Visualization of Surfactant Dynamics to and along Oil-Water Interfaces using Solvatochromic Fluorescent Surfactants – Supplemental Information

Christopher V.H.-H. Chen[1], Ying Liu[2], Howard A. Stone[2], Robert K. Prud'homme[1]

[1]Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ 08544

[2]Department of Mechanical and Aerospace Engineering, Princeton University, Princeton, NJ 08544

* Correspondence should be addressed to:

Prof. Robert K. Prud'homme

Department of Chemical and Biological Engineering

Princeton University, Princeton, NJ 08544

Phone: (609)258-4577

E-mail: prudhomm@princeton.edu

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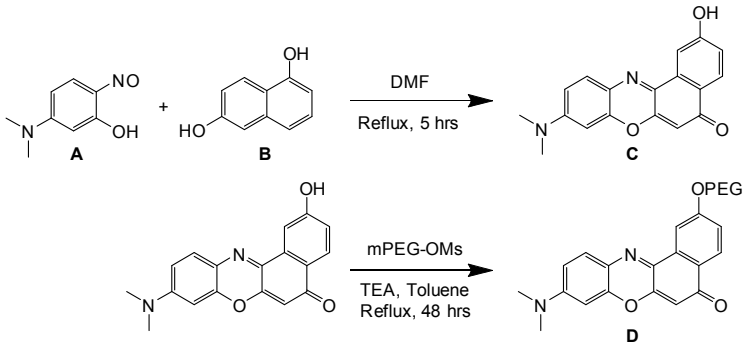
SI.1. Synthesis of NR-Based Surfactant-Dyes

Materials. All reagents used were obtained from commercial sources and used without further purification, unless specified. 1,6-dihydroxynaphthol (99%) was purchased from Acros Organics (Geel, Belgium), and 5-dimethylamino-2-nitrosophenol hydrochloride (>98%) was purchased from TCI (Mountain View, CA) as precursors to our hydroxy-Nile Red derivative (9-dimethylamino-2-hydroxy-5H-benzo[a]phenoxazin-5-one). Triethylamine (TEA, 99.5%), methanesulfonyl chloride (>99.7%), polyethylene glycol methyl ether (M_n 5k), acrylic acid, anhydrous (99%) and benzophenone (99%) were all obtained from Sigma-Aldrich (St. Louis, MO). TEA was distilled, under vacuum, to remove impurities at 90 ^{o}C before use. Nile Red (NR, >99%), toluene, anhydrous extra dry (>99.8%) were purchased from Acros. Anhydrous dimethylformamide, Drisolv (DMF, >99.8%) was obtained from EMD (Darmstadt, Germany). All non-dry solvents, drying salts, salts used to generate aqueous buffers, and mineral acids and bases used were of ACS grade (or higher) and were purchased from Fisher Scientific (Waltham, MA). Column chromatography was performed using Fluka basic alumina, Brockmann activity I (60-350 mesh) from Sigma. Silicon oil (DC 200) and mineral oils (light and heavy) used in imaging experiments were obtained from Sigma. Paroil 10NR – a chlorinated oil with a density of 1.11 g/mL – was obtained from the Dover Chemical Corporation (Downers Grove, IL).

Synthesis of 5-dimethylamino-2-nitrosophenol hydrochloride (HONR, C). In the first step toward NR-based PEGylated surfactant-dyes described in Scheme SI.1, 1,6-dihydroxynaphthol (**B**) (208 mg, 1.026 mmol) was reacted with 5-dimethylamino-2-nitrosophenol hydrochloride (**A**) (246 mg, 1.54 mmol) under reflux in 20 mL DMF in a 50 mL round bottom flask for 5 hours, as described by Chen et al.[1] DMF was then removed by adding n-heptane, via heterogeneous azeotropic distillation[2], to near dryness, before purification via flash chromatography with 2:1 ethyl acetate-isopropanol to yield a bright red solution that dries to a dark green solid (95.1 mg, 28% mass recovery). Fluorescence spectra of dried product in toluene and methanol were found to be similar to that of pure NR and commercially available

2-hydroxy Nile Red (available from TimTec) when excited at 514 nm. See Figure SI.1 for ^{1}H NMR in DMSO-d6.

Scheme SI.1. Synthesis of NR-PEG (**D**) via the nucleophilic substitution of mPEG-mesylate with 9-Dimethylamino-2-hydroxy-5H-benzo[a]phenoxazin-5-one (HONR, **C**).



