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PLURIZYMES AS ALTERNATIVE FOR CASCADE REACTIONS: A TRANSAMINASE-ESTERASE PLURIZYME AS STUDY CASE

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PURPOSE OF THE ABSTRACT

Nowadays, efficiency and environmental sustainability are major issues of the circular economy. One-pot cascade reactions are the preferable choice for the synthesis and assembly of numerous molecules, as they can minimize chemical waste, save time and money and simplify technical aspects. However, they require the engineering of the biocatalysts of each internal chemical reaction that conform the cascade reaction. Enzymes are the key to this handicap, as they are selective, specific and a greener option than chemical catalysts, and through protein engineering and machine-learning methods these biocatalysts properties can be adjusted. In this regard, a novel alternative to the use and engineering of several enzymes for one-pot reactions would be PluriZymes, enzymes that maintain their native active site whilst having others artificially introduced ones supporting similar [1] or complementary [2] chemistry.

In this study we designed TR2E2, a PluriZyme with amine transferase and esterolytic activity [3]. In brief, a Ser-Glu-His catalytic triad (which provides esterase activity) was introduced, unprecedentedly, into a class III ω -transaminase, to produce the first transaminase-esterase PluriZyme that could perform one-pot bioinspired cascade reactions that allow the one-pot synthesis of chiral amino acids from keto-esters. This is achieved through the conversion of a β -keto ester into a β -keto acid at the artificially introduced hydrolytic site and its subsequent conversion into a β -amino acid (e.e. >99 %) at the native transaminase site. Some limitations and challenges related to cascade reactions and lessons learnt from the recent design of PluriZymes for non-cascade and cascade

reactions are discussed. We also provided biochemical and structural information demonstrating that new active sites supporting esterolytic activity can be effectively introduced into transaminase scaffolds to design PluriZymes for bioinspired cascade reactions of interest.

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FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

PluryZymes | Enzyme design | Biocatalysis | Transaminase-esterase

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