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Review

Molecular glue-mediated targeted protein degradation: A novel strategy in small-molecule drug development

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SUMMARY

Small-molecule drugs are effective and thus most widely used. However, their applications are limited by their reliance on active high-affinity binding sites, restricting their target options. A breakthrough approach involves molecular glues, a novel class of small-molecule compounds capable of inducing protein-protein interactions (PPIs). This opens avenues to target conventionally undruggable proteins, overcoming limitations seen in conventional small-molecule drugs. Molecular glues play a key role in targeted protein degradation (TPD) techniques, including ubiquitin-proteasome system-based approaches such as proteolysis targeting chimeras (PROTACs) and molecular glue degraders and recently emergent lysosome system-based techniques like molecular degraders of extracellular proteins through the asialoglycoprotein receptors (MoDE-As) and macroautophagy degradation targeting chimeras (MADTACs). These techniques enable an innovative targeted degradation strategy for prolonged inhibition of pathology-associated proteins. This review provides an overview of them, emphasizing the clinical potential of molecular glues and guiding the development of molecular-glue-mediated TPD techniques.

INTRODUCTION

In modern medicine, small-molecule drugs and biologics are two major drug classes.[1] Biologics, such as insulin, are derived from living cells or produced through biological processes. They are typically complex molecules with a mass exceeding 1,000 Da. Conversely, small-molecule drugs are chemically synthesized compounds. They are smaller in size and usually have molecular weights under 1,000 Da.[1] Small-molecule drugs have several prominent advantages over biologics. Their small molecular weights endow them with potential oral bioavailability and the ability to cross biological barriers. Furthermore, small-molecule drugs are readily available through chemical synthesis and can be structurally modified in the process. As a result, small-molecule drugs have dominated the modern drug market, accounting for as much as 90% of global sales.[2]

Small-molecule drugs can function as enzyme inhibitors and receptor antagonists by occupying the active binding sites of the targets.[3] However, only 15% of the pharmacologically exploitable proteins encoded in the human genome can be targeted by small-molecule drugs.[4] The remaining proteins usually have shallow and broad active sites that are intractable for small molecules, or smooth surfaces with few sites to bind to, thus they are deemed as "undruggable" proteins.[5] The inability of small-molecule drugs to target undruggable proteins has significantly limited their application and development, as many proteins significant in cancer and disease pathology are undruggable proteins. Fortunately, the emergence of molecular glues has provided new strategies for small-molecule drugs to target undruggable proteins.

Unlike conventional small-molecule drugs that target a single protein, molecular glues are a type of small molecules that function by enhancing the affinity between two proteins, thus inducing protein-protein interaction (PPI) between them. The mechanism of molecular glues is prevalent in nature, with abscisic acid (ABA) being one of the best-known examples. ABA is a sesquiterpene phytohormone that promotes leaf abscission and dormancy and has an inhibitory effect on seed germination.[6] ABA can bind to pyrabactin resistance 1-like (PYL), a monomeric receptor of ABA, to form an ABA-PYL complex. This complex subsequently recruits protein phosphatase type 2Cs (PP2Cs) to form a PYL-ABA-PP2Cs ternary complex, which inhibits the activity of PP2C and results in the regulation of downstream genes, eventually producing the biological functions of ABA. Additionally, most post-translational modifications of proteins, including methylation, acetylation, and ubiquitination, can also be regarded as molecular glues that influence biological processes through protein recruitment.[7]

PPIs play a significant role in maintaining the proper functioning of various cellular processes, such as metabolism and gene expression. At the same time, many diseases and cancers result from abnormal PPIs.[8] The emergence of molecular glues has provided new strategies for

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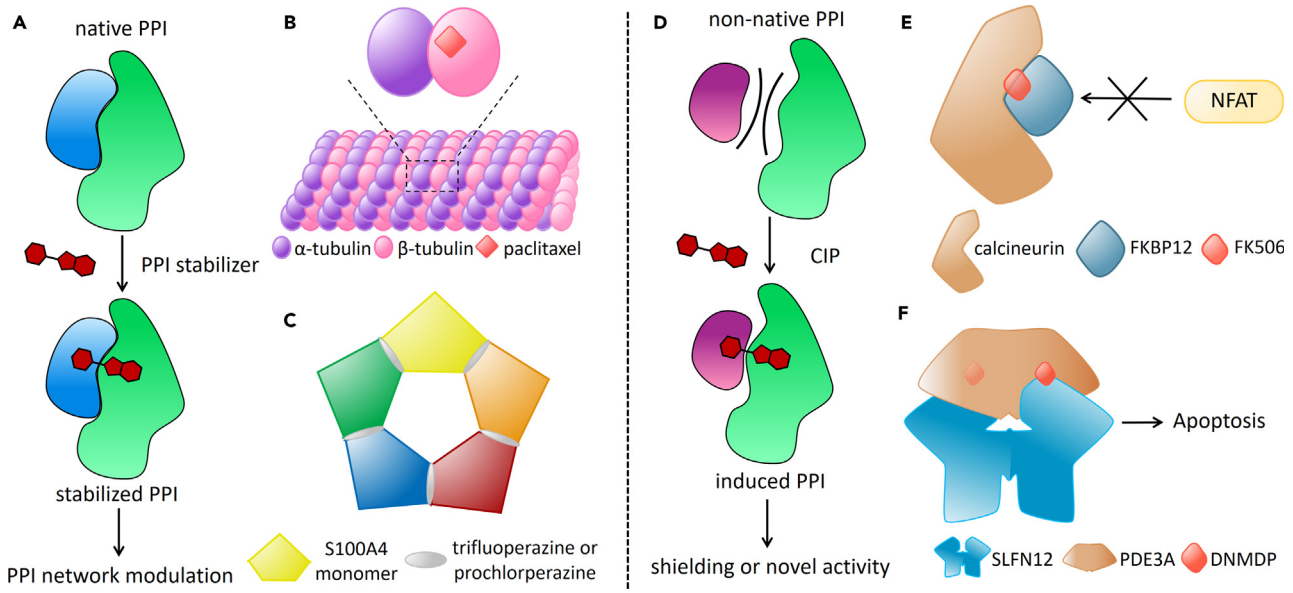


Figure 1. Schematic representation of molecular glues

(A) Molecular glues that stabilize protein-protein interactions (PPIs).

(B) Paclitaxel-induced stabilization of microtubules and the interaction between α-tubulin and β-tubulin.

(C) Stabilization of the S100A4 pentamer by phenothiazines.

(D) Schematic representation of chimeric inducer of proximity (CIPs).

(E) Inhibition of the calcineurin-NFAT interaction through FK506-induced FKBP12-calcineurin interaction.

(F) DNMDP (6-[4-(diethylamino)-3-nitrophenyl]-5-methyl-4,5-dihydro-3(2H)-pyridazinone) induces SLFN12-PDE3A interaction, leading to apoptosis.

small-molecule drug applications in the treatment of pathologies caused by abnormal PPIs. Furthermore, molecular glues can target proteins without high-affinity active binding sites for small molecules, thereby overcoming the limitation of conventional small-molecule drugs in target selection and making many undruggable proteins no longer intractable.[9]

Molecular glues encompass two major categories: PPI stabilizers and chemical inducers of proximity (CIPs).[10] PPI stabilizers, a subset of PPI modulators,[11] modulate PPI networks by enhancing the thermodynamic stability of native PPIs (Figure 1A). Notably, paclitaxel illustrates this mechanism by stabilizing the microtubule interaction between α-tubulin and β-tubulin (Figure 1B), preventing microtubule depolymerization and inducing mitotic arrest.[12] Phenothiazine drugs, trifluoperazine and prochlorperazine, disrupt S100 calcium binding protein A4 (S100A4)-myosin-IIA interaction by stabilizing S100A4 oligomers, inhibiting S100A4 activity, and preventing associated diseases (Figure 1C).[13]

CIPs induce non-native PPIs, exploiting the principle of proximity in biological systems (Figure 1D). Proximity-inducing molecules, like scaffold proteins and covalent modifications, non-covalently or partially covalently participate in molecular recognition, enhancing the specificity and rate of biological processes.[9] CIP-induced PPIs may be inactive, blocking participant activity for selective endogenous protein inhibition. For instance, FK506-induced FKBP12-calcineurin interaction inhibits the calcineurin-NFAT interaction, leading to immunosuppression (Figure 1E).[14] Alternatively, some non-native PPIs exhibit novel activities, as seen in DNMDP-induced interaction between phosphodiesterase 3A (PDE3A) and Schlafen12 protein (SLFN12), triggering SLFN12 dephosphorylation and inducing apoptosis (Figure 1F).[15] Notably, certain non-native PPIs induced by CIPs are able to trigger protein degradation, especially those involving E3 ubiquitin-ligases. For instance, auxin is able to regulate gene expression by inducing the interaction between transport inhibitor response 1 (TIR1) and SKP1/Cullin1/F-box (SCF) E3 ubiquitin-ligase, which leads to fast proteasomal degradation of TIR1.[16] In the case of indisulam, its anticancer activity is attributable to its ability to induce the interaction between RNA binding motif protein 39 (RBM39) and DDB1 and CUL4 associated factor 15 (DCAF15) E3 ligase, which results in the proteasomal degradation of RBM39.[17]

The unique mechanism of action of molecular glues holds promise for applications in biological research and drug development, prompting the rapid emergence of various molecular glue-mediated techniques, including anchor-away, apoptosis through targeted activation of caspase 8 (ATTAC), and chromatin *in vivo* assay.[18–20] Among these techniques, molecular glue-mediated targeted protein degradation (TPD) stands out, utilizing molecular glues to harness endogenous protein-degrading systems, namely ubiquitin-proteasome system (UPS) and lysosome system (LYS) for eliminating proteins associated with human pathologies. This review provides an overview of molecular glues' applications in TPD, offering insights into the development, recent advances, and clinical applications of two major UPS-based techniques, namely proteolysis targeting chimeras (PROTACs) and molecular glue degraders. Additionally, we delve into the emerging LYS-based techniques, molecular degraders of extracellular proteins through the asialoglycoprotein receptors (MoDE-As) and macroautophagy degradation targeting chimeras (MADTACs). Finally, we elucidate the complementarity and provide a comparative analysis between UPS-based techniques and LYS-based techniques.

