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REVIEW

Expanding the horizons of targeted protein degradation: A non-small molecule perspective

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Abstract Targeted protein degradation (TPD) represented by proteolysis targeting chimeras (PROTACs) marks a significant stride in drug discovery. A plethora of innovative technologies inspired by PROTAC have not only revolutionized the landscape of TPD but have the potential to unlock functionalities beyond degradation. Non-small-molecule-based approaches play an irreplaceable role in this field. A wide variety of agents spanning a broad chemical spectrum, including peptides, nucleic acids, antibodies, and even vaccines, which not only prove instrumental in overcoming the constraints of conventional small molecule entities but also provided rapidly renewing paradigms. Herein we summarize the burgeoning non-small molecule technological platforms inspired by PROTACs, including three major trajectories, to provide insights for the design strategies based on novel paradigms.

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1. Introduction

Over the last two decades, targeted protein degradation (TPD) has garnered escalating attention from both academic and industrial spheres. The conceptualization that proteins' destinies can be modulated by co-opting the endogenous protein degradation apparatus constitutes a groundbreaking paradigm shift in the realm of drug discovery[1,2]. Proteolysis targeting chimeras (PROTACs), an exemplar within the TPD strategies predicated upon the ubiquitin−proteasome system (UPS), has evinced remarkable efficacy. Among the myriad of small molecule PROTACs documented hitherto, more than a dozen have commenced clinical translational endeavors[3−6]. Functioning as heterobifunctional entities, PROTACs comprise three integral constituents: a ligand that interfaces with the protein of interest (POI), a compound that enlists the ubiquitin E3 ligase, and a linker that effectuates their convergence. By concurrently engaging the E3 ligase and POI, thereby culminating in the establishment of a stable ternary complex, PROTACs effectuate the co-optation of the cellular UPS, consequently instigating the polyubiquitylation and subsequent proteasomal degradation of POI[7].

The salient attributes characterizing PROTAC molecules encompass their distinctive event-driven pharmacology and sub-stoichiometric catalysis mechanism, conferring upon them advantages unattainable by conventional small molecule inhibitors[8−12]. Notably, the proficient degradation of POIs engendered by PROTACs is not contingent upon robust interactions between the ligands and the POIs, thereby enabling the degradation of proteins conventionally deemed "un-druggable", inclusive of those harboring shallow and expansive binding clefts. Furthermore, after the degradation of targeted proteins, a protracted state of "knockdown" endures until nascent proteins are synthesized. These pharmacological attributes obviate the occurrence of compensatory upregulation of target proteins post-inhibition, a predominant antecedent of resistance to small molecule inhibitors. Furthermore, the sub-stoichiometric catalysis mechanism imparts a superior pharmacodynamic performance to PROTACs, endowing them with the capacity for potent and sustained exposure at minimal doses, thus contributing to enhanced drug delivery and mitigated dose-associated toxicity.

Concomitant with the swift progress of PROTACs, molecular glues represent an alternative avenue in the domain of small molecule degraders. Molecular glues effectuate a restructuring of the surface of E3 ligases, instigating interactions with their newfound substrates, potentially broadening the purview of druggable protein targets[13,14]. Additionally, notwithstanding their encompassment of more intricate molecular architectures, molecular glues garner clinical favoritism due to their favorable physicochemical properties[3]. In parallel, other TPD modalities that have come to fruition within this same epoch encompass hydrophobic tagging (HyT), which constitutes an initial endeavor to broaden the degradation mechanism[15].

In light of the TPD modalities delineated above, a profusion of innovative techniques has surfaced in recent years, poised to transcend extant degradation methodologies and potentially unlock functionalities beyond degradation. Notably, non-small molecule-based approaches have witnessed substantial progress, assuming a pivotal role in this domain. While conventional PROTAC strategies have been predominantly employed based on small molecules for the intracellular degradation of proteins require considerable exertion in novel target exploitation and physicochemical property optimization, the ambit of non-small molecule-based PROTACs transcends these confines. A diverse array of agents spanning a wide chemical spectrum, encompassing peptides, nucleic acids, antibodies, and even vaccines, has been instrumental in effecting the degradation of a plethora of proteins hitherto impervious to small-molecule degraders, including transcription factors (TFs), membrane proteins, protein aggregates, and viral proteins[16−18]. These modalities confer additional benefits, including heightened metabolic properties and tissue specificity. Furthermore, the exploitation of the lysosomal degradation pathway signifies a significant milestone in the domain of TPD, broadening the spectrum of degradation mechanisms and augmenting substrate diversity. Predominantly, macromolecular entities predicated upon antibodies and nucleic acids have been instrumental in modulating the endocytosis-lysosome pathway, which exhibits rapid renewal kinetics, thereby affording diverse avenues for the degradation of extracellular and membrane proteins[19,20]. Similarly, the employment of peptide-based entities leverages chaperone-mediated autophagy (CMA) as a complementary pathway for the degradation of protein aggregates[21]. Additionally, the heterobifunctional paradigm, which has been rigorously validated by the aforementioned TPD entities, furnishes a blueprint for the development of induction-proximity entities transcending degradation, including protein stabilizers and modulators of post-translational modifications (PTMs)[22]. Macromolecular entities may manifest analogous advantages in these broader domains, akin to their contributions within the realm of TPD, such as an expanded target spectrum and a diverse array of effects[23].

In this review, we have elucidated the burgeoning technological platforms inspired by PROTAC in recent years. The accrued knowledge not only provides novel TPD strategies but also heralds an era of breakthroughs beyond the confines of degradation, giving rise to a panoply of diverse effects. Our emphasis rests on non-small moieties, encapsulating three primary avenues of developmental trajectory. Finally, we expound upon the distinctive attributes and attendant challenges intrinsic to these methodologies, with the aspiration of furnishing insights for the discovery of paradigm-based pharmaceutical agents.

2. Macromolecular PROTACs

The initial iteration of PROTACs emerged in the form of peptide-based constructs (peptide PROTACs)[24,25]. However, their clinical utility has been hampered by factors including immunogenicity and subdued activity. In contradistinction, small-molecule PROTACs offer enhanced absorptive capacity and improved drug formulation attributes, thereby propelling the gradual ascendancy of small-molecule variants to the forefront[26]. Nevertheless, certain limitations persist, notably suboptimal physicochemical properties culminating in diminished cellular permeability and subpar metabolic performance[27,28]. Over the past decade, the landscape of targeted protein degradation has been revolutionized by the rapid proliferation of macromolecular PROTACs, facilitated by advancements in structural biology, click chemistry and coupling methodologies. This surge in research activity has engendered a diverse array of platform technologies underpinned by the extensive deployment of biomolecular agents. Macromolecular PROTACs, categorized by the biomolecules harnessed, encompass antibody-based, nucleic acid-based, and peptide-based iterations[16]. Recent literature even hints at the potential integration of live attenuated vaccines within this

paradigm[18]. Collectively, these macromolecular PROTACs embody a constellation of attributes hitherto unattainable by their small-molecule counterparts, including an expanded target repertoire, enhanced metabolic profiles, reduced toxicity, and heightened specificity for tumor targets.

2.1. *Antibody-based PROTACs*

In tandem with the rapid advancement of PROTAC technology, the domain of antibody−drug conjugates (ADCs) have undergone three successive technological iterations in the past two decades, culminating in notable achievements within both academic and industrial spheres[29,30]. ADCs, achieved through the covalent linkage of cytotoxic agents to monoclonal antibodies (mAbs) *via* meticulously designed chemical linkers, enable the targeted delivery of cytotoxic payloads to specific tumors or tissues in an antigen-dependent manner[31]. Representing a promising therapeutic modality, ADCs exhibit both precise targeting capabilities and potent cytotoxic effects, thereby mitigating off-target effects and widening the therapeutic window[32,33]. It is envisaged that conjugation with mAbs could similarly confer novel functionalities and attributes to PROTACs. This concept was initially substantiated in 2020 and has swiftly evolved into a nascent technological frontier, subsequently denominated as degrader−antibody conjugates (DACs)[34,35]. As envisioned, DAC technology offers a potent means to refine the physicochemical and pharmacokinetic properties of conventional small molecule PROTACs. Furthermore, DAC affords superior tissue specificity through an antigen-dependent approach and may circumvent certain *in vivo* delivery challenges, presenting a viable solution for clinical implementation. Alternatively, owing to its distinctive catalytic mechanism, PROTACs hold the capacity to effect sustained and potent degradation of targets at exceedingly low doses, aligning well with the stringent cytotoxicity prerequisites of ADC payload[36]. Consequently, PROTAC molecules emerge as prime candidates for ADC payloads. In summation, DAC technology amalgamates the favorable pharmacodynamic attributes of PROTACs with the heightened tissue specificity characteristic of ADCs, thus amalgamating the principal advantages of each modality.

Broadening the array of accessible E3 ubiquitin ligases for identification and amalgamation stands poised to propel the evolution of TPD technology[37]. Among presently known E3 ubiquitin ligases, the majority are intracellularly situated, constituting a prevailing strategy in degradation methodologies grounded in the UPS[38]. Recently, a bivalent antibody-based technique known as proteolysis targeting antibodies (PROTABs) has been devised, extending the purview of targeted degradation to cell-surface E3 ligases harboring exposed extracellular domains (ECDs)[39]. Through simultaneous engagement with E3 ligases and membrane receptors, PROTABs instigate ubiquitination and degradation of targeted proteins *via* a dual-pathway mechanism hinging on both proteasomal and lysosomal pathways. PROTABs furnish a robust and tissue-selective approach for degrading cell-surface targets, thereby potentially catalyzing the discovery of novel therapeutic entities influencing the functions of membrane receptors.

2.1.1. *DACs*

DACs represent trifunctional macromolecular entities wherein PROTACs serve as payloads tethered to mAbs *via* purposefully designed chemical linkers (Fig. 1A). Analogous to the paradigm of ADCs, DACs operate through a triphasic mechanism encompassing internalization, lysosomal trafficking, and the subsequent liberation of active PROTAC moieties[34,36]. Upon administration, DACs traverse the circulation in a stable conjugated state. Subsequently, the mAb portion of DAC exhibits specific recognition of tumor-associated antigens present on the surface of target cells, instigating receptor-mediated internalization of the entire conjugate[40]. The internalized DAC is subsequently trafficked to an activated lysosome, where it released PROTACs being stable to the lysosomal environment *via* the cleavage of chemical linkers or the catabolization of the mAb[41]. Ultimately, these liberated, active PROTACs traverse to the cytoplasm, thereby inducing target protein degradation (Fig. 1B)[40,42].

The pivotal consideration in synthesizing such trifunctional entities lies in the judicious selection of conjugation methodologies and the meticulous design of chemical linkers[33,43,44]. Within the domain of DACs, the predominant conjugation approach entails the attachment of linker−payload fragments to cysteine residues situated at distinct positions of the antibody, effected *via* electrophilic substitution reactions to yield conjugates characterized by a relatively stable drug antibody ratio (DAR). Notably, the latter methodology facilitates the generation of nearly homogeneous conjugates[33,43].

A primary consideration in linker design pertains to its susceptibility to cleavage within the lysosomal environment. Based on their release mechanisms, cleavable linkers may be categorized into two subclasses: chemo-sensitive linkers (predominantly disulfide bonds) and enzyme-sensitive linkers (comprising peptide and pyrophosphate bonds). Cleavable linkers afford the advantage of minimizing interference with the biological properties of active PROTACs. In contrast, non-cleavable linkers (notably thioether bonds) exhibit heightened stability in plasma[43,44]. A secondary consideration pertains to the release of active PROTACs. Typically, free functional groups within PROTACs, such as hydroxyl or amino groups, serve as incorporation sites. Recent disclosures, however, have showcased the incorporation of aniline as a "chemical handle"[45,46]. Additionally, a spacer (optional) forms an integral component of the chemical linker, facilitating the subsequent release of free PROTACs through a process of self-immolation[34]. Further intricacies will be expounded upon in subsequent cases.

The inaugural reported DAC featured GNE-987, a highly potent chimeric bromodomain-containing protein 4 (BRD4) degrader with picomolar cell potencies, yet encumbered by unfavorable drug metabolism and DMPK attributes (Fig. 2). In a strategic endeavor to optimize its pharmacokinetic profile, six GNE-987 molecules were attached as payloads to a single C-type lectin-like molecule-1 (CLL1) targeting antibody *via* an innovative cleavable linker (DAR = ∼6.0)[35]. Subsequent evaluations in HL-60 and EOL-1 xenografted murine models revealed that conjugate **2** demonstrated favorable and dose-dependent tumor growth inhibition post single intravenous (iv) administrations. Notably, conjugate **2** demonstrated commendable stability, prolonged half-lives, and attenuated clearance rates in pharmacokinetic assessments conducted in non-tumor-bearing mice, affirming that conjugation with CLL1 mAb indeed imparts GNE-987 with pharmacokinetic properties akin to monoclonal antibodies.

Shortly thereafter, a second DAC, predicated upon a BRD4-degrader, emerged. This particular study entailed the conjugation of a BRD4 degrader analogue, MZ1, with trastuzumab *via* an alternative conjugation strategy termed "Thio-Bridge"[47]. Notably, the copper-free, strain-promoted azide-alkyne cycloaddition (SPAAC) reaction was instrumental in connecting

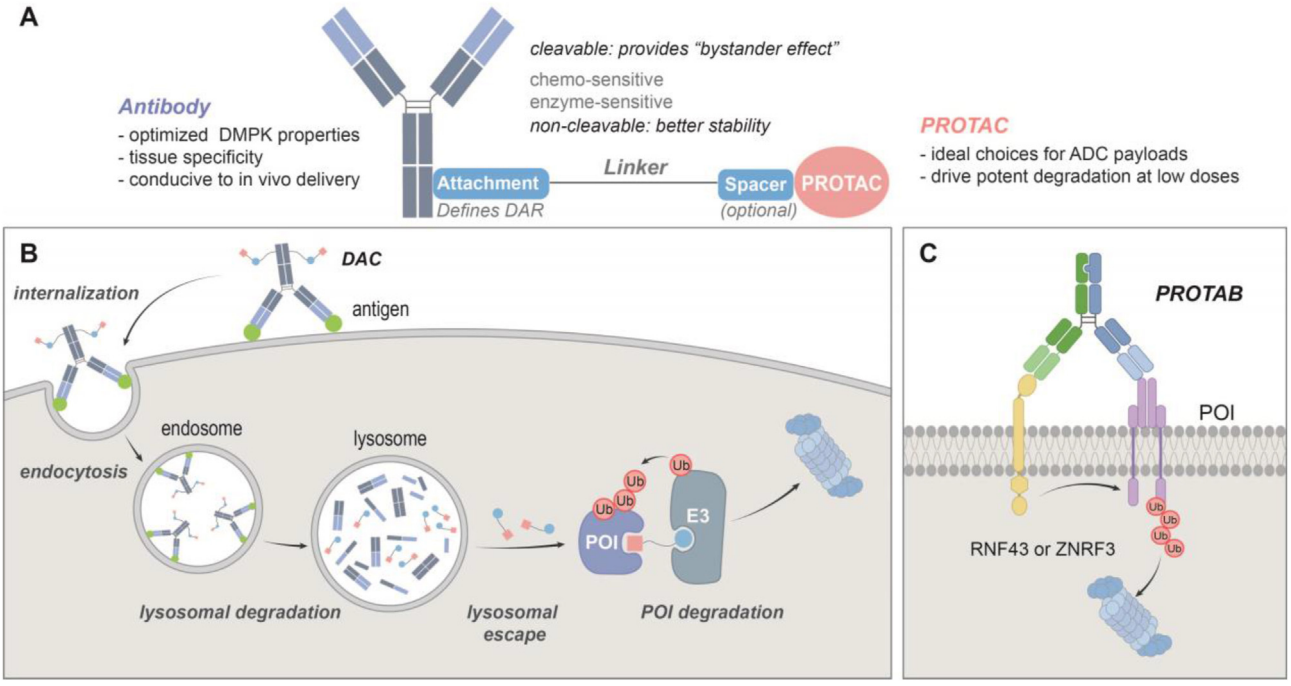


Figure 1 Constructional considerations and mechanisms of antibody-based PROTACs. (A) Schematic representation of DAC construction. (B) Details of DAC mechanism-of-action. (C) Ligase-based PROTAB-mediated membrane POI degradation.

