Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Information

Flower-like droplets by self-emulsification of a phase-separating (SEPS) aqueous film

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Movie S1. A typical video showing the evolution of aqueous "mother droplets" containing 0.50 mM and 2.00 mM CTAB deposited on an aqueous substrate, followed by fast spreading and fragmentation. The droplet and substrate phases are 20 wt% PEG (Mw = 8000)-rich and 5 wt% Na_2CO_3-rich aqueous phases, extracted from the top and bottom phases of an equilibrated 20 wt% PEG-5% Na_2CO_3 ATPS solution, respectively. The 0.02 wt% rhodamine 6G is added to improve the optical contrast. The volume of deposited droplet is 6 μL, and the movie is played in real time. The scale bar is 5 mm.

Movie S2. A close-up video showing the hole nucleation, hole expansion, and fragementaion of a surfactant-laden (0.5 mM CTAB) aqueous film. The droplet and substrate phases are 20 wt% PEG (Mw = 8000)-rich and 5 wt% Na_2CO_3-rich aqueous phases, extracted from the top and bottom phases of an equilibrated 20 wt% PEG-5% Na_2CO_3 ATPS solution, respectively. The volume of deposited droplet is 6 μL, and the movie is played in real time.

Movie S3. A typical video showing the fragmentation of an aqueous film in the case of a non-equilibrium ATPS. The droplet and substrate phases are pure 5 wt% dextran (Mw = 10 kDa) aqueous solutions containing 10 mM SDS and pure 30 wt% PEG (Mw = 35 kDa) aqueous solutions, respectively. The volume of deposited droplet is 7 μL, and the movie is played in real time. The scale bar is 550 μm.

Movie S4. A typical video showing the fragmentation of a aqueous film in the case of a non-equilibrium A3PS. The droplet and substrate phases are the aqueous mixture of 6 wt% dextran (Mw = 10 kDa) and 4 wt% PEG (Mw = 35 kDa) containing 2 mM SDS, and the 30 wt% PEtOx (Mw = 50 kDa) concentrated solution, respectively. The volume of deposited droplet is 5 μL, and the movie is played in 8X real time. The scale bar is 400 μm.

Movie S5. A typical video showing the fragmentation of a nanoparticle laden aqueous film in the case of a non-equilibrium A3PS. The droplet and substrate phases are the aqueous mixture of 6 wt% dextran (Mw = 10 kDa) and 4 wt% PEG (Mw = 35 kDa) containing 5 mg/mL nanoparticles and 2 mM SDS, and the 30 wt% PEtOx (Mw = 50 kDa) concentrated solution, respectively. The volume of deposited droplet is 5 μL, and the movie is played in 2X real time. The scale bar is 400 μm.

1) Materials

i) Chemicals for AMPSs: Polyethylene glycol (PEG, molecular weight Mw = 600 Da, 8 kDa or 35 kDa) and sodium carbonate anhydrous (Na_2CO_3) are purchased from Aladdin, Shanghai, China; Dextran (Mw = 10 kDa or 40 kDa) is purchased from CASB, Shanghai, China. Choline chloride ($[N_{111(2OH)}]Cl$, ≥ 98%) and Ficoll (Mw = 70 kDa) are purchased from Sigma-Aldrich, St. Louis, USA. PEG and Na_2CO_3, PEG and dextran, or $[N_{111(2OH)}]Cl$ and PEG are used to prepare the three different types of ATPSs, including polymer/salt, polymer/polymer, and inonic liquid-based systems. PEG, dextran, and Ficoll are used to form the aqueous three-phase system (A3PS).

ii) Hydrosoluble surfactants: Three different types of hydrosoluble surfactants are used to lower the surface tension of the aqueous droplet phase, including: i) the cationic hexadecyltrimethylammonium bromide (CTAB, BioXtra, ≥ 99%); ii) the anionic surfactant sodium dodecyl sulfate (SDS, ACS reagent, ≥ 99%); both of which are purchased from Sigma-Aldrich, St. Louis, USA; and iii) the nonionic surfactant, Tween 20, which is purchased from Promega Corporation, Madison, USA.

