ISSN 0006-2979, Biochemistry (Moscow), 2026, Vol. 91, No. 1, pp. S118-S132 © Pleiades Publishing, Ltd., 2026.
Russian Text © The Author(s), 2026, published in Uspekhi Biologicheskoi Khimii, 2026, Vol. 66, pp. 159-178.

REVIEW

Targeted Protein Degradation: Methods and Prospects

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Received August 19, 2025
Revised September 23, 2025
Accepted October 9, 2025

Abstract—In recent years, targeted proteolysis systems have emerged as powerful tools for directed degradation of pathogenic proteins, offering novel therapeutic strategies for cancer, neurodegenerative disorders, and infectious diseases. This review systematizes key mechanisms and recent advances in inducible targeted proteolysis, including targeted proteasomal degradation (PROTACs, AbTACs, molecular glues), lysosome-mediated degradation (LYTACs, AUTACs, ATTECs) via endocytosis or autophagy, and targeted proteolysis in bacteria (BacPROTACs), which extends degradation technologies to prokaryotic systems. The structural features, advantages, and limitations of each platform are discussed in detail, along with key publications demonstrating their preclinical and clinical efficacy. Special attention is given to the prospects for translating these technologies into therapeutics, including overcoming challenges such as selectivity and *in vivo* delivery.

DOI: 10.1134/S0006297925604034

Keywords: PROTAC, LYTAC, AUTAC, ATTEC, BacPROTAC, AbTAC, Homo-BacPROTAC, AUTOTAC, GlueTAC

INTRODUCTION

Proteolytic mechanisms are natural regulators of cellular homeostasis. In recent years, these mechanisms have been deliberately harnessed for therapeutic purposes, particularly in cancer treatment. Targeted protein degradation is based on selective tagging of proteins of interest, recruitment of cellular degradation machinery (such as proteasomes), and acceleration of proteolysis through specialized small molecules that bind the target protein. Over the past 25 years, several classes of molecules and associated technologies have been developed to induce intracellular protein degradation, including PROteolysis TArgeting Chimeras (PROTACs), antibody-based proteolysis targeting chimeras (AbPROTACs), and bacterial proteolysis targeting chimeras (BACPROTACs), as well as so-called molecular glues. Collectively, these approaches not only significantly expanded the application of targeted protein degradation, but have opened new avenues in drug discovery. The aim of this review was to examine the development of these targeted proteolysis strategies and to compare and discuss their therapeutic applications.

Protein degradation and proteolysis are integral components of proteostasis, i.e., the cellular protein homeostasis that regulates the composition and quality of the proteome [1]. Proteolysis is the cleavage of proteins and peptides into smaller peptides or amino acids via hydrolysis of the polypeptide backbone by proteolytic enzymes, also known as proteases, proteinases, or peptidases. In living cells, proteolysis is highly regulated and selective, with specific proteins targeted for degradation. In eukaryotic cells, protein degradation is primarily mediated by proteasomes and lysosomes [2, 3]. Proteasomes are mainly responsible for the degradation of soluble, short-lived, and misfolded proteins, whereas lysosomes degrade long-lived proteins, as well as protein complexes, macromolecules, and entire organelles [4]. In bacterial cells, proteolysis is carried out by ATP-dependent protease complexes, such as ClpP and Lon proteases, as well as by bacterial 20S proteasome, which together ensure the removal of damaged or unnecessary proteins [5].

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TARGETED PROTEIN DEGRADATION BY PROTEASOMES

Targeted proteolysis is based on selective tagging of defective proteins leading to their degradation. In eukaryotic cells, this is most commonly the covalent attachment of ubiquitin molecules. Importantly, ubiquitination not only serves as a signal for proteasomal degradation but can also mark the protein for other cellular pathways, including endocytosis and autophagy [6]. Proteasomes are components of the ubiquitin–proteasome system, which also includes ubiquitin ligases and deubiquitinating enzymes. Ubiquitination is carried out by three enzymes: ubiquitin-activating enzyme E1, ubiquitin-conjugating enzyme E2, and ubiquitin ligase E3. First, E1 binds ubiquitin in an ATP-dependent manner and transfers it to E2. Next, E3 catalyzes the transfer of ubiquitin from E2 to the target substrate. The repeated action of these three enzymes results in the substrate polyubiquitination. Ubiquitin molecule has seven lysine residues (K6, K11, K27, K29, K33, K48, and K63) and the amino group of the N-terminal methionine, which act as attachment points for forming different types of polyubiquitin chains, each directing proteins to distinct cellular fate. The K48 and K63 chains are most common (about 80%) polyubiquitin chains in mammalian cells. Proteins with K48 chains are primarily targeted for proteasomal degradation, whereas proteins with K36 chains play a key role in the regulation of lysosomal functions and inflammatory response [7-9]. The principles of the ubiquitin–proteasome system form the foundation for the function of PROTACs.

PROTACs. PROTAC molecules consist of two ligands connected by a linker: one ligand binds to E3 ligase; the other binds the protein of interest (POI) targeted for ubiquitination. Simultaneous binding of the E3 ligase and the POI induces ubiquitination of the latter, leading to its proteasomal degradation, whereas the PROTAC molecule itself is not degraded