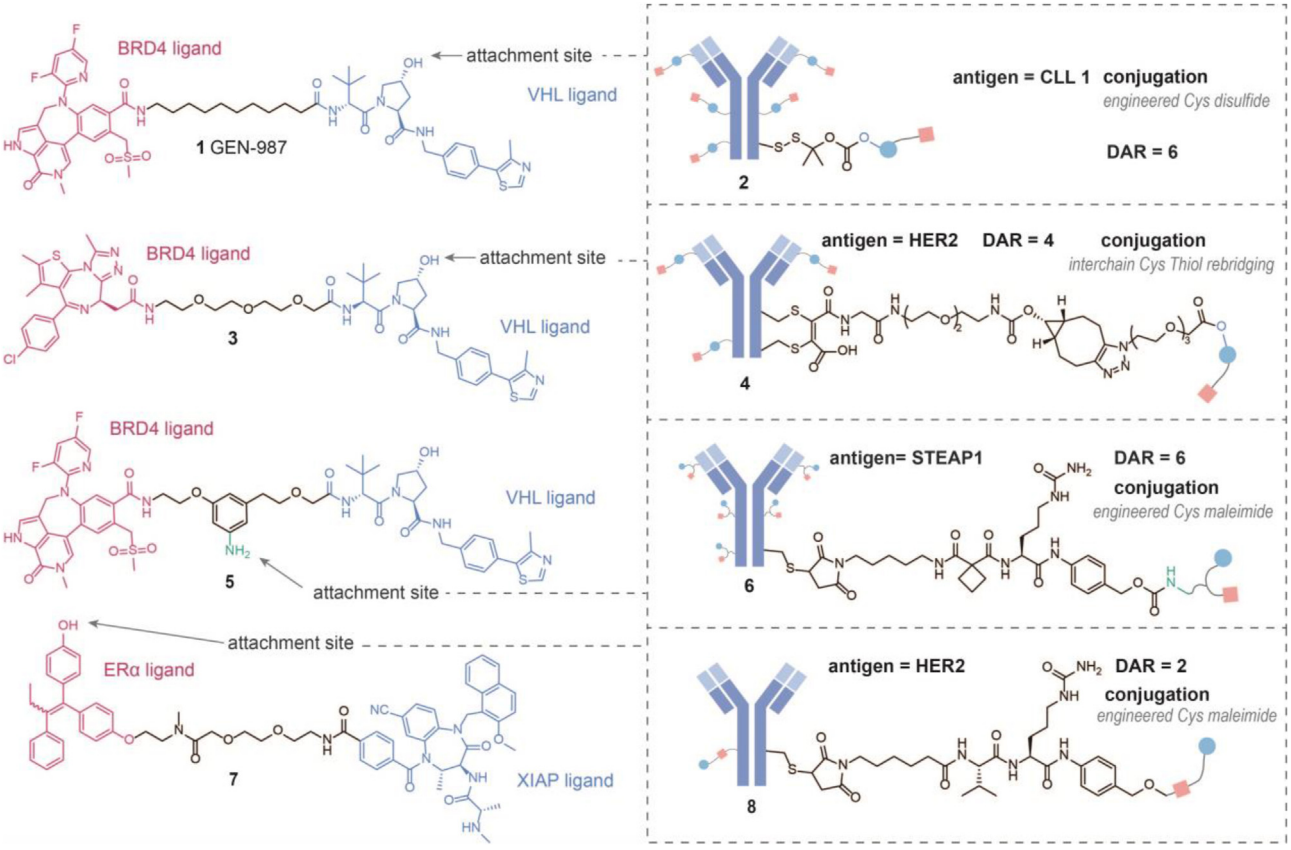


Figure 2 Structure of DACs and their corresponding small molecule PROTACs. The POI ligands are marked in magenta, while the E3 ligands are highlighted in blue. DAR, drug antibody ratio.

the molecules. *In vitro* investigations demonstrated that exposure to conjugate **4** led to selective degradation of BRD4 solely in the human epidermal growth factor receptor 2 (HER2) positive breast cancer cell lines, underscoring the potential for selective protein degradation by DAC in specific cellular contexts.

A recent comprehensive study focused on BRD4-based DACs has significantly advanced our understanding of DAR values, linking and attachment methodologies, and warheads, with the overarching aim of enhancing BRD4 degradation activity. Consequently, this endeavor holds promise for augmenting *in vitro* antiproliferative efficacy and *in vivo* anti-tumor potency[45,46]. This investigation underscores the pivotal role of formulating higher-DAR conjugates in heightening BRD4 degradation activities. Furthermore, the integration of more potent BRD4-binding fragments emerges as imperative in elevating the *in vitro* antiproliferative activity of BRD4-based DACs. For instance, compound **5**, a chimeric BRD4 degrader featuring a novel BRD4-binding fragment and an aniline group as the "chemical handle", was attached to the six transmembrane epithelial antigen of the prostate 1 (STEAP1) targeting antibody. This conjugation, employing a peptidomimetic-containing protease-cleavable linker, achieved a high DAR value (conjugate **6**). Noteworthy, conjugate **6** demonstrated antigen-dependent and potent *in vitro* BRD4 degradation activities, alongside pronounced antiproliferative properties when assessed in the PC3-S1 cell line. In addition, in a study employing a PC3-S1 xenograft model, compound **6** also showed dose-dependent and antigen-dependent *in vivo* tumor growth inhibition efficacy.

In conjunction with the targeted degradation of BRD4, recent studies have unveiled diverse targets susceptible to degradation through DACs, employing corresponding chimeric degraders as the pivotal payloads. A notable instance involves compound **7**, a PROTAC that recruits the X-linked inhibitor of apoptosis protein (XIAP) E3 ligase and selectively degrades estrogen receptor alpha (ERα). Compound **7** was chosen as the payload and attached to a HER2-targeting mAb[48]. Upon assessment in MCF7-neo/HER2 cells, conjugate **8** exhibited potent and antigen-dependent capabilities in reducing ERα protein levels. This case underscores the criticality of judiciously selecting the conjugation site, as DACs prepared using a similar strategy at the amine group of the XIAP ligand moiety incurred pronounced aggregation effects. Furthermore, recent patent publications have disclosed several non-BRD4 targets amenable to degradation. These encompass transforming growth factor beta receptor 2 (TGFβR2)[49], Brahma homolog (BRM) protein[50], and G1 To S Phase Transition 1 (GSPT1) protein[51], thus attesting to the prospective utility of DAC platform technology in industrial development.

2.1.2. *PROTAB*

The E3 ubiquitin ligases, zinc and ring finger 3 (ZNRF3), alongside its homologue ring finger protein 43 (RNF43), have been identified as cell-surface entities capable of regulating the Wnt signaling pathway *via* negative feedback mechanisms. In pathological states, such as colorectal cancer (CRC), aberrant Wnt signaling leads to heightened expression of these ligases[52,53]. This prompts speculation regarding their potential to instigate degradation of non-native targets within the same milieu, thereby conferring therapeutic efficacy. The tyrosine kinase insulin-like growth factor 1 receptor (IGF1R), a cell-surface modulator integral to growth factor signaling, was selected as a model to substantiate this concept, given its demonstrated compatibility with several extensively investigated antibodies, including cixutumumab (Cixu)[54]. Subsequently, a pool of 65 anti-RNF43 and 40 anti-ZNRF3 antibodies, characterized by desirable affinities, were screened and individually paired with Cixu to fabricate the corresponding PROTABs (Cixu*ZNRF3 and Cixu*RNF43)[39]. In a comprehensive array of CRC cell lines exhibiting varying endogenous ligase levels, the resultant PROTABs demonstrated pronounced efficacy in inducing the degradation of IGF1R *via* RNF43 or ZNRF3-mediated ubiquitination pathways. Mechanistic elucidations underscored the capacity of PROTABs to engage both the ubiquitin−proteasome pathway and the autophagy−lysosome pathway (Fig. 1C). Subsequent *in vivo* investigations underscored the efficacy and tumor selectivity of PROTABs. Notably, a single dose of Cixu*ZNRF3 (1 mg/kg) following intravenous injection into mice bearing subcutaneously transplanted SW48 colorectal tumors elicited sustained degradation of IGF1R. Furthermore, IGF1R degradation induced by PROTABs was exclusively observed in multiple patient-derived CRC organoids, exerting no discernible effect on normal colon organoids. Significantly, this study also divulged endeavors to expand the spectrum of PROTAB-addressable targets and ligases. A broader spectrum of cell-surface E3 ligases could be assimilated into the application purview of the PROTAB platform. Additionally, certain membrane-associated therapeutic targets, such as HER2 and programmed death-ligand 1 (PD-L1), could similarly undergo PROTAB-mediated degradation[39]. Finally, it also investigated the effect of molecular design on the clearance efficiency of targets, demonstrating that either the addition of an extra anti-E3 ligase or the adoption of single-armed structures would considerably enhance the clearance rate of targets compared to the standard bispecific antibody. Hence it is proposed that optimization of antibody affinity, molecular form and antigenic epitopes are critical for optimizing the efficiency of target degradation.

Collectively, the PROTAB platform not only possesses catalytic mechanism analogous with PROTAC and desirable tissue selectivity, but also achieves high efficiency through a dual-pathway degradation approach. Despite being in its infancy, with the modular synthesis strategy, this platform holds promising prospects for modulating membrane receptor function and exhibits the potential for broader applications across diverse therapeutic realms within the ambit of cancer treatment.

2.2. *Nucleic acid-based PROTACs: Expanding target accessibility in TPD*

A fundamental challenge in TPD research pertains to circumventing the constraints imposed by traditionally deemed "undruggable" targets. Recently, nucleic acid-based therapeutics have emerged as a promising therapeutic modality, alongside small molecules and antibodies, offering a prospective avenue for historically refractory proteins devoid of conventional ligandable sites. This category encompasses TFs, RNA-binding proteins (RBPs), and G-quadruplex (G4) binding proteins, demonstrating potential accessibility to nucleic acid sequences. This revelation opens avenues for the construction of targeted chimeras tailored to these proteins[55,56]. The seminal demonstration of the TPD strategy employing nucleic acid sequences as a warhead materialized in 2021 with the unveiling of transcription-factor-targeting chimeras (TRAFTACs)[57]. Subsequently, an array of nucleic acid-based TPD platforms have swiftly emerged, constituting four principal categories: TF-PROTACs[58−61], RNA-PROTACs[62], G4-PROTACs[63], and aptamer-PROTACs[64].

Nucleic acid-based PROTACs present several notable advantages. Foremost, the design and development of nucleic acid warheads afford relative independence in optimizing chemical modifications and metabolic properties, in contrast to their small molecule and antibody counterparts. This expedites the preparatory phases and screening endeavors for potential nucleic acid warheads[55,56]. Additionally, the integration of aptamers into PROTAC construction, whether as an adjunct or as the warhead itself, bestows the resultant protein-degrading chimeras with commendable physicochemical attributes, coupled with tumor-specific targeting proficiency[16,64]. Cumulatively, nucleic acid PROTACs manifest a promising trajectory, serving as emerging tools in chemical biology, with the potential to evolve into therapeutic entities.

2.2.1. *Targeted degradation of transcription factors*

TFs represent a crucial class of proteins orchestrating the transcriptional process through specific DNA sequence binding. Their aberrations are closely entwined with diverse pathological states, ranging from cancer to diabetes and autoimmune disorders, rendering targeted TF degradation an appealing therapeutic avenue[65,66]. While certain TFs possess ligand binding pockets (*e.g.*, nuclear receptors) or well-defined dimerization domains (*e.g.*, BCL-6, MYC, and STAT3) amenable to small molecule-based PROTAC-mediated degradation with commendable efficacy[67−69], a considerable portion of TFs exhibit structurally disordered, ligand-deficient pockets, thereby impeding the development of small molecule-driven targeting strategies.

To transcend these limitations, innovative technology platforms founded on alternative chemical entities have been extensively harnessed to expand the spectrum of druggable TF targets. Seminal work culminated in the conception of TRAFTACs (Fig. 3A). These chimeric oligonucleotides comprise a double-stranded DNA (dsDNA) moiety, which engages the transcription factor of interest (TOI), and an RNA segment that serves to anchor the dCas9-Halotag7 fusion protein[57]. By virtue of HaloPROTAC involvement, TRAFTACs have demonstrated remarkable efficacy in effecting the degradation of NF-κB and brachyury through the recruitment of TOI and the von Hippel Lindau (VHL)-E3 ligases, facilitated by the intermediate protein dCas9-HT7. However, the therapeutic application of this technique is markedly circumscribed by its reliance on the formation of the dCas9-HT7:TRAFTAC complex, warranting further clinical evaluation.

In subsequent strides, refined technology platforms have emerged, notably those obviating the necessity for CRISPR-guided methodologies and instead directly linking TFs to E3 ligases[58−61]. A prominent exemplar is OligoTRAFTAC, a second-generation technique that builds upon the TRAFTAC foundation (Fig. 3B)[58]. Concretely, two oligonucleotide sequences were meticulously chosen to serve as TF-recruiting elements, targeting c-Myc and brachyury, respectively. Additional flanking nucleotides were appended to both ends of the TF-recruiting oligonucleotide to confer structural flexibility to the composite entity. An orthogonal alkyne handle was subsequently affixed at each terminus to facilitate a Copper-catalyzed alkyne-azide cycloaddition (CuAAC) click reaction with the azide-modified VHL ligand. The double-stranded structure of the TOI recruiting element was obtained by annealing reactions. Remarkably, at a concentration of 50 nmol/L in HeLa cells and HEK293T cells, compounds **OT3** and **OT7** precipitated significant, sequence-dependent TOI degradation *via* the proteasomal pathway after a 20-h post-transfection period. Analogous outcomes were replicated in UM-Chor1 cells, where 60 nmol/L of **OT3** engendered ~70% brachyury degradation. It is noteworthy that the replacement of the phosphodiester backbone in **OT3** into phosphorothioate (PS) backbone markedly enhanced its nuclease stability, thereby amplifying its brachyury degradation efficacy (15 nmol/L in UM-Chor1 cells and 30 nmol/L in JHC-7 cells to generate significant degradation). Consistent findings were gleaned from *in vivo* experiments employing zebrafish as a model organism. Microinjection of the PS-modified iteration of **OT3** led to tail deformation in approximately 70% of embryos, whereas the majority of zebrafish embryos treated with **OT3** evinced no such aberrations. Furthermore, the TF-PROTAC platform has been instrumentalized for the targeted degradation of NF-κB and E2F1[61]. Specifically, two series of DNA oligomers underwent azidation, with VHL ligands (VH032) conjugated to bicyclooctyne-containing linkers of varying lengths. The ultimate conjugation of these two entities was executed *via* a SPAAC click reaction under near-physiological conditions. Post-transfection into HeLa cells *via* liposomes, both series of TF-PROTACs demonstrated the capacity to catalyze ubiquitination-mediated degradation of TOIs in a concentration-dependent fashion, thereby exhibiting commendable anti-proliferative and anti-tumorigenic effects on HeLa cells.