iii) Fluorescent Dyes and Nanoparticles: Rhodamine 6G, fluorescein isothiocyanate-dextran (FITC-dextran, Mw = 500 kDa), amine modified polystyrene fluorescent particles (mean diameter: 100 nm) are purchased from Sigma-Aldrich, St. Louis, USA. Rhodamine B-Ficoll (Mw = 70 kDa) is purchased from Nanocs, New York, USA.

iv) Photo-crosslinking: Poly(ethylene glycol) diacrylate (PEGDA, Mw = 575 Da) and photoinitiator, 2-hydroxy-2-methylpropiophenone are purchased from Sigma-Aldrich, St. Louis, USA.

iv) Polyelectrolytes and polysaccharides: Poly(allylamine hydrochloride) (PAH, Mw = 50 kDa) and sodium alginate (BioReagent) are purchased from Sigma-Aldrich, St. Louis, USA.

2) Experimental characterization

i) Density, viscosity, interfacial tension, surface tension and osmolality measurements: The viscosity, density, interfacial tension, and osmolality of the two aqueous phases are measured by a rheometer (MCR 302, Anton Paar), a density meter (DA-100M, Mettler Toledo), a spinning drop tensiometer (KRÜSS, SITE100) and a micro-omometer (Model 3250, Advanced Instruments), respectively. The surface tension of all aqueous solutions is obtained experimentally by the pendant drop method[1, 2] with a custom MATLAB code. All experiments are performed at room temperature (~25 °C).

ii) Microscope observation: The bright field images are captured by a high-speed camera (Phantom V9.1, FASTCAM SA4, Photron) coupled with an inverted microscope (Motic AE2000). The fluorescence images are captured by a fluorescence microscope (DMIL LED Fluo, Leica) equipped with a camera (Infinity 3, Lumenera). The captured images are processed using ImageJ (NIH) software.

iii) Scanning electron microscope (SEM): Hydrogel microparticles are washed by deionized (DI) water for more than 5 times, and then freeze-dried. The dried samples are sputtered with gold before imaging under scanning electron microscope (Hitachi S3400N VP, 20.0 kV).

3) Preparation of aqueous multi-phase systems (AMPSs)

i) The equilibrated ATPSs: We prepare the equilibrium ATPS by dissolving three different pairs of solutes, 20 wt% PEG (Mw = 8 kDa)-5 wt% Na_2CO_3, 10 wt% dextran (DEX, Mw = 40 kDa)-5 wt% PEG (Mw = 35 kDa), and $[N_{111(2OH)}]Cl$ and PEG (Mw = 800 Da), into DI water. To facilitate dissolution, the aqueous solutions are vigorously mixed in a vortex (Vortex V-1 plus, Boeco, Germany). The well-mixed solutions are centrifuged in a laboratory centrifuge (Thermo Scientific, Germany) at 8000 r.p.m for 2 hours and then allowed to phase-separate for more than 48 hours. Finally, the two immiscible aqueous solutions, PEG-rich and Na_2CO_3-rich phases, PEG-rich and DEX-rich phases or $[N_{111(2OH)}]Cl$-rich and PEG-rich phases, are collected from the top and bottom phases of the equilibrated ATPS, respectively, as shown in Figure S1.

