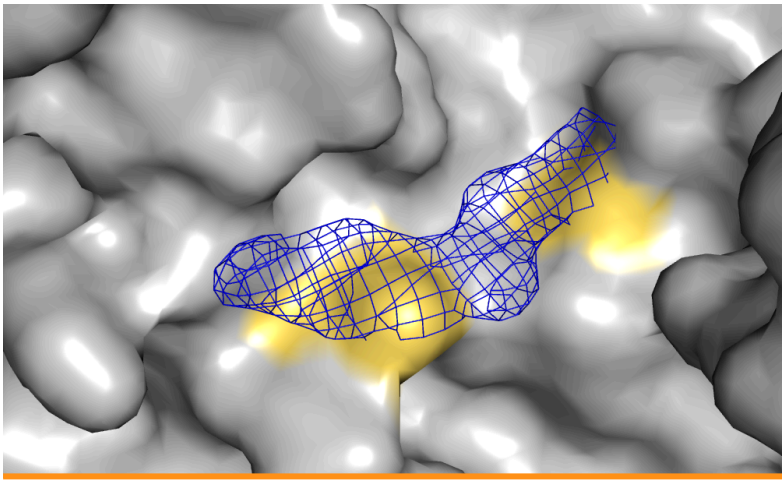
A Structure-Enabled Approach to PPI Drug Discovery

Accessing “Undruggable” Targets with Covalent Compounds

The therapeutic targets of today are more complex than ever before. As the biotech industry moves toward harder-to-drug mechanisms, particularly protein–protein interactions (PPIs), traditional sources of hit matter often fall short. These targets typically involve large, shallow, and flexible interfaces, with few defined binding pockets. In short, they are poorly ligandable.

However, with the right chemistry, platform, and expertise, even highly challenging targets can be accessed.



Above: Crystal structure of a covalent compound simultaneously conjugated to two proximal cysteines on the target. A fragment screen failed to yield hits that were progressable, likely due to the flat, featureless nature of the desired binding site. Two cysteines flank the targeted binding site and one of the compounds bound both. The chicken wire denotes the surface of the compound, the S atoms of the Cys residues are shown in yellow and the remainder of the solvent accessible protein surface is shown in grey.

Covalent Chemistry: Overcoming the Ligandability Challenge

Covalent compounds form an irreversible (or slowly reversible) bond with their protein target, which allows them to “lock in” even modest initial binding interactions. The only requirement for the target is a nucleophilic residue (for example, Cys, Lys or Ser) - residues that are frequently present at the desired ligand binding site. By screening a library of covalently reactive compounds, you can generate a variety of biologically active, structurally enabled, progressable starting points for a small molecule campaign where other sources of non-covalent hit matter are unlikely to succeed or have already failed.

What makes PPI targets so difficult to drug?

Protein-Protein Interactions (PPIs) are central to nearly all biological functions, including immune response, apoptosis, transcription, and more. However, the interfaces involved typically span 1,000–2,000 Å^2 and are often flat or dynamic. These characteristics often make them unamenable to traditional small-molecule binding, and hence very tough to tackle with standard libraries or non-covalent approaches.

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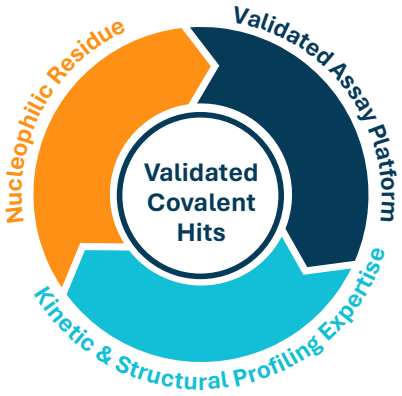
Why Covalent Compounds Are Different



Screening covalent compounds for hits goes beyond traditional reversible binding by leveraging chemistry that forms a permanent (or slowly reversible) bond between a compound and its protein target. This approach depends on two key elements:

- **A warhead:** A reactive functional group on the compound designed to form a covalent bond.
- **A nucleophilic residue:** Most commonly cysteine (Cys), lysine (Lys), or serine (Ser), located at or near the binding site on the target protein.