In contrast to preceding examples, the creation of O'PROTACs involved the solid-phase synthesis method, demonstrating efficacy in degrading two TFs, lymphoid enhancer-binding factor 1 (LEF1) and ETS-related gene (ERG)[59,60]. This synthesis procedure entailed the attachment of phosphoramidites, endowed with diverse linkers, to the E3 ligase ligands, followed by their addition to the DNA synthesizer and conjugation to the reverse strand in adherence to conventional phosphoramidite protocol. Ultimately, the double-stranded structure of TOI recruitment elements was realized through annealing with the forward strand. The VH032-based O'PROTAC, **OP-V1**, designed to target LEF1, exhibited robust efficacy both *in vitro* and *in vivo*. At a concentration of 25 nmol/L, OP-V1 elicited dose-dependent degradation of LEF1 protein in PC-3 cells. Moreover, **OP-V1** substantially curtailed the proliferation of PC-3 and DU145 cells *in vitro*, and restrained the growth of PC-3 and DU145 tumors in xenografted mice. It is worth noting that, in the realm of pomalidomide-based O'PROTACs, the hydrolysis of the pomalidomide moiety during the deprotection of the reverse strand posed a notable challenge to the solid-phase synthesis methodology. In response, an innovative strategy was devised, involving the identification of 3-amino phthalic acid as a novel CRBN ligand. Subsequently, ERG-targeting O'PROTACs were synthesized through a similar phosphoramidite synthesis approach. The resultant **C-P1** demonstrated proteasome-based degradation of ERG in a CRBN-dependent manner, with a DC_{50} of 172 nmol/L in VCaP cells. Furthermore, **C-P1** not only impeded the transcriptional activity of ERG, but also attenuated the growth and invasiveness of VCaP cells.

Recent developments in PROTAC technology have led to the creation of ARE-PROTACs, which capitalize on the antioxidant response element (ARE) to facilitate the co-degradation of heterodimeric Nrf2-sMaf transcriptional complexes[70]. Concretely, the alkyne-modified double-stranded oligonucleotide ARE sequence (5′-TCACAGTGACTCAGCAGAATC-3′) was linked to azide-modified E3 ligands with varied linkers through a CuAAC click reaction. The representative compound **14** demonstrated robust Nrf2 degradation in A549 cells (DC_{50} = 1.85 nmol/L).

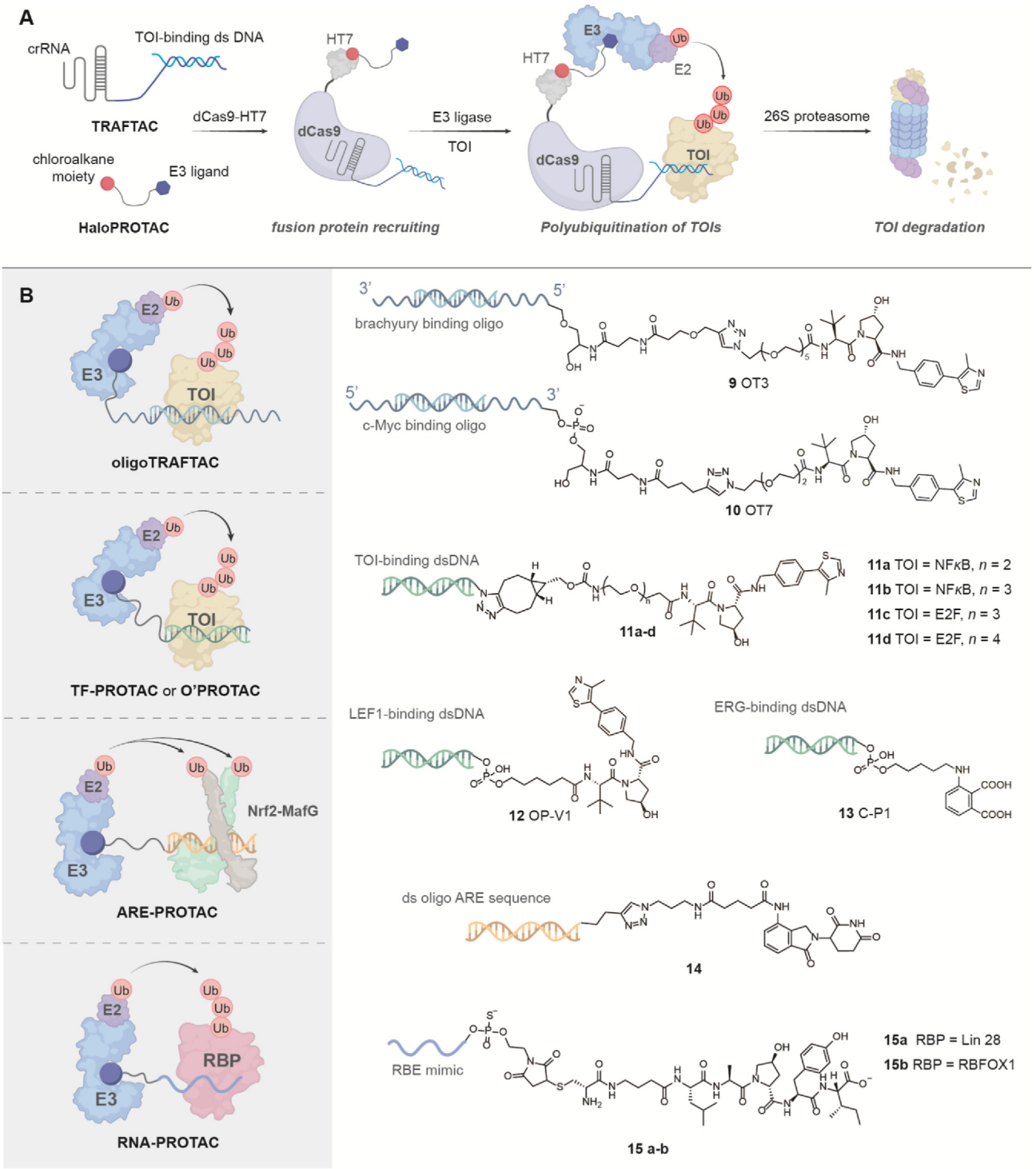


Figure 3 Structures and mechanisms of TF-PROTACs and RNA-PROTACs. (A) Schematic diagram of TRAFTAC and its degradation mechanism. Heterobifunctional TRAFTAC consists of an RNA moiety recruits the dCas9-HT7 engineered fusion protein, while the dsDNA sequence binds to the transcription factor of interest (TOI). In the presence of HaloPROTAC, TRAFTAC recruits the E3 ligase complex to the vicinity of the TOI, leading to its degradation by mediating proximity-induced ubiquitination. (B) Diagram illustrating the design strategies and chemical structures of representative TF-PROTACs and RNA-PROTAC.

Notably, MafG, a binding partner of Nrf2 that shares the same ARE binding sequence, was also significantly downregulated by **14** in A549 cells (DC_{50} = 66 nmol/L). Importantly, this down-regulation was contingent on Nrf2, as **14** failed to reduce MafG protein levels in Nrf2 knockdown cells. Furthermore, dose- and time-dependent degradation of Nrf2-MafG by **14** was observed in various non-small cell lung cancer (NSCLC) cell lines. Mechanistically, compound **14** impedes the Nrf2 signaling pathway by

suppressing the transcriptional activity of Nrf2-ARE, rendering NSCLC cells more susceptible to ferroptosis and conventional therapeutic agents.

2.2.2. *RNA-PROTACs: Targeted degradation of RNA binding proteins*

RBPs play pivotal roles in diverse regulatory functions through their specific binding to RNA, forming ribonucleoprotein (RNP) complexes[71,72]. Dysfunctions in RBPs have been implicated in the onset of various diseases, underscoring the importance of targeting these proteins[73,74]. However, due to the shared homologous domains among RBPs and the absence of readily ligandable pockets, targeting this class of proteins has proven to be a formidable challenge[75,76]. A promising strategy for targeting RBPs involves employing a short oligonucleotide warhead designed to align with the RNA consensus binding element (RBE) of the specific RBP. This concept has led to the development of RNA-PROTACs, a novel class of RBP-targeted chimeric degraders capable of effecting protease-mediated degradation of RBPs. Notably, this approach has successfully enabled the degradation of two distinct RBP targets, namely Lin28 and RBFOX1[62]. In targeting Lin28, a conserved sequence 5′-AGGAGAU-3′ ($L28_{RBE}$) was utilized. Two oligonucleotide modification techniques, diastereoisomeric PS linkages and 2′-*O*-methoxy-ethyl (MOE) modifications, were employed to optimize the *in vivo* stability. Similarly, the oligonucleotide sequence 5′-UGCAUGU-3′ (RBE of RBFOX1, FOX_{RBE}) was subjected to a similar protocol to serve as a warhead for targeting RBFOX1. The resultant RNA-PROTACs were obtained by conjugating the modified derivatives to VHL peptide ligands, respectively. Western blot analyses demonstrated that the two RNA-PROTACs individually induced protease-dependent degradation of Lin28 (in NT2/D1 embryonic cells) and RBFOX1 (in HEK293T embryonic kidney cells) with a DC_{50} of 2 μmol/L. This proof-of-concept presents a promising avenue for the targeted degradation of RBPs, a protein class previously considered intractable.

2.2.3. *Aptamer-PROTAC: Targeted degradation utilizing aptamers*

Aptamers, synthetic single-stranded oligonucleotides known for their unique secondary structures, share a parallel mode of target engagement with antibodies, folding into distinctive three-dimensional conformations to bind target proteins with high specificity and affinity[77−80]. Moreover, aptamers present advantages including facile large-scale synthesis, favorable tissue permeation, low immunogenicity, and commendable *in vivo* safety profiles[78,79,81]. Notably, AS1411, an ssDNA aptamer rich in guanine bases, has demonstrated remarkable tumor-targeting properties and has been employed extensively in targeted degradation chimera development (Fig. 4A)[82−84]. The initial foray into aptamer−PROTAC conjugates (APCs) involved the union of AS1411 with a potent BET-targeted PROTAC, achieved through a cleavable linker featuring an ester-disulfide moiety (Fig. 4B, compound **16**)[85]. This conjugation preserved BET degradation potency while enhancing specificity in nucleolin-overexpressing MCF-7 cells and mitigating cytotoxicity, ultimately leading to heightened *in vivo* tumor targeting and enhanced anti-tumor efficacy in MCF-7 xenograft models.

Alternatively, strategies have emerged for employing AS1411 directly as a warhead in the construction of nucleolin-targeted degradation chimeras. One such approach involved the click reaction between DBCO-labeled AS1411 and an azide-modified VHL ligand, yielding the inaugural nucleolin-targeted PROTAC[86]. ZL216, through selective binding and internalization of the nucleolin−PROTAC complex, facilitated the degradation of nucleolin proteins, effectively suppressing the proliferation and migration of breast cancer cells. This tumor cell-specific nucleolin degradation was further observed in MCF-7 and BT474 xenograft models upon treatment with ZL216.

A recent advancement introduced a photoswitchable aptamer-based PROTAC with a focus on mitigating systemic toxicity[87]. Initial construction of dNCL#T1, a CRBN-dependent PROTAC, leveraged a click reaction between thalidomide-*O*-amido-propargyl and azide-modified AS1411. dNCL#T1 exhibited affinity for nucleolin and drove its ubiquitin−proteasome-dependent degradation, resulting in the inhibition of nucleolin-associated breast cancer cell proliferation and migration. Subsequently, hybridization with a photolabile complementary oligonucleotide yielded opto-dNCL#T1, a light-controlled nucleolin degrader. In MCF-7 cells, opto-dNCL#T1 demonstrated UVA dose-dependent ubiquitin−proteasome-dependent degradation of nucleolin. Furthermore, UVA irradiation effectively suppressed the viability, proliferation, and migration of MCF-7 and MDA-MB-231 cells transfected with opto-dNCL#T1. This exemplifies the potential application of aptamer based PROTACs in precision medicine, offering a light-controllable approach.

2.2.4. *G4-PROTAC: Targeted degradation utilizing G-quadruplex structures*

The -G4 structure, characterized by a distinctive four-stranded arrangement of nucleic acids through stacked guanine (G)-rich DNA or RNA repeats, plays pivotal roles in various cellular processes encompassing transcription, replication, recombination, and more[88,89]. Numerous proteins interacting with G4 structures have been successfully identified, presenting a wealth of potential therapeutic targets[90−92]. Among these, the DEAH box helicase RHAU, renowned for its G4 binding capability, emerges as a critical therapeutic target, particularly due to its heightened expression in tissues of C9orf72-associated amyotrophic lateral sclerosis (ALS) patients[93−95]. Recently, RHAU-targeted degradation chimeras, employing G4 as the warhead, have been engineered[63]. In this innovative approach, the nucleic acid sequence T95-2T was strategically linked to two extensively studied E3 recruiters (pomalidomide and VH032) *via* a $Cu_{(II)}$-TBTA-mediated alkyne-azide click reaction (Fig. 5). In HeLa adenocarcinoma cells, both G4-PROTACs induced >50% RHAU protein degradation at 50 nmol/L. Notably, the observed "hook effect" validated through dose-dependent assessments and Western blot experiments following bortezomib pretreatment, underscored that G4-PROTACs facilitated degradation *via* the proteasomal pathways. The introduction of G4-PROTACs not only attests to the feasibility of designing targeted degradation chimeras hinged on atypical nucleic acids but also opens new avenues for treating conditions associated with G4-interactions.

