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Combinatorial biosynthesis of nonribosomal peptide antibiotics

Nonribosomal peptide synthetases (NRPSs) produce numerous biologically active peptides, including potent antibiotics, and are suitable for biosynthetic engineering due to their modular molecular architecture. NRPS modules typically consist of adenylation (A), condensation (C), and thiolation (T) domains organized in huge multimodular proteins like beads on a string. These modules elongate the nascent peptide chain one amino acid at a time. Several NRPS engineering approaches have been proposed in the past that suffer from various shortcomings. Binding pocket mutagenesis can be laborious and insufficient for larger changes in specificity and module or domain swaps can disrupt interdomain contacts.

In this project, a streamlined process for combinatorial biosynthesis of nonribosomal peptides will be developed based on ‘subdomain swapping’. These subdomains encompass known specificity determining factors of the A domain and are short enough for cost-effective gene synthesis. When subdomains are retrieved from sequence databases and synthesized, subdomain swapping can potentially produce a much larger number of module permutations than previous approaches. Strategies for combinatorial subdomain swapping will be developed to change multiple residues in one nonribosomal peptide. Using a novel microfluidics platform for antibiotic discovery, the resulting NRPS libraries will be screened for production of antibiotic peptides in collaboration with another PhD student. Directed evolution will be applied to overcome potential activity losses and to optimize peptide production. If successful, subdomain screening may accelerate the biosynthetic engineering of peptide natural products and contribute to the

development of tailor-made drugs.

Publications

Huang HM, Stephan P, Kries H (2021) Engineering DNA-Templated Nonribosomal Peptide Synthesis. *Cell Chem Biol* 28(2), 221-227.e7. Details PubMed

Guo H, Schmidt A, Stephan P, Raguž L, Daniel Braga D, Kaiser M, Dahse HM, Weigel C, Lackner G, Beemelmanns C (2018) Precursor-directed Diversification of Cyclic Tetrapeptidic Pseudoxylallemycins. *Chembiochem* 19(21), 2307-2311. Details PubMed

Supervisor

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