Ultrahigh-throughput screening in microfluidic droplets – a faster route to new enzymes

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Biocatalysis is an environmentally friendly, cost-efficient alternative to chemocatalytic synthesis. Yet improvements in specificity and efficiency are needed, and have to be generated quickly to meet the fast pace of product development in industry. This ambition is met by droplet microfluidics, a powerful tool to scan vast libraries at kHz speed with >10^7/day throughput. Instead of µL robotic liquid handling systems, pL water-in-oil emulsion droplets are generated in PDMS chips replacing the use of plasticware.

Novel detection devices and coupled reactions complement direct optical readout and broaden the range of target reactions to enable functional metagenomics and directed evolution campaigns. The combination of droplet screening with UMIC-seq, high quality nanopore sequencing at a cost of <1.1 cent per sequence, creates maps in sequence space, possibly a future basis for applying AI/ML to extrapolate trajectories beyond the experimental output. The success of combinatorial campaigns in droplet formats suggests that this technology is coming of age.

Advantages and challenges

Advantages

- Selections are based on *direct* measurement of product, not a proxy.
- Lower costs: (i) pL instead of >µL assay volumes (i.e. ~10^8-fold ↓); (ii) low capital expenditure: droplet rig >20-fold cheaper than screening robots.
- Screening of >10^6 variants per hour: (i) more likely to beat the odds in directed evolution and functional metagenomics**,** low probability events captured, large accelerations per round; (ii) higher throughput more likely to avoid evolutionary dead ends: escape from local fitness plateaus facilitated
- Versatile and modular assay toolbox: (i) microfluidic chip design emulates screening workflows; (ii) a wide range of enzyme classes can be assayed; (ii) double emulsions can be sorted in a flow cytometer, as an alternative to all-on-chip workflows.

Challenges

- Commercial availability and setup: (i) 'black box' instrument rarely commercially available but modules for droplet formation, chip design etc. available on the market. (ii) microfluidic expertise required.
- Sensitivity: fluorescence-activated droplet sorting (FADS) has a detection threshold of < 0.1 µM (~3000 molecules in a pL droplet), but higher detection thresholds for absorbance-activated droplet sorting (AADS; 10 µM), dielectrophoretic droplet sorting (DEDS, 1 µM) and mass-activated droplet sorting (MADS, 30 µM).
- More assays needed – synthetic access to assayable substrates important.
- Reaction product must not escape from the droplet compartment – if it does, the screening is compromised.