2.2.5. *Other PROTACs based on nucleic acids*

The recent advent of telomere-targeting chimeras (TeloTACs) has demonstrated remarkable potential in the selective degradation of telomeric repeat-binding factors 1 and 2 (TRF1/2). This development underscores the feasibility of degrading DNA-binding proteins, diverging from the conventional TF-PROTAC strategy[96]. TRF1/2 are pivotal constituents of the shelterin complex, a hexasubunit assembly known for its role in suppressing the DNA damage response of telomeres. This complex is indispensable for

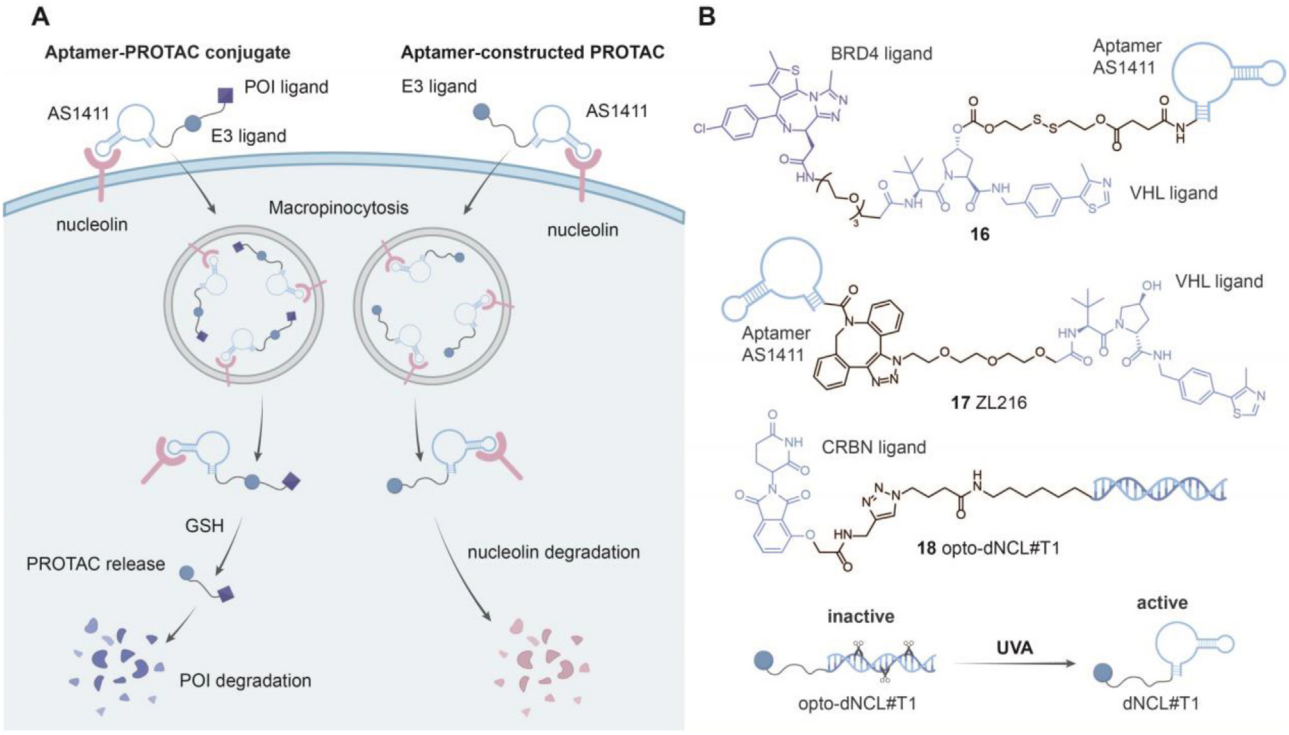


Figure 4 Schematic representation of the mechanism-of-action and representative structures of aptamer-conjugated and aptamer-constructed PROTACs. (A) Degradation of POI or nucleolin triggered by aptamer-conjugated or aptamer-constructed PROTACs. (B) Representative structures of aptamer-conjugated and aptamer-constructed PROTACs.

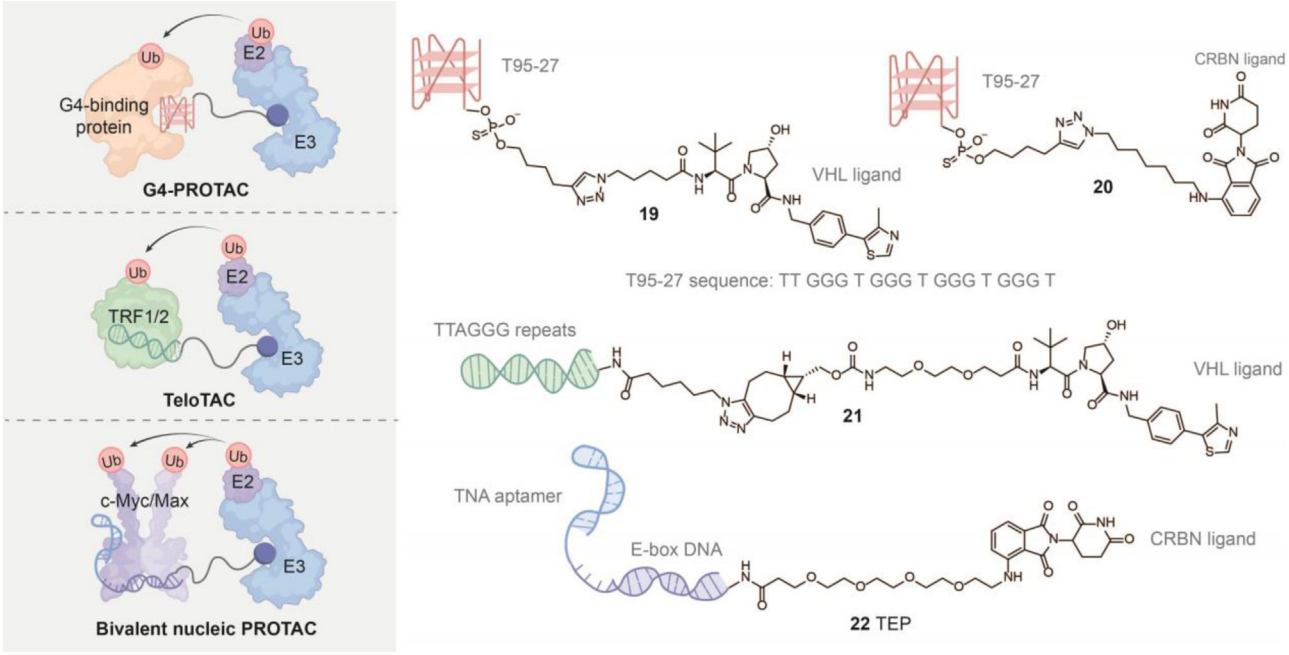


Figure 5 Chemical structures and design rationale of G4-PROTACs, TeloTAC and Bivalent nucleic PROTAC.

preserving telomere length and genome integrity[97]. Functionally, TRF1/2 bridges the telomeric TTAGGG repeat sequence and recruits telomerase, thus mediating essential telomere–telomerase interactions[98]. Consequently, orchestrating the disintegration of the shelterin complex through TRF1/2 degradation presents a viable approach to reverting the infinite replicative potential characteristic of cancerous targets[99,100]. In structural terms, the TRF1/2 ligand comprises three TTAGGG tandem repeats. The incorporation of a "TTT-short linker" engenders a stabilizing stem-loop structure within the DNA oligomer. Subsequently, a

range of TeloTACs, featuring diverse linker types, were synthesized *via* a SPAAC click reaction, uniting the oligonucleotide ligands with VHL E3 ligands. Notably, the representative compound **21** elicited substantial degradation of TRF1/2 at 5 μg/mL in A431, MDA-MB-231, and HeLa cells. This was followed by the inhibition of cellular proliferation and the shortening of telomere length[96]. It is worth highlighting that the IC_{50} of **21** on normal non-cancerous cells was approximately tenfold higher than that observed for cancer cells overexpressing TRF1/2. This compelling selectivity profile offers a promising therapeutic window. In summary, TeloTACs represent an innovative DNA-based targeted degradation strategy capable of shortening telomeres and impeding cancer cell proliferation. This groundbreaking approach holds significant promise for the targeted degradation of non-transcription factor DNA-binding proteins.

Additionally, a bivalent PROTAC simultaneously utilizing a threose nucleic acid (TNA) aptamer and a natural DNA ligand for the degradation of the c-Myc/Max heterodimer complex has been recently reported[101]. c-Myc is a kind of TFs with wide effects such as regulation of cell proliferation, differentiation and metabolic. It forms a heterodimer with its associated factor X (Max), which recognizes and binds to the DNA enhancer element E-box sequence, thereby activating the corresponding transcriptional targets[102,103]. Furthermore, overexpression or amplification of c-Myc could be observed in a wide range of cancers, suggesting that targeted degradation of c-Myc might be a potential option for the treatment of multiple cancer types[104,105]. In this study, a series of candidate TNA aptamers were generated by DNA library technology, followed by the attachment of an E-box sequence to obtain TNA-DNA sequences through DNA primer extension reaction. This was followed by four rounds of *in vitro* screening to obtain high-affinity chimeric TNA-DNA ligands. *In vitro* selectivity experiments show the two parts binds to different epitopes, confirming the sequence as a bivalent binding ligand. In addition, the obtained TNA-DNA sequence exhibited improved biostability and enhanced binding affinity (K_d = 22 nmol/L) compared to the TNA aptamer (K_d = 56 nmol/L) or the E-box sequence (K_d = 189 nmol/L)[101]. The bivalent PROTAC was subsequently constructed based on the E3 ligand pomalidomide. Single-stranded chimeric TNA-DNA and complementary DNA attached with pomalidomide at the 3′ end was prepared separately, with the two parts assembled by DNA hybridization. In Hs578T cells, TEP degrade endogenous c-Myc with an IC_{50} value of 53 nmol/L. Notably, the TNA aptamer was essential for enhancing binding affinity as well as stability to the nuclease, since the E-Box-pomalidomide coupling did not lead to a reduction in c-Myc protein levels, furthermore the stability of the all-DNA version was significantly reduced. In conclusion, the design of bivalent ligands contributes to improved affinity and selectivity, and the introduction of TNA aptamer enhances resistance to nucleases, providing a novel and promising strategy for the construction of nucleic acid PROTACs.

2.3. *Peptide PROTACs*

Peptide PROTACs have emerged as a compelling approach for targeted protein degradation, offering notable advantages in terms of affinity, specificity, and safety despite challenges related to stability and permeability[106,107]. Innovative strategies, including the utilization of cell-penetrating peptides, conformational constraints, and precise delivery systems, have been employed to mitigate these limitations[108–111]. Furthermore, advances in structural biology, phage display, and yeast display technologies have facilitated the identification of peptide warheads with high affinity for POI epitopes[112,113]. Therefore, peptide PROTACs remain a promising avenue for the development of targeted protein degraders, with recent examples summarized in Table 1.

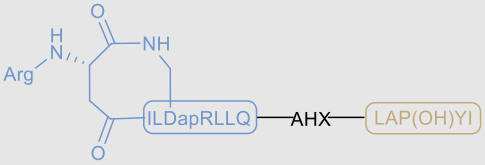
In scenarios involving conventionally "undruggable" targets, peptide warheads offer an alternative to the creation of targeted degraders. One such example is CREPT, an oncogene associated with RNA polymerase II, with significant implications for cell proliferation and tumorigenesis[124,125]. The absence of small molecule ligands has posed challenges in investigating CREPT oncoproteins. Nevertheless, a CREPT-targeting peptide PROTAC was successfully designed based on its dimerization interaction motif, leading to the selective degradation of CREPT in pancreatic cancer cells. This achievement underscores CREPT's potential as a therapeutic target in cancer therapy[114]. β-Catenin, a central player in the canonical Wnt/β-catenin signaling pathway, holds intrinsic importance in cell proliferation and differentiation and is an attractive intracellular target for cancer therapy[126–128]. Overcoming challenges related to the efficacy of small molecule ligands and rapid β-catenin accumulation in cancer, peptide PROTACs have been developed based on highly specific stapled helical peptides. These peptide PROTACs demonstrate effective degradation of β-catenin, along with potent inhibition of Wnt signaling in cancer cells and intestinal organoids. This attenuation translates into reduced cell proliferation, organoid growth, and tumor formation[115].

In the context of protein−protein interactions (PPIs), peptide PROTACs present notable advantages over small molecule counterparts due to their expansive and flexible backbones[129]. For instance, a series of MDM2/MDMX stapled peptide degraders have been devised, leveraging the MDM2/MDMX−p53 interaction peptide inhibitor PMI (TSFAEYWALLSP). By employing conformational constraints, these peptide PROTACs have shown promise[121,130]. A recent development, PMIBcr/Abl-R6, a peptide PROTAC designed to degrade Bcr/Abl, a pivotal player in Philadelphia chromosome-positive leukemia (LK-Ph+), was achieved by grafting PMI to the N-terminal helix region of Bcr/Abl tetramerization domain[122,123]. Notably, ^{PMI}Bcr/Abl-R6 plays a dual role as it participates in the innate tetramerization process of Bcr/Abl while also acting as an MDM2 recruiter and an MDM2−p53 PPI inhibitor. This multifunctional approach tandemly connects two PPIs, Bcr/Abl tetramerization and MDM2−p53 interaction, exerting multifunctional effects beyond TPD through simultaneous induction of Bcr/Abl degradation and activation of the p53 pathway.

2.4. *Live attenuated PROTAC vaccine*

Live attenuated vaccines represent a pivotal platform for vaccine advancement, eliciting robust and comprehensive humoral and cellular immune responses, surpassing the immunity generated by inactivated vaccines[131,132]. Nonetheless, progress in this domain has been constrained by challenges like virulence reversion, restricted immunogenicity, undesirable safety profiles, and intricate manufacturing processes. To surmount these hurdles, various strategies have been devised, including enhanced replication fidelity, codon deoptimization, and viral genome engineering[133,134]. Recently, a groundbreaking influenza A vaccine rooted in PROTAC technology has emerged, providing a fresh avenue to enhance safety and mitigate the virulence of live attenuated vaccines (Fig. 6A)[18,135]. For the design of the viral sequence, a

Table 1 Recently reported peptide PROTAC sequences.

