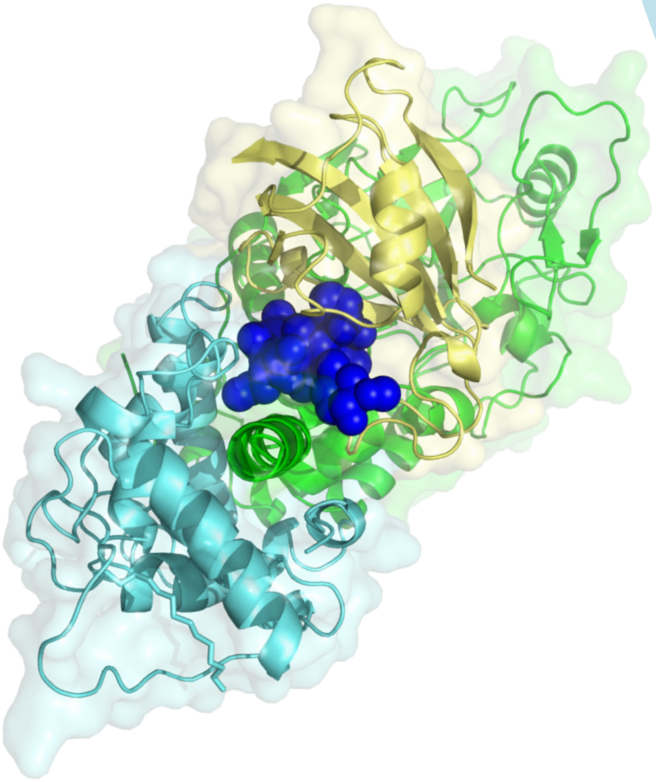
White Paper

Discovering & Advancing Molecular Glues Using a Cooperativity-Based Approach

Molecular glues represent an exciting modality in drug discovery, offering the potential to drug 'undruggable' targets, and create alternative ways to modulate established targets. However, molecular glues can be challenging to identify and develop.

The key differentiator between a molecular glue and traditional small molecule inhibitors is the concept of "cooperativity". Discover how our cooperativity-focused approach is helping researchers successfully identify and advance molecular glues.



Above: *Crystal structure of a protein complex induced by a molecular glue. The glue, FK506 in blue, binds to the cyclophilin (yellow) and brings both calcineurin A and B (green and cyan) into the complex, thereby inhibiting the phosphatase activity.*

Introduction

While molecular glues (MGs) represent an exciting avenue in drug discovery, they are notoriously difficult to discover, given their complex mode of action. These agents can induce new protein-protein interactions, or enhance innate ones, allowing novel ways to modulate or degrade a target. Through their ability to bring two proteins together, molecular glues can facilitate interactions that would otherwise have been considered undruggable by small molecules.

Given the need to glue together two protein surfaces, it is reasonable to expect that such compounds would tend to be large and complicated. However, many profoundly effective molecular glues are small molecule fragments.

Our unique combination of deep expertise in ternary complexes and purpose-built biophysics platform enables a cooperativity-driven approach to discover, characterize, and evolve molecular glues. Supported by our proprietary fragment library, expertise in protein science, and structural biology platform encompassing all 3 atomic resolution techniques, we are ideally positioned to assist drug hunters in successfully identifying and characterizing advanceable molecular glues.

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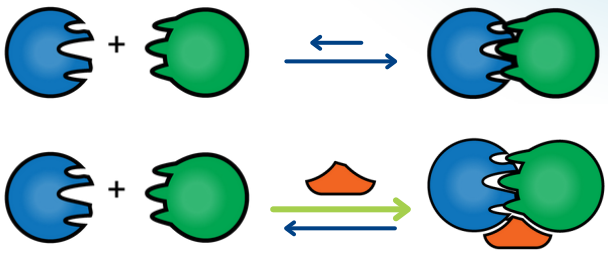
Cooperativity: A fundamental parameter of molecular glue discovery

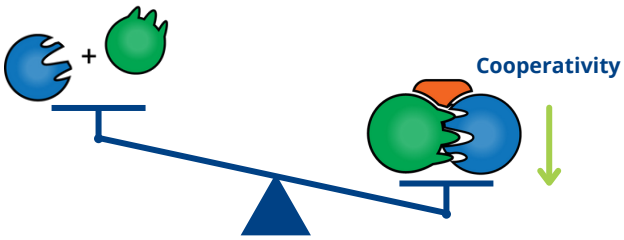
In contrast to traditional small molecule inhibitor discovery, molecular glues create a three component (ternary) complex. The potency of the MG is defined by the multiplication of the affinity constants (as opposed to addition) of the two binary complexes plus an additional factor coming from the resulting protein-protein interaction. This extra affinity is referred to as the "cooperativity" factor of the MG and is the defining characteristic of the mechanism of action.

