Electronic Supplementary Information

Selective droplet coalescence using microfluidic systems

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Supplementary Note 1. Interfacial tension measurements using pendant droplet tensiometry

Surfactant adsorption experiments were performed with a commercial pendant droplet tensiometry apparatus (DSA100S, Krüss), equipped with drop shape analysis software (DSA Version 1.90.0.14). Initially a hanging droplet of FC-40 fluorinated oil (with or without surfactant) was formed on the bevelled tip of a 22-gauge needle (Hamilton) immersed in a glass cell (Hellma) filled with MQ-water. The time required to create a drop was about 1-2 seconds. For surfactant solutions ranging from 10^{-9} to 10^{-2} % (w/w) we formed $2.0 \pm 0.1 \,\mu\text{L}$ drops and for 0.1 to 5 % (w/w) solutions we formed $1.0 \pm 0.05 \mu$ L size drops. Immediately after hanging drop formation digital droplet profiles were recorded over time and automatically fitted to a numerical solution of the Young-Laplace equation included in the DSA software. The interfacial tension between FC-40 oil and water was independent of droplet size (surface area ranging from 5 to 14 mm² tested) and found to be 52.07 ± 0.66 mN m⁻¹. The temperature of the room was maintained at 23°C by an air-conditioning system. Following the same procedure we performed interfacial tension measurements with 10%, 40% and 96% ethanol solutions in surfactant-free FC-40 oil. The obtained values are indicated in Table S1.

Supplementary Note 2. Interfacial tension measurements of microfluidic droplets

Following Taylor's theory (1, 2) the interfacial tension γ of a droplet in an unbounded extensional flow fields can be obtained by measuring the transient deformation D of a droplet:

$$\kappa \eta_c \left(5 \frac{\dot{\varepsilon}_1 - \dot{\varepsilon}_2}{4\hat{\eta} + 6} - \frac{dD}{dt} \right) = \gamma \frac{D}{2R_0}, \ [1]$$

where η_c and η_d are viscosities of continuous and dispersed phases, $\hat{\eta} = \eta_d / \eta_c$ is viscosity ratio, R_0 is a radius of spherical droplet, the term $\kappa \eta_c$ is an "effective viscosity" and κ being a function of viscosity given by:

$$\kappa = \frac{(2\hat{\eta} + 3)(19\hat{\eta} + 16)}{40(\hat{\eta} + 1)}$$
[2]

The extensional flow field is generated when the flow accelerates or decelerates due to expansion(s) or constriction(s) in the channel consequently deforming the droplet interface. In unidirectional flow along the *x*-axis the extensional rate is described by:

$$\dot{\varepsilon} = \frac{du_d}{dx} = -\left(\frac{dt}{dx}\right)^{-2} \frac{d^2t}{dx^2}, \qquad [3]$$

where *t* is the transit time and u_d is droplet velocity (3). The scalar droplet deformation parameter *D* defined as D = (A - B)/(A + B), where *A* and *B*, the major and minor principal radii of the spheroid droplet, can be calculated from the moments of inertia or, as used in our approach, by directly measuring the major and minor drop axes. A detailed characterization of two-phase microfluidic interfacial tensiometry, as well as sources of errors has been described recently (4-6).



Fig. S1 Schematic and design of the microfluidic device. (a) Schematic of the microfluidic system showing the main operations including: on-chip droplet generation (clear circles), emulsion reinjection (black circles), droplet pair formation, spacing of droplet pairs, sliding and droplet coalescence. Black arrows indicate the direction of the flow. (b) Design of the droplet coalescence device. Individual microfluidic modules are indicated. The red square indicates the channels used for emulsion reinjection, droplet production on-chip, droplet pair formation and coalescence. The dimensions of each channel are given in μ m, where *w* is width and *L* is length of the channels. All channels were 20 μ m deep.



Fig. S2 Selective droplet coalescence in asymmetric and symmetric regimes. Reinjected emulsion droplets (volume V_2) are black and droplets generated on-chip (volume V_1) are clear. (a) Droplet coalescence corresponding to the symmetric case when $V_1 = V_2 = 20$ pl. (b) Droplet coalescence corresponding to the asymmetric case when $V_1 > V_2$; $V_1 = 20$ pl and $V_2 = 16$ pl. (c) Droplet coalescence corresponding to the asymmetric case when $V_1 < V_2$; $V_1 = 20$ pl and $V_2 = 60$ pl. (d) Droplet coalescence corresponding to the asymmetric case when $V_1 > V_2$; $V_1 = 25$ pl and $V_2 = 10$ pl. The red arrows track a surfactant-stabilized droplet, which ultimately fuses with a droplet generated on-chip. Black arrows indicate direction of the flow. Scale bars 50 µm. The time step between images is given inside the gray boxes.



Fig. S3 Surfactant adsorption analysis using pendant droplet tensiometry. (a) Dynamic interfacial tension measurements of adsorption of EA-surfactant onto a water-fluorinated oil (FC-40) interface at different surfactant concentrations over 20 hours of incubation. The concentration of surfactant C was 10^{-9} (1), 10^{-6} (2), 10^{-5} (3), 10^{-4} (4), 10^{-3} (5), 10^{-2} (6), 10^{-1} (7), 1 (8) and 5 (9) % (w/w) of EA-surfactant, where 1% = 1.47 mM. Numbers on the right side of each curve correspond to the list of surfactant concentrations as indicated above. (b) Equilibrium interfacial tension values γ_e for FC-40 oil and water interface as a function of EA-surfactant concentration C. Data points were obtained from the intercept of linear plot γ versus t $^{1/2}$. The red solid line represents a least square curve fit to the Langmuir model. The fit parameters were $\Gamma_{\infty} = 4.475 \times 10^{-6} \text{ mol/m}^2$ and $a_L = 6.7 \,\mu\text{M}$ (4.56×10^{-3} % mass fraction). The intercept of the red and black lines corresponds to bulk surfactant concentration in fluorinated FC-40 oil reaching the CMC at ~ 0.03% (~ 44μ M), which is in good agreement with DLS measurements (CMC = 40μ M). The slight reduction in interfacial tension above the CMC may indicate relative polydispersity in the molecular mass of the polymer, structural changes within the adsorbed layer in the nearly saturated state and distribution of interfacial activity of EA-surfactant.



