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Supporting Information

An Oil Droplet Division–Fusion Cycle

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Contents

Materials and Methods. Beta-carotene, decanoic acid (DA)decyltrimethylammonium bromide (DTAB), dimethyldioctadecylammonium bromide (DDAB), hexadecyl-trimethyl-ammonium bromide (CTAB), iodine, myristoleic acid (MO), nitrobenzene, oil red O, oleic acid (OA), sodium chloride, sodium hydroxide, sudan black B were obtained from Sigma Aldrich (Denmark). Glass microscope slides with a 1.5 cm diameter concave depression were purchased from VWR (Italy). Glass bottom culture dishes of 35 mm diameter were supplied by MatTek Corporation (USA).

Negatively charged surfactants in water. Neat fatty acid oil was added to alkaline water (NaOH) to make 5 mM micelles at pH 12.

Positively charged surfactants in water. Solid surfactant was added to alkaline water (NaOH) and sonicated for 5 min to make 20 mM micelles at pH 12.

Oil phase. Nitrobenzene was used as provided from the supplier. For fission, positive surfactant powder was added directly to the oil and heated to 55°C for 15 minutes to dissolve the surfactant into the oil. For the negatively charged surfactants, the neat oil was added directly to the nitrobenzene and vortexed. To distinguish droplets by color, sudan black B or oil red O was added to nitrobenzene to a final concentration of 0.2 mg/ml to make blue and red droplets respectively.

Tensiometry. Interfacial tension of the oil water interface was determined using a PAT1D tensiometer (Sinterface) by the pendent drop method using the Sinterface software. The samples were prepared with nitrobenzene as the internal phase and an external aqueous phase containing varying surfactants. To measure the interfacial tension under the fission conditions, a nitrobenzene oil droplet with 20 mM CTAB was added to 5 mM oleate at pH 12. All values were taken after the tension reached a steady state whenever possible and each condition was tested in triplicate.

The maximum volume of oil droplets in surfactant was determined by pendant drop tensiometry. The volume of a hanging droplet was manually increased until the droplet fell from the capillary. The maximum droplet volume indicates the volume of the oil droplet held under tension at the tensiometer capillary before the force of gravity overcomes the tension and the droplet falls from the capillary. This data was then plotted against the measured interfacial tension for each condition. Measurements were repeated at least in triplicate.

Droplet fission. A droplet of nitrobenzene oil impregnated with surfactants and dye was transferred by hand pipette to a concave glass slide containing 100 μl of surfactant at pH 12 or to a glass bottom culture dish 35mm in diameter containing 300 μl of surfactant pH 12. See Table S1 for the combinations of surfactants tested. The system was monitored using an iSight camera and BTV Pro software.

Droplet fission fusion cycle. Nitrobenzene containing sudan black B for color (blue) was impregnated with CTAB, as described above. A 20 μl droplet is added to the convex impression of a glass slide containing 100 μl 5 mM oleate pH 12. After droplet fission, 10 μl of the divided droplets are transferred to a proximal chamber containing 100 μl 20 mM CTAB pH 12 and a 10 μl nitrobenzene droplet with oil red O added for color (red). Fusion between the droplets is induced by adding 5 M NaCl as described. After fusion, 20 μl of the fused oil phase is transferred to a chamber containing 5 mM oleate pH 12. After fission, 10 μl of divided droplets are transferred to a chamber containing 20 mM CTAB pH 12. 5 M NaCl is again added to induce fusion of the droplets. System was monitored with an iSight camera and BTV Pro software.

Droplet fusion. Droplets (usually of 5-10 μl) of either nitrobenzene or nitrobenzene impregnated with a surfactant and dye were added to a convex glass slide containing 100 μl

20 mM CTAB pH 12 by hand pipette. 1 µl 5 M NaCl was then added to the sample as a fusagen. The system was monitored using an iSight camera and BTV Pro software.

Iodination assay. Nitrobenzene was saturated with solid iodine to produce a dark orange oil. For use in the fusion experiments, the saturated oil was diluted in nitrobenzene 1:10 to produce a more lightly colored orange oil. Independently beta-carotene was added to pure nitrobenzene and mixed to obtain a yellow oil at 2 mg/ml. Droplets of 5 µl of each preparation were added to 100 µl 20 mM CTAB pH 12 on a glass slide with concave impression as described above. Fusion and color change to green, indicating the iodination reaction, was monitored using an iSight camera and BTV Pro software.

Supplementary Table S1. Conditions that support spontaneous droplet fission. Several different surfactant pairs were tested where one surfactant was solvated in the aqueous phase (at 5 mM) and the other in the oil phase (at 20 mM). All aqueous solutions were prepared at pH 12. No fission was observed where the same surfactant was solvated in both phases as a control. No fission was observed in cationic-cationic or anionic-anionic combination controls. Fission was found with DTAB (aq) with all anionic surfactants (oil). Similarly fission was found with CTAB (oil) with all anionic surfactants (aq). Reciprocity was demonstrated for DTAB and MO combination where it did not matter into which phase the catanionic amphiphiles were partitioned. Results are summarized in Table S1. Final molecular ratio of aqueous phase surfactant to oil phase surfactant was held constant at 15:1.

