A novel absorbance-activated droplet sorting platform for enzyme evolution

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The past few decades have seen a dramatic increase in the use of biocatalysts in commercial chemical processes, shifting the emphasis from energy-intensive traditional chemistry to sustainable chemistry. Unsurprisingly, significant effort has gone into modifying and improving the characteristics of naturally occurring enzymes for use in specific biotechnological applications. Current enzyme engineering techniques, such as directed evolution, require the production and testing of large libraries of mutations to identify commercially valuable variants. Unfortunately, traditional screening approaches are unable to screen such large mutagenesis libraries in a robust and timely manner [1,2]. Droplet-based microfluidic systems are able to produce, process and sort picoliter droplets at kilohertz rates and have emerged as a potentially powerful and high-throughput tool for library screening. However, the reliance of these screening approaches on inline fluorescence detection either restricts their use to a limited number of natural substrates and enzyme classes or involves the use of surrogate substrates, which bias the enzyme optimization process [3,4]. Accordingly, enlarging the "detection toolbox" to include additional optical techniques is a recognized priority. Absorbance-detection, being the most widely used bulk detection method for bioanalytical measurements, is an obvious and potentially powerful option. However, unlike fluorescence, absorbance-based detection is compromised when optical pathlengths are small. This poses problems for it use in microfluidic devices, where optical pathlengths are on the order of tens or hundreds of microns. Moreover, in droplet-based microfluidic systems, scattering at the droplet and oil interface further complicates the detection, and reduces signal-to-noise ratios [5]. Consequently, the existing absorbance-activated droplet sorting platforms require a complicated optical assembly for absorbance detection and are often limited in throughput due to the use of droplet with large volumes [6-8].

We present a novel absorbance-activated droplet sorting platform (iAADS) that allows the direct measurement of absorbance signals from pL-volume droplets using a lithographic mask and refractive index matched fluids, avoiding the use of complicated optical-fibers and external light sources, and allowing the sensitive interrogation and sorting of droplets at kilohertz rates. Additionally, we use an impedance-based detection to identify sorted droplets, which obviates the need for optical monitoring of the microfluidic system. Utilizing this platform, we show a rapid screening of a 10^5-member aldehyde dehydrogenase library towards D-glyceraldehyde using a NADH mediated coupled assay that results in the formation of WST-1 formazan. In a 2x coverage of the library, we successfully obtained four mutant variants with increased V_{max} and two with improved K_m. The most successful variant showed a 51% improvement in catalytic efficiency for the conversion of D-glyceraldehyde, and a notable increase in overall activity was observed for a broad substrate spectrum.

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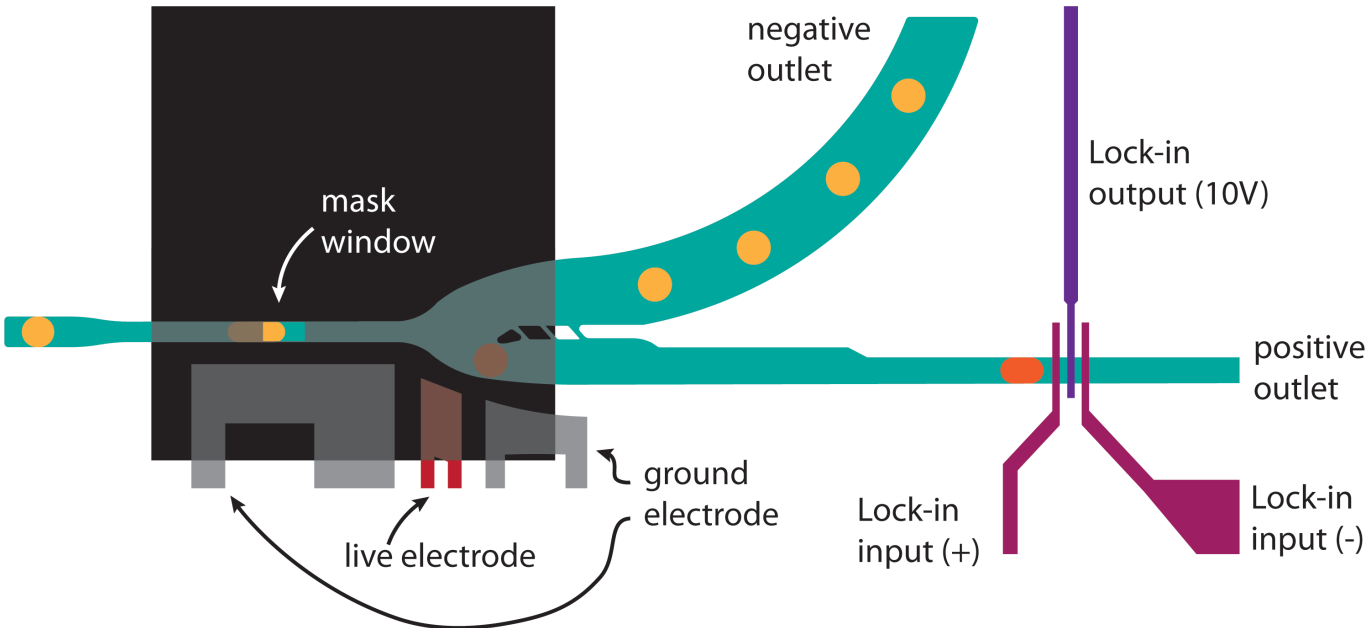
negative
outlet
Lock-in
output (10V)
mask
window
positive
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ground
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Lock-in
input (+)
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