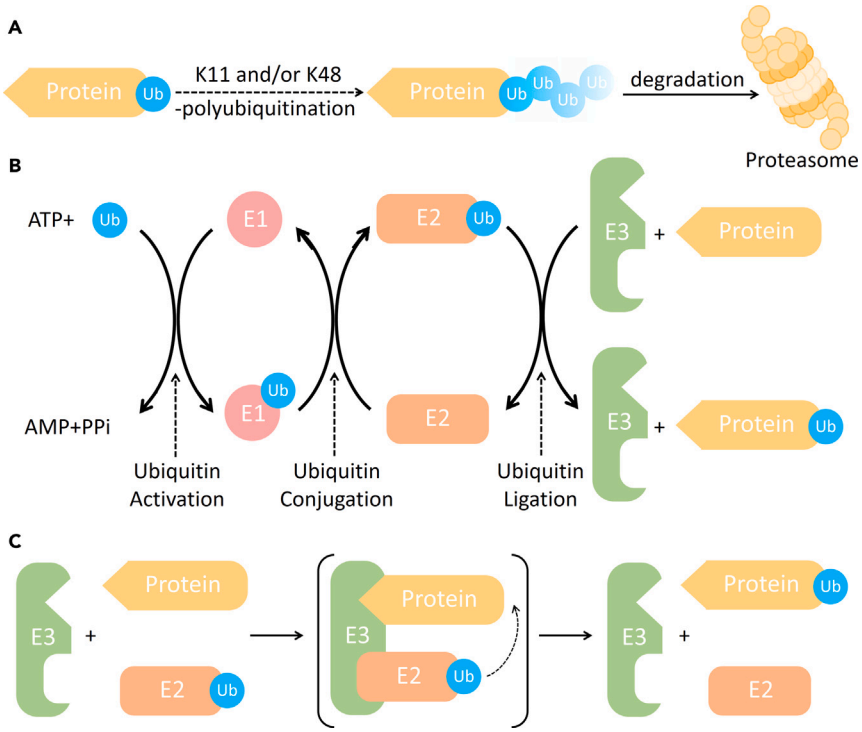


Figure 2. Schematic representation of the ubiquitin-proteasome system

(A) Proteins with K11 and/or K48-polyubiquitination can be recognized and degraded by the proteasome.

(B) Schematic representation of the three-step ubiquitination cascade.

(C) The process of ubiquitin ligation catalyzed by RING type or U-Box type E3 ligase.

UPS-BASED TECHNIQUES

In eukaryotic cells, protein elimination occurs through either the proteasomal or lysosomal pathway. The former primarily degrades intracellular proteins, encompassing damaged, misfolded, and nonfunctional proteins. In contrast, the latter is responsible for removing long-lived, extracellular, membrane-located proteins, as well as insoluble protein aggregates. Additionally, lysosomal pathways manage the disposal of other biomacromolecules and damaged organelles. The proteasomal pathway, accounting for 80–90% of cellular protein degradation in mammalian cells, plays a pivotal role in cellular function regulation and protein homeostasis.[21] UPS orchestrates protein disposal, consisting of ubiquitin, ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), ubiquitin ligases (E3s), and the proteasome. Ubiquitin is a 76-amino acid protein with seven lysine residues. Notably, in cellular protein-degrading systems, K11, K48, and K63 involved in ubiquitin linkages can function as degradative labels, namely K11 and K48 for proteasomal pathway and K63 for lysosomal pathway (Figure 2A).[22]

The ubiquitination process, a three-step enzymatic cascade including ubiquitin activation, ubiquitin conjugation and ubiquitin ligation, involves all three types of ubiquitinating enzymes as depicted in Figure 2B.[23] Ubiquitin is first activated by E1 in an ATP-dependent manner, and then the activated ubiqutin is transferred from E1 to E2. In the final step, the E3 ligase binds simultaneously to the ubiquitin-carrying E2 and its substrate protein and catalyzes the transfer of ubiquitin from E2 to the substrate proteins. Notably, the ubiquitin transfer process varies with the type of E3 ligases.[24] The E3 ligases can be subdivided into four types; namely homologous to E6AP C-terminus (HECT) type, really interesting new gene (RING) type, U-box type, and RING-between-RING (RBR) type; among which RING type E3 ligases are the major type. For RING type and U-box type E3 ligases, the ubiquitin is transferred from E2 to the substrate protein directly (Figure 2C), and for HECT type and RBR type E3 ligases, the ubiquitin is first transferred to a catalytic cysteine on the E3 ligase and the RING2 domain respectively, then to the substrate protein. By leveraging molecular glues to modify the binding preferences of E3 ligases, a non-native PPI between E3 ligase and a neo-substrate can be induced, potentially reprogramming the E3 ligases for the ubiquitination of a pathology-associated protein. This presumption was validated in 2001,[25] laying the foundation for PROTACs and molecular glues for protein degradation (molecular glue degraders).[26]

PROTACs: proteolysis targeting chimeras

PROTACs, heterobifunctional molecules, consist of an E3-recruiting ligand, a warhead targeting the protein of interest (POI), and a linker tethering the ligand and the warhead. As depicted in Figure 3A, when a PROTAC molecule binds to the corresponding E3 ligase and the POI with

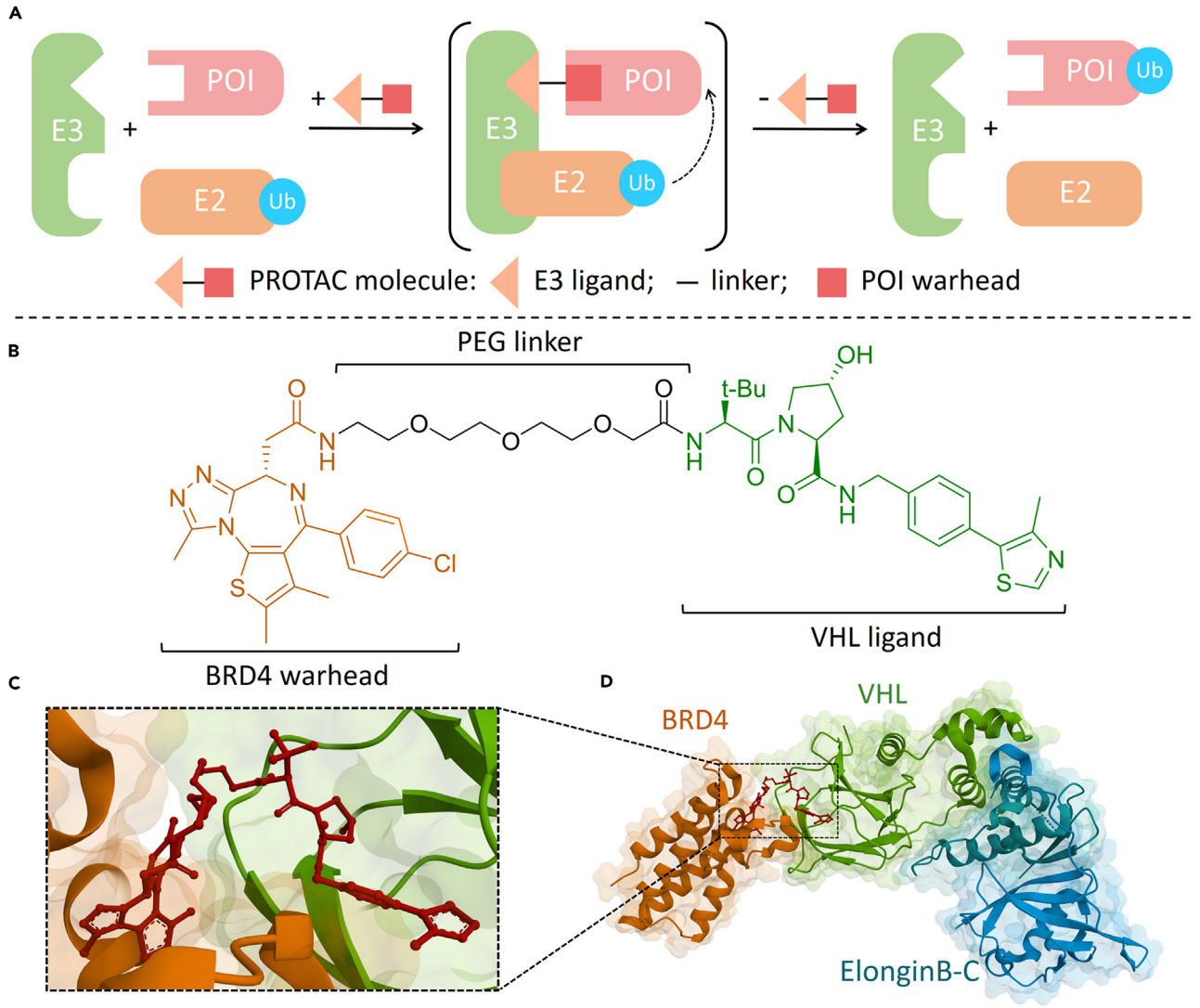


Figure 3. Illustration of the mechanism of action of PROTACs

(A) Schematic representation of PROTAC-induced POI ubiquitination.

(B) Chemical structure of the VHL-based PROTAC molecule targeting BRD4. BRD4 warhead and VHL ligand are respectively marked in orange and green.

(C) Magnified interaction interface of BRD4-PROTAC-VHL interaction.

(D) Illustration of the BRD4-PROTAC-(VHL-ElonginB-C) ternary complex. (PDB code: 5T35). BRD4: bromodomain-containing protein 4; PEG: polyethylene glycol; VHL: von Hippel Lindau protein; ElonginB-C: subdomain of VHL ligase.

the ligand and the warhead, an E3-PROTAC-POI ternary complex (Figures 3B–3D) is formed. Subsequently, the POI undergoes ubiquitination by the E3 ligase, leading to its proteasomal degradation. The removal of POI induced by PROTAC results in the inhibition of its function. Notably, the formation of the E3-PROTAC-POI ternary complex in this process is reversible, making the ubiquitination of the POI catalytically induced by the PROTAC molecule[27]

PROTACs offer several advantages over conventional small-molecule drugs due to their unique mechanism of action (MoA). Firstly, the catalytic nature of PROTACs enables effective action at low concentrations, contrasting with conventional small-molecule drugs that require high stoichiometrical doses, potentially leading to unexpected side effects.[28] Secondly, PROTACs achieve POI inhibition through degradation, requiring new protein synthesis for recovery, providing longer-term effectiveness compared to conventional small-molecule drugs whose effects diminish with decreasing plasma concentration.[26] Lastly, as molecular glues, PROTACs enjoy more freedom in target selection than conventional small-molecule drugs. PROTACs only necessitate an appropriate surface-exposing lysine residue for ubiquitination and a warhead binding pocket, while conventional small-molecule drugs rely on high-affinity active binding sites.[29] Consequently, PROTACs can target conventionally undruggable proteins, such as scaffold proteins and transcriptional factors, as long as their ligands have been identified.

