Supporting Information for:

Creating Biocompatible Oil-Water Interfaces without Synthesis: Direct Interactions between Primary Amines and Carboxylated Perfluorocarbon Surfactants

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Supporting Information (SI) Contents:

Supplementary Materials.

Materials for fabrication of the microfluidic emulsion generator (**Fig. S-2**) are included here. SU-8 2035 photoresist was obtained from Microchem. Silicon wafers were purchased from Silicon Inc. 0.02-in I.D. Tygon tubing and blunt needles were obtained from Small Parts. Aquapel was obtained from Pittsburgh Glass Works. Polydimethylsiloxane (PDMS) precursors were obtained from Dow Corning (Sylgard 184 elastomer and curing agent).

DNA sequences are included herein. Amine-labeled DNA: 5'- /5AmMC6//iSp18//iSp18//iSp18/TCA GCC ATT CGA ATC GTA CT -3' ; fluorescent complementary sequence: 5'- /56-FAM/AGT ACG ATT CGA ATG GCT GA -3'; Rox-labeled DNA for reference droplets: 5'-/56-ROXN/ CTT TCC TAC ACC TAC G, template DNA for PCR float tests (cDNA obtained by RT-PCR from mouse RNA): 5'-**AAG CAG GTC ATT GTT TCA ACA TGG** CCC TGT TGG TGC ACT TCC TAC CCC TGC TGG CCC TGC TTG CCC TCT GGG AGC CCA AAC CCA CCC AG GCT TTT GTC AAA CAG CAT CTT TGT GGT CCC CAC CTG GTA GAG GCT CTC TAC CTG GTG TGT GGG GAG CGT GGC TTC TTC TAC ACA CCC AAG TCC CGC CGT GAA GTG GAG GAC CCA CAA GTG GAA CAA CTG GAG CTG GGA GG**A AGC CCC GGG GAC CTT CAG ACC TTG GCG**-3' and corresponding double-quenched ZEN probe (Taqman format): 5'- TGT TGG TGC ACT TCC TAC CCC TG -3'; template DNA for droplet PCR: 5'-**TCC ACT CCT TTT CAT CTG CC**T TCC TTT TCT CCA TCG AGG TCC AGG TGA CCA TTG GTT TCG GCG GG**C GCA TGG TGA CAG AGG AAT**-3' and corresponding double-quenched ZEN probe (Taqman format): 5'- CCT GGA CCT CGA TGG AGA AAA GGA -3'; RPA forward primer (RPA_F-primer): 5'- GGT AAA GGT GTC GTG GAA CTA TCT AGC GGT GTA C -3' ; RPA reverse primer (RPA_R-primer) : 5'- TTT GTT TGA TAC CTT AGC CTA ATA CCC GAT T -3'; and RPA template sequence: 5'- TCG TGG AAC TAT CTA GCG GTG TAC GTG AGT GGG CAT GTA GCA AGA GGG TCA TCA TTC GAA TCG TAC TGC AAT CGG GTA TTA GGC TA -3') were obtained from Integrated DNA Technologies (IDT). RPA probe: 5'- GTA CGT GAG TGG GCA TGT AGC AAG AGG GTC A3C HT1 CGA ATC GTA

CTG CAT CGT TCT CCC AGT AGT AAG -3' was obtained from Biosearch Technologies, where "3" = dT-FAM fluorescent label, "H" = tetrahydrofuran, and "1" = dT-BHQ1 quencher label.

Supplementary Methods.

Krytox Preparation. Krytox 157 FSH ("Krytox") was converted to its carboxylate salt form. 20 g of Krytox and 180 g of methanol were combined in a glass dish and gently stirred. Concentrated NH_4OH was then added drop wise until the solution cleared. The mixture was placed on a microplate shaker at 240 rpm for approximately 10 min then filtered by vacuum filtration to remove residual solvent, rinsed with fresh solvent, and allowed to dry over the vacuum for 5 min. The product was dissolved in pure HFE 7500 oil at a previously-determined optimal concentration of 1.8 % w/w. The mixture was passed through a 0.2 μm pore syringe filter prior to use.

Ionic Strength Test. Emulsions were generated by vortexing, using two types of oil/surfactant combinations: 1.8% w/w Krytox in HFE 7500 oil or Jeffamine-bound Krytox in HFE 7500 oil. Several dilutions of PBS were made from a stock solution (100 mM Phosphate, 150 mM NaCl). Jeffamine was added to each dilution at a concentration of 0.75% w/v. A second set of PBS dilutions were made at the same concentrations, and 1.5 mM $MgCl_2$ was added to each solution along with 0.75% w/v Jeffamine. Emulsions were made from each PBS solution by adding 100 μL oil/surfactant and 50 μL of aqueous solution to a micro-centrifuge tube and vortexing for 30 s. 150 μL of mineral oil were then added to the top of each emulsion, and the tubes were heated to 95 °C for 10 min on a heating block. Digital images of each emulsion in tubes were captured immediately before and after heating. Images were captured again after allowing emulsions to sit at room temperature overnight. (**Figure 3A**)

