Supplementary information:

Reliable microfluidic on-chip incubation of droplets in delay-lines

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1. Optical system for droplet observation and fluorescence detection.

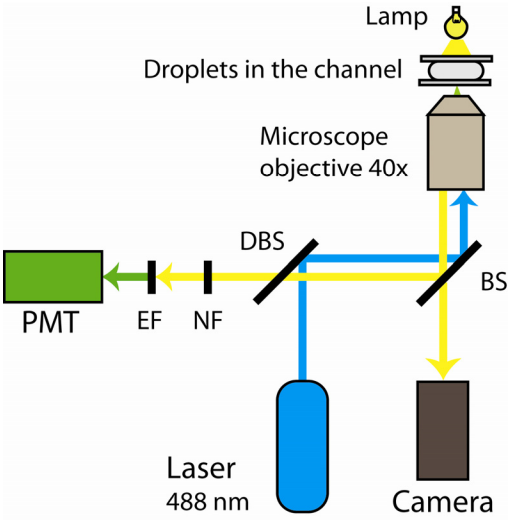

Fig. SI 1 Schematic representation of the optical setup. The 488 nm laser is reflected by a dichroic beamsplitter (DBS) into the microscope. Inside the microscope the laser is reflected at a beamsplitter (BS) and focused into the microfluidic channel by a 40x objective. The emitted fluorescent light and the light of the lamp pass back through the microscope and reach either the highspeed camera or pass through the filters (Notch filer NF and emission filter EF). The emission filter is a bandpass filter transmitting 504 ± 20 nm to the PMT which records the light intensity.

As illustrated in Fig. SI 1 a 488 nm laser source was used to excite the fluorophores contained in the droplets. The laser was focused in the channels through a 40x microscope objective (Leica). Fluorescence emission was filtered with an appropriate set of filters (Semrock Inc.) in the 484-524nm range (fluorescein detection) and then collected with a photomultiplier tube (Hammamatsu). Fluorescence detection was driven by a data-acquisition system (Labview, National Instruments) that also allowed signal processing and statistical analysis. Additionally, a high speed camera (Phantom V4.2 at $2 - 10 \times 10^3$ frames per second) recorded sequences of images of the droplet movement in the channels.

2. Cloning, expression and purification of β-lactamase.

In order to produce purified β-lactamase for the enzymatic assay, His-tagged β-lactamase was expressed in the periplasm of *E. coli* and subsequently purified from periplasmic extracts using a Ni^{2+}-NTA column.

The plasmid used is a derivative of the plasmid pAK400 (Krebber *et al*., *J. Immunol. Methods,* 1997, **201**, 35–55), which already codes for a C-terminal His-tag. The plasmid contains the strong RBS T7G10 and a pelB signal peptide for periplasmic expression, which is flanked by an upstream *Xba*I and a downstream *Nco*I site. In contrast to pAK400, which possesses a *lac* promotor, the derivative used here contains the arabinose inducible promotor of the pBAD series of plasmids (Invitrogen, Cergy Pontoise, France). To obtain this new plasmid the *lac* promotor region had been replaced with a DNA fragment coding for the araC repressor and the *ara*BAD promotor. Furthermore, an *EcoRI* site had been introduced before the C-terminal His-tag. For the cloning of β-lactamase a pUC based plasmid having ampicillin resistance (pIVEX series; Roche Applied Science, Meylan, France) was used as the PCR template. β-lactamase was amplified together with its signal peptide using the primers bla_forw_Xba 5`- GCTCTAGAGAAGGAGATATACA-TATGAGTATTCAACATTTCCGTG-3` and bla_rev_EcoRI 5`-GGAATTCCCAATGCTTAATCAGTGAGG-3`. The PCR fragment was purified, cut with *Xba*I and *Eco*RI and cloned into the pAK400 derivative thereby replacing the pelB signal sequence. The new plasmid was verified by sequencing.

The plasmid was transformed into the *E. coli* K12 strain TB1 (New England Biolabs, Frankfurt, Germany). The cultures for the purification were grown at 25 °C in 400 ml of SB medium (20 g l^{-1} tryptone, 10 g l^{-1} yeast extract, 5 g l^{-1} NaCl, 50 mM K_2HPO_4) containing 30 µg ml^{-1} chloramphenicol. This culture was inoculated from a 20 ml preculture to OD_{600}=0.1. Expression was induced with 0.02 % arabinose at an OD_{600} between 1.0 and 1.5. The cells were harvested 3 h after induction by centrifugation at 5000 g and 4° C for 10 min.