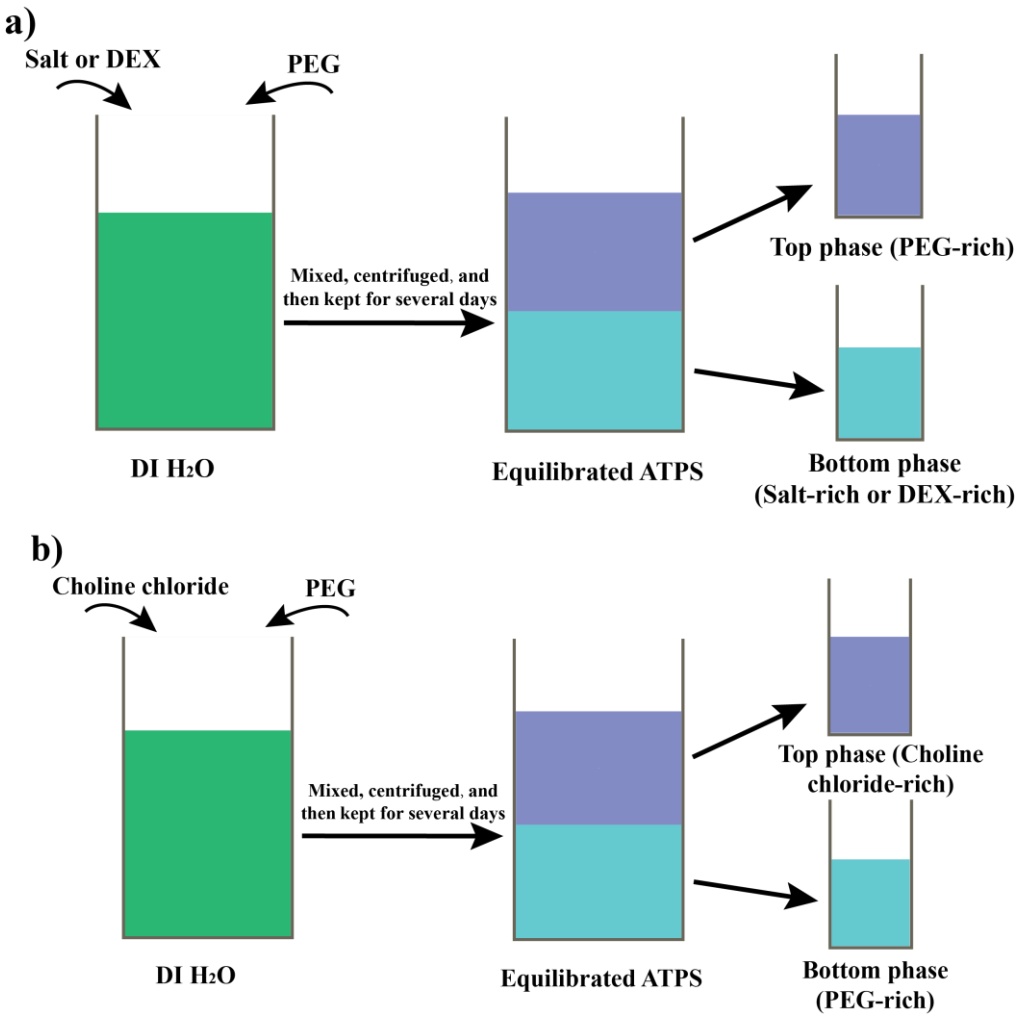

Figure S1. Schematics showing the typical preparation of the equilibrated ATPSs: a) polymer-salt (PEG-Na_2CO_3), polymer-polymer (PEG-DEX); b) ionic liquid-polymer ($[N_{111(2OH)}]$Cl-PEG) systems.

ii) The non-equilibrated AMPSs: We prepare the solutions by directly dissolving the respective solutes in deionized (DI) water. The procedures of phase-separation and collecting the respective phases (the top and bottom phases), which usually takes at least several hours, are not needed, as shown in Figure S2.

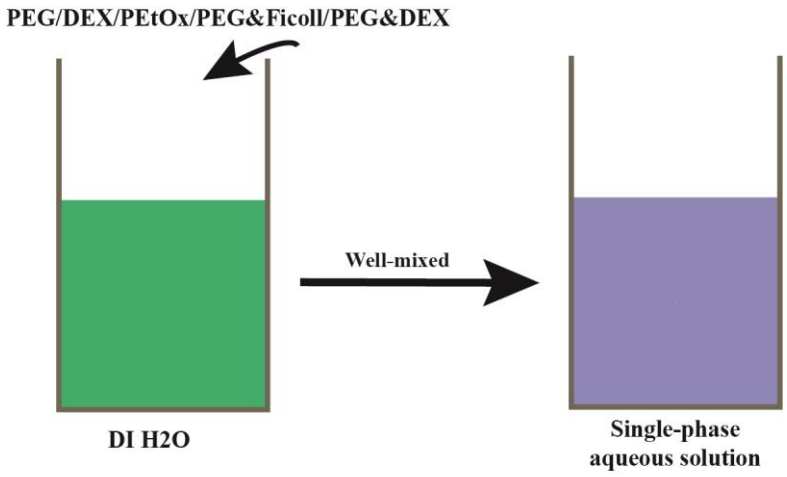

Figure S2. A schematic showing the typical preparation of non-equilibrated AMPSs.

