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Review Article

**PROTEOLYSIS TARGETING CHIMERAS (PROTACS):
MECHANISTIC BASIS, COMPUTATIONAL AND
ARTIFICIAL-INTELLIGENCE-DRIVEN DESIGN,
PRECISION ENGINEERING, AND CLINICAL
TRANSLATION****K. Priyaranjan¹, Chetan Kasyap Vepachedu² ***¹Department of Pharmaceutics, Gokaraju Rangaraju College of Pharmacy, Hyderabad, India² Department of Pharmaceutics, Gokaraju Rangaraju College of Pharmacy, Hyderabad, India**Abstract:**

Targeted protein degradation (TPD) broadens the druggable proteome beyond classical occupancy-based inhibitors. Proteolysis targeting chimeras (PROTACs) are heterodimeric small-molecules that link the protein of interest (POI) and an E3 ubiquitin ligase into a ternary complex which repurposes the ubiquitin-proteasome system to degrade the target. Since a single PROTAC molecule can trigger multiple rounds of degradation, this event-driven pharmacology operates under sub-stoichiometric dosing and is capable of disabling scaffolding activity that is out of reach for inhibition. This review discusses five related themes. First, we introduce PROTAC structure and mechanism, focusing on ternary-complex cooperativity and the hook effect. Second, we present the use of computational and AI-assisted tools, such as docking (Rosetta, PROsettaC), deep learning structure prediction (AlphaFold2/3, RoseTTAFold-All-Atom), and machine learning linkers design and degradation prediction. Third, we discuss light-, radiation-, ultrasonic-, and bioorthogonal click-release responsive platforms enabling spatiotemporally controlled PROTAC delivery. Fourth, we highlight the approaches towards brain-penetrant PROTACs addressing neuro-oncology and neurodegenerative diseases. Finally, we present the clinical development status of late-stage androgen- and estrogen-receptor degraders as well as lysosomal modalities allowing TPD to go beyond intracellular targets.

Keywords: PROTACs; Targeted protein degradation; Ubiquitin-proteasome system; Computational drug design; Artificial intelligence; E3 ubiquitin ligase

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1. INTRODUCTION:

The field of small-molecule drug discovery has been characterized by a pharmacologic model of occupancy in which a ligand requires sufficient binding affinity to occupy an active site or pocket in order to interfere with protein catalysis and binding for as long as the drug binds to its receptor over three decades. This model has yielded considerable benefits for human health; however, it inherently precludes 80% of the human proteome transcription factors, scaffolding proteins, and targets acting via protein-protein interaction mechanisms and not enzyme catalysis due to lack of accessible pockets. Occupancy pharmacology also relies on constant target occupation, requiring high dosing regimens, and is prone to developing resistance mechanisms through overexpression and mutation of the target-binding sites.

Targeted protein degradation (TPD) is the opposite approach to this problem. Degradation does not bind the target but utilizes the protein quality control system of the cell to destroy the target protein. For this, the endogenous system called the ubiquitin-proteasome system (UPS) can be used: an E1 activator enzyme modifies the ubiquitin, an E2-conjugating enzyme transports it to the substrate, and any of hundreds of E3 ubiquitin ligases ensures substrate specificity by binding the E2-ubiquitin complex and the substrate protein at once and catalyzing the ubiquitination of the target protein's lysine residues. Iterations of this reaction result in the formation of a K48-linked polyubiquitin chain which is then recognized by the 26S proteasome, unfolding the protein and degrading it to short peptides. Heterobifunctional small molecules called proteolysis targeting chimeras (PROTACs) were created to utilize this mechanism: the first moiety ("warhead") of the PROTAC binds the protein of interest (POI), the second binds E3 ligase, while the linker connects them, forming an induced proximity complex which does not have to occupy a functional site on the POI at all but just binds it long enough to perform ubiquitination.

The event-driven nature of PROTACs presents a number of pharmacological benefits compared to classical inhibition. For one, since the PROTAC is not used up in the reaction, it can undergo multiple rounds of catalysis, where the ternary complex, ubiquitination, and target degradation can take place; hence, it would be possible for a drug to have a pharmacodynamic effect without being stoichiometrically bound to the target site an aspect known as "catalytic pharmacology". Target degradation allows the elimination of scaffolds and non-catalytic functions that would not be addressed by inhibitors, and since degradation targets the whole protein and not just a particular binding site,

there would be no possibility for resistance mutations that disrupt binding but preserve stability.

The conceptual basis of chemically induced protein degradation is provided by a patent in 1999 on regulation of protein levels inside the cells [2]; the first proof-of-principle molecule was reported in 2001 as the chimera of a MetAP-2-ligand derived from ovalicin and an I κ B α -phosphopeptide recognized by SCF- β -TrCP E3 ligase complex [6]. Further transition of peptidic E3 recruiters into small molecules targeting cereblon (CRBN) and von Hippel-Lindau (VHL) protein enabled the transition of the PROTACs from laboratory tools incompatible with oral administration into drug candidates, and in the middle of 2010s CRBN-degraders like dBET1 were already demonstrated to be able to efficiently degrade bromodomain proteins in the cellular context [7]. Androgen-receptor degrader ARV-110 was the first PROTAC entered human clinical trials in 2019 [8]; estrogen-receptor degrader ARV-471 has since been progressed to registrational Phase III study in breast cancer [2,5] which has spurred an interest in this modality in both industry and academia [1,10].

However, in order to exploit the potential of PROTACs, overcoming some unique design difficulties that have no precedence in traditional medicinal chemistry must be overcome: PROTACs are usually big and flexible structures that do not obey Lipinski's rules of five and belong to the "beyond rule of five" (bRo5) chemical space; their bioavailability, membrane permeability, and pharmacokinetics are much more unpredictable and difficult to optimize compared to those of the regular inhibitors. Degradation efficacy not only relies on affinity of each binary interaction but on the cooperativity of the ternary complex as well, an aspect which cannot be predicted based on binary affinity data alone and is highly dependent on the length, composition, and linkage geometry of the PROTACs. In addition, at high PROTAC concentration, the efficiency of protein degradation may decrease due to the saturation of the POI and E3 ligase by the PROTACs in the form of binary complex separately; this phenomenon is called the "hook effect." Such design difficulties have rapidly resulted in the convergence of structural biology, computational chemistry, and artificial intelligence (AI) in designing PROTACs in combination with efforts towards development of tissue-selective, stimulus-responsive, and CNS-penetrating degraders.

