovial sarcomas and autoimmune diseases^{12,161,163–171} (Table 1).

Despite their history of prenatal toxicities, thalidomide analogues, with adequate procedural safeguards, are widely used for several haematological malignancies, and their clinical success makes a strong case for TPD as a means to address otherwise unattainable pharmacology.

The numerous ongoing clinical trials will address whether the pharmacokinetic advantages that molecular glue degraders enjoy over PROTACs (such as superior bioavailability or tissue penetrance) outweigh the wider range of clinical applicability afforded by ease of PROTAC design. Furthermore, although PROTACs are generally thought to be highly target specific, some studies are characterizing unexpected off-target effects⁸². Some of these include the engagement of a different E3 ligase or target substrate¹³⁴, whereas we and others have found that thalidomide derivative-based PROTACs can still lead to degradation of SALL4 and other molecular glue targets^{34,82,84,85,172}. These findings serve as a warning that some PROTACs might harbour residual toxicities, particularly in relation to fetal development, which is compounded by the fact that commonly used toxicology model organisms such as rats and dogs are immune to most thalidomide derivative degradation targets^{85,173}.

TPD represents a highly differentiated modality with certain advantages but it also carries multiple challenges that need to be addressed to achieve full clinical benefit. In some cases, degradation alone is insufficient to achieve lasting clinical effects without the inhibition or antagonism of a given pathway. Although the event-driven pharmacology degraders¹⁷⁴ (in which the biological effect is driven by the level of protein depletion) has distinct benefits over the occupancy-driven pharmacology of inhibitors (in which a biological effect is driven by a drug binding to modulate the function of a protein), it is still unclear under what circumstances TPD is therapeutically superior over pathway inhibition. As previously mentioned, sometimes difficult pharmacokinetics properties of PROTACs, and the potential off-targets of both molecular glues and PROTACs, might make it difficult to target specific tissues. Further chemical work might be needed to optimize the pharmacokinetic properties of these molecules, and studies looking into engaging tissue-specific E3 ligases for PROTACs might ameliorate some of these off-target effects.

Some mechanisms of resistance are unique to thalidomide derivatives and PROTACs. Lenalidomide resistance in multiple myeloma focuses on pathway mutations (*MAPK*, *TP53*, *WNT* signalling), decreased expression or mutation of CRBN and its downstream

Box 3

PROTACs and molecular glue degraders in basic research

In addition to their therapeutic potential, the discovery of small molecules capable of degrading specific intracellular proteins has sparked the development of a series of innovative strategies for use as basic research tools (reviewed elsewhere¹³). Both PROTACs and molecular glue degraders offer the unprecedented possibility of the selective removal of proteins from the cell in a dose-dependent and time-dependent manner, enabling exploration of rapid biological processes and functions that remained unexplored by inhibitors (such as the scaffolding functions of proteins). A defining feature of the modality is the induction of tight protein–protein interactions that further boost selectivity of degrader molecules, making them excellent tools to study the consequences of protein depletion. Mass spectrometry proteomics further enables the evaluation of degradation selectivity, which is again impossible for inhibitors at the proteome level. As such, although it is important to keep in mind that PROTACs and some molecular glues can act as inhibitors of protein function, proteomic assessment has thoroughly classified this class of molecules and they are therefore suited for use as chemical probes.

Another TPD-derived tool is protein tags, collectively known as degron tags, which are paired with molecules known to efficiently bind and degrade them. One such example is the dTAG system, in which FKBP12^{F36V} is rapidly (within minutes) degraded by a CRBN-based or VHL-based PROTAC (dTAG13 or dTAGV1, respectively)²¹⁵. The FKBP12 tag can be fused to a protein of interest, generating a fully degradable fusion. The dTAG system has been successfully used *in vitro* and *in vivo* to validate or invalidate biological hypotheses.

pathway (*IRF4* and *IKZF1*), and increased myeloma cell adhesion (CD44). In del(5q) MDS, mutations in *TP53*, *RUNX1* and *GATA2* have been documented^{175–181}. Interestingly, resistance to CRBN-based and VHL-based PROTACs has been shown to be caused by genomic alterations to *CRBN* or *CUL2*, respectively¹⁷⁵, as well as the upregulation of the efflux pump MDR1 (ref. 182).

Moving beyond cancer, TPD has been trialed for other clinical indications². Given their, at times, overlapping set of targets, a handful of PROTACs developed for oncology have been repurposed for inflammatory diseases. IRAK4 is a serine–threonine kinase that is a target in diffuse large B cell lymphoma but is also implicated in many inflammatory and autoimmune diseases. PROTACs against IRAK4 are therefore a novel therapeutic strategy that could encompass several indications^{183–185}. Neurodegenerative diseases are perhaps one of the most logical indications for TPD, as a chief hallmark of their pathogenesis is protein misfolding and insoluble aggregation. Multiple groups have reported targeted degradation of Tau, Huntingtin, and alpha-synuclein via PROTACs and other-directed small molecules^{186–192} (Box 2).

Finally, TPD has been investigated in numerous infectious diseases^{193,194}; PROTACs have been developed to target pathogen-specific

proteins, such as the NS3/4A protease in hepatitis C¹⁹⁵, and influenza proteins (polymerase subunit PA and neuraminidase)^{196,197}. These novel molecules are conjugates of anti-viral molecules such as telaprevir and oseltamivir combined with CRBN or VHL warheads, and they have been shown to be more resilient in treating telaprevir-resistant and oseltamivir-resistant strains^{195,197}. Thus, TPD has potential applications towards hard-to-treat and resistant viral strains.