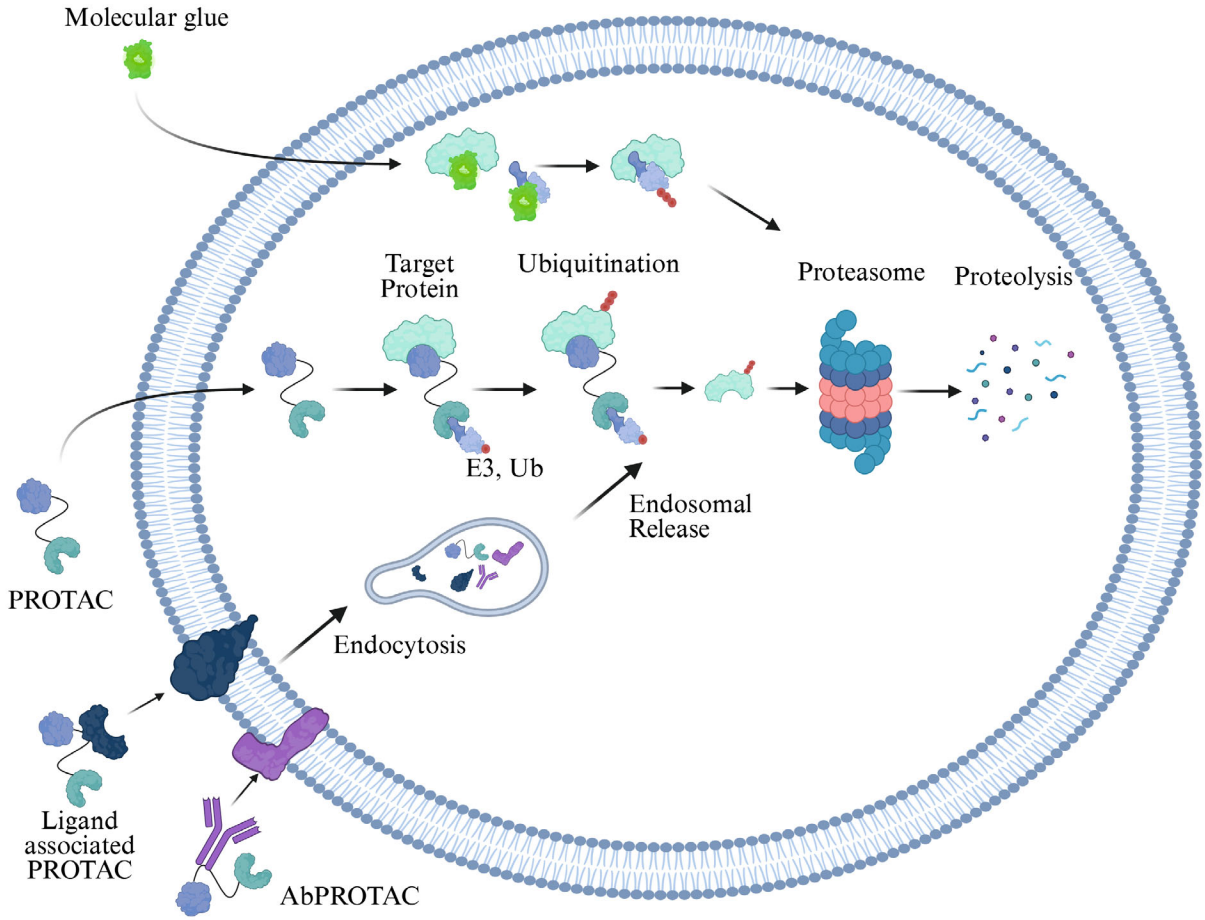

Fig. 1. Mechanism of PROTAC action. A PROTAC molecule consists of a ligand binding E3 ligase, a linker, and a ligand interacting with POI. Simultaneous binding of PROTAC with E3 ubiquitin ligase and POI induces polyubiquitination of POI and its degradation by the proteasome. The molecular glue also induces the interaction between the POI and the E3 ubiquitin ligase through binding with them, but compared to PROTACs, molecular glues contain no linker and have a lower molecular weight. AbPROTACs and ligand-based PROTACs (LiPROTACs) penetrate into cells via endocytosis and then into the cytoplasm through the endocytic release.

during this process (Fig. 1). In contrast to small-molecule inhibitors, PROTACs are recyclable and act in multiple catalytic cycles; therefore, they are expected to be effective at lower doses. Theoretically, PROTACs can be designed to target virtually any intracellular or transmembrane protein, including proteins previously considered resistant to targeted cleavage, such as nonenzymatic proteins [10].

Modern PROTAC research has undergone significant changes since the emergence of the PROTAC concept in 2001. PROTAC1 employed the E3 ligase and SCF complex consisting of SKP1 (S phase kinase-associated protein 1), cullin 1, and F-box protein and was initially tested in *Xenopus laevis* egg extracts [11]. Subsequent advances transformed PROTACs from early peptide-based constructs into fully synthetic small molecules capable of using alternative E3 ligase complexes to induce proteasomal degradation [12, 13]. However, only 13 of the approximately 600 known E3 ligases have been successfully employed for the PROTAC design, highlighting an opportunity for further innovation [14]. PROTACs possess inherent limitations, including relatively high molecular weights (0.6-1.3 kDa) compared to traditional small-molecule inhibitors (<0.5 kDa) and large polar surface areas, which negatively affect their solubility and membrane permeability, thus limiting the overall bioavailability of these molecules in clinical applications and upon oral administration. Moreover, because PROTACs rely on the ubiquitin–proteasome system, their activity is largely restricted to intracellular and transmembrane proteins, leaving secreted proteins and certain monotypic membrane proteins inaccessible to conventional PROTAC strategies [15]. Despite these limitations, more than 20 PROTAC-based therapeutics have entered phase I and II clinical trials for both solid tumors (e.g., ARV-766, KT-333) and hematologic malignancies (e.g., NX-2127, DT2216) [16]. PROTACs targeting the androgen receptor (ARV-110; NCT03888612) and estrogen receptor (ARV-471; NCT05654623) have progressed to phase II and III clinical trials for prostate and breast cancers, respectively, emphasizing the growing clinical promise of PROTACs as a novel anticancer therapeutic strategy.

One of the most attractive features of PROTACs as therapeutic agents is their ability to target historically "undruggable" oncoproteins, such as MYC and signal transducer and activator of transcription 3 (STAT3). PROTACs offer other advantages, including enhanced targeting specificity and sustained degradation of target proteins, even in cells that have developed resistance to conventional inhibitors [17]. Thus, a $KRAS^{G12C}$-targeting PROTAC (LC-2) has been developed as an alternative to the small-molecule inhibitor MRTX849. LC-2 selectively targeted oncogenic $KRAS^{G12C}$ and enhanced its degradation, even in inhibitor-resistant cell lines. A distinctive feature of LC-2 is its high selectivity for the mutant KRAS, with no interaction with the wild-type protein [18, 19]. Consequently, PROTACs that target mutant oncoproteins are expected to exert limited side effects on normal cells. Furthermore, the recyclable nature of PROTACs enables sustained protein degradation at relatively low effective doses compared with traditional small-molecule inhibitors. As a result, PROTAC-based therapies may minimize the adverse effects commonly associated with conventional chemotherapeutic agents

Another critical challenge in oncology is the development of therapeutic resistance. PROTACs offer a promising strategy to address this problem by targeting and eliminating proteins involved in resistance mechanisms. Importantly, PROTACs may be applied preventively to suppress the pathways underlying drug resistance. This approach is particularly relevant for malignancies with poor prognoses, such as metastatic colorectal cancer resistant to BRAF V600E inhibitors, for which median survival is often less than one year [20]. For instance, PROTAC-mediated degradation of bromodomain-containing protein 4 (BRD4) implicated in resistance to doxorubicin, has been shown to enhance doxorubicin efficacy in both cancer cell lines and mouse models [21]. Although these findings are still at an early stage of development, they indicate the potential of PROTACs to prevent or overcome resistance to existing therapies and provide compelling evidence for their capacity to restore drug sensitivity across diverse oncological contexts.

Molecules capable of simultaneously targeting multiple proteins are also under active development. Due to their enhanced specificity and effectiveness at lower concentrations compared with conventional inhibitors, PROTACs represent promising therapeutic tools for difficult-to-treat tumor cell subpopulations. For example, a dual-target PROTAC (PROTAC 753B) capable of degrading both B-cell lymphoma extra-large (BCL-XL) and B-cell lymphoma 2 (BCL-2) proteins has been developed to alleviate chemotherapy-induced cellular senescence in acute myeloid leukemia [22]. Beyond dual-target chimeras, multifunctional PROTACs are emerging. One such example is Y-PROTAC, which targets anaplastic lymphoma kinase (ALK) and is linked via a glutathione-cleavable disulfide bond to a cyclin-dependent kinase 4/6 (CDK4/6) inhibitor [23]. This molecule exhibits the antitumor and antiproliferative activities *in vitro* not only through ALK degradation and CDK4/6 inhibition, but also through the simultaneous degradation of both ALK and CDK4.

