Ultrahigh-throughput screening in drop-based microfluidics for directed evolution.

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Comments

In this paper, the authors blended several techniques and developed a valuable microfluidic system for directed evolution of enzymes relying on encapsulation of an enzyme variant and its substrate in water microdroplets and its subsequent rapid sorting based on activity.

Encapsulation of an individual enzyme variant and its coding gene in aqueous microdroplets dispersed in oil represents one of the ways to link genotype and phenotype in directed evolution experiments {1}. This link was successfully used in several selection experiments, where an active enzyme catalyzes a reaction on its own gene's handle, allowing for recovery of the gene from a pool of variants upon breaking of the water-in-oil emulsion. Unlike selection, in screening experiments, where the activity of each individual clone can be read, more insight into the statistical distribution of the variant's activities can be obtained, resulting in better control over the experiment. Indeed, the limited speed of the screening is the major trade-off for this higher degree of control. The technique described in the present paper is able to screen and sort droplets based on fluorescence at speeds of over 2000 droplets/sec, allowing screening of 10^8 variants in about 10 hours. The enzyme in question (here, horseradish peroxidase) is displayed on yeast and the individual yeast cell is encapsulated in a microdroplet together with the enzyme's substrate. During in-line incubation, the enzyme converts the substrate into fluorescent product and droplets are subsequently actively sorted based on their fluorescence. The whole process from droplet formation to sorting can take place on the same chip. Similar sorting of water-in-oil droplets by flow cytometry (FACS) has been demonstrated before {2}, but, in this case, the whole process is somewhat cumbersome, as emulsion has to be converted into double emulsion, water-in-oil-in-water, prior to sorting. From the information presented in the paper, the described sorting device can be relatively inexpensive ($10,000, including computer and software). In addition, the sorting chip is disposable, thus helping to prevent sample cross-contamination.

References: {1} Taly et al. Chembiochem 2007, 8:263-72 [PMID:17226878]. {2} Mastrobattista et al. Chem Biol 2005, 12:1291-300 [PMID:16356846].

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