Electronic Supplementary Material (ESI) for Lab on a Chip

Electronic Supplementary Information for

A Simple Method to Evaluate the Biochemical Compatibility of Oil/Surfactant Mixtures for Experiments in Microdroplets

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1. ImageJ analysis

Droplet analysis in ImageJ requires the following processing steps:

Step 1 – Calibrate scale of images

Open image of micrometer, draw a line of known length, go to Analyze > Set Scale, enter the length of the line and the units, tick “global”.

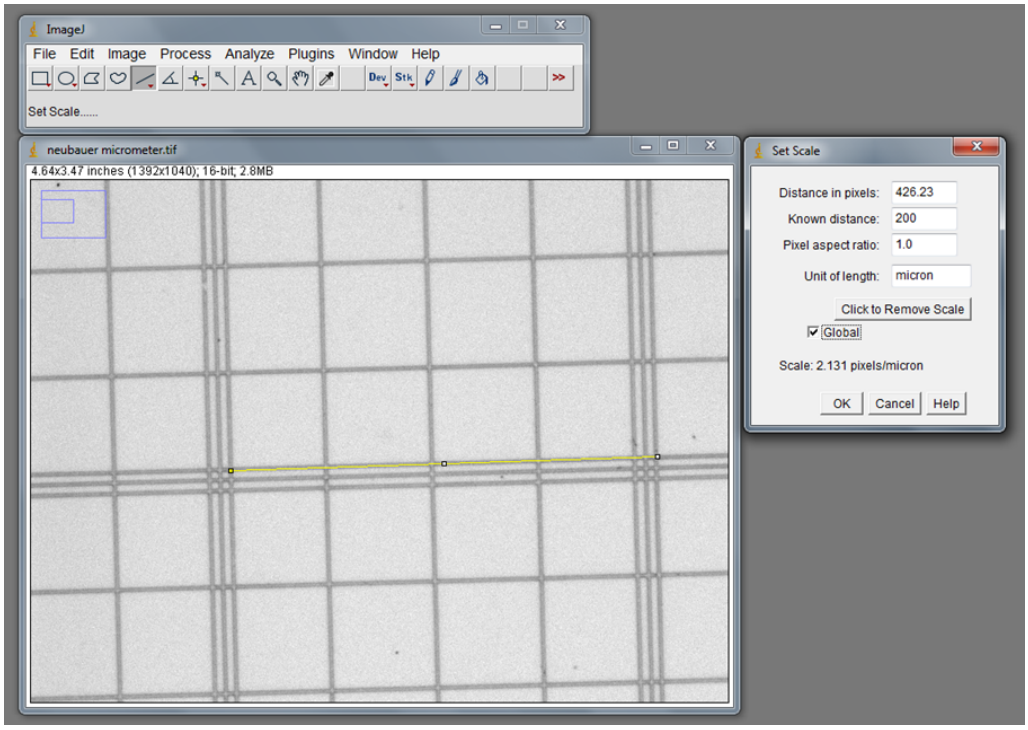

Figure S1. Microdroplet analysis in ImageJ – Screenshot of processing step 1. Calibrate scale.

Step 2 – Confirm global scale calibration

Open file, untick global scale warning, select “Disable these Messages”.