Although many currently known PROTACs are highly effective protein-degrading agents, they generally lack tissue specificity because of their reliance on E3 ligases with broad expression profiles. A major

obstacle to the clinical translation of PROTAC technology is the lack of methods for efficient and selective delivery to the desired tissues and cell types. Their unfavorable biophysical properties of PROTACs, including relatively high molecular weight and polarity, limit their cellular permeability. Moreover, nonspecific distribution and activity of PROTACs may lead to adverse effects, particularly when wild-type oncogenic proteins are targeted [24]. Depending on the POI, other deleterious side effects are possible, including formation of malignant neoplasms, as aberrant expression levels of certain genes, such as tumor protein p53 (TP53) and RAD51 recombinase (RAD51), are themselves pathogenic [25, 26].

Several strategies have been developed to address these limitations, including AbPROTACs, LiPROTACs (Fig. 1), and nanoparticle-encapsulated PROTACs (nanoPROTACs) [24, 27]. AbPROTACs are aimed to achieve cell type-specific delivery via antibody/antigen-mediated endocytosis, followed by endosomal escape to avoid premature lysosomal degradation. This strategy requires the presence of a cancer-specific surface antigen and a corresponding high-affinity antibody. For example, trastuzumab (Herceptin), which targets HER2 tyrosine kinase receptor, has been successfully incorporated into PROTAC constructs, resulting in improved delivery to HER2-positive breast cancer cells [28]. However, AbPROTACs suffer from significant drawbacks, including large molecular size and limited stability upon systemic administration, which constrain their clinical applicability. One more interesting approach to enhance the tissue specificity is conjugating PROTACs with small-molecule ligands whose receptors are overexpressed in tumor cells. For example, folate receptor alpha (FOLR1) is frequently upregulated in multiple cancer types. The conjugation of folate group with a ligand of ubiquitin ligase VHL E3 increased the specificity of PROTAC, while reducing its off-target activity [29]. However, despite an enhanced efficacy, such modifications increase the molecular weight of already large PROTAC molecules, which may have a negative effect on their pharmacokinetic properties and limit their clinical applications. To overcome the limitations associated with large antibody or ligand conjugates, smaller peptide simulating antibody–ligand recognition sites have emerged as an attractive alternative. In particular, cross-linked (stapled) peptides, which contain covalent constraints such as hydrocarbon bridges, can maintain α-helical structure and exhibit enhanced stability compared to unmodified peptides. Incorporating cross-linked peptides that selectively bind either the target protein or the E3 ligase may reduce the overall size of PROTAC constructs while preserving their specificity and efficacy [30]. Although the application of cross-linked peptides in PROTAC design is still under active investigation, this approach holds promise for the development of more stable, selectively delivered PROTACs.

The nanoparticle technology has attracted considerable interest due to its potential to enhance therapeutic efficiency and specificity, as well as to simplify molecular delivery. Based on their cellular uptake mechanisms, nanoparticles can be classified as passive or active. *Passive* targeted nanoparticles exploit the hypoxic and highly angiogenic environment of malignant tumors, which increases vascular permeability and facilitates diffusion or endocytic uptake by cancer cells. In contrast, *active* targeted nanoparticles rely on the receptor-mediated endocytosis, analogous to PROTACs conjugated with antibodies or ligands [27]. The first nanoPROTACs, developed in 2022, were targeted at a Lewis lung carcinoma model *in vivo*. Specifically, the BRD4-targeting PROTAC dBET6 was encapsulated in nanoparticles composed of a pH/glutathione-responsive polymer (DS-PLGA). To further enhance the specificity, two additional molecules were generated, one of which included the macrophage-specific CRV peptide, to enable the targeting of tumor-associated macrophages. All three nanoPROTAC formulations demonstrated prolonged circulation times *in vivo* and induced significantly greater tumor volume reduction (~75-90%) compared with the same PROTAC administered without nanoparticle encapsulation (~50%). Analysis of the nanoparticle biodistribution revealed that membrane-bound PROTACs accumulated predominantly in tumor cells; however, non-targeted accumulation in the liver and spleen was also observed [31].

Despite these advances, the development of resistance to PROTACs remains a concern. Evidence suggests that resistance to PROTACs is primarily driven by alterations in the recruited E3 ubiquitin ligase rather than by changes in the target protein itself [32, 33].

Molecular glues. Molecular glues promote the dimerization or colocalization of two proteins, resulting in the formation of a ternary complex [34]. Through this mechanism, they can regulate a wide range of biological processes, including transcription, chromatin remodeling, protein folding, localization, and degradation. The earliest examples of molecular glues were the immunosuppressants cyclosporin A (CsA) and FK506. CsA induces the formation of a cyclophilin–CsA–calcineurin complex, whereas FK506 promotes the assembly of the FKBP12 (FK506-binding protein 12)–FK506–calcineurin complex. These discoveries laid the foundation for the term *molecular glues* [35]. Later, another immunosuppressant, rapamycin, was identified, which stabilizes the FKBP12–rapamycin–FRB (mTOR) complex. In addition to their immunosuppressive effects, rapamycin and its analogs display the antifungal and antitumor activities [36].

Molecular glues used for targeted proteolysis induce interactions between an E3 ubiquitin ligase and a target protein, leading to ubiquitination and subsequent proteasomal degradation [37] (Fig. 1). Like PROTACs, molecular glues exploit the ubiquitin–proteasome system; however, there are several important distinctions between these two systems. PROTACs are heterobifunctional molecules that simultaneously bind an E3 ligase and a target protein, whereas molecular glues typically bind to only one component – most often the ligase – and promote or stabilize its interaction with the substrate. Moreover, molecular glues generally have a lower molecular weight because they lack a linker, which contributes to improved oral bioavailability and enhanced cell permeability compared with PROTACs. Despite these advantages, molecular glues are more challenging to develop, although rational design strategies are now beginning to emerge.

Notable examples of molecular glues employed in targeted proteolysis include thalidomide, lenalidomide, and pomalidomide. These compounds had been approved by the FDA for the treatment of various malignancies long before their mechanisms of action were fully understood. Years later, it was discovered that their antitumor activity arises from their function as molecular glues: they promote interactions between the cereblon E3 ligase (a *substrate-recognition subunit of the E3 ligase complex)* and transcription factors IKZF1 and IKZF3, leading to their ubiquitination and degradation. Given their drug-like properties, molecular glues are expected to attract an increasing interest from both the scientific community and the pharmaceutical industry [38].