Careful balance between reactivity and specificity is key. The warhead must be reactive enough to form a bond, yet selective enough to avoid off-target effects. Presence and positioning of a nucleophilic residue are critical, but selectivity also depends on non-covalent compound–protein interactions. Success hinges on the ability to characterize and control these parameters.

Performed correctly, a covalent strategy allows for strong, durable engagement with modestly ligandable or even flat binding surfaces, making covalent screening a powerful tool for challenging targets like PPIs.

Covalent vs Non-Covalent Hits: Selecting the Right Approach for Your Target

Feature	Covalent	Non-Covalent
Binding Interaction		
Bond type	Irreversible / slowly reversible + non-covalent interactions	Reversible, non-covalent interactions
Requirement	Nucleophilic residue	Defined binding pocket
Binding relies on	Covalent bond + complementary non-covalent interactions	Complementary non-covalent interactions
Dissociation	Minimal or none (after covalent bond)	Typically rapid; depends on affinity
Screening Efficiency		
Sensitivity to pocket quality	Low, but high sensitivity to nucleophilic residue local to the binding site	High, requires at least transient well-formed binding site
Hit quality	Good, if screen is run correctly	Low with weak affinity for PPI-like targets
Assay requirements	Kinetic (k_{inact} / K_I)	Standard biochemical / biophysical assays
Hit validation	Complex (kinetics + structure)	More straightforward
Strategic Advantages		
Use for "undruggable" targets	Ideal for poorly ligandable, flat PPIs	Limited success
Selectivity potential	High (if (k_{inact} / K_I optimized)	Moderate to high
Clinical precedent	FDA-approved covalent drugs rising YoY	Vast
Progressability	Rapid access to structural data for DTMA	High but can stall if potency not achievable
When to Consider a Covalent Approach		
Consider a covalent screening approach when:	• No well-formed binding pocket exists on your target, or you do not wish to target existing binding pockets (such as the ATP pocket) • Targeting PPIs with large, featureless surfaces • Exquisite selectivity is required and a nucleophilic residue is uniquely present at the desired binding site on the target (such as the kRas G12C mutation) • Screens of non-covalent hit matter have failed to yield hits • It is known that long residence time is required for therapeutic benefit	

