Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Cross-talk between emulsion drops: How are hydrophilic reagents transported across oil phases?

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Influence of pH on leakage

To test the influence of the pH on the leakage of water-perfluorinated oil-water double emulsions, we produce fluorescein-loaded double emulsions stabilized with FSH_2-Jeffamine900 and measure the fluorescence of their cores as a function of the incubation time if immersed in an aqueous solution whose pH is 7 and pH=8.5. The leakage is significantly retarded if the pH is increased to 8.5, as shown in Figure S1. However, we still observe a continuous leakage even at higher pHs, as shown by the red curve.

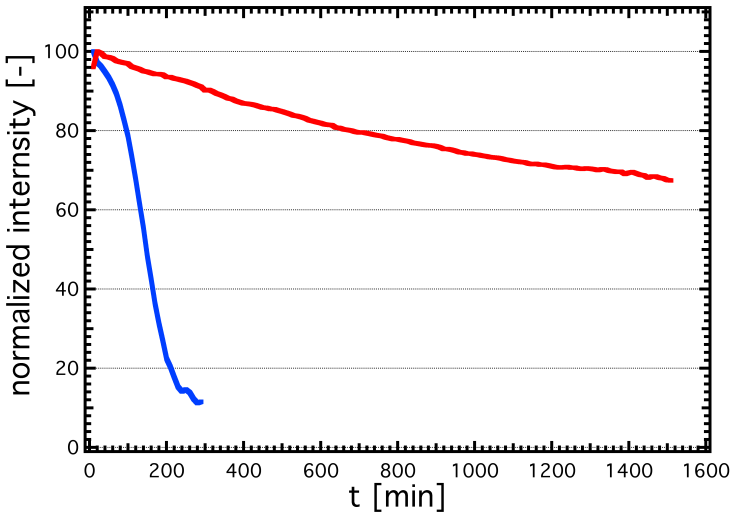

Figure S1. Influence of the pH on the leakiness of double emulsions. Water-perfluorinated oil-water double emulsions stabilized with FSH_2-Jeffamine900 and loaded with fluorescein are incubated in an aqueous solution at pH = 7 (blue curve) and pH=8.5 (red curve).

Critical micelle concentration

To determine the critical micelle concentration (CMC), we measure the mean count rate of HFE-7500 containing different concentrations of surfactants using dynamic light scattering (DLS), as exemplified for FSH_2-Jeffamine900 in Figure S2a. Additionally, we measure the interfacial tension between the surfactant containing HFE-7500 and water at different surfactant concentrations using pendant drop measurements, as shown in Figure S2b.

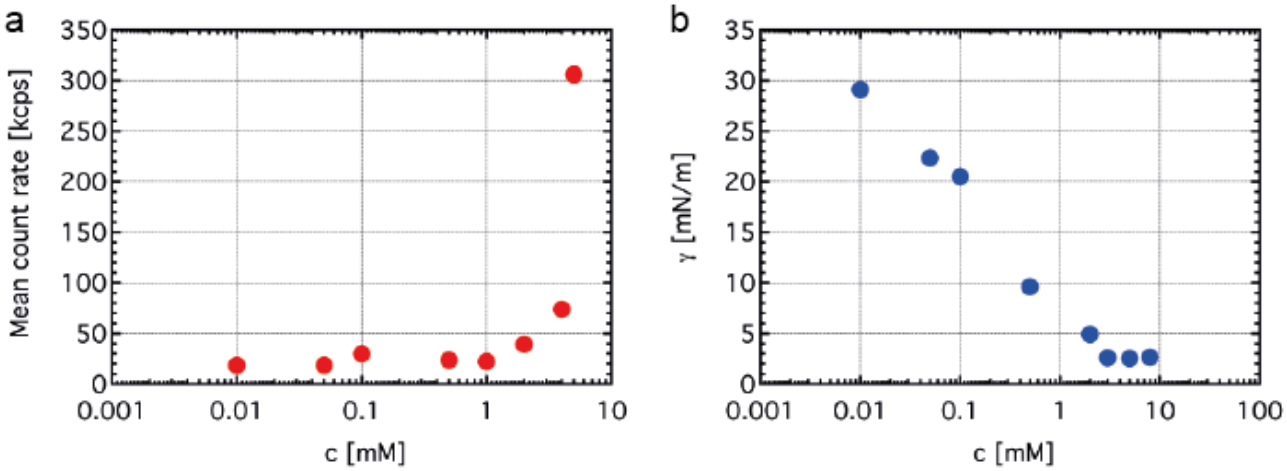

Figure S2. Quantification of the critical micelle concentration. (a) The mean count rate, determined with DLS, is shown as a function of the concentration of FSH_2-Jeffamine900 contained in HFE-7500. (b) The interfacial tension, γ, is measured as a function of the concentration of FSH_2-Jeffamine900 contained in HFE-7500.

