FROM PROTACS TO DACS: HOW TARGETED PROTEIN DEGRADERS ARE BREAKING RULES AND BOUNDARIES IN DRUG DESIGN

Approximately 80% of proteins are considered "undruggable." These proteins may lack enzymatic activity or an obvious active site for traditional small-molecule drugs to block, thwarting the development of therapies for diseases associated with these proteins.[1] As researchers take on these undruggable proteins, they are turning to complex drug structures like proteolysis-targeting chimeras (PROTACs) and other chimeric targeted protein degraders that challenge the traditional rules of drug design.

Targeted protein degraders work by hijacking the natural protein degradation processes of the cell to destroy disease-causing proteins rather than inhibiting protein activity. By turning previously undruggable and hard-to-drug proteins into druggable targets, protein degraders are paving the way to novel therapeutics for conditions such as cancers and neurodegenerative diseases.[2]

"It's very exciting to see the wide range of degraders being explored," says Fabienne Charrier-Savournin, senior product manager life sciences at Revvity. "Each new approach potentially broadens the range of diseases we'll be able to treat."

Most degraders currently target proteins of interest that have well-characterized binding sites. However, unlike small-molecule inhibitors, degraders can remove proteins that have disease-causing roles beyond enzymatic or receptor activity. For example, Kymera Therapeutics has begun clinical trials with an interleukin-1 receptor-associated kinase 4 (IRAK-4) degrader.[3] IRAK-4, a protein involved in inflammatory pathways, has two functions: it phosphorylates other proteins in these pathways and acts as a scaffold for a larger protein complex involved in inflammation. In contrast to using small-molecule kinase inhibitors, removing IRAK-4 with a degrader prohibits both functions.

Over the past decade, pharmaceutical companies have started to invest significant funds in targeted protein degraders, and numerous well-funded start-ups have entered this field. PROTACs are among the most explored degraders—and the most advanced. The first PROTAC entered clinical trials in 2019, and many more are now being tested in humans.[4] "There are over 30 different clinical trials already," says Ivan Đikić, director of the Institute for Biochemistry II at Goethe University Frankfurt, Germany, and member of the steering board for PROXIDRUGS, a German academic-industry consortium that develops targeted protein degraders and related drugs. The most clinically advanced PROTAC is ARV-471, also known as vepdegestrant (Figure 1), which was developed by Arvinas and is currently in Phase 3 trials against breast cancer. The US Food and Drug Administration (FDA) also granted fast track designation to investigate vepdegestrant for treatment of breast cancer in February 2024. Other types of targeted protein degraders are in earlier stages of development and are showing promise (Figures 3 and 4).

