Target-Directed Stapled Peptides through Dynamic Combinatorial Chemistry

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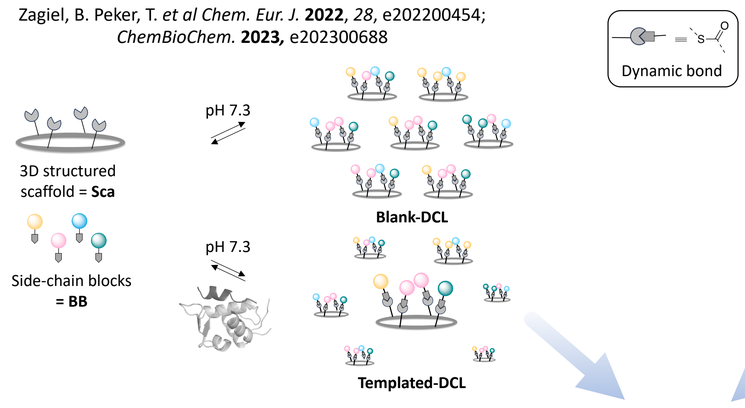
Introduction

Protein–protein interactions (PPIs) are challenging targets for conventional small-molecule drug discovery. Peptides offer a promising alternative, but short linear peptides are highly flexible and susceptible to proteolytic degradation, limiting their pharmacological potential. Conformational constraint can overcome these limitations by stabilizing the bioactive conformation. Two complementary strategies can be considered: (i) constraining the native peptide framework, or (ii) grafting key residues onto a rigid scaffold to reproduce their optimal spatial arrangement. Both aim to recapitulate protein interaction hotspots, but their optimization remains challenging.

Dynamic Combinatorial Chemistry (DCC) enables the rapid exploration of chemical space through reversible library formation and target-driven selection and amplification of high-affinity ligands.[1] DCC has recently been extended to peptide discovery.[2] Building on this concept, we developed target-directed DCC (tdDCC) for both strategies: (i) side-chain selection on defined peptidic scaffolds,[3,4] and (ii) selection of conformational constraints (“staples”) on flexible peptide sequences.[5] Thiol–thioester exchange enables reversible library formation under biologically compatible conditions. The p53–hDM2 interaction was used as a model PPI.[6] Here, we focused on the target-directed optimization of stapled peptides.

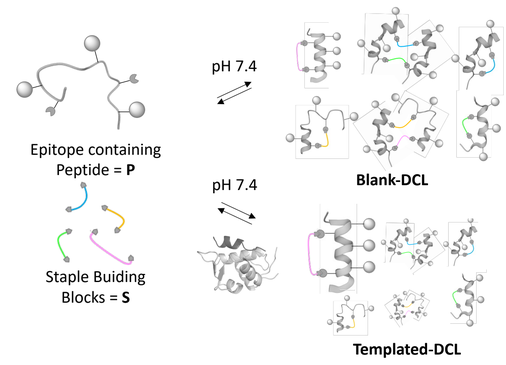
Target-Directed Side-Chain Grafting

Zagiel, B. Peker, T. *et al Chem. Eur. J.* **2022**, *28*, e202200454; *ChemBioChem.* **2023,** e202300688

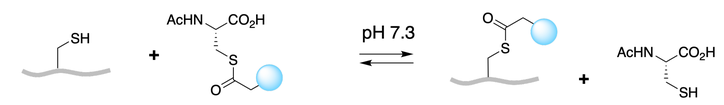


Target-Directed Peptide Stapling

Rodrigues *et al submitted* (Preprint on ChemRxiv)



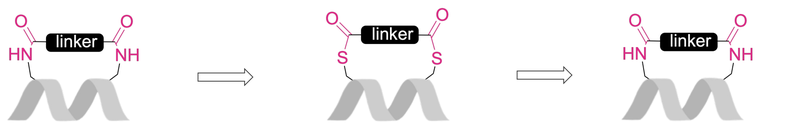
Reversible Chemistry: Thiol to thioester Exchange



- ✓ Reversible in physiological conditions
- ✓ Biocompatible
- ✓ Freezable at acidic pH

tdDCC for Peptide Stapling

Concept

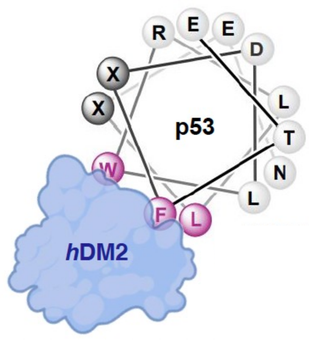


Bis-amide stapled peptides
Inouye and coworkers
Chem. Eur. J. **2008**, *14*, 857–863