Leakage from double emulsions

To determine the influence of the surfactant concentration on the leakage of double emulsions, we measure the fluorescent intensities of double emulsion cores containing fluorescein as a function of time. The leakage of double emulsions decreases with decreasing surfactant concentration, as shown in Figure S3.

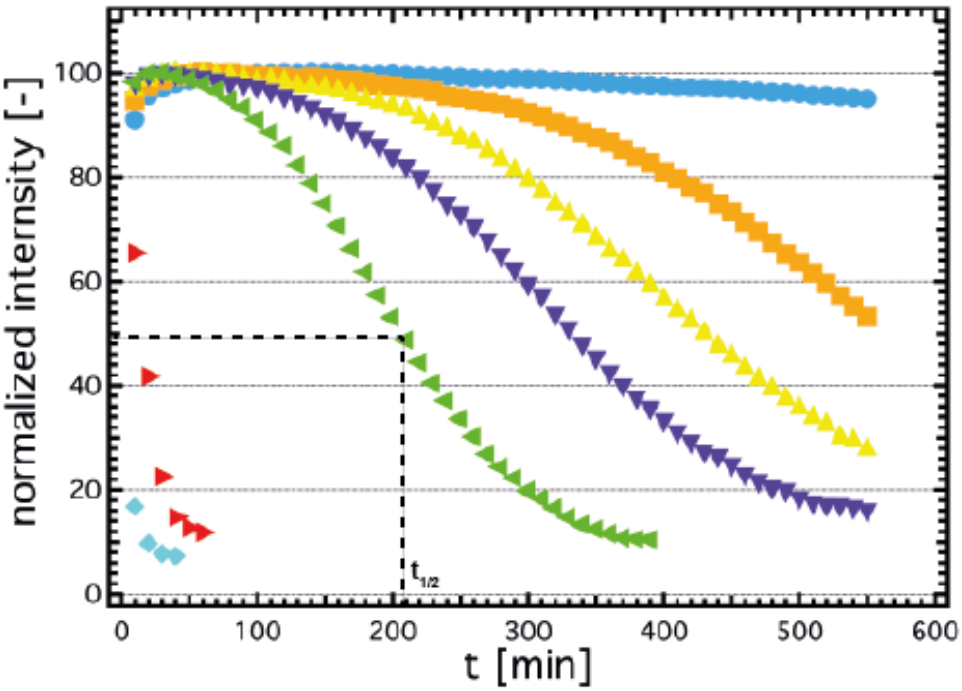

Figure S3. Quantification of the permeability of double emulsions. The normalized fluorescence intensity of cores of double emulsions stabilized with 5 mM (◆), 3 mM (▶), 1 mM (◀), 0.7 mM (▼), 0.5 mM (▲), 0.3 mM (■), and 0.1 mM (●) FSH_2-Jeffamine900 is shown as a function of time. The time when 50% of the fluorescein is released, $t_{1/2}$, is shown.

Spontaneous formation of aqueous drops

To test the influence of the interfacial tension on the spontaneous formation of small aqueous drops in the oil phase, we dissolve 5 mM FSH_2-Jeffamine2000 in HFE-7500 and add water on top of the oil. The turbidity of the oil is measured as a function of time by acquiring time-lapse photographs. The turbidity of the oil containing 5 mM FSH_2-Jeffamine2000, whose interfacial tension is 23 mN/m, remains within experimental error unchanged, as shown in Figure S4. This is in stark contrast to oils containing 5 mM FSH_2-Jeffamine900, whose interfacial tension with water is 2.5 mN/m, where the turbidity strongly increases within 50 h, as shown in Figure 3 in the main text. These results indicate that the spontaneous formation of aqueous drops is delayed or even suppressed if the interfacial tension is sufficiently high.