4) Typical experimental procedures to form microdroplets via film bursting

In a typical trial, for instance, in the case of the phase-separated 5 wt% Na_2CO_3-20 wt% PEG (Mw = 8 kDa) ATPS, 15 mL 5 wt% Na_2CO_3-rich aqueous solutions fill a Petri dish (100 mm diameter, ISO Lab) and the resulting depth of the liquid bath is around 2 mm. The 20 wt% PEG-rich droplets with a volume of 6 μL and containing a small amount of surfactants, are carefully deposited on the liquid substrate via a micropipette (2-10 μL, Thermo Scientific). The prepared surfactant-laden PEG-rich aqueous solutions, with surfactant concentration varying from 0 mM to 10.00 mM, are diluted from stock solutions containing 10.00 mM surfactants. No surfactants are added into the Na_2CO_3-rich substrate phase. We monitor the whole dynamic behaviors using a Canon 70D(W) camera from above (5472×3072 pixels) at 30 fps with an LED panel illuminating from below, and the local bursting of thin films is recorded by a high-speed camera (Phantom V9.1, FASTCAM SA4, Photron) coupled with an inverted microscope (Motic AE2000).

5) Criteria to determine the fate of a liquid droplet deposited on a liquid substrate

The fate of the droplets upon deposited on a liquid substrate is determined by the spreading coefficient S, where $S > 0$, the droplet will spread on the substrate. For $S < 0$, the droplet will sit on the surface, as schematically shown in Figure S3.

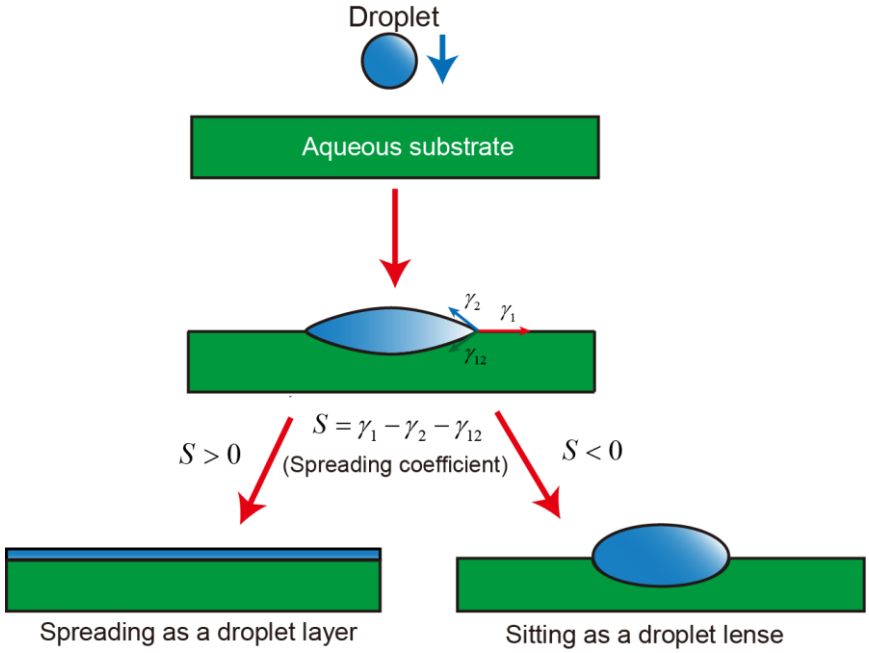

Figure S3. Schematic showing the final configuration of a liquid droplet deposited on a liquid substrate estimated by the spreading coefficient, defined as $S = \gamma_1 - \gamma_2 - \gamma_{12}$.

6) Shape of resulting droplets demonstrated by PEGDA microparticles

To capture the shape of resulting droplets, we adopt the phase-separated 15 wt% PEGDA (Mw= 575)/ 10 wt% Na_2CO_3 ATPS, where 15 wt% PEGDA-rich phase with 10 mM CTAB and 5 wt% photoinitiator, and 10 wt% Na_2CO_3-rich aqueous phases are used as the "mother droplet" and aqueous substrate, respectively. After the formation of the "daughter droplets", we illuminate with UV to initiate the cross-linking reaction to fabricate microgels. The UV light was supplied via a UV curing equipment (SP-VI Spot Cure, USHIO).