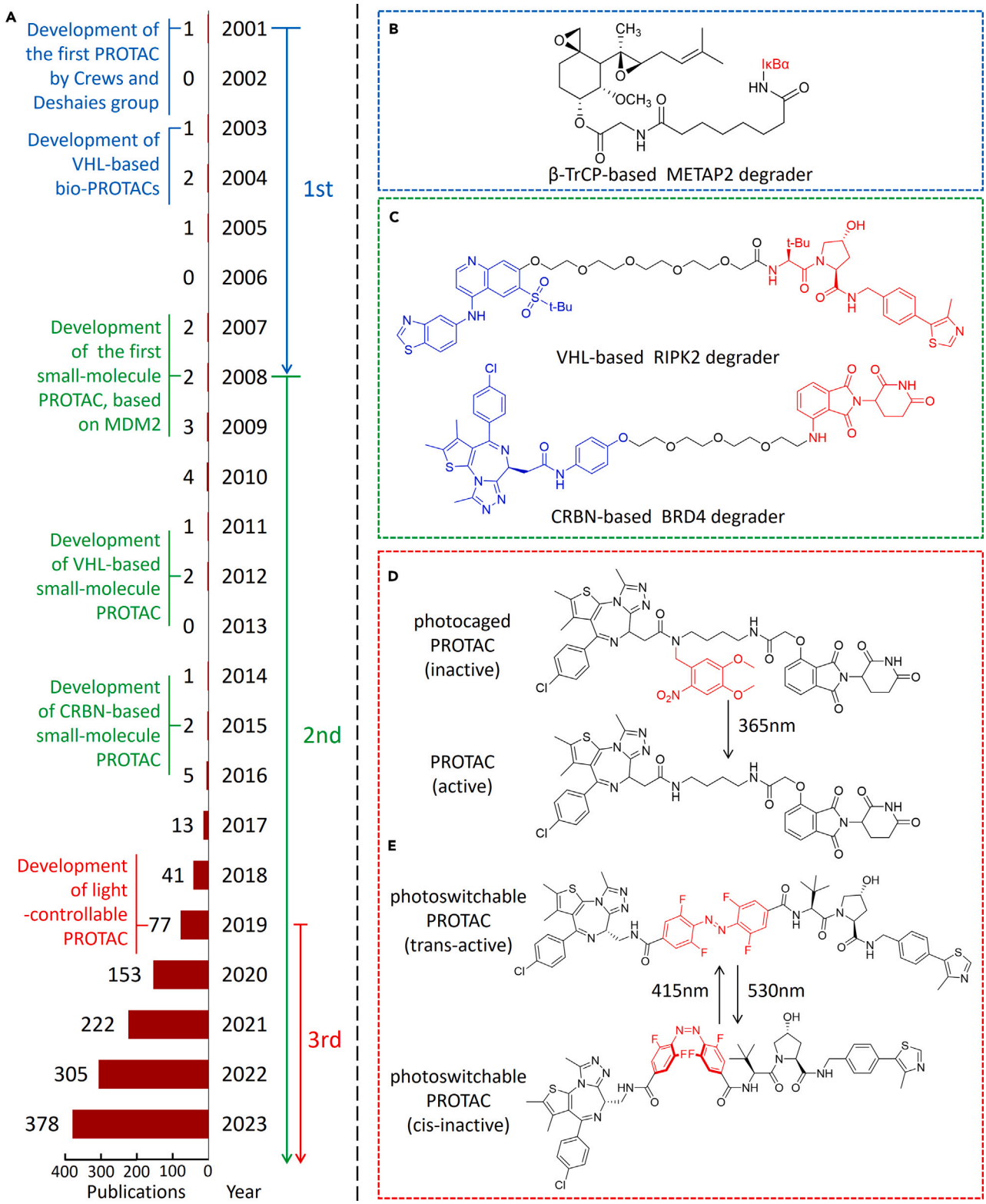


Figure 4. Illustration of the three generations of PROTACs

(A) Timescale illustration of the number of PROTAC publications and significant events in PROTAC development. Blue, green, and red respectively represent the first, second, and third-generation PROTACs. Data obtained from a January 2024 Web of Science search for "PROTAC" not "Protein C".

Figure 4. *Continued*

(B) Chemical structure of a representative first-generation PROTAC: β-TrCP-based bioPROTAC degraders of METAP2. Peptide ligand is marked in red.

(C) Chemical structures of the representative second-generation PROTAC:VHL and CRBN-based small-molecule PROTAC degraders. Peptide ligands are marked in red.

(D) Schematic illustration of photocaged PROTAC. The cage group is marked in red.

(E) Schematic illustration of photoswitchable PROTAC. The ortho-F4-azobenzene linker is marked in red. RIPK2: receptor-interacting protein kinase 2.

Simultaneously, conventionally druggable proteins are also amenable to PROTACs using corresponding small-molecule drugs as warheads.[30]

In order to demonstrate the development process of PROTACs over time, we define in this review that PROTACs have evolved through three generations since their initial conceptual proposal (Figure 4A).[31] In the first generation, as no E3-binding small molecules were known, polypeptides served as ligands to E3 ligases, leading to the classification of the first-generation PROTACs as bio-PROTACs.[5] The inaugural PROTAC, PROTAC-1 utilized a phosphopeptide derived from IκBα (IPP) recognizable by β-transducin repeat-containing (β-TrCP), linking it to ovalicin, a small-molecule methionyl aminopeptidase 2 (METAP2) inhibitor (Figure 4B, left) The strategically designed molecule induced the interaction between β-TrCP and METAP2, resulting in METAP2 ubiquitination and proteasomal degradation.[25] Subsequent IPP-based PROTACs exhibited degradative activities toward androgen receptor (AR) and estrogen receptor (ER).[32] Although first-generation PROTACs were pioneering, their use of peptides as E3 ligands resulted in poor cell permeability and limited clinical potential due to potential immunogenicity.[33]

The second generation, small-molecule PROTACs, emerged with the discovery of small-molecule E3 ligands, overcoming the limitations of bio-PROTACs. This approach remains popular in current TPD drug development. Small-molecule PROTACs primarily leverage four RING type E3 ligases with known ligands for POI ubiquitination, namely nutlin derivatives for mouse double minute 2 (MDM2), peptidomimetics of the hypoxia-inducible factor-1α (HIF-1α) binding peptide for von hippel-lindau (VHL) (Figure 4C, down), thalidomide derivatives for cereblon (CRBN) (Figure 4C, up), and bestatin derivatives for cellular inhibitor of apoptosis (cIAP).[25] CRBN and VHL are most frequently utilized, accounting for 60.1% and 30.1% of developed PROTACs over the past 20 years, while cIAP constitutes 5.5%.[34] Although other E3 ligases like DCAF15,RING finger protein 4 (RNF4), and kelch-like ECH-associated protein 1 (KEAP1) have been applied in PROTACs, the exploration of E3 ligases in PROTACs remains limited considering the vast number encoded in the human genome.[35]

Many second-generation PROTACs are currently under clinical investigation, particularly as anticancer drugs. The first PROTAC to enter clinical trials, ARV-471 developed by Arvinas, demonstrated degradation activity toward ER, showing potential for metastatic breast cancer treatment and helping advance the drug candidate to phase III trials.[36] Arvinas has also developed other PROTAC anticancer drugs, such as ARV-110[37] and ARV-766,[38] both being AR degraders in phase II evaluation with potential for treating metastatic castration-resistant prostate cancer. Other PROTACs in clinical evaluation for cancer therapy include KT-333 developed by Kymera Therapeutics, demonstrating selective degradation of the transcriptional regulator signal transducer and activator of transcription 3 (STAT3) for the treatment of cutaneous T cell lymphoma and peripheral T cell lymphoma.[39] Another example is HSK29116 developed by Haisco Pharmaceutical, a Bruton tyrosine kinase-targeting PROTAC drug for relapsed or refractory B cell malignancies.[40] Both KT-333 and HSK29116 are currently in phase I trials.

Off-tissue effects are persistent issues of modern inhibition-based therapies, so are for the second-generation PROTACs. Aiming to achieve higher controllability and tissue selectivity and avoid off-tissue effects, a recent advance in the field is represented by the third-generation PROTACs, featured by the introduction of controlling groups such as photo cage group, photoswitchable linker and enzyme-responsive cage group, which allow precise control of PROTACs' on-target degradation activity. The most notable example of third-generation PROTACs is light-controllable PROTACs, which can be further classified into photo-caged PROTACs and photoswitchable PROTACs. In a photo-caged PROTAC, a photo-removable group is introduced to the E3 or POI binding domain to inhibit the degradative activity of the PROTAC. Upon reaching the target tissue, the cage group can be cleaved under light irradiation at a specific wavelength, releasing the active PROTAC to eliminate the target protein.[41] Conversely, photoswitchable PROTACs, as illustrated in Figure 4E, introduce the ortho-F4-azobenzene moiety as the linker to reversibly control the topological distance between the warhead and ligand. The azo-trans-isomer induces targeted degradation of the POI, while the azo-cis-isomer is inactive due to the reduced distance, prohibiting the formation of the E3-PROTAC-POI ternary complex. This allows the switch of degradative activity through light-induced conformational transition of the ortho-F4-azobenzene linker and tissue-specific activation of the photoswitchable PROTAC.[42] Additional examples of third-generation PROTACs include enzyme-responsive PROTACs, which achieve tissue-selective antitumor efficacy by introducing enzyme-responsive cage group to the E3 or POI binding domain.[43–46] Though having not been evaluated clinically, these emerging third-generation PROTACs hold promise for improving the controllability and tissue selectivity and avoiding the potential toxicity of second-generation PROTACs associated to off-tissue effects.

Despite the promise of second-generation PROTACs in clinical applications, several key challenges remain to be addressed. Firstly, the molecular weight of PROTACs is generally above 700 Da due to their complex construction, which includes an E3 warhead, a ligand for the POI, and a linker.[47] The relatively larger size, compared to conventional small-molecule drugs, poses a major obstacle for optimizing oral bioavailability and ADME (absorption, distribution, metabolism, and excretion) properties.

In addition, the E3-PROTAC-POI ternary system may exhibit the "hook effect" at high PROTAC concentrations. This phenomenon involves the hindrance of the formation of the E3-PROTAC-POI ternary complex due to the simultaneous formation of E3-PROTAC and PROTAC-POI binary complexes.[48] However, though the occurrence of the "hook effect" has been observed in *in-vitro* studies,[49–51] resulting in a decreased efficiency of the PROTACs, "hook effect" has not been observed in *in-vivo* studies. Therefore, whether or not "hook effect" has a negative impact on PROTAC efficiency in clinical applications is to be further investigated.

