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Covalent Peptide Evolution: Redefining Protein–Protein Interaction Inhibition Through Phage Display.

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ABSTRACT

Covalent cyclic peptides represent a transformative approach for targeting challenging protein-protein interactions (PPIs) characterized by flat, extensive binding surfaces. Recent advances in electrophilic phage display now enable the evolution of these peptides through integrating sulfur(VI) fluoride exchange (SuFEx) chemistry with functional selection strategies. This innovative platform combines genetic encoding with site-specific cyclization and warhead incorporation to generate high-affinity, irreversible binders. When targeting the SARS-CoV-2 Spike-ACE2 interface, the approach produced sub-100 nM inhibitors with >10-fold improved potency over non-covalent analogues. The methodology's success against this clinically relevant target underscores its potential to address longstanding challenges in PPI modulation, particularly for high-value targets in oncology and neurodegeneration. By combining covalent engagement with phage display's evolutionary power, this technology establishes a new paradigm for developing mechanistically validated peptide therapeutics against previously intractable interactions.

Keywords: covalent peptide inhibitors, cyclic peptide therapeutics, phage display evolution, protein-protein interaction inhibition, SuFEx chemistry, irreversible binders, SARS-CoV-2 inhibitors, Spike-ACE2 disruption, electrophilic warheads, undruggable targets, functional selection, peptide macrocycles, PPI drug discovery, covalent phage display

INTRODUCTION

Targeting protein-protein interactions (PPIs) remains a major challenge in drug discovery, largely due to their broad and topologically flat interfaces, which are poorly suited to conventional small-molecule inhibitors.[1] Thanks to their conformational stability and selective binding, cyclic peptides are increasingly recognized as valuable tools for modulating PPIs.[2] For example, the c-Myc/Max interaction—a pivotal oncology target with a flat, extended interface—has resisted decades of small-molecule discovery efforts.[1] However, transitioning from reversible to covalent binding—advantageous for its prolonged engagement and selectivity—remains constrained by the incompatibility of electrophilic chemistry with standard genetic encoding systems.[5]

Recent advances by Wang et al. [6], underscored in a commentary by Jin[7], introduce a novel approach to phage display that incorporates electrophilic elements to evolve covalent peptide binders.

A Dual-Layered Evolutionary Strategy

Wang et al. engineered phage-displayed peptide libraries incorporating dibromoaryl fluorosulfate linkers,[6] introducing sulfur(VI) fluoride exchange (SuFEx) electrophiles that react selectively with nucleophilic amino

acid residues (Tyr, Lys, His) near PPI interfaces. The AXCX$_7$CG format enables two cysteines for cyclization via dibromoaryl fluorosulfate linkers while installing a SuFEx warhead—a sulfur(VI)-based electrophile that reacts selectively under physiological conditions.[6]

The peptides were genetically encoded and fused to the N-terminus of the filamentous phage protein pIII. They were chemically cyclized in situ upon phage assembly, yielding a covalent cyclic peptide–phage library.