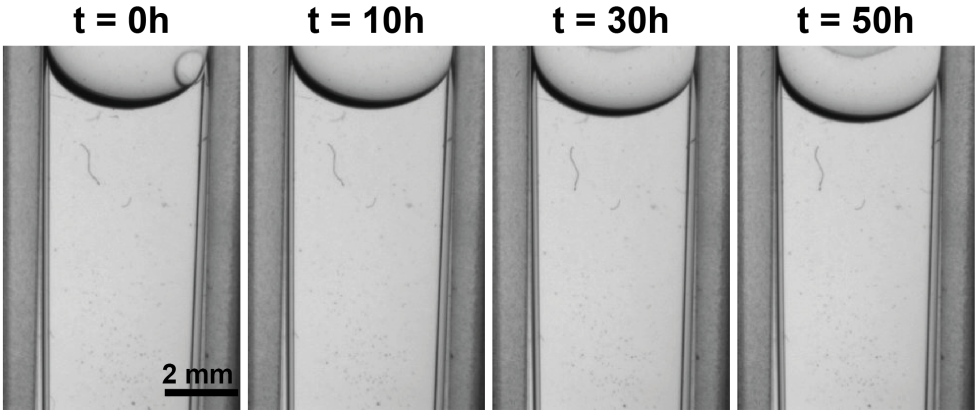

Figure S4. Time-lapse photographs of a cuvette containing HFE-7500 encompassing 5 mM FSH_2-Jeffamine2000 and a layer of water. Images were acquired 0, 10, 30 and 50 h after the sample was prepared. We could not observe any significant change in the turbidity of the oil indicating that only very small amounts of aqueous drops with diameters similar to the wavelength of the visible light spontaneously form within this time frame.

Quantification of the size of scattering objects

To quantify the average size average of scattering objects formed in HFE-7500 containing different surfactants, we perform dynamic light scattering (DLS) on HFE7500-based solutions containing 4 mM of surfactants, as exemplified for FSH_2-Jeffamine900, in Figures S5a and b. These results reveal that the scattering objects have an average diameter of order 100 nm, as summarized in Table S1.

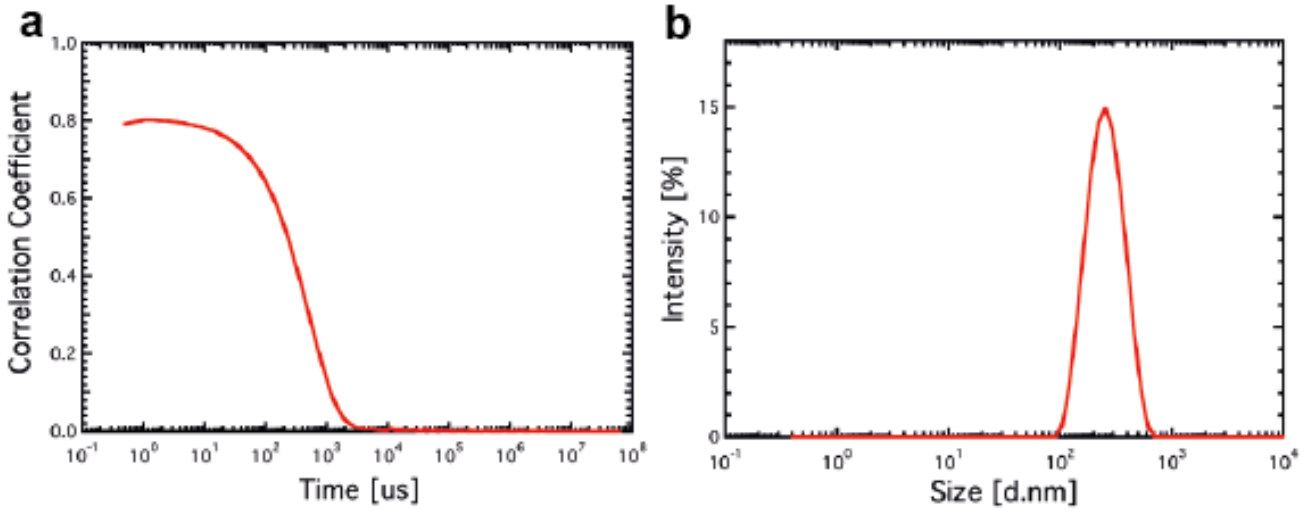

Figure S5. Size average of scattering objects formed in HFE7500 containing 4 mM FSH_2-Jeffamine900 measured with DLS. (a) Correlation function and (b) intensity weighted size distribution of scattering objects formed HFE7500 containing 4 mM FSH_2-Jeffamine900 if in contact with an aqueous layer.

Table S1: Intensity weighted average size of scattering objects formed in HFE-7500 containing 4 mM of different surfactants measured with DLS.