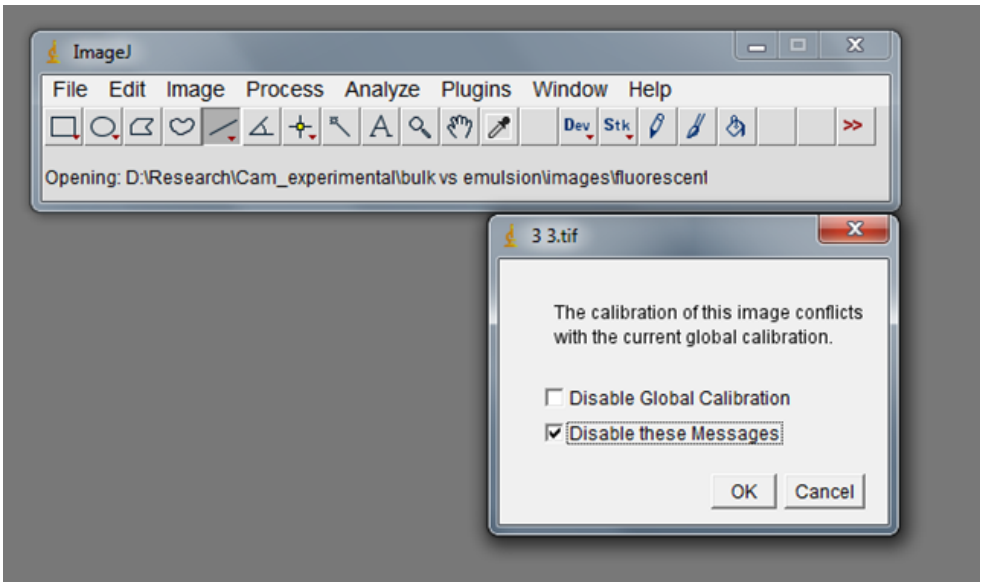

Figure S2. Microdroplet analysis in ImageJ – Screenshots of processing step 2.

Step 3 – Identify droplets

Smooth (ctrl shift s), Duplicate (ctrl shift d) and Threshold (ctrl shift t). Tick “dark background”. Adjust top slider to the left so that as many droplets as possible are highlighted without picking up background. Apply and close window.

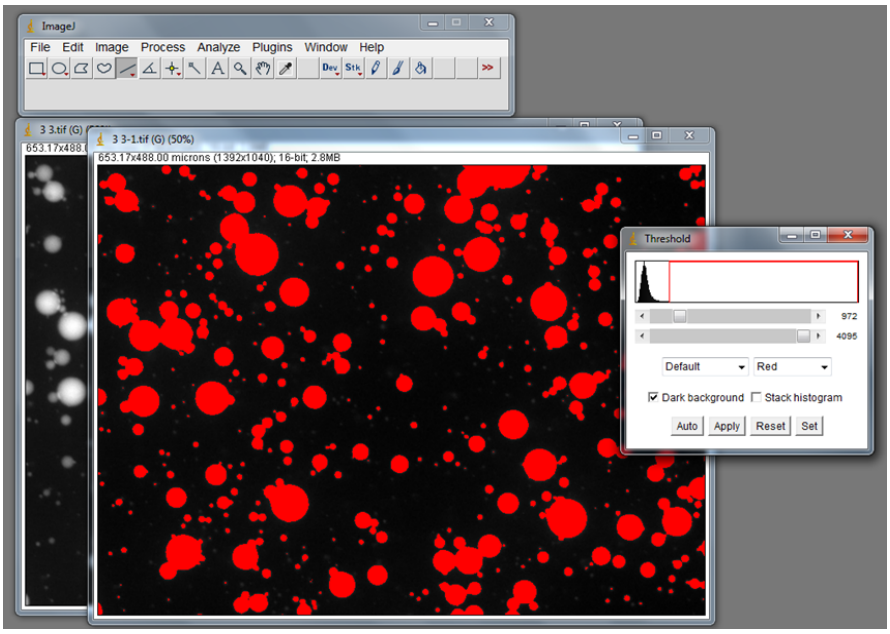

Figure S3. Microdroplet analysis in ImageJ – Screenshot of processing step 3.

Step 4 – Split touching droplets

Go to Process > Binary > Watershed – this will split touching droplets where there is an identifiable 'waist'.

