This proposal aimed to miniaturize cell lysate screening assays to the single cell level in order to perform high throughput screening of metagenomic libraries in search for new biocatalysts and to perform directed enzyme evolution. As we described in the proposal the project had three milestones: (1) The set-up and optimization of the screening system. (2) Generation of libraries. (3) Screening the resulting libraries to isolate new enzymes and perform directed evolution. All three steps have been completed in the two years of the project, and we have published a peer-reviewed research article on the screening procedure, a review and a book chapter on the state-of-the-art of screening in microdroplets. Experiments for an additional research article, including the whole project up to the third milestone, is in preparation and will be published in the following year.

(1. milestone) We set up workflows that translate conventional screening procedure using *E.coli* cell lysate from microtitre plate to the microfluidic droplet scale (Figure 1A). The final procedure that was optimised in the first year of the fellowship is shown in Figure 1B. The key feature of this technology is a fluorescence-activated dielectrophoretic droplet sorter that enables the quantitative discrimination of enzyme variants in these artificial reaction compartments at a rate of 1000 s^{-1}. Currently two spin-off companies (Drop-Tech, Sphere Fluidics Limited) utilize the device at the University of Cambridge and we are building a partnership with a leading company in industrial enzymes, to exploit the potential in the technology. We have also developed an electroporation procedure which enable us to use the sample purified from the droplets directly to transform *E. coli* cells (step 5), and there is no need for PCR amplification and cloning. This feature makes the system much faster compared to previous protocols. Using an alternative droplet formulation, in which a

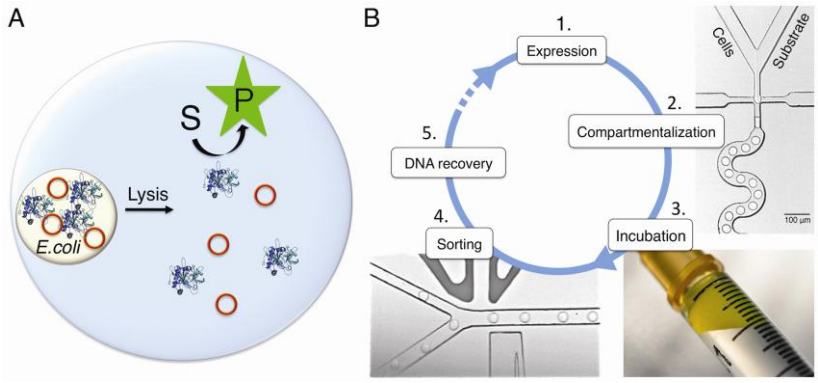

Figure 1. The screening workflow starts with intracellular expresssion of an enzyme library in *E. coli* liquid culture (step 1). Single cells compartmentalised into monodisperse emulsion droplets along with the substrate and cell lysis agents (step 2). The sample is collected in a syringe (10^7 droplets) and incubated to let the reaction progress until the fluorescent product have accumulated (step 3). Subsequently the droplets are reinjected into the fluorescence activated droplet sorter (FADS) (step 4). After releasing the aqueous phase of the sorted droplets, plasmid DNA is purified from the sample and electroporated into *E. coli*.

second emulsification step is applied to form water-in-oil-in-water double emulsion droplets, the screening procedure has been adapted to sort the libraries with conventional cell sorters (Figure 2).

To test the screening ability of the system we performed directed evolution of *Pseudomonas aeruginosa* arylsulfatase to improve one of its promiscuous activities, the ability to degrade phosphonate monoester substrates (with fluorescein-methylphosphonate k_{cat}/K_M is $2x10^3$ $M^{-1}s^{-1}$). A random mutagenesis library of the gene was constructed and screened in droplets. The results show a frequency-increase of clones with improved activity about 2000-fold after three rounds of screening, indicating the system is suitable for directed evolution. These results are being summarised in the first research article, which is one of the deliveries of the proposed project.

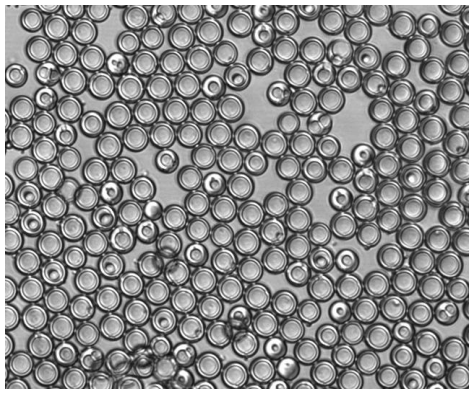

Figure 2. Water-in-oil-in-water double emulsion droplets generated in microfluidic device. These artificial reaction compartments are compatible with conventional fluorescent activated cell sorters

(2. milestone) We constructed the metagenomic libraries from DNA prepared from bovine rumen microbiome (by Diego Morgavi (INRA, Clermont-Ferrand)) and also obtained libraries using DNA from goose pond, sandy soil and marine sludge (Gabor, E. et al., *FEMS Microb. Ecol.* **2003**, 44: 153-163). Being able to isolate a few hydrolytic enzymes by screening a fraction of the libraries with conventional cell lysate assays in the microtitre plates revealed the quality of the libraries are suitable for testing the droplet screening system with them.

(3. milestone) Prior to screening the metagenomic libraries the isolated positive hits (see in 2. milestone) were assayed in droplets to see how to adapt the screening procedure for these specific activities. We found that the low efficiency of heterologous expression from metagenomic DNA required high sensitivity for the assay. When droplet volumes were decreased to 50 fL (10 μm in diameter) the resulting activity of the enzyme expressed by a single cell was within the same range as that achieved with conventional cell lysate assays on the macroscale. The first library screening experiments in droplets shows that miniaturization to the single cell level is fully compatible with metagenomic screening and provide extraordinary throughput for these experiments. Screening of further libraries and the characterization of the system is being performed and will be published in the following year in the form of a peer reviewed research paper.