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

Biocompatible fluorinated polyglycerols for droplet microfluidics as an alternative to PEG-based copolymer surfactants

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Supporting Information

This supplemental information contains details on the synthesis of polyglycerol surfactants, their characterization and the fabrication of stamped microfluidic devices. In addition, we provide information on the formation of surfactant-stabilized water-in-oil (W/O) emulsions, their mechanical stability, and their leakage behavior.

General experimental details: All reagents and chemicals were used as received unless otherwise noted. Poly(perfluoropropylene glycol) (PFPE) with carboxylic acid functionality was purchased as Krytox FSH (DuPont®) from Costenoble GmbH & Co. KG. A commercial in vitro transcription/translation (IVTT) kit was purchased from 5PRIME (RTS 100 Escherichia coli HY kit). The DNA of approximately 3,500 base pairs was a plasmid encoding the green fluorescent protein based on the pRSET vector. Milli-Q water was obtained by filtering water through a set of Millipore cartridges from Milli-Q plus with a

minimum resistivity of 18.0 MΩ. Dialysis was conducted with regenerated cellulose Spectra/Por® dialysis tubes from spectrum europe. NMR spectra were recorded on a Joel ECX 400 or a Jeol Eclipse 500 MHz spectrometer. Proton and carbon NMR were recorded in ppm and were referenced to the indicated solvents. Mass spectrometry was performed on a Bruker ultrafleXtreme MALDI-TOF/TOF mass spectrometer in either a linear or reflection mode using a cyano-4-hydroxycinnamic acid (HCCA) matrix for polyglycerol and a 2,4-bis(trifluoromethyl)benzoic acid (TFMBA) matrix for triblock surfactants. To determine the exact channel height of the microfluidic channel network, differential interference contrast (DIC) microscopy was performed on a Wyco NT1100 optical profiler (Bruker, USA). Bright-field microscopy images were obtained on an IX41 microscope (Olympus) equipped with a 10x, 20x, and 40x objective (air) and a Phantom MIRO ex2 high-speed camera (Vision Research Inc., USA). Confocal microscopy measurements of green fluorescent protein (GFP) synthesis during IVTT were performed using an Olympus IX81 confocal microscope with an Andor iXon3 camera, Andor 400-series solid-state lasers, and a Yokogawa CSU-X1 spinning disk unit. GFP was excited at 488 nm and fluorescence emission detected employing a 520/55 nm band pass filter.

Synthesis of polyglycerol surfactants: For the formation of triblock surfactants, two poly(perfluoropropylene glycol) (PFPE) chains with carboxylic acid functionality, and one linear polyglycerol (LPG) with amine functionality on both ends were coupled together.[1]

The linear polyglycerols LPG(OMe) as well as LPG(OEE), which is the precursor for LPG(OH), were prepared by living anionic polymerization based on the procedure of Hans et al. with 3-dibenzylamino-1-propanol as initiator via reaction with the respective oxirane monomer.[2] 3-Dibenzylamino-1-propanol was synthesized according to the literature.[3] The initiator (1 eq.) and KO*t*Bu (1 eq.) were dissolved in dry dimethoxyethane (DME) under argon atmosphere and heated to 80˚C for 30 min until full deprotonation of the functionalized alcohol occurred. The generated *t*-BuOH and the solvent were removed in vacuo. The remaining alcoholate initiator was completely dried in high vacuum, redissolved in dry DME, and heated to 110˚C under argon atmosphere. The freshly distilled monomers, glycidyl methyl ether (GME) for in LPG(OMe) or ethoxyethyl glycidyl ether (EEGE) for LPG(OEE) (eq. depending on the desired molecular weight), were added to the alcoholate DME solution and polymerized for 24 h under argon atmosphere. The reaction was quenched by the addition of water, concentrated under reduced pressure, and subsequently dried in high vacuum. For purification, the obtained yellow oil was dissolved in Et_2O and centrifuged to separate the insoluble salts. The Et_2O from the decanted top layers was removed in vacuo, and the dibenzylaminopropanol-functionalized LPG was obtained as a slightly yellow oil (80%).

