OPTOFLUIDIC TECHNIQUES FOR DIRECTED EVOLUTION OF ENZYMES

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Enzymes are highly versatile and ubiquitous biological catalysts. They can greatly accelerate large variety of reactions, while ensuring appropriate catalytic activity and high selectivity. These properties make enzymes attractive biocatalysts for a wide range of industrial and biomedical applications. Over the last two decades, directed evolution of enzymes has transformed the field of protein engineering. We have devised microfluidic systems for directed evolution of haloalkane dehalogenases in emulsion droplets. In such a device, individual bacterial cells producing mutated variants of the same enzyme are encapsulated in microdroplets and supplied with a substrate. The conversion of a substrate by the enzyme produced by a single bacterium changes the pH in the droplet which is signalized by pH dependent fluorescence probe. The droplets with the highest enzymatic activity can be separated directly on the chip by dielectrophoresis and the resultant cell lineage can be used for enzyme production or for further rounds of directed evolution. The developed platform is applicable for ultra-fast screening of large libraries in directed evolution experiments requiring mutagenesis at multiple sites of a protein structure. In a system for evaluatoin of the enzyme kinetics of the dehalogenase reactions, we use Raman microspectroscopy and surface enhanced Raman spectroscopy (SERS) in microfluidic systems for detection of the reaction substrate and metabolites.

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