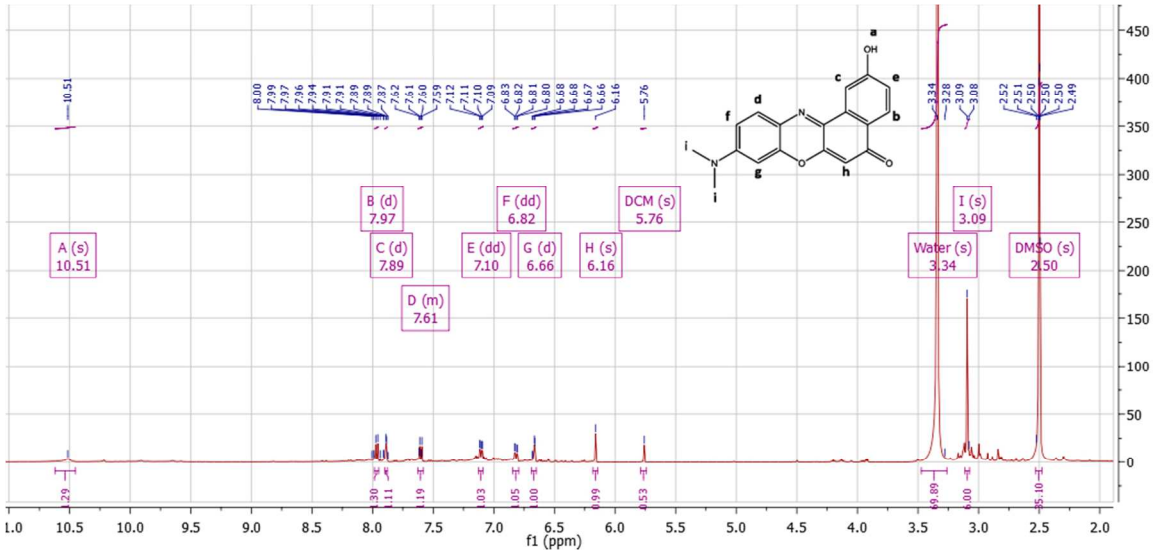


Figure SI.1. ^{1}H NMR of 9-Dimethylamino-2-hydroxy-5H-benzo[a]phenoxazin-5-one (**C**) in DMSO-d6.

Synthesis of Mesyl-functionalized Monomethyl Polyethylene Glycol (mPEG-OMs). To synthesize NR-PEG via hydroxy-Nile Red, the methanesulfonyl (mesyl) derivative of monomethyl PEG (mPEG) was synthesized. 1000 mg of mPEG (5k) was added to 20 mL toluene in a 50 mL round bottom flask. mPEG was dried via azeotropic distillation at around 130 ^{o}C. After 15 mL of solvent was removed, 10 mL anhydrous toluene was added to the flask and distillation continued until another 18 mL of liquid is distilled. The reaction flask was brought back down to room temperature and sealed with a rubber

septum. 3 equivalents of distilled TEA (84 µL) in 6 mL anhydrous toluene was then added, and the reaction flask was purged with argon for 15 minutes. 5 equivalents of methanesulfonyl chloride (MsCl, 77 µL) were added after purging dropwise, and the reaction was carried out for 24 hours, stirring at room temperature. The reaction solution was then filtered with a 0.22 µm nylon filter to remove TEA salts, and then concentrated to 2 mL by rotary evaporation. The concentrated reaction solution was then added dropwise to 45 mL hexanes (at room temperature) in a 50 mL Falcon tube to precipitate the product. The precipitated solution is shaken for 20 minutes before centrifuging to pellet the solids at 2000 rcf for 8 minutes. The hexane supernatant was decanted, and the precipitate is dried under nitrogen for 30 minutes, and under vacuum overnight to produce a white powder with quantitative yield. See Figure SI.2 for ^{1}H NMR in $CDCl_3$.