The UV light intensity around the liquid-air interface of the aqueous substrate is measured by an intensity meter (ABM Inc., USA), which is in the range of 10~25 mW/cm^2 (at 365 nm) for all presented results. The UV light illuminates for 120 s for each trial. Due to the low density of the droplet phase (ρ_d = 1.088 g/cm^3) relative to that of the aqueous substrate (ρ_s = 1.133 g/cm3) as well as the low interfacial tension of the w/w interface ($\gamma_{ATPS} \approx$ 1.39 mN/m), the droplet templates float and therefore the crosslinked PEGDA microparticles adopt a saucer-like shape, as shown in Figure S4.[3]

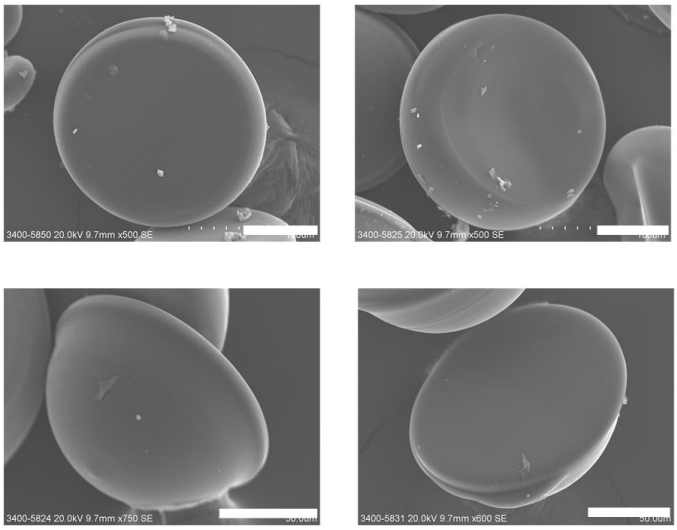

Figure S4. SEM images showing the fabricated PEGDA microparticles. The droplet and substrate are 15 wt% PEGDA-rich phase with 10 mM CTAB and 10 wt% Na_2CO_3-rich aqueous phases. The volume of "mother droplet" is 6 μL. Scale bars: 50 μm.

7) Estimation of the height of the "daughter droplets" formed

The height of the "daughter droplets" formed is calibrated against the corresponding fluorescence intensity measured from fluorescence microscope imagea of an aqueous droplet (Volume: V_s = 1 μL) with the same composition as that of "daughter droplets"on a glass slide.[4] The droplet is assumed to be a spherical cap; by measuring its diameter (d), we can calculate the height of the droplets (h), which is given as:

$$V_s = \frac{1}{6}\pi h\left(3a^2 + h^2\right) \quad (1)$$

where V_s, a, and h are the volume, diameter, and height of the spherical cap.

After the calibration, the height of droplet cap can be estimated from the measured fluorescence intensity, as shown in Figure S5(a-c). Using this approach(Figure S5c), the height (h_m) of a typical "daughter droplet" with radius (r_m) of around 50 μm is estimated to be around 55 μm, as shown in Figure S5(d, e).