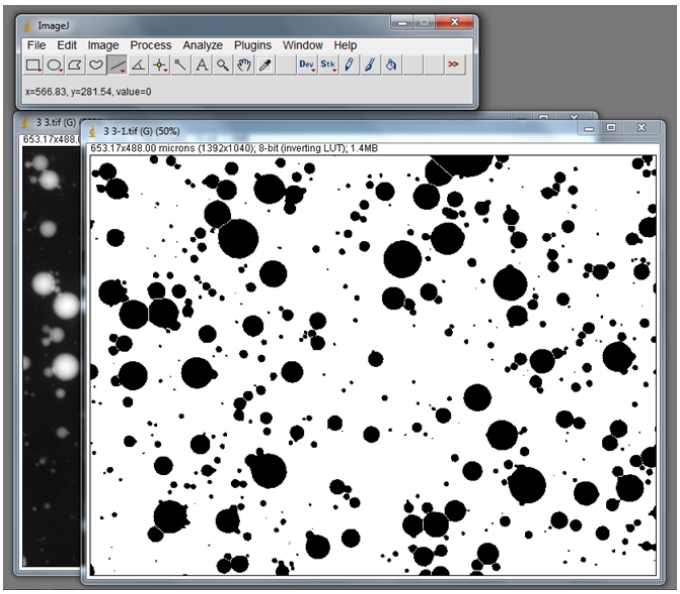

Figure S4. Microdroplet analysis in ImageJ – Screenshot of processing step 4.

Step 5 – Set measurements

Go to Analyze > Set Measurements – redirect to original image (i.e. the image not converted to binary), tick shape descriptors option.

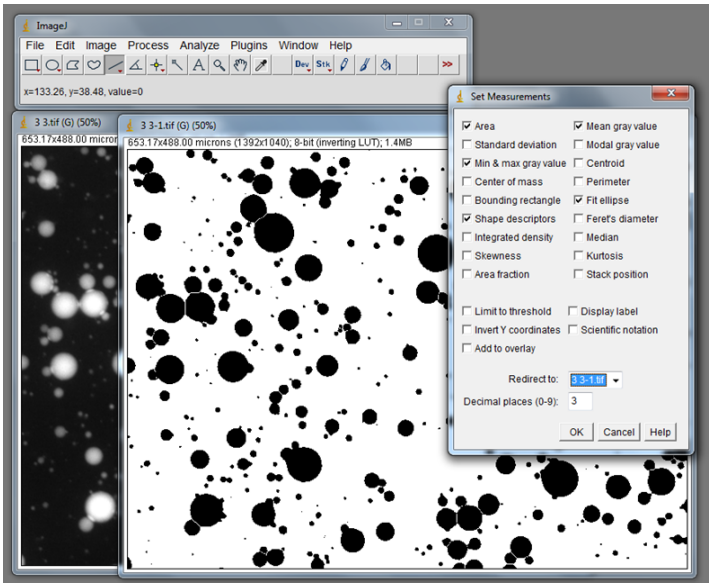

Figure S5. Microdroplet analysis in ImageJ – Screenshot of processing step 5.

Step 6 – Analyze particles

Go to Analyze > Analyze Particles. Select “Show outlines” from the dropdown, tick “Display results” and “Exclude on edges”. This command can also filter the droplets on circularity but it is easier to do this afterwards. It is convenient to set a minimum area at this point to exclude very small objects, although this can also be done later in a spreadsheet program.

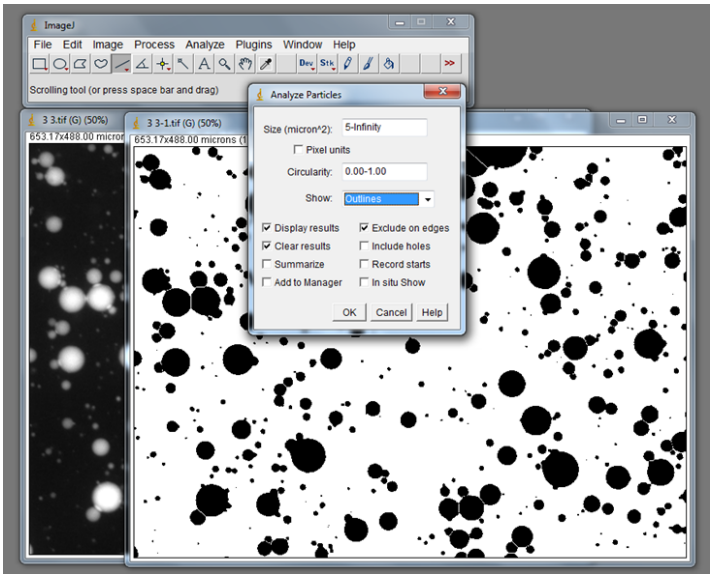

Figure S6. Microdroplet analysis in ImageJ – Screenshot of processing step 6.

