Access to thermostable enzymes and their application in flow biocatalysis

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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 T_{50} 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.

[1] M. Maier *et al.* (2018) Angew. Chem., Int. Ed., 57, 5539.

[2] M. Peng *et al.* (2019) Biol. Chem., 400, 1519.

[3] M. Peng *et al.* (2019) Chem. - Eur. J., 25, 15998.

[4] M. Peng *et al.* (2022) ChemBioChem, 23, e202100468.

[5] G. Li *et al.* (2019) ACS Synth. Biol., 8, 1411.