This review highlights the current trends in the literature on this rapidly developing convergence [1–5]. First, we provide an overview of the structural and functional principles of degradation by PROTACs. Next, we consider the growing arsenal

of computational and AI approaches to the design of PROTACs, stimuli-responsive PROTACs, brain-penetrating degrader design strategies, and degradation pathways involving the lysosome as a mechanism for expanding TPD to targets outside the cell.

2. METHODOLOGY:

The current review was prepared in accordance with a narrative-synthesis methodology as opposed to a systematic review protocol in view of the goal of synthesizing the conceptual, mechanistic, and translational advances of a rapidly evolving research field as opposed to a quantitative synthesis of primary results. The evidence base includes, as its principal sources, five recent full-text review articles dealing with in silico and computational design of PROTAC molecules [1], PROTAC drug discovery and its practice in the clinical setting [2], brain-penetrant PROTAC design for delivering PROTAC molecules into the central nervous system [3], physical and chemical triggers for precision-engineering PROTACs in cancer treatment [4], and enhanced with artificial intelligence PROTACs drug discovery [5]. Additional primary sources have been included in view of direct reference to the relevant data found in the five source review articles.

The information has been arranged based on themes, rather than chronology, within six broad categories, namely: (i) PROTAC structure, function, and SAR;

(ii) E3 ligase biology and degrader modalities; (iii) computational and artificial intelligence-based design techniques; (iv) precise activation and responsive activation approaches; (v) PROTAC delivery to the central nervous system; and (vi) clinical translation and applications of PROTACs. In each category, information obtained from the source reviews has been checked for convergence, and only those aspects which showed convergent evidence have been discussed here. All the figures used in this review paper have been created by the author specifically for this review paper and are not copies of the figures from the source reviews.

3. RESULTS AND DISCUSSION:

3.1. PROTAC Structure and Mechanism of Action

All PROTACs have a three-component modular structure: the ligand for binding with POI (warhead, usually a modified version of known inhibitor or fragment), the ligand for recruiting the E3-ligase (anchor), and the linker which connects the two through covalent bonds (Figure 2A). Since warhead is required to be able to bind with POI with enough affinity and duration to allow ubiquitination process to happen but not necessarily to bind within the active site of POI, it is theoretically possible to use ligands which cannot inhibit the target protein on their own.

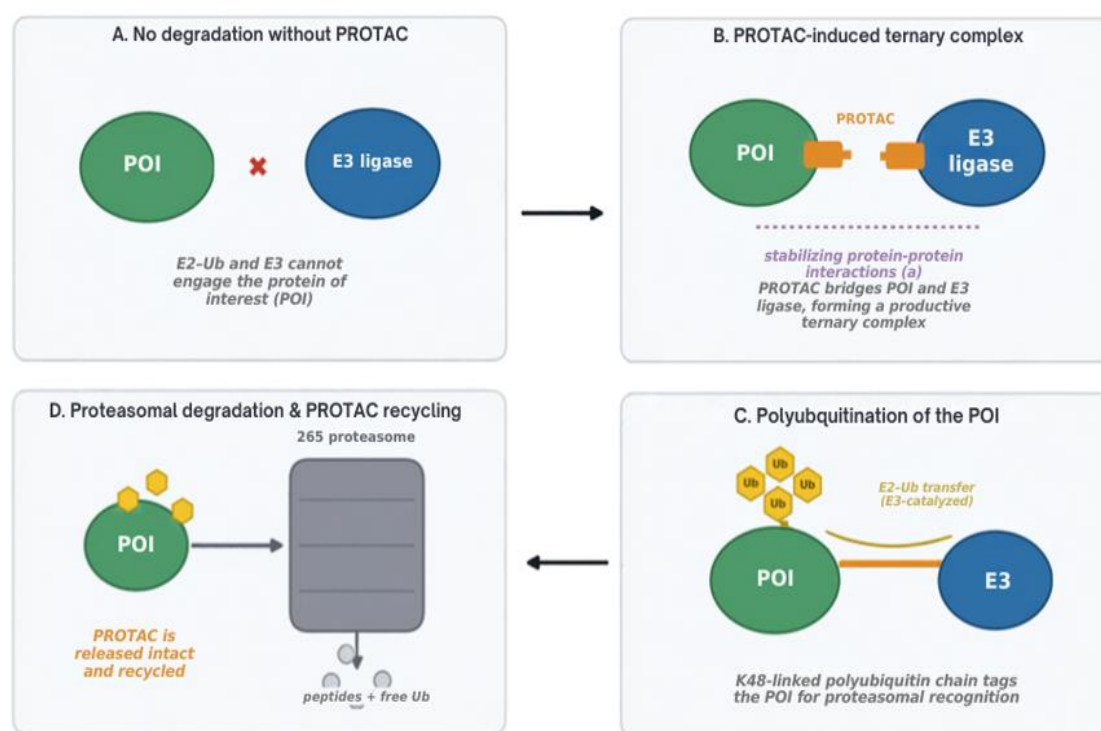


Figure 1. PROTACs bridge a POI and an E3 ligase to form a ternary complex, driving K48-linked polyubiquitination and proteasomal degradation of the POI, after which the PROTAC is recycled for further rounds.

