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Medicinal Chemistry and Chemical Biology Highlights

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Fantastic Molecular Glues and Where to Find them

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Identifying protein pockets suitable for small-molecule engagement is a critical part of early drug discovery. Enzymes that operate on or with small molecule cofactors and G-protein coupled receptors (GPCRs) are the quintessential 'druggable' proteins because their biological functions require a hydrophobic pocket. Extending the druggable proteome to include enzymes operating on macromolecules, transcription factors, and scaffolding proteins represents a significant challenge in drug discovery – but novel approaches are beginning to bear fruit. Drugging transient pockets formed by biomacromolecular interactions is one strategy in expanding the druggable proteome and I will outline some success stories here.

The term 'molecular glue' has been coined to describe a series of molecules that bind to one protein (with weak or strong affinity), and through this interaction a new affinity with a second protein is created.[1] Such ternary complexes can influence protein function in several ways. In some cases, the complexes serve to inhibit the function of one or both proteins,[2] while in others the complex stimulates degradation of one of the proteins.[3] In fact, molecules that act through both of these mechanisms are marketed drugs – it's important to recognize, though, that the mode-of-action of currently approved molecular glues was unknown at the time of their discovery. In fact the field began with a series of natural products whose profound biological effects (immunosuppression and cell growth inhibition) presented a mechanistic mystery.[2] Cyclosporin, rapamycin, and FK506 are all macrocyclic molecular glues that simultaneously bind two proteins and inhibit the function of one of the proteins. Molecular glues can also promote protein degradation. In the plant world, this phenomenon is well known since there are several plant proteins whose stability is controlled by a protein–protein interaction mediated by a small molecule. Auxin, for example, induces the interaction of an E3 ubiquitin ligase with protein substrates bearing a specific peptide sequence known as the auxin inducible degron (AID).[4] The induced interaction leads to ubiquitin conjugation and ultimately degradation of the tagged protein through the ubiquitin proteasome pathway (UPP). This plant system is now used as a portable degron system applicable in a wide range of cells to chemically induce protein degradation (Fig. 1).[4a–c]