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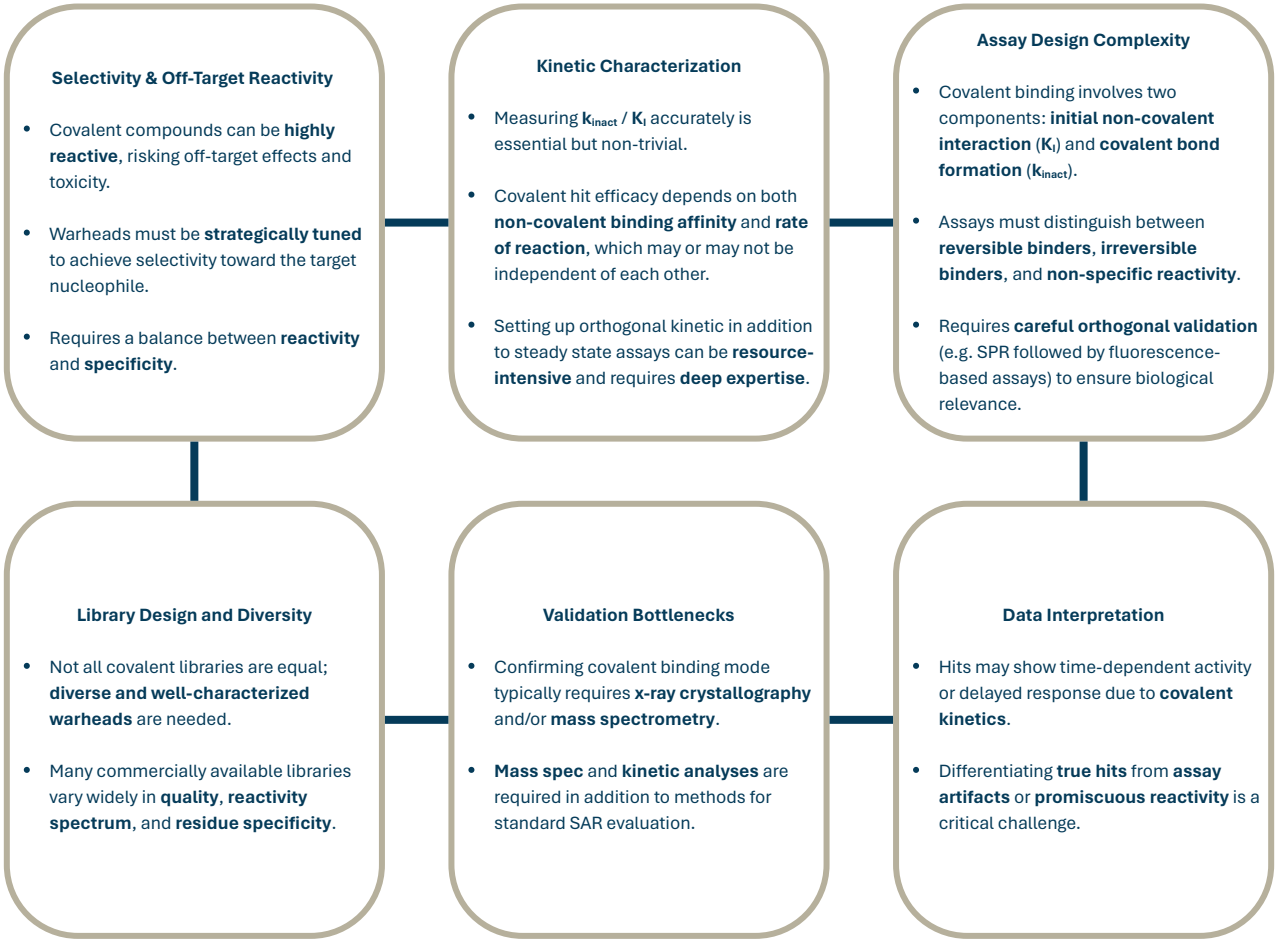
The Challenges Behind Covalent Hit Discovery

While covalent screening offers powerful advantages for targeting difficult proteins, it also presents unique technical challenges that require specialized expertise across assay design, kinetics, and structural validation:



Three-Step Approach to Screening Covalent Compounds

Our integrated covalent screening approach has been designed specifically to address the complexities of working within this rewarding yet challenging area of drug discovery. From high-quality protein production to assay development, screening, and structural validation, our approach combines deep scientific expertise and experience with proven technologies to deliver validated, progressable starting points.

1 Screening Strategy

Getting the Biology Right

- **Biology-first design:** We build assays that report directly on the desired biological activity (e.g., disruption of PPIs), not just reactivity.
- **Validated for robustness:** Every screen is anchored in well-characterized biology, using biophysical techniques such as SPR to validate specificity before scaling.
- **Built for covalent kinetics:** We design screens to capture compounds with true biological activity, not artifacts. We then characterize hits for both reversible binding (K_I) and covalent bond formation (k_{inact}), revealing true, mechanism-led actives.

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2 Hit Discovery

From Target to Screen

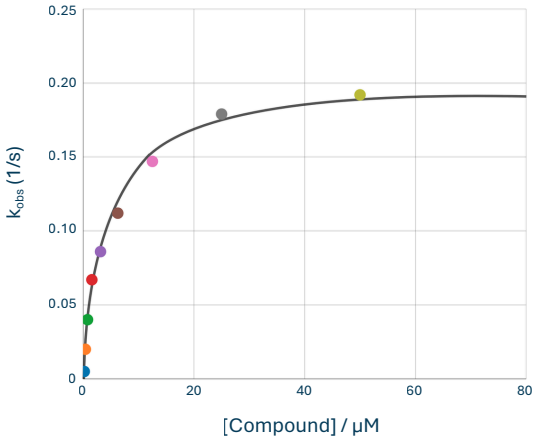
- **Structure-grade protein:** Expressed in E. coli or HEK, tailored to your target for reliable downstream characterization.
- **Fit-for-purpose assays:** Functional assays like FP or tr-FRET built on SPR insights ensure biological relevance.
- **Smart screening workflow:** Pilot → full screen of a curated covalent library, with time points and conditions optimized for each warhead class.