Binding Optimization. To determine the concentration of additive needed to allow PCR biocompatibility, two PCR amplification tests were designed. Jeffamine was used as a PCR additive at final concentrations of 7.5% (75 mg/mL), 0.75%, 0.075%, or 0.0075% w/v by diluting in D.I. H_2O and adding to the PCR amplification mixture. 20 μL of each PCR solution (D.I. H_2O, Jeffamine additive,

1% BSA, 42 pM template, 50 nM Taqman Probe, 0.2 μM Fwd/Rev Primer, 1.5 mM $MgCl_2$, 1X Platinum *Tfi* Reaction Buffer, 0.2 mM dNTPs, 0.1 U/μL Platinum *Tfi* DNA Polymerase) were loaded into individual PCR wells on a 96-well plate. The 96-well plate was covered with plastic film, and the following temperature cycling program was performed: initial denaturation at 94°C for 2 min; cycles of 94°C for 15 s and 60°C for 1 min; total of 50 cycles. Amplification without Jeffamine as an additive was monitored as a positive control, and amplification without additive or template was monitored as the negative control (**Figure 3B**, top).

The second test monitored amplification in the presence of two oil/surfactant combinations: 1.8% w/w Krytox in HFE 7500 oil, and pure HFE 7500 oil. 20 μL of each oil/surfactant combination were loaded into individual PCR wells on a 96-well plate. 20 μL of amplification mixture containing either 0.75% or 0.075% w/v Jeffamine PCR additive were carefully layered on top of this oil, avoiding emulsion generation. 30 μL of mineral oil were layered on top of the aqueous phase. The 96-well plate was covered with plastic film, and the following temperature cycling program was performed: initial denaturation at 94°C for 2 min; cycles of 94°C for 15 s and 60°C for 1 min; total of 50 cycles. Amplification without oil/surfactant present was monitored as a positive control. (**Figure 3B**, bottom)

pH-dependence of Interaction. Tests were performed using either pure HFE 7500 oil or 1.8% w/w Krytox in HFE 7500 oil and 75 mg/mL Jeffamine in either 0.01 M HCl (pH ≈ 2), 0.01 M NaCl (pH ≈ 7), or 0.01 M NaOH (pH ≈ 12). FT-IR spectroscopy and mass spectrometry were used to determine the extent of direct binding in different pH environments. (**Figure 3C**)

Proximity FRET Protein Assay (pFRET). The pFRET assay, a FRET version of the proximity ligation assay[30,31] was conducted by combining 94 nM fluorophore- and quencher-labeled antibody-oligonucleotide probes, 94 nM insulin, and 560 nM connector oligonucleotide, for a final volume of 60 μL. The assay response was measured on a Filter Max F5 Multimode Microplate Reader (Beckman Coulter and Molecular Devices). The plate wells either contained no surfactant with 110 μL HFE 7500

oil, 110 μL HFE 7500 oil with 1.8% Krytox surfactant, or 110 μL HFE 7500 with 1.8% Jeffamine-saturated Krytox. (**Figure 4A**)

Droplet PCR. Droplets for performing DNA amplification by PCR were generated by pulling a vacuum of approximately 70 kPa to a single-channel emulsion generating microfluidic device (**Fig. S-2**), using 1.8% w/w Krytox in HFE 7500 oil as the carrier phase. The aqueous inlets were filled with amplification mix for PCR (D.I. H_2O, 0.75% w/v Jeffamine, 1% BSA, template DNA, 50 nM Taqman Probe, 0.2 μM Fwd/Rev primer oligos, 1.5 mM $MgCl_2$, 1X Platinum *Tfi* Reaction Buffer, 0.2 mM dNTPs, 0.1 U/μL Platinum *Tfi* DNA Polymerase). Solutions contained either none, 160 fM, or 16 pM template. A separate solution of amplification mix, containing 16 pM template and no Jeffamine, was also made as a negative control to show that Krytox inhibits PCR. Separate microfluidic chips (**Fig. S-2**) were used for each template concentration. After emulsifying all of the solution, droplets were transferred from the device outlet to individual PCR tubes. 60 μL mineral oil was layered on top of each emulsion, and PCR products were generated using a CFX1000 Thermal Cycler with CFX96 qPCR detection system (BioRad) and the following temperature cycling program: initial denaturation at 94°C for 2 min; cycling at 94°C for 15 s and at 60°C for 1 min; total of 35 cycles. An aqueous solution of amplification mixture (no oil) containing 16 pM template was used as a positive control, and a solution containing no template was used as a negative control. After thermal cycling, sample droplets were mixed with reference droplets by pipetting, and they were transferred to a single microfluidic channel for imaging via confocal microscopy. Reference droplets (50 nM ROX-labeled DNA, 50 nM Taqman probe; 1% BSA; 1X Platinum *Tfi* Reaction Buffer) were generated using a separate droplet-generating device. (**Figure 4B-C**)

Droplet RPA. RPA solution was prepared (700 nM Fwd & Rev primers, 200 nM RPA probe, 1.9 mg/mL BSA, and