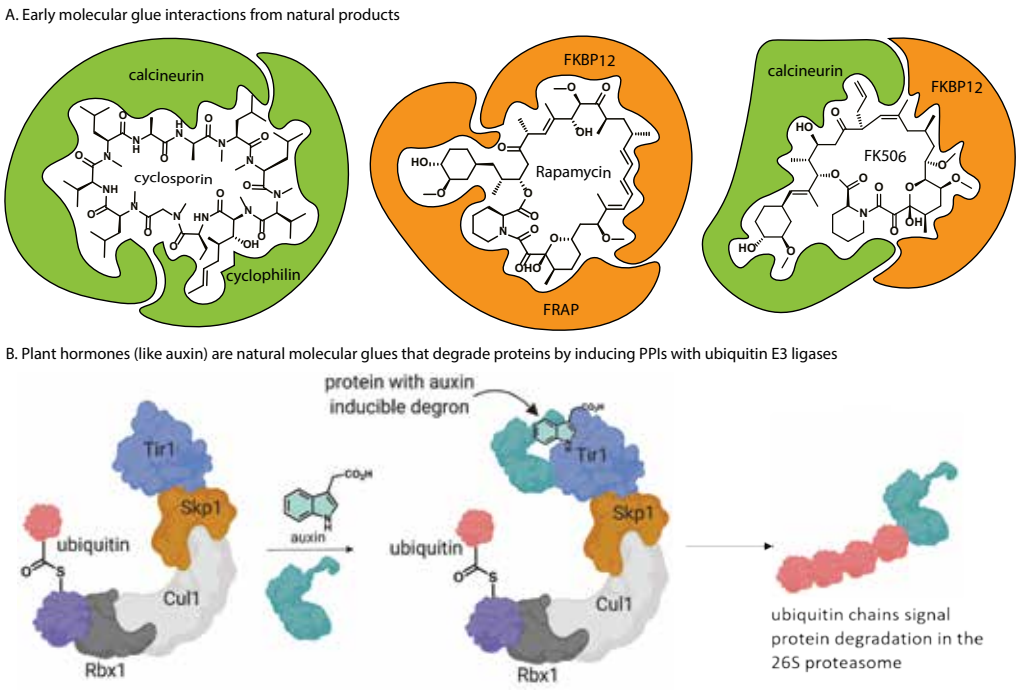

Fig. 1. A. Natural products provided the first examples of molecular glues. B. Plants regulate protein stability with small molecule glues.

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Man-made molecular glues have also been discovered.[3,5] These too though, were all discovered by happenstance[6] or phenotypic screening[7] and only later was their mechanism as molecular glues unveiled. Thalidomide and its derivatives (collectively referred to as immunomodulatory drugs, or IMids) degrade zinc fingers by engaging the E3 ligase substrate receptor cereblon (see Fig. 2A). Certain arylsulfonamides degrade the essential splicing factor RBM39 by recruiting it to the E3 ligase substrate receptor DCAF15 (see Fig. 2B). And, most recently, a series of molecules were identified in several labs[6,8] that promote cyclin K degradation by recruiting CDK12/cyclin k directly to the cullin scaffolding protein DDB1 (see Fig. 2C) – causing cyclin K degradation.

As medicinal chemists continue to harvest the fruits of unintended discoveries from the past, an important question is how we do this rationally. Systematic screens for degradation-type molecular glues is one strategy that has recently been demonstrated.[8b] In this work a hypomorphic mutant of an essential activator of one clade of UPS proteins (cullin-ring ligases (CRLs) was created and then small molecule screening against these cells in comparison to wild-type could identify small molecules whose mode-of-action operated somewhere in the CRL clade of ubiquitin ligases. In another approach systematic profiling of the proteins degraded by the IMiDs revealed a specific zinc finger motif as a minimal degron.[9] With this information binding assays between IMiDs, specific disease-relevant zinc fingers (often transcription factors), and the involved E3 ligase substrate receptor (cereblon) could now be imagined.

For glues that do not promote protein degradation (such as the molecules in Fig. 1A) innovative high-throughput screening (HTS) will be important. Typically HTS assays are developed with a primary read-out assay, which is followed up with counter screens that eliminate false positives. Molecules like the IMiDs or rapamycin would not have been discovered by such screens because they would have either not been amenable to primary assay development, or counter screens would have filtered out these 'weirdos'. Of course screens cannot capture everything, but glue mechanisms are worthwhile to think about at this early screening stage. For example reliable, genetically defined, cellular assays is one thing to consider. While deciding to induce a fundamentally new glue interaction is an immense challenge (what should one glue together?), a couple of examples of recent molecular glues point to some pathways for rational glue discovery.

Some biomolecular interactions occur transiently or only form in response to a signal – these might be the perfect candidates for novel molecular glues. For example, inhibitors of the important DNA damage response protein poly(ADP-ribose) polymerase 1 (PARP-1) are not potent simply because they inhibit PARP-1 catalytic activity (in fact genetic PARP knockouts do not show the same toxicities as PARP inhibitors in homologous recombination deficient cells), but rather because they trap PARP-1 as its DNA-bound form. Persistent DNA-binding of PARP-1 is particularly toxic in cells that lack normal homologous recombination and this mechanism underlies the recent emergence of PARP inhibitors in the clinic.

Another example involves inhibitors of the Bloom syndrome protein (BLM) – a helicase with important roles in the DNA damage response. A primary HTS screen that measured the ability of molecules to inhibit the helicase activity of BLM discovered several interesting hits.[10] However, more recent reanalysis of the publically deposited screening data along with crystallographic analysis of binding,[11] revealed that one potent compound series inhibited BLM by binding an induced pocket that forms at a domain interface as BLM translocates along DNA.