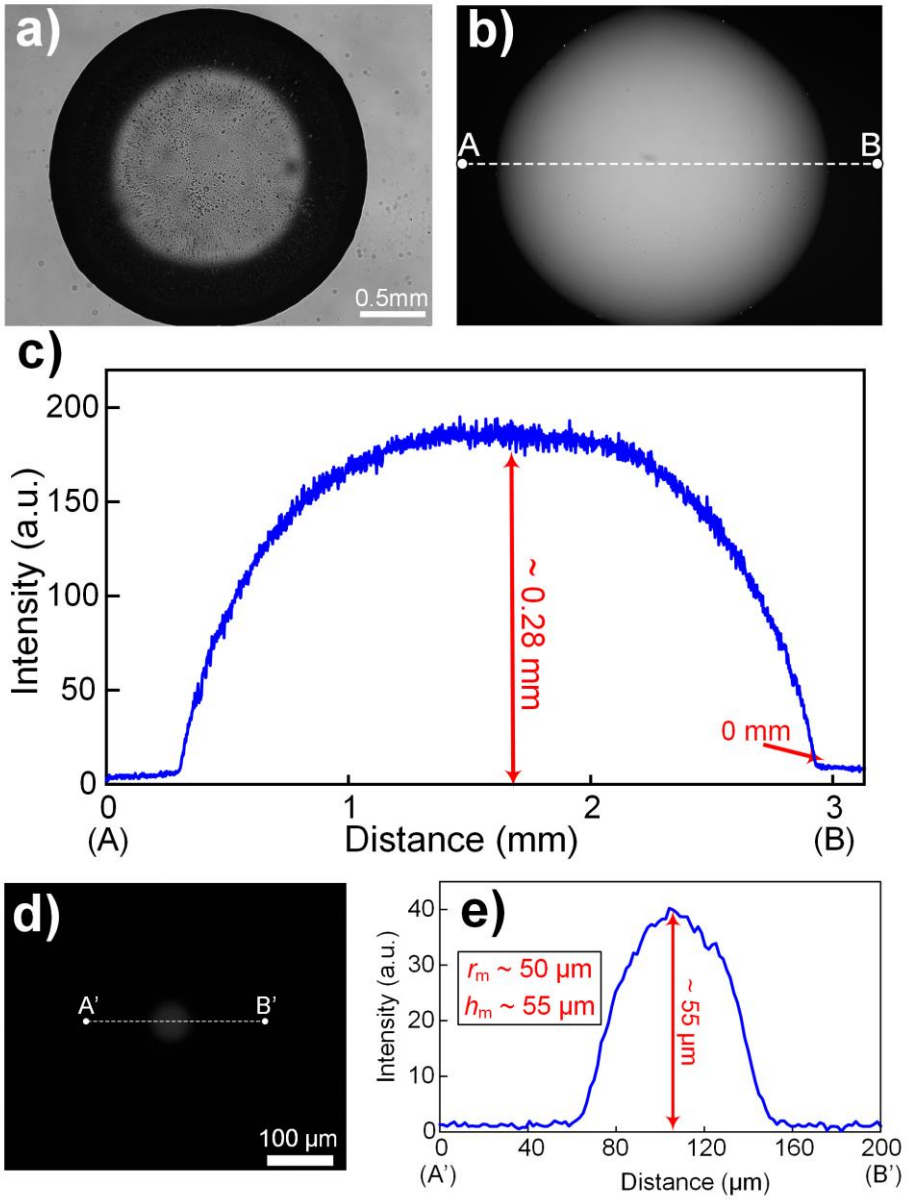

Figure S5. Estimation of the height of the "daughter droplets" formed. a) Bright-field and b) fluorescence microscope images showing an aqueous droplet (1 μL) deposited on a glass slide. 0.02 wt% Rhodamine 6G is added into the droplet phase. c) The distribution of fluorescence intensity from points "A" to "B" that cross the droplet cap. d) Typical fluorescence images of a "daughter droplet". e) The distribution of fluorescence intensity from points "A'" to "B'" that cross a typical "daughter droplet". In this case, the height (h_m) of "daughter droplet" with radius (r_m) of around 50 μm is estimated to be around 55 μm.

8) Extension to other ATPSs

In the case of equilibrium ATPS to produce the "daughter droplets", we mainly use the salt-poymer ATPS, i.e., 5 wt% Na_2CO_3-20 wt% PEG (Mw = 8 kDa) system. However, other types of ATPS also exhibit a similar phenomenon, such as polymer-polymer and inonic liquid-based systems: i) The equilibriated 5 wt% PEG (Mw = 35 kDa)-10 wt% dextran (Mw = 40 kDa) ATPS. The aqueous "mother droplet" is 5 wt% PEG (Mw = 35 kDa)-rich phase with 0.5 mM CTAB and the substrate phase is 10 wt% dextran (Mw = 40 kDa)-rich phase, respectively. Typical experimental images are shown in Figure S6(a). ii) The equilibriated

[$N_{111(2OH)}$]Cl and PEG (Mw = 600 Da) ATPS. The aqueous "mother droplet" is 37.5 wt% PEG-rich phase containing 10 mM CTAB and the substrate phase is 37.5 wt% [N111(2OH)]Cl-rich phase, respectively. Typical experimental images as shown in Figure S6(b).

