An Immobilized Silicon-Carbon Bond-Forming Enzyme for Anaerobic Flow Biocatalysis

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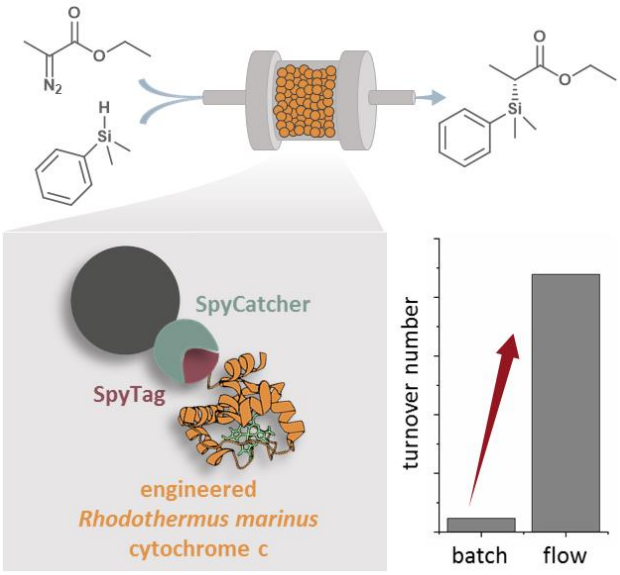
Introduction

The key to establish successful biocatalytic and sustainable applications lies in a holistic design of the reaction processes in suitable reactor systems, which includes the immobilization of enzymes and thus enables simplified product separation and reusability. The recent development of tailored cytochrome enzymes via directed evolution has enabled “new-to-nature” reactivities, such as the biocatalytic formation of carbon-silicon bonds using the cytochrome c from *Rhodothermus marinus*.[1]

Objective

To maximise the potential of this remarkable biocatalyst by increasing its turnover numbers (TON) and to enable its application in continuous biocatalysis, we recently reported the use of the SpyTag/SpyCatcher (ST/SC)[2] bioconjugation system to immobilise this enzyme.[3]



Results

In our work, we successfully attached the ST to the enzyme without significantly affecting its catalytic activity. Even after immobilization on agarose microparticles, the enzyme retained 60% of its activity, as determined using a newly developed heme-

specific analytical methods to quantify the amount of bound enzyme. When optimizing the conditions of the enzyme reaction, we found that using 25% acetonitrile as a co-solvent resulted in increased product formation, but the yield of the reaction was restricted by the reduced lifetime of the diazoester substrate and the solubility of the silane substrate in aqueous solutions. To overcome these limitations, we utilized a continuously operated packed-bed reactor with inline mixing under anaerobic conditions, in which the substrates are supplied as a solution in pure acetonitrile. This minimized preincubation time with buffer and ensured optimal cosolvent concentration for the production of the organosilicon resulting in up to 6-fold TONs in continuous flow reactions over a total period of 10 days compared to the free enzyme reaction previously reported[1], and thus in much higher space-time-yields. However, we observed a drop in stereoselectivity under these conditions.

Conclusion

We demonstrated that the silicon-carbon bond-forming cytochrome c can be immobilized efficiently via the ST/SC system and employed in sequential batch reactions as well as in continuous flow reaction processes leading to improved TONs. Further optimization of the enzyme, the reaction conditions as well as the flow setup might enable improvements towards a more economically feasible application of this and similar heme-containing enzymes.

[1] S. B. Kan, R. D. Lewis, K. Chen, F. H. Arnold (2016) "Directed evolution of cytochrome c for carbon-silicon bond formation: Bringing silicon to life", *Science*.

[2] B. Zakeri, J. O. Fierer, E. Celik, E. C. Chittock, U. Schwarz-Linek, V. T. Moy, M. Howarth (2012) "Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin", *P Natl Acad Sci USA*, *109*.

[3] S. Gallus, E. Mittmann, A. J. Weber, M. Peng, C. M. Niemeyer, K. S. Rabe (2023) "An Immobilised Silicon-Carbon Bond-Forming Enzyme for Anaerobic Flow Biocatalysis", *ChemCatChem*, *15*.