Degradation occurs via four stages conceptually (Fig. 1). First, in the absence of the PROTAC, there is no reason for the POI and E3 ligase to come together. The PROTAC binds to both the proteins together, causing a formation of a ternary complex. Unlike addition of two individual protein-ligand interactions, this step is different from a thermodynamic perspective due to the possibility of the formation of a new protein-protein interaction between the POI and the E3 ligase which cannot be formed in isolation in a binary complex (Fig. 2C). This is quantified using a cooperativity factor α [9,10], where the binary dissociation constant is divided by the dissociation constant of the ternary complex. If α exceeds 1, the formation of the ternary complex is favored thermodynamically compared with the binary complex formation (cooperativity); this is usually associated with high efficacy and selectivity of the degrading process; if α is lower than 1, there is a destabilization of the ternary complex due to steric clash/electrostatic repulsion between the POI and the E3 ligase (negative cooperativity), resulting in inefficient degrading; usually, the affinity values for both binary interactions are favorable. The concept of cooperativity, and not only binary affinity, turned out to be crucial for the degrader's efficacy and selectivity. The crystallographic data on the ternary

complex structure mediated by the PROTAC molecule (BRD4-PROTAC-VHL) shed light on the nature of the interaction [9].

Third, after ternary complex formation, the E3 ligase together with the cognate E2 conjugating enzyme mediates multiple rounds of ubiquitin transfer on the surface-exposed lysines of the POI to create a K48-linked polyubiquitin chain that acts as the canonical degradation signal for the proteasome. Fourth, the polyubiquitinated POI is bound by the 26S proteasome, unfolded, translocated inside the proteolytic chamber, and degraded to peptides, whereas the PROTAC itself dissociates and can form a new ternary complex with another POI and E3 ligase. This is the reaction mechanism that enables the catalytic sub-stoichiometric action of degraders versus their occupancy-based counterparts and it provides an explanation for one of the most frequently encountered non-monotonic effects in the PROTAC field: the hook effect, where degradation is increased with PROTAC concentration until a maximum and then decreases above optimal PROTAC concentration due to increasing saturation of the POI and E3 ligase with free PROTAC forming non-productive binary complexes rather than productive ternary complexes [1,2].

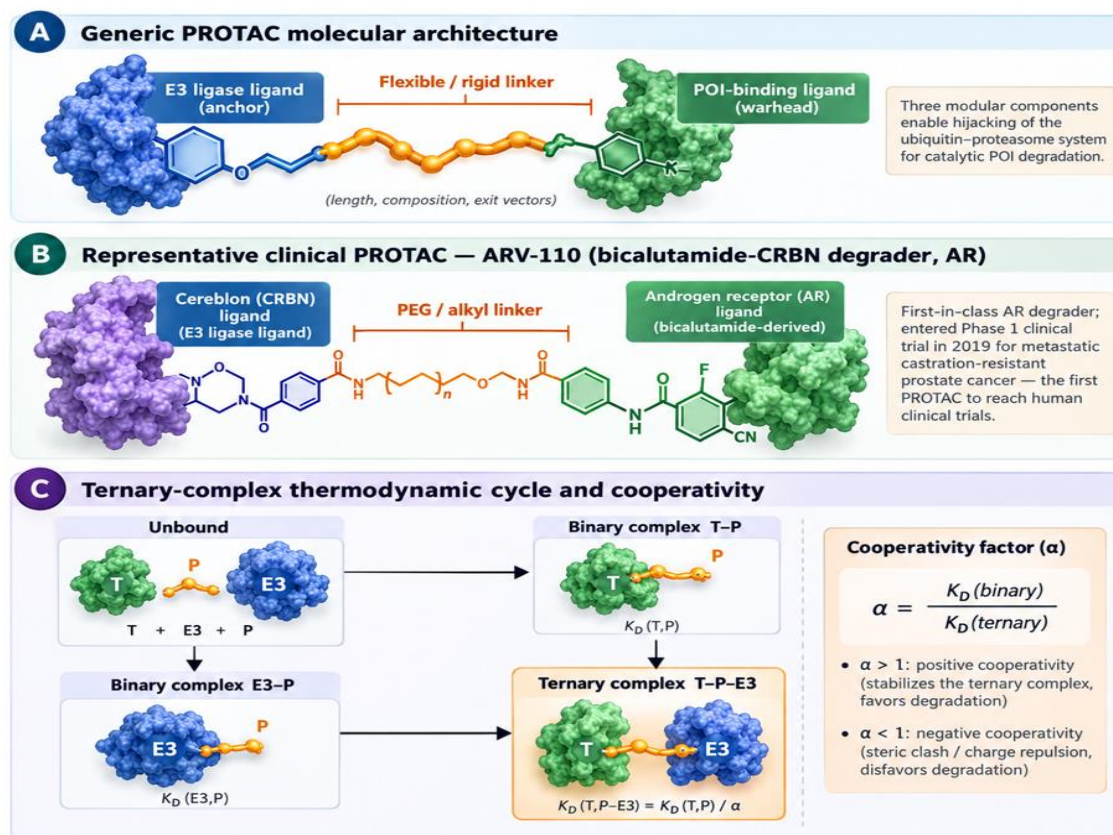


Figure 2. PROTAC scaffold (A), ARV-110 as a clinical example (B), and the thermodynamic cycle defining cooperativity α in ternary-complex formation (C).

3.2. Structure-Activity Relationships: The Linker and Beyond-Rule-of-Five Chemical Space

Given the fact that the linker dictates the precise relative positioning of the warhead and the E3 ligand, the length, stiffness, structure (which is typically made up of polyethylene glycol, alkyl, or piperazine chains), and vectors of attachment to each of the ligands become crucial factors in the successful generation of a cooperative ternary complex. Linkers that are too short might cause a steric clash between the POI and the E3 ligase; linkers that are too long or flexible can result in the reduced effective concentration of the productive closed conformation, thereby decreasing the potency of the system and increasing the entropic penalty of ternary complex formation. This step, which has traditionally involved the synthesis and evaluation of analog matrices, has become a key target for computational and generative-AI acceleration (Section 3.4).

The resulting PROTACs are invariably large molecules compared to the usual criteria of medicinal chemistry, with molecular weights exceeding 700-1000 Da, and hence are way beyond the threshold of Lipinski's rule of five and in so-called beyond-Rule-of-Five (bRo5) chemical space [1,2,3]. This has very significant implications for drug design, since bioavailability, solubility, and membrane permeability for such compounds become much harder to achieve, and much more unpredictable from standard physicochemical parameters, than for conventional small molecule inhibitors. Nonetheless, a few PROTACs, among them ARV-110 and ARV-471, have managed to attain oral bioavailability for clinical development, proving the viability of bRo5 chemical space with the proper structure-activity optimizations [2,3].

