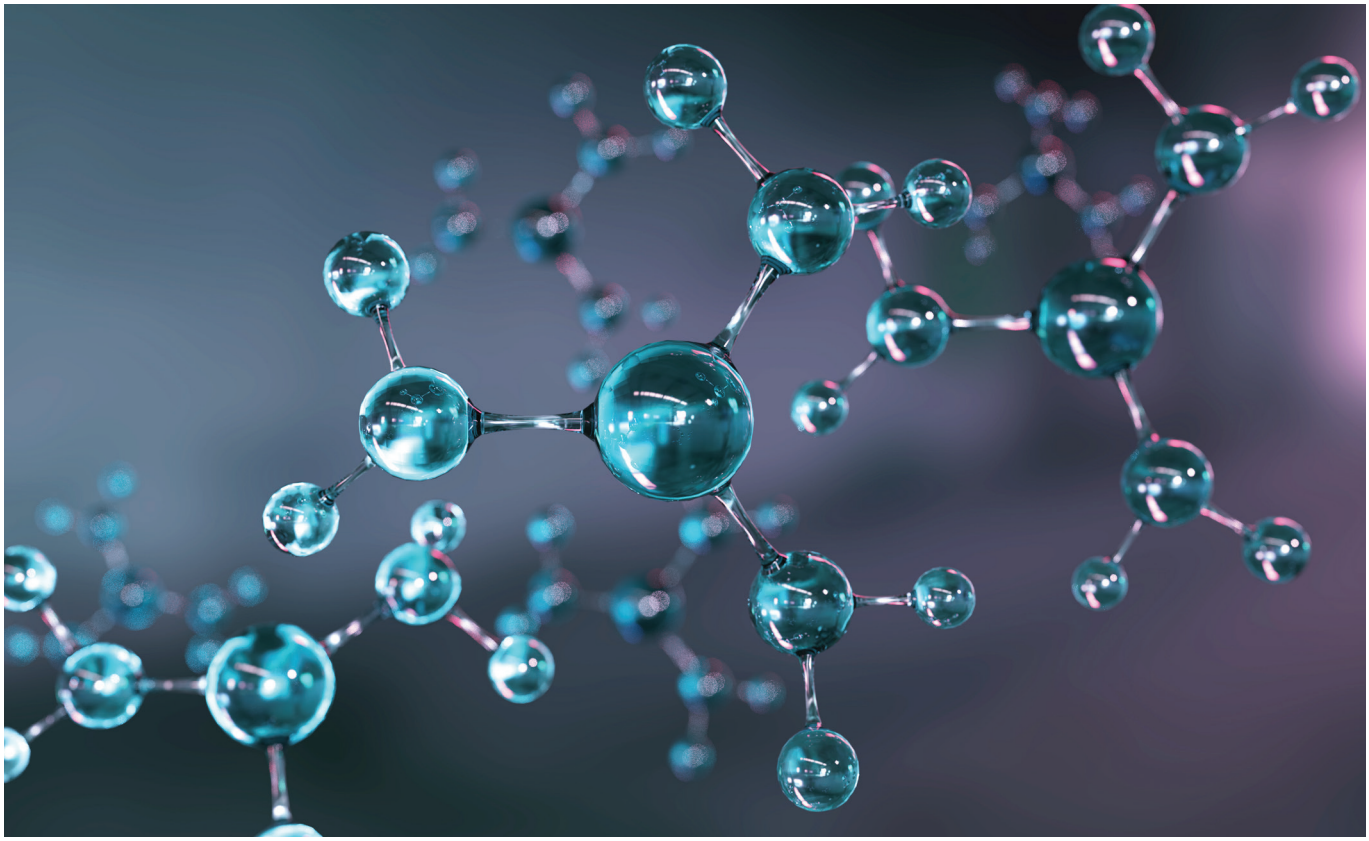

# **Bifunctional Molecules:** A Diverse New Modality That Unlocks New Target Classes

Bifunctional molecules are opening up once-inaccessible targets and enabling development of drugs that are less susceptible to target protein mutations. By bringing two proteins together, molecules with this emerging modality can modulate degradation, signalling, protein folding and cellular events that are hard to control with traditional small molecules. However, as with any new modality, bifunctional molecules face challenges and continue to benefit from the development and application of new tools.