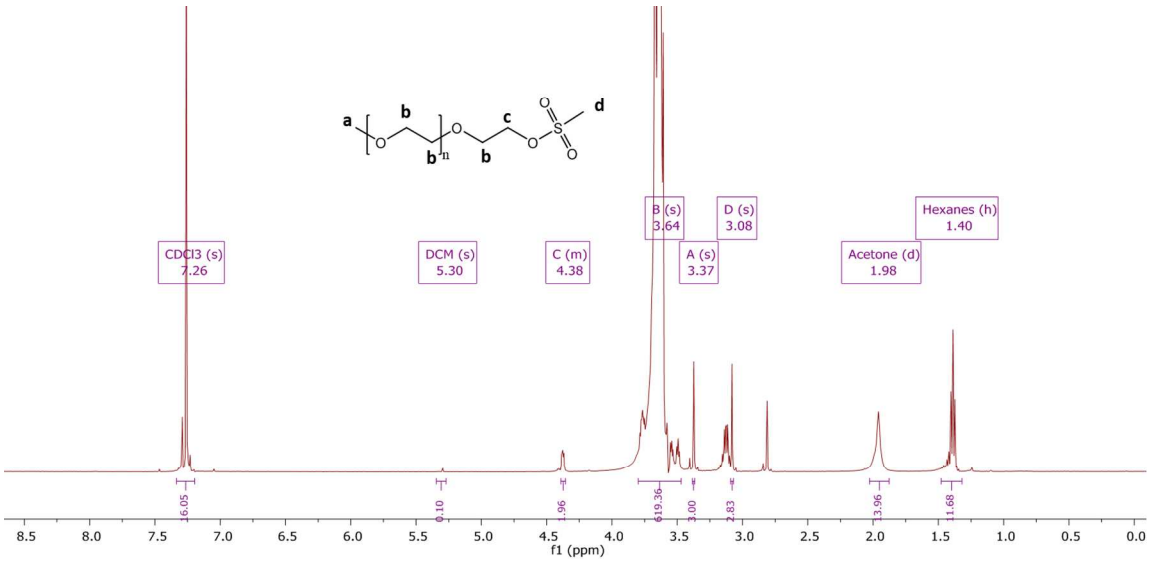


Figure SI.2. Example ^{1}H NMR of 5k mPEG-OMs in $CDCl_3$.

Synthesis of Nile Red-PEG Surfactant-Dye (D). 30 mg mPEG-OMs (5k) was added to 15 mL toluene in a 25 mL round bottom flask and dried via azeotropic distillation at around 130 ^{o}C. After 10 mL of solvent was removed, 5 equivalents (4 µL) of TEA and 2.5 equivalents (5 mg) of HONR were added to the flask. The reaction was stirred for 48 hours under toluene reflux. The solution was then

concentrated to 1 mL by rotary evaporation before being added dropwise to 15 mL cold ether in a 20 mL Falcon tube to precipitate the product. The precipitated product in cold ether was then pelleted at 2000 rcf for 8 minutes. The ether was then decanted and the pellet was resuspended in 15 mL cold ether twice more to remove excess HONR. The final wash should be colorless. The precipitate dried in vacuo overnight, producing a dark red solid with 60% reaction yield.

SI.2. Fabrication of Glass Microfluidic Droplet Generators

Fabricated devices were built using the "off-the-shelf" capillary microfluidic device design shown in Figure SI.3 and described by Benson et al.[3] Instead of the flow-focusing device depicted by Benson et al., here we used a co-flow device that was assembled from PEEK chromatography tees and unions (IDEX Health & Science, P-713 and P-703), a 1 mm OD and a 0.87 mm OD round glass capillaries (VitroCom, CV8010 and CV7087), and soft 1/8" OD PVC tubing (Tygon, R-3603). An acrylic device holder was also constructed to hold these glass microfluidic devices to flat when conducting confocal imaging.

To construct these devices, first, three 1 cm long pieces of Tygon tubing were placed on the larger round capillary. These pieces of tubing helped to seal the capillaries the chromatography fittings and were positioned where the connecting fittings were to be placed. The larger round capillary was inserted through one end the chromatography tee, but not through to the other side. A flangeless ferrule, part of the PEEK chromatography assembly, was then placed on the larger round capillary and moved to be at the end of the tubing piece closest to the chromatography tee. The Tygon tubing and ferrule were then moved to be flush against the tee's connecting port, and a screw was used to seal the capillary in place. Another screw was then placed on the capillary before placing the ferrule on the next piece of tubing. The larger round capillary was then inserted through the chromatography union, and the screw in between the tee and union was used to seal the middle end of the capillary to the union. The final ferrule and screw was then placed on the other end of the union to seal the remaining union-

capillary connection. Tygon tubing was then connected to the open end of the larger round capillary to carry the produced emulsion away from the device and microscope. This capillary assembly carried the continuous, oil phase from inlet A and the resulting emulsion from outlet C (Figure SI.3).