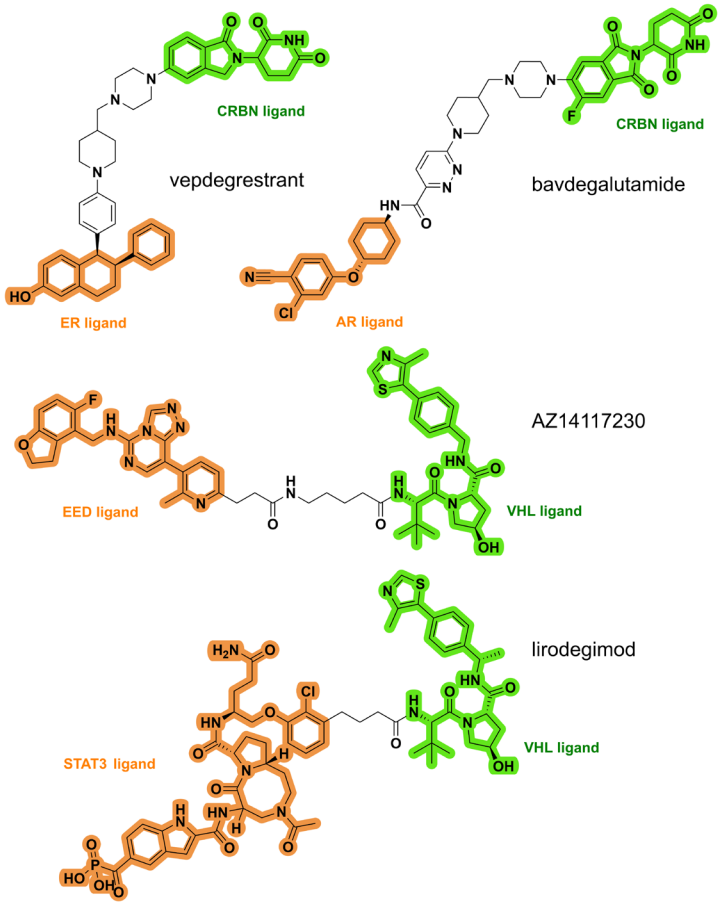


Figure 1: Chemical structures of four different PROTACs showing the ligand of the protein of interest in orange (ER: estrogen receptor, AR: androgen receptor, EED: embryonic ectoderm development, STAT3: signal transducer and activator of transcription 3) and that of the E3 ligase in green (CRBN: cereblon, VHL: von Hippel-Lindau). Image generated with Signals ChemDraw.
Source: "PROTACs, molecular glues and other degraders," IUPHAR/BPS Guide to Pharmacology, accessed Dec. 17, 2024, https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=1030

Credit: Revvity Signals

Collaborations are essential to accelerating targeted protein degraders toward the clinic. Arvinas, a spin-off from Craig Crews's laboratory at Yale University, for example, has been partnering with Pfizer since 2021 to develop vepdegestrant. Such collaborations are enabling researchers to establish design and development principles for these novel drugs and are leading to rapid advances.

PROTACS PRIMED FOR SUCCESS

PROTACs are long molecules consisting of two ligands connected by a chemical linker. One ligand interacts with a target protein while the other binds an E3 ubiquitin ligase. Once bound, the proximity between the target protein and the ubiquitin ligase enables the ligase to tag the protein with ubiquitin, marking it for degradation by the cellular proteasome (Figure 2).

The potential therapeutic advantages of PROTACs go beyond enabling previously undruggable disease-causing proteins to be removed. In theory PROTACs are catalytic, which allows them to be effective at very low doses, enabling the destruction of multiple target proteins.

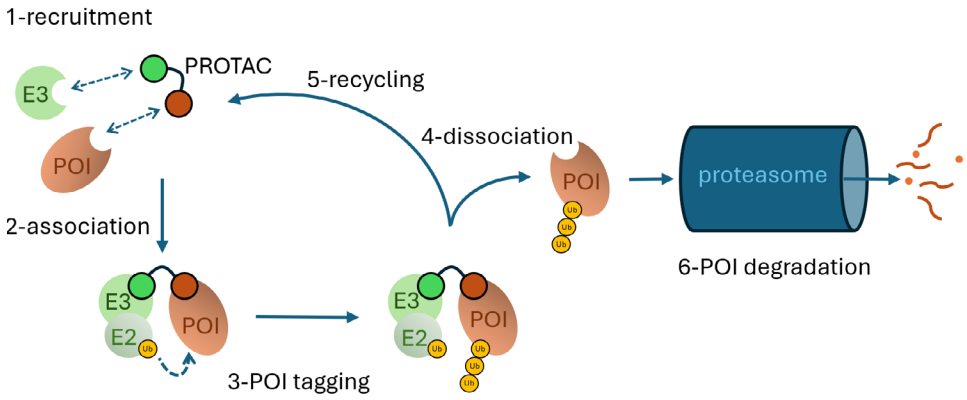


Figure 2. PROTACs enable proteins of interest (POIs) to be tagged by ubiquitin ligases (E2, E3) for degradation by the proteasome.

Credit: Revvity Signals

Their chimeric three-part design means that PROTACs are often much larger and more complex than traditional small-molecule drugs.[6] Traditional strategies around what makes drug candidates viable for oral dosing (such as Lipinski's rule of 5) or other favorable properties do not necessarily apply. "PROTACs don't follow Lipinski's rule, posing some pharmacokinetic challenges," explains Đikić. The principles governing bioavailability of PROTACs still need to be explored. The lack of robust principles and their overall size and complexity mean that PROTACs can be very challenging to design.

