Materials, Microbes and Microfluidics

Data-based and Evolutionary Methods for Biotechnology

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The targeted conversion of molecules is an essential technology to ensure life as it is currently possible for humans. In the chemical industry, a large number of reaction routes with a wide variety of catalysts are used for this purpose. Increasingly, biotechnological processes with the corresponding biological catalysts are also providing relevant additions. Especially applications employing such catalysts in fluidic, cascaded setup can have significant impact on synthetic chemistry.[1] In order to realize the great potential of individual enzymes, cascades of enzymes or entire cells in biotechnology, these biocatalysts need features such as high long-term process stability, as well as high catalytic activity and productivity.

Thermostable enzymes for example can offer solutions to these challenges and techniques are needed to stabilize unique enzymes or to identify novel thermostable enzymes. We have recently employed several methods such as guided protein evolution or computational prediction to provided several classes of enzymes such as alcohol dehydrogenases,[2] esterases,[2] ketoisovalerate decarboxylases,[2] phenolic acid decarboxylases[3] and benzaldehyde lyases[4], which were then either immobilized and implemented into flow reactors using encapsulation in 3D printed, agarose-based thermoreversible hydrogels or immobilization on epoxy-modified beads. The biocatalytic conversions in the resulting flow reactors converted the corresponding substrates continuously for several days with high efficiency and stereoselectivity.

Futhermore whole cell biocatalysts also need to by optimized to work under process conditions for the production of chemicals. Here, microfluidic devices offer the opportunity to grow populations to spatially organized stressor gradients, which can promote their adaptation to complex phenotypes that are difficult to achieve with conventional experimental setups. In such experimental setups for microbial adaptive laboratory evolution (ALE) - a key tool for studying evolution in basic and applied contexts - microbial cells can be exposed to a defined profile of stressors such as antibiotics. Utilizing a newly developed chip-based ALE setup[5] led to the discovery of previously unknown mutations in *Escherichia coli* that cause resistance to nalidixic acid. Such microfluidic devices can amplify the occurrence of mutations under defined microenvironmental conditions and thus enable cells to adapt to biotechnologically or biomedically relevant conditions.

These works emphasize that the combination of computer-based and evolutionary methods are a powerful approach to create novel biocatalytic flow processes and expand the availability of biocatalysts for stable continuous production.

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