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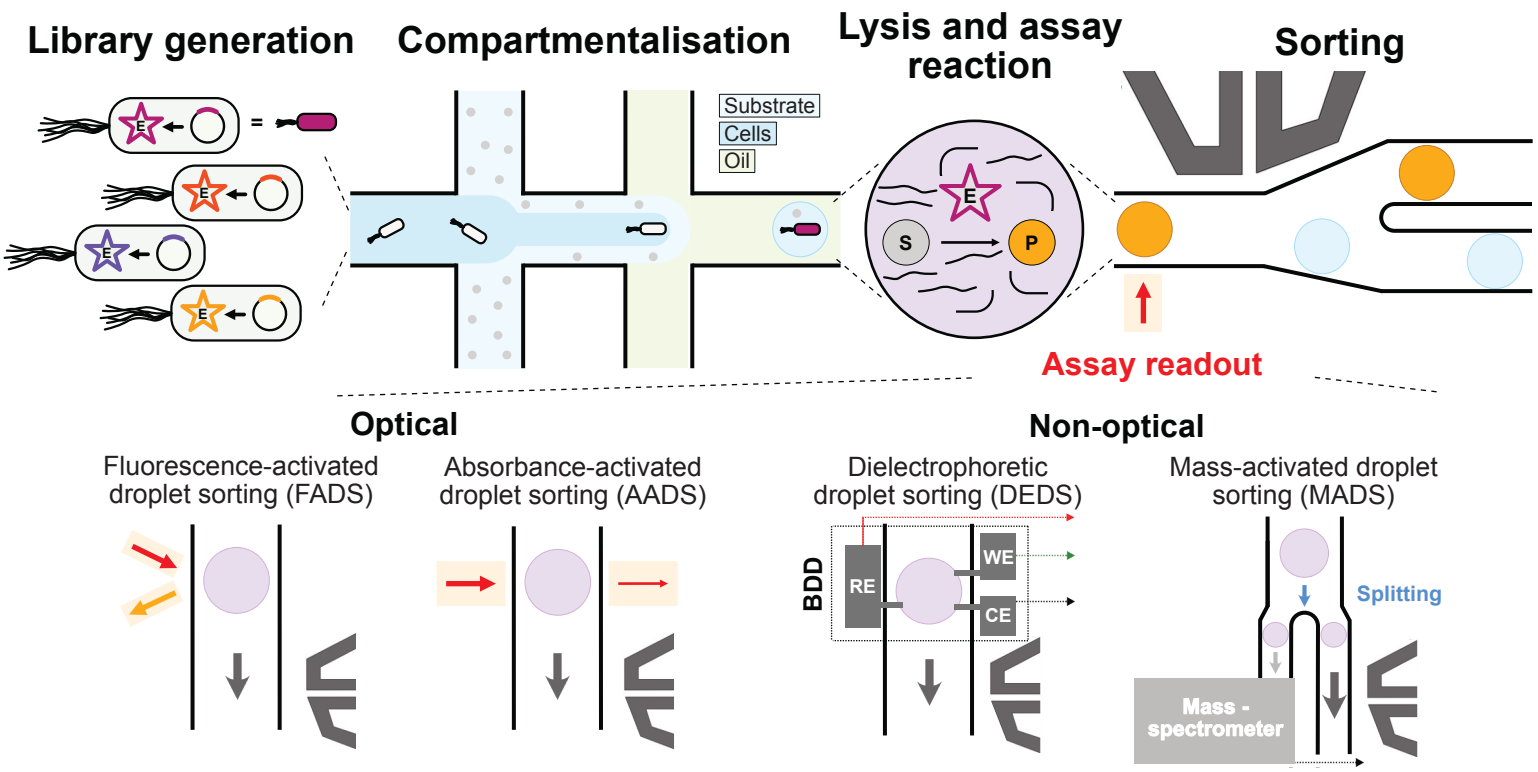
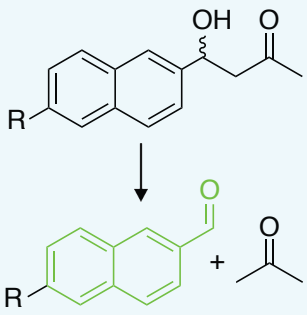

Library generation
Compartmentalisation
Lysis and assay reaction
Sorting
Substrate
Cells
Oil
E
S
P
Assay readout
Optical
Non-optical
Fluorescence-activated droplet sorting (FADS)
Absorbance-activated droplet sorting (AADS)
Dielectrophoretic droplet sorting (DEDS)
Mass-activated droplet sorting (MADS)
BDD
RE
WE
CE
Splitting
Mass - spectrometer

Fluorescence

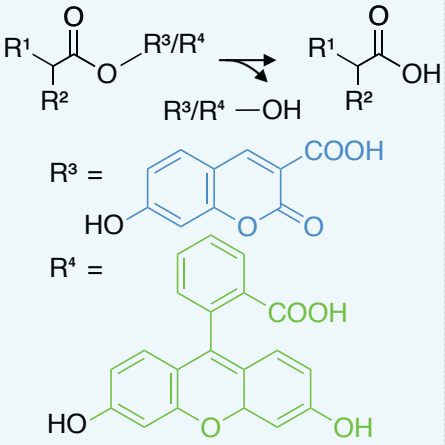
Aldolase [8]



k_{cat} **30-fold** ↑

direct

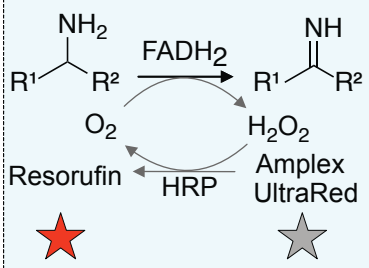
Esterase [6]



enantioselectivity **700-fold** ↑

coupled

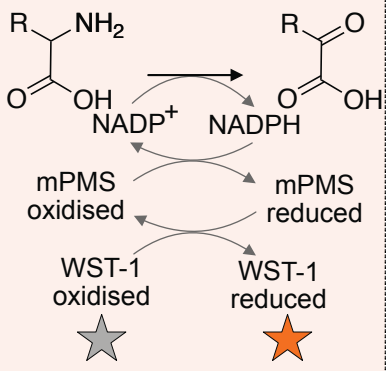
Amine oxidase [2]



k_{cat}/K_M **960-fold** ↑

Absorbance

Amine dehydrogenase [10]



total turnover number **3.3-fold** ↑