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Self-Assembling Enzyme Materials and Additive Manufacturing for Flow Biocatalysis

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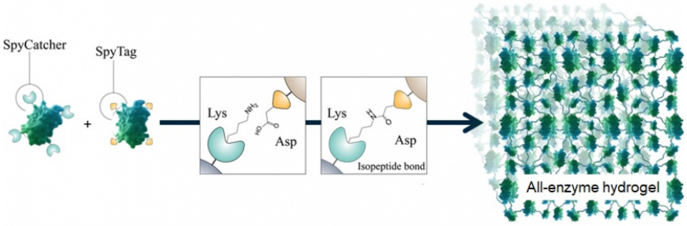
PURPOSE OF THE ABSTRACT

Applications employing catalysts in fluidic, cascaded setup are becoming increasingly relevant.[1] In order to also incorporate biocatalysts in such setups, efficient immobilization strategies are in demand. Employing the genetically encoded, covalent SpyTag/SpyCatcher (ST/SC) system, we have recently developed an all-enzyme hydrogel (Figure 1), which can be prepared from many enzymes, enabling the continuous conversion of a wide variety of substrates.[2-5] In such carrier-free formulations the enzymes themselves form a material, which for example stabilizes the enzymes and also offer the retention of expensive cofactors such as NAD(P)H. Depending on the application, the same immobilization strategy using the ST/SC system can also be used to immobilize enzymes onto epoxy-modified beads[6,7] or fully biogenic SC-modified magnetosomes.[7] In all cases the biocatalytic conversions in the resulting flow reactors converted the corresponding substrates continuously for several days with high efficiency.
As an alternative approach, biocatalysts can also be implemented in flow biocatalysis employing additive manufacturing. Such bioprinting applications call for thermotolerant organisms and proteins. We have recently showed reported how to employ guided protein evolution for engineering biocatalysts for such applications.[8-10] In this context new methods using correlation analysis and machine learning have been developed which greatly aid the identification of thermostable enzymes.[11, 12] With this approach, we have successfully evolved enzyme variants that reveal significantly increased stability and activity at elevated temperatures. These improved variants along with naturally thermostable proteins were then used for direct 3D printing of bioinks to manufacture reaction modules (Figure 2).[8,9] These modules can be produced on demand from bioinks, which are stable over weeks inside the printing cartridge. Reactor modules containing different enzymes can be arranged into cascades and show a tunable behavior. Different enzyme classes have been successfully utilized including alcohol dehydrogenases, esterases, ketoisovalerate decarboxylases, phenolic acid decarboxylases and benzaldehyde lyases.

FIGURES



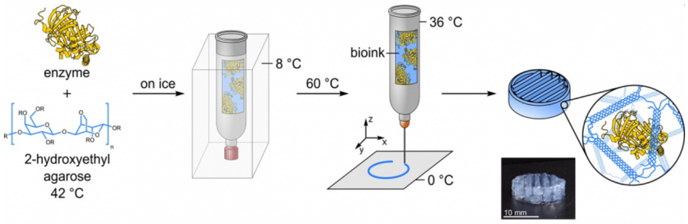


FIGURE 1

All-enzyme hydrogel formation

Genetically encoded ST/SC system enables the formation of defined all-enzyme hydrogels.

FIGURE 2

3D printing of biocatalytic reaction modules

Thermostabile enzyme allow the direct printing of biocatalytic reaction modules.

KEYWORDS

flow biocatalysis | enzyme immobilization | thermostabilization | additive manufacturing

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