Step 7 – Save analysis results

Save drawing and results. Open results in a spreadsheet, sort by roundness and delete those below the chosen threshold (we use 0.9). If necessary sort by area and remove very small objects (we deleted those with an area below 5 µm^2). The results are now ready to be graphed and further analysed.

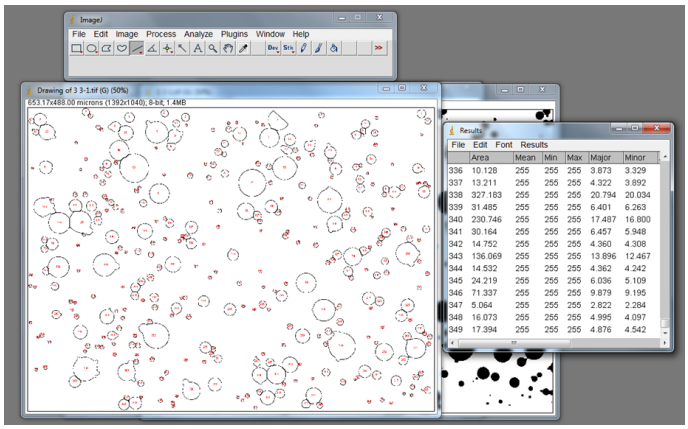

Figure S7. Microdroplet analysis in ImageJ – Screenshot of processing step 7.

2. Correlation between fluorophore concentration and y-intercept obtained by ImageJ analysis

Figure S8 shows the correlation between PAH concentration inside the droplets and y-intercept of the fluorescence/diameter curves obtained by ImageJ analysis. The weaker linear correlation compared to the slope/concentration curves can be rationalised as follows: the mean fluorescence is an uncalibrated measure, so the intercept of the fluorescence/diameter curves is arbitrary and a given fluorescence value cannot be used as an absolute measure of concentration. The exact value of the intercept depends on the background fluorescence, which could depend on the optical properties of the oil, which would therefore frustrate efforts to compare different oils. The background brightness also depends on brightness and density of the droplets present in the image, leading to variation even for comparison of images for a single type of oil.