3.3. E3 Ligases and Emerging PROTAC Modalities

Despite there being more than several hundreds of E3 ligases coded by the human genome, few have been explored in the context of PROTAC development, which is more due to the availability of small molecules for binding these E3 ligases than a biochemical limitation. The use of cereblon (CRBN), a substrate-receptor subunit in the cullin-RING ligase complex as well as a target for thalidomide-like immunomodulatory compounds, is the most common E3 anchor since the glutarimide-like ligands for it are readily synthesized, characterized, and orally active. Nonetheless,

CRBN-based PROTACs have the limitation of CRBN's expression dependence in different tissues and cells [2,5]. Von Hippel Lindau (VHL) protein recruited through hydroxyproline-mimicking ligands is the second most common anchor and constitutes a structurally different and validated anchor with precedent in ternary complex crystal structures [9]. Ligands for MDM2 (nutlins) and inhibitor-of-apoptosis-proteins (IAP ligands derived from bestatins or SMAC mimetics) are two additional anchoring chemistries that are less explored and each with unique tissue expression and off-target liabilities; MDM2 binding, for instance, may activate the p53 pathway [2,5]. A common constraint that applies to all four types of ligases is that they exhibit heterogeneity or tumor-specific expression in many cases, which necessitates further exploration in order to discover new E3 ligases with better expression patterns [2,5,19].

In addition to the classical heterobifunctional structure, several alternative structures of PROTACs have been developed. Homo-PROTACs are designed to couple two copies of an E3 ligand to allow the degradation of an E3 ligase by another copy of itself, which can be applied to study the biology of E3 ligases or for degrading an oncogenic E3 ligase. Both trivalent and bivalent types of PROTACs are characterized by a coupling of two warheads, POI-binding (or E3 ligand-binding), on one scaffold to increase its cellular potency due to higher avidity, especially when working with POIs with tandem domains, such as BTK [5,19]. The degrader platform based on nucleic acid extends the idea of ternary complex from small molecules to non-proteinaceous moieties: TRAFTAC-type complexes contain a POI-binding oligonucleotide (RNA aptamer) and E3 ligand, thus degrading transcription factors and other nucleic acid-binding proteins lacking the small molecule-binding pockets. AbTAC-type of complexes contain a bispecific antibody scaffold to bring the surface E3 ligase (ubiquitin ligase) and cell-surface protein-of-interest into a proximity [2,5].

A different type of degrader called molecular glue, which is both mechanistically similar but structurally very different from PROTACs and involves the use of monovalent molecules that stabilize the interaction between an E3 ligase and a neosubstrate through a native or near-native protein-protein interface without having the linker-bridged two-part structure as seen in PROTACs, has been gaining a similar level of interest in tandem with PROTACs in the field of targeted protein degradation [20].

Table 1. Major E3 ubiquitin ligases exploited in PROTAC design, their representative recruiting chemotypes, and principal advantages and limitations.

E3 Ligase	Representative ligand chemotype	Key advantages	Key limitations
Cereblon (CRBN)	Thalidomide-/lenalidomide-/pomalidomide-derived glutarimides	Most widely used anchor; synthetically accessible; oral bioavailability precedent (e.g., ARV-110, ARV-471)	Tissue- and cell-type-dependent expression; potential immunomodulatory neosubstrate off-target effects
Von Hippel-Lindau (VHL)	Hydroxyproline-mimetic peptidomimetics	Extensive ternary-complex structural precedent; second most widely validated anchor	Larger, more peptidic ligands; historically more limited oral bioavailability
MDM2	Nutlin-derived cis-imidazolines	Well-characterized binding pocket; concurrent p53-pathway activation in some contexts	p53 activation may confound interpretation; utility largely restricted to p53-wild-type tumors
Inhibitor-of-apoptosis proteins (cIAP1/XIAP)	Bestatin-/SMAC-mimetic-derived	Structurally distinct chemotype broadens accessible target and tissue space	Less extensively validated clinically; possible interaction with apoptotic signaling

3.4. Computational and Artificial-Intelligence-Driven PROTAC Design

The design space of potential PROTAC designs, including warhead selection, E3 ligand selection, linker length, linker chemistry, and attachment vector, is much too large to explore through iterative synthesis and screening alone. In recent years, this has led to a convergence on computational structure-based design, structure prediction through deep learning, and property prediction via machine learning into a unified design workflow (Figure 3).

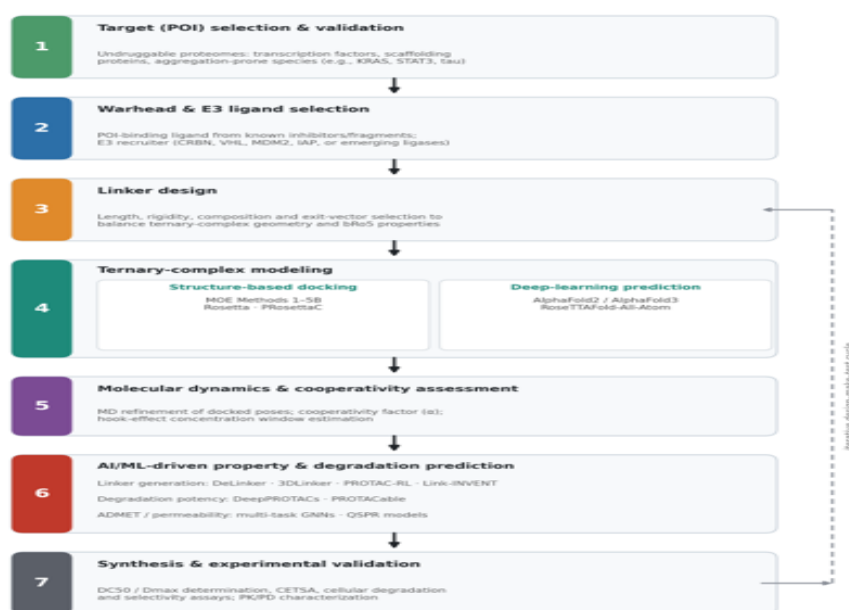


Figure 3- Integrated AI and computational PROTAC design: from ternary-complex modeling to potency/ADMET prediction, closed by iterative design-make-test-analyze cycles.

