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Access to thermostable enzymes and their application in flow biocatalysis

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PURPOSE OF THE ABSTRACT

The immobilization of biocatalysts in a continuous fluidic setup is one way to achieve compartmentalization and thus precise control over artificial reaction cascades for synthetic chemistry. We recently demonstrated the encapsulation of unmodified thermostable enzymes in a 3D printed, agarose-based thermoreversible hydrogel to enable multi-step sequential biotransformations.[1] To test the feasibility of the encapsulation strategy, we used a naturally thermostable alcohol dehydrogenase (ADH) as well as a ketoisovalerate decarboxylase (KIVD) from a mesophile organism as exemplary biocatalysts. KIVD was thermostabilized by different computational or evolutionary methods to increase the T50 value by up to 9°C.[1,2] After the successful proof-of-concept study, we further expanded the scope of this system by integrating phenacrylate decarboxylases (PAD) into this microfluidic system.[3] As an alternative for the hydrogel based immobilization strategy, thermostable enzymes can be covalently attached onto beads in a packed-bed reactor. In this context thermostable enzymes offer improved process stability and we selected a benzaldehyde lyase (BAL) as an example, since only one enzyme had been biochemically characterized before, which was rather instable.[4] To this end, we employed a computational prediction tool[5] for the identification of a novel thermostable benzaldehyde lyase and employed the enzyme for the continuous production of α-hydroxy-ketones. A homology-model based approach was used to create enzyme variants with altered substrate scope, which also showed further increased thermal stability.

FIGURES

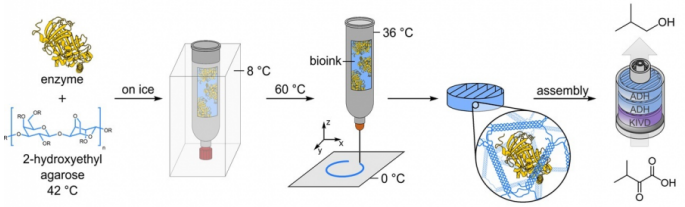

FIGURE 1
3D-Printed flow reactor modules

FIGURE 2

KEYWORDS
thermostability | flow biocatalysis | protein-engineering | protein immobilization

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