The term "bifunctional molecules" covers a collection of related modalities with different but similar strategies. Some small molecules that act as a molecular glue can be considered as bifunctional compounds. These molecules, which include immunomodulatory drugs (IMiDs) such as thalidomide and lenalidomide, can interact with an E3 ligase and promote proteasomal degradation of various substrates.

However, bifunctional molecules are typically more complex structures than the molecular glues. A class of such molecules of particular interest currently are the proteolysis targeting chimera molecules, or PROTACs. These molecules are composed of a warhead that targets the protein of interest, a ligand that recruits an E3 ligase and a linker that connects these two ligands. While PROTACs are structurally different from molecular glues, they can have a similar functional effect by bringing a target protein close to an E3 ligase, leading to ubiquitination of the target protein and subsequent degradation by the proteasome.

The breadth of designs of bifunctional molecules creates opportunities to use the drugs in a range of applications. Molecules may feature linkers that can affect immune system direction, act on transcription elongation and inhibit protein-protein interactions

(PPIs). Further bifunctional molecules can modulate the interactome, or inhibit transporter function.

There is a growing body of evidence that PROTACs have certain advantages over other modalities. Researchers have often found that PROTACs have greater selectivity than their constituent parts, enabling the preferential degradation of target proteins. For example, small molecule ligands with low selectivity among kinase targets, when built into a PROTAC, may often act as a more selective ligand and trigger the degradation of only a limited pool of kinases.

Other studies have shown PROTACs can overcome point mutations in the target protein active sites, pointing to the potential to use such molecules as second-line therapies in patients who have developed resistance to other therapeutic agents. However, it remains to be seen whether mutations that render particular proteins resistant to being targeted by PROTACs can develop.

As with any modality, researchers need a deep understanding of the selectivity of a molecule and its downstream consequences when designing bifunctional molecules. The unintended tagging and degradation of a useful protein can cause negative outcomes and experience with IMiDs shows that stabilizing ligand-protein interactions can trigger a range of clinical effects, only some of which are beneficial.

## The Tools That Enable Bifunctional R&D

Identifying and working with bifunctional molecules creates new challenges. PROTACs move researchers away from familiar "rule of 5" chemistry and require them to work with multicomponent molecules that bind to at least two proteins. Biophysical events between the components drive the process and need to be understood, both individually and in aggregate. Faced with those new challenges, researchers are developing novel assay technologies to understand how bifunctional molecules behave and what 3D structures they adopt, alone and in complex with cognate proteins.

Successful bifunctional R&D requires mastery of multiple technologies and workflows. An early step in the process may be the use of DNA-encoded libraries (DEL) to identify binders to target proteins. The DEL strategy enables researchers to make and screen libraries of up to trillions of DNA-tagged molecules. Exposing the library to a protein of interest and sequencing the DNA barcodes of any molecules that show affinity to the target protein enables identification of new ligands.

Fragment-based drug design (FBDD) is another powerful tool for identifying new ligands for the target protein. FBDD comprises the development of high-affinity compounds from low molecular weight fragments that make it possible to cover a wide chemical space with a relatively small number of molecules. In the context of PROTACs against non-traditional drug targets, the ability to optimize low affinity ligands using biophysical techniques is particularly valuable.

Affinity selection mass spectrometry (ASMS), another binding-driven assay, is also a key part of the R&D toolkit. ASMS assesses the binding of large numbers of candidate molecules to the target protein to rapidly identify those that show affinity.

These affinity-based screening platforms enable identification of moderate-to-high affinity ligands. No functional activity is needed for either the target protein ligand, or the ligand for the E3 ligase, unlike in classical pharmacology. Further optimization of the individual ligand, or the bifunctional molecule, has been successfully carried out using computational chemistry platforms to optimize ligand-target interactions and linker design.