Name	***Size at 4 mM [d.nm]***
FSH-PEG220	189
FSH-Jeffamine600	100
FSH-Jeffamine1000	143
FSH-Jeffamine2000	34
FSH_2-PEG310	171
FSH_2-Jeffamine600	67
FSH_2-Jeffamine900	114
FSH_2-Jeffamine2000	21

Influence of the surfactant composition on the stability of double emulsions

To test the influence of the surfactant composition on the stability of double emulsions, we prepare double emulsions stabilized with 1 mM surfactants. Double emulsions are placed in a well containing water that has the same osmotic pressure as the aqueous phase that forms the core of the double emulsions. All the double emulsions are imaged and counted at room temperature. The samples are subsequently heated to 95°C for 10 min before images are acquired to determine the number of intact double emulsions. From these measurements, we determine the percentage of double emulsions that remains intact during this incubation at elevated temperatures. The majority of the double emulsions remains intact, independent of the surfactant composition. We cannot observe any clear influence of the PEG molecular weight on the stability of double emulsions, as shown in Figure S6a. As a result, the stability of the double emulsions is not directly correlated to the interfacial tension as quantified with pendant drop measurements, as shown in Figure S6b.

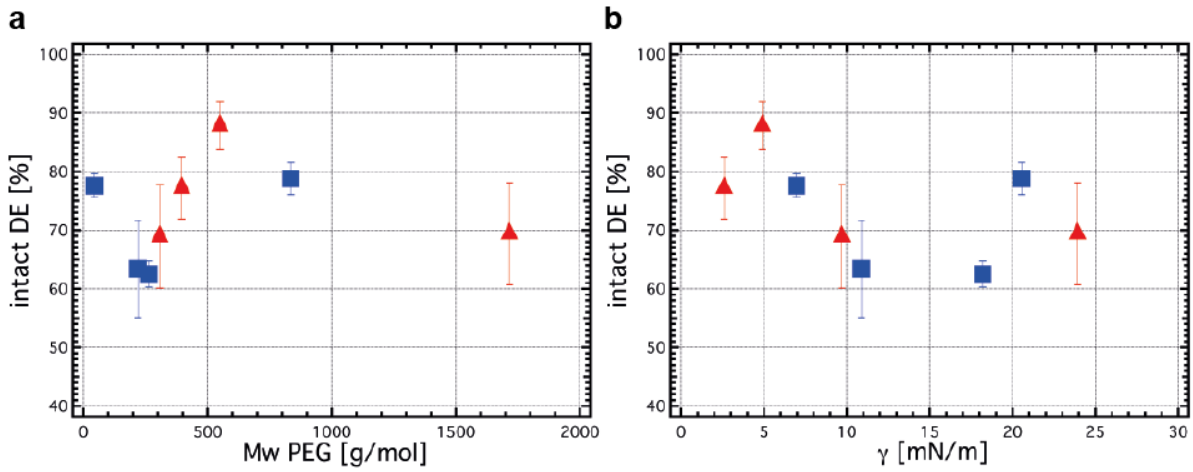

Figure S6. Influence of surfactant composition on the stability of double emulsions. (a) The influence of the PEG molecular weight of diblock (■) and triblock (▲) copolymer surfactants (M_w, PEG) and (b) the interfacial tension, γ, on the stability of double emulsions, measured as the percentage of intact double emulsions after they have been incubated at 95°C for 10 min. Double emulsions are stabilized with 1 mM of surfactant.

Permeability of double emulsions using commercial surfactants

To test if the permeability of water-oil-water double emulsions observed here is related to the surfactants we synthesized, we produce double emulsions using a commercial oil containing surfactants. Double emulsions are loaded with fluorescein or DNA strands with 17 base pairs and their permeability is quantified using fluorescence microscopy. Double emulsions whose shell is composed of the droplet generation oil EvaGreen (BioRad, USA) display a similar leakage as that observed with our FSH_2-Jeffamine900 surfactant, as shown in Figure S7. By contrast, drops whose shell is composed of the partitioning oil (10X genomics, USA) are less leaky and the transport of fluorescein across this shell is similar to the transport observed with our optimized surfactant FSH_2-Jeffamine2000, shown by the diamonds, or if we reduce the shell thickness to 0.33 µm, as shown by the triangles in Figure S7.