TARGETED PROTEOLYSIS IN LYSOSOMES

Lysosomes mediate intracellular degradation of proteins and organelles through three major pathways: endocytosis, phagocytosis, and autophagy [39]. During endocytosis, cells internalize extracellular materials or membrane-associated proteins. Phagocytosis involves the recognition and engulfment of large particles, such as viruses and bacteria. Autophagy is a cellular process responsible for the removal of misfolded or aggregated proteins, damaged organelles, and intracellular pathogens. Autophagy occurs through three distinct routes: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). In macroautophagy, defective proteins or organelles are selectively recognized by autophagy receptors and sequestered into vesicles known as autophagosomes [40]. Autophagosomes fuse with lysosomes, where their contents are degraded. In microautophagy, lysosomes directly engulf cytosolic cargo, leading to its degradation [41]. In contrast, CMA involves selective recognition of substrate proteins by cytosolic chaperones, followed by their direct translocation across the lysosomal membrane for degradation. CMA possesses two distinguishing features: first, it selectively degrades soluble proteins rather than organelles; second, it does not require autophagosome formation [42]. Several strategies for targeted proteolysis in lysosomes exploit these pathways. Unlike proteasome-based targeted degradation, which is largely restricted to specific intracellular proteins, lysosome-based targeted proteolysis enables clearance of protein aggregates, damaged or excess organelles, membranes, and extracellular proteins.

Lysosomal proteolysis of proteins. LYTACs and their analogs. LYTACs (LYsosome-TArgeting Chimeras) induce the degradation of extracellular and membrane proteins through the endosomal–lysosomal pathway. Because extracellular and membrane proteins account for approximately 40% of all encoded proteins and play key roles in neurodegenerative, autoimmune, and cancer diseases, the LYTAC approach represents an important complement to the PROTAC-based strategies. LYTAC molecules are designed to simultaneously bind an extracellular domain of a membrane protein or a soluble extracellular protein and a lysosome-targeting receptor (LTR), such as cation-independent mannose 6-phosphate receptor (CI-MPR), expressed on the cell surface. Formation of this ternary complex triggers receptor-mediated endocytosis, directing the target protein to lysosomes for degradation [43]. In late endosomes, the acidic environment promotes dissociation of the targeted protein from CI-MPR followed by its degradation, while CI-MPR is recycled back to the Golgi apparatus and then to the cell surface. The first reported LYTAC molecules were based on CI-MPR, also known as insulin-like growth factor 2 receptor (IGF2R). These constructs consisted of a small molecule or an antibody conjugated to a synthetic glycopeptide ligand that binds CI-MPR (Fig. 2) [43]. This strategy demonstrated strong potential for the degradation of multiple therapeutically relevant proteins. For example, a LYTAC generated by covalent conjugation of a CI-MPR ligand to the anti-EGFR antibody Cetuximab was shown to selectively degrade EGFR in various cell lines [43].

The bispecific aptamer chimera composed of a DNA aptamer targeting CI-MPR and a transmembrane target protein can be regarded to as a LYTAC analog. Such aptamer chimeras can direct membrane proteins, including receptor tyrosine kinases MET and PTK-7, to lysosomes for degradation, while exerting minimal effects on non-target proteins. Overall, this strategy represents a powerful, efficient, and broadly applicable platform for inducing selective degradation