Reversible bis-thioester stapled peptide
This work

Transposition into a stable bis-amide stapled peptide surrogate

Peptide Design

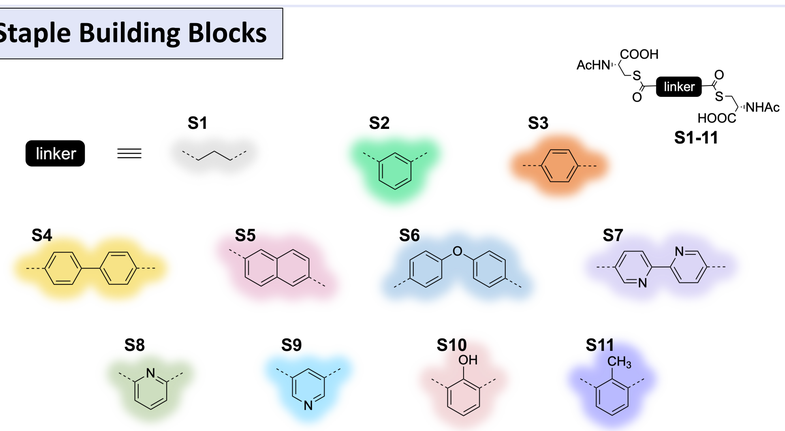


p53$^{17-29}$: ETFSDLWKLLPEN

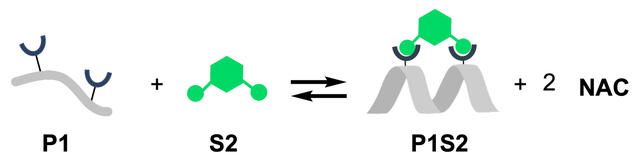
P1-2: Ac-ETF**X**DLWRLL**X**EN-NH_2

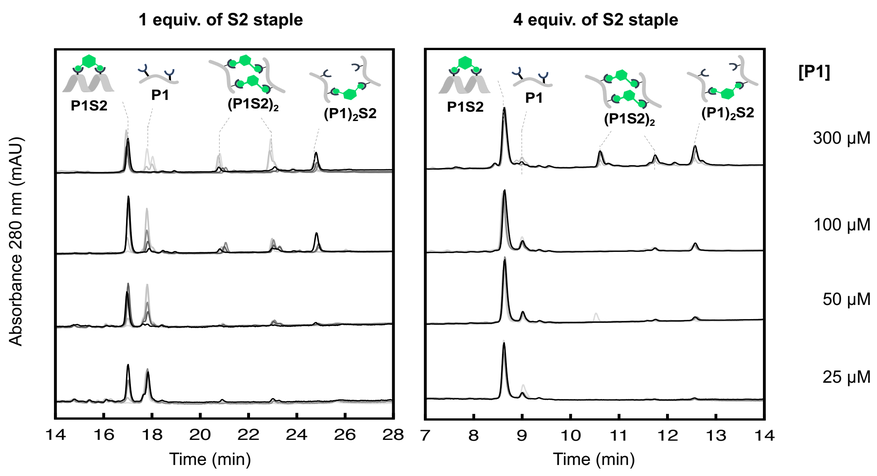
P1: X=Cys and **P2**: X=hCys

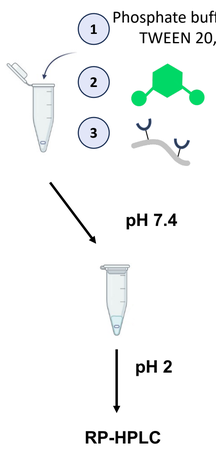
Staple Building Blocks



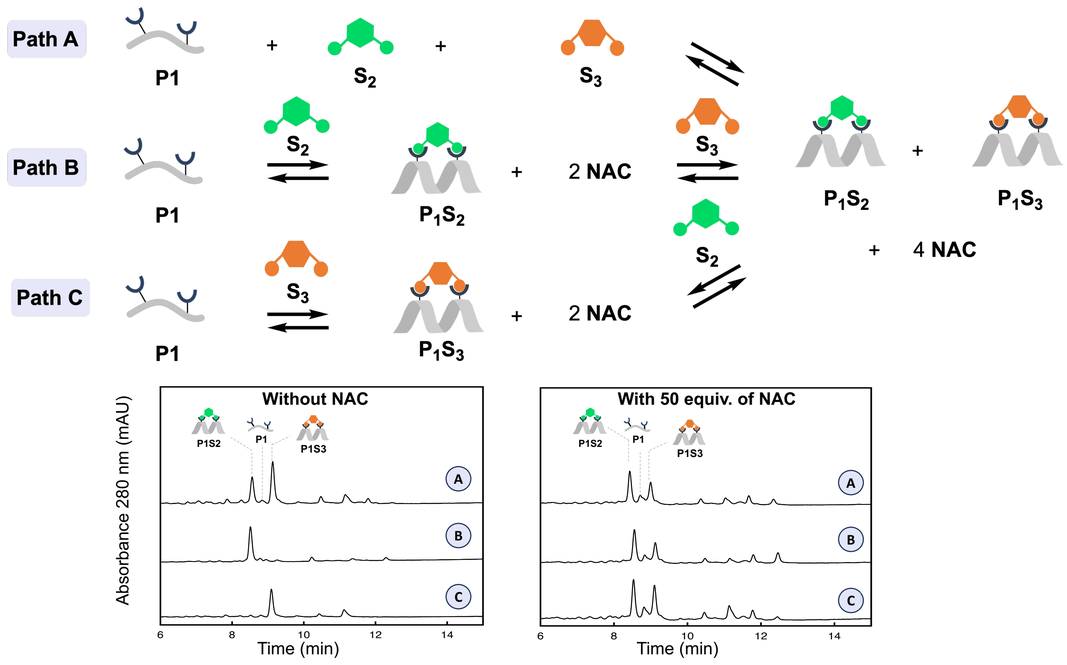
Reaction Optimization







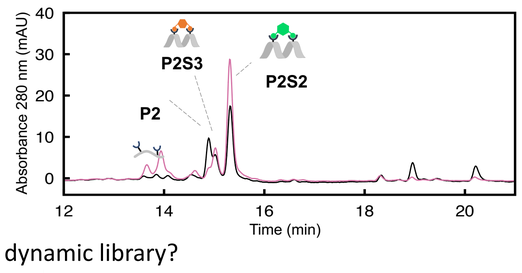
Reversibility



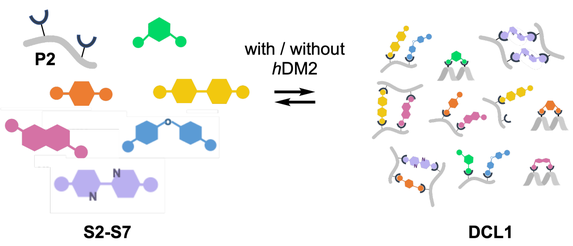
Target-Directed DCC Screening

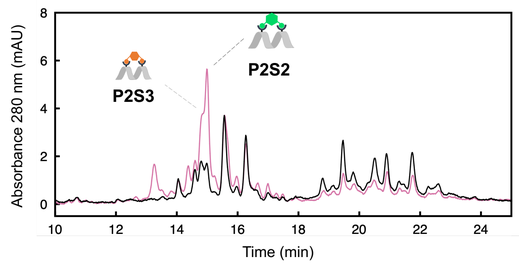
❖ Can tdDCC identify target-stabilized stapled peptides?



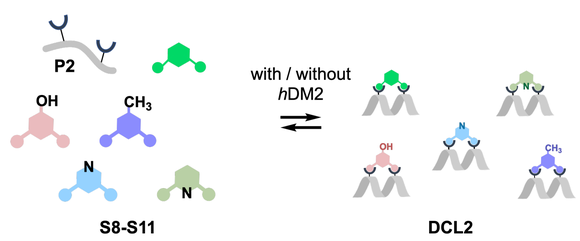


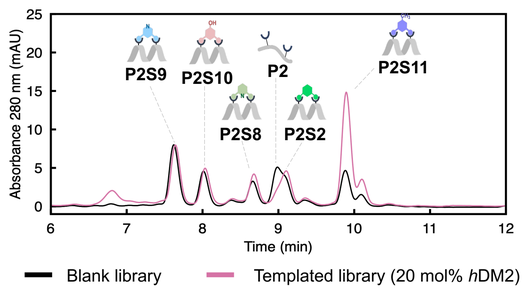
❖ Can tdDCC select the preferred stapling geometry from a larger dynamic library?





❖ Can tdDCC reveal target-dependent chemical preferences within the selected m-phenylene stapling scaffold?





DCC Hit Validation

Competition fluorescence polarization binding curves: tdDCC hit translate into tighter binder

Circular dichroism analysis

Hit Translation

P3: Ac-ETF**Dab**DLWRLL**Dab**EN-NH_2

Acknowledgements

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Key Takeaways