**Table S1. Conditions that support spontaneous droplet fission.**

Aq phase (5mM)	Oil phase (20 mM surfactant)					
	CTAB	DTAB	DDAB	OA	MO	DA
CTAB	-	-	-	-	-	-
DTAB	-	-	-	+	+	+
DDAB	-	-	-	-	-	-
OA	+	-	-	-	-	-
MO	+	+	-	-	-	-
DA	+	-	-	-	-	-

Supplementary Table S2. Fission conditions with varying ratios of the catanionic systems. Cationic and anionic surfactants were tested in both the aqueous and oil phases at varying final molecular ratios calculated for the entire volume of the system to determine the parameters that support spontaneous droplet fission, see Methods. The ratios and results of the fission assays are reported in Table S2. Fission occurs only when the ratio of anion to cation is skewed. Final equimolar ratios of the two surfactants results in no fission in the system. Fission was also observed with other catanionic pairs such as MO in excess over DTAB (ratio 15:1). The DDAB system does not promote fission for any ratio tested. This may be due to the fact that DDAB is very hydrophobic and once assembled at the oil water interface, it does not readily leave the interface as determined by real time pendant drop tensiometry (data not shown). Reciprocity of catanionic partitioning at the start of the experiment can be shown with different pairs (e.g. DTAB and MO as shown in Table S1) as long as the molecular ratios are highly skewed (Table S2). We hypothesize that the fission processes rely upon the formation of a catanionic supramolecular system, which depends selectively on the type and numbers of molecules in the system

Table S2. Catanionic ratios tested for oil droplet fission.

Aqueous Phase	Volume (µl)	Oil Phase	Volume (µl)	Ratio anion/cation	Fission?
OA (mM)		CTAB (mM)			
1	300	60	5	1	NO
2	300	120	5	1	NO
5	300	300	5	1	NO
5	300	60	5	5	YES
5	300	20	5	15	YES
CTAB (mM)		OA (mM)			
20	100	5	10	0.025	YES
20	1000	5	100	0.025	YES

5	300	20	5	0.07	NO
5	300	60	5	0.2	NO
5	300	120	5	0.4	NO
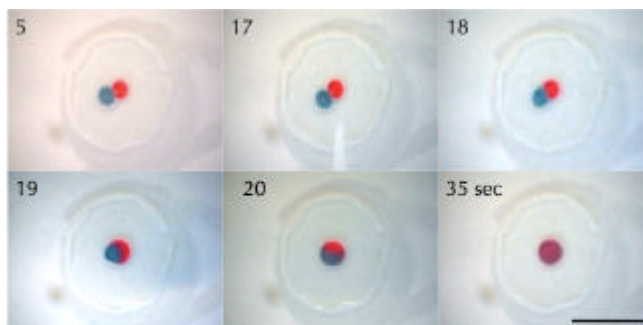
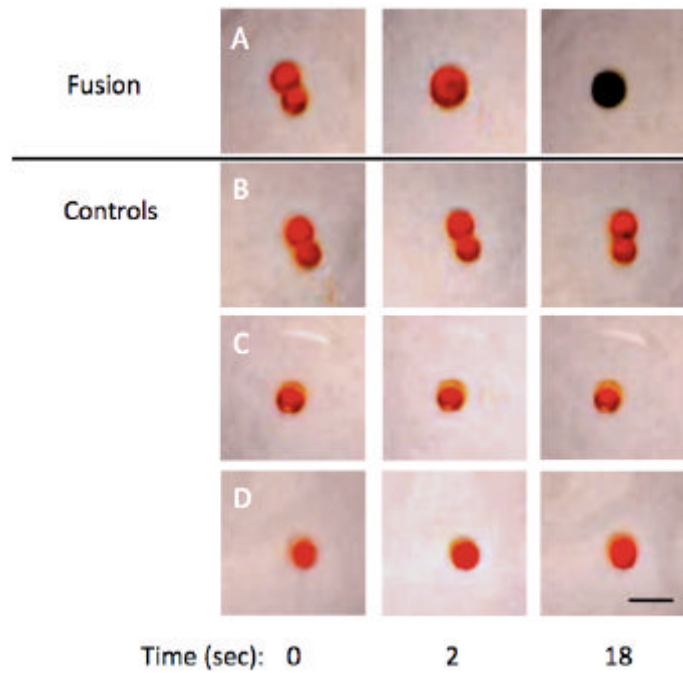**Figure S1.** Droplet fusion time course. Two droplets of 5 µl (red and blue) were added to 100 µl 20 mM CTAB pH 12. 1 µl of 5 M NaCl was then added next to the droplets (time 17 sec, pipette visible at center bottom of image) triggering fusion. See Movie S2. Size bar: 1cm.
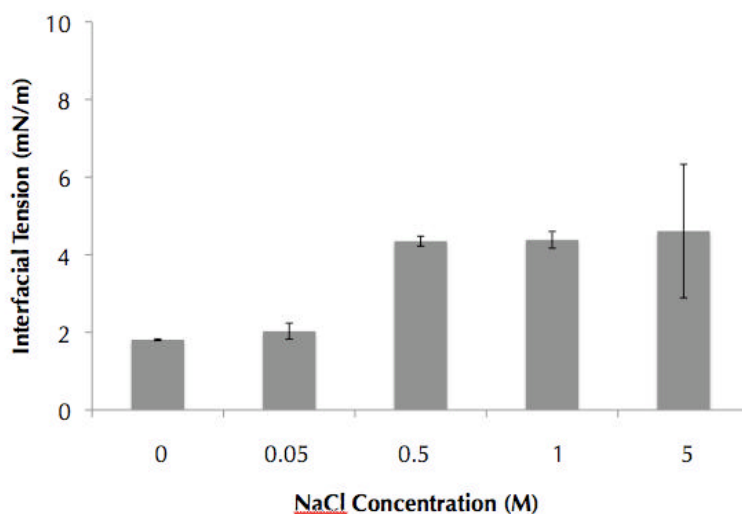
Figure S2. Interfacial tension versus salt concentration. Oil droplets were placed in aqueous phase containing 20 mM CTAB, pH 12. Different samples were measured with increasing concentrations of NaCl. Errors bars represent the standard error over three independent readings.