Bn_2N-LPG(OMe)-OH: ^{1}H-NMR: (400 MHz, $CDCl_3$, TMS): δ (ppm) = 1.73-1.80 (m, 2H, $CH_2CH_2CH_2$), 2.48 (t, 2H, J = 7.02 Hz, NCH_2), 3.31-3.62 (m, 8H, OCH, OCH_3, 2x OCH_2), 7.19-7.35 (m, 10H, Bn_2N).
MS (MALDI-TOF): M_n / M_W = 1.04, Mn = 1334.9 $[M (n = 13)+Na]^+$, M_W = 1525.
Bn_2N-LPG(OEE)-OH: ^{1}H-NMR: (400 MHz, MeOD, TMS): δ (ppm) = 1.19 (t, J = 7.04 Hz 3H, OCH_2CH_3), 1.28 (d, J = 5.24 Hz, 3H, OCH_2CH_3), 1.73-1.80 (m, 2H, $CH_2CH_2CH_2$), 2.51 (t, 2H, J = 6.9 Hz, NCH_2), 3.31-3.68 (m, 8H, 2x OCH, 3x OCH_2), 4.71-4.75 (m, 2H, OCH, OCH_2), 7.21-7.37 (m, 10H, Bn_2N).

MS (MALDI-TOF): M_n / M_W = 1.06, M_n = 1885.2 $[M (n = 12)+Na]^+$, M_W = 1846.

The quenched hydroxyl terminus of the LPGs was further functionalized to create LPG with two terminal amino groups. The hydroxyl group originating from quenching was mesylated followed by substitution with an azide group according to the literature.[4] In the resulting Bn_2N-LPG(OR)-N_3, the azide terminus was reduced to an amine, and the dibenzylamino terminus was deprotected in one step.

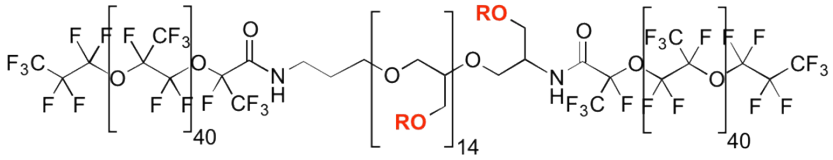
Bn_2N-LPG(OMe)-N_3 and Bn_2N-LPG(OEE)-N_3 were dissolved in MeOH and Pd/C (10% w/w) was added. This reaction mixture was transferred to a pressure cylinder and allowed to proceed under 5 bar hydrogen atmosphere at room temperature for 3 days. The mixture was filtered through celite to remove the catalyst, and the filtrate was concentrated under reduced pressure. The diamino-functionalized LPG(OEE)-$(NH_2)_2$ and LPG(OMe)-$(NH_2)_2$ were obtained as slightly yellow, viscous oils in quantitative yield after drying in high vacuum.

LPG(OMe)-$(NH_2)_2$: ^{1}H-NMR: (500 MHz, MeOD-d4, TMS): δ (ppm) = 1.73-1.78 (m, 2H, $CH_2CH_2CH_2$), 2.78 (t, 2H, J = 6.85 Hz, NCH_2), 3.31-3.79 (m, 8H, OCH, OCH_3, 2x OCH_2).
LPG(OEE)-$(NH_2)_2$: ^{1}H-NMR: (400 MHz, MeOD, TMS): δ (ppm) = 1.19 (t, J = 7.04 Hz 3H, OCH_2CH_3), 1.28 (d, J = 5.24 Hz, 3H, OCH_2CH_3), 1.70-1.77 (m, 2H, $CH_2CH_2CH_2$), 2.74 (t, 2H, J = 6.94 Hz, NCH_2), 3.51-3.68 (m, 8H, 2x OCH, 3x OCH_2), 4.71-4.75 (m, 2H, OCH, OCH_2).

The coupling of LPG(OEE)-$(NH_2)_2$ and LPG(OMe)-$(NH_2)_2$ and perfluoropolyether (PFPE) to form the triblock copolymer was performed in a two-step process. First, the PFPE carboxylic acid was converted to the acid chloride, as described previously.[1,5] In a second step, 0.5 eq. with a small excess of LPG diamine was dissolved in dry pyridine and added to the PFPE acid chloride. The mixture was allowed to react overnight. Pyridine and pyridium salt were removed under high vacuum and repeatedly washed with methanol to give the coupled products, LPG(OMe)-$(PFPE)_2$ and LPG(OEE)-$(PFPE)_2$, respectively.