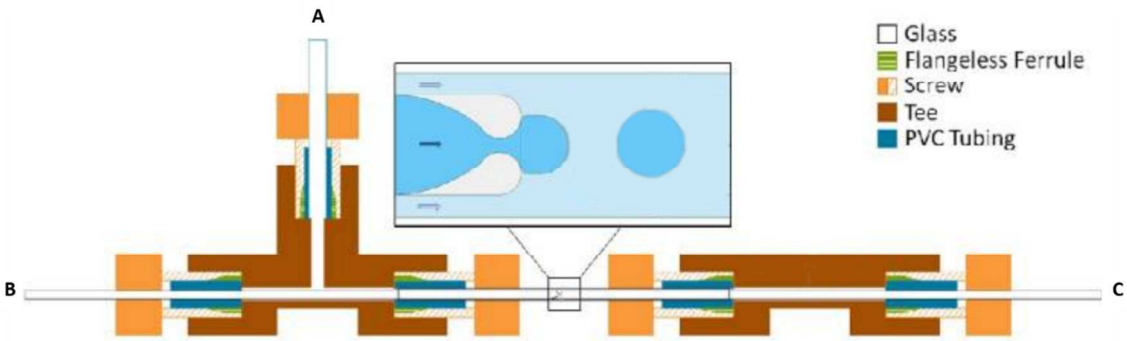


Figure SI.3. Schematic of co-flow, glass capillary microfluidic device used to test the ability of NR-PEG surfactant-dyes to dynamically visualize surfactant at oil-water interfaces. (**A**) Inlet for the oil external phase – a density-matched solution of mineral oil. (**B**) Inlet for aqueous, NR-PEG-containing, dispersed phase. The resulting emulsion was collected at outlet (**C**). Devices were produced both with flamed tip (depicted in the **inset**) and pulled tip capillaries. Fabrication is described by (and figure is adapted from) Benson et al.[3]

The round glass capillary was used to inject the dispersed phase (from inlet B), and was prepared by flame-tipping, as depicted in the inset of Figure SI.3 or tapering by pulling the round capillary after heating over a flame. This reduced the ID treated end of the capillary to approximately 100 microns. A length of Tygon tubing, with connected flangeless ferrule, was then place at other end of the round capillary, which was then inserted, treated end first, through the unsealed side of the capillary tee and into larger round capillary. A screw was then placed over the Tygon tubing and used to seal the capillary to the tee. Tygon tubing was placed at inlet A, sealed in a similar fashion using a screw and ferrule to the remaining opening in the tee.

SI.3. Fabrication of Microchannel Epoxy Devices

Norland optical adhesive, the epoxy used in device fabrication, was purchased from Norland Products, Inc. (Cranbury, NJ). Sylgard 185 Silicone Elastomer and Curing Agent (poly(dimethyl siloxane) (PDMS) kit) was obtained from Dow Corning (Midland, MI). Acrylic acid, anhydrous (99%) and

benzophenone (99%) were obtained from Sigma. All solvents used were of ACS grade or higher and were purchased from Fisher. MilliQ (MQ) water was used for water in all instances without further purification. Glass slides and cover slips (both at standard 25 x 75 cm) were purchased from VWR International (Radnor, PA).

The epoxy microfluidic devices, manufactured using the microfluidic "sticker" technique41, required the production of two PDMS molds: The first mold was used to pattern the grooves, or microchannels, which were fabricated using degassed PDMS solution with 10 w% curing agent and cast onto a silicon mold containing the 50 microchannel grooves with dimensions of 36 mm x 9 µm x 9.8 µm. The PDMS was then cured for 90 min at 70 oC, before being peeled away from the silicon mask. The molded PDMS was then placed under vacuum until use so that any residual air from the epoxy used would be absorbed by the PDMS mold. The second PDMS mold, used to scaffold the main channel and inlets of the epoxy device was fabricated in a similar fashion.

The glass slide used as the backing of the main channel and to create the inlets into the device, needed to be pre-drilled prior to fabrication of the epoxy device. To do so a plastic sheet containing holes for the device inlets are marked on the glass. The glass slide was then drilled using a 3 mm diamond tipped bit purchased from THK Diamond Tools. The drilling was conducted under about 2 cm of water in a petri dish in order to keep the glass cool while drilling. The drilled glass was then rinsed with isopropanol and then water, before dried by blowing with air and incubation in the oven (70 oC for at least 10 min).