3.4.1. Structure-based ternary-complex docking

The oldest, as well as the most commonly used computational technique for designing PROTACs, involves structure-based docking of the ternary complex. The most common form of such structure-based docking involves protein-protein and protein-ligand docking techniques provided by platforms such as the Molecular Operating Environment (MOE). The latter provides a collection of algorithms involving rigid body protein-protein docking, linker-restrained conformational sampling and flexible optimization of the complete ternary complex. This technique results in a collection of candidate ternary complex structures, which are further scored and filtered on the basis of geometric and energy constraints. For example, one of such criteria is the possibility of bridging the docked binary structures with a linker that has a realistic length and torsion angle without any steric hindrance.

The alternative set of protocols derived from the Rosetta software, exemplified by the popularly used PROsettaC protocol, integrates protein-protein docking with the explicit sampling of linkers and all-atom refinement to build ternary-complex models, and has achieved reasonably good performance with the known crystallography and cryo-electron microscopy ternary-complex structures [11]. Some other Rosetta-derived scoring functions have also been created especially to rank and score the predicted ternary-complex structures in terms of their stability, serving as a computational approach for the cooperativity factor α even prior to the synthesis of the compounds [12]. The structure-based approaches are most reliable in case a crystal or cryo-EM structure of at least one binary complex is known.

3.4.2. Deep-learning structure prediction

Structure-based PROTAC design is now far more widely applicable thanks to the emergence of protein structure prediction through deep learning approaches. The most popular tool for such an approach is AlphaFold2 [13] with its ability to accurately predict the protein structure solely based on its sequence. This algorithm was further improved with the introduction of AlphaFold3 [14]. Moreover, there is a very similar algorithm called RoseTTAFold-All-Atom [15], which can be used to directly predict ternary assemblies of proteins-proteins, as well as proteins-small molecules. Thus, this method may also be used to predict the structure of ternary complexes of the POI-PROTAC-E3 system. Although the predictions using this approach are less reliable when predicting protein-

protein-small molecule assemblies, the experiments still recommend doing them, if possible.

3.4.3. Machine learning for linker generation and degradation/ADMET prediction

The third and novel aspect of the computational frontier lies in the direct application of generative and predictive machine learning to the process of linker design and molecular property prediction, instead of merely predicting the structure of the ternary complex. The fragment-linking generative models include DeLinker and its three-dimensional version, 3DLinker, which learn to generate new linker designs based on the three-dimensional coordinates and exit vectors of two anchor fragments, thus making the process of linker enumeration more automated based on medicinal chemistry intuitions [16]. The reinforcement-learning models like PROTAC-RL and generative models (also including the Link-INVENT-like architecture), on the other hand, optimize the generated linkers based on multi-objective reward functions [1,5].

Regarding property prediction, graph neural network approaches like DeepPROTACs leverage physicochemical features of the warhead, linker, and E3 ligand to directly predict degradation potency (such as DC50, the concentration that produces half-maximal degradation) from the molecular structure without having to model the ternary complex structure in the early stages of triage [17]. Multi-task graph neural networks and QSPR approaches have been used to predict several other PROTAC-specific ADMET properties, including cellular permeability and, for PROTACs designed for brain-directed degradation, blood-brain barrier penetration (further explored in Section 3.6) [3,5]. Curated databases of PROTACs that include structures, linker chemistries, and degradation/binding information have simultaneously become an essential part of training and testing these models. Together with structure-based docking and deep-learning structure prediction, the above AI methods are combining into the overall iterative design-make-test-analyze loop presented in Figure 3.

3.5. Precision-Engineered, Stimuli-Responsive PROTACs

Given that the action of PROTACs is catalytic and can result in very effective depletion of the target protein, unregulated activity poses the threat of on-target toxicity in healthy tissue that expresses the same POI and E3 ligase. Hence, an increasingly popular field of PROTAC design is the development of controlled release, which ensures that the active PROTAC is released primarily at the site of the

tumor (or disease) but not the whole body (Figure 4) [4].