Most approaches to molecular glue discovery (as with single targets) focus on the binding affinity of the binary complexes of the small molecule to either of the proteins and may overlook promising glue molecules that exhibit high cooperativity but relatively weak initial affinity. Using our deep understanding of cooperativity, as demonstrated in multiple peer reviewed publications, we have built a biophysics platform designed to specifically leverage cooperativity to multiply the signal output only when a ternary complex is formed. This allows us to screen directly for ternary complex formation using, for example, our fragment library as a source of hit matter.



Above: *Schematic view of binary vs ternary complex formation. In the absence of a MG, the two proteins (green and blue shapes) form a binary complex, typically with weak or even undetectable affinity. In the presence of the glue (orange), a tight, readily detectable complex forms.*

Below: *Schematic view of the concept of cooperativity. The higher binding energy (weaker affinity) of the binary complex is shown on the left. The lower energy of the MG complex (higher affinity) is shown on the right. The difference in energy levels is the cooperativity obtained from ternary complex formation.*

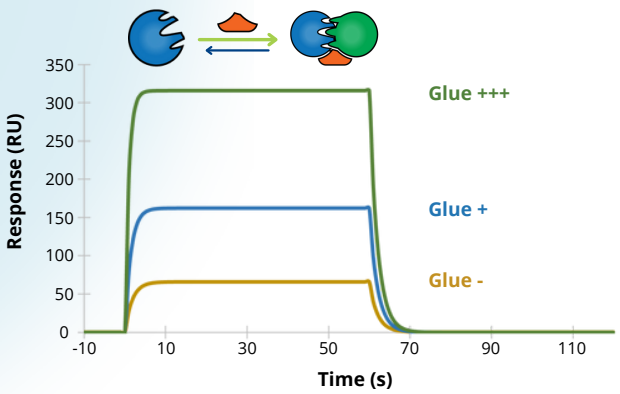


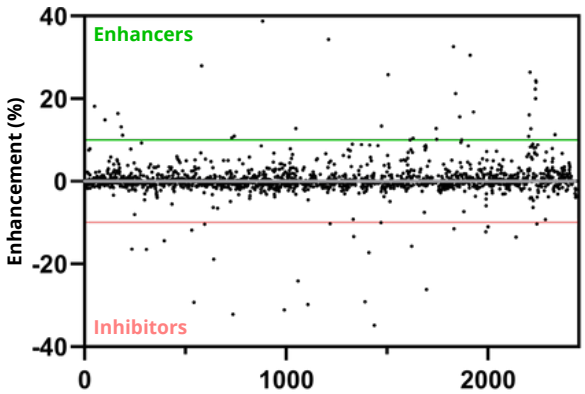
The molecular glue discovery workflow

1

Screen for molecular glue hits

To discover new molecular glues, we screen a compound library using surface plasmon resonance (SPR). While our proprietary fragment library has delivered MGs across multiple screens, we can also screen alternative libraries, including collections which are covalently reactive. The SPR assay involves immobilizing one protein while keeping the other in solution. This methodology is particularly adept at identifying molecular glues as our assay design ensures that compounds binding only to the immobilized protein do not generate a response. However, compounds that act as a glue by recruiting the non-immobilized protein generate a response sufficient to score as a positive hit. In this way, our discovery team have screened a wide variety of targets, finding hits for proteins ranging from very small enzymes to very larges scaffold proteins.





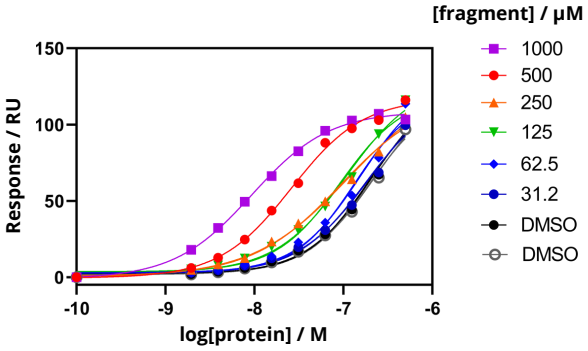
Left: *Titration of an active MG. In this titration, the amount of host is held constant and only the concentration of MG is increased. Formation of the ternary complex on the surface results in 100's of RUs whereas binding of the compound alone to the target would result in only a few RUs.* ***Right:*** *SPR screen of our proprietary fragment library for molecular glues. Each compound is represented by a single point. Points below the 0 line denote fragments that inhibit the protein-protein interaction while those above the line enhance it, representing molecular glue hits.*

2 De-risk via careful characterization of complex formation

After molecular glue hits have been identified, careful characterization is an essential opportunity to de-risk. We use dose-response titrations to determine the binding affinity of identified molecular glues for each of the individual proteins. We then use a custom, truncated 2D titration to quantitatively determine the cooperativity (the *α* factor). This ensures that all starting points for development are genuine MGs.

Next, we use orthogonal confirmation techniques to detect and eliminate compounds that induce protein aggregation. This step is crucial for distinguishing true molecular glues from artifacts caused by co-aggregation, ensuring that only the most promising candidates progress in the drug discovery pipeline.