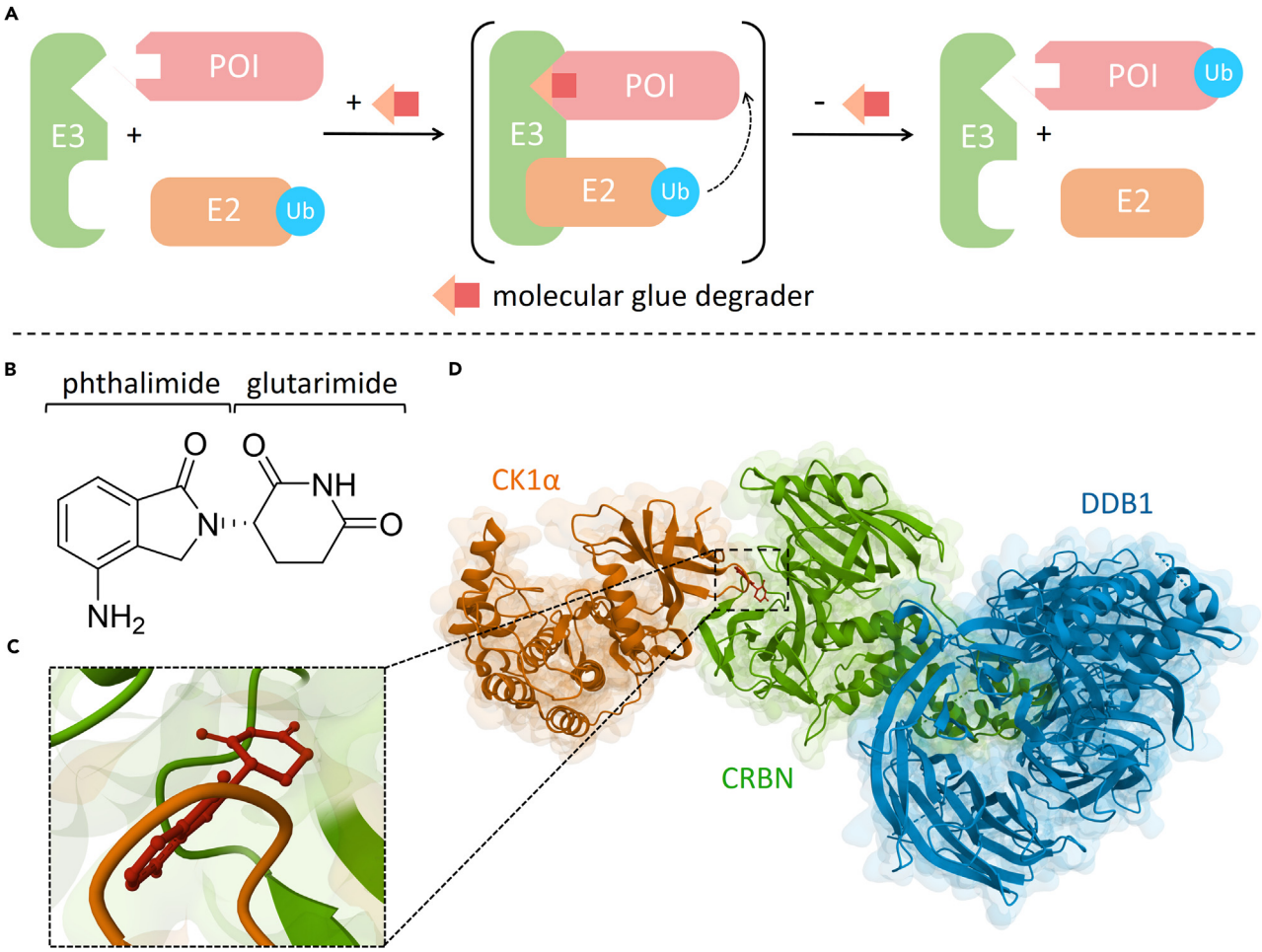


Figure 5. Illustration of the mechanism of action of molecular glue degrader

(A) Schematic representation of molecular glue degrader-induced POI ubiquitination.

(B) Chemical structure of lenalidomide.

(C) Magnified interaction interface of CK1α-lenalidomide-CRBN interaction.

(D) Illustration of CK1α-lenalidomide-(CRBN-DDB1) ternary complex. (PDB code: 5FQD). DDB1: subdomain of the CRBN E3 ligase.

Moreover, reports indicate that tumor cells can develop resistance to CRBN-based and VHL-based PROTACs. In contrast to resistance toward conventional small-molecule drugs, often attributed to secondary mutations in targeted proteins, PROTAC resistance is caused by genomic alterations that downregulate the expression of the corresponding E3 ligase or its subunit. The reduced intracellular concentration of the E3 ligase inhibits the formation of the E3-PROTAC-POI ternary complex, which is essential for the degradative activity of PROTACs, contributing to the acquired resistance of tumor cells.[52] Addressing these challenges is crucial for advancing the efficacy and applicability of PROTACs in therapeutic settings.

Molecular Glue Degraders

In the realm of TPD, molecular glue degraders are defined to denote a class of molecular glues that exhibit proteasomal degradative activity similar to PROTACs. Like PROTACs, molecular glue degraders operate by inducing the interaction between E3 ligase and the protein of interest (POI) (Figure 5A). The formation of the E3-molecular glue degrader-POI ternary complex brings the E3 ligase and POI into proximity, initiating POI ubiquitination and subsequent proteasomal degradation. However, in contrast to PROTACs, molecular glue degraders induce PPI in a distinct manner. A standalone molecular glue degrader molecule may only have an affinity for either the E3 ligase or the POI.[53] The molecule first binds to the corresponding protein with which it has an affinity. Subsequently, the remaining part of the molecule exposed outside the binding pocket creates a new interaction surface with the surrounding residues to recruit the other partner of the PPI.[54] For example, lenalidomide, a compound featuring a phthalimide ring and a glutarimide ring (Figure 5B), induces the interaction between CRBN and casein kinase 1α (CK1α). The glutarimide ring of lenalidomide binds to a hydrophobic pocket on CRBN's surface, while the exposed phthalimide ring of lenalidomide forms an interaction surface together with the surrounding residues of CRBN, which is complementary to a

β-hairpin loop of CK1α and able to recruit CK1α to form CRBN-lenalidomide-CK1α ternary complex (Figures 5C and 5D).[27] Unlike PROTACs, the surface properties of proteins play a significant role in molecular glue degrader-induced ternary complex formation.[55]

Molecular glue degraders share a similar catalytic MoA with PROTACs, enabling them to exert effects at low concentrations and achieve prolonged inhibition of the POI. However, they possess distinct advantages over PROTACs, including improved oral bioavailability and pharmacokinetic properties owing to their smaller size.[56] Moreover, they can target proteins that are challenging for both conventional small-molecule drugs and PROTACs. Unlike PROTACs, which require a ligandable pocket on the POI, they can fit into shallow hydrophobic grooves on the surface of the POI or E3 ligase, utilizing surrounding amino residues to create a protein recruiting surface.[56] Although an appropriate lysine residue remains necessary, the potential target spectrum of molecular glue degraders is significantly broader compared to PROTACs. Conversely, the lack of necessity for ligandable pockets and the unique MoA of molecular glue degraders, coupled with limited knowledge of PPI interfaces, present challenges for their rational design.[57] As a result, the discovery of new molecular glue degraders often arises from serendipitous or rational findings, limiting their development (Figure 6A).

The serendipitous discovery of molecular glue degraders often stems from the investigation of known drugs, such as immunomodulatory drugs (IMiDs) (Figure 6B). Thalidomide, the first IMiD recognized as a molecular glue degrader, was initially prescribed to treat morning sickness in pregnant women and led to one of the most severe medical disasters due to its teratogenicity.[58] In 2010, thalidomide's teratogenicity was linked to CRBN,[59] and in 2014, structural evidence demonstrated its molecular glue degrader nature.[60] Derivatives of thalidomide, namely lenalidomide and pomalidomide, were identified as molecular glue degraders as well.[61] and IMiDs are now applied clinically for the treatment of multiple myeloma. Another case of serendipitous molecular glue degrader discovery is aryl sulfonamides (Figure 6C), resulting from exploring the mechanism behind their antitumor activity. Indisulam, an aryl sulfonamide derivative, was discovered as an antitumor drug in 2001.[62] Initially thought to produce antitumor efficacy as carbonic anhydrase inhibitors,[63] indisulam and its analogs, tasisulam, and CQS were later identified as chemical inducers of the interaction between RNA binding motif protein 39 (RBM39) and DCAF15 E3 ligase in 2017, leading to RBM39 ubiquitination.[16] Indisulam was further confirmed as a molecular glue degrader through X-ray crystallography and cryoelectron microscopy analysis.[64–66]

Despite the potential for TPD revealed in the serendipitous discovery of molecular glue degraders and subsequent research,[67] their serendipitous identification and lack of rational design principles have significantly limited systematic therapeutic assessment and development. Therefore, various strategies for rational discovery, including high-throughput screening and chemogenomic screening, have been employed in molecular glue degrader identification.[56] For instance, (R)-CR8, a cyclin-dependent kinase (CDK) inhibitor, was identified as a molecular glue degrader through systematic database mining. The Ebert group analyzed the correlation between the cytotoxicity of 4,518 clinical and preclinical small molecules and the expression levels of E3 ligase components across hundreds of human cancer cell lines.[68] This analysis led to the discovery of the association between the cytotoxicity of (R)-CR8 (Figure 6D) and the mRNA levels of damage-specific DNA binding protein 1 (DDB1) E3 ligase, suggesting that (R)-CR8 likely acted as a CDK degrader, a hypothesis further validated through experiments.[68] Another example of rational discovery is dCeMM1∼4 (Figures 6E and 6F), identified through comparative chemical profiling. The Winter group conducted a screen on a library of approximately 2,000 cytostatic/cytotoxic small molecules in hyponeddylated versus neddylation-proficient cells, identifying dCeMM1 and dCeMM2-4, an aryl sulfonamides analog and DDB1-based molecular glue degrader degraders of CDK, respectively.[69]

While the rational design of molecular glue degraders is challenging, significant progress has been made based on research into their MoA, structure-effect relationships, and protein-molecular glue degrader interactions, enabling structure-based rational optimization of them.[70] An illustrative example is NRX-103094, derived from NRX-1532 which induces the interaction between β-catenin and β-TrCP. Understanding the potential movement of the N-terminus of the β-catenin peptide, NRX-103094 was designed by introducing a diarylthioether group into NRX-1532, resulting in increased affinity.[71] Another case is CC-90009, derived from CC-885, where difluoro substitution in the urea linker (Figure 6H) was identified as a crucial factor for high *in vitro* selectivity and activity. Further optimization led to the development of CC-90009, a potent clinical candidate.[72]

Before their MoA as molecular glue degraders was elucidated, IMiDs and aryl sulfonamides had already been Food and Drug Administration (FDA)-approved for clinical therapies, saving many lives. Meanwhile, newly developed molecular glue degraders are making their way to clinical trials. A promising category is cereblon E3 ligase modulating drugs (CELMoDs) derived from thalidomide, with candidates such as CFT7455 targeting ikaros zinc finger 1/3 (IKZF1/3) and TMX-4116 targeting CK1α entering clinical trials.[73,74] E7820, an aryl sulfonamide analog used in clinical treatment of solid tumors, is undergoing a phase II clinical trial for the treatment of elapsed or refractory myelodysplastic syndromes.[75,76] Recently emerging molecular glue degraders, including (R)-CR8 and NRX-252114, are also in various stages of clinical evaluation.[68,71]

However, unlike PROTACs, which have been explored for treating various human diseases and cancers, molecular glue degraders' clinical applications primarily focus on cancer treatment.[77] The development of molecular glue degraders for non-oncoprotein degradation remains to be achieved. Furthermore, resistance to lenalidomide has been observed in the treatment of multiple myeloma, which is largely attributable to genomic alteration-associated suppression of CRBN.[78] This indicates that molecular glue degraders also face the problem of acquired resistance similar to PROTACs, emphasizing the need for the development of innovative molecular glue degraders. To address these challenges and expand the spectrum of molecular glue degrader applications, advancing the understanding of PPI interfaces and protein-small-molecule interactions is crucial for developing rational design principles for molecular glue degraders.

