UNLOCKING BIOCATALYTIC ACYLATIONS BY ENZYME REPURPOSING AND ENGINEERING FOR AMIDE SYNTHESIS

Christian Schnepel, KTH Royal Institute of Technology, Stockholm, Sweden
schnepel@kth.se
Laura Rodríguez-Pérez, The University of Manchester, Manchester, United Kingdom
Yuqi Yu, The University of Manchester, Manchester, United Kingdom
Martin A. Hayes, AstraZeneca, Gothenburg, Sweden
Nicholas J. Turner, The University of Manchester, Manchester, United Kingdom
Sabine L. Flitsch, The University of Manchester, Manchester, United Kingdom

Key Words: Multistep cascades, enzyme repurposing, amide formation, targeted mutagenesis, coenzyme A

Acylation reactions, especially amide bond formations, account for a vast number of transformations in organic synthesis and are the predominant reaction in pharmaceutical synthesis. Such reaction types are of utmost importance in medicinal chemistry and fine chemical synthesis enabling the assembly of complex molecular scaffolds. Consequently, there is a huge incentive in the development of green methodologies that allow for the efficient formation of amides, whereas conventional approaches typically suffer from hazardous conditions, low atom economy and require toxic reagents. Enzyme-catalysed approaches that facilitate direct activation and coupling of carboxylic acids to amides are most sought after but remain scarce until now. In metabolism, thioesters play a central and unique role as acyl carriers in enzyme catalysis due to their chemical properties as sufficiently stable, but highly reactive acyl building blocks. They are particularly relevant for the biochemistry of coenzyme A (CoA-SH), that functions as an essential cofactor for biocatalytic *N*-, *O*-, and *C*-acylations. A vast number of acyltransferases is available from natural sources, yet their applications are prohibited by restricted access to thioester substrates. Cognate coenzyme A (CoA-SH) ligases that are able to provide acyl-S-CoA substrates are typically rather specialised for their native carboxylic acid substrate and of limited use in biocatalysis, not allowing for promiscuous applications.

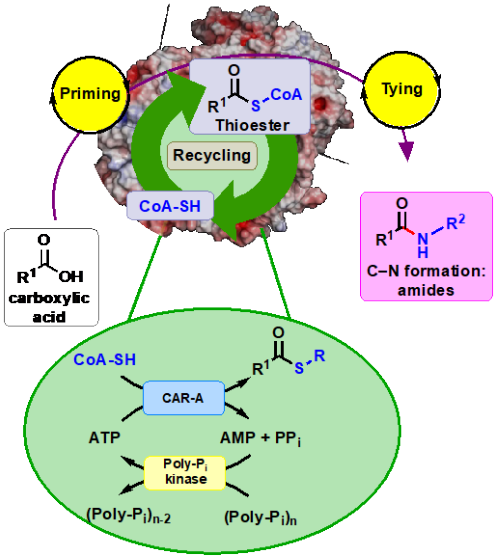

Figure 1 – Modular biocatalytic platform for the generation and recycling of thioesters enabling direct functionalisation of carboxylic acids

This dilemma demands for a more generic thioester generation and recycling system that can be applied in amide synthesis, for example. We found that the adenylation domain of a carboxylic acid reductase can function as a generic thioester synthetase, which can be utilised for the synthesis and recycling of acyl-S-CoA and other thioesters (Figure 1). Exploiting this viable platform opens up manifold applications towards thioester-dependent, enzyme-catalysed C–N couplings and beyond that will be presented here. Firstly, we demonstrate enzyme repurposing towards a new catalytic entity enabling generic thioester recycling. Secondly, implementation of thioester recycling for challenging amide formations in water is being presented. Thirdly, recent developments of rational engineering of a modular enzyme cascade towards a range of bioacylations are shown.

1. M. Lubberink, C. Schnepel, J. Citoler, S. R. Derrington, W. Finnigan, M. A. Hayes, N. J. Turner, S. L. Flitsch, ACS Catal. 2020, 10, 10005–10009.
2. M. Lubberink, W. Finnigan, C. Schnepel, C. Baldwin, N. J. Turner, S. L. Flitsch, Angew. Chem. Int. Ed. 2022, 61, e202205054
3. C. Schnepel, L. R. Pérez, Y. Yu, A. Angelastro, R. S. Heath, M. Lubberink, F. Falcioni, K. Mulholland, M. A. Hayes, N. J. Turner, S. L. Flitsch, Nat Catal 2023, 6, 89–99.