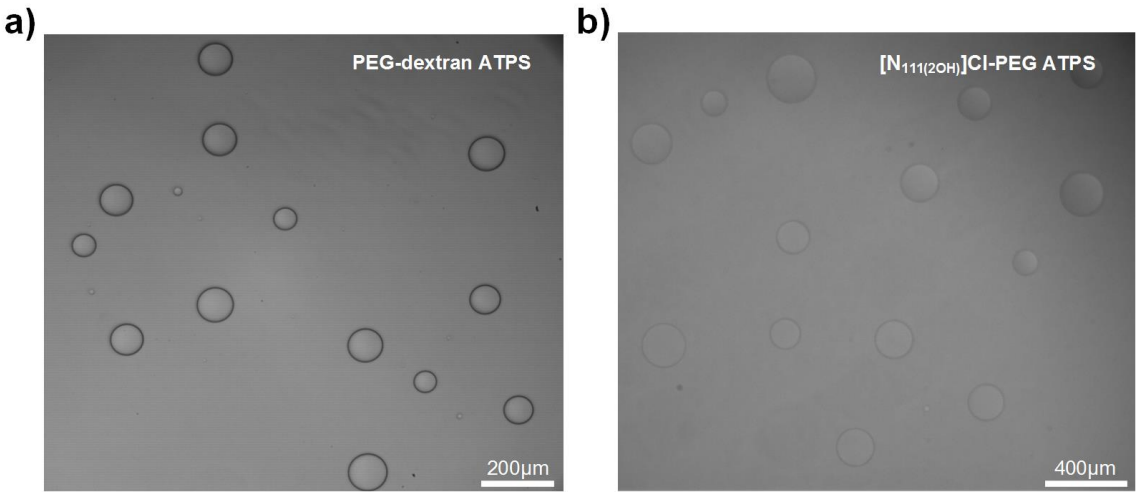

Figure S6. Typical optical microscope images showing the resulting droplets of: a) A PEG-rich aqueous droplet containing 0.50 mM CTAB deposited on the dextran-rich aqueous substrate. The two phases are extracted from the equilibriated 5 wt% PEG (Mw = 35 kDa)-10 wt% dextran (Mw = 40 kDa) ATPS, respectively. b) A PEG-rich aqueous droplet ccontaining 10 mM CTAB deposited on the [$N_{111(2OH)}$]Cl-rich aqueous substrate. The two phases are extracted from the equilibriated 37.5 wt% [$N_{111(2OH)}$]Cl - 37.5 wt% PEG (Mw = 600 Da) ATPS, respectively. The volume of deposited droplet is 6 μL.

9) Extension to other water-soluble surfactants

In the main text, we use mainly CTAB as water-soluble surfactants, which is cationic. We could expect that the bursting of thin aqueous films should also be observed for the anionic surfactant (such as SDS) and nonionic surfactant (such as Tween 20), as shown in Figure S7(a) and S7(b), respectively.