Sequence	POI	E3 ligase	Ref.
KRRR VRALKQKYEELKKEKESLVDK —AHX— LAP(OH)YI	CREPT	VHL	114
RRWPRS$_5$ILDS$_5$HVRRVWR —AHX— ALAPYIP	β-Catenin	VHL	115
YQQYQDATADEQG GSGS LDPETGEYL RRRRRRRR	Tau	Keap1	116
YGRKKRRQRRR GVLYVGSKTR RRRG	α-Synuclein	TRIP12 STUB1 UHRF1	117
TRCMISYGGADYKCIT GSGS ALAPYIP RRRRRRRR	PD-1	VHL	118
CGIQDTNSKKQSDTHLEET GSGS ALAPYIP RRRRRRRR	PD-L1	VHL	118
YLC SSNNNRERDK FRR GGSGGT SFEQFWAWLWP	AR-V7	MDM2	119
	ER AR	VHL	120
TSFR$_8$EYWALLS$_5$ —PEG— LAHypYHIeP	MDM2/MDMX	VHL	121
MVDP TSFAEYWAALSP DSEPPRMELRSVGDIEQELERAKASIRRL EQEVNQERFRMIYLQTLLAKEKKSYDR RRRRRR	Bcr/Abl	MDM2	122,123

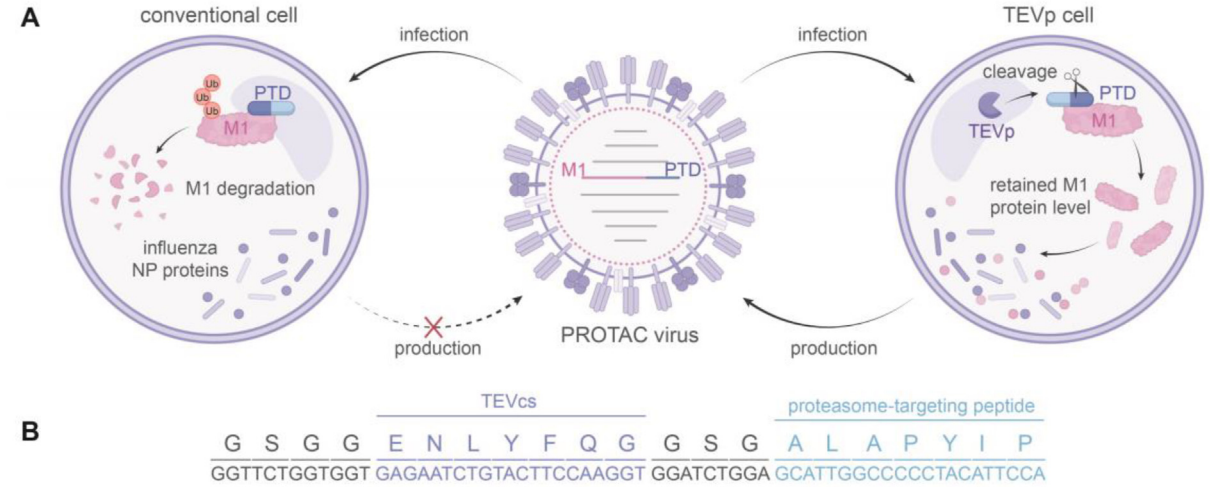


Figure 6 Schematic illustration of the mechanism of attenuation and critical genome of PROTAC virus. (A) Schematic illustration of the PROTAC virus production system. PROTAC viruses replicate efficiently in TEVp-expressing stable cells, which cleaves the PTD. Whereas in conventional cells, deficient protein synthesis and attenuation of replication are triggered by proteasome-mediated viral protein destabilization, leading to live attenuated PROTAC viruses. Ub, ubiquitin; M1, matrix protein; PTD, proteasome-targeting domain. (B) Diagrammatic representation of the PTD genome.

proteasome-targeting domain (PTD) was conceived, encompassing a VHL recruitment peptide (ALAPYIP) and a tobacco etch virus cleavage site (TEVcs) linker (ENLYFQG), aimed at inducing the degradation of influenza viral proteins (Fig. 6B). Subsequently, the PTD was integrated into the M1 gene fragment of the influenza A virus to form the genome of the PROTAC virus. In the production phase, the engineered cell lines, which stably express tobacco etch virus protease (TEVp), were transfected with the designed PTD-containing PROTAC virus genome. The subsequent excision of the PTD by TEVp ensures the accurate synthesis of all virally encoded proteins, thus activating the viral replication life cycle. Conversely, in conventional cells, PROTAC viruses leverage the host cell's endogenous ubiquitin–proteasome system to achieve targeted degradation of the viral M1 protein. This leads to faulty viral protein synthesis, consequently attenuating viral replication[18].

As a potential vaccine candidate, the safety of the PROTAC virus was initially assessed through replication kinetics experiments in MDCK-TEVp engineered cells and MDCK.2 cells. At a multiplicity of infection (MOI) of 0.01, the PROTAC virus demonstrated efficient replication exclusively in MDCK-TEVp cells. Moreover, the restored viral replication competence and reversed M1 protein expression were induced by the proteasome inhibitor MG-132 and the VHL ligase inhibitor VH298. This affirmed that the mechanism of viral attenuation is proteasome- and E3 ligase-dependent. Further *in vivo* investigations in mice and ferrets were conducted to appraise safety, immunogenicity, and protective effects[18]. The findings underscored a substantial reduction in replication capacity compared to the wild-type virus, without any noticeable weight loss or mortality. Furthermore, PROTAC vaccines were demonstrated to evoke a potent and extensive immune response in both mice and ferrets, along with providing cross-protection against homologous and heterologous viral challenges. This robust immune response arises from the fact that the conserved epitopes of the PROTAC vaccine are adept at eliciting a wide spectrum of humoral, mucosal, and cytotoxic T-lymphocyte immune responses.

3. Degraders engaging lysosomal pathways

Addressing potential therapeutic targets such as aggregates, non-intracellular proteins, lipid droplets, and organelles presents a notable challenge for proteasomal degradation strategies[136,137]. In contrast to the ubiquitin−proteasome pathway, the lysosomal degradation pathway constitutes another vital protein degradation route in eukaryotic cells. Its principal function involves the degradation of long-lived proteins, aggregates, surplus organelles, and extracellular proteins[138−140]. Recently, an array of emerging degradation strategies harnessing the lysosomal pathway have swiftly emerged to exploit the diverse substrate pool of lysosome-mediated degradation. These strategies furnish novel avenues for targeting "undruggable" entities and complement the existing PROTAC technology[19,20,141].

3.1. Endocytosis-based degraders

Lysosomes, serving as the primary intracellular degradation compartment, participate in the catabolism of intracellular and extracellular materials through various pathways, including endocytosis, phagocytosis, and autophagy[138]. Lysosome-centric degradation strategies primarily leverage endocytosis and autophagy. Upon endocytosis, membrane proteins or extracellular substrates are enclosed by the plasma membrane, entering the early endosome where sorting is predominantly initiated. Chosen cargo may be recycled to the cell surface or other organelles, contributing to the maintenance and alteration of the cell-surface proteome.

Conversely, other cargoes are translocated to the lysosome for degradation[138,142,143]. Presently, several methodologies have been devised to achieve lysosome-targeted degradation, comprehensively detailed in extant literature. Broadly, three principal strategies have been employed:

A) Construction of bispecific entities, wherein one terminus binds to POI while the other end identifies cell surface receptors linked with internalization. This prompts receptor-mediated endocytosis and subsequent degradation of the POI. Illustrative examples encompass lysosome-targeting chimera (LYTAC)[144,145], bispecific aptamer chimera (BAC)[146], dendronized DNA chimera (DENTAC)[147], and cytokine receptor-targeted chimera (KineTAC)[148].
B) Mimicking lysosomal sorting signals, *e.g.*, encompass GlueBody targeting chimera (GlueTAC)[149] and signal-mediated lysosome-targeting chimera (SignalTAC)[150].
C) Ubiquitination of cell surface proteins and/or extracellular proteins, which instigates endocytosis and subsequent lysosomal degradation, as observed in antibody-based PROTAC (AbTAC)[151,152].

The first strategy predominates and continues to see rapid expansion. This article offers a comprehensive summary and update on pertinent reports pertaining to such entities (Fig. 7).

3.1.1. LYTAC and MoDE-A

LYTACs, introduced in 2020, represent a significant stride in achieving lysosomal degradation of extracellular and membrane proteins, garnering considerable attention and inspiring the exploration of related strategies[145,153]. LYTACs operate on a bifunctional blueprint, wherein one structural domain houses an antibody, peptide, or small molecule as the POI-binding element, while the other serves as a ligand for the lysosome-targeting receptor (LTR)[144,153−155]. Mechanistically, the LTR-LYTAC−POI ternary complex undergoes endocytosis through endoplasmic reticulum-mediated processes and subsequently undergoes acidification within the endosomes. This prompts dissociation of the LTR, which is then recycled to the cell membrane. Simultaneously, the LYTAC−POI complex progresses to the lysosome for degradation.

The initial LTR commandeered for LYTACs is the cation-independent mannose-6-phosphate receptor (CI-M6PR), and its corresponding ligand comprises glycopolypeptides bearing multiple serine-*O*-mannose-6-phosphonate residues (M6Pn)[145,153]. Through covalent coupling of poly-M6Pn with specific antibodies, M6P-LYTAC has demonstrated the ability to induce the lysosomal degradation of diverse extracellular and membrane proteins, including epidermal growth factor receptor (EGFR), transferrin receptor-1 (CD71), and PD-L1[153].

An additional LTR harnessed for LYTACs is the asialoglycoprotein receptor (ASGPR)[144,154]. In comparison to CI-M6PR, ASGPR exhibits hepatocyte-specific expression and swift recycling, facilitating liver-specific TPD. Triantenerrary *N*-acetylgalactosamine (tri-GalNAc), an ASGPR ligand with nanomolar affinity, has been employed in the assembly of LYTACs[155]. By conjugating cetuximab or Pertuzumab to tri-GalNAc *via* click reactions, GalNAc-LYTACs have successfully achieved degradation of EGFR and HER2 in hepatocellular carcinoma cell lines (HCC) in a GalNAc-dependent manner. Additionally, a 3.4 kDa polyspecific integrin-binding peptide (PIP)—a synthetic peptide ligand targeting cellular integrins with a much lower molecular weight compared to antibodies—was also coupled to tri-GalNAc[144]. In HepG2 cells, PIP-GalNAc-mediated degradation of $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins led to inhibition of cell proliferation. Noteworthy advancements include the conjugation of biotin to tri-GalNAc, enhancing the uptake of NeutrAvidin to the lysosome for degradation while retaining specificity for liver tissues, thereby suggesting potential simplifications in LYTAC structure[154].

Subsequently, a small molecule variant of LYTACs, termed molecular degraders of extracellular proteins through

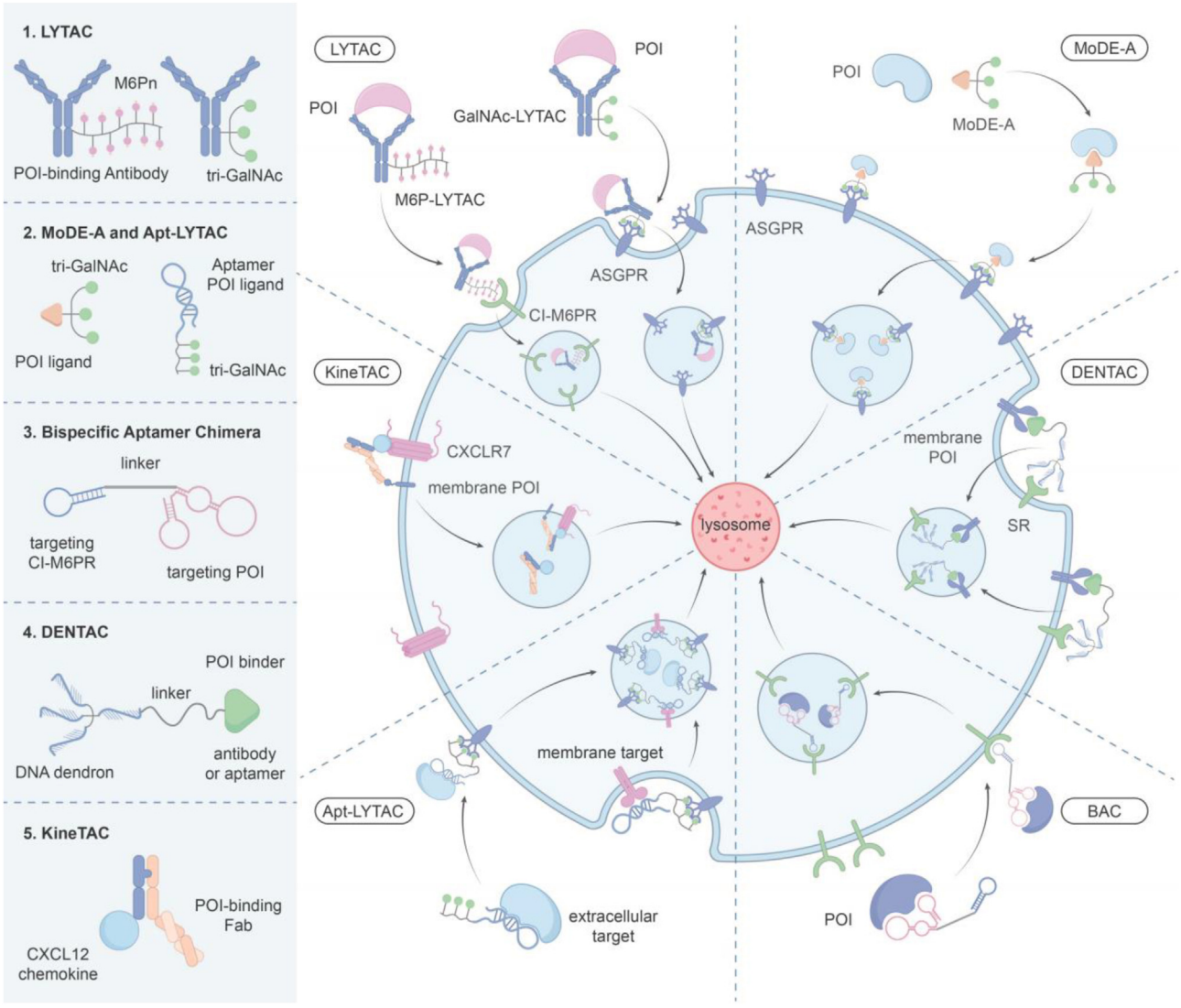


Figure 7 Summary of Endocytosis-based lysosomal degraders. The construction of LYTAC, BAC, DENTAC and KineTAC is summarized in the left column. LYTACs and BACs tame CI-M6PR and ASGPR as the lysosome-targeting receptor (LTRs) to achieve the degradation of membrane proteins and extracellular proteins. KineTAC acquires lysosomal targeting properties by binding CXCLR7 and CXCL12, with the ability to degrade membrane targets. DENTAC utilizes SR as a novel kind of LTRs to promote the lysosomal degradation of membrane POI.

asialoglycoprotein receptors (MoDE-As), was reported[156]. MoDE-As have been demonstrated to swiftly eliminate the migration inhibitory factor (MIF) from circulation *via* a lysosomal process, effectively preventing autoimmune reactions. These monodisperse small molecules hold promise for mitigating concerns associated with antibody-based LYTACs, including laborious synthesis, inefficient internalization, and limited homogeneity.

Furthermore, aptamers have recently emerged as a versatile tool for LYTACs preparation (Apt-LYTACs), enabling lipocyte-specific degradation of both extracellular and membrane proteins[157]. The attachment of the ASGPR binding motif to the POI-targeted aptamer is achieved through a pivotal solid-phase synthesis approach, wherein three consecutive GalNAc phosphoramidites are added to the 5′ terminus of the aptamer sequence. With a shift in aptamer types, Apt-LYTACs have enabled liver-specific lysosomal degradation of platelet-derived growth factor (PDGF) and protein tyrosine kinase-7 (PTK7). Moreover, Apt-LYTACs exhibit lower immunogenicity, offering a potential resolution to challenges encountered with antibody-based LYTACs, including intricate synthesis, suboptimal internalization, and limited uniformity.

3.1.2. Bispecific aptamer chimeras (BACs)

Much like LYTACs, the core mechanism behind lysosomal delivery facilitated by BACs centers on the appropriation of LTRs[146]. Structurally, BACs integrate two aptamers with distinct functionalities: one that binds to the POI, and the other designed to target the CI-M6PR. These aptamers are connected by a dsDNA linker, which serves the dual purpose of bridging and stabilizing the two aptamers. The ensuing interaction with membrane proteins prompts the rapid internalization of the POI *via* CI-M6PR-mediated processes, leading to its lysosomal translocation. Notably, BACs have exhibited proficient degradation of therapeutically pertinent cell-surface proteins, such as Met and PTK-7, demonstrating both efficacy and selectivity. In summary, BACs stand out for their precise and relatively simplified

synthesis, providing a nucleic acid-based approach for achieving lysosome-targeted degradation.

