Supplementary Material for

Static Microdroplet Arrays: A Microfluidic Device for Droplet Trapping, Incubation and Release for Enzymatic and Cell-based Assays

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Cloning and expression of YFP ("Venus")

Cloning

The "Venus" gene (700 bp) was isolated from the pIVEX-Venus[1, 2] plasmid (pCS2-Venus) by digestion with NcoI and BamHI using (1 µg pIVEX plasmid, *BamHI* buffer (NEB), BSA (10x), deionized water (MQ), *BamHI* (NEBs), *NcoI* (NEBs) in a total volume of 30 µL). The mixture was incubated at 37 °C for 2 h, then additionally 0.5 µL of each enzyme were added and the mixture was incubated further at 37 °C for 1 h.

The desired fragment was isolated from a 2% agarose gel followed by purification (High Pure PCR Product Purification Kit, Roche) and cloned into a vector (pBAD/myc HIS, Invitrogen) that allows tight expression control regulated by arabinose. The Vector was prepared via *NcoI* and *BgIII* digestion (1 µg vector-DNA, NEBuffer #3 (NEBs), MQ, *BgIII* (NEBs) and *NcoI* (NEBs), in a total volume of 20 µL. Again the mixture was incubated at 37 °C for 2 h, then an additional quantity (0.5 µL) of each enzyme were added and the mixture was digested at 37 °C for 1 h.

After complete digestion the solution was purified via Roche's High Pure PCR purification kit. For ligation 80 ng of digested pBAD vector and 200 ng of the insert were combined. The reaction mixture was cooled on ice for 20 min prior to addition of one unit T4 Ligase (Promega). Afterwards, the mixture was incubated at 16 °C for 16 h followed by purification of the pBAD/myc HIS-YFP plasmid using the Roche kit.

Transformation and protein production

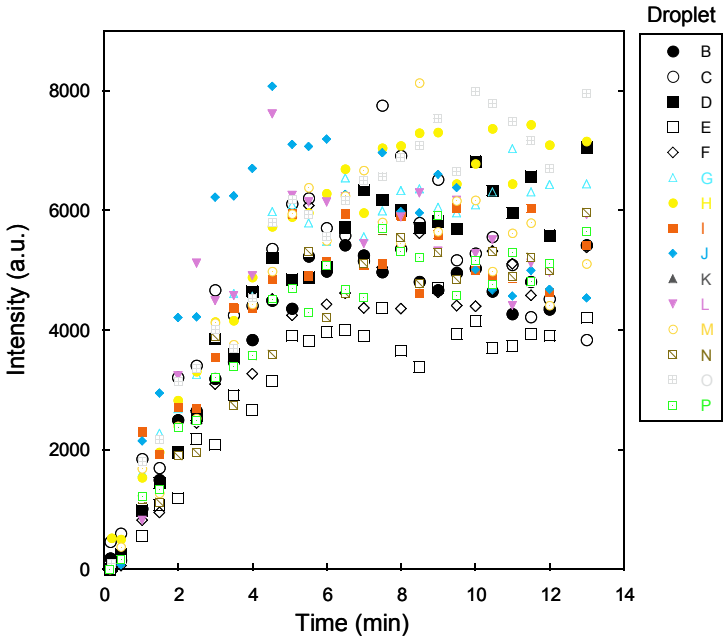
The ligation mixture (1 µL) was transformed into *E. coli* XL 1 Blue electro-competent cells. After one hour incubation time at 37 °C the cell suspension was plated on agar plates containing ampicillin (200 µg/mL) and incubated 16-20 h at 37 °C. DNA sequencing (Department of Biochemistry, University of Cambridge) of plasmid pBAD/myc HIS-YFP (QIAGEN, QIAprep Spin Miniprep Kit) confirmed the correct sequence for the YFP gene.

The vector was transformed into *E. coli* cells (BL21 DE3) and after growing for 24 h on an agar plate a single colony was picked and used to inoculate 10 mL of 2 × Luria Bertani broth (LB, containing 0.1 mg mL^{-1} ampicillin), then grown overnight in a shaker at 37 °C. This overnight culture was used to inoculate 1000 mL of 2 × LB media containing of 0.1 mg mL^{-1} ampicillin, and grown to a cell density of ~0.6 A^{600nm} in a shaker at 37 °C at 225 rpm. Protein expression was induced by the addition of arabinose (0.2%,w/v) and allowed to proceed overnight at 23 °C leading to a yellow broth. Cultures were harvested by using a centrifuge (15 min, 5,000 rpm, 4 °C, Beckham Coulter Avanti J-20XP) and resuspended into 50 mL of lysis buffer (50 mM Tris, 2 mM TCEP, pH 8.0) containing half a tablet of protease inhibitor cocktail (complete Mini, Roche). The cells were lysed by feeding the cell suspension into a high pressure homogenizer (EmulsiFlex C5, ~400-550 kPa, three cycles, Avestin) and the lysate was centrifuged (17,500 rpm, 90 min, 4 °C, Beckham Coulter Optima LE-80K Ultra-zentrifuge). The supernatant was then pooled, filtered (Millex® GP, 0.22 µm syringe filter, Millipore) and loaded onto a Q-Sepharose (Pharmacia) column at 0.45 mL min^{-1} until the yellow protein was visibly bound to the top of the column. After washing for 10 column volumes with 5 mL min^{-1} (50 mM Tris, 2 mM TCEP, pH 8.0) Venus was eluted with a 5 column volume salt gradient (100-300 mM NaCl) and the purity assessed by SDS-PAGE (12%, v/v). The pure yellow fractions were pooled and concentrated (Amicon® Ultra-15, 10,000 NMWL, Millipore) then loaded onto a HiLoad 26/60 Superdex G75 column (Amersham Biosciences), pre-equilibrated in buffer (50 mM Tris, pH 6.0, 150 mM NaCl) and run at 2 mL min^{-1}. Fractions of interest were pooled and concentrated (Amicon® Ultra-15, 10,000 NMWL, Millipore). Venus was either kept in the dark at 4 °C or flash frozen and stored at -80 °C until use.