Once the PDMS mask was prepared, the epoxy solution was removed from refrigeration and poured into a small plastic dish. A 1 mL syringe with a 25 ga needle was then used to pull up 500 µL of epoxy. Air was then evacuated from the epoxy by inversion of the syringe and tapping to remove all the bubbles. In small droplets, about 100 µL of epoxy was placed onto both sides of the groove pattern of the PDMS mold. A cover glass, cleaned with tape to remove dust, was then placed – not pushed – on

top of the epoxy droplets, allowing the epoxy to flow into and around the mold. The molding epoxy was then allowed to sit for 90 min – to allow the PDMS to absorb any residual air in the epoxy – before placing the setup into the Uvitron International Intelli-Ray 400 UV oven for 80 s to cure the epoxy.

To form the rest of the device, the PDMS mold for the main channel and a glass slide were cleaned with tape. The PDMS mold was then placed onto the cleaned glass, where the two pieces should naturally adhere. Two pieces of plastic tubing were then placed on through the two holes in the PDMS mold, and the mold is filled with epoxy. The drilled glass, previously prepared, was then placed on top of the PDMS pushing the tubing through the two holes in the glass. This assembly was then placed in the UV oven with the drilled glass face up for 45 s, and then with the drilled glass face down for 65s. Once cured, the bottom (clear) glass slide and then the PDMS mold were removed, leaving the molded epoxy channel on the drilled glass slide. The holes were then widened and cleaned by using a 25 ga needle, and excess epoxy was removed using a razor blade.

In order to make the epoxy channels more hydrophilic, the two epoxy pieces were plasma treated – using a Harrick Plasma plasma cleaner – for 5 minutes prior to sealing of the device. Once treatment was completed, the grooved epoxy was placed onto the larger channel epoxy piece, making sure that the grooves are aligned parallel to the long side of the main channel. The adjoined epoxy device was then cured in the UV oven for 20 s. This began the adhesion between the two pieces, at which point the device was pressed with a pair of tweezers to connect the epoxy. Darker coloration appeared at this point – showing that the connecting was occurring. The device was then returned to the UV oven for 200s to complete bonding.

As a final step, two PDMS square blocks are connected to the PDMS device so allow for connection to tubing for our experiments. These 1”x1” PDMS chips are cut from a larger cured piece of PDMS in a petri dish prepared similarly to the PDMS molds. Once cut, a 1.5 mm diameter punch was used to create holes in the middle of each block. To adhere the PDMS to the device, the PDMS blocks

were plasma treated and placed on top of the drilled glass, aligning the punched PDMS holes with that of the device. The resulting epoxy microchannel devices contained 50 microchannel grooves along the bottom of the device with dimensions of 36 mm x 9 μm x 9.8 μm. The overall device dimensions measure 45 mm x 7 mm x 180 μm.

In order to increase the hydrophilicity of these devices post-fabrication, a poly(acrylic acid) (PAA) layer was grafted onto the epoxy interface, adapted from Schneider et al.42 To generate this coating, a 10 w% of benzophenone in 50 v% acetone-ethanol solution was generated and injected into the finished microchannel device (less than 300 μL of solution is needed). The solvent in this solution swells the epoxy and allows the benzophenone to be absorbed into the device substrate. After 2 minutes, the solution was flushed out with air from a 1 mL syringe. This process was then repeated for the second inlet. A freshly generated solution of 10 w% acrylic acid in water was then injected into each of the inlets of the device, and the inlets were sealed with clear tape to prevent leakage of the solution. The device was then treated in the UV oven for 2-3 minutes at maximum intensity to graft the PAA to the epoxy surface. This time can vary depending on the desired PAA coverage and the real lamp intensity of the UV oven, which may change over time. If too short of a UV treatment was used, devices were not adequately hydrophilic, and aqueous solution would dewet from the microchannels.

After the PAA coating has been polymerized, the tape was removed, and the remaining solution was flushed out with ethanol. A pH 10 solution (0.1 mM NaOH in water) was then used to flush the channels to remove any unreacted acrylic acid. Finally, water was used to rinse any final residual material from the channels. If the devices were not used right away, the devices were filled with water and sealed with clear tape to help maintain the PAA containing prior to testing. However, most experiments were carried out on the same day that the devices were coated (the day after the devices were first produced).

