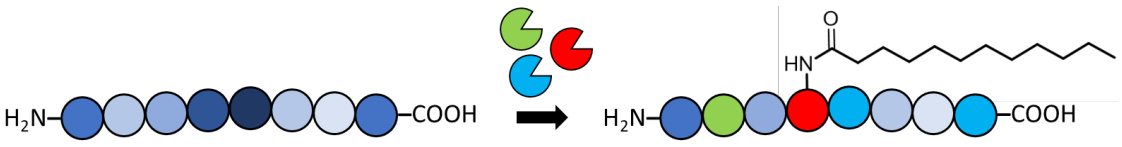
Exploiting post-translational biocatalysts to generate lipopeptide diversity

Anna L. Vagstad[1], Johannes Eckert[1], Nina M. Bösch[1], Florian Hubrich[1], Daniel Richter[1], Annina Meyer[1], Serina Robinson[2], and Chris Field[1]

[1] Institute of Microbiology, ETH Zurich, Vladimir-Prelog-Weg 4, 8093 Zürich, Switzerland
[2] Eawag, Überlandstrasse 133, 8600 Dübendorf, Switzerland
avagstad@ethz.ch

The incorporation of unnatural amino acids, conformational constraints, and conjugates can improve the physiochemical properties of peptide therapeutics.[1] Maturation enzymes from the biosynthesis of ribosomally synthesized and post-translationally modified peptide (RiPP) natural products offer a rich resource for peptide-modifying biocatalysts.[2] Genome mining such pathways led to the discovery of novel enzyme functions: for example, radical-mediated peptide epimerases that irreversibly install D-amino acids[3,4], atypical arginases that hydrolyze peptidyl arginine to ornithine[5,6], and N-acyltransferases that conjugate diverse fatty acid moieties from endogenous lipid pools to the sidechain of ornithine/lysine residues[7]. Such naturally promiscuous enzymes offer a promising mechanism for the diversification of gene-encoded peptide libraries for drug development as the substrate peptide sequence is easily altered by standard codon mutagenesis. The sidechain *N*-acyltransferases are of particular interest since lipidation is known to fine-tune the bioactivity of peptide therapeutics—increasing serum half-life and in some cases facilitating cell permeability or improving function against membrane targets.[8] To lay the foundation for the use of these enzymes in biotechnology applications, we surveyed the natural diversity of *N*-acyltransferases using a targeted genome mining approach with heterologous production of prioritized lipopeptides in *Escherichia coli*, revealing C10-C18 fatty acyltransferases. Mutagenesis studies have helped to elucidate the underlying principles of substrate-specificity and demonstrate tolerance to diverse peptide substrates while maintaining the fidelity of the attached fatty acid. Furthermore, natural and artificial lipopeptide pathways are being reconstructed to allow rapid recombineering with additional post-translational enzymes in different bacterial hosts. Insights garnered from these studies will help establish branched lipopeptides as a new source of natural and engineered bioactive agents.



[1] M. Muttenthaler, G. F. King, D. J. Adams, P. F. Alewood, *Nat Rev Drug Discov* **2021**, *20*, 309.
[2] M. Montalbán-López,et al., *Nat Prod Rep* **2021**, *38*, 130.
[3] B. I. Morinaka, A. L. Vagstad, M. J. Helf, M. Gugger, C. Kegler, M. F. Freeman, H. B. Bode, J. Piel, *Angew Chem Int Ed Engl* **2014**, *53*, 8503.
[4] M. F. Freeman et al., *Science* **2012**, *338*, 387.
[5] S. Mordhorst, B. I. Morinaka, A. L. Vagstad, J. Piel, *Angewandte Chemie International Edition* **2020**, *59*, 21442.
[6] S. Mordhorst, T. Badmann, N. M. Bösch, B. I. Morinaka, H. Rauch, J. Piel, M. Groll, A. L. Vagstad, *ACS Chem Biol* **2023**, *18*, 528.
[7] F. Hubrich, N. M. Bösch, C. Chepkirui, B. I. Morinaka, M. Rust, M. Gugger, S. L. Robinson, A. L. Vagstad, J. Piel, *PNAS* **2022**, *119*.
[8] P. Kurtzhals, S. Østergaard, E. Nishimura, T. Kjeldsen, *Nat Rev Drug Discov* **2023**, *22*, 59.