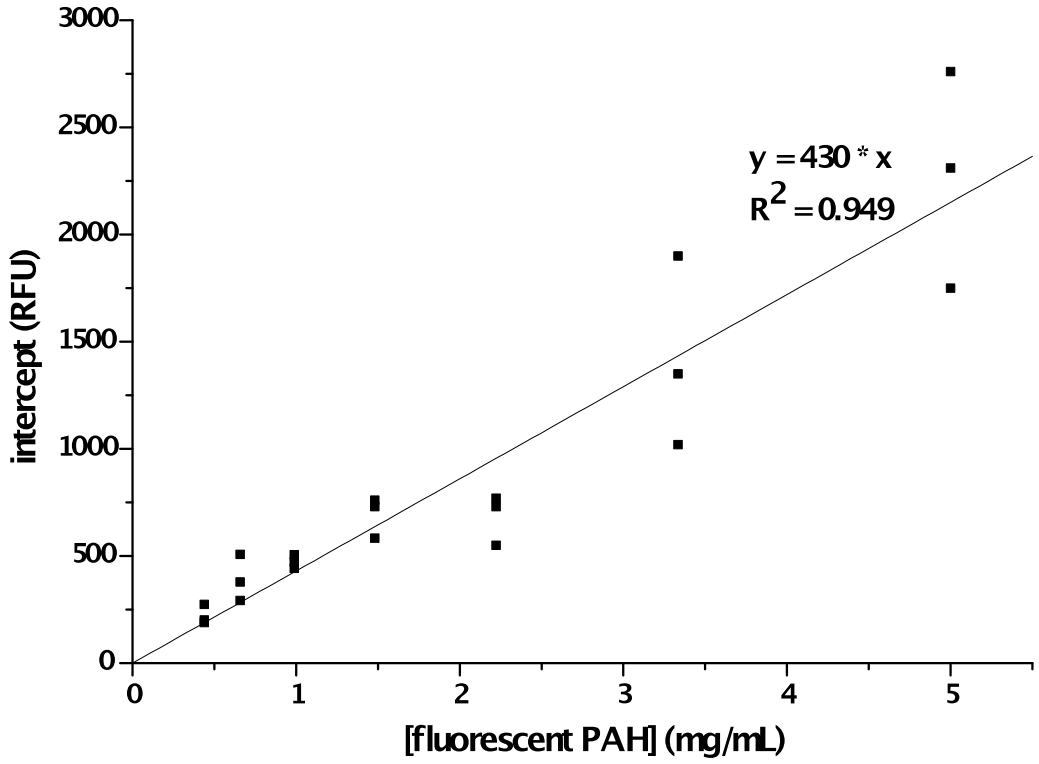

Figure S8. Combined data from Figure 1 of the main text fit to a linear function of intercept against fluorophore concentration.

3. Droplet size distributions

Histograms of droplet size distributions are shown for the different droplet generation methods (Figure S9) as well as for the different oil/surfactant mixtures used (Figure S10).

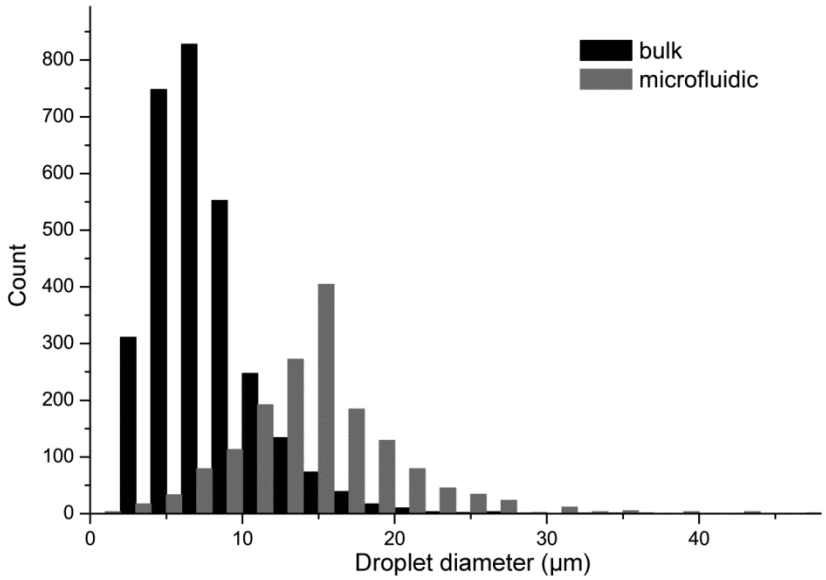

Figure S9. Histogram of size distribution of droplets produced for comparison of bulk emulsion and microfluidic droplet generation shown in Figure 2. The bin size is 2 µm.