To obtain the final product LPG(OH)-$(PFPE)_2$, the acetal-protected side chains of LPG(OEE)-$(PFPE)_2$ were deprotected. Under acidic conditions, the ethoxyethyl group, attached to the side chain oxygen of each repeating unit, was cleaved off as Et_2O. The deprotection afforded the hydroxy side-chain linear polyglycerol.[6] Therefore, LPG(OEE)-$(PFPE)_2$ was dissolved in HFE-7100 and hydrochloric acid (6M) was added. A white precipitate was observed immediately. The mixture was further stirred for two hours at room temperature, the supernatant was decanted, HFE was removed in vacuo, and the remaining white product dried at 40˚C overnight. The product was further purified by washing with HFE-7100/MeOH (2:1), dialysis in regenerated cellulose membrane with 10 kDa M_W cut-off in HFE-7100/MeOH (5:1), and drying in high vacuum to give a turbid white oil (80%).

LPG(OMe)-$(PFPE)_2$: ^{1}H-NMR: (400 MHz, C_6F_6, HFE7500, TMS): δ (ppm) = 3.30-4.2 (m, 8H, OCH, OCH_3, 2x OCH_2), 8.22 (s, 2H, CONH).
MALDI-TOF analysis showed exclusively molecular weights above 10 kDa confirming the triblock structure.
LPG(OH)-$(PFPE)_2$: ^{1}H-NMR: (400 MHz, THF-d8, HFE7100, TMS): δ (ppm) = 3.51-3.62 (m, 5H, OCH, 2x OCH_2), 8.22 (s, 2H, CONH).



LPG(OH)-PFPE$_2$ R = H
LPG(OMe)-PFPE$_2$ R = Me

Fig. S1. Chemical structure of the triblock copolymer surfactants.

The surfactants were dissolved in HFE-7500 and filtrated with 0.45 µm syringe filters before all the subsequent procedures.

Interfacial tension measurement via pendant drop

Surface tensions were measured at different surfactant concentrations using the pendant drop method (DataPhysics OCA). The setup consisted of a 3-15 µl drop of HFE7500 (3M) surfactant solution on a syringe tip, inside de-ionized water. The measurements took several minutes to several hours until equilibrium was reached, depending on the surfactant concentration. The resulting IFTs were plotted against the surfactant concentration showing the CMC as shown in Figure S2.

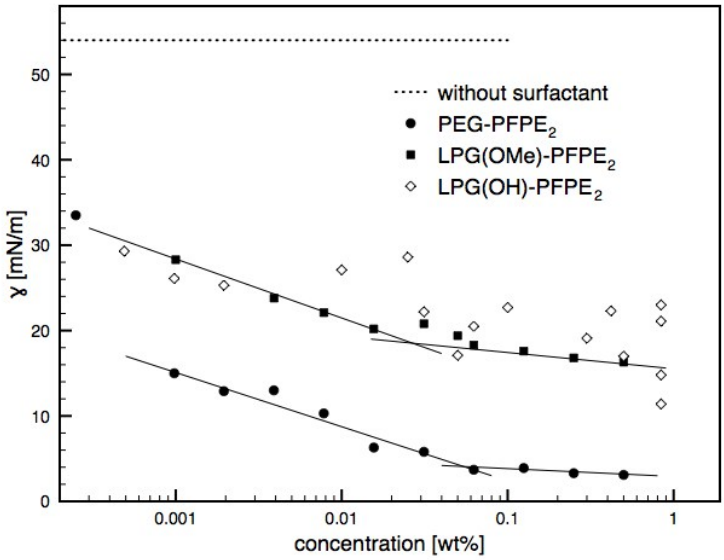


Fig. S2. Interfacial tension measurement of a fluorinated oil drop in de-ionized water via pendant drop method.

Dynamics Light Scattering

Dynamics Light Scattering (DLS) measurements were conducted with a Malvern Zetasizer instrument. We measured the scattered intensity of the HFE-7500 surfactant solution as a function of concentration for each surfactant. The Zetasizer software Version 6.32 provided by the manufacturer displayed the recorded number of particles per second as a *derived count rate* in kilo counts per second (kcps). The resulting values were plotted as a function of concentration including a zero baseline of pure HFE-7500 solvent measurements. A linear

growth of measurement counts can be seen in the double logarithmic presentation, starting at the estimated aggregation concentration shown in Figure S3.