LYS-BASED TECHNIQUES

Lysosome is a single membrane-enclosed cytoplasmic organelle containing over 60 types of acid hydrolases, including lipases, proteases, and glycosidases.[79] In eukaryotic cells, the lysosome primarily serves to degrade biomacromolecules such as proteins, lipids, nucleic acids,

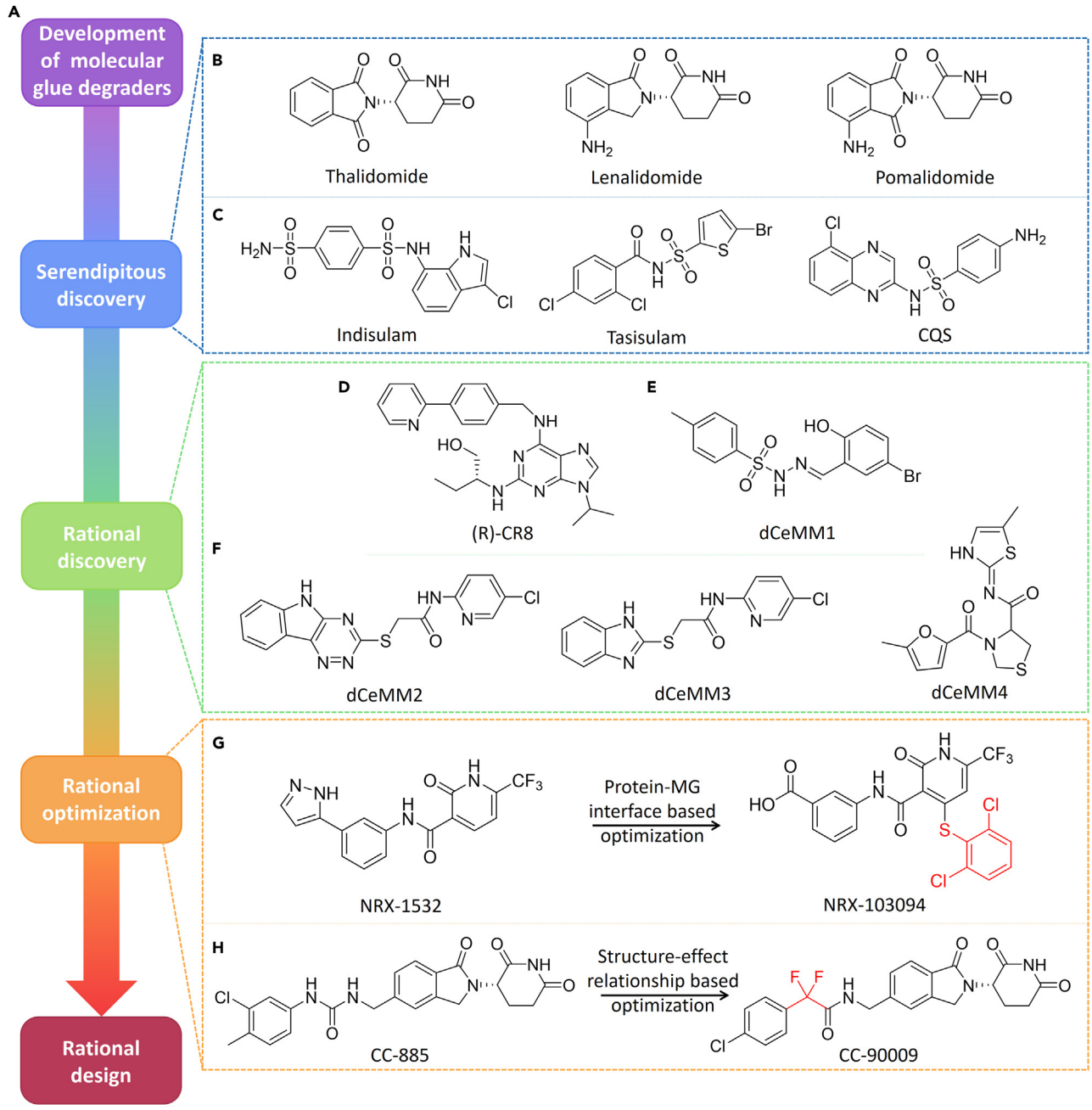


Figure 6. Illustration of the development of molecular glue degraders

(A) Overview of the development of molecular glue degraders.

(B) Chemical structures of IMiDs.

(C) Chemical structures of aryl sulfonamide molecular glue degraders.

(D) Chemical structure of (R)-CR8.

(E and F) Chemical structures of dCeMM1-4.

(G) Schematic illustration of NRX-1532 optimization. Diarylthioether group is marked in red.

(H) Schematic illustration of CC-885 optimization. Difluoro-substituted linker is marked in red.

extracellular particles, and dysfunctional organelles.[80] Illustrated in Figure 7, materials for degradation are transported to the lysosome through endocytosis, phagocytosis, and autophagy.[81]

Endocytosis and phagocytosis handle the transportation of extracellular cargoes, with endocytosis internalizing cytoplasmic proteins, including signaling receptors and ion channels, as well as extracellular molecules.[82] Larger particles with diameters exceeding 0.5 μm are

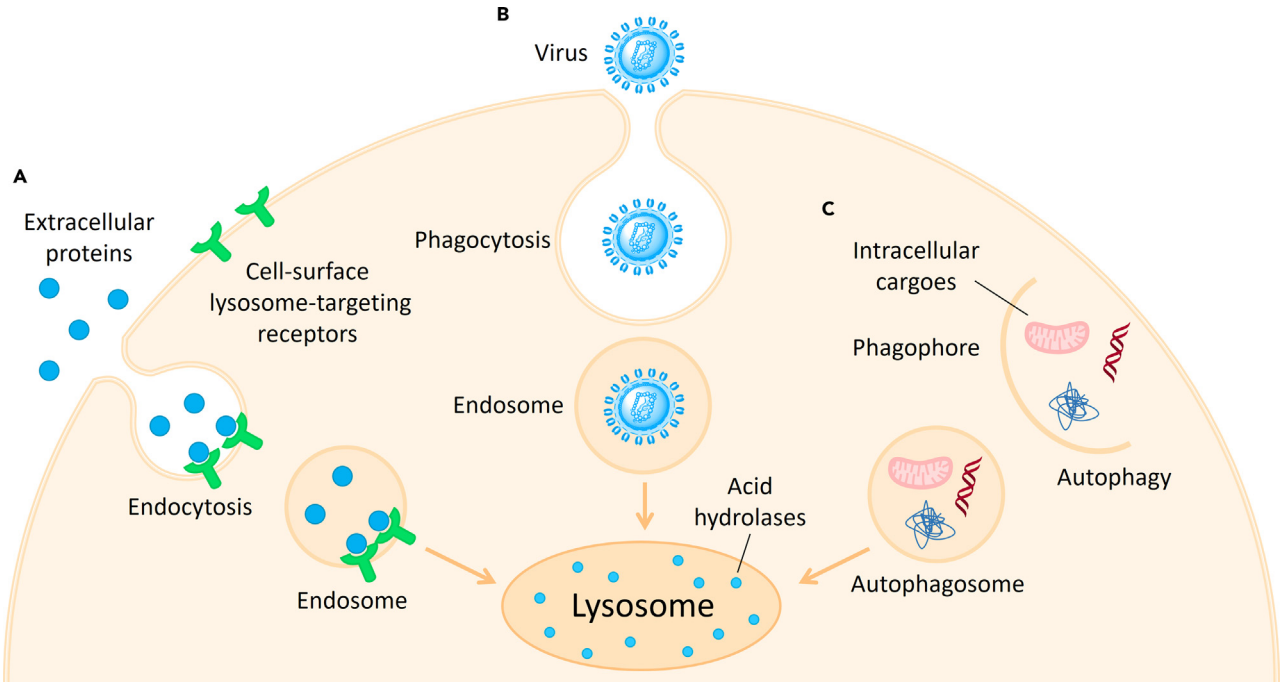


Figure 7. Schematic representation of transportation pathways to lysosome

(A) Overview of the endocytosis pathway.

(B) Overview of the phagocytosis pathway.

(C) Overview of the autophagy pathway.

internalized through phagocytosis. Additionally, apoptotic cells and pathogens are directed to the lysosome for elimination through phagocytosis, playing a crucial role in immune defense.[83] On the other hand, autophagy is categorized into macroautophagy, microautophagy, and chaperone-mediated autophagy, responsible for the transportation of intracellular cargoes. These cargoes mainly include damaged or misfolded proteins, other biomacromolecules, and dysfunctional organelles.[84] Based on the physiological function of LYS depicted previously, LYS-based techniques including MoDE-As and MADTACs are developed.

MoDE-As: molecular degraders of extracellular proteins through the asialoglycoprotein receptors

Extracellular and membrane proteins, constituting 40% of the proteins encoded in the human proteome, play pivotal roles in critical physiological processes such as signal transduction, nutrient sensing, and cell polarity maintenance.[85] They are also implicated in various diseases, particularly aging, neurodegenerative diseases, and cancers.[82] Therefore, targeting these proteins is of great significance for TPD techniques. However, as an intracellular system, UPS is ineffective for these extracellular and membrane proteins.[5] To address this limitation, researchers have turned to the lysosome, developing lysosome-based techniques as a complementary approach to UPS-based techniques.[86]

In recent years, two biologics-based techniques, antibody-based PROTACs (AbTACs) and lysosome-targeting chimaeras (LYTACs) have emerged as novel strategies specifically designed for targeting extracellular and membrane proteins. AbTACs employ bispecific antibodies to mimic the MoA of PROTACs. Bispecific antibodies have two binding sites directed at either two different antigens or two distinct epitopes on the same antigen.[87] The recombinant bispecific IgGs used in AbTACs induce the colocalization of the POI and transmembrane E3 ligase, leading to POI internalization and subsequent lysosomal degradation.[88] On the other hand, by conjugating cell-surface lysosome-targeting receptors (LTRs) ligands to the antibody of POI, LYTACs are able hijack LTRs to induce POI internalization and lysosomal degradation. The two precursors have illustrated that lysosome system can be utilized in the TPD of extracellular and membrane proteins. Based on the findings in the studies of LYTACs, MoDE-As are developed as molecular glue-based technique for extracellular proteins.

