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Expanding the enzyme universe: Exploration of novel scaffolds for artificial enzymes

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PURPOSE OF THE ABSTRACT

Biocatalysis is a sustainable alternative to environmentally damaging and energy-intensive chemical transformations.[1,2] The use of enzymes has become an attractive approach for the chemical and pharmaceutical industries because they can catalyze reactions with exquisite chemo-, regio- and enantioselectivity, along with fulfilling the requirements of green chemistry. However, biocatalytic strategies are often restricted to metabolic reactivities. Whereas, asymmetric chemical approaches, such as the use of organocatalysts; while allowing performing abiological reactions, tend to be less effective exhibiting relatively low turnover numbers.[3,4] These drawbacks can be addressed by combining both systems through the expansion of the genetic code. In the last 20 years, this methodology has emerged as a powerful strategy to incorporate non-canonical amino acids (ncAAs) with new-to-nature chemical groups into proteins (alloproteins/alloenzymes).[5,6] Inspired by well-studied organocatalysts,[3,4] we aimed to expand the repertoire of protein functional groups by incorporating ncAA-bearing secondary amines-based residues. In this work, we (re)designed a set of biomolecular scaffolds for the Michael addition of nitromethane into cinnamaldehyde. This is an abiological reactivity with relevance in the pharmaceutical industry for synthesizing valuable enantiopure γ-nitroaldehydes.[7] First, we proposed a panel of putative alloenzymes by inspecting their natural binding for cinnamaldehyde. Then, we defined a restricted amount of mutations for the amber stop codon (for the cellular incorporation of ncAA). The putative alloenzymes were expressed, purified and the in-house-ncAA cellualr incorporation was confirmed by LC-MS. Finally, the catalytic performance of the alloproteins was investigated upon the iminium catalysis model reaction (Michael addition of nitromethane to cinnamaldehyde). The in vivo incorporation of functional secondary amines showed a biotechnologically attractive potential, expanding the available toolbox for protein engineering and sustainable, abiological biocatalysis.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Biocatalysis | Genetic Code Expansion | Non-canonical amino acids | Iminium catalysis

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