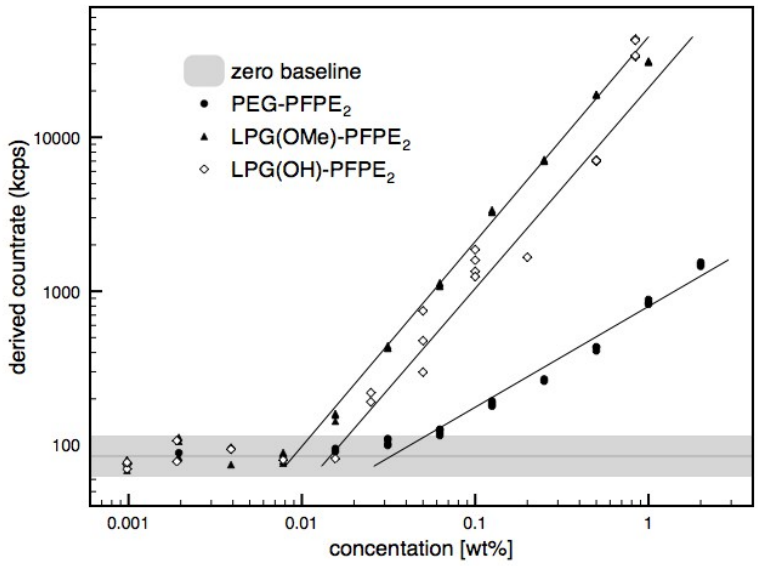


Fig. S3. Dynamics Light Scattering (DLS) measurement results of the HFE7500 surfactant solution as a function of concentration for each surfactant. The grey area marks the zero baseline of pure HFE7500 solvent.

Fabrication of microfluidic devices

Stamped microfluidic devices were fabricated using a combination of photo and soft lithography, as shown in Figure S4.[5,7] A negative photoresist (SU-8 25 Microchem Co., USA) was spin-coated onto a silicon wafer (2 inch, SI-MAT, Germany). A mask aligner (MJB3, Süss MikroTec, Germany) was then used to expose the photoresist-coated wafer with UV light (365 nm) through a transparent photomask (JD Phototools, UK) containing the desired microchannel structure. We optimized the master device fabrication employing DIC microscopy to obtain microchannels with a height of approximately 23 µm. A poly(dimethylsiloxane) (PDMS) replica of the channel design was formed by mixing PDMS oligomer and cross-linker in a ratio of 10 : 1 w/w (Dow Corning, Germany) and curing the homogeneous, degassed mixture at 65 °C for 2 hours. Thereafter, access ports for tubing were bored into the replica with a biopsy needle (outer diameter: 1.0 mm, Pfm, Medical Workshop, USA). The microfluidic device was assembled by bonding the PDMS replica to a glass slide via oxygen plasma treatment. The bonding process was completed in an oven at 90 °C for approximately 1 h.

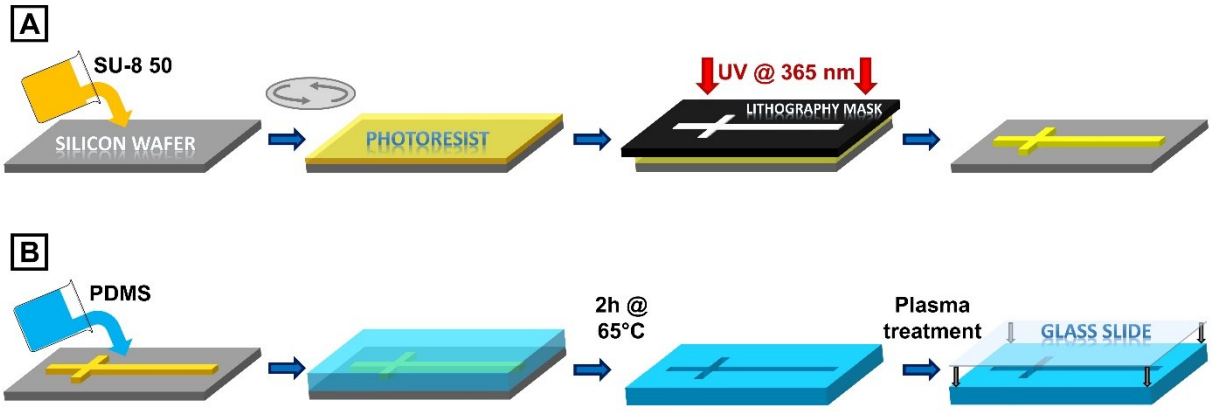


Fig. S4. Microfluidic device fabrication via a combination of (A) photolithography and (B) soft lithography, adapted from [9].