3.1.3. Dendronized DNA chimera (DENTAC)

In addition to the well-explored CI-M6PR and ASGPR, DENTACs have broadened the scope of potential LTRs by incorporating cell surface scavenger receptors (SRs) into the realm of applicable targets for cell surface protein depletion[147]. SRs, recognized as anionic ligand binding receptors prevalent on cancer cells, have previously been harnessed by nucleic acid nanodevices for various applications[158,159]. To exploit SRs and facilitate modular synthesis, researchers employed a solid-phase synthesis approach to engineer a covalently branched DNA ligand. This construct consists of a poly-thymine DNA dendron (PTDD) adorned with DBCO moieties at the terminus, providing a pivotal component for biological orthogonal reactions. Additionally, an essential module involves the POI ligand, such as a monoclonal antibody or aptamer, furnished with an azide-modified linker[147]. These two modules were seamlessly integrated through a SPAAC click reaction, yielding the final product, DENTAC.

The functionality of DENTAC was validated in A549 cells employing an anti-IgG antibody as the POI binder. Notably, a substantial lysosomal delivery of cytosolic IgG was observed, substantiating its efficacy. By substituting the POI ligands with the nucleolin aptamer AS1411 and cetuximab, DENTACs proficiently induced the lysosomal degradation of oncogenic membrane nucleolin (NCL) and EGFR, respectively. In essence, through the strategic engagement of SRs—abundantly expressed and widely distributed LTRs—DENTACs offer a valuable complement to existing degradation strategies targeting cell surface proteins. Furthermore, the adaptable nature of POI ligands opens up new avenues for target diversification.

3.1.4. Cytokine receptor-targeted chimera (KineTAC)

The cytokine CXCL12, exhibiting high selectivity for the decoy recycling receptor CXCR7, initiates internalization through β-arrestin recruitment, culminating in its ultimate delivery to the lysosome for degradation[160]. Leveraging this intrinsic cellular process, KineTACs have been engineered. KineTACs represent genetically encoded recombinant bispecific antibodies, composed of a cytokine arm and a POI-binding arm[148]. By capitalizing on internalization mediated by cognate cytokine receptors, KineTACs facilitate robust and extensive lysosomal delivery, thereby enabling the depletion of both membrane-bound and extracellular proteins.

To establish a proof-of-concept, KineTACs targeting PD-L1 were initially constructed. One arm was fused with the human CXCL12 chemokine, while the second arm harbored an antigen-binding fragment (Fab) antibody sequence specific for atezolizumab. In MDA-MB-231 cells, KineTACs achieved a notable 70 % maximum degradation of PD-L1 through sequestration to CXCR7 for internalization. Employing analogous strategies, the degradation of four cell surface proteins (HER2, EGFR, CDCP1, and TROP2) as well as two extracellular soluble proteins (VEGF and TNF-α) was successfully realized. Furthermore, the cytokines incorporated into KineTACs were interchangeable with CXCL11 and vMIPII (affinitive to CXCR7), or interleukin-2 (IL-2, engaging the IL-2 receptor complex), underscoring the versatility and broad applicability of the KineTAC platform.

In summation, KineTACs present a versatile and modular genetically encoded strategy, adept at effecting lysosomal degradation of both extracellular and cell surface proteins by harnessing cytokine receptors. This innovative approach holds promise for diverse applications in the realm of targeted protein degradation.

3.2. Autophagy-based protein degradation strategies

Autophagy, an essential cellular process, involves the targeted transport of cargos to lysosomes for degradation. It plays a pivotal role in maintaining cellular and organismal homeostasis by eliminating dysfunctional organelles and damaged biomolecules[161]. Currently, autophagy is classified into three main types: macroautophagy, microautophagy, and CMA[161−163]. Recent advancements in protein degradation techniques primarily leverage macroautophagy and CMA (Fig. 8)[21,164].

Macroautophagy entails the formation of a double-layered delimiting membrane that isolates cytosolic cargos. Subsequently, this membrane seals to create autophagosomes, which are then translocated to lysosomes for degradation[161,165,166]. The fusion of autophagosomes with lysosomes facilitates the degradation of the enclosed cargo. By interacting with key proteins in macroautophagy, such as microtubule-associated protein 1A/1B light chain 3 (LC3) or the SQSTM1/p62 complex, several degradation strategies have been developed to achieve lysosomal degradation of diverse potential pathological factors. These strategies include AUtophagy-TArgeting Chimeras (AUTACs)[167], autophagosome-tethering compounds (ATTECs)[168,169], and AUTOphagy-TArgeting Chimeras (AUTOTACs)[170].

CMA is characterized by the selective lysosomal degradation of proteins harboring a KFERQ-like motif, mediated by the chaperone HSC70 and its cochaperones. Lysosomal translocation relies on the mutual recognition between the HSC70-substrate complex and lysosome-associated membrane protein type 2A (LAMP2A)[163,171]. Currently reported CMA-based degraders adopt a multifunctional structural design, comprising at least a POI-binding region and a CMA-targeting motif[21,172].

In summary, autophagy-based protein degraders demonstrate the capacity to target not only oncogenic proteins, but also pathogenic factors associated with neurodegenerative diseases, as well as non-protein targets. This underscores their versatile and universal applicability[164,173].

3.2.1. Macroautophagy-based degraders

Small molecules are preferentially emerged macroautophagy-based degraders with three distinct mechanisms represented. The recognition of selective xenophagy, involving *S*-guanylation as a pivotal tag for initiating K63-linked polyubiquitination, laid the foundation for the development of AUTACs, marking a significant milestone in autophagy-based protein degradation[174]. An AUTAC typically comprises a substrate binding fragment, a linker, and a guanine-based degradation tag (FBnG). This construct exhibits the capacity to traverse the cell membrane, subsequently instigating K63-linked polyubiquitination of the substrate. This, in turn, culminates in lysosomal degradation facilitated by p62/SQSTM1-mediated autophagosome recruitment. AUTACs have demonstrated proficiency in the degradation of various cytoplasmic proteins, including methionine aminopeptidase 2 (MetAP2) and FK506-binding protein (FKBP12). Notably, AUTAC technology holds promise in addressing mitochondria-related disorders, as exemplified by its interaction with the translocator protein (TSPO) on the outer mitochondrial membrane, leading to the efficient clearance of dysfunctional mitochondrial fragments and the restoration of mitochondrial homeostasis[174].

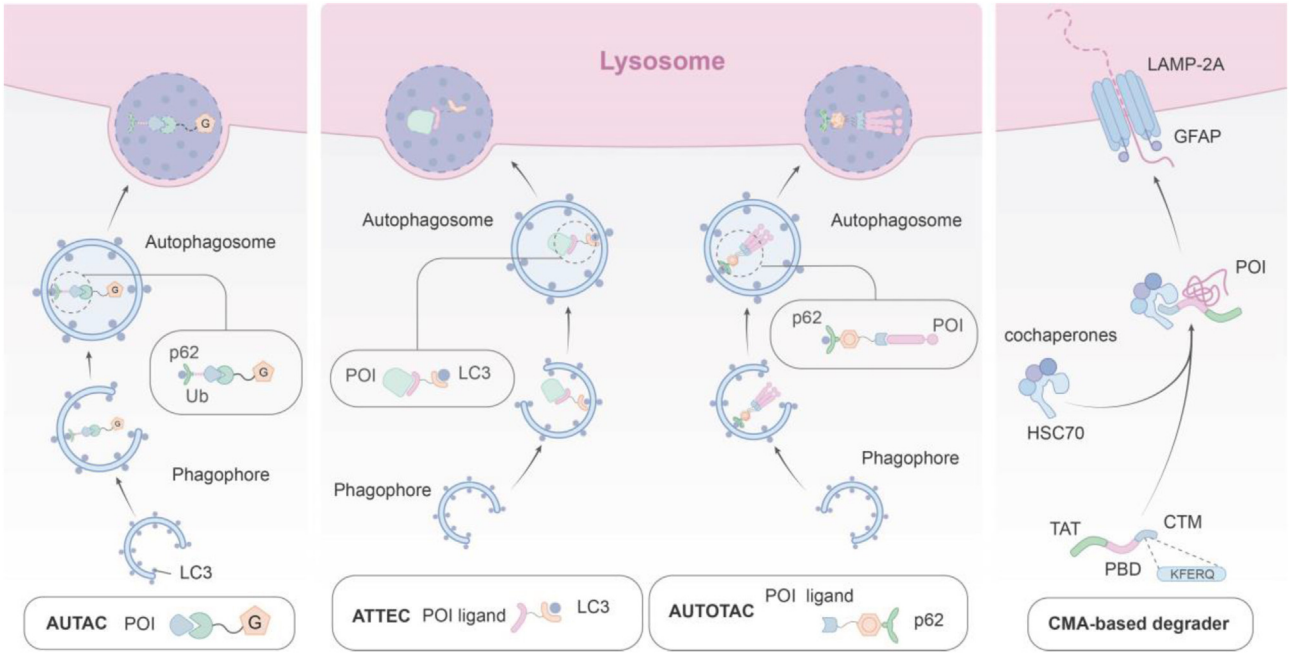


Figure 8 Schematic diagram of the design rationale of AUTAC, ATTEC, AUTOTAC and CMA-based degrader. AUTAC, ATTEC and AUTOTAC promote the degradation of POI *via* the formation of POI-specific autophagosome. CMA-based degrader mimics KFERQ motifs thereby capturing HSP70, facilitating POI degradation *via* the chaperone-mediate process.

LC3, an instrumental protein in phagophore expansion, persists in its binding to the membrane post-autophagosome formation, rendering it an ideal tethering anchor for integration into the autophagic machinery[175]. By simultaneously engaging target proteins and LC3, ATTECs instigate phagophore encapsulation of target proteins, subsequently facilitating their degradation through autophagosome-lysosome fusion. Noteworthy achievements in this domain include the selective degradation of intracellularly aggregated mutant Huntington proteins (mHTT) without affecting wild-type HTT proteins[176]. This group of compounds acts through a mechanism similar to that of molecular glues. Additionally, ATTECs have been extended to non-protein targets, exemplified by their proficiency in degrading lipid droplets (LDs), ubiquitous intracellular lipid-storing organelles[177]. LD-ATTECs are structurally close to PROTACs, *i.e.*, the LC3-binding warhead (GW or DP) is connected to the LD-binding moiety (Sudan III or Sudan IV) by a simple linker. The therapeutic potential of ATTECs has been further demonstrated in targeting oncoproteins like BRD4 and nicotinamide phosphoribosyl transferase (NAMPT)[178,179].

AUTOTAC, a pioneering autophagic degradation technology, leverages the autophagy cargo receptor SQSTM1/p62 for targeted protein degradation. Unlike its precursor, AUTACs, which employ polyubiquitination to recruit SQSTM1/p62, AUTOTACs directly interact with the ZZ domain of SQSTM1/p62 (p62zz). This interaction triggers a conformational activation of SQSTM1/p62, unveiling the LIR motif and the PB1 domain. Subsequently, target proteins undergo degradation through PB1-mediated p62 self-polymerization and LC3-mediated autophagosome recruitment, circumventing the polyubiquitination process[180,181]. Structurally, AUTOTACs are composed of target-binding ligands (TBL) linked to autophagy-targeting ligands (ATL) through multiple linkers. Notably, AUTOTACs have demonstrated efficacy in the clearance of established oncoproteins, including MetAP2, ERβ, and AR[180]. A distinctive strength of AUTOTACs lies in their capability to eliminate misfolded protein aggregates including Tau and α-Syn, offering a valuable complement to PROTAC technology and introducing novel avenues for neurodegenerative disease treatment[180–182].

In essence, AUTAC, ATTEC, and AUTOTAC herald a new era in autophagy-based protein degradation, showcasing remarkable versatility and potential for therapeutic intervention. These strategies not only signify significant strides in targeted protein degradation but also illuminate promising avenues for the treatment of various disorders.

Recently reported peptide-based chemical biology strategies have hijacked the macroautophagy process in an ATTEC-like mechanism, filling the gap of non-small molecule-based macroautophagy-dependent degraders. An intriguing non-small molecule ATTEC, Q14, has emerged as a therapeutic candidate against Parkinson's disease (PD) by augmenting mitophagy through two synergistic mechanisms[183] (Fig. 9). By targeting the mitochondria-anchored deubiquitinase ubiquitin-specific protease 30 (USP30), Q14 operates both as an allosteric inhibitor of USP30 and as an ATTEC that degrades mitochondria. This multifaceted approach holds promise for mitigating mitochondria-related diseases. Furthermore, a strategy termed autophagosome-anchored chimera (ATACC) employs the structural design of a multifunctional peptide chimera, linking the protein-binding domain (PBD) to the LC3 interaction region (LIR) and enabling efficient extracellular delivery *via* cell-penetrating peptides (CPPs)[184]. Currently, ATACCs have been demonstrated to possess LC3B- and autophagosome-dependent α-Syn degradation ($DC_{50} = 46.8 \pm 3.7$ μmol/L in HEK293T cells) as well as *in vivo* neuroprotective activity, making it an alternative strategy for coping with synucleinopathies.