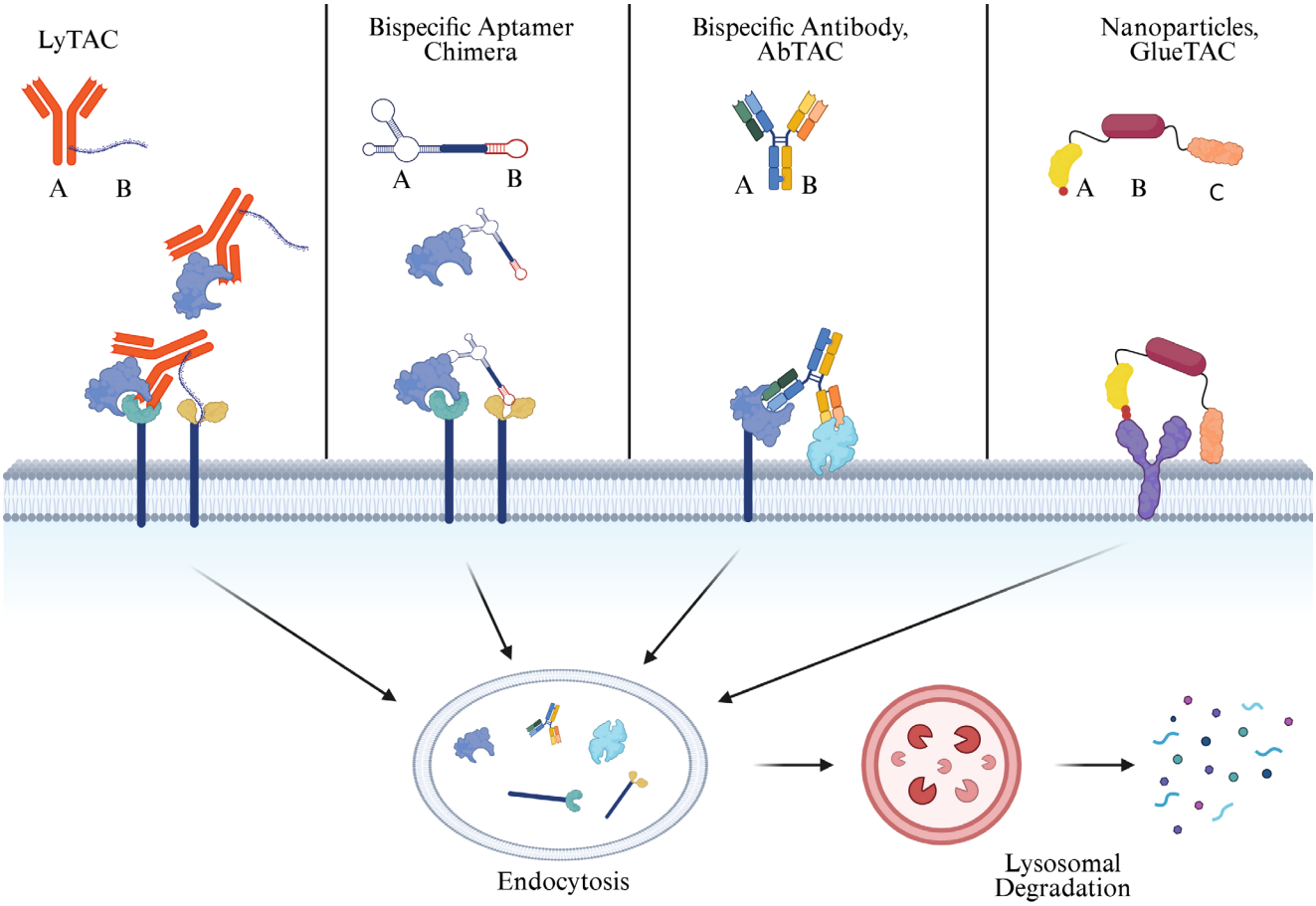

Fig. 2. Lysosomal proteolysis of proteins mediated by LYTACs and their analogs. LYTAC consists of a small molecule or an antibody (A) conjugated with a ligand that binds to the lysosome-targeting receptors (LTR) such as CI-MPR (B). CI-MPRs are internalized by endocytosis together with the LYTAC molecules and the POI. Whereas the POI is degraded in the lysosomes, the ligand is returned to the plasma membrane for reuse. The bispecific aptamer chimera uses a DNA aptamer for targeting the POI (A) to the LTR (B). AbTAC uses a recombinant bispecific antibody to attract the membrane POI (A) and the membrane-bound E3 ligase RNF43 (B). The POI is degraded in the lysosomes (and not by proteasomes). GlueTAC consists of a covalently modified nanoparticle (A), a cell-penetrating peptide (CPP) (B), and a lysosomal sorting sequence (C). The nanoparticle is responsible for binding the POI, while CPP induces endocytosis of the GlueTAC–POI complex, followed by lysosomal degradation.

of membrane proteins. Nucleic acid aptamers offer several advantages over antibodies, such as ease of synthesis, high structural precision, and superior stability [44] (Fig. 2).

Unlike antibody-based approaches, GlueTACs employ nanoparticles conjugated with a cell-penetrating peptide (CPP) and a lysosome sorting sequence (LSS). The nanoparticles enhance the cellular uptake, while the CPP-LSS promotes lysosomal trafficking and subsequent degradation. To overcome a relatively low binding affinity and to reduce off-target effects, the nanoparticles and antigens are linked by covalent bonds. For instance, a designed GlueTAC molecule effectively reduced PD-L1 levels in cells and suppressed tumor growth in immunodeficient mice, outperforming the FDA-approved anti-PD-L1 antibody atezolizumab [45] (Fig. 2).

Noncanonical bispecific AbTACs also exploit the lysosomal degradation pathways. Unlike conventional PROTACs, AbTACs are capable of targeting membrane proteins, thereby substantially expanding the repertoire of substrates amenable to modern targeted proteolysis strategies [46]. Although classified as PROTACs, AbTACs are more closely related to LYTACs. AbTACs use bispecific antibodies, with one arm recognizing a cell-surface POI and the other engaging a transmembrane E3 ubiquitin ligase, such as RNF43 [47]. After AbTAC binding, the resulting ternary complex is internalized and trafficked to lysosomes, where the target protein undergoes degradation. However, the mechanism of action of AbTACs remains less well understood than that of LYTACs. In particular, it is unclear whether ubiquitination of the intracellular domain of the target protein occurs

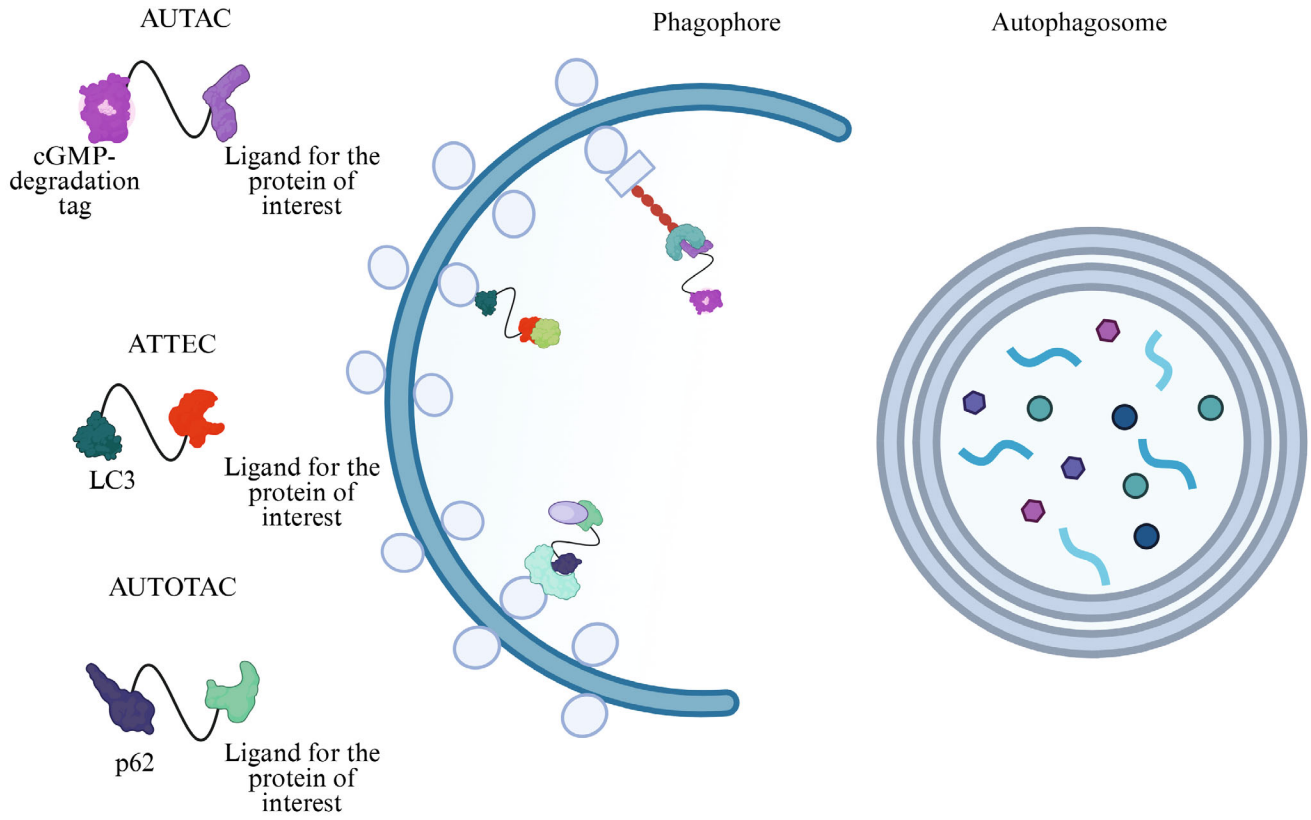

Fig. 3. Targeted protein degradation via autophagy. AUTAC molecule consists of a ligand targeting the POI, a linker, and a cGMP-based degradation tag. The degradation tag recruits autophagosomes for the degradation of cytoplasmic proteins and cell organelles. ATTEC molecule simultaneously binds LC3 and POI, while AUTOTAC molecule binds p62 and POI. The binding induces autophagosome formation, and the subsequent fusion of autophagosomes with lysosomes leads to the POI degradation.

prior to endocytosis and, if so, how this ubiquitination facilitates lysosomal trafficking. Moreover, it remains unknown whether RNF43 is recycled and reused following degradation, as observed for LYTAC receptors and other endocytic receptors (Fig. 2).

