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Expanding the enzyme universe: Incorporation of functional secondary amines through stop codon suppression

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PURPOSE OF THE ABSTRACT

Enzymes are immensely powerful catalysts. Their unmatched rate accelerations and impressive selectivities have inspired chemists to unlock their catalytic potential in industrial applications. This industrial biotechnology revolution is of great importance since enzymes can represent an environmentally friendly, “green" alternative to multi-step chemical syntheses, which often require harsh conditions, utilize toxic reagents and solvents, and show poor atom economy [1]. Notably though, natural enzymes are only able to catalyze a fraction of the reactions routinely employed by synthetic chemists. As a result, creating designer biocatalysts with the ability to efficiently catalyze transformations that cannot be found in nature's repertoire remains a long-standing goal in chemical biology [2]. Efforts toward this goal typically consider only canonical amino acids in the initial design process. However, natural amino acids contain limited functional groups covering relatively limited chemistry and as a result, posttranslational modifications and cofactors are often required for proteins to gain their full functionality.

In this study, a panel of ncAAs with functional secondary amines was designed, synthesized, and characterized, and a library of designer enzymes was prepared by incorporating these newly prepared ncAAs into an evolvable protein scaffold. (Figure 1) The catalytic properties of the resulting artificial enzymes were evaluated using the Michael addition of nitromethane to cinnamaldehyde. Variants displayed increased conversion and were able to access (S)-product with modest enantioselectivity. These findings highlight the potential of ncAAs in expanding the reaction scope of designer enzymes and provide a promising avenue for the development of more efficient and versatile biocatalysts.

FIGURES

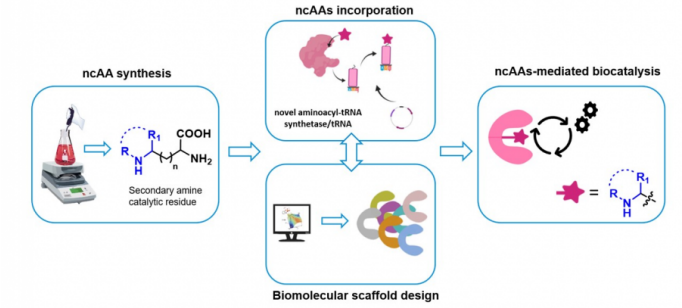

FIGURE 1

Figure 1.

Schematic representation of the presented project focused on the investigation of various secondary amine-based non-canonical amino acids as catalytic residues.

FIGURE 2

KEYWORDS

non-canonical amino acids | artificial enzymes | biocatalysis | new-to-nature reactivities

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