BacPROTACs¹⁹⁸ are PROTACs that hijack and bind to the bacterial substrate receptor of the ClpC–ClpP (ClpCP) protease to degrade bacterial proteins of interest. Using this method to target the specific stress response factors ClpC1 and ClpC2 in *Mycobacterium tuberculosis* proved to be a more effective mechanism than the parent antibiotic, cyclomarin A^{198,199}.

Future perspectives

The aforementioned modes of TPD have illustrated the diversity of mechanisms that can be used for substrate removal, and hint at others that are yet to be determined. There is an inspiring diversity in novel approaches for TPD, such as antibody targeting chimeras (AbTACs)⁹, autophagy targeting chimeras (AUTACs)¹⁰, lysosome-targeting chimeras (LYTACs)⁵, autophagosome-tethering compounds (ATTECs)¹¹ and antibody–drug conjugates that utilize degrader molecules as cargo²⁰⁰, which are beyond the scope of this Review (Box 2). It is, however, probable that in the near term, the majority of TPD therapeutics entering clinical development will broadly belong to PROTACs or molecular glue degraders.

Although PROTACs and molecular glues are mechanistically similar in that they lead to the degradation of their targets, they face different challenges. Molecular glue degraders, as a result of their smaller size, often have superior bioavailability, and are frequently exquisitely potent^{12,107,160,201}. Unfortunately, as no readily available methods exist yet to rationally design and discover molecular glues, only a few are in clinical development at present¹² (Supplementary Box 1). However, as most glues have been found to bind to new targets, these clinical programs are unique in that they often focus on novel targets such as transcription factors. Additional complexities arise from the often steep structure–activity relationship, with single-atom changes capable of changing substrate specificity²⁰².

By contrast, PROTACs can be rationally designed by building upon a number of E3 ligase ligands and leveraging existing small molecules for attractive targets, such as hormone receptors, in a mix and match fashion. This has led to a rapid increase in the number of PROTACs (and degradation targets), especially if known ligands exist for those targets (for example, oestradiol and ER). These PROTAC targets span a number of clinical indications and include multiple target families, including nuclear hormone receptors, kinases, growth receptors, interleukin receptors, bromodomains and BCL family proteins. However, unlike glues, these targets have mostly been previously drugged using more conventional approaches and therefore the burden to prove clinical superiority is often higher. Finally, the trade-off of the large size of PROTACs has led to a different set of issues around sub-optimal chemical properties, which necessitates often a long and empirical period of pharmacological optimization extending development timelines¹².

The rapid growth of the TPD field has ignited broad interest in proximity-based pharmacology leading to novel concepts that might lead to future therapeutics²⁰³. These include TCIPs (transcriptional/epigenetic chemical inducers of proximity)²⁰⁴, RIPTACs (regulated induced proximity targeting chimaeras)²⁰⁵, AceTAG (acetylation tagging)²⁰⁶, DEPTACs (dephosphorylation-targeting chimaeras)²⁰⁷, PhoRCs (phosphate-recruiting chimaeras)²⁰⁸, PhosTACs

(phosphorylation-targeting chimaeras)²⁰⁹, PHICs (phosphorylation inducing chimaeras)²¹⁰ and DUBTACs (deubiquitinase-targeting chimaeras)²¹¹. These molecules lead to target inhibition, deubiquitination, phosphorylation and acetylation, expanding the functionality and clinical applications of small molecule-induced proximity beyond degradation.

As previously discussed, protein degradation is versatile and can be achieved by a multitude of mechanisms catalysed by small molecules, including direct ligase recruitment, protein polymerization, templated labelling, intramolecular dimerization, or recruitment to E2 or the proteasome. Many of the small molecules that result in degradation still await the discovery of their mechanisms of action, which might bring new strategies for rational development of degraders. Finally, researchers continue to take inspiration from nature, which uses small molecules in the modulation of protein levels across all living kingdoms, and which might illuminate further ligases and targets amenable to induced neo-substrate interactions. Both the bifunctional approach, in which combinations of existing ligands offer plug-and-play functionality, and the discovery of molecular glue degraders, present tangible mechanisms to modulate cellular states with the potential to yield even more impactful therapies for patients and for furthering our understanding of fundamental biology (Box 3). Although it will take years for some of these mechanisms to reach clinical utility, their versatility and the creativity of approaches in creating or discovering new molecules will undoubtedly expand the druggable target space and ultimately lead to patient benefit. Most importantly, although not intentionally discovered, the clinical benefit provided by lenalidomide and other FDA-approved degraders targeting transcription factors intractable to other small molecule approaches is providing benefit to patients every day and clearly demonstrates the future potential.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

E.S.F. is a founder, member of the scientific advisory board and equity holder of Civetta Therapeutics, Proximity Therapeutics and Neomorph, Inc. (also board of directors). He is an equity holder and scientific advisory board member for Avilar Therapeutics and Phytos Therapeutics, an equity holder in Lighthouse Therapeutics and a consultant to Novartis, Sanofi, EcoR1 Capital, Ajax, Odyssey and Deerfield. The Fischer lab receives or has received research funding from Deerfield, Novartis, Ajax, Bayer, Interline and Astellas. B.L.E. has received research funding from Celgene, Deerfield, Novartis and Calico and consulting fees from GRALL. He is a member of the scientific advisory board and shareholder for Neomorph Inc., TenSixteen Bio, Skyhawk Therapeutics and Exo Therapeutics. R.P.N. and J.M.T. declare no competing interests.

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