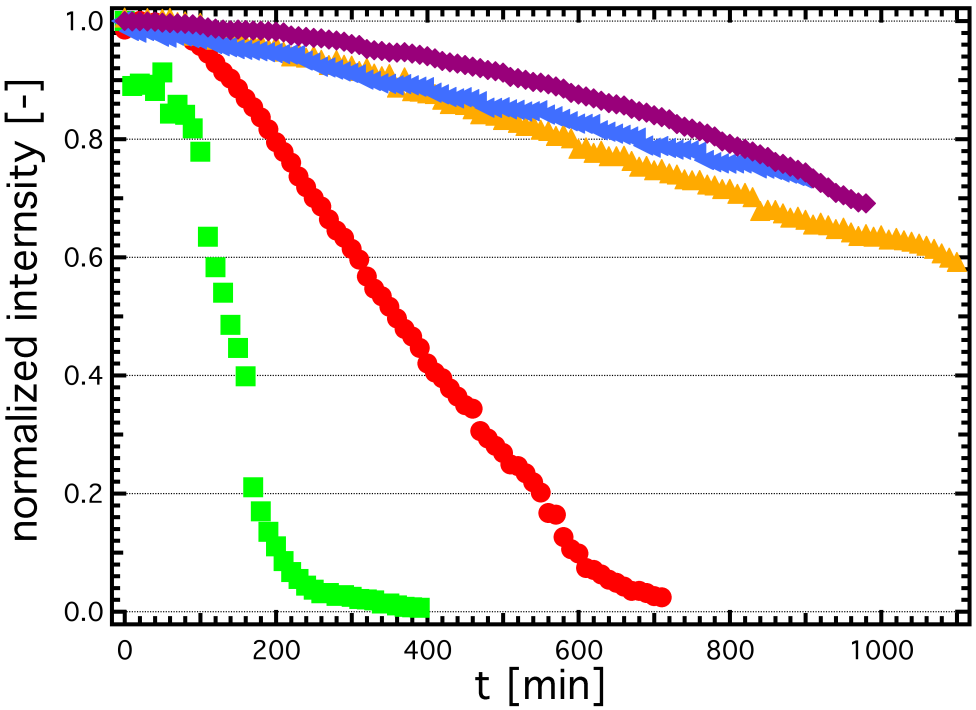

Figure S7. Transport of encapsulants across the shell of water-oil-water double emulsions whose shell is composed of commercial oils. Evolution of the fluorescence intensity of the core of double emulsions with shells composed of the droplet generation oil Eva Green (BioRad). Double emulsions contain fluorescein (●), or fluorescently labelled DNA with 17 base pairs (■) in the core. In addition, the permeability of double emulsions with shells composed of a surfactant-containing fluorinated oil from 10X genomics that contain fluorescein in their cores (▲) is shown. The permeability is compared to optimized double emulsions reported in the main paper, namely double emulsions with shell thicknesses of 0.33 µm (◀) and those with 12 µm thick shells that are stabilized with FSH_2-Jeffamine2000 (◆).

Characterization fluorescent polystyrene beads

To test if the small aqueous drops that form in the shell of double emulsions can also transport larger solid objects across their shells, we load double emulsions with fluorescently labelled polystyrene (PS) beads. To quantify their size, we image them with scanning electron microscopy (SEM) and measure their hydrodynamic diameter using dynamic light scattering (DLS). The average diameter of these particles is approximately 100 nm as shown in the SEM image and the DLS results in Figure S8a and S8b.