Furthermore, autophagy-targeting nanobody chimera (ATNC), a versatile and multifunctional autophagy-based targeted degradation platform technology was presented *via* the adoption of

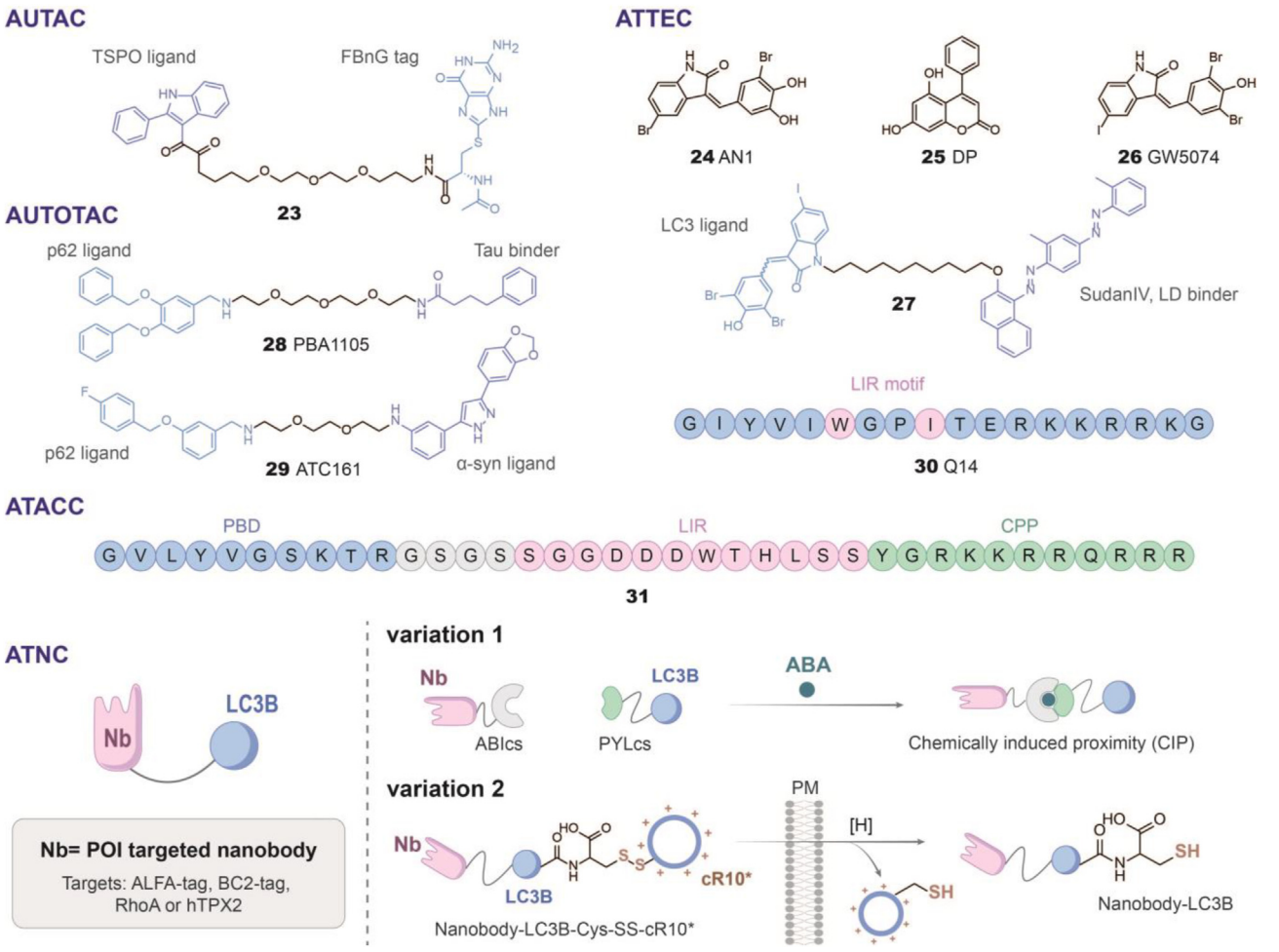


Figure 9 Chemical structures and construction considerations of autophagy-based degraders.

modular construction strategies[185]. The fundamental construction of ATNC was conceived by directly fusing the POI-targeting nanobody to the LC3B protein. Building upon the proof-of-concept related to the degradation of various fluorescent-tagged proteins, ATNC was then validated as an easy-to-implement and modular system for the degradation of endogenous substrates, comprising soluble proteins and organelles. A remarkable characteristic of ATNC is that it does not perturb endogenous LC3 levels, instead relying on additional LC3B supplementation. Endeavors towards further extending the modularity characteristics also bore fruit, consisting primarily in two variants offering two distinct benefits, controlled degradation and cell permeability. The first form integrates the (+)-abscisic acid (ABA)-based chemically induced proximity (CIP) system with ATNC. More specifically, PYLcs/ABIcs, a pair of proteins that are dimerizable in the presence of ABA, were conjugated respectively to LC3B and nanobodies. Tunable PYLcs/ABIcs dimerization could be accomplished *via* shifting ABA concentrations, hence the POI degradation degree could be finely tuned. The second variant is designed for triggering nonendocytotic intracellular delivery. One prime illustration is HE4 nanobody-LC3B-Cys-SS-cR10* (HLR), a cell-permeable ATNC drug for the degradation of human epididymis protein 4 (HE4), an endogenous protein which remains "undruggable" hitherto. HLR features an HE4-targeted ATNC and a cyclic cell-penetrating peptide (cR10*), with the two parts conjugated *via* a cleavable disulfide bridge, which assures adequate intracellular delivery and active ATNC liberation. *in vitro* assays demonstrated a dose-dependent HE4 degradation triggered by HLR, achieving inhibition of ovarian cancer cell proliferation and migration. Overall, ATNC provides a promising strategy for the construction of autophagy-dependent degradation platforms, with its ease of implementation and versatility based on modular construction, which enables considerable potential applications in biological research and disease treatment.

3.2.2. *CMA-based protein degradation strategies*

CMA-based degraders encompass peptide chimeras with three essential domains: a cell membrane-penetrating sequence for effective passage through the blood−brain barrier (BBB) and plasma membrane, a CMA-targeting motif (CTM) emulating the KFERQ motif, and a PBD. Upon intracellular entry, the PBD engages the target protein and leverages the CMA's lysosomal transport machinery by co-opting HSC70. This culminates in hydrolysis within the lysosome. Presently, CMA-based degraders have exhibited proficiency in degrading a diverse array of endogenous proteins, including death-associated protein kinase 1 (DAPK1), scaffolding protein PSD-95, α-synuclein, and Cyclin-dependent kinase 5 (CDK5)[186,187]. Furthermore, their efficacy in eliminating protein aggregates, such as mHTT and amyloid β oligomers (Aβ), has been well established[188,189]. Additionally,

akin degradation strategies have been elucidated for the lysosomal turnover of membrane protein PD-L1, founded on the structural-functional attributes of HIP1R. This negative regulator of PD-L1 relies on a PD-L1 binding motif and a lysosomal sorting sequence akin to the KFERQ motif (MDFSGLSLIKLKKQ)[190]. In summary, although CMA-based degraders grapple with distinct challenges relative to other TPD technologies, including stability and delivery efficiency, they epitomize a distinctive peptide-driven approach within the TPD landscape.

4. Proximity-inducing modalities: Beyond TPD

Since the early 1990s, the concept of inducing proximity through heterobifunctional chimeras has been pivotal in reshaping protein−biomolecule interactions[209]. Among these innovations, the breakthroughs achieved by PROTACs and molecular glues have been nothing short of revolutionary[22,210]. Building on this foundation, recent years have witnessed the emergence of various induced-proximity entities that extend their functionality beyond degradation (Table 2), encompassing targeted protein stabilization and the modulation of PTMs.

Notably, technologies rooted in small molecules have garnered substantial attention for their immense potential in reshaping the landscape of drug discovery. Deubiquitinase-targeting chimeras (DUBTACs), for instance, stand as monumental milestones in this trajectory[193]. Consequently, these proximity-inducing entities, which transcend the realm of small molecules, present a significant expansion of target spectra and cellular machinery, albeit with a somewhat measured pace of development[23,211,212]. In this section, we furnish a comprehensive overview of emerging proximity-inducing modalities that surpass the boundaries of small molecules, classified based on their underlying mechanisms.

4.1. *Targeted protein stabilization*

In contrast to PROTACs, which orchestrate the ubiquitination of the POI, DUBTACs facilitate the close proximity between deubiquitinases (DUBs) and target proteins. This innovative approach offers a supplementary avenue for the restoration of proteins that have been aberrantly degraded, presenting a novel perspective for the stabilization of diverse tumor suppressors. Among the DUBs, OTUB1 has emerged as the preferred deubiquitinase in DUBTAC strategies due to its widespread expression and a repertoire of diverse substrates. Notably, OTUB1 demonstrates a distinct proficiency in specifically cleaving K48-linked polyubiquitin chains[213,214]. This unique enzymatic activity lays the foundation for the targeted stabilization of proteins.

Leveraging the identification of the covalent ligand EN523 for OTUB1, small-molecule DUBTACs have been successfully employed to stabilize the ΔF508-mutant of the cystic fibrosis transmembrane conductance regulator (CFTR) and WEE1 kinase[193]. Moreover, building upon the EN523 scaffold, a transformative platform, TF-DUBTAC, was engineered (Fig. 10A). This innovative construct involved the conjugation of EN523 to the 5′ terminus of various oligonucleotide ligands *via* a SPAAC click reaction[194]. *In vitro* investigations substantiated the efficacy of TF-DUBTACs in selectively stabilizing a spectrum of tumor suppressor transcription factors endowed with well-defined DNA-binding motifs. Notable examples include FOXO3A, p53, and IRF3. This successful demonstration underscores the versatility of TF-DUBTAC as a broadly applicable platform. It is

Table 2 Recently reported proximity-inducing modalities beyond targeted protein degradation.

Biological effect	Acronym	Effector	Target	Indication	Ref.
Acetylation	AceTAG	p300/CBP	$FKBP12^{F36V}$ tagged histone H3.3, RelA, p53	Cancers; neurodegenerative disorders	191
	AceTAC	p300/CBP	The p53Y220C mutant	Cancers	192
Deubiquitination	DUBTAC	OTUB1	ΔF508-CFTR, WEE1	Cancers, cystic fibrosis	193
	TF-DUPTAC	OTUB1	FOXO3A, p53, IRF3	Tumors	194
Phosphorylation	PHICS	PKC	BRD4, BTK, BCR-ABL, FKBP12	Cancers, viral infection	195
Dephosphorylation	PHORC	PP1; PP5	p-$ASK1^{T838}$, AKT, EGFR	Cancers	196,197
	PhosTAC	PP2A-A	Tau, PDCD4, FOXO3a	Tauopathies, *i.e.*, AD; cancers	198,199
	DEPTAC	PP2A-Bα	Tau	Tauopathies, *i.e.*, AD	200
	RIPR	CD45	PD-L1, CD28, CTLA-4, SIRPα	Cancers	201
RNA destruction	RIBOTAC	RNase L	MicroRNA (Pri-miR-21)	Breast cancer, Alport syndrome	202
	PINAD	Multiple ribonucleases	G4 and pseudoknot RNA in SARS-CoV-2 genome	SARS-CoV-2	203
Glycosylation	Nanobody-OGT	OGT	GFP-fused Jun B, cJun, Nup 62, α-Syn	Biological research	204
	DS RNA aptamers	OGT	β-Catenin	Biological research	205
Deglycosylation	Nanobody-split OGA	OGA	c-Jun, c-Fos	Biological research	206
	Antibody-sialidase	Sialidase	Sialoglycan	Cancer immunotherapy	207,208

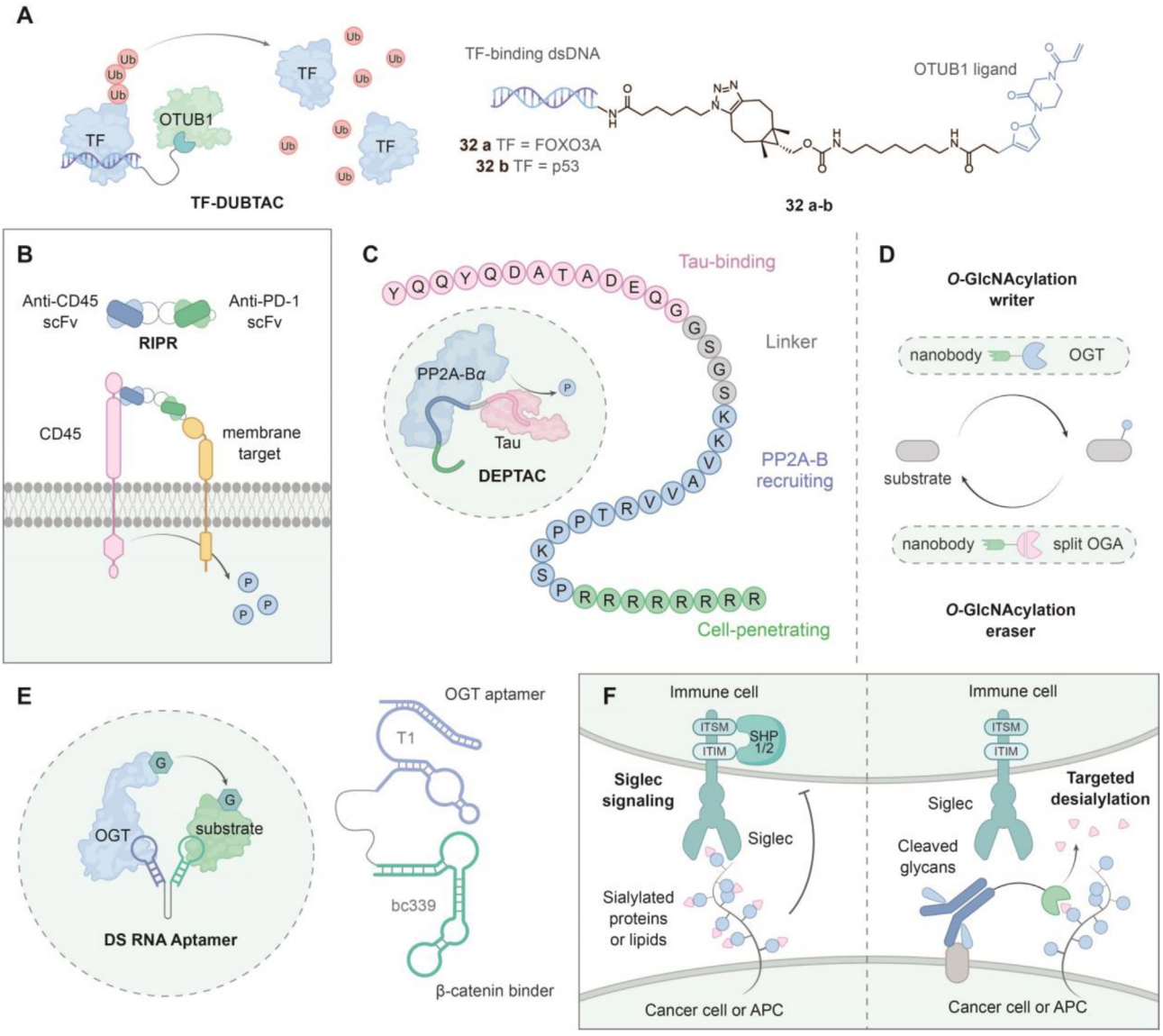


Figure 10 Summary of non-small bifunctional chimeras with the functions beyond TPD. (A) Chemical structure and design rationale of TF-DUBTAC. (B) RIPR promotes the dephosphorylation of targeted proteins by simultaneous binding with POI and CD45. (C) Design rationale and amino acid sequence of DEPTAC. (D) Bidirectional regulation of *O*-GlcNAcylation generated by nanobody−enzyme conjugates. (E) Targeted *O*-GlcNAcylation based on modular dual-specificity (DS) RNA aptamer. (F) HER2 antibody−sialidase conjugates induce catalytic degradation of sialoglycans in a tumor-specific manner.

important to underscore that this technology is in its nascent stage, and subsequent endeavors will be imperative to enhance its specificity through meticulous medicinal chemistry optimization.

4.2. *Targeted protein post-translational modification (PTM)*

PTMs constitute a pivotal facet of cellular biology, regulating protein structure, dynamics, and function through covalent modifications with functional groups or regulatory subunits[215]. PTMs exert profound influence over myriad cellular processes and pathological states. Recent strides in proximity-inducing modalities have introduced an innovative dimension to PTM regulation, empowering the precise control of processes such as phosphorylation/dephosphorylation, acetylation/deacetylation, and glycosylation/deglycosylation[23,209,210,212].