SI.4. Videos of Microfluidic Experiments

A total of nine representative videos can be found attached to this Supplemental Information, the first two of which are for the glass microfluidic droplet generator experiments, and the other seven for the epoxy microchannel experiments. The glass microfluidic droplet generator experiments were conducted using a 10 mg/mL 5k NR-PEG aqueous phase with a density-matched Paroil-Mineral oil external phase, at 2.5x magnification, under a 1:50 dispersed-to-continuous phase flow rate ratio. Video 1 shows a composite of the brightfield, and aqueous (green) and oil (yellow) fluorescent channels when excited at 514 nm (630-650 nm and 560-580 nm, respectively). An image from this video is used to produce Figure 5 in the main article. Some interesting behavior is also observed when the droplet is formed, as shown in the breakdown of the video shown in Figure SI.4.

Here, we were able to show presence of surfactant-dye in the continuous phase prior to the appearance of the continuous phase front (Figure SI.4b and c). The residual fluorescence observed the aqueous channel is due to the overlap of the fluorescence in the oil phase into the spectra window set at 650-670 nm, observed in the fluorescence spectra shown In Figure 2 in the main article. This ejection of NR-PEG prior to droplet generation is likely due to convection by oil that refills the capillary after a droplet is generated while in the dripping regime for this device (in which we operate), as suggested by residual fluorescence in the oil phase in the capillary in Figure SI.4e that is no longer imaged in f.