Formation of surfactant-stabilized water-in-oil emulsions

All fluids were loaded into gastight syringes (Hamilton 1000 series) mounted onto high-precision syringe pumps (Cetoni® neMESYS, 14.5 gear). The syringes and the microfluidic device with a flow-focusing unit, 25 µm in height and width at the droplet-forming nozzle, were connected via PE tubing (HSE Harvard Apparatus GmbH, inner diameter = 0.38 mm, outer diameter = 1.09 mm). We formed microdroplets by injecting water as the inner phase and fluorinated oil (HFE 7500, 3M®) with surfactant (2% w/w) as the outer phase at typical flow rates of 200 and 600 µL h^{-1}, respectively. For testing the biocompatibility of the surfactants, the inner phase of the W/O emulsion was composed of an in vitro gene expression mixture made from premixed solutions of amino acids (36 µL), reaction buffer (15 µL), cell lysate extract (36 µL), methionine (3µL), and reaction mixture (30 µL), as well as 10 µL DNA solution (3 nM L^{-1}). The outlet tubing of the microfluidic device was fed into a Parafilm-sealed Eppendorf tube for further characterization or the emulsion was directly collected on a glass slide. Droplet stability on-chip was investigated at the droplet-forming nozzle and the exit microchannel.

Determining emulsion stability

To determine the mechanical stability of our W/O emulsions formed with different polyglycerol surfactants at varying concentrations, a sample of the thus formed emulsion was collected on a glass slide mounted on a brightfield microscope. A microneedle (25G x 1”, 0.5 x 50 mm, Terumo) was then used for each experiment to deform microdroplets between the glass slide surface and the tip of the needle. The process was recorded with a high-speed camera. An emulsion was considered to be stable, if it remained monodisperse, and deformed droplets did not coalesce. In Figure S5, the image sequence of a stability test of microdroplets stabilized with LPG(OMe)-$(PFPE)_2$ surfactant at 1.8% (w/w) that was mechanically stable is shown in the upper row. For comparison, the lower row displays two images of individual emulsion samples that coalesced under mechanical stress by a needle tip.