LTRs play a crucial role in lysosomal delivery systems by recognizing glycoproteins with specific glycosylation. When an LTR binds to its corresponding glycosyl ligand, endocytosis is triggered. The LTR, along with the glycoprotein cargo, is internalized and transported to the lysosome. In the acidic lysosomal environment, the LTR dissociates from the glycosyl ligand, releasing the glycoprotein cargo, while the LTR is recycled and returns to the cell surface.[89] MoDE-As leverage this mechanism by utilizing conjugations of LTR ligands and POI ligands, while LYTACs by conjugations of LTR ligands and antibodies, to induce the colocalization of a cell-surface LTR and the target extracellular or membrane protein, leading to POI transportation to the lysosome.[90]

LTRs currently employed in LYTACs include the cation-independent mannose-6-phosphate receptor (CI-M6PR) and the asialoglycoprotein receptor (ASGPR). Additionally, ASGPR is employed in MoDE-As as well. CI-M6PR, expressed in various tissue types, participates in the

mannose-6-phosphate pathway and recognizes glycoproteins with M6P recognition markers, transporting them to the lysosome via endocytosis.[91] ASGPR, specific to liver tissues, recognizes glycoproteins with N-acetylgalactosamine (GalNAc) markers and delivers them to the lysosome through the endocytic pathway.[92]

The earliest LYTACs developed by the Bertozzi group employ CI-M6PR ligands and antibodies for POI transportation and recognition, as depicted in Figure 8A. Instead of utilizing the phosphatase-sensitive mannose-6-phosphate (M6P) as CI-M6PR ligand, a phosphatase-inert analog, is utilized.[93] In detail, the LYTACs comprises an antibody specific to the POI and a glycopolypeptide bearing multiple serine-O-mannose-6-phosphonate (M6Pn) residues. These LYTACs were demonstrated to exhibit degradative activity apolipoprotein E4, epidermal growth factor receptor (EGFR), transferrin receptor-1, and programmed death ligand 1.[93] To achieve tissue-specific degradation, ASGPR-based LYTACs, known as GalNAc-LYTACs, were developed with triantenerrary N-acetylgalactosamine (tri-GalNAc) serving as the ASGPR ligand (Figure 8B). A GalNAc-LYTAC comprises a tri-GalNAc domain and an antibody targeting the POI. GalNAc-LYTACs employing antibodies like cetuximab, pastuzumab, and polyspecific integrin-binding peptide exhibited tissue-selective degradative activities toward EGFR, human epidermal growth factor receptor 2 (HER2), and integrin.[94,95]

Intriguingly, in the study of CI-M6PR-based LYTACs, it was discovered that a poly(M6Pn)-bearing glycopolypeptide and biotin conjunction was able to induce the proximity between CI-M6PR and neutravidin, triggering the internalization of neutravidin.[93] Building on this discovery, as depicted in Figure 8D, A molecule composed of tri-GalNAc and biotin was designed. This LYTAC demonstrated degradative activity toward neutravidin, suggesting the possibility that LTR ligand and POI warhead conjunction could induced the lysosomal degradation of POI in a similar manner to LYTACs.[95] In 2021, the Spiegel group reported a novel sort of bifunctional synthetic molecules called Molecular Degraders of Extracellular proteins through the Asialoglycoprotein receptor (MoDE-As) (Figure 8C).[96] MoDE-As shares a similar ligand-linker-warhead structure with PROTACs, comprising a tri-GalNAc ligand and a POI-targeting warhead. MoDE-As function by inducing the interaction between ASGPR and the POI, forming an ASGPR-MoDE-A-POI ternary complex (Figure 8E). Subsequently, the POI is endocytosed and trafficked to lysosome for degradation. The Spiegel group designed two MoDE-As respectively targeting dinitrophenol antibody and cytokine macrophage migration inhibitory factor. They were demonstrated to exhibit degradative their corresponding target proteins both *in vitro* and *in vivo*.[96] Notably, both target proteins are extracellular proteins, therefore, the ability of MoDE-As to mediate the lysosomal degradation of membrane proteins.

MADTACs: macroautophagy degradation targeting chimeras

While the endocytic pathway has been harnessed in LYTACs for degrading extracellular and membrane proteins,[97] the autophagic pathway, responsible for intracellular cargo transportation, holds promise for lysosomal degradation of intracellular proteins. Macroautophagy, a major type of autophagy, can be categorized into non-selective autophagy and selective autophagy.[98] Non-selective autophagy, or bulk autophagy, occurs in response to nutrient deficiency, degrading various cytoplasmic substances, including proteins, lipids, nucleic acids, and organelles, to provide essential nutrients.[84] In contrast, selective autophagy recognizes and degrades specific cargoes, such as mitophagy (mitochondria), xenophagy (invading pathogens), aggrephagy (protein aggregates), lipophagy (lipid droplets), etc.[99] In selective autophagy, K63-ubiquitin-marked cargoes are identified by the ubiquitin-associated domain of autophagy receptors, such as p62. These receptors then interact with microtubule-associated protein light chain 3 (LC3) via LC3-interacting region domains, ultimately triggering the selective autophagy of the cargoes.[100]

A novel class of lysosome-targeting molecular glues, known as MADTACs, has been developed to exploit macroautophagy for the lysosomal degradation of intracellular proteins. MADTACs comprise two subtypes: autophagy-targeting chimeras (AUTACs and AUTOTACs) and autophagosome-tethering compounds (ATTECs).

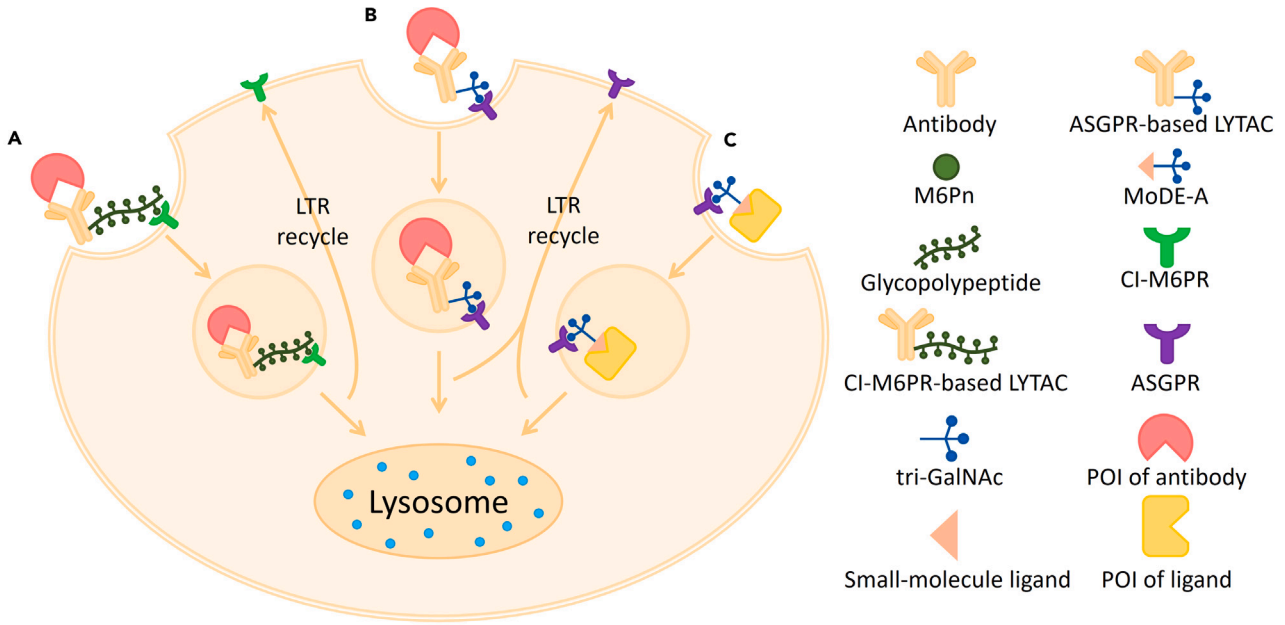
AUTACs: autophagy-targeting chimeras

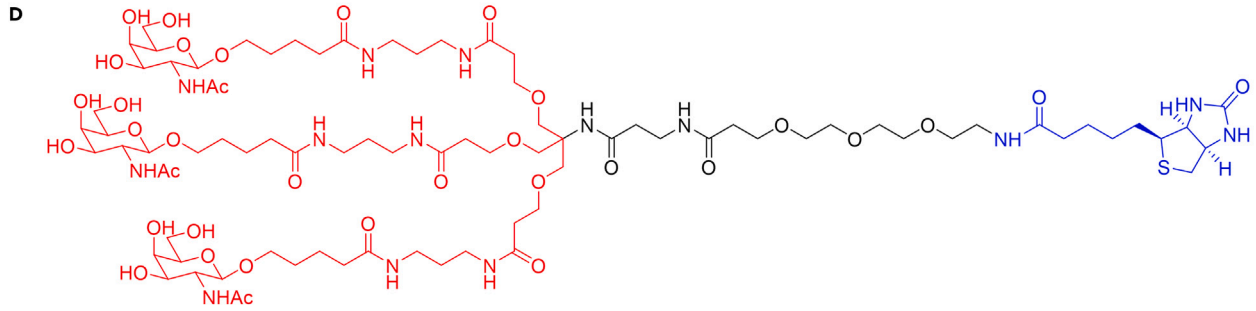
The development of AUTACs was inspired by the discovery that the cysteine residue of invading cytoplasmic group A streptococci (GAS) could be S-guanylated by endogenous nucleotide 8-nitroguanosine-cyclic monophosphate, leading to GAS K63-polyubiquitination and subsequent lysosomal clearance during xenophagy.[101] Subsequent research revealed the colocalization of S-guanylated proteins with K63-ubiquitin, p62, and LC3B, key players in selective autophagy.[102]

AUTACs, illustrated in Figure 9A, consist of a warhead for the POI, a linker, and a *p*-fluorobenzylguanine (FBnG) tag with similar activity to 8-nitroguanosine-cyclic monophosphate, mitigating side effects.[103] Researchers designed AUTAC1-3 targeting METAP2, FK506 binding protein 12 (FKBP12), and BRD4, respectively (Figure 9B), validating their ability to induce lysosomal degradation of the corresponding POIs. Interestingly, AUTACs also demonstrated the capability to expedite the degradation of dysfunctional, fragmented mitochondria. AUTAC4, utilizing 2-phenylindole-3-glyoxyamide as the warhead for the translocator protein on the outer mitochondrial membrane, delivered a guanine tag to the mitochondrial membrane, inducing mitophagy (Figure 9A). AUTACs exhibited significant clinical potential, particularly in inducing the mitophagy of dysfunctional, fragmented mitochondria.[103] However, the mechanism of autophagy induction via AUTACs requires further elucidation through additional investigations.

