ABSORBANCE-ACTIVATED-DROPLET SORTING FOR DIRECTED ENZYME EVOLUTION

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The successful creation of custom-made enzymes by directed evolution relies in no small part on screening as many variants as possible. Massive scale-down of assay volumes by compartmentalization of library members in water-in-oil emulsion droplets has recently led to the development of ultrahigh-throughput screening platforms that use small volumes (typically picoliters) and allow sorting of more than 10^6 variants per hour [1,2]. The key technical module to make this possible is a microfluidic droplet sorter that has so far relied exclusively on fluorescent readouts.

To extend the range of assays amenable to this approach, we developed a highly efficient microfluidic absorbance-activated droplet sorter (AADS)[3]. Using this module, microdroplets can be sorted based on absorbance readout at rates of up to more than a million droplets in 3 hours. To validate this device, we implemented a miniaturized coupled assay for an NAD^+- dependent amino acid dehydrogenase. The detection limit (10 μM in a coupled assay producing a formazan dye) enables accurate kinetic readouts and sorting experiments showed that the AADS successfully enriched active variants up to 2,800-fold from an overwhelming majority of inactive ones at ≈ 100 Hz. Furthermore, improved variants showing increased solubility (up to 60%) and thermostability (up to 12 °C) were identified after two rounds of directed evolution, thereby demonstrating the usefulness of this sorting module for enzyme engineering. This AADS makes the most widely used optical detection format amenable to screens of unprecedented size, paving the way for the implementation of chromogenic assays in droplet microfluidics workflows. We are currently expanding its range of applications towards the monitoring of cell growth for the development of survival assays and the detection of weak enzymatic reactions.

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