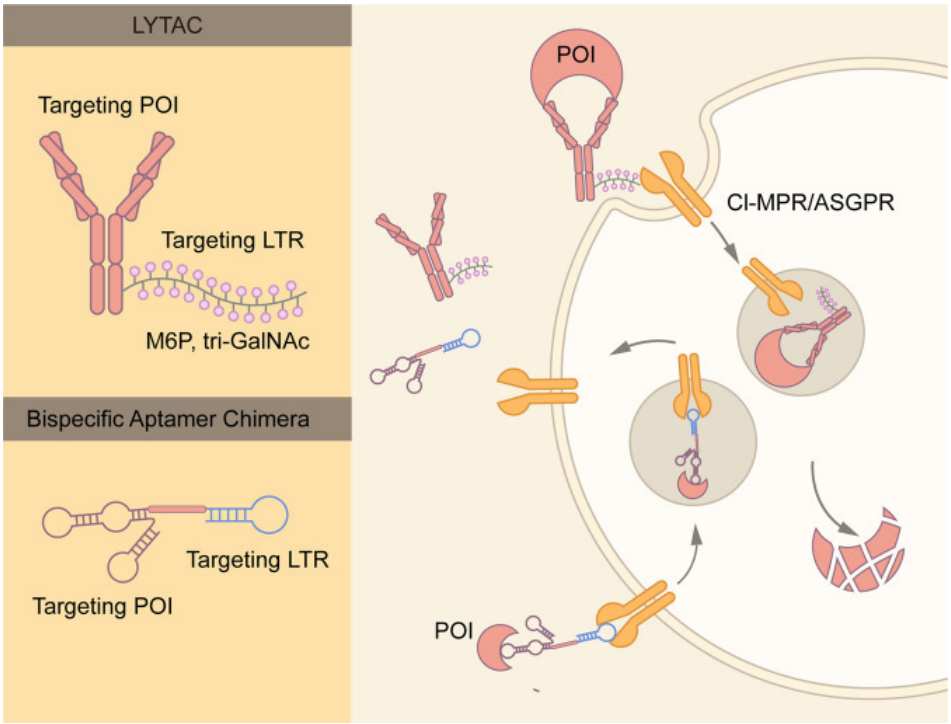
PROTACs that can be taken orally tend to be designed in a way that minimizes breaking Lipinski's rule. For example, vepdegestrant meets three out of the five Lipinski criteria.[6]

The lack of established guidelines, however, isn't noticeably slowing progress.[7] Đikić is particularly excited about the development of new oral PROTACs that can penetrate the blood-brain barrier to degrade the leucine-rich repeat kinase 2 (LRRK2) protein. LRRK2 has been implicated in neurodegenerative diseases, including Parkinson's and progressive supranuclear palsy. "It took less than 2 years to go from finding a PROTAC head that binds to LRRK2 to making a PROTAC that can go to the brain," Đikić says. The first patient was dosed with Arvinas' LRRK2-degrading PROTAC—known as ARV-102—in February 2024.

PROTAC-based antibiotics are also being explored in academic laboratories. These "BacPROTACs" may be able to selectively target and destroy disease-mediating proteins in bacteria, a unique mechanism of action that could help combat antimicrobial resistance encountered with traditional antibiotics. The first evidence of this happening in vivo was in 2022.[8] In this example, after a BacPROTAC enters a *Mycobacterium*, one head binds a target mycobacterial protein. The second head induces proximity between the captured protein and the mycobacterial protein degradation machinery that is analogous with the proteasome in eukaryotic cells. Diseases caused by *Mycobacteria* include tuberculosis and leprosy. "I think this can become one of the complementary anti-bacterial treatments to antibiotics in the future," says Đikić.

EXPLOITING THE LYSOSOME

The proteasome isn't the only cellular protein degradation machinery drug developers can commandeer. "The lysosome is a big degradation 'bag' that can degrade proteins, organelles, and pathogens," Đikić says. Several lysosome-related mechanisms are being hijacked for targeted protein degradation.[7]



Source: Da Jia et al., "Targeted protein degradation: mechanisms, strategies and application," *Signal Transduct. Target Ther.*, 7 no. 1 (April 2022): 113, https://doi.org/10.1038/s41392-022-00966-4.

Lysosome-targeting chimeras (LYTACs) target a different group of proteins than PROTACs: the 40% of proteins that function outside of cells.[9] They capture and tag disease-causing proteins with a receptor that traffics them across the cell membrane and delivers the protein to the lysosome.[10] LYTACs are similar to PROTACs, with a head that binds target proteins, a linker, and a second head that binds lysosome-trafficking receptors. The first iterations of LYTACs use an antibody to target the POI (Figure 3). They are the brainchild of Carolyn Bertozzi, Nobel laureate and professor of chemistry at Stanford University, who launched Lycia Therapeutics in 2019 to commercialize this idea. Autophagy-targeting chimeras (AUTACs) also exploit the lysosome.[11]

Autophagy is a natural pathway that targets proteins, protein aggregates, and some non-protein substrates, even organelles, to lysosomes for destruction. Like PROTACs and LYTACs, AUTACs have one head that binds to a specific cellular target. With AUTACs, the other head consists of guanine derivatives that recruit the autophagy machinery and initiate a degradation chain reaction. The ability of AUTACs to target protein aggregates makes them particularly exciting, as aggregates of misfolded proteins play a role in most Neurodegenerative conditions, including Alzheimer's and Parkinson's diseases. However, this technology is still in its infancy, says Đikić, and it is currently limited to laboratory research, not clinical research.

