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Tiny droplets make a big splash

Micrometer-sized emulsion droplets form the foundation for an innovative technology that could change how scientists approach high-throughput screening.

In every cell, proper function depends on segregation of molecules within specific organelles or smaller subcompartments. This organizational model was an inspiration for Andrew Griffiths and Dan Tawfik, two scientists at the MRC Laboratory in Cambridge, UK, who wanted to develop an *in vitro* compartmentalization (IVC) system in which individual gene sequences could be contained with the enzyme variant they encode. "We came to the idea of compartmentalizing using emulsions," explains Griffiths, "because basically compartmentalization in cells is the way nature links genotype to phenotype." They began working with water droplets dispersed in oil, forming water-in-oil emulsions, and developed a useful experimental strategy for directed evolution.

However, this approach proved impractical for high-throughput screening via fluorescence-activated cell sorting (FACS). "There was one small problem, and that was that FACS machines were invented to work with water and not mineral oil," explains Tawfik. "One week, [we] took the guy who was running the FACS machine in Cambridge, and 'invested' several pints in him, and convinced him that he should let us try and run the machine on mineral oil. And in doing so, we gathered a large number of enemies for a very long time, because it took something like two or three weeks to fix that machine!"

A colleague, chemist Shlomo Magdassi, suggested a safer solution—using a second emulsion, so that each experiment is now contained within a water-in-oil-in-water (w/o/w) emulsion. The principle proved sound, and the two found that this external water phase put FACS back on the table. Now, Griffiths and Tawfik have moved forward to demonstrate two new strategies in which FACS analysis of w/o/w droplets can greatly accelerate directed evolution studies.

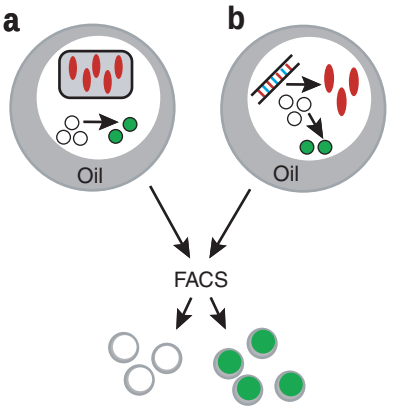

Figure 1 | Directed evolution using w/o/w emulsion droplets. (**a**) Captured bacteria produce enzyme (red ovals) that can convert inactive (white) substrate to fluorescent (green) state. (**b**) *In vitro* reactions produce enzyme from compartmentalized gene copies to catalyze substrate conversion. Increased fluorescence allows FACS separation of efficient enzyme variants.

Tawfik's group, now at the Weizmann Institute in Israel, used droplets to capture individual bacteria (Aharoni *et al.*, 2005), each expressing a variant of paraoxonase (PON1), an enzyme that processes a variety of substrates. Thiobutyrolactones (TBLs) are normally hydrolyzed by PON1 with very low efficiency. Tawfik's group transformed a library of mutant PON1 variants into bacteria, which were individually embedded in droplets, along with TBL substrate and a fluorescent dye that detects hydrolysis (**Fig. 1a**). Even after just one round of stringent sorting for increased fluorescence, they identified several variants with up to 100-fold increased activity, representing what may be the most efficient TBLases yet identified.

Rather than embedding cells in droplets, Griffiths' team turned droplets into cells, using them as reaction vessels for transcription and translation of compartmentalized genes (Mastrobattista *et al.*, 2005). They worked with Ebg, a mysterious *E. coli* protein related to β-galactosidase, but with negligible β-galactosidase activity and no known function. Earlier studies, however, have shown that certain mutations can bestow β-galactosidase activity on Ebg. Griffiths' group sorted droplets containing mutated Ebg variants along with the components for *in vitro* transcription and translation, and a fluorescent β-galactosidase substrate (**Fig. 1b**); these were then subjected to multiple rounds of FACS-based selection. Within a few rounds, they isolated several clones with β-galactosidase activity at least 300-fold greater than wild-type Ebg, and equivalent to previously described 'activated' mutants.

Each approach has its virtues. Whole-cell studies may be better for targets with low enzymatic activity. "The yield of enzyme that you can get from a single bacterium in a single droplet can be much higher than you can get with *in vitro* translation systems, which improves the dynamic range," says Griffiths. Conversely, *in vitro* screens bypass the problems of working with live cells. "If you take all the reading frames of *E. coli* and you try to overexpress them in *E. coli*, 50% of them will be toxic to the cell," explains Tawfik. "In a cell-free system, you can express a much wider diversity."

Griffiths is now working with microfluidics, which allow the generation and sorting of highly stable and monodisperse droplets, and sees potential in these systems for evolving enzymes for use as therapeutics or as catalysts for biofuel cells. Tawfik also likes the idea of automation and expresses interest in applications in functional genomics and DNA analysis. Both men agree that this is only the beginning, and Griffiths concludes, "[I]n the end, almost anything that you can do in a microtiter plate, I think you'll be able to put into droplets for an emulsion-based high-throughput screening system."

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RESEARCH PAPERS

Aharoni, A. *et al.* High-throughput screening of enzyme libraries: thiolactonases evolved by fluorescence-activated sorting of single cells in emulsion compartments. *Chem. Biol.* **12**, 1281–1289 (2005).

Mastrobattista, E. *et al.* High-throughput screening of enzyme libraries: in vitro evolution of a β-galactosidase by fluorescence-activated cell sorting. *Chem. Biol.* **12**, 1291–1300 (2005).