AUTOTACs: autophagy-targeting chimeras

Proteins' *in vivo* half-lives are associated with degradation signals, mainly determined by N-terminal residues, termed N-degrons.[102] The selective autophagy receptor p62 functions as an N-recognin, binding to type-1 or type-2 N-degrons via a ZZ-type zinc finger domain. Upon binding, p62 undergoes conformational activation and polymerization, inducing selective autophagy of the protein.[104]





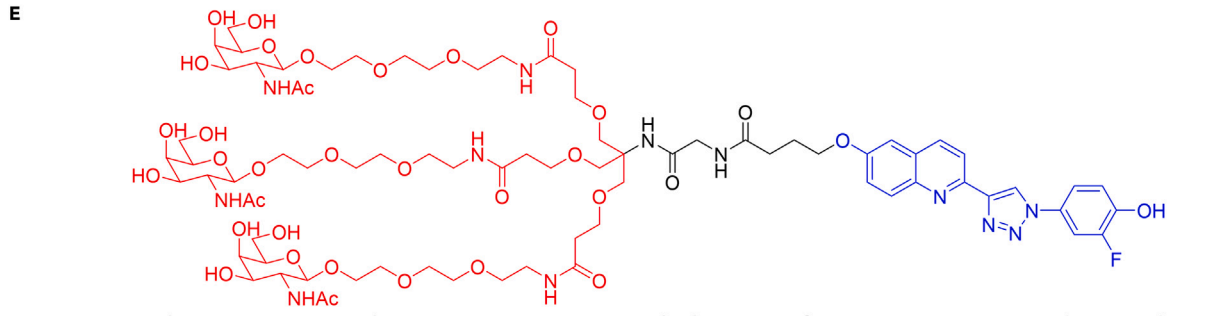


Figure 8. Illustration of the mechanism of action and chemical structures of LYTACs and MoDE-As

(A) Schematic representation of CI-M6PR-based LYTACs.

(B) Schematic representation of ASGPR-based LYTACs.

(C) Schematic representation of MoDE-As.

(D) Chemical structure of tri-GalNAc-biotin-LYTAC.

(E) Chemical structure of MoDE-A degrader of cytokine macrophage migration inhibitory factor. Tri-GalNAc and POI warheads are respectively marked in red and blue.

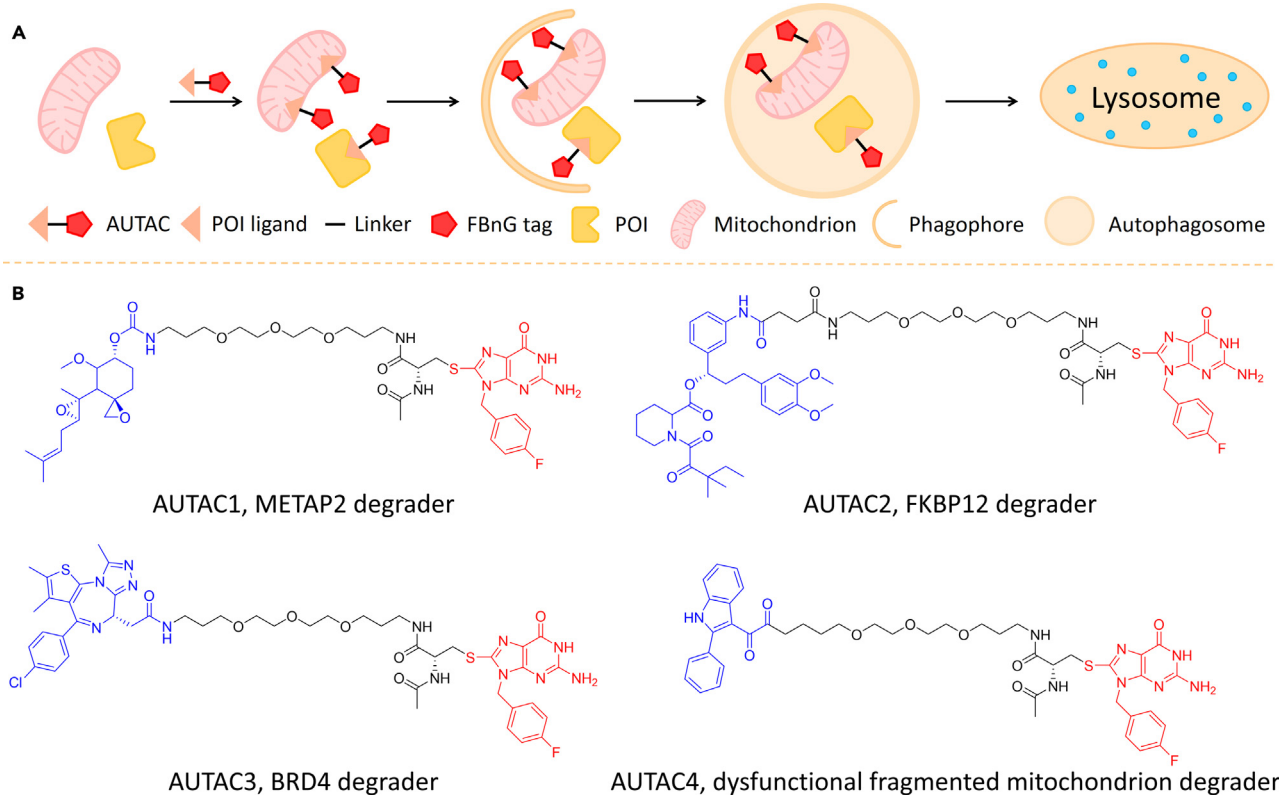


Figure 9. Illustration of MoA and chemical structures of AUTACs

(A) Schematic representation of AUTACs.

(B) Chemical structures of AUTAC1-4. FBnG and POI warheads are respectively marked in red and blue.

Building upon p62's role as an N-recognin, the Kwon group developed a novel class of lysosome-based bifunctional molecules for TPD, termed AUTOTACs. As depicted in Figure 10A, AUTOTACs share a PROTAC-like structure, comprising a POI warhead, a polyethylene glycol (PEG) linker, and an activated ligand for the ZZ domain of p62. AUTOTACs with warheads targeting ERβ, AR, and METAP2 have demonstrated lysosomal degradative activity against their respective proteins (Figures 10B–10D).[105] Notably, AUTOTACs can also induce the degradation of misfolded and aggregated proteins. Using Anle138b, a warhead for neurodegenerative proteinopathy-associated protein oligomers and aggregates, and 4-phenylbutyric acid (PBA), a selective recognizer of exposed hydrophobic regions commonly found in misfolded proteins, AUTOTACs successfully degrade UPS-resistant misfolded proteins, their oligomers, and neurodegenerative disease-relevant proteins like P301L tau mutant and mutant huntingtin (Figures 10E–10G).[105] However, the lack of selectivity in Anle138b and PBA-based AUTOTACs may lead to unexpected side effects, necessitating the development of more selective warheads.

ATTECs: autophagosome-tethering compounds

Huntington's disease (HD) is a presently incurable neurodegenerative disorder caused by a variation in the huntingtin gene on chromosome 4, involving the repetition of a dominantly inherited CAG trinucleotide.[106] The expression of the mutant huntingtin gene results in the production of the mutant huntingtin protein (mHTT), characterized by an abnormally expanded repeat of polyglutamine (polyQ). mHTT tends to aggregate, and these aggregates may contribute to neuronal dysfunction and programmed cell death. Consequently, targeted clearance of mHTT is crucial for HD treatment. *In vivo*, mHTT is degraded through autophagy, with the autophagosome protein LC3 playing a pivotal role. The Lu group hypothesized that compounds exhibiting high affinity for both LC3 and mHTT, while showing little affinity for normal huntingtin protein (HTT), could serve as mHTT-LC3 linkers, inducing selective autophagic clearance of mHTT.[107]

Two candidate compounds, 10O5 and 8F20, through small-molecule microarray-based screening were identified by the Lu group.[107] Building on the common features of 10O5 and 8F20, another screening was conducted, leading to the identification of two additional hit compounds, AN1 and AN2 (Figure 11B).[107] These four compounds were then investigated as mHTT-ATTECs, demonstrating lysosome-dependent degradative activity toward mHTT as hypothesized (Figure 11A). Moreover, the mHTT-ATTECs exhibited degradative activity toward a long expanded polyQ stretch bearing green fluorescent protein (GFP), which suggested the specificity of mHTT-ATTECs toward mHTT was attributed to their specific recognition of long expanded polyQ tracts.[107] Furthermore, mHTT-ATTECs were found to function as LC3 ligands. By conjugating LC3 ligands to warheads for BRD4[108] and nicotinamide phosphoribosyltransferase (NAMPT),[109] PROTAC-