The pursuit of small-molecule induced-proximity entities for PTMs initially centered on phosphorylation/dephosphorylation, a quintessential and therapeutically pertinent class of PTMs[23,195]. Two notable exemplars emerged: phosphorylation-inducing chimeric small molecules (PHICS) and phosphatase recruitment chimeras (PHORCs)[195−197]. These innovative constructs leverage allosteric activators of kinases (writers) or phosphatases (readers) linked to targeted protein ligands, endowing precise regulation of phosphorylation/dephosphorylation cascades without necessitating engineered fusion proteins. A significant leap in the field came with the advent of Acetylation Targeting Chimeras (AceTACs), representing a pioneering approach to targeted acetylation[192]. For instance, MS78 effectively engages histone acetyltransferase p300/CBP to induce lysine acetylation in p53Y220C mutants, thereby rectifying p53 dysfunction. This burgeoning

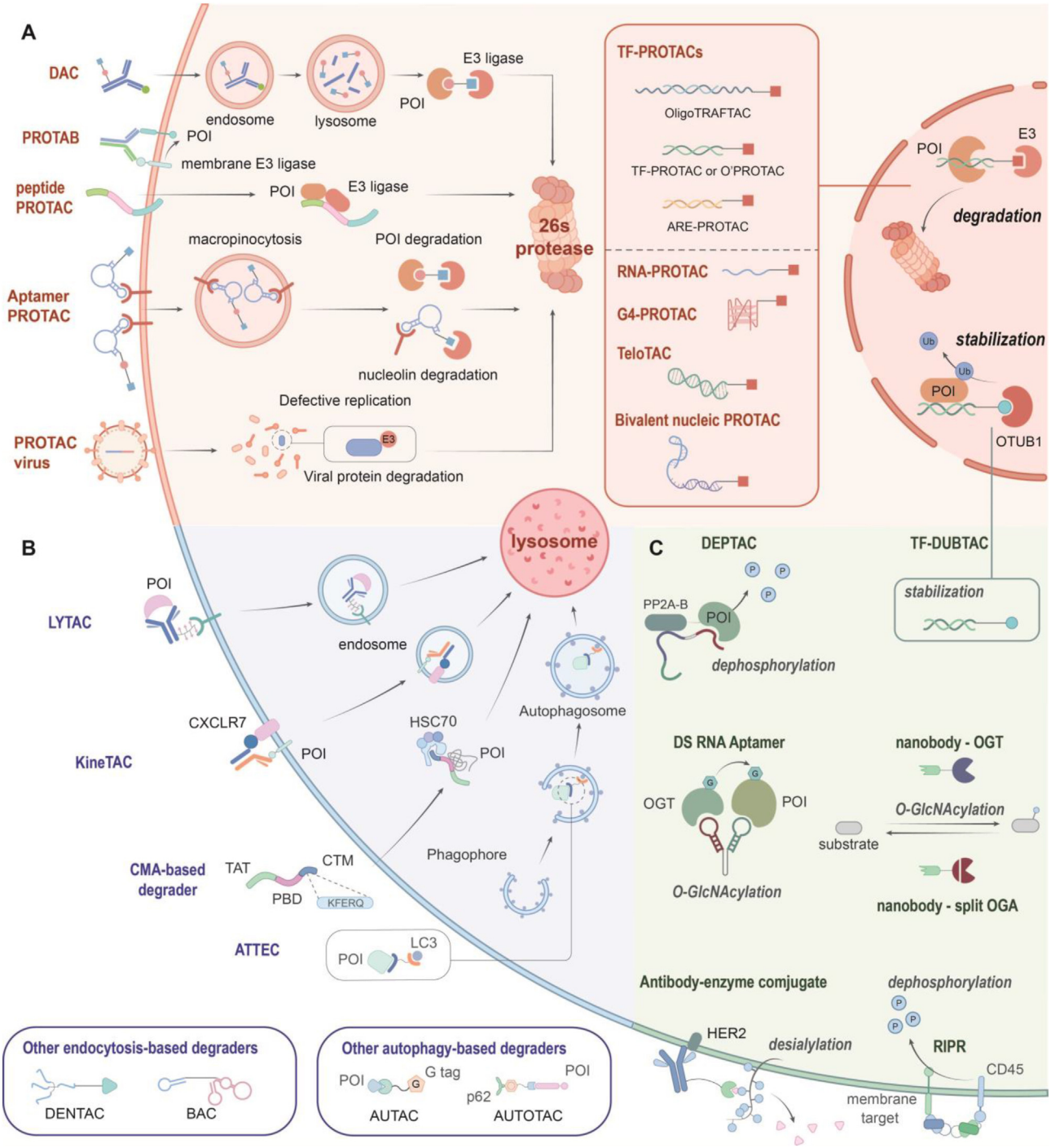


Figure 11 Graphical summary of the mechanism of bifunctional non-small molecule entities to induce degradation and other proximity-mediated effects. (A) The mechanism of the non-small molecule PROTACs. POIs are ubiquitin-tagged *via* recruitment of the E3 ligase complex and subsequently degraded *via* the 26S protease. (B) Lysosomal degraders utilizing endocytosis or autophagy processes. (C) Macromolecular entities for triggering proximity-mediated effects to regulate multiple post-translational modifications (PTMs).

domain of small molecule-induced PTMs holds transformative promise, heralding a new era in protein editing. Nevertheless, certain challenges persist, including the intricacies in ligand design and screening, grappling with "undruggable" targets, and the imperative for engineered fusion proteins[23,212].

In parallel, non-small molecule modalities in TPD might be transposed to PTMs, harnessing the advantages of facile platform design, swift modular construction, access to a broader spectrum of targets and PTMs, and adaptability to intricate cellular environments. A compelling illustration is dephosphorylation, where two platform techniques have garnered widespread attention: Receptor Inhibition by phosphatase recruitment (RIPR) and dephosphorylation targeting chimeras (DEPTAC)[200,201]. RIPR, akin to PROTAB, orchestrates the construction of antibody-based bispecific entities, culminating in the simultaneous binding of the extracellular domains of target proteins and cell-surface

phosphatases, facilitating the dephosphorylation of specific targets (Fig. 10B). Notably, CD45, a widely expressed cell surface tyrosine phosphatase, has been recruited for the targeted dephosphorylation of PD-L1, a key modulator of T-cell function[216,217]. PD-L1 RIPRs present a distinct approach to enhancing T cell activity, ensuring sustained inhibition of intracellular PD-1 signaling, thereby distinguishing it from PD-1/PD-L1 antagonist antibodies. In mouse models of small-cell lung cancer and colon adenocarcinoma, significant tumor growth inhibition could be induced by PD-L1 RIPR. This approach has the potential to be generalized as a generic strategy to enforce the repression of kinase-activated receptors, as it has been shown to modulate other immune receptors, including CD28, CTLA-4 and SIRPα[201]. Beyond targeted dephosphorylation, DEPTAC capitalizes on a chimeric framework comprising a target protein binding motif, a cell-penetrating sequence, and a phosphatase recruiting sequence (Fig. 10C)[200]. In its inaugural disclosure, DEPTAC selectively recruited Bα subunit-containing protein phosphatase 2A (PP2A-Bα), the preeminent Tau phosphatase in the brain, leading to dephosphorylation of several Alzheimer's disease-associated sites on tau proteins[218,219]. Both *in vitro* and *in vivo* assessments underscored the impressive penetration and selectivity of DEPTAC for neuronal cells. Noteworthy, DEPTAC substantially ameliorated the morphology and functional status of neurons in transgenic mice overexpressing human Tau protein[200]. Despite attendant stability concerns, DEPTACs exhibit striking selectivity, offering a novel perspective on the aberrant phosphorylation of protein aggregates.

Protein glycosylation, encompassing diverse modifications, plays pivotal roles in fundamental cellular processes including signaling and immune recognition[220]. One prominent form, O-linked β-*N*-acetylglucosamine (*O*-GlcNAc) glycosylation, is finely regulated by *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA) enzymes[220,221]. To effectuate precise bidirectional control and functional assessment of substrate proteins, a pair of sophisticated proximity-inducible systems have been engineered using fusion nanobodies in conjunction with OGT/OGA (Fig. 10D)[204–206]. Notably, the fusion of nanobodies recognizing the EPEA sequence with OGT led to the selective glycosylation of endogenous α-synuclein, signifying therapeutic potential in Parkinson's disease[204]. Likewise, split OGA, comprising a simplified structure, was fused with distinct nanobodies, resulting in heightened selectivity and steadfast deglycosidase activity[206]. This system facilitated site-selective deglycosylation of two pivotal members of the AP-1 transcription factor complex, namely c-Jun and c-Fos.

Notwithstanding, some considerations persist, such as the requisite expression of epitope-tagged POIs and potential unintended impacts on target protein functionality. Concurrently, a novel approach rooted in modular dual-specificity (DS) RNA aptamers has shed new light on targeted *O*-GlcNAcylation[205]. In a proof-of-concept demonstration, a DS aptamer, tethering OGT at one terminus and binding endogenous β-catenin at the other, engendered protein-specific *O*-GlcNAcylation alongside other OGT substrates, with OGT/OGA levels remaining unaffected (Fig. 10E). This tool unveiled the pivotal role of *O*-GlcNAcylation in the stabilization and augmented transcription of β-catenin through interactions with the E3 ligase β-TrCP, as well as several epigenetic modifiers including EZH2, KAT2A, and EP300. In sum, DS aptamers stand as versatile and efficacious facilitators of *O*-GlcNAcylation on discrete proteins, promising broad applicability in regulating diverse intracellular PTMs.

Sialylation, another prominent facet of protein glycosylation, has emerged as a critical factor in immune evasion, particularly observed in heightened sialoglycans on tumor cell surfaces[222–225]. However, conventional sialoglycan ligands pose challenges for the assembly of desialylation chimeras due to extensive fusion with cell surface proteins and lipid scaffolds, as well as notable chemical heterogeneity[208]. In lieu of this, contemporary strategies have pivoted towards the construction of HER2 antibody−sialidase conjugates for catalytic sialoglycan degradation in a tumor-specific manner. For instance, the coupling of α-HER2 antibody to Vibrio cholerae sialidase (T-Sia 1) facilitated the dissociation of sialoglycans from diverse HER2-positive breast cancer cells, augmenting NK cell cytotoxicity (Fig. 10F)[207]. An enhanced iteration, predicated on Salmonella typhimurium sialidase (T-Sia 2), exhibits diminished off-target activity and heightened chemical stability. T-Sia 2 has demonstrated efficacy in delaying HER2+ tumor growth in syngeneic breast cancer models, and prolonged the survival of trastuzumab-resistant breast cancer mice[208].

In summary, the delineation of dynamic protein glycosylation landscapes and the strategic targeting thereof hold immense promise for therapeutic interventions in various disease contexts. The concerted efforts in understanding the nuanced interplay of glycosylation patterns with cellular processes pave the way for innovative and precisely tailored therapeutic modalities.

5. Conclusions

In recent years, the emergence of novel entities inspired by PROTACs has marked a significant stride in drug discovery. These entities not only revolutionize the landscape of TPD but also extend their impact beyond mere protein degradation. Although non-small molecules are not the predominant choice, they have proven instrumental in overcoming the constraints of conventional small molecule entities. and have resulted in three major trajectories: macromolecular PROTACs, lysosomal degraders and other proximity-inducing modalities (Fig. 11).

Macromolecule-based modalities, while varying in mechanisms, offer several distinct advantages. Notably, click reactions and biocoupling techniques empower the construction of bispecific macromolecule-small molecule heterodimers or macromolecule-macromolecule chimeras, offering a rapid and modular synthetic approach with broad applicability across diverse domains including peptides, nucleic acids, antibodies, and bacterial enzymes. This methodology largely circumvents conventional trial-and-error techniques reliant on high-throughput screening. Fundamentally, the versatility of the "targeting + effect" paradigm stems from the independent identification of ligands for POIs or non-protein targets, coupled with the recruitment of enzymes possessing specific functionalities. Additionally, advances in biotechnology have introduced supplementary construction strategies, exemplified by bispecific antibodies and aptamers, crucial for targeting membrane-associated proteins and facilitating processes like ubiquitination degradation, lysosomal degradation, dephosphorylation, and deglycosylation. Furthermore, macromolecular ligands vastly expand the range of potential targets, encompassing secreted proteins, membrane-associated proteins, protein aggregates, lipids, glycans, and even entire organelles.

Several distinct benefits exist as well beyond these aforementioned commonalities. For macromolecular PROTACs, the utilization of aptamers or antibodies as a delivery vehicle for small-molecule PROTACs improves their targeting and pharmacokinetic

properties considerably. Moreover, well-designed nucleic acid/peptide ligands contribute not only to the construction of highly selective entities, but also offer a potential to finely modulate biological effects, *e.g.*, achieving co-degradation or modulating specific PPIs. Besides, the concept of macromolecular PROTAC can be extended to living organisms (*e.g.*, viruses), hinting at its potential for deep multidisciplinary cross-fertilization. However, as this field is still nascent, numerous challenges persist, primarily attributed to the inherent properties of macromolecular ligands. Peptides and nucleic acids exhibit suboptimal stability and limited cell permeability, while antibodies and non-native glycans may evoke immunogenic responses. Interdisciplinary collaborations have yielded strategies to address these limitations. For instance, conformational restriction techniques and the emergence of cell-penetrating peptides have enhanced the stability and delivery efficiency of peptide ligands. Similarly, chemical modifications targeting the oligonucleotide backbone have bolstered resistance to nucleases. Moreover, drug delivery systems offer a promising avenue to enhance the therapeutic efficacy of nucleic acid-based entities.

Apart from remarkably extended target spectra, one of the major strengths in lysosomal degraders is the generalizability towards diverse cell types, which outperforms proteasome-dependent entities that is restricted by E3 ligase expression levels. Furthermore, the structural design of endocytosis-based degraders is more flexible compared to PROTACs since cell permeability need not be considered. Additionally, it is noteworthy the potential of lysosomal degraders in the treatment of metabolic and neurodegenerative diseases. Devising novel ligands to hijack the more diversified LTRs could be the focal point for the evolvement of endocytosis-based degraders, whereas autophagy-based macromolecular degraders are just in their infancy, with more efforts needed to be deployed on the identification of novel paradigms as well as generalizability research.

Despite lagging its evolvement compared to small molecule entities, macromolecular induction-proximity agents offer unique benefits in terms of modular construction platforms, regulatory accessibility to a wider array of PTMs, and applicability to complex cellular environments. However, the compositional complexity of macromolecule-based entities poses greater challenges compared to conventional small-molecule PROTACs. Further exploration is imperative, particularly in devising standardized approaches for pharmacokinetic and safety assessments. It is noteworthy that pioneering paradigms often traverse a protracted trajectory from proof-of-concept to emerging as leading candidates for potential translational research.

Although macromolecule-based heterobifunctional entities are still in their formative stages, ongoing research holds the promise of significantly broadening their potential applications, endowing them with remarkable utility in biomedical research and drug discovery. Anticipating a continuous stream of groundbreaking revelations in this field, we foresee an enhanced understanding of challenging targets and cellular interactions that remain incompletely understood, providing novel therapeutic avenues for diseases currently lacking effective treatments.

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Author contributions

Gu He: Project administration, Supervision, Funding acquisition. Xiang Li: Conceptualization, Methodology, Supervision. Xiaowei Huang: Data curation, Writing-Original Draft, Visualization. Fengbo Wu: Writing-Review & Editing, Visualization. Jing Ye: Data Curation. Lian Wang: Visualization. Xiaoyun Wang: Supervision.

Conflicts of interest

The authors declare no conflicts of interest.

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