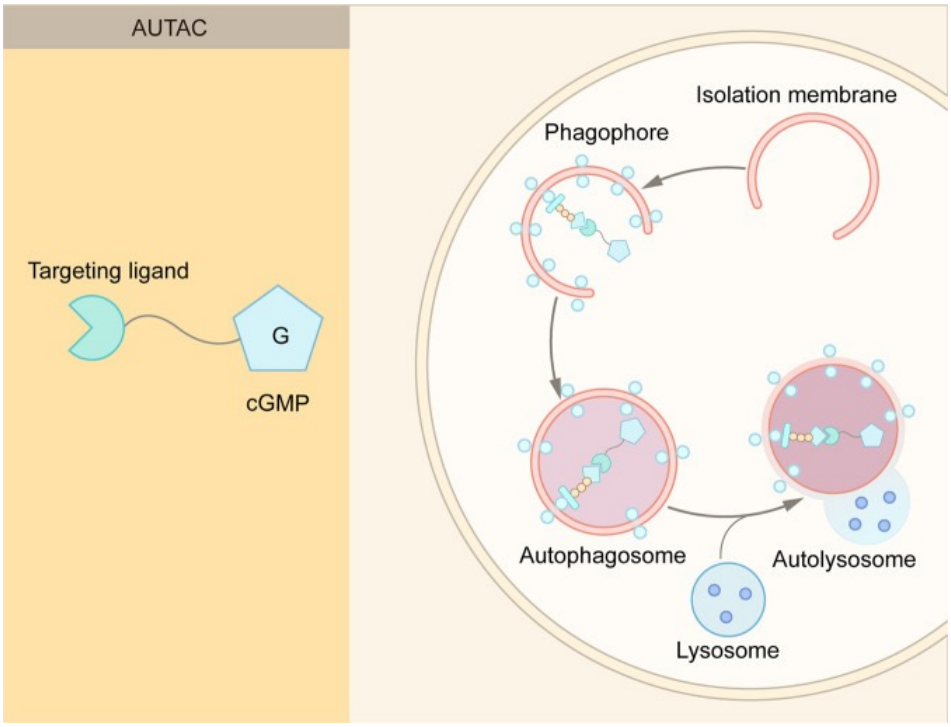


Figure 4. Mechanism of action of an AUTAC (cGMP: cyclic guanosine monophosphate). Source: Da Jia et al., "Targeted protein degradation: mechanisms, strategies and application," *Signal Transduct. Target Ther.* 7, no. 1 (April 2022): 113, https://doi.org/10.1038/s41392-022-00966-4.

UPPING COMPLEXITY WITH MULTIMODAL DEGRADERS

As the targeted protein degrader field has advanced, researchers are setting their sights on even more complex degrader modalities. A prominent example is degrader-antibody conjugates (DACs), which couple antibodies with protein degraders for more precision and specificity.[12]

DACs are inspired by antibody-drug conjugates (ADCs). ADCs themselves are a hot area within the anticancer drug field, with more than 10 drugs approved by the US FDA between 2017 and 2024. ADCs are made of a monoclonal antibody attached by a chemical linker to a cytotoxic drug. The antibodies bind specifically to antigens expressed on cancer cell surfaces and deliver the payload directly to cancer cells. This targeted mechanism is efficient at treating the cancer while limiting harm to healthy cells.

DACs are similar but instead carry protein degrader payloads. DAC development is a fast-advancing field. Genentech researchers first published on DACs in 2019[13], and by late 2022, the first DAC entered human clinical trials. This candidate, ORM-5029, is being developed by Orum Therapeutics and is currently in Phase I trials against breast cancer. ORM-5029 incorporates a molecular glue degrader, but DACs that use other types of protein degraders are also in development.

The extremely complex structure of DACs means that partnerships are critical in this field. In September 2023, Nurix Therapeutics, which specializes in targeted protein degraders, and Seagen, with ADC expertise, agreed to a multiyear deal to develop DACs. In February 2024, another partnership in this space was announced: Firefly Bio emerged from stealth mode after receiving significant funding from a group that includes Eli Lilly and Company.