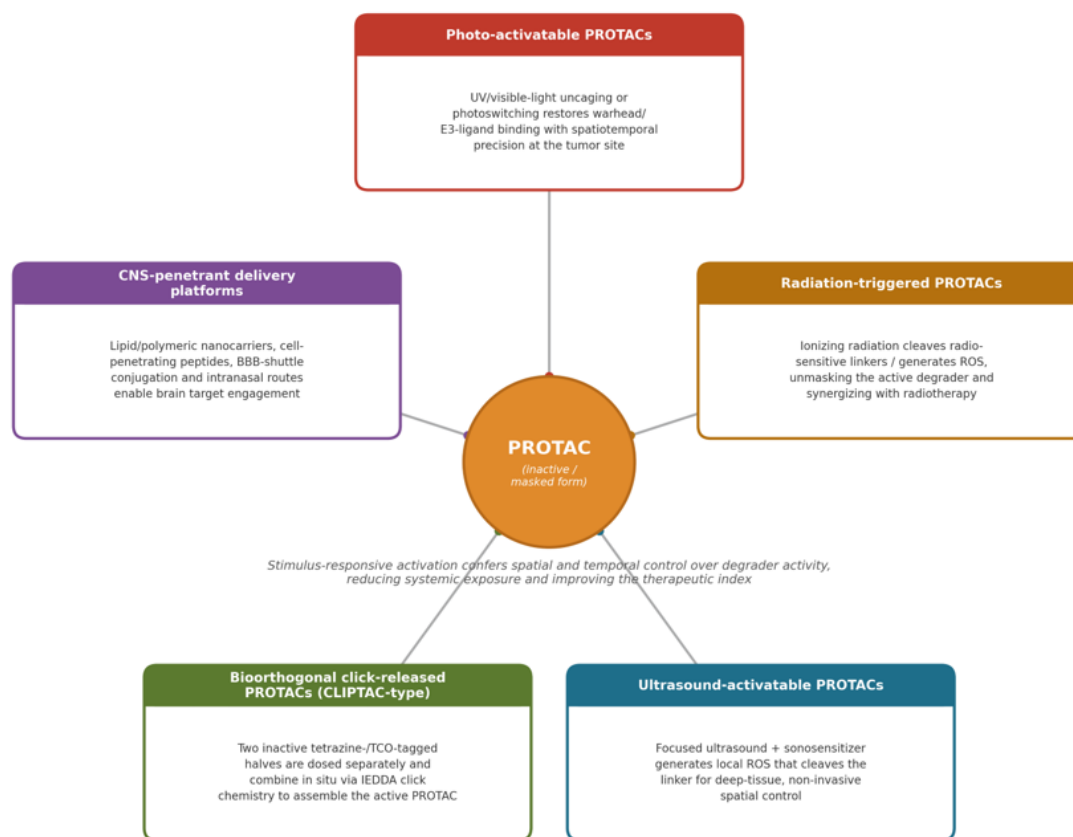


Figure 4. Controlled PROTAC activation (light, radiation, ultrasound, click chemistry) and blood-brain-barrier delivery platforms extend degrader reach.

Photopharmacology methods involve blocking the binding capabilities of the PROTAC's POI ligand or E3 ligand through a photolabile protecting group or incorporating a photoswitch within the linker itself (for example, azobenzene), such that the drug becomes pharmacologically inactive until irradiated with light at the desired site, after which the uncaging/photoisomerization reaction renders the warhead or E3-ligand capable of its binding functions and allows for degradation on a spatially and temporally controlled manner. Ionizing radiation-activated PROTACs follow a similar principle but utilize ionizing radiation as the activating signal, with either radiation-sensitive linker structures or radiation-induced generation of reactive oxygen species (ROS) as the mechanism for activation of the active degrader specifically inside the radiotherapy field, providing the opportunity to incorporate radiotherapy along with targeted protein degradation therapy and obtaining pharmacological synergy within the radiotherapy field [4]. Sonodynamic PROTACs make use of focused ultrasound alongside a sonosensitizer to generate ROS inside the tissue in order to induce cleavage of

a radiation-sensitive linker, thus providing a method of non-invasive and deep-tissue penetrable activation [4].

Another approach that is based on chemistry, like click-release and CLIPTAC technologies, does not require any physical source of energy but utilizes bioorthogonal chemistry: two inert precursor molecules, which contain a bioorthogonal reactive handle (e.g., tetrazine and trans-cyclooctene, which interact via inverse electron-demand Diels-Alder, IEDDA, chemistry), are delivered separately and come together in situ, usually inside a tumor microenvironment or in an intracellular compartment, to form the active, full-length PROTAC when both precursors meet [4]. Regardless of the type of the stimulus, the common design concept here is to dissociate the pharmacological "decision" regarding degradation of the target from the pharmacokinetic properties of the drug in the systemic circulation. These methods are less advanced compared to the first-generation PROTACs and also have a number of issues related to their translation into clinical practice: the

penetration depth of light or ultrasound, the spatial accuracy of the radiotherapy-activated method, and proper pharmacokinetics of two-component click-release systems [4].

3.6. CNS-Penetrant PROTACs and Blood-Brain-Barrier Delivery

For the successful adaptation of PROTAC technology to the field of neuro-oncology and neurodegenerative disease, PROTACs that can traverse the blood brain barrier (BBB) must be developed, an issue made particularly problematic by the bRo5-like physicochemical characteristics of heterodimeric degraders (see section 3.2), in that high molecular weight, high polar surface area, and high counts of hydrogen bond donors/acceptors have each been independently correlated with low BBB permeability, with PROTACs often having each of these disadvantages at once [3].

Medicinal Chemistry Approach The approaches involve reducing the length and polarity of the linker, making the linker more rigid to overcome the penalty of entropy and polar surface area, choosing small E3 recruiting chemotypes, and optimizing the number of hydrogen bonds, which is based on the existing principles of multiparameter optimization of the central nervous system adopted for designing small-molecule drugs for the central nervous system [3]. Some degraders targeting CRBN have shown enough brain penetration to be used in clinical trials, even the BTK degrader designed for CNS lymphomas and other B-cell malignancies of the CNS [3].

On the delivery-platform side, complementary approaches focus on getting these generally less permeable degraders across the BBB, as opposed to being limited solely to their passive permeability characteristics. Such complementary strategies involve the use of nanocarriers made of lipids or polymers designed to allow for their BBB transcytosis, attachment to cell-penetrating peptides or BBB shuttle ligands (like targeting the transferrin receptor for receptor-mediated transcytosis), and non-invasive nasal delivery methods making use of direct delivery pathways through olfactory and trigeminal nerves, thereby bypassing systemic circulation and the BBB altogether [3]. Predictive tools using AI have also been adapted to this particular task, whereby multi-task graph neural networks and other machine-learning models were used to predict BBB permeability from PROTAC structures themselves, allowing for early screening for brain-penetrant PROTACs without the need for chemical synthesis of candidates in a manner similar to degradation potency and general ADMET predictive tools discussed in Section 3.4.3 [3,5]. Overall, medicinal chemistry and delivery platform approaches are increasingly combining to create a

distinct subfield of PROTAC engineering for CNS targets.

3.7. Lysosome-Based and Extracellular Targeted Protein Degradation

While PROTACs target intracellular protein degradations through the ubiquitination-proteasome pathway, an alternative class of degrader technologies has been designed to target extracellular, membrane-bound, or aggregating proteins which may be poor candidates for proteasomal degradation. Lysosome-targeting chimeras (LYTACs) are bispecific conjugates that consist of a POI-specific antibody or a ligand, and a glycan ligand for the lysosome-targeting receptor such as the cation-independent mannose-6-phosphate receptor, facilitating the transport of extracellular or cell surface proteins to the endolysosomal degradation pathway; LYTACs enable targeted protein degradations in secreted proteins and cell-surface receptors that are difficult to access by intracellular degraders such as PROTACs [18].