Periplasmic extracts were prepared according to a protocol included in the manual for the Ni^{2+}-NTA columns (Qiagen, Courtaboeuf, France). The extracts were dialyzed against loading buffer (50 mM sodium phosphate pH 8.0, 300 mM NaCl, 10 mM imidazole) and loaded onto the Ni^{2+}-NTA column equilibrated with loading buffer. The column was washed with 30 column volumes of loading buffer and 5 column volumes of a washing buffer (50 mM sodium phosphate pH 8.0, 300 mM NaCl, 30 mM imidazole). Elution was achieved by adding 5 column volumes of elution buffer (50 mM sodium phosphate pH 8.0, 300 mM NaCl, 200 mM imidazole). The eluted material was dialyzed against phosphate buffered saline (PBS; 10 mM Na phosphate, pH 7.4, 137 mM NaCl, 2.7 mM KCl) and concentrated using Ultrafree-4 (Millipore, Molsheim, France). The purity was confirmed by SDS-PAGE. The concentration of β-lactamase was determined by measuring the absorbance at 280 nm. The extinction coefficient was calculated using the program Vector NTI (Invitrogen). Finally, the concentration was adjusted to 1 mg ml^{-1} (corresponding to 32.6 μM) and the protein was stored in aliquots at -80 °C.

3. Multiple parallel channels as an additional strategy to reduce dispersion.

In addition to the delay-line with constrictions presented in the main text we tested an alternative layout. The strategy was not to reduce dispersion but to prevent dispersion from occurring by removing the possibility for droplets to overtake each other. The design consists of multiple parallel narrow channels (see Fig. SI 2a), that are not wider than twice the droplet diameter. As a result, there can not be any fast central stream of droplets within these channels. Provided that the flow rates in the different channels are equal, the expectation is that there would not be any dispersion.

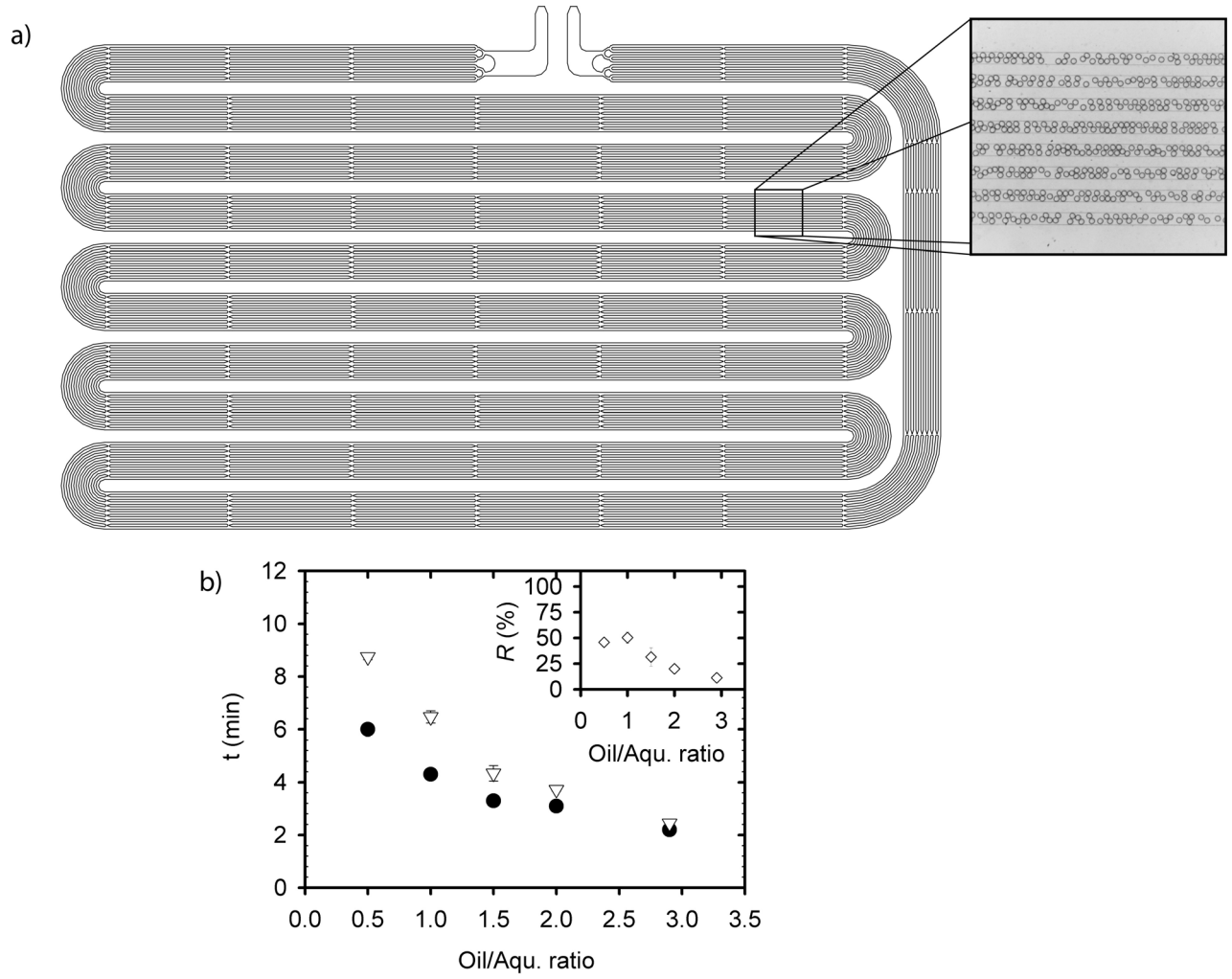

Fig. SI 2 Multiple channel delay-line. a) The wide channel which forms the delay-line is divided into eight parallel channels of 100 μm width and 75 μm depth. The design includes connections between the channels every 3 cm. Since the width of these channels is slightly smaller than twice the droplet diameter the droplets cannot overtake each other and all droplets have contact with the walls. b) Result of the dispersion measurements analogous to Fig. 2c in the main article.