Droplet fusion and encapsulated chemical reaction. When one droplet is loaded with beta-carotene as a reactant, and another droplet is loaded with iodine, fusion can induce a chemical reaction. Iodination of beta-carotene will produce a spectral shift in absorbance resulting in a dark green color[20]. As shown in Figure S3 A, two droplets containing

reactants are fused in CTAB with NaCl. The fused droplet quickly changes color from light orange to dark green, indicating the iodination reaction occurs. Figure S3 B-D shows the controls where no color change is seen in droplets with either reactant alone (C and D) or in droplets placed together without fusion (B).

Time (sec): 0   2   18

Figure S3. Time course of a droplet fusion event that triggers iodination of beta-carotene. Droplets of 5 ml each are introduced to 100 ml of 20 mM CTAB pH 12 placed into wells on glass slides. A: one droplet containing iodine and one containing beta-carotene. This well also includes NaCl and therefore represents the fusion condition. B: contains the same as A but without NaCl is a no fusion control. C, D: single droplets with beta-carotene and iodine respectively as controls. The diameter of each frame is 2.0cm.

Movies. Fission (S1), fusion (S2), replication cycle (S3), fusion-fission (S4).

Thermodynamics of droplet fission

If we assume the droplet fission process is mainly shaped by the competing gravitational (g) – and interfacial (i) energies, we may express Gibb's free energy for the droplet system as:

$G = G_i + G_g$.   (1)

Assuming we have a total oil droplet volume of V (~5 µl, assumed to for a sphere), we can express the free energies G_i and G_g in the droplet system as a function of the number of oil droplets n present

$$G_i(n) = g\,[3V\,(4p)^{1/3}]^{2/3}\,n^{1/3} = g\,C_i\,n^{1/3}, \tag{2}$$

where g denotes the interfacial tension and

$$G_g(n) = Dr\,V\,g\,[3V/4p]^{1/3}\,n^{-1/3} = C_g\,n^{-1/3}, \tag{3}$$

where Dr denotes the density difference between nitrobenzene and water (~0.2 g/cm^3) and g denotes the gravitational acceleration (~9.82 m/s^2).

Since the free energy G is minimal in equilibrium, we can determine how G is minimized as a function of the number of droplets n, by differentiating G with respect to n:

$$\partial G/\partial n = 0 \quad \hat{U} \tag{4}$$

$$n = (C_g/g\,C_i)^{2/3} = [(Dr\,g\,V^{2/3}/g)(1/144p^2)^{1/3}]^{3/2} \tag{5}$$

Keeping V, Dr and g constant in each experiment (values give above) we can use equation (5) to determine how the number of droplets n varies with the interfacial tension g assuming thermodynamic equilibrium and equal droplet sizes.

In particular we can determine when a single droplet (n=1) is expected to break into two identical smaller droplets (n=2) as a function of the reduction in the interfacial tension. For instance, for the droplet fission process depicted in figure 1 where we see n=1 → n=2, we can from equation (5) determine, that the value of g after the division lies in the interval between 0.39 mN/m (n = 1.5) and 0.28 mN/m (n = 2.5), with an average of 0.335 mN/m. If g was smaller than 0.27 mN/m we would have seen a droplet fission into 3 new smaller droplets (n=3).

References

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