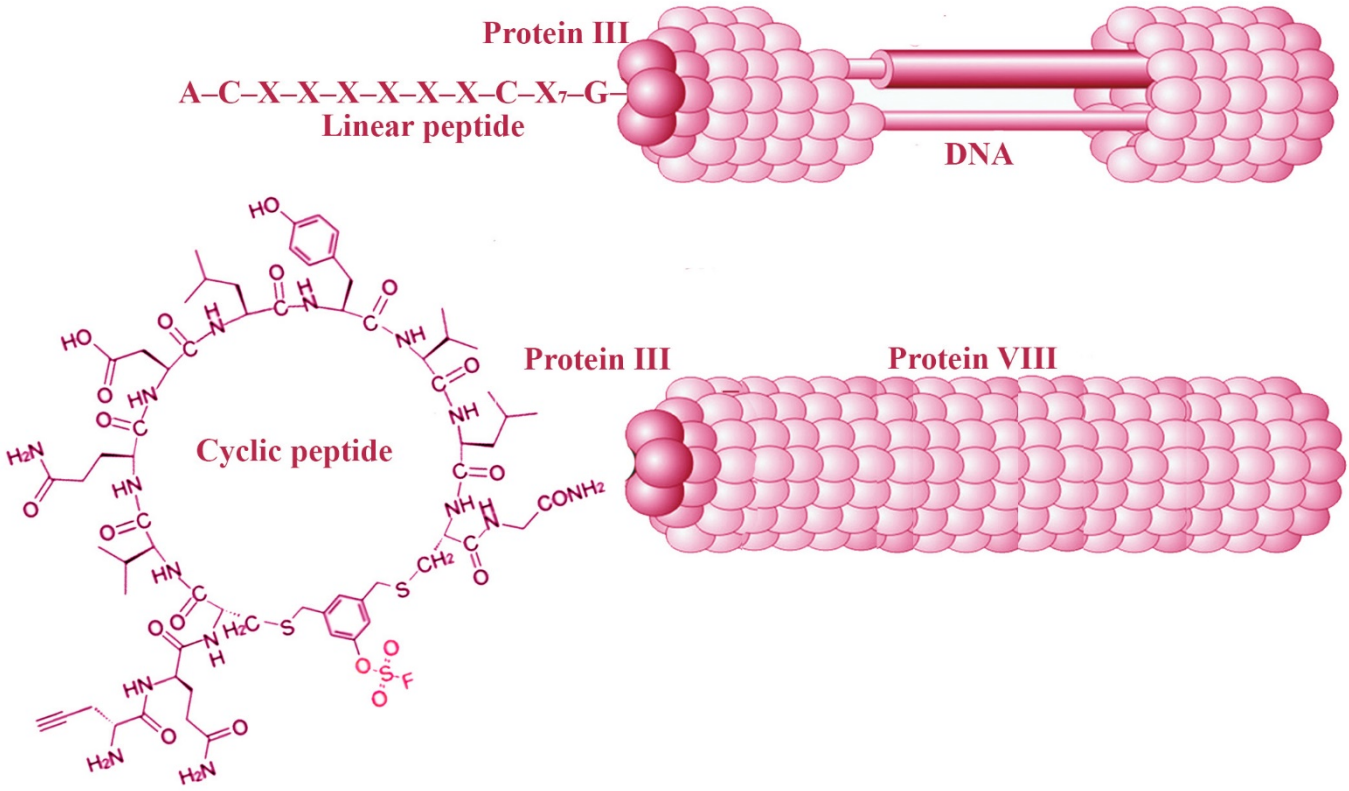

Figure 1. Workflow for Covalent Cyclic Peptide Evolution via Electrophilic Phage Display. Schematic representation of th e library generation and selection process described by Wang et al.[6] Peptides with the sequence AXCX$_7$CG are genetically encoded and fused to the N-terminus of the filamentous phage protein pIII. The linear peptides are displayed on the phage surface and contain two cysteine residues, enabling chemical cyclization via a dibromoaryl fluorosulfate linker. This linker forms a thioether bridge between the cysteines and installs a SuFEx electrophilic warhead (red). The resulting covalent cyc lic peptide-phage library undergoes iterative rounds of (1) positive selection on immobilized target protein (e.g., SARS-Co V-2 Spike), followed by (2) functional counterselection with a competitor (e.g., ACE2 or a covalent nanobody),
To enrich for high-affinity, functional binders. Selected clones are sequenced and validated for covalent target engagement and biological activity. [Adapted from Wang et al. [6] and Jin et al. 2025 [7]]

Applied to the SARS-CoV-2 Spike–ACE2 interaction, the platform employed three rounds of positive selection followed by two rounds of functional counterselection using ACE2 and a covalent nanobody competitor. This ensured the enrichment of peptides binding specifically at the functional interface. The lead peptide exhibited irreversible binding and an IC$_{50}$ of 72 nM against the Omicron BA.2 variant, representing a >10-fold improvement compared to non-covalent peptide inhibitors of Spike–ACE2, such as the linear mini protein 2P2 (IC$_{50}$ ≈ 700 nM).[15]

Beyond Affinity: A Function-First Paradigm

The innovation lies not just in chemistry but in evolutionary logic. Implementing counterselection against non-functional binders shifts the strategy from passive affinity enrichment to mechanistically driven selection. This mirrors Wang et al.'s counterselection strategy, where functional pressure (ACE2 competition) was critical to isolate interface-binding peptides—only 0.1% of initial hits passed this stringent filter.[6]

The framework builds on prior work with cyclic peptide libraries for therapeutic applications, including our efforts using proapoptotic cyclic peptides to impair CK2-mediated phosphorylation and induce antitumor effects.[4] Future developments might include:

- In silico electrophile pre-screening to optimize chemical reactivity[5,9]
- Modular warhead libraries to expand target scope[5,6]
- Orthogonal counterselection against off-targets or homologous proteins[6,7]

For example, counterselection against BRD4 BD1 could yield inhibitors selective over BD2, addressing a key challenge in BET protein targeting.[8,12]

Structural and Therapeutic Advantages of Cyclic Peptides

Conformational Rigidity: Cyclization reduces the entropic penalty of binding by ~5 kcal/mol compared to linear analogs.[3]
Proteolytic Stability: Half-lives exceed 24 hours in serum vs. <1 hour for linear versions.[11]
Permeability: Despite chemical engineering advances, only ~20% of cyclic peptides achieve intracellular concentrations >1 µM without modifications such as N-methylation or stapling.[12]
Drug-like Properties: Cyclic peptides combine the specificity of biologics with small-molecule permeability, enabling them to modulate previously intractable PPIs.[10, 12]

Therapeutic Horizons

This technology opens new doors to previously "undruggable" targets:

- In oncology, covalent inhibition of c-Myc could address its "undruggable" status in over 70% of human cancers.
- In neurodegeneration, targeting Tau's oligomerization interfaces may help block early aggregation events in Alzheimer's disease.[1,12]

Its applications extend across oncology, virology, and neurodegenerative disease and support the development of chemical biology tools for dissecting transient or disordered PPIs.[6,7,12]

CONCLUSIONS

The strategy presented by Wang et al. represents a significant evolution in covalent ligand engineering, merging chemical reactivity with selective evolutionary pressure within the phage display framework. By embedding electrophilic chemistry and functional selection pressure into phage display, this approach offers a powerful platform to generate mechanistically active covalent cyclic peptides, poised to unlock the therapeutic potential of PPIs long considered intractable.

In addition to its therapeutic potential, this methodology may facilitate the development of specialized chemical probes, aiding the validation of complex protein interaction targets in biomedical research.

Ethics statement (even if not applicable):

This article contains no studies with human or animal subjects performed by the author.

Conflict of interest:

N.S.V. is affiliated with 1st Clinical Biotec SL. The author declares no other competing interests.

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