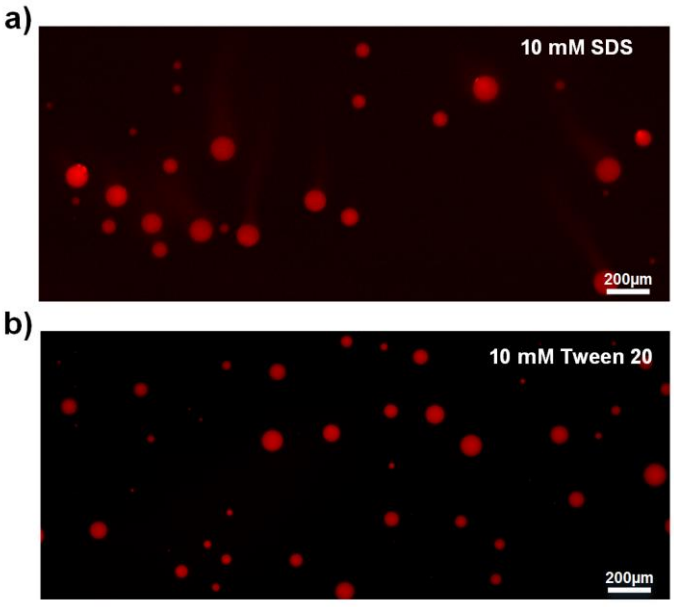

Figure S7. Typical fluorescence images showing the resulting droplets of different water-soluble surfactants: a) A PEG-rich aqueous droplet containing 10 mM SDS is deposited on the Na_2CO_3-rich aqueous substrate. b) A PEG-rich aqueous droplet containing 10 mM Tween 20 is deposited on the Na_2CO_3-rich aqueous substrate. The two phases are extracted from the equilibriated 5 wt% Na_2CO_3 - 20 wt% PEG (Mw = 8 kDa) ATPS respectively. The volume of deposited droplet is 5 μL, and 0.05 wt% Rhodamine 6G is added into the droplet phase.

10) Fabrication of microparticles via electrostatic complexation

As a demonstration, we firstly dissolve two typical hydrophilic species, including polycations, poly(allylamine hydrochloride) (PAH, Mw = 50 kDa) and polyanions, sodium alginate. Initially, we dissolve PAH and sodium alginate with concentrations of 2 mg/mL, 5 mg/mL, or 10 mg/mL for both phases into the 10 wt% dextran (Mw = 10 kDa) aqueous solutions and the 20 wt% PEG (Mw = 35 kDa) aqueous substrate respectively. We also add 1.00 mM Tween 20 into the droplet phase to induce the formation and bursting of thin liquid film. Here, Tween 20 is adopted, instead of CTAB or SDS, to avoid interaction between the charged species and surfactants. The fabricated microparticles from the fragmentation of the thin films with 2 mg/mL PAH and alginate are shown Fig. S8a. To realize the formation of microparticles, the concentrations of the PAH and alginate should not be too high, such as in the case of 2 mg/mL; otherwise the irregular hydrogel sheets will form, such as in the cases of 5 mg/mL and 10 mg/mL (see Fig. S8b,c), indicating that the competition between spreading and breakup of the "mother droplet" with the complexation is also very important to achieve microparticles.

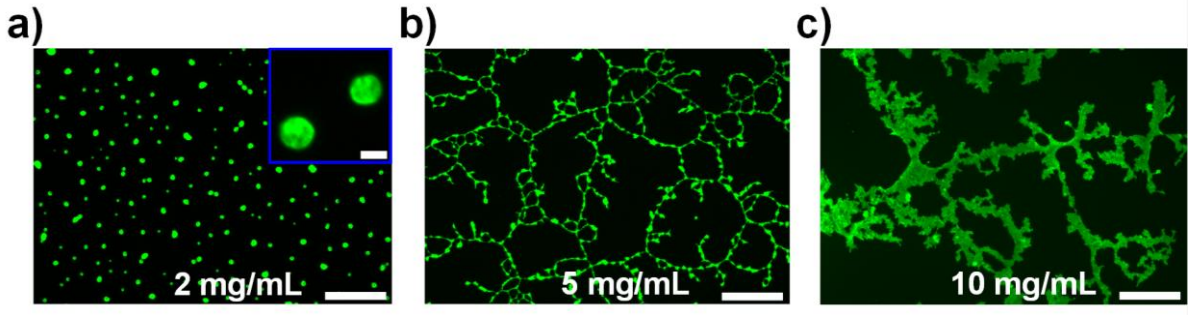

Figure S8. (a-c) Fluorescence images showing the fabricated PAH~alginate microparticles with a magnification (inset). PAH and alginate with concentrations of 2 mg/mL (a), 5 mg/mL (b) and 10 mg/mL (c), are added to the droplet and substrate phases. The droplet and substrate are 10 wt% dextran with 1 mM Tween 20 and 20 wt% PEG aqueous phases. The volume of "mother droplet" is 6 μL. Scale bars: 200 μm (20 μm in inset).

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3. M. Abkarian, S. Protière, J. M. Aristoff and H. A. Stone, *Nat. Commun.*, 2013, **4**, 1895.
4. Z. Zheng, M. A. Fontelos, S. Shin and H. A. Stone, *Phys. Rev. Fluids*, 2018, **3**, 032001.