Initial designs allowed an exchange of flow between the narrow channels only at the beginning and at the end of the delay-line. As a consequence, any irregularity (dirt, channel depth fluctuations, etc.) present in one of the channels completely destabilized the system. In order to reduce this problem we added bridges between the channels every 3 cm. This strategy improved the system but a completely homogenous flow across all the channels could not be achieved. For this reason the relative dispersion ratio, as seen in Fig. SI 2b, still reaches values above 50%. Although it is a clear reduction of the dispersion compared to the conventional delay-line (Fig. 2c – max R >

90%), this layout is outperformed by the delay-line with constrictions (Fig. 3b - $R < 10\%$). Additionally, this multiple channel approach requires more real-estate on the chip to get the same volume as a single wide channel of the same length. Furthermore, the fluidic resistance is higher for multiple channels (equation (2)), which limits the practical length of the delay-line (hence the maximum incubation time). In summary, the multiple channels approach has the potential to prevent dispersion, but further investigation is required to understand the factors at play in this system.

4. Optimizations of the constriction designs.

In general, all tested layouts with constrictions in the delay-line proved to be useful in reducing dispersion. However, we extracted certain design rules which improved the system. Fig. SI 3a shows a design with posts inside the channel. In this case, droplets are forced to pass the obstacle on either side. We observed that droplets can get temporary trapped at the tip of the obstacle where the stream splits, and that the droplet stream would confine them to this spot (see Fig. SI 3a). The second design depicted in Fig. SI 3b is an asymmetric constriction with a corner. The flow rates in this corner are low and droplets get pushed and trapped into this spot especially at high droplet densities.

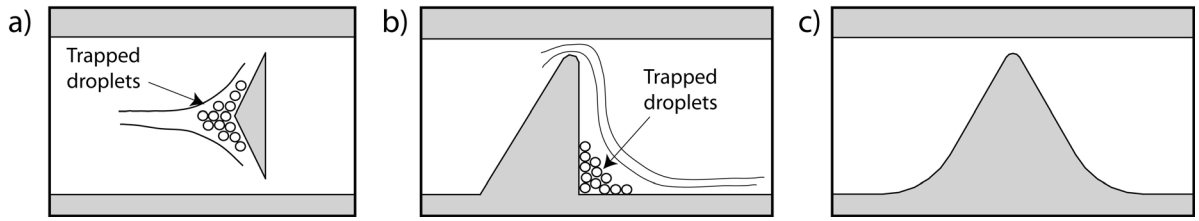

Fig. SI 3 Characteristics of different constriction designs. In all cases the width of the main channel is 1000 µm wide and is reduced to 60 – 100 µm at the constriction. Droplets flow from left to right. a) Obstacles in the channel split the stream and trap droplets temporary. b) Sharp corners, especially after a constriction lead to dead volumes with low flow rates. Droplets get temporary caught in the corner. c) Design avoiding any possibilities for droplets to get trapped.

It is therefore favorable to avoid designs, which split the stream – meaning that obstacles in the middle of the channel should be avoided. Furthermore sharp corners, especially after a constriction should be rounded out. These findings led to two designs, which resulted in very low dispersion values. The first design is shown in Fig. SI 3c and the second design in Fig. 3a of the main text.

5. Supplementary movies.

- Movie M1 shows the low droplet density regime (oil/aqu. ratio of >3) in a 1000 µm wide, 75 µm deep channel (delay-line). All of the droplets remain in the faster streamlines and do not touch the walls, which results in almost equal travelling speeds.
- Movie M2 show the medium droplet density regime (oil/aqu. ratio of ~0.7-3) in a 1000 µm wide, 75 µm deep channel (delay-line). At this density some droplets get pushed into the slower stream lines next to the walls. As a result, these droplets are overtaken by more central droplets.
- Movie M3 shows the high droplet density regime (oil/aqu. ratio of <0.7) in a 1000 µm wide, 75µm deep channel (delay-line). Droplets pack so densely that it is no longer possible for them to pass each other. They travel at almost equal speed.
- Movie M4 shows the mixing of the droplets after a constriction in the delay-line. The movie was recorded in the medium density regime (oil/aqu. ratio of 1). The channels are 1000 µm wide, 75 µm deep and the constriction has a width of 60 µm. The three arrows in the video help to follow certain droplets. The droplets marked by the green and blue arrow flow initially close to the wall and end up more central in the channel. The droplet marked by the red arrow gets redistributed from the channel center to the side.
- Movie M5 shows the measuring loops of the delay-line for the kinetic analysis. The loops are 50 µm wide and 25 µm deep. The droplets go back and forth between the shallow (25 µm) and deep channels (75 µm) at the connection points.