What conclusions can we make from the molecular glues discovered so far?

- Small molecule induced protein–protein interactions can have profound biological consequences;
- Several marketed medicines and many molecules that have entered clinical trials likely operate by a molecular glue mechanism;
- Innovative screens will be essential in finding the next generation of molecular glues;
- Transient biomolecular interactions can be trapped by small molecules, offering a rational path to glue development.

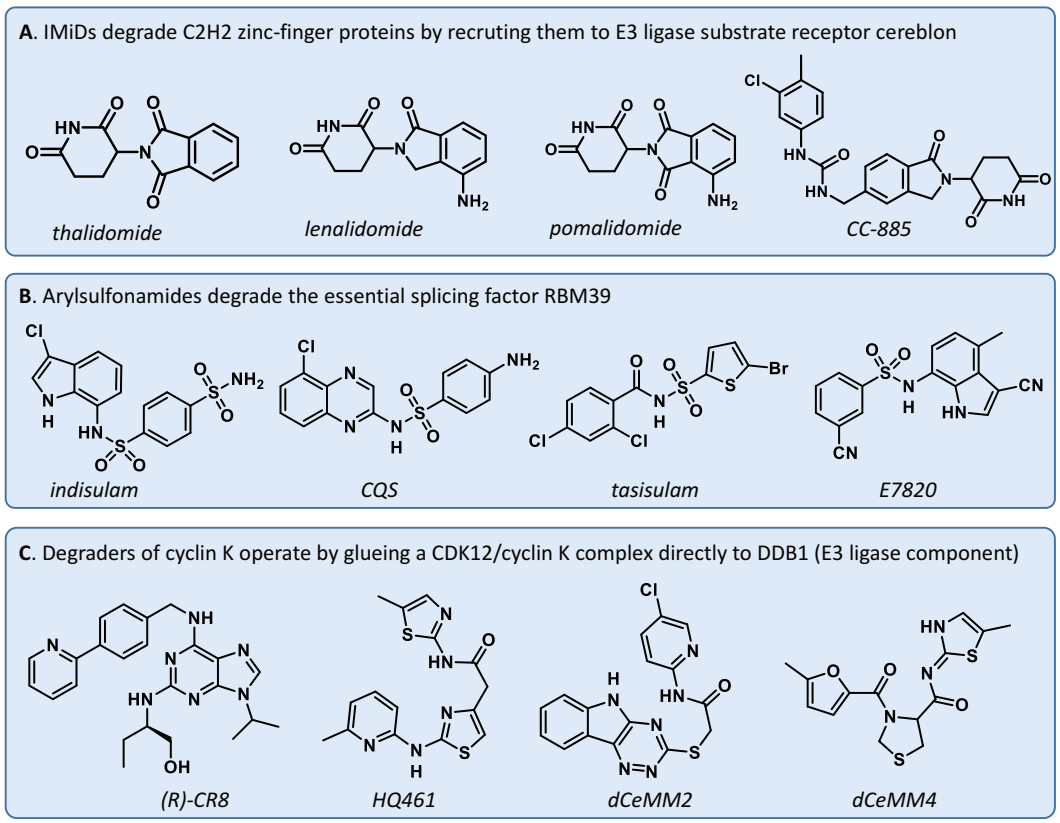

Fig. 2. Examples of validated molecular glue degraders that target proteins to the UPP. A. The IMiDs degrade zinc-finger proteins. B. Arylsulfonamides degrade the splicing factor RBM39. C. A variety of molecules can cause a CDK12/cyclin K complex to associate with DDB1, leading to cyclin K degradation.

While the molecular glue field is still in its infancy, it represents an exciting new way that small molecules can treat disease. Given that this modality was clinically validated before it was understood, a great deal of academic and industrial effort has been unleased toward discovering the next generation of molecular glues. Hence we should expect the next decade to be full of exciting developments in this area.

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