Protein degradation via autophagy. A molecule of autophagy-targeting chimera (AUTAC) (Fig. 3) consists of three elements: a cGMP-based degradation tag, a linker, and a small ligand molecule that binds either a target protein or a specific organelle. AUTACs induce K63 polyubiquitination, thereby triggering selective autophagy and subsequent lysosomal degradation. This mechanism contrasts with that of PROTACs, which promote K48 polyubiquitination and proteasomal degradation. Notably, AUTACs can target not only cytosolic proteins but also entire cellular organelles, such as mitochondria. For example, AUTAC4 contains a ligand that binds a transporter located on the outer mitochondrial membrane, enabling selective mitophagy. Treatment with AUTAC4 has been shown to restore mitochondrial membrane potential and ATP production [48].

Similar to AUTACs, AuTophagy-TEthering Compounds (ATTECs) (Fig. 3) promote selective protein degradation by using the autophagosomal machinery. Whereas AUTACs recruit autophagosomes indirectly, ATTECs directly bind microtubule-associated protein light chain 3 (LC3), a key component of autophagosomes. For example, small molecules have been developed that simultaneously bind LC3 and pathogenic mutant huntingtin protein (mHTT) containing expanded polyglutamine tract that causes the Huntington's disease. Notably, these ATTECs selectively recognize mHTT while sparing the wild-type protein, despite the two differing only in the polyglutamine tract length. This high specificity opens up new opportunities for treating the Huntington's disease [49].

AUTOTAC molecules (Fig. 3) consist of two functional modules: one that binds the ZZ domain of the autophagy receptor p62 and another that recognizes the POI [50]. By linking the target protein to p62 independently of ubiquitination, AUTOTACs induce p62 oligomerization and activation, thereby promoting degradation via the autophagy. AUTOTACs enable the targeted degradation of both monomeric and aggregation-prone proteins. In mouse models overexpressing pathological human tau, AUTOTAC treatment effectively cleared misfolded tau protein. In contrast,

proteasome-based strategies such as PROTACs and molecular glues, are generally ineffective against protein aggregates. Beyond tau, AUTOTACs effectively removed various oncoproteins, including the androgen receptor (AR) [50].

Chaperon-mediated autophagy. In CMA, heat shock protein 70 (HSC70) recognizes soluble proteins containing the pentapeptide KFERQ. This motif functions as a degradation signal, analogous to ubiquitination, and may become exposed on the protein surface as a result of unfolding, post-translational modifications (e.g., acetylation or phosphorylation), or other structural perturbations. The HSC70–substrate complex binds to the lysosome-associated membrane protein 2A (LAMP2A) on the lysosomal membrane, enabling translocation of the protein substrate into the lysosomal lumen for degradation. Based on this mechanism, chimeric peptides incorporating the KFERQ motif together with the sequence that binds a target protein can be used to induce selective degradation of pathogenic or misfolded proteins. Chimeric molecules targeting proteins for CMA consist of three functional domains: a cell-penetrating sequence, a target protein-binding sequence, and a CMA-targeting motif. Upon cellular uptake, these molecules bind the target protein and direct it to lysosomes for degradation [51]. However, compared with PROTACs and LYTACs, CMA-based chimeras exhibit limited stability and inefficient intracellular delivery. Consequently, this strategy has not yet led to effective therapeutic applications.

TARGETED PROTEOLYSIS IN BACTERIA

Protein degradation in bacteria is carried out by proteases such as Clp and Lon, as well as by bacterial 20S proteasome, all of which contain AAA+ ATPase domains. As a general principle, proteolytic complexes consist of an ATPase that unfolds polypeptide substrates and a protease that catalyzes hydrolysis of peptide bonds. In addition, bacteria possess numerous other proteases with specialized functions and diverse intracellular or extracellular localizations [52]. The gene encoding the caseinolytic protease ClpP is present in most bacterial genomes, with the exception of mycoplasmas [53]. ClpP has also been found in eukaryotes, primarily in chloroplasts and mitochondria. It is an ATP-dependent serine protease that associates with AAA+ chaperones. Structurally, ClpP assembles into a tetradecameric, barrel-shaped complex composed of two stacked heptameric rings. In some bacteria harboring two paralogous *clpP1* and *clpP2* genes (e.g., Mycobacteriaceae, Listeriaceae, and Pseudomonaceae), ClpP1 and ClpP2 form separate homoheptameric rings that stack on top of each other to generate a functional protease complex. The active sites of the 14 subunits face inward toward the central proteolytic chamber. Because of the small diameter of the entrance pore, ClpP alone can degrade only unstructured proteins and short peptides. Efficient degradation of folded proteins requires cooperation with AAA+ chaperones, which recognize substrates and actively unfold them prior to the translocation into the proteolytic chamber. In general, the subunits of processive proteases exhibit low substrate specificity; therefore, substrate selection is mediated by short recognition sequences known as degrons. Degrons are recognized by AAA+ subunits either directly or via adaptor proteins that facilitate substrate delivery to the proteolytic subunits. Both C-terminal and N-terminal degrons exist in bacteria. Incompletely synthesized proteins stalled on ribosomes are typically tagged with C-terminal degrons, such as the ssrA peptide, and are subsequently degraded by the ClpXP protease complex [54]. Most often, bacterial N-degrons are generated through endoproteolytic processing or by the attachment of primary destabilizing residues to specific N-terminal amino acids through the action of amino acid transferases [55].

There are very few known examples of targeted proteolysis in bacteria, causing a substantial methodological gap between bacterial and eukaryotic systems. This disparity largely arises from the absence of the ubiquitin–proteasome system in bacteria, which serves as the central mechanism for targeted protein degradation in human cells. Nevertheless, the diversity of proteases and degradation pathways discussed in this review highlights the potential for developing strategies to target specific bacterial proteins toward controlled degradation.

Bacterial PROTACs (BacPROTACs). The first example of a small molecule causing specific protein degradation in bacteria through protein destabilization was pyrazinamide. This compound inactivates aspartate 1-decarboxylase PanD (pantothenate biosynthesis gene D), an enzyme essential for coenzyme A (CoA) biosynthesis in *Mycobacterium tuberculosis*. Pyrazinamide was originally classified as a conventional enzyme inhibitor; however, recent studies have demonstrated that it promotes PanD degradation by ClpC1P [56]. Pyrazinamide exposes the C-terminal degron of PanD and alters the multimeric assembly of the PanD complex, thereby triggering its proteolysis. Pyrazinamide represents the first antimicrobial agent shown to exploit targeted protein degradation as its primary mechanism of action.

In addition, so-called Homo-BacPROTACs (homobifunctional BacPROTACs) have been developed. These molecules consist of two cyclic heptapeptides derived from cyclomarins (biologically active marine cyclopeptides), that bind to the mycobacterial unfoldase ClpC1.

