Combining Flow-Based Chemical Peptide Synthesis and Biochemical Tools to Investigate Post-Translationally Modified Peptides with a Focus on Lasso Peptides

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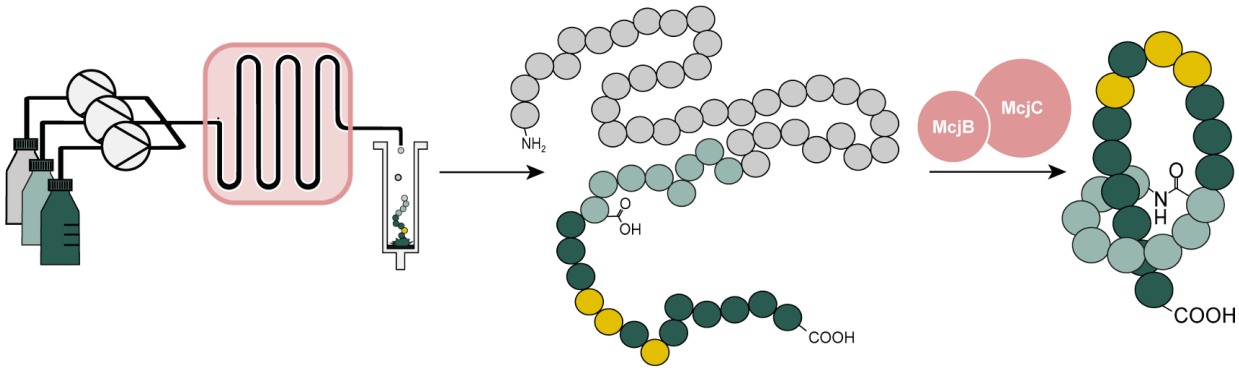
Peptides are versatile biomolecules whose functions are strongly influenced by post-translational modifications (PTMs) and higher-order structures. However, accessing complex peptide architectures and precisely installed modifications remains a major challenge. This work demonstrates how automated fast-flow peptide synthesis (AFPS), combined with enzymatic maturation, enables rapid access to structurally and functionally diverse peptides.

Lasso peptides, mechanically interlocked natural products, served as a model system. While purely chemical approaches failed to produce the threaded topology, they revealed key structural constraints governing lasso formation. We therefore developed a hybrid chemoenzymatic approach in which AFPS-generated precursor peptides with non-canonical residues and backbone modifications were processed by native biosynthetic enzymes. This approach revealed remarkable enzyme promiscuity and enabled the rapid generation of diverse lasso peptides, linking chemical modification to biological activity.



Overall, this work establishes AFPS as a versatile platform for integrating chemical synthesis with biological catalysis, providing new opportunities for the design, diversification, and functional study of complex peptide architectures, opening new avenues in peptide engineering and drug discovery.

[1] K. Schiefelbein, J. Lang, M. Schuster, C.E. Grigglestone, R. Striga, L. Bigler, M.C. Schuman, O. Zerbe, Y. Li, N. Hartrampf, *J. Am. Chem. Soc*, **2024**, *146(25)*, 17261-17269.