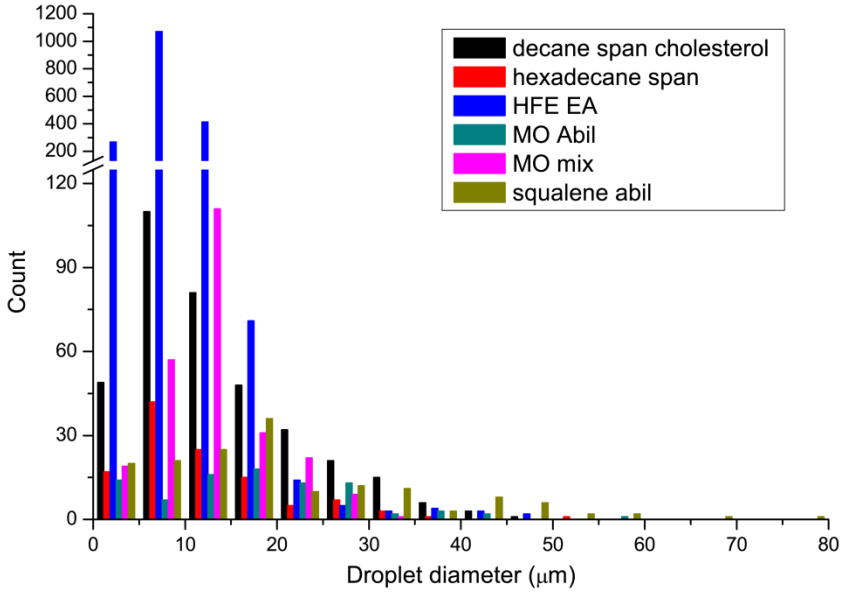

Figure S10. Histogram of droplet size distributions for data shown in Figure 4. The bin size is 5 µm. Note the broken y-axis and the different scales above and below the break.

4. Loss of GFP fluorescence in mineral oil mix

Single images of droplets containing purified GFP in 0.5% Tween80, 4.5% Span80 in mineral oil were taken at approximately one minute intervals for 10 minutes and the gradient of the mean fluorescence/droplet diameter curve determined for each sample. The gradients were replotted and fitted with an exponential decay model.

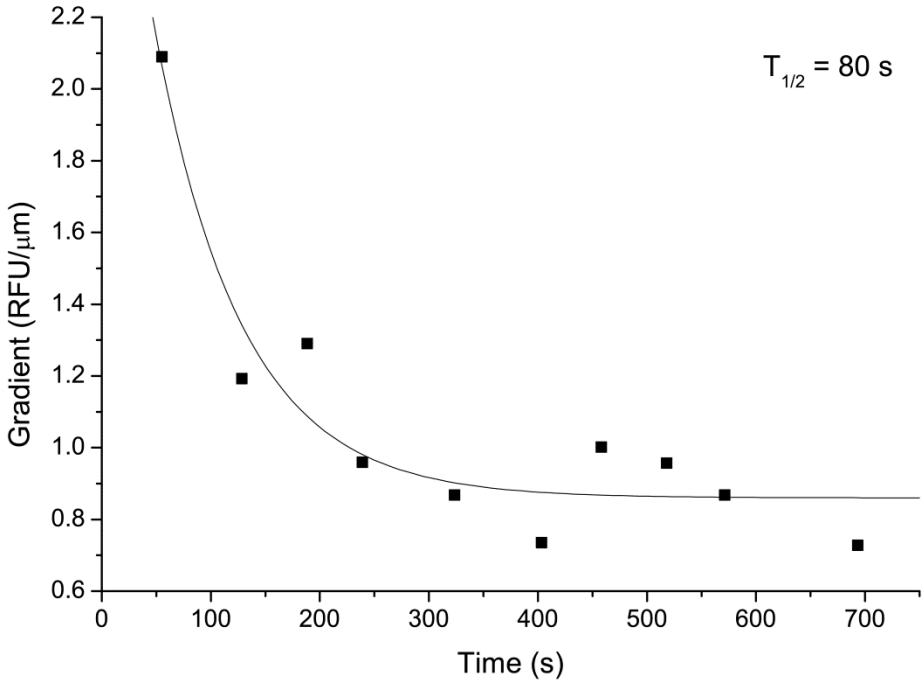

Figure S11. Loss of GFP fluorescence in 0.5% Tween80, 4.5% Span80 in mineral oil shows a half life of approximately 80 s.