We demonstrate target-directed dynamic combinatorial chemistry (tdDCC) as an adaptive strategy for the discovery and optimization of stapled peptides with target-dependent recognition properties. By enabling peptides to dynamically sample multiple staple architectures, tdDCC first identifies the preferred stapling geometry and subsequently reveals target-dependent chemical preferences within the selected scaffold. This hierarchical selection provides a streamlined strategy for staple optimization from a single peptide sequence.

References:

[1] P. Frei, R. Hevey, B. Ernst, *Chem. Eur. J.* **2019**, *25*, 60–73; [2] A. Rodrigues, L. Rocard, R. Moumné, *ChemSystemsChem* **2023**, e202300011 [3] B. Zagiel, T. Peker, R. Marquant, G. Cazals, G. Webb, E. Miclet, C. Bich, E. Sachon, R. Moumné, *Chem. – Eur. J.* **2022**, *28*, e202200454, [4] T. Peker, B. Zagiel, L. Rocard, C. Bich, E. Sachon, R. Moumné, *ChemBioChem* **2023**, *24*, e202300688; [5] A. Rodrigues, J. G. Coll, D. Ravault, B. Zagiel, L. Carlier, L. Rocard, R. Moumné, **2026**, ChemRxiv; [6] L. Rocard, S. Mehdi, M. Dogrusoz, A. Rodrigues, R. Moumné, “Peptide Constraining and Scaffold Grafting for Protein Mimetics Design: Lessons from the p53–hDM2 Interaction.” *RSC Medicinal Chemistry* **2026**, *accepted*.