



DROPLET-BASED SCREENING FOR THE ACCELERATED EVOLUTION OF OPTIMIZED ENZYMES

Task

Biotechnological processes based on enzymatic catalysis are increasingly replacing large-scale chemical processes since they are not only more ecological and economical, but they also make it possible to produce completely new types of substances, such as bioplastics. Before such biotechnological production is feasible, novel, optimized technical enzymes are needed. Making them available, however, is tedious and expensive.

Method

Based on an existing enzyme gene, therefore, one million different enzyme variants shall be generated by genetic engineering and subsequent cell-free enzyme expression. In this case, droplets with a diameter of less than 10 μm serve as micro-expression systems in which the genes are isolated and enzyme is produced from them using a cell extract. After addition of a substrate, which metabolizes the enzymes to a fluorescent product, the activity of the enzymes can be determined by the fluorescence intensity. Particularly active and thus promising production variants have a high fluorescence signal. In order to select the best enzymes for production, Fraunhofer ILT has developed a droplet-based

screening method in which microdroplets with enzyme genes, cell extract and substrate are injected into an oily phase and examined in a sorting chip for their fluorescence. The best enzyme candidates are sorted out on a microfluidic branch with highly focused laser light and deposited on the chip. An iteration of the procedure described results in optimized technical enzymes.

Results

Selective Laser-Induced Etching (SLE) was used to develop fused-silica microfluidic systems that can generate 5 to 10 μm droplets at generation rates above 10 kHz. The developed screening and sorting platform screens droplets with rates of greater than 1 kHz and separates optical and non-contact droplets with fluorescence signals above the threshold.

Applications

The screening and sorting platform can be used in biotechnology for the accelerated, directed evolution of enzymes. Moreover, it can also be used in personalized medicine for the detection and separation of circulating tumor cells in blood.

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3 Droplet generation with diameters of less than 10 μm .

4 Droplet generator on carrier chip.