Autophagy-targeting chimeras (AUTACs) and the autophagosome-tethering compounds utilize, instead, an approach wherein the ligand binding to the POI is joined to another moiety, which is capable of nucleating the creation of autophagosomes around the target, making use of the lysosome-autophagy pathway in order to facilitate the degradation of protein aggregates, damaged organelles, and other substrates not effectively degraded by the proteasome [2]. Another autophagy-based strategy utilizing autophagosome-tethering compounds directly interacting with LC3 (ATTEC molecules) has proven especially promising for degradation of aggregation-prone proteins of relevance to neurological disease that cannot be effectively addressed via conventional proteasome-targeting PROTACs [2,3]. Thus, all together these strategies do not compete with, but rather supplement PROTACs in terms of increasing the number of available target localizations.

3.8. Clinical Development and Therapeutic Applications

The progress made by PROTACs into clinical development has been impressively fast compared with that of most other first-in-class drug platforms (Figure 5). After the original theoretical studies done during the late 1990s and early 2000s and the determination of structural cooperativity in ternary complexes during the mid-2010s, ARV-110 (bavdegalutamide), the first PROTAC to make it into human clinical trials, was developed in 2019 to treat patients with metastatic castration-resistant prostate cancer and showed some clinical efficacy,

including PSA responses in molecularly defined subsets of patients [8]. ARV-471 (vepedegestrant), another estrogen-receptor degrader, is now in Phase

III clinical testing in ER-positive, HER2-negative advanced or metastatic breast cancer and is the most advanced PROTAC to date.

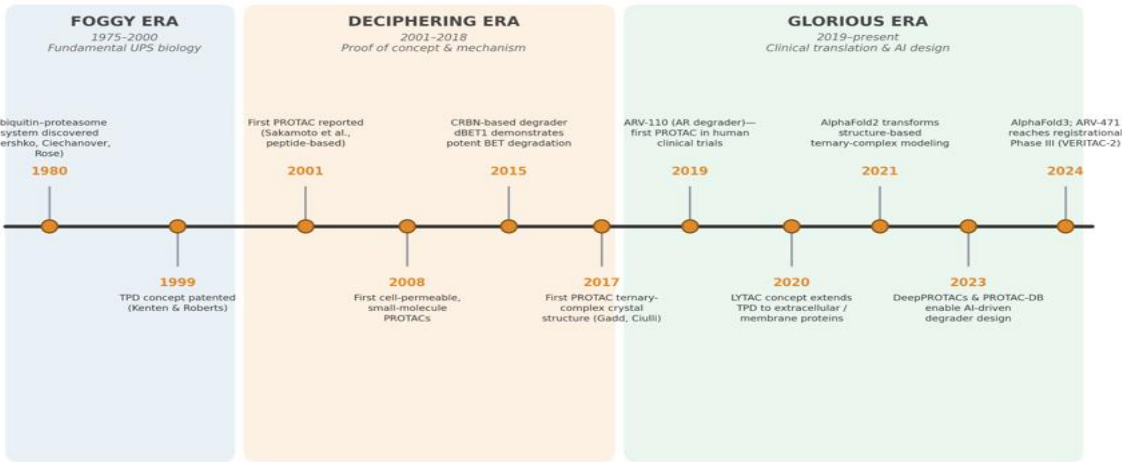


Figure 5. Milestones in targeted protein degradation, from ubiquitin-proteasome discovery to clinical PROTACs and AI-driven design.

Apart from the androgen and estrogen receptor-targeting PROTACs discussed above, the clinical development pipeline of PROTACs has significantly diversified to include BTK (along with brain-penetrating BTK degraders to treat B-cell malignancies involving the CNS), IRAK4, STAT3, and other difficult-to-drug oncology targets that have entered preclinical and early-to-mid phase clinical trials, along with ongoing preclinical exploration of other transcription factors and scaffolding proteins (Table 2) [2,3,5]. Such diversification is indicative of increased confidence that the design guidelines set for the first-in-class CRBN or VHL-targeting PROTACs, with regards to the design of effective linkers and optimization of physicochemical properties and cooperative ternary complex design principles, can be generalized for other classes of targets, although challenges specific to each class of target or organ/tissue type need to be addressed (Section 4).

Table 2. Representative PROTACs and related targeted protein degraders in clinical or late preclinical development.

Degrader (code name)	Target	E3 ligase	Primary indication	Development stage
ARV-110 (bavdegalutamide)	Androgen receptor (AR)	Cereblon (CRBN)	Metastatic castration-resistant prostate cancer	Phase 1/2
ARV-471 (vepedegestrant)	Estrogen receptor (ER)	Cereblon (CRBN)	ER-positive/HER2-negative advanced or metastatic breast cancer	Phase III (registrational)
NX-2127	Bruton tyrosine kinase (BTK)	Cereblon (CRBN)	Relapsed/refractory B-cell malignancies	Phase 1
NX-5948	BTK (brain-penetrant)	Cereblon (CRBN)	B-cell malignancies with CNS involvement	Phase 1
ARV-102	LRRK2 (brain-penetrant)	Cereblon (CRBN)	Parkinson's disease / neurodegeneration	Phase 1
KT-474	IRAK4	Cereblon (CRBN)	Atopic dermatitis, hidradenitis suppurativa	Phase 1/2

Various (preclinical)	STAT3, KRAS, Tau and other aggregation-prone or transcription-factor targets	CRBN, VHL, others	Oncology; neurodegeneration	Preclinical
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Alongside intracellular PROTACs, lysosomal- and extracellular degradation strategies (Section 3.7) are making their way through early stage preclinical and clinical testing for cell surface receptors and secreted disease-causing proteins, such as cancer and neurological targets, expanding the therapeutic potential of the targeted protein degradation approach from the early stage cancer-focused PROTAC pipeline to immunological, neurological diseases, and others [2,18].

