Exploring the Chemical Space of Lasso Peptides by Merging Flow Synthesis and Enzymatic Transformation

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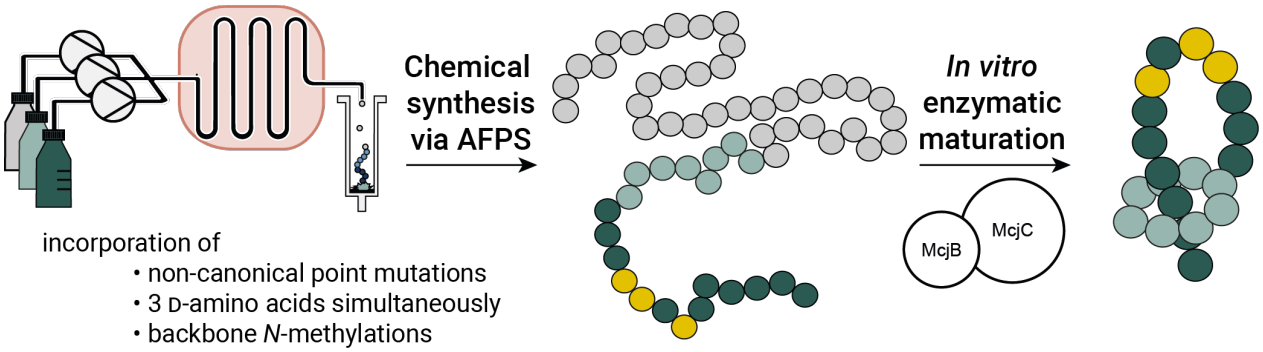
Lasso peptides are a subclass of ribosomally synthesized and post-translationally modified peptides (RiPPs) classified by their conformationally constrained lariat knot-like structure. Their remarkable enzymatic, thermal, and chemical stability combined with a variety of biological activities make them attractive scaffolds for drug development.[1] The total chemical synthesis of lasso peptides such as microcin J25 (MccJ25) remains elusive. Therefore, expanding the chemical space of MccJ25—an antagonist of Gram-negative bacteria—beyond the range of biological methods is challenging.[2] The in vitro transformation of non-natural precursor peptides could enable the production of MccJ25 derivatives.[3] **In this study, we employ flow-based peptide synthesis to obtain chemically modified precursor peptides and enzymatically transform them, *in vitro*, into correctly folded lasso peptides.**



The recombinant expression of the processing enzymes, McjB and McjC, was extensively optimized to facilitate the transformation of modified precursor peptides to multiple analogs of MccJ25, including the incorporation of non-canonical tyrosine and histidine residues. A biologically active analog with three D-amino acids and an MccJ25 derivative containing backbone N-methylations were produced, showcasing the versatility of our chemoenzymatic strategy. This method affords access to chemically modified lasso peptides previously inaccessible by strictly biological methods, thus increasing the scope of potential modifications to improve therapeutic properties.

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