Optimization and characterization of bifunctional molecules such as PROTACs are very often carried out using a combination of biophysical assay platforms and structural biology. Techniques such as microscale thermophoresis (MST) and surface plasmon resonance (SPR) are commonly used to interrogate both ligand binding and bifunctional molecule binding, as well as to measure the co-operativity of binding to the ternary complex of ligase + target protein + PROTAC that bifunctional molecules can enable.

Structural biology assessments are enabled by protein X-ray crystallography and, increasingly, by CRYO-EM. Using these technologies to reveal high-resolution ternary complex crystal structures can

facilitate rational, efficient optimization of complex molecules. By deploying these technologies, researchers have discovered and advanced bifunctional molecules with the potential to address major unmet medical needs.

"Successful bifunctional R&D requires mastery of multiple technologies and workflows."

## The Future Of The Field

There is scope to build on the successes achieved to date. Expanding the repertoire of ligases to include further examples with different substrate profiles, and diverse temporal and spatial expression profiles, could enable the identification of molecules with different efficacy and safety profiles. New tools are needed to drive further development, but the rewards for doing the work could be significant.

To date only a handful of the 600 or so proteins predicted to be members of the E3 ligase gene family have been employed to create PROTAC molecules. Substantial groups of ligases such as the RING domain E3 ligases remain largely unexplored yet potentially represent new opportunities for cell-type selective or tissue selective targeting of candidate substrate proteins. For example, research into the RING-domain E3 ligase APC could unlock opportunities to degrade CDC20, a regulator of cell division that is essential to all cancer types but only expressed at low levels in most human tissues.

PROTAC research could also advance through the targeting of further members of the HECT family of E3 ligases, which comprises enzymes that form a thioester bond with ubiquitin. Research has linked HECT E3 ligases to cancers, cardiovascular disease and neurological disorders, creating another avenue for drug developers to pursue.

There are also opportunities to move beyond ubiquitination and hijack other cellular processes to drive degradation of proteins of interest. Lysosome targeting chimeras (LYTACs), for example, use glycan tags to mark extracellular proteins for intracellular lysosomal degradation. By exploiting the lysosome pathway, LYTACs may be able to drive degradation of extracellular and membrane-bound proteins that are resistant to intracellularly focused PROTACs.

Researchers are also starting to explore the potential of autophagy-targeting chimeras (AUTACs) and autophagosome-tethering compounds (ATTEC). AUTACs, like PROTACs, use ubiquitination to drive the degradation of target proteins. However, AUTACs trigger a different form of polyubiquitination that is recognized by the selective autophagy pathway. ATTECs, in contrast, degrade proteins through direct binding to the target and a key autophagosome protein. Ubiquitination is not involved.

The growing breadth of therapeutic opportunities open to bifunctional molecules is testament to the expansion of knowledge of intracellular and extracellular degradation pathways. Having honed tools and techniques on PROTACs, researchers are equipped to translate knowledge of pathways into new medicines.

Equipped with tools for rapidly identifying affinity ligands, techniques for characterizing the role of kinetics and structure information to facilitate in silico design techniques, R&D groups are poised to discover and develop a range of bifunctional molecules that unlock previously inaccessible biology. The field, like all emerging areas of drug development, will face challenges and setbacks, but progress made to date suggests bifunctional molecules have the potential to address major unmet medical needs.

Scrip
Informa Pharma Intelligence

药明康德
WuXi AppTec

WuXi AppTec provides a broad portfolio of R&D and manufacturing services that enable companies in the pharmaceutical, biotech and medical device industries worldwide to advance discoveries and deliver groundbreaking treatments to patients.

As an innovation-driven and customer-focused company, WuXi AppTec helps our partners improve the productivity of advancing healthcare products through cost-effective and efficient solutions.

With industry-leading capabilities such as R&D and manufacturing for small molecule drugs, cell and gene therapies, and testing for medical devices, WuXi AppTec's open-access platform is enabling more than 4,100 collaborators from over 30 countries to improve the health of those in need – and to realize our vision that "every drug can be made and every disease can be treated."

**For more information, please visit: https://www.wuxiapptec.com/**

View PROTAC Webinar:

Contact WuXi AppTec RSD:

Follow WuXi AppTec RSD on LinkedIn: