Engineering DNA templated nonribosomal peptide synthesis

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Nonribosomal peptide synthetases (NRPSs) that protect microorganisms against environmental threats by producing siderophores or antibiotics, for instance, are predisposed for biosynthetic engineering because of their modular molecular structure. We have explored several strategies for the redesign of NRPS specificity. Notable examples are the incorporation of a clickable amino acid through targeted binding pocket mutagenesis or specificity transfer through swapping of small protein fragments. Incorporation of clickable amino acids has further enabled a strategy for high-throughput sorting of mutagenized NRPSs leading to a remarkable switch in substrate specificity from alpha- to beta-phenylalanine. However, compared to the ease by which the ribosomal code can be rewritten to generate novel peptide and protein sequences, NRPS engineering remains cumbersome.

Here, we demonstrate the addition of DNA templates to nonribosomal peptide synthetases to facilitate NRPS reprogramming. We have deconstructed the NRPS for the cyclic decapeptide gramicidin S into modules that were later reassembled on a DNA template. For this purpose, we added zinc finger proteins to each module that mediate specific DNA binding to 9 bp recognition sites on the DNA template. Partially disabled docking domains installed on the termini of the modules only mediate efficient module-communication in the presence of the DNA strand. The amounts of various peptide products reacted strongly to the module sequence encoded on the DNA. Several unnatural peptide sequences could be synthesized with the same set of engineered modules, although turnover rates were compromised. In the future, DNA programmable NRPSs honed by laboratory evolution could provide facile access to natural product-like peptides.