3 Hit Characterization

From Raw Hits to Real Starting Points

- **Structural confirmation:** High-throughput crystallography pinpoints binding mode and elucidates non-covalent interaction.
- **Kinetic profiling:** Quantitative measurement of k_{inact} and K_I ensures hits are both selective and optimizable.
- **Covalent verification:** Intact mass spec confirms stoichiometry and rules out promiscuous reactivity.

Right: *Kinetic profile of a covalently reactive compound. The rate of covalent bond formation at different concentrations of the compound is plotted. This data allows us to abstract k_{inact} and determine k_{inact} / K_I, a key parameter to be optimized during hit evolution.*



Results You Can Move Forward With

What You Receive	What It Means To You
Structurally enabled hits	You'll receive diverse hit series with validated binding modes and clear molecular mechanisms of action, supported by crystallography and kinetic profiling.
Biologically active compounds	All hits are confirmed to disrupt your target's biological function, giving you confidence in their relevance from the start.
Mechanistic clarity	With k_{inact} / K_I data, stoichiometry by mass spec, and orthogonal assay validation, you will develop a robust understanding of how your compounds engage the target.
Rapid progression to leads	Our platform is designed for efficient DMTA cycles, enabling rapid structure-guided optimization and focused library design.
Reduced risk, higher confidence	You start your small molecule campaign on solid ground with meaningful, validated, and progressable starting points.

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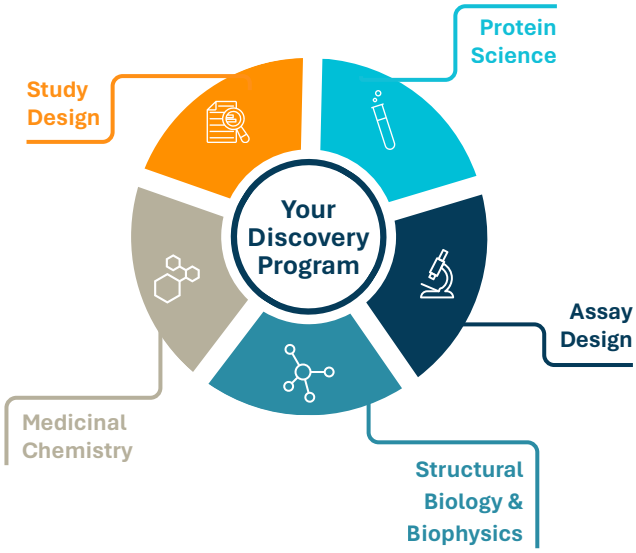
Why Partner with ZoBio?

Choosing the right partner is critical when working with covalent screening. Success depends not only on access to diverse libraries, but also on the expertise to design meaningful assays, interpret complex kinetics, and validate true hits with structural and biophysical precision. Here's what sets our approach apart:

- **Integrated Discovery Platform:** We drive the entire process, from in-house protein production and assay design to screening and structural biology, ensuring quality and consistency throughout.
- **Expertise in Covalent Chemistry:** Our team understands the subtleties of covalent screening, including warhead selection, nucleophile positioning, and the kinetic considerations needed for effective hit triage and optimization.
- **Structure-Driven Decision Making:** We leverage high-throughput crystallography and orthogonal biophysical methods to validate hits and generate atomic-level insight early, enabling smart, efficient progression.
- **Tailored to Tough Targets:** Our platform was built with difficult targets in mind, including protein–protein interactions and poorly ligandable proteins. We succeed where standard approaches fall short.
- **Collaborative and Customizable:** Every project is shaped in close partnership with our clients, with data checkpoints, transparent decision-making, and respect for your autonomy integral to the process.



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:

- **ZoBio.com**
- **BD@zobio.com**

More information

ZoBio is a structure-based drug discovery company supporting pharmaceutical and biotech partners from gene to lead. We facilitate strategically driven hit identification through lead optimization projects for complex, challenging and novel targets. Our teams combine scientific excellence with collaborative delivery to solve tough scientific challenges, de-risk early discovery programs, and accelerate high-quality small molecule candidates into preclinical development.

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