5. Substrate turnover by PTE in different oil/surfactant mixtures

Representative images of substrate turnover by PTE as well as linear regression curves obtained by ImageJ analysis of five images for each oil/surfactant mixture are given below.

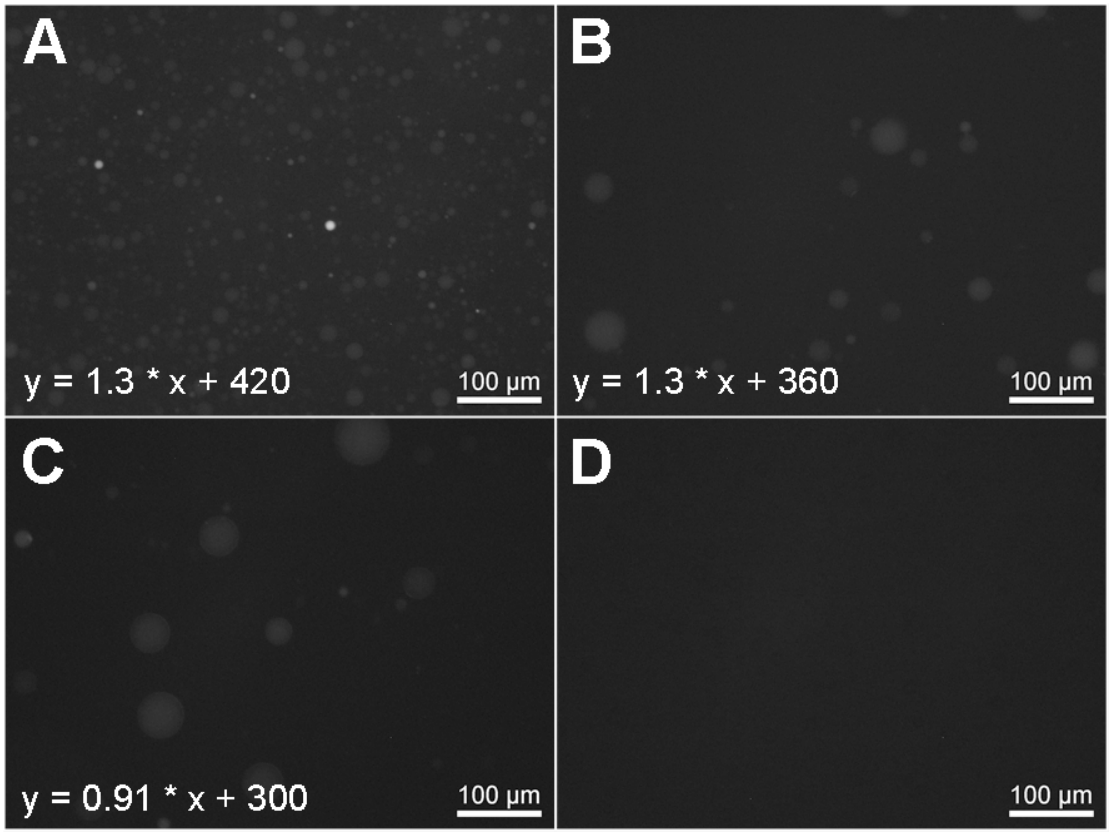

Figure S12. Substrate turnover by PTE in selected oil/surfactant mixtures. (A) 0.5% Raindance in FC7500. (B) 3% Span80 in hexadecane. (C) 3 % AbilEM90 in squalene. (D) 0.5% Tween80, 4.5% Span80 in mineral oil. Samples were incubated at 25 °C and images taken 1h after emulsification when the enzymatic reaction was still in the linear phase. For R^2 values for the fits, see Table S5, ESI.

6. Description of mutant PTE used in this study

We used a mutant phosphotriesterase from *P. diminuta* with reduced activity for the native substrate paraoxon compared to the wild type (k_{cat}/K_M = 50 $M^{-1}s^{-1}$). This mutant contains the following mutations relative to a previously published, wild-type like variant (PTE-S6, reference 1): T45A, A49V, K77E, A80V, S102T, I106L, S111R, L130V, F132L, M138I, E144V, T172I, A176M, T199I, L140M, Q180H, A204G, D233E, H254R, S269T, L271F, L272M, I274S, F306I, I313F, M314T, V341T.