ssβG-Galactosidase production

The plasmid encoding ssβG-galactosidase plamid was transformed into chemical competent *E. coli* cells BL 21(DE3) and plated overnight on an agar plate (37 °C). Single clones were picked and grown in LB medium containing kanamycin (50 μM, 37 °C) to an absorbance of 0.6 (A^{600nm}). The target protein was induced by addition of IPTG (0.1 mM). Cells were harvested by centrifugation, resuspended in 1/10 volume of the column loading buffer (5 mM imidazole, 20 mM Tris, 0.5 mM NaCl, pH 7.8) and sonicated. The suspension was centrifuged again to pellet cell debris (10,000 rpm, 30 min) and purified by His_6-Ni-chelation chromatography (washing buffer: 60 mM imidazole, 20 mM Tris, 0.5 mM NaCl, pH 7.8; elution buffer: 300 mM imidazole, 20 mM Tris, 0.5 mM NaCl, pH 7.8, HisTrapTM FF, GE Healthcare). The eluted protein peak was dialyzed against sodium phosphate buffer (50 mM, pH 6.5, 25 °C) and stored at 4 °C. Kinetic parameters in bulk experiments were determined at pH 6.4: K_M was 65 μM and k_{cat} = 0.32 sec^{-1} (data not shown).

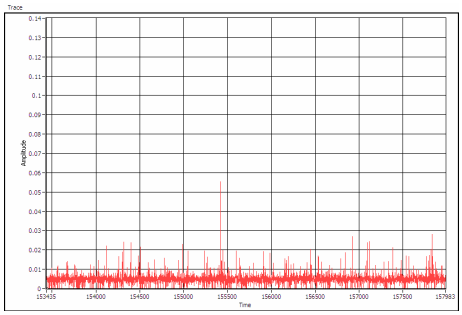
Supplementary Figure 1:



S1- Data traces for enzymatic reactions in individual droplets

Time-integrated fluorescence intensity as a function of time for 15 droplets recorded simultaneously (droplets are lettered in sequence). Figure 6B displays the averaged signal from these data.

Supplementary Figure 2:



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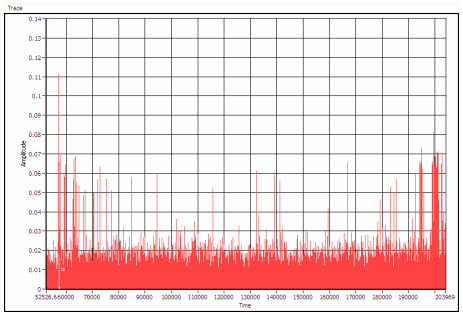

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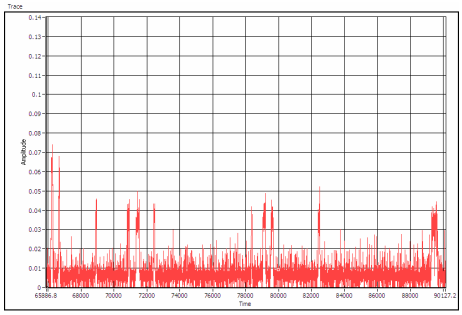

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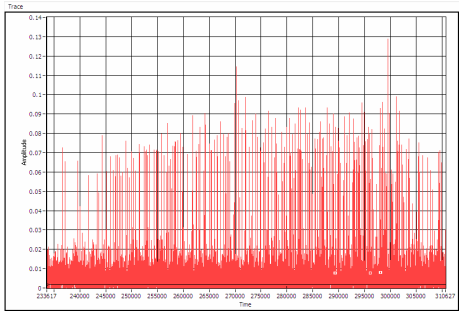

t = 40 min

S2 - Data traces used to derive the data in Figure 7.

The plots shows the laser-induced fluorescence measurements of approximately 90 droplets at each time point. The longer the reaction time, the higher the observed fluorescence signal in each droplet due to progression of the biochemical reaction. The background signal increases over time due to a small amount of product leaking into the oil. This was found to be less than 10% of the fluorescence intensity of the droplets.

Supplementary Movie 1

The movie shows the recovery of trapped droplets and was deposited at http://www-microdroplets.ch.cam.ac.uk/ref/trap.html . As the oil flow is reversed (from flowing to the left to flowing to the right) droplets are pushed out of the traps (indicated by the time clock on the lower left of the movie). The movie was recorded with a Phantom V7.2 fast-camera mounted onto a microscope under 10x magnification (XI 71, Olympus).

References

1. A. Huebner, M. Srisa-Art, D. Holt, C. Abell, F. Hollfelder, A. J. deMello and J. B. Edel, *Chemical Communications*, 2007, 1218-1220.
2. T. Nagai, K. Ibata, E. S. Park, M. Kubota, K. Mikoshiba and A. Miyawaki, *Nat Biotechnol*, 2002, **20**, 87-90.