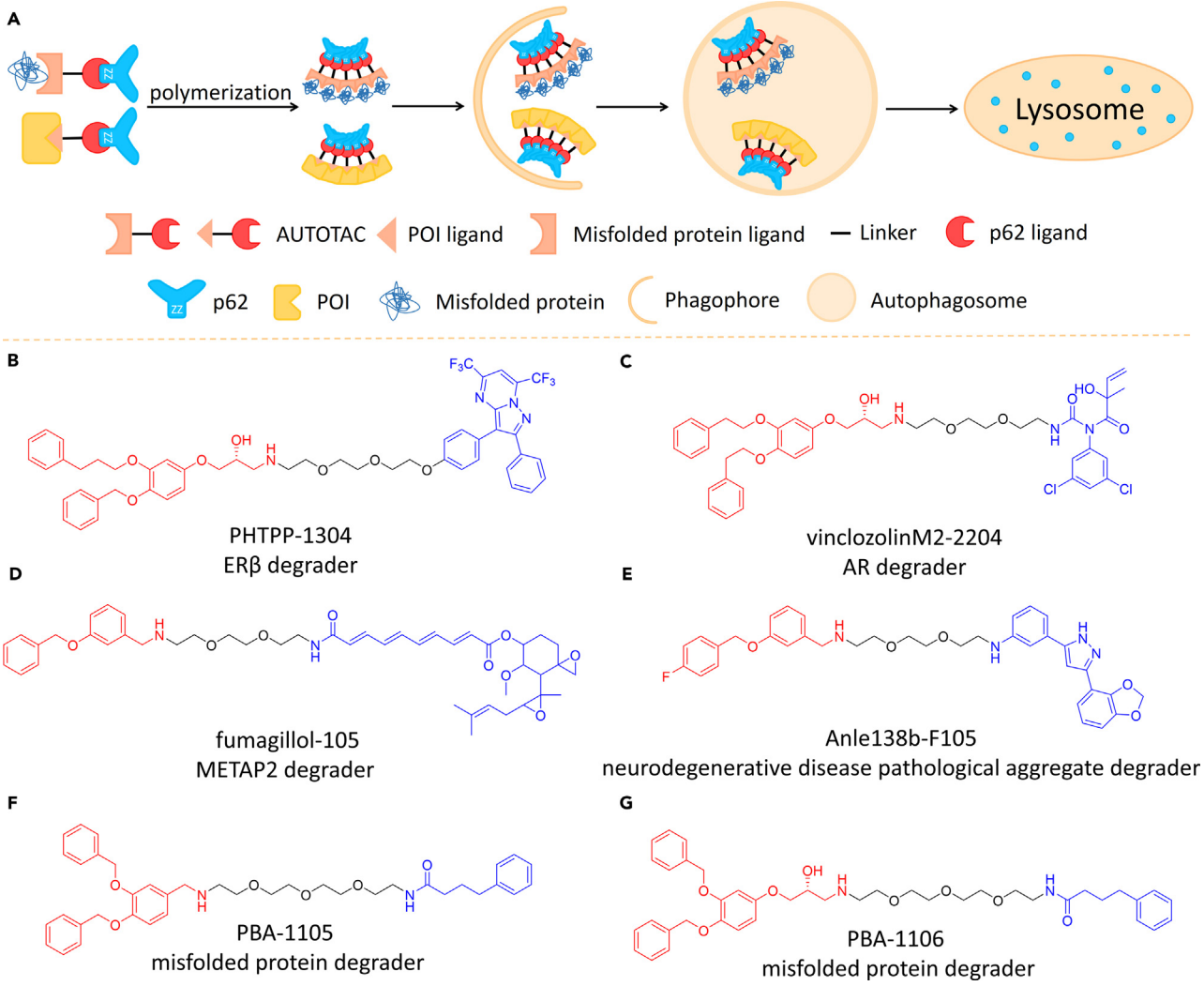


Figure 10. Illustration of MoA and chemical structures of AUTOTACs

(A) Schematic representation of AUTOTACs.

(B–D) Chemical structures of AUTOTAC degraders of ERβ, AR, and METAP2.

(E) Chemical structure of Anle138b-based AUTOTAC.

(F and G) Chemical structures of PBA-based AUTOTAC. p62 ligands and POI warheads are respectively marked in red and blue.

like ATTECs were designed to induce the targeted degradation of corresponding proteins, showcasing the potential of ATTECs as degraders for other intracellular disease-associated proteins (Figure 11C). Interestingly, ATTECs were also capable of inducing lipophagy by utilizing Sudan III and Sudan IV as ligands for lipid droplets (Figure 11D), suggesting the broadened potential of ATTECs to target non-protein biomacromolecules.[110]

SUMMARY AND OUTLOOK

Molecular glues represent a novel class of small-molecule drugs, offering a targeted degradation strategy for inhibiting pathology-associated proteins. This approach allows molecular glue degraders to achieve prolonged inhibition compared to conventional occupancy-driven methods. Their catalytic mechanism enables inhibitory effects at low concentrations, minimizing side effects and acquired resistance during extended administration. Crucially, molecular glues overcome the limitations of conventional small-molecule drugs, making many traditionally undruggable proteins accessible. In conclusion, the molecular glue-mediated targeted protein degradation strategy holds promise for treating diseases associated with intractable proteins.

PROTACs, as an early UPS-based technique, have undergone three generations of development. The second generation is in clinical trials for cancer treatment, with the third generation focusing on controllability, originality, and novel modalities. However, limitations such as high molecular mass, the hook effect observed in *in-vitro* studies, and the risk of acquired resistance hinder the application of PROTACs.

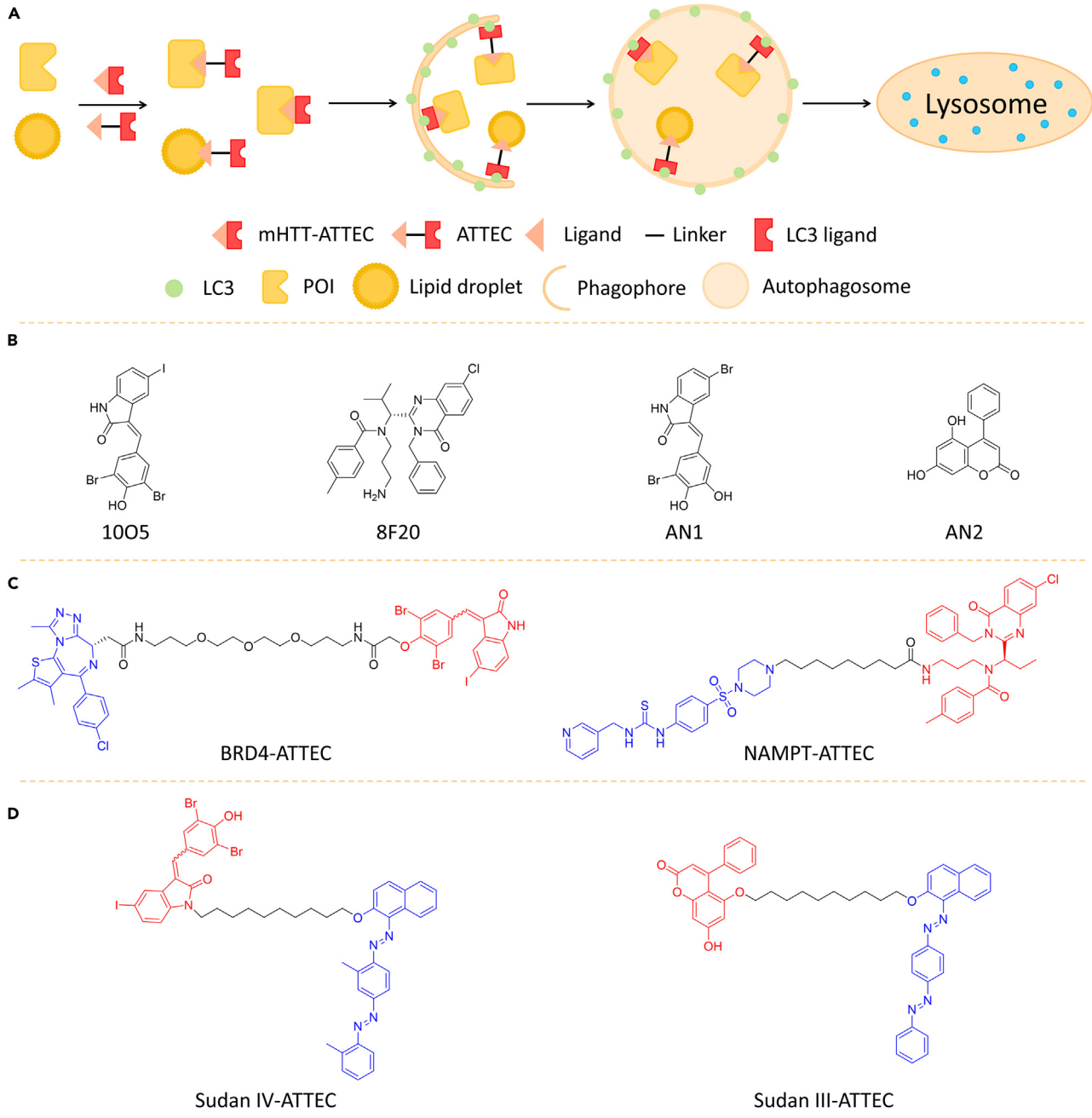


Figure 11. Illustration of the mechanism of action and chemical structures of ATTECs

(A) Schematic representation of ATTECs.

(B) Chemical structures of mHTT-ATTECs.

(C) Chemical structures of ATTEC degraders of BRD4 and NAMPT.

(D) Chemical structures of ATTEC degraders of lipid droplets. LC3 ligands and POI warheads are respectively marked in red and blue.

Addressing these challenges requires further exploration of rational design and optimization principles and the development of novel E3 ligase-based PROTACs. Molecular glue degraders, sharing modalities similar with conventional small-molecule drugs, have advantages in molecular weight. Despite the newly developed molecular glue degraders entering clinical trials for cancer treatment, the lack of rational design principles impedes molecular glue degrader development, necessitating further exploration in this area.

Though LYS-based techniques are still in their infancy, they show potential in targeting proteins challenging for UPS-based techniques. MoDE-As broaden the spectrum to extracellular proteins, demonstrating tissue-selective degradative activity as well. MADTACs are able to target

protein oligomers, aggregates, and even non-protein targets. Particularly, AUTACs exhibit degradative activity toward dysfunctional fragmented mitochondria, and ATTECs can induce autophagy of lipid droplets. In addition, intracellular protein monomers are included in MADTACs' spectrum of targets as well. While UPS-based techniques, especially PROTACs, offer more ligand selections due to diverse E3 ligases, MADTACs provide an alternative for UPS-based techniques. In conclusion, LYS-based techniques complement UPS-based techniques effectively.

Most small molecules utilized in MoDE-As and MADTACs have a PROTAC-like structure, allowing design insights from PROTACs. However, they also possibly face challenges such as the hook effect and issues associated with large molecular weights. Conversely, molecular glue degrader-like molecules, namely mHTT-ATTECs, offer advantages in bioavailability and ADME properties due to their smaller sizes. Despite also facing the lack of rational design principles, mHTT-ATTECs are able to provide a reference for the development of novel molecular glue degrader-like molecules for LYS-based techniques. However, the mechanism of action of LYS-based techniques is not fully elucidated, for instance the function of S-guanine tag in AUTAC-induced autophagic degradation, necessitating further investigation. Comprehensive exploration of pharmacological properties, therapeutic index, and efficacy in disease models is essential as well. Considering the complexity of the LYS compared to UPS, many mechanisms may be harnessed for developing novel LYS-based techniques based on molecular glues. In conclusion, ongoing research is vital to optimize existing LYS-based techniques and develop novel LYS-based techniques.

Over the past two decades, molecular glue-mediated TPD techniques have emerged as a novel strategy for small-molecule drugs, sparking significant interest in the TPD field. These techniques enable the hijacking of cellular degradation systems for the targeted degradation of proteins, with the potential to target non-protein entities and expand the application spectrum of small-molecule drugs. Recent reports even suggest that molecular glues can be utilized to modulate the posttranslational modification of proteins,[111] hinting at the prospect of precisely influencing other essential cellular processes via molecular glues. The ongoing exploration of the potential of molecular glues opens exciting avenues for future research and applications.

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AUTHOR CONTRIBUTIONS

Z.H. and X.T. are responsible for writing the article and have the equal contribution. X.T. revised and edited the article. Z.H made the drawings and adjustments to the references. H.P., J.Z., and Z.J supervised and polished the article.

DECLARATION OF INTERESTS

The authors declare no conflicts of interest.

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