Split inteins for generating combinatorial nonribosomal peptide libraries

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Antibiotics such as Penicillin, Vancomycin, Daptomycin, and Colistin are natural products produced by complex biosynthetic pathways involving non-ribosomal peptide synthetases (NRPS). These enzymes are able to incorporate structural features like cyclizations, acyl chains, D- or other non-proteinogenic amino acids or N-methylations, which confer favorable drug-like properties like high affinity, target selectivity, proteolytic stability, and cell permeability[1].

Despite longstanding efforts to engineer NRPS, modified peptides were often only produced in very low amounts or only small changes in the peptide backbone could be achieved. However, recent breakthroughs in megasynthetase engineering, such as the XU and XUT concepts, have renewed interest in using these systems for compound library generation[2, 3].

In this study, we introduce inteins as a novel molecular tool for NRPS engineering. Using the xenotetrapeptide synthetase as a model system, we demonstrate the robust and efficient generation of recombinant NRPS from up to three different natural systems. We have created a library of more than 200 new-to-nature peptides that can be biocatalytically produced in *E. coli* using combinatorial transformation of only 21 plasmids.

In contrast to synthetic macrocycle libraries, such genetically encoded compound libraries make the production and screening of natural product-like macrocycles easier and more broadly accessible to the research community. We are currently applying this library in various target-based and bioactivity screens to explore its potential as an entirely new source of antimicrobial compounds.

References

1. Süssmuth, R.D. & Mainz, A. Nonribosomal Peptide Synthesis—Principles and Prospects. *Angewandte Chemie International Edition* **56**, 3770-3821 (2017).
2. Bozhüyük, K.A.J. et al. De novo design and engineering of non-ribosomal peptide synthetases. *Nature Chemistry* **10**, 275-281 (2018).
3. Bozhüyük, K.A.J. et al. Evolution-inspired engineering of nonribosomal peptide synthetases. *Science* **383**, eadg4320 (2024).