7. Synthesis of PTE substrate

The leakage-proof oligonucleotide-conjugated substrate for PTE was prepared by adding doubly esterified FITC phosphotriester **1** (20 µL of a 50 mM solution in DMF) to amine-terminated oligonucleotide **2** (5'-H_2N-CTTTGTCGTATTAATTTCGCGGGA, Thermo Scientific, 50 µL of a 1 mM solution) in borate buffer (50 µL of 50 mM, pH 8.5) followed by incubation in the dark for 2.5 h (Figure 9). Phosphotriester **1** was a generous gift from Dr. S. Patil (Department of Chemistry, University of Cambridge). The reaction was then diluted with 200 µL water and purified on a G25 PD10 desalting column (GE Lifesciences) that had been pre-equilibrated with water. The first fraction, which contained the product **3**, was collected, lyophilised to give a yellow solid and dissolved in 100 µL DMSO to afford a stock solution with an oligonucleotide concentration of 5.1 µg/µL.

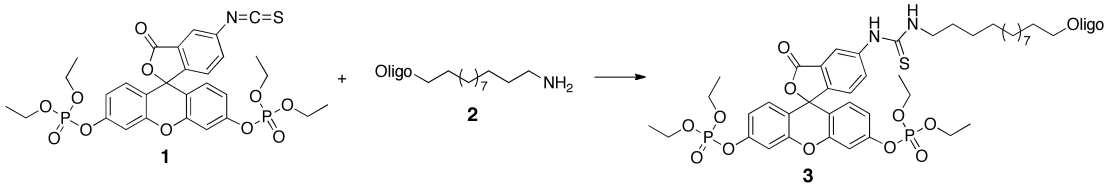

Figure S13. Synthesis of oligonucleotide-conjugated fluorescent substrate for PTE.

8. Quality of the fits

The R^2 values for all fits are given below.

Table S1. R^2 values for fits shown in Figure 1 of the main text.

Sample	R^2
5 mg/mL PAH	0.94
3.3 mg/mL PAH	0.93
2.2 mg/mL PAH	0.93
1.5 mg/mL PAH	0.92
1 mg/mL PAH	0.91
0.66 mg/mL PAH	0.95
0.44 mg/mL PAH	0.91

Table S2. R^2 values for fits shown in Figure 2 of the main text.

Sample	R^2
bulk	0.60
microfluidic	0.69

Table S3. R^2 values for fits shown in Figure 4 of the main text.

Sample	R^2
A. 0.5% Tween80, 4.5% Span80 in mineral oil	0.89
B. 3% Abil EM 90 in mineral oil	0.48
C. 3% Abil EM 90 in squalene	0.80
D. 0.5% EA surfactant in fluorous oil (HFE7500)	0.90
E. 1% Span60, 1% cholesterol in decane	0.95
F. 3% Span80 in hexadecane	0.98

Table S4. R^2 values for the fits shown in Figure 6 of the main text.

Sample	R^2
A. 0.5% EA surfactant in fluorous oil (HFE7500)	0.96
B. 3% Span80 in hexadecane	0.85
C. 3% Abil EM 90 in mineral oil	0.64
D. 3% Abil EM 90 in squalene	0.74
E. 0.5% Tween80, 4.5% Span80 in mineral oil + BSA	0.84

Table S5. R^2 values for the fits shown in Figure S11.

Sample	R^2
A. 0.5% EA surfactant in fluorous oil (HFE7500)	0.35
B. 3% Span80 in hexadecane	0.54
C. 3% Abil EM 90 in squalene	0.52

9. References

1. C. Roodveldt and D. S. Tawfik, *Protein Eng Des Sel*, 2005, **18**, 51-58.