4. Challenges and Limitations

Though significant advances have been made on the clinical front, several interconnected issues persist which impede further advancements in PROTAC technology, and which need to be resolved in order for PROTACs to reach their full therapeutic potential. The bRo5 physicochemical properties of PROTACs (see Section 3.2) still pose issues with respect to oral bioavailability, solubility, and formulation, and though some candidates have successfully demonstrated sufficient oral exposure, this usually involves a laborious compound-specific structural modification process, and not one guided by general principles. The “hook effect,” which was mentioned in Section 3.1, makes it difficult to choose the right dose and necessitates a thorough characterization of the drug’s PK/PD profile across an extensive range of doses.

As the E3 ligases used for PROTAC development are expressed differently in distinct tissues and cell types, even when the POI is not present, there will be great variability between tumors in the efficiency of protein degradation, thus hindering patient selection and possibly resulting in different therapeutic outcomes; there have been attempts to find other E3 ligases with a selective or broader distribution in tumors. Acquired resistance to E3 ligases has also been seen both in clinical practice and preclinical studies, which occurs due to various reasons such as mutational inactivation of the E3 ligase itself (which leads to resistance to all CRBN- and VHL-based drugs targeting various POIs), mutation of the POI within the PROTAC recognition site, and activation of other survival pathways – resistance mechanisms that have some common points with those of inhibitors, but not identical to them [2,5]. CMC challenges for PROTACs as opposed to simple small molecules would be more complex due to increased size, multiple synthetic moieties and requirements of

controlled linker chemistry which directly translate to cost of goods and scalability considerations [1,2]. Lastly, stimuli responsive or CNS delivery platforms (sections 3.5-3.6) are highly promising but relatively nascent approaches in which challenges of achieving spatial precision, systemic safety before activation and increased CMC complexities for multiple component systems continue to be explored [3,4].

5. Future Perspectives

A number of convergent trends appear poised to influence the future course of PROTAC research. Firstly, further integration of structure-based docking, deep-learning structure prediction, and generative machine learning in fully automated design-make-test-analyze pipelines (Figure 3) is expected to steadily diminish the amount of synthetic iterations required to find degraders with desirable cooperativity, efficacy, and physicochemical characteristics, and eventually allow for the rational design of novel POI-E3 combinations without known structural precedence. Secondly, an increased focus on expanding the arsenal of validated E3 ligases beyond CRBN, VHL, MDM2, and IAP proteins, possibly assisted by AI-guided ligand discovery and profiling of tissue expression [5,19], might lead to improved tumor/tissue selectivity and help address the issues of resistance and heterogeneous response raised in Section 4. Third, the field is likely to see the development of precise engineering of stimuli-responsive activation approaches and CNS delivery technologies for application in cases in which the risk posed by systemic administration of a permanently active degrader or the inability to reach a pharmacological sanctuary site, such as the CNS, through another means presents a problem. Fourth, as lysosome- and autophagy-based therapeutics (LYTACs, AUTACs, ATTEC) [18] and nucleic acid- and antibody-based degrader systems [5,19] advance alongside small-molecule PROTACs, there will be an expansion of the clinical pipeline into fields other than oncology, such as immunology and neurodegeneration. Finally, the development of first-in-class PROTACs and the associated accumulation of pharmacological information on dosing protocols to prevent hooking, resistance and rational sequencing/combinations, and the long-term safety concerns associated with the sustained target depletion will feedback into the preclinical phase and early development process.

6. CONCLUSION:

The PROTAC approach has made tremendous advances within just two decades from a proof of concept using peptides to a well validated therapeutic approach with several programs in advanced stages of development, thanks to the growing sophistication of our knowledge of ternary complex cooperativity [9,10], quick adoption of computer and artificial intelligence driven design approaches [1,5] along with simultaneous advancement of precision engineering and stimulus-responsive [4] and brain-penetrant [3] degrader technologies. The event-driven and catalytic pharmacology inherent to protein degradation offers truly complementary mechanisms of action for

targeting historically undruggable targets, scaffolding activities, and resistance mechanisms in disease biology. While realization of the potential of the modality will entail solving the inter-related problems of improving the physicochemical properties of bRo5 molecules, E3 ligase diversity, resistance management, and manufacturing scale-up, the integration of structural biology, computational chemistry, artificial intelligence, and clinical pharmacology described in this review presents a realistic path towards achieving those goals, setting PROTACs and other degrader modalities up to become a mainstream drug discovery approach on par with small molecule and biologic therapies.

List of Symbols and Abbreviations

Abbreviation	Full Form
AbTAC	Antibody-based PROTAC
ADMET	Absorption, distribution, metabolism, excretion, and toxicity
AI	Artificial intelligence
AR	Androgen receptor
ATTEC	Autophagosome-tethering compound
AUTAC	Autophagy-targeting chimera
BBB	Blood-brain barrier
bRo5	Beyond rule of five
CMC	Chemistry, manufacturing, and controls
CNS	Central nervous system
CRBN	Cereblon
DC50	Half-maximal degradation concentration
Dmax	Maximal degradation
E1/E2/E3	Ubiquitin-activating/conjugating/ligating enzymes
ER	Estrogen receptor
GNN	Graph neural network
IAP	Inhibitor of apoptosis protein
IEDDA	Inverse electron-demand Diels-Alder
LYTAC	Lysosome-targeting chimera
MDM2	Mouse double minute 2 homolog
MOE	Molecular Operating Environment
PK/PD	Pharmacokinetics/pharmacodynamics
POI	Protein of interest
PROTAC	Proteolysis targeting chimera
QSPR	Quantitative structure-property relationship
ROS	Reactive oxygen species

Abbreviation	Full Form
TPD	Targeted protein degradation
Ub	Ubiquitin
UPS	Ubiquitin-proteasome system
VHL	von Hippel-Lindau protein

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Conflict of interest

Authors doesn't have conflict of interest with the publication of manuscript.

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