Characterizing the formation of binary and ternary complexes is essential to understand the mechanistic details of molecular glue activity. As MGs progress, in addition to the *α* factor, we also perform kinetic analyses to measure the rates of association (k_{on}) and dissociation (k_{off}) for the binary and ternary complexes. This is powerful information that can be used to select chemotypes for further advancement and drive SAR.



Above: *2D SPR titration of partner protein in solution with increasing concentrations of a MG. Each curve represents titration of the partner protein in solution at a fixed concentration of the MG. Increasing concentration of the glue shifts the affinity for the partner protein to the left, proving the cooperativity. From this data the alpha (α) factor can be extracted using mathematical models.*

3 Generate structural data

3D structures enable rational, structure-guided optimization of small molecules. However, X-ray crystallography, the workhorse of structural biology, is much less successful with the weak ternary complexes that are typical of an early-stage MG project.

In-house, we have expertise in all 3 atomic resolution structural techniques: crystallography, NMR and Cryo-EM. This capability enables access to the optimal method, on a project-by-project basis, to derive the necessary structural information for your MG. For example, we might use crystallography to resolve the structure of a glue in complex with one protein, and NMR to map the binding interface between the two proteins. However, if the complex is very large, Cryo-EM is typically the method of choice.

As your molecular glue advances from fragment hit to lead and beyond, structural data can be leveraged to streamline medicinal chemistry and ultimately strengthen an IP strategy.

Risk reduction strategies

Our molecular glue discovery program has been meticulously designed to help you manage risk and support investor confidence. Our team serves as your drug discovery partner, preserving your autonomy while providing access to our experience, expertise, and facilities. Some key ways we help you mitigate risk include:

- **Open and nimble communication at all stages**
- **Pre-agreed stop / go decision milestones**
- **Bespoke protein science to ensure the highest-quality protein, reduce artefacts in hit discovery, and enable structural biology**
- **Tailored validation via orthogonal screening**
- **Structural data to enable rational, guided optimization of hits, streamline medicinal chemistry timelines, and support and strengthen IP strategy**

Review our publications

- de Vink, P. J. et al (2019). **Cooperativity basis for small-molecule stabilization of protein–protein interactions.** *Chemical Science, 10*(10), 2869–2874.
- Sijbesma, E. et al (2019). **Site-directed fragment-based screening for the discovery of protein–protein interaction stabilizers.** *Journal of the American Chemical Society, 141*(8), 3524–3531.
- Wolter, M. et al (2020). **Selectivity via cooperativity: preferential stabilization of the p65/14-3-3 interaction with semisynthetic natural products.** *Journal of the American Chemical Society, 142*(27), 11772–11783.
- Stevers, L. M. et al (2018). **A thermodynamic model for multivalency in 14-3-3 protein–protein interactions.** *Journal of the American Chemical Society, 140*(43), 14498–14510.
- Geertjens, N. H. J. et al (2022). **Straightforward model construction and analysis of multicomponent biomolecular systems in equilibrium.** *RSC Chemical Biology, 4*(4), 252–260.
- Andrei, S. A. et al (2018). **Rationally designed semisynthetic natural product analogues for stabilization of 14-3-3 protein–protein interactions.** *Angewandte Chemie International Edition, 57*(41), 13470–13474.
- de Vink, P. J. et al (2022). **Cooperativity as quantification and optimization paradigm for nuclear receptor modulators.** *Chemical Science, 13*(9), 2744–2752.
- de Vink, P. J. et al (2017). **A binary bivalent supramolecular assembly platform based on cucurbit[8]uril and dimeric adapter protein 14-3-3.** *Angewandte Chemie International Edition, 56*(33), 8998–9002.

Why work with our team?

- Take advantage of our deep expertise in molecular glue discovery. For further insight, review our peer reviewed publications (left).
- Leverage our purpose-built biophysics platform and cooperativity-driven approach to detect ternary complexes without overlooking potentially promising hits due to weak initial affinity.
- Comprehensive structural enablement for rational, guided optimization of hits, streamlined medicinal chemistry timelines, and a stronger IP strategy.

Next steps

When you're ready to learn more, you can request an informal conversation with our team, in which we'll learn a little more about your objectives, answer any questions you might have, and provide you with the information you need to decide whether to move forward.

Reach out to your relationship manager or contact us at:

- oncodesign-services.com
- contact@oncodesign-services.com

More information

Oncodesign Services is a leading CRO specializing in drug discovery and preclinical services. In 2024, it acquired ZoBio, a boutique CRO with gene-to-lead expertise in small molecules. The Oncodesign-ZoBio group contributes to the development of innovative therapies from target to preclinical candidates through stand-alone and integrated capabilities in medicinal chemistry, computer-assisted drug design, protein production, biophysics, structural biology, DMPK, in vitro / in vivo pharmacology, and in vivo pharmaco-imaging.

The group supports a global portfolio of clients from laboratories based in Dijon (France), Leiden (The Netherlands), Montreal (Canada) and Paris (France).

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