Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary Information for

Protein-protein interaction analysis in single microfluidic droplets using FRET and fluorescence lifetime detection

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Analyte mixing within microdroplets was investigated by observing on-chip mixed droplets of aqueous fluorescein and sulforhodamine B solutions at three detection positions (Fig. 2b). Microphotographs were taken using a triple filter for FITC/Cy3/Cy5, which results in display of fluorescein emission in the green and sulforhodamine B emission in the red image channel. Images shown in Fig. S1 were processed using ImageJ with the following procedure: RGB image channels were split and a ratio image was constructed from green channel divided by red channel. Amplification of contrast and false colour imaging showed relative fluorescein fluorescence on a scale from red (low) to black (high), which is presented in Fig. S1b-d. At the first designated position the excess of fluorescein at the bottom of the droplet is visible (Fig. S1b). After movement along the channel to the second position circulation inside the droplet is induced resulting in a nearly homogeneously mixed microdroplet (Fig. S1c). Microdroplet mixing can be regarded as complete at the third detection position (Fig. S1d) after the serpentine channel.

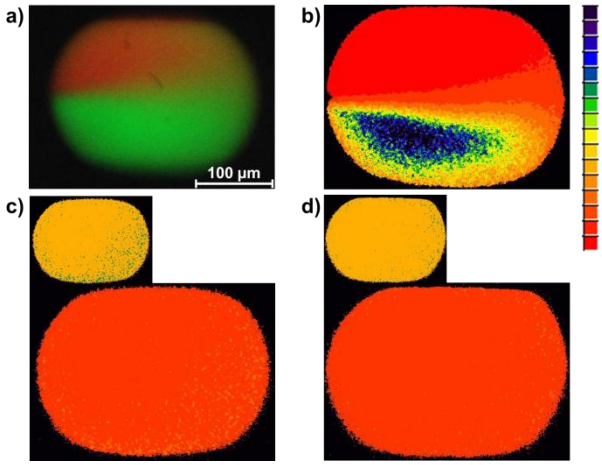

Fig. S1 Mixing progress of fluorescein and sulforhodamine B in a droplet including false colour palette from high (blue) to low (red) excess of fluorescein on black background. B. (a) Photographic image of on-chip mixed solutions at position 1. (b)-(d) False colour ratio images of red and green channel of mixing states at positions 1 to 3 showing distribution of compounds (10-fold enhanced contrast on top of c and d).

Protein binding was investigated by on-chip mixing and observation at the detection positions on the chip (Fig. 2b). A pronounced drop in fluorescence lifetime of BSA-biotin is visible when mixing BSA-biotin and streptavidin from two different streams directly in front of the flow-focusing cross. Flow rates of aqueous phases were kept constant at 300 nL/min (0.75 Hz) overall and were adjusted to produce different concentration ratios of 1:1, 1:2 and 1:3 BSA-biotin to streptavidin. Fig. S2 shows the lifetime of four and 140 successive droplets (four runs with 35 droplets each) of these solutions at the three aforementioned positions on the chip. Protein binding was easily detectable by observing only four droplets.

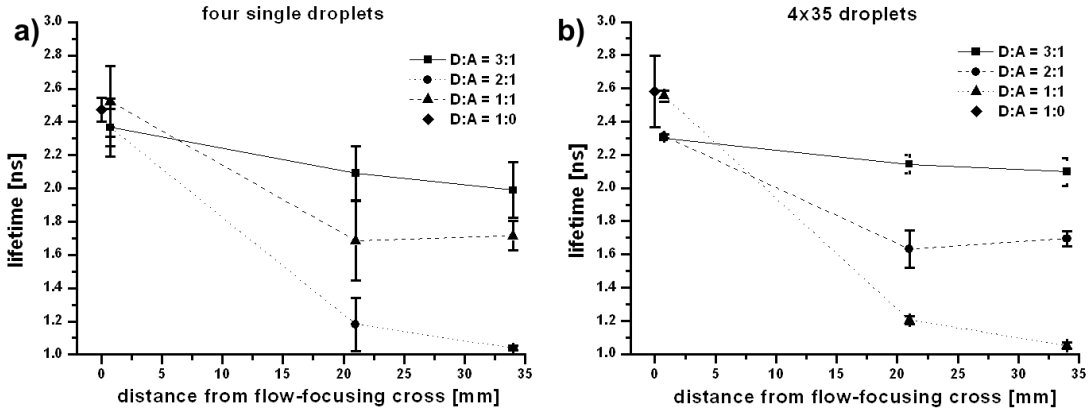

Fig. S2 Binding progress probed via fluorescence lifetime at three different positions in the microfluidic chip.

Displayed in Fig. S3 is a typical fluorescence intensity trace in the AF488-BSA-biotin channel of a single microdroplet in a competitive assay format showing large variations of fluorescence intensity along a single microdroplet.

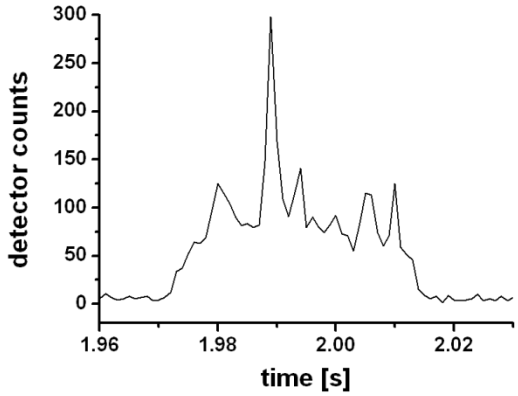

Fig. S3 Fluorescence trace of a single droplet from a competitive microdroplet assay with an avidin-to-streptavidin ratio of 1:2 at a droplet frequency of 7.9 Hz.