COLLABORATE TO LEARN AND MOVE FAST

Designing and developing targeted protein degraders is a highly multidisciplinary affair. It's rare for even the largest pharmaceutical companies to have all the necessary expertise in-house. "This area is prone to collaboration," says Kerstin Koch, scientific manager and PROXIDRUGS project leader at Goethe University Frankfurt.

PROXIDRUGS is an initiative funded by the German federal government that is bringing academics together with major pharmaceutical partners to rapidly advance technology transfer in the targeted protein degradation space and advance modalities like PROTACs and DACs towards clinical trials. The cluster started in 2021, and the German federal government is investing up to €45 million (approximately $46 million) over 9 years in 3 funding phases to support it. It started with nine partners and has recently increased to 21 partners within the second funding phase. One PROXIDRUGS collaborator, Revvity, has codeveloped assays with cluster members that will support the protein degrader community's search for new degraders.[14]

The purpose of PROXIDRUGS is to pool together expertise, says Đikić. Sharing knowledge to advance the field faster "was the central objective of the project when we started", he says. "There are many different questions that we still need to resolve before this becomes a mainstream drug development area."

PROXIDRUGS projects cover a broad range of topics in the field and aim to (i) develop innovative technologies and assays to aid degrader discovery and validation, (ii) develop and test novel degraders and their chemical modules, (iii) explore alternative modalities, and (iv) tackle challenges related to delivery and carrier systems.

Koch adds that PROXIDRUGS and other targeted protein degrader developers are also bringing onboard smaller partners with expertise in artificial intelligence and machine learning. In the future, such tools could allow degrader researchers to run hundreds of virtual experiments to identify promising leads before investing time and resources in the lab. "We're seeing more and more in silico-driven approaches that enrich the discovery space and have great potential to accelerate the development of PROTACs or glues," Koch says, "this is a field where often smaller companies drive innovation."

REVVITY SIGNALS PERSPECTIVE: SOFTWARE TOOLS CAN EASE R&D COLLABORATIONS

"One of the challenges of large, multi-institutional R&D collaborations is being able to share data and other information in a timely and secure environment," says Zev Wisotsky, senior principal marketing manager at Revvity Signals. "We smooth this process by providing software that breaks down silos and speeds up development of targeted protein degraders."

A unified software solution

Drug discovery is becoming increasingly complex, with demanding and sophisticated new approaches to research and development. Revvity Signals provides Signals Research Suite, an integrated software solution to help scientists tackle critical challenges.

Breaking down silos for collaboration

One of the greatest obstacles in modern R&D is siloed information—data scattered across teams, departments, or external partners. Revvity Signals removes these barriers with a unified, cloud-native SaaS R&D platform that creates a single source of data. Researchers, contract research organizations, and academic collaborators can work together more effectively, securely, and in real time by centralizing experimental, assay, and study data. This unified platform ensures alignment across the entire drug discovery pipeline, accelerated timelines, and reduction in costly delays.

Turning data into decisions
The platform combines tools that simplify how scientists interact with their data.

- **Comprehensive data capture and management:** With integrated tools for tracking assays, in vivo studies, and experimental outcomes, the software streamlines workflows and makes it easy to locate and share critical information.

- **Intelligent data visualization and mining:** Complex datasets are transformed into insights through intuitive visualizations and advanced analytics. Scientists can explore patterns, predict outcomes, and make data-driven decisions faster.

- **HELM-powered innovation:** By leveraging hierarchical editing language (HELM) and a structured database, researchers can iterate on macromolecular structures' modifications, unlocking new opportunities to optimize candidates and modalities.

Focus on science
Addressing the challenges of cross-functional collaboration, data management, and decision-making ensures that scientists can maximize the value of their work and bring transformative therapies to patients faster.

BREAKING BOUNDARIES AND UNLOCKING UNTAPPED POTENTIAL

Targeted protein degraders are redefining what is possible in drug discovery. Their diverse structures and mechanisms offer incredible versatility, but targeted protein degraders also have unique challenges. Collaboration is proving to be essential in overcoming these obstacles, with initiatives like PROXIDRUGS creating a shared framework to accelerate the progress of degraders toward the clinic.

By combining partnerships, advanced in silico approaches, and strategic investments, the drug discovery enterprise can make significant strides in developing targeted protein degraders to meet critical medical needs. Ultimately, overcoming traditional limits and boundaries in drug discovery and development will offer new opportunities for patients waiting for solutions.

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14. Revvity Signals is the software arm of Revvity and is not directly affiliated with PROXIDRUGS.

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