By dimerizing ClpC1, Homo-BacPROTACs redirect the Clp protease machinery to degrade this regulatory subunit, effectively removing the cell's degradation machinery and leading to bacterial death. Homo-BacPROTACs have been shown to induce degradation of the ClpC1 N-terminal domain *in vitro*, as well as endogenous full-length ClpC1 in *Mycobacterium smegmatis* cells. Notably, compared with monomeric analogues, Homo-BacPROTACs display enhanced antibacterial activity against wild-type *M. tuberculosis*, intracellular bacteria residing in macrophages, and drug-resistant strains [57].

The application of the PROTAC technology to bacteria could, in principle, enable the development of more potent antibiotics with novel mechanisms of action. However, bacteria lack the E3 ligase–proteasome system, which prevents the direct application of this strategy for antibiotic discovery [5].

The first antibiotic functioning through the targeted proteolysis was discovered serendipitously. Pyrazinamide, a cornerstone of first-line tuberculosis therapy for more than six decades, is a prodrug that is converted by bacterial pyrazinamidase into its active form, pyrazinoic acid (POA) [58]. Remarkably, the mechanism of action of POA had remained elusive for many years. The studies of pyrazinamide- and POA-resistant *M. tuberculosis* strains *in vitro* and in a specialized mouse model have identified mutations in PanD [59, 60]. These findings demonstrated that POA suppresses CoA biosynthesis by binding to PanD [59, 61], but does not directly inhibit its enzymatic activity. Further investigations revealed mutations in ClpC, a component of the Clp complex [62]. The studies in reporter strain showed that PanD contains a C-terminal degradation tag and represents a substrate of the ClpCPprotease complex, indicating that PanD levels are regulated post-translationally by Clp complex. The binding of POA to PanD induces conformational changes that promote recognition of the C-terminal degradation tag by the ClpC1 complex, leading to the targeted proteolysis of PanD [56].

Later, BacPROTAC-1 and BacPROTAC-3 were developed. BacPROTAC-1 consists of a biotin moiety linked to a polyarginine motif, which functions as a ligand for monomeric streptavidin selected as the model POI. BacPROTAC-1 successfully mediated the biochemical degradation of monomeric streptavidin by a purified ClpCP protease complex derived from *B. subtilis* [63, 64]. In 2022, the first fully functional BacPROTAC platform was reported, demonstrating highly specific retargeting of the ClpCP protease toward new substrates. ClpCP is found in Gram-positive bacteria and mycobacteria, where it recognizes phosphorylated arginine residues as degradation signals. Engineered BacPROTAC molecules consist of three components: a ligand for the target protein, a chemical linker, and an anchor that binds the N-terminal domain of ClpC (ClpCNTD) (Fig. 4). Originally, this anchor was a phosphorylated peptide derivative that mimicked the native bacterial degradation tag. This BacPROTAC efficiently induced the degradation of four distinct engineered proteins, with the highest activity observed for the substrate with the least structured C-terminal region. The experiments were conducted in *B. subtilis* and *M. smegmatis*. Detailed structural analysis of the ClpCP complex revealed that BacPROTAC binding triggered a reorganization of ClpCP from its inactive decameric state into the active assembly of four hexameric rings. This finding suggests that BacPROTACs not only recruit substrates to the protease but also promote conformational activation of ClpCP, thereby facilitating proteolysis [65].

Structural analysis of the ClpCP complex from *B. subtilis* demonstrated that the BacPROTAC-1 binding induces a conformational transition of ClpC from its inactive decameric state to an active higher-order oligomer capable of forming a functional complex with ClpP. This observation suggests that BacPROTAC molecules directly promote substrate engagement by triggering ClpCP activation. BacPROTAC-1 induced the degradation of monomeric streptavidin (POI) by the purified ClpCP complex from *M. smegmatis*, indicating that the BacPROTAC strategy may be applicable to other bacterial species as well [65]. Despite these promising results, the polyarginine motif of BacPROTAC-1 limits its cellular permeability, and the molecule itself is chemically unstable. Moreover, validation of BacPROTAC activity in intact cells using the biotin–streptavidin system is complicated by competition of intracellular biotin for binding with monomeric streptavidin. To overcome these limitations, the authors redesigned both the protease-recruiting element and the POI-targeting ligand, leading to the development of BacPROTAC-3 (BacPROTAC-2 displayed activity similar to BacPROTAC-1 and did not represent a substantial improvement). BacPROTAC-3 consists of the JQ1 ligand, which binds the protein of interest BRDTBD1 (bromodomain-1 of the bromodomain testis-specific protein) and a protease-recruiting motif derived from the antibiotic cyclomarin A (CymA), known to bind ClpC in mycobacteria [66, 67].

Interestingly, BacPROTAC-3 induces the ClpCP-dependent degradation of POI both *in vitro* and in *M. smegmatis* cells expressing the target protein. Proteomic analysis confirmed that treatment with BacPROTAC-3 resulted in the selective degradation of the target protein [65]. These findings indicate that CymA-based BacPROTACs can mediate highly selective protein degradation in intact mycobacterial cells. Although current BacPROTACs have been designed primarily to engage ClpC, future BacPROTACs may be developed to recruit alternative bacterial proteases,

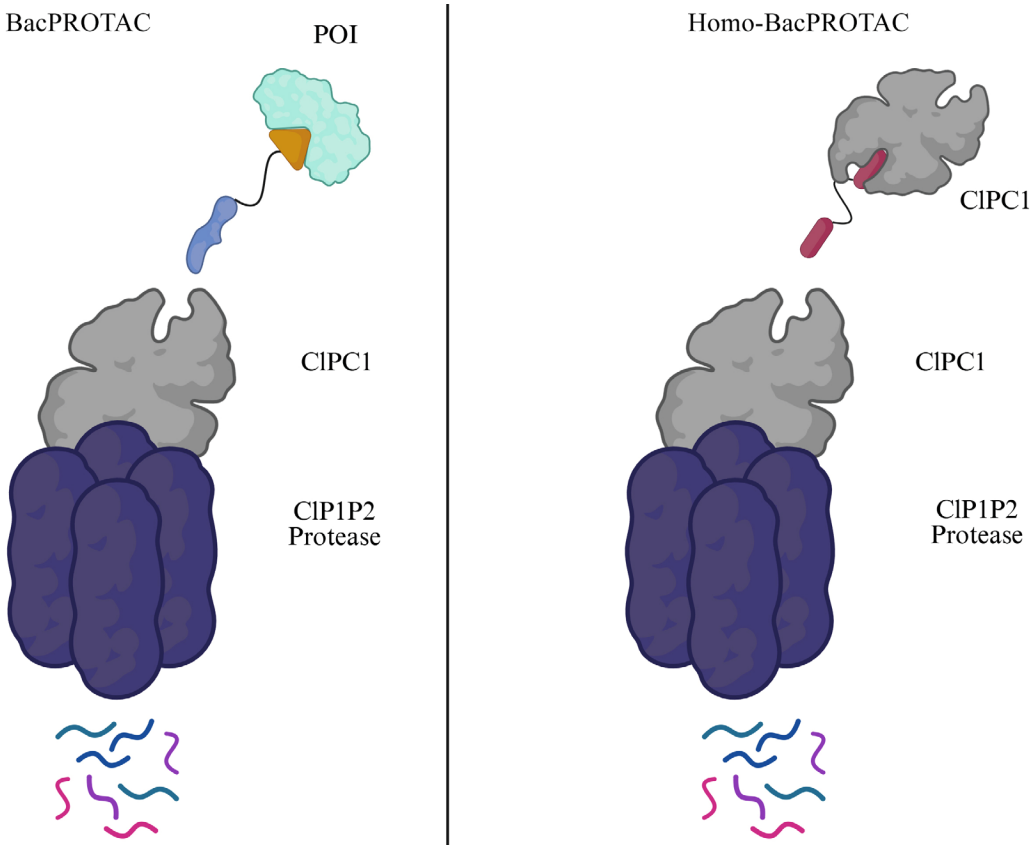

Fig. 4. The mechanism of action of BacPROTACs and Homo-BacPROTACs. BacPROTACs bind the POI, while the polyarginine region is recognized by ClPC1, targeting the POI to the protease complex. Homo-BacPROTACs induce the degradation of ClPC1, which in this case is the POI.

such as the serine protease Lon or metalloprotease FtsH.

The previously synthesized polypeptide deoxycyclomarin-C (dCym) was utilized in the next generation of Homo-BacPROTAC molecules. CymA is a potent inhibitor of ClpC1 [66], and its analog dCym significantly disrupts the mycobacterial proteome, inducing a ~600-fold increase in the ClpC2 levels [68]. ClpC2, which shares structural similarity with the receptor domain of ClpC1, can bind dCym and thereby compete with ClpC1 for ligand binding. This competition reduces the cytotoxicity of dCym approximately fourfold. In *M. smegmatis*, ClpC2, like the recently described ClpC3, functions as a regulatory component of the Clp protease, possessing the same ligand-binding site as ClpC1. Through competition for substrate binding, these paralogs act to limit excessive proteolysis and thereby protect the cell [68, 69].

Cyclic peptide dimers designed and synthesized to disrupt the “protective” effect against dCym- and CymA-mediated inhibition through competitive binding to ClpC2/ClpC3 were named **Homo-BacPROTACs**. They feature the dCym motifs at both ends, connected via linkers of varying lengths in order to simultaneously target ClpC1, ClpC2, and the ClpC1P1P2 complex. Using *M. smegmatis* as a model system, Homo-BacPROTACs were shown to reduce ClpC1 and ClpC2 levels to approximately 40% and 45-60%, respectively, compared to monomeric dCym after 24 h of treatment. The antimicrobial activity was assessed in *M. tuberculosis* H37Rv, where the minimum inhibitory concentrations (MICs) of HBP-6 and HBP-7 were 0.34 μM and 0.26 μM, respectively, compared to 39 μM for dCym. Both compounds effectively reduced ClpC1 and ClpC2 levels and demonstrated activity against dormant *M. tuberculosis* cells [68] (Fig. 4).

The accidental discovery that pyrazinamide, a first-line antituberculosis drug, exerts its effect through targeted proteolysis has highlighted the potential of this approach for antibacterial drug discovery. However, this finding alone has not enabled the development of a rational, broadly applicable strategy to direct any POI to degradation. Achieving this requires bifunctional molecules capable of simultaneously engaging the target protein and the cellular degradation machinery. Since bacteria lack the E3 ligase–proteasome system, the conventional PROTAC approach cannot be directly applied to bacterial cells. Recent studies have addressed this challenge by inducing proximity between the POI and bacterial

proteasome using bifunctional molecules known as BacPROTACs. This strategy, guided by structural and biochemical insights, has successfully demonstrated the possibility of targeted protein degradation in bacterial systems.

CONCLUSIONS

Molecules designed for targeted proteolysis represent a unique class of compounds with a pseudocatalytic mechanism of action. Although they are not catalysts in the classical sense, their ability to trigger multiple cycles of target protein degradation (through the recycling of E3 ligases or lysosomal receptors) makes them exceptionally efficient tools for precise modulation of the proteome.

A defining feature of these molecules is their dual functionality: one region binds specifically to the POI, while the other links it to the cellular machinery responsible for degradation, such as the proteasome, lysosome, chaperones, or bacterial enzymatic complexes. Once bound, the molecule effectively tags the target protein for degradation and facilitates its delivery to the appropriate proteolytic system. One of the most important advantages of this approach is the "recycling" capability of these compounds. Most molecules remain active throughout multiple biochemical cycles, returning to their original state after each degradation event. This property allows drugs based on targeted proteolysis to maintain efficacy at low doses and sustain long-lasting therapeutic effects.

Early generations of PROTACs faced challenges such as low membrane permeability and limited bioavailability. Recent advances, particularly in small molecule-based PROTACs, have improved the pharmacokinetics and enabled activity against both membrane-bound and intracellular targets. The emergence of heterobifunctional platforms – such as LYTACs, AUTACs, and ATTECs – has broadened the spectrum of degradable targets to include extracellular proteins, protein aggregates, and even organelles. In bacterial systems, BacPROTACs have adapted the principles of targeted proteolysis to prokaryotic degradation mechanisms, for example, by recruiting ClpXP proteases, thus paving the way for next-generation antibiotics.

In oncology, PROTACs have already shown efficacy against traditionally "undruggable" targets, including BRD4 and ERα, while LYTACs address the challenge of degrading ligand–receptor complexes, such as PD-L1.

Systems targeting tau protein and α-synuclein (e.g., ATTECs) hold promise for the management of neurodegenerative diseases.

Despite their high specificity, these technologies can still exhibit off-target effects, e.g., unintended activation of the immune response by LYTACs, highlighting the need for further optimization. Overcoming the blood–brain barrier for neurodegenerative applications and achieving tissue-specific targeting remain critical for clinical translation. Additionally, cases of resistance to PROTACs have been reported, often due to mutations in E3 ligases or in target proteins themselves.

Future directions in this field include integrating targeted proteolysis with complementary approaches, such as CRISPR-based target validation or nanocarrier-mediated delivery, and developing multispecific degraders capable of simultaneously modulating multiple oncogenes. Even now, targeted protein degradation represents a transformative paradigm in therapeutic development, seamlessly combining chemistry, structural biology, and cell biology.

Abbreviations

AbPROTAC	antigen-based proteolysis targeting chimera
ATTEC	autophagy-tethering compound
AUTAC	autophagy-targeting chimera
BacPROTAC	bacterial proteolysis targeting chimera
CPP	cell-penetrating peptide
LYTAC	lysosome-targeting chimera
PROTAC	proteolysis targeting chimera

Contributions

I.V.S. conducted the literature search, analyzed the materials, and wrote the text of the article; A.M.R., A.P.S., and E.G.K. developed the study concept and edited the manuscript.

Funding

This study was supported by the Ministry of Science and Higher Education of the Russian Federation.

Ethics approval and consent to participate

This work does not contain any studies involving human or animal subjects.

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

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