Fig. S4 Time series of droplet break-up. The re-injected emulsion droplet is black and droplet generated on-chip is clear. The time step between images is 244 μ s. The continuous phase was FC-40 oil with ~ 2 mM EA-surfactant. The black arrow indicates the direction of the flow and the red arrow indicates the satellite droplet produced during break-up of an emulsion droplet. After break-up the satellite droplet remains attached to the interface of the on-chip droplet. Scale bar 20 μ m.



Fig. S5 The dynamics of interfacial tension for EA-surfactant adsorption at the FC-40 oil and water interface using the pendant droplet technique. The dispersed phase (1.0 μ l) was FC-40 fluorinated oil containing 7.5 mM (5% [w/w]) EA-surfactant and continuous phase was pure water. The interfacial tension values (mN/m) were obtained by fitting the Young-Laplace equation to a droplet profile with Droplet Tensiometry Apparatus DSA software (Krüss). The surface tension value after 1 hour of incubation was $\gamma \sim 6.60$ mN/m. Temperature during the measurements was 23°C.



Fig. S6 Critical micelle concentration measurements using dynamic light scattering. The CMC for EA-surfactant in different fluorinated oils (FC-40, FC-77 and Galden HT-135) was determined by performing dynamic light scattering measurements on a Malvern Zetasizer Nano-S apparatus equipped with a 4.0 mW laser operating at $\lambda = 633$ nm and at a scattering angle 173°. Different amount of EA-surfactant were dissolved in fluorinated oils and equilibrated for 24 hours at room temperature (25°C) prior to measurement. For each sample, 10 measurements were recorded and the mean sizes with standard deviations are plotted in the graph. The CMC values obtained in FC-40, FC-77 and Galden HT-135 oils were as following: CMC_{FC40} = 40 μ M (0.027%), CMC_{FC77} = 30 μ M (0.022%) and CMC_{HT135} = 105 μ M (0.076%).

Supplementary references:

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- 4. Cabral JT & Hudson SD (2006) Microfluidic approach for rapid multicomponent interfacial tensiometry. *Lab on a Chip* 6(3):427-436.
- 5. Hudson SD, Cabral JT, Goodrum WJ, Beers KL, & Amis EJ (2005) Microfluidic interfacial tensiometry. *Applied Physics Letters* 87(8):081905(081903).
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ESI Movie Captions



Movie S1. Droplet reinjection and pairing with droplets generated on-chip. Emulsion (reinjected) droplets (15pl) are black and droplets generated on-chip (25 pl) are clear. Carrier oil is FC-40 containing 3 mM EA-surfactant. Black droplets (containing 50 mM Methylene Blue) were introduced into the microfluidic device at 25 μ L/hr, spaced with carrier oil at 60 μ L/hr and brought into contact with droplets generated on-chip that were produced at flow rates 25 μ L/hr for the aqueous phase and 125 μ L/hr for the carrier oil.



Movie S2. Passive droplet coalescence inside a straight channel. Emulsion droplets are black and droplets generated on-chip are clear. Inside the coalescence channel, emulsion droplets slide over the surface of droplets generated on-chip until the position where they become pulled apart by the hydrodynamic flow leading to coalescence. Black droplets were introduced into the microfluidic device at 25 μ L/hr and spaced with carrier oil at 60 μ L/hr. Droplets generated on-chip were produced using flow rates of 25 μ L/hr for the aqueous phase and 125 μ L/hr for the carrier oil. Droplets pairs entering the coalescence channel were spaced by carrier oil at a flow rate of 100 μ L/hr.



Movie S3. Passive droplet coalescence with an excess of droplets generated on-chip. Only those droplets generated on-chip (clear) that were paired with emulsion droplets (black) experienced fusion. When spatiotemporal order inside the coalescence channel is disrupted, droplets begin to collide resulting in irregular spacing between droplets, perturbation of the flow surrounding the droplets and variability in coalescence time point and angle. Black droplets were introduced into the microfluidic device at 20 μ L/hr, spaced with carrier oil at 60 μ L/hr. Droplets generated were generated on-chip using a flow rate of 25 μ L/hr for the aqueous phase and 125 μ L/hr for the carrier oil. To trigger collision between multiple droplets the flow rate of carrier oil in the coalescence channel was reduced to 10 μ L/hr.



Movie S4. Fused droplet collection off-chip. Fused droplet (40 pl) are collected off-chip in the form of an emulsion.



Movie S5. Passive droplet coalescence with an excess of emulsion droplets. Emulsion droplets (black) are fusing selectively one-to-one with droplets generated on-chip (clear) even when the emulsion droplets introduced into the device are in large excess.



Movie S6. Selective 2:1 passive droplet fusion. Two emulsion droplets (black) are fusing selectively to a single droplet generated on-chip (clear). Emulsion droplets are 5 pl and on-chip generated droplets are 15 pl volume.



Movie S7. Selective 3:1 passive droplet fusion. Three emulsion droplets (black) are fusing selectively to a single droplet generated on-chip (clear). Emulsion droplets are 3 pl and on-chip generated droplets are 15 pl volume.