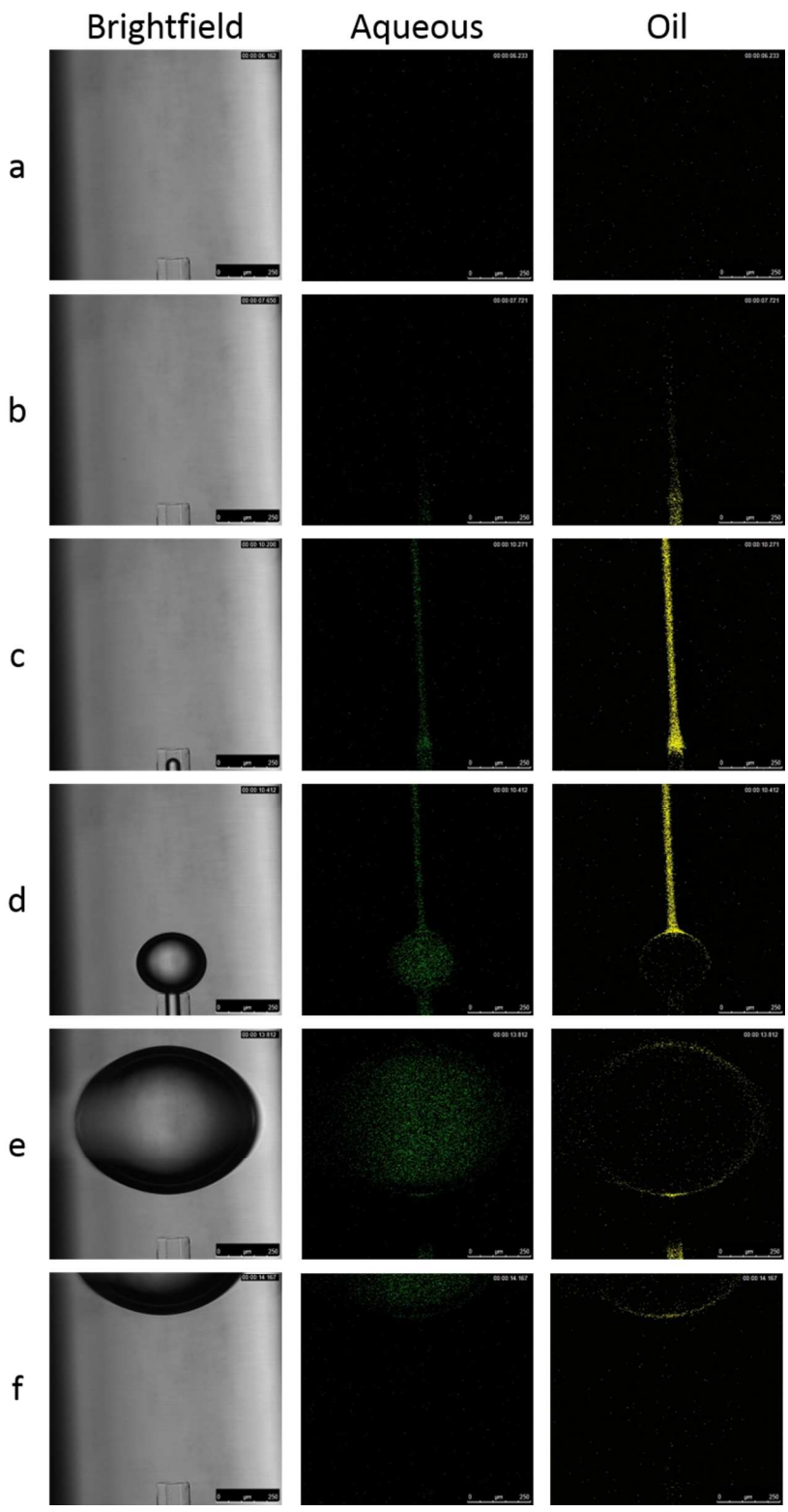


Figure SI.4. Brightfield, and fluorescence aqueous (650-670 nm) and oil (560-580 nm) example images taken by confocal of 1 mg/mL 5k NR-PEG droplets being produced in a flow-focusing, glass capillary microfluidic device by a mineral oil-Paroil mixture for the external phase at 10x magnification. The flow direction is toward the top of the page. (**a**) An example image between drops. No fluorescence is observed in either spectra window and no solution is observed by brightfield in the capillary. (**b** and **c**) Before the aqueous solution front appears, NR-PEG surfactant dye in oil is ejected from the capillary. (**d** and **e**) The aqueous droplet is produced, where the NR-PEG in the droplet can be seen in the aqueous spectral window, and surfactant-dye is found to be localized at the droplet-oil interface as seen in the oil fluorescence images. NR-PEG can be seen to be localized at the bottom of the newly formed droplet. (**f**) The droplet leaves the frame, and oil fills the dripping capillary, taking some residual NR-PEG back into the capillary with this oil. The NR-PEG that was concentration at the bottom of the droplet immediately after formation becomes less localized, and the fluorescence at the droplet surface increases as surfactant diffuses along and adsorbs to the interface. The cycle for droplet production then returns to where no fluorescence is observed and repeats (**a**).

At these concentrations, we image how the surfactant-dye accumulates at and diffuses along the forming droplet interface (Figure SI.4d-f). The former behavior is seen qualitatively when comparing d and e, where the fluorescence on the surface increases, and the latter is seen between e and f, where the concentrated fluorescence at the back of the droplet becomes more diffuse.

Videos 3-9 were used to produce the plots in Figure 8 of the main article. Images from Videos 3 and 4 were used to produce Figure 7 of the main article. Videos 3-8 were generated using 0.1 mg/mL 5k NR-PEG in water solution as the aqueous phase and silicone oil as the external phase with flow rates of 0.1 to 0.5 mL/hr within untreated epoxy devices. Video 9 was captured using a 0.1 mg/mL 5k NR-PEG in 67 wt% light corn syrup (glucose)-water solution as the aqueous phase and mineral oil as the external phase, with a flow rate of 1 mL/hr.

SI.5. References

1. Chen, C. V. H. H.; Triana, B. P.; Prud'homme, R. K., Investigation of the Local Environment of Hydrophobic End Groups on Polyethylene Glycol (PEG) Brushes Using Fluorometry: Relationship to Click Chemistry Conjugation Reactions on PEG-Protected Nanoparticles. *ACS Macro Letters* **2015,** *4* (5), 521-525.
2. Gmehling, J.; Menke, J.; Krafczyk, J.; Fischer, K.; Fontaine, J.-C.; Kehiaian, H. V., Azeotropic Data for Binary Mixtures. In *CRC Handbook of Chemistry and Physics*, 95th ed.; Haynes, W. M., Ed. CRC Press/Taylor and Francis: Boca Raton, FL, 2013.
3. Benson, B. R.; Stone, H. A.; Prud'homme, R. K., An "off-the-shelf" capillary microfluidic device that enables tuning of the droplet breakup regime at constant flow rates. *Lab on a Chip* **2013,** *13*, 4507-4511.