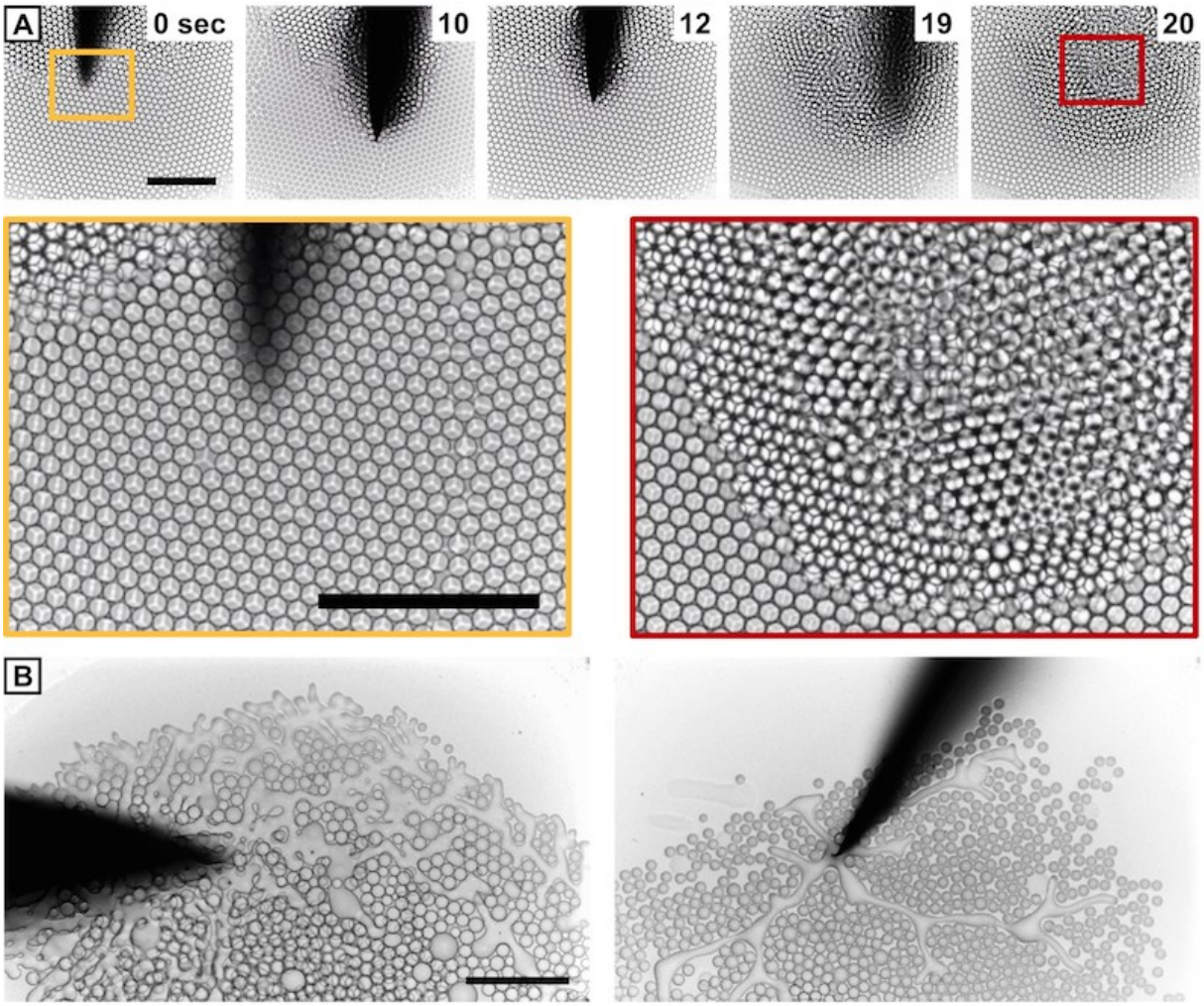


Fig. S5. Microdroplet stability test using mechanical force by a needle. (A) W/O microemulsion stabilized with LPG(OMe)-$PFPE_2$ that remained stable when mechanical stress was applied. (B) Two examples of an LPG(OH)-$PFPE_2$ emulsion that coalesced during mechanical deformation (lower row). The scale bars denote 250 µm.

FITC-dextran leakage tests

To study whether microemulsions exhibited enhanced partitioning of droplet cargo into the surrounding oil phase, when stabilized with our surfactants, we microfluidically fabricated water droplets loaded with fluorescein isothiocyanate (FITC)-labeled dextran (500,000 g mol^{-1}) in fluorinated oil (HFE7500, 3M). As a proof of principle, we stabilized the FITC dextran-emulsion with 2% (w/w) of LPG(OMe)-$PFPE_2$. The emulsion was then loaded into a microfluidic chamber fabricated in PDMS that enabled fluorescence imaging over several hours without significant droplet shrinkage due to evaporation. As shown in Figure S6A, the droplets were monodisperse and aligned into a hexagonal package. The fluorescence image taken after four hours also showed that the fluorescence was homogenous throughout the droplet volume without any noticable accumulation of the FITC dextran at the surfactant-stabilized droplet interface. Both the fluorescence of the microdroplets as well as the oil phase did not distinctly change over the course of the experiment, which indicated the surfactant

layer was not porous enough to allow for leakage of the fluorescent droplet cargo, as shown in Figure S6B.

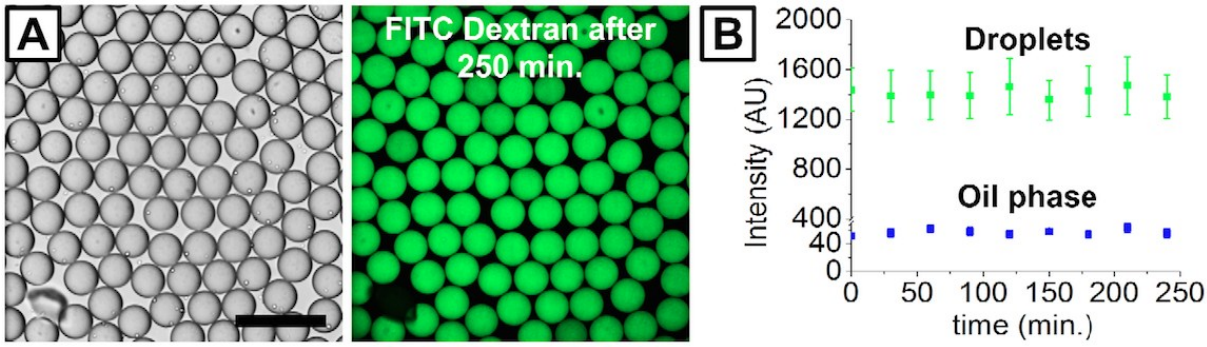


Fig. S6. (A) Brightfield (left) and fluorescence (right) microscopy images of water-in-oil microdroplets loaded with 1 µM FITC dextran (500,000 g mol^{-1}). The scale bar denotes 150 µm. (B) Fluorescence intensity inside FITC-dextran-loaded droplets (green squares) and in the surrounding fluorinated oil (blue circles) over time.

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