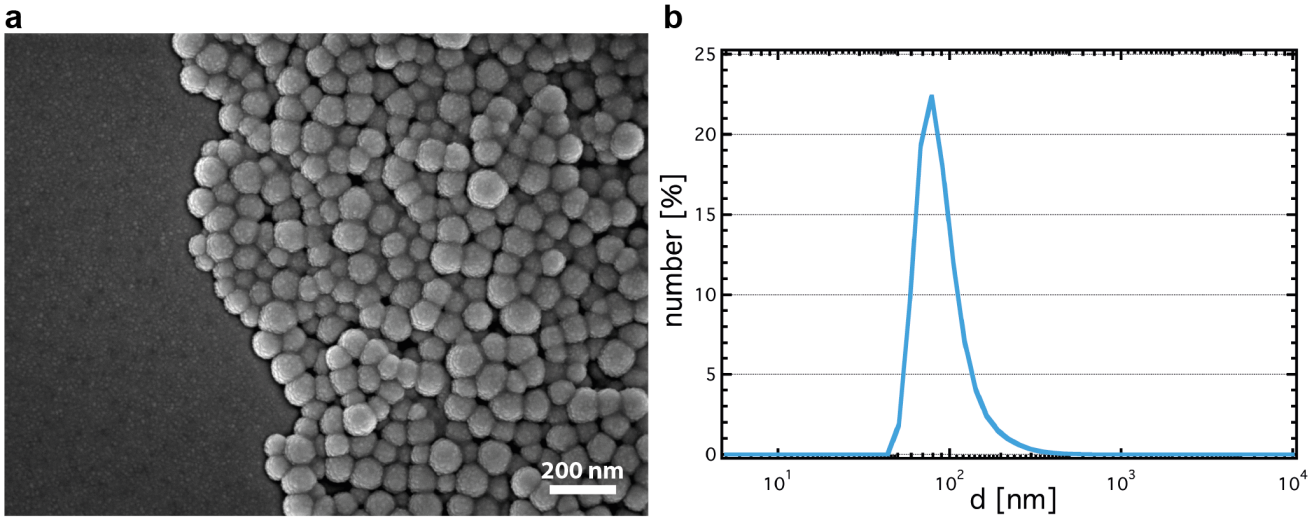

Figure S8. Characterization of fluorescently labelled polystyrene beads. (a) Scanning electron microscopy (SEM) image of the fluorescently labelled polystyrene beads and (b) the size distribution of these beads measured with DLS.

Permeability of single emulsion drops

To test if the transport of fluorescently labeled 100 nm polystyrene beads across oil phases is limited to double emulsions, we produce water in oil single emulsion drops where the drops contain fluorescently labelled PS beads. If single emulsion drops loaded with PS beads are mixed with empty drops, the fluorescence of the PS containing drops decreases over time, as shown in Figure 5b in the main paper. By contrast, if only single emulsions containing PS beads are dispersed in the oil phase and these drops are imaged under the same conditions as have been employed to acquire the images in Figure 5b, the fluorescence of the drops remains unchanged, as shown in Figure S9. These results indicate that the decrease in fluorescence, observed in Figure 4b, is related to an exchange of PS beads between PS-loaded and empty drops and it is not related to bleaching.

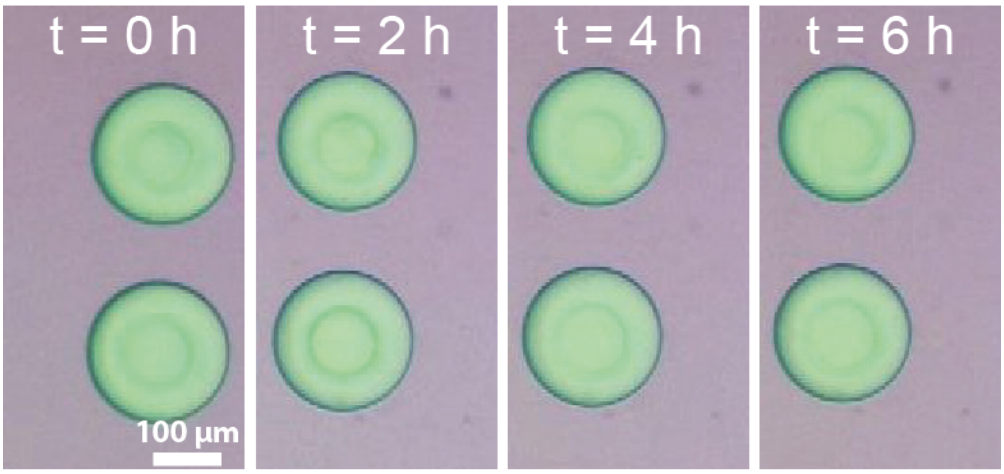

Figure S9 Time-lapse microscopy images of trapped single emulsion drops containing 100 nm polystyrene beads. Compared to Figure 5b in the main paper, where a mixture of empty and PS bead-loaded drops was incubated, here only drops containing the fluorescently labelled PS beads are incubated.

Supporting Movies

Movie S1: Evolution of fluorescence in the cores of double emulsions with 12 µm thick shells, stabilized with 1 mM and 0.1 mM FSH_2-Jeffamine900 that contain fluorescein in their cores.

Movie S2: Time-lapse photographs of a cuvette containing HFE-7500 and water is imaged over 4190 min. The oil in the left cuvette contains no surfactant. By contrast, the oil in the right cuvette contains 5 mM FSH_2-Jeffamine900. Over time, objects whose size is of order of the visible light become apparent in the right cuvette, rendering it turbid, while no visible change is observed in the left cuvette.

Movie S3: Leakage of fluorescein from double emulsions with shell thicknesses of 9 µm and 0.29 µm, respectively. Double emulsions are stabilized with 1 mM FSH_2-Jeffamine900.