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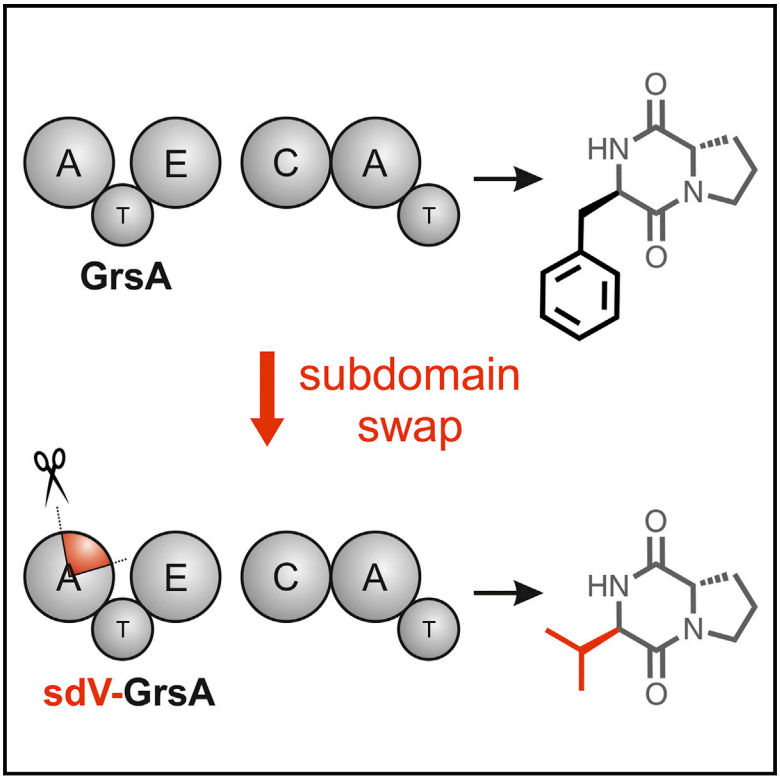
A Subdomain Swap Strategy for Reengineering Nonribosomal Peptides

Graphical Abstract



Authors

Hajo Kries, David L. Niquille, Donald Hilvert

Correspondence

hilvert@org.chem.ethz.ch

In Brief

Nonribosomal peptide synthetases (NRPSs) produce a plethora of bioactive natural products that can be further diversified by enzyme engineering. Kries et al. successfully employ swapping of short subdomains to transfer specificity from one NRPS module to another. This approach complements other engineering strategies and may facilitate combinatorial biosynthesis of novel peptides.

Highlights

- Specificity of nonribosomal peptide synthetases (NRPSs) is encoded on subdomains
- Subdomain swaps can be used to reprogram the specificity of a dipeptide synthetase
- An engineered construct successfully incorporates valine into a dipeptide
- Transplanting short subdomains may be advantageous for combinatorial screening

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A Subdomain Swap Strategy for Reengineering Nonribosomal Peptides

Hajo Kries,[1,2,3] David L. Niquille,[1,2] and Donald Hilvert[1,*]
[1]Laboratory of Organic Chemistry, ETH Zurich, 8093 Zürich, Switzerland
[2]Co-first author
[3]Present address: Department of Biological Chemistry, The John Innes Center, Norwich NR4 7UH, UK
*Correspondence: hilvert@org.chem.ethz.ch
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SUMMARY

Nonribosomal peptide synthetases (NRPSs) protect microorganisms from environmental threats by producing diverse siderophores, antibiotics, and other peptide natural products. Their modular molecular structure is also attractive from the standpoint of biosynthetic engineering. Here we evaluate a methodology for swapping module specificities of these mega-enzymes that takes advantage of flavodoxin-like subdomains involved in substrate recognition. Nine subdomains encoding diverse specificities were transplanted into the Phe-specific GrsA initiation module of gramicidin S synthetase. All chimeras could be purified as soluble protein. One construct based on a Val-specific subdomain showed sizable adenylation activity and functioned as a Val-Pro diketopiperazine synthetase upon addition of the proline-specific GrsB1 module. These results suggest that subdomain swapping could be a viable alternative to previous NRPS design approaches targeting binding pockets, domains, or entire modules. The short length of the swapped sequence stretch may facilitate straightforward exploitation of the wealth of existing NRPS modules for combinatorial biosynthesis.

INTRODUCTION

The evolutionary history of proteins provides a rich source of ideas worthy of imitation by protein engineers (Glasner et al., 2007). These ideas extend beyond single point mutations. Protein evolution "one amino acid at a time" (Bloom and Arnold, 2009; Tracewell and Arnold, 2009) alone cannot account for the vast diversity of proteins found in the biological world. In addition to incremental sequence optimization, genetic events that perturb protein folds more drastically present shortcuts to remote areas of the fitness landscape (Grishin, 2001; Lupas et al., 2001). For example, gene fusions, circular permutations, and illegitimate recombinations between unrelated genes (Lupas et al., 2001) mix and match folding units of proteins. Comparisons of bacterial genomes show that protein domains from one species are sometimes found as freestanding proteins in another species (Enright et al., 1999). In other cases, the freestanding progenitors may no longer exist but can be inferred from phylogenetic analyses and structural studies. The widespread $(\beta/\alpha)_8$ fold of TIM barrel enzymes, for instance, is believed to have arisen from fusion of two no longer extant half barrels (Farías-Rico et al., 2014; Lang et al., 2000). Insertion-deletions can also alter protein topology in a dramatic fashion (Grishin, 2001).

Protein engineering can be very successful when designers turn to nature for inspiration. For instance, introduction of point mutations, screening, selection, and recombination in directed evolution experiments is nothing more than a recapitulation of Darwinian evolution in the laboratory (Jäckel and Hilvert, 2010). In analogy to homologous recombination in nature, family shuffling of genes takes advantage of the natural diversity in a protein family to boost activity in laboratory evolution (Crameri et al., 1998; Minshull and Stemmer, 1999). Circular permutation, too, is a tool that has been used for protein engineering, for example to modulate substrate specificity of the ene-reductase old yellow enzyme (Daugherty et al., 2013). In another impressive design effort, a $(\beta/\alpha)_8$ barrel was illegitimately recombined with a $(\beta/\alpha)_4$ motif excised from a flavodoxin fold (Bharat et al., 2008), thereby swapping four strands of the barrel. Only a few mutations were needed to restore stability and ligand binding capability to the artificial fusion protein (Eisenbeis et al., 2012). Mixing and matching of protein fragments has also been successfully applied to multimodular natural product synthetases (Calcott and Ackerley, 2014; Cane et al., 1998; Williams, 2013).

Domains of modular nonribosomal peptide synthetases (NRPSs) (Tanovic et al., 2008), polyketide synthetases, and fatty acid synthetases (Smith and Tsai, 2007) lend themselves to engineering by fragment recombination because they are connected like beads on a string. NRPSs, for example, are multienzyme clusters in which the number and order of domains usually determines the sequence of the synthesized peptide. Adenylation (A), condensation (C), and thiolation (T) domains work together in modules that elongate nascent peptides by successive addition of amino acids. Permutation of NRPS domains and individual modules has shown great promise for the purposeful, combinatorial biosynthesis of novel peptides (Calcott and Ackerley, 2014; Cane et al., 1998; Kries and Hilvert, 2011; Williams, 2013).

In a seminal study, Stachelhaus et al. engineered surfactin A synthetase by transplanting A domains to alter amino acid specificities (Stachelhaus et al., 1995). Module deletions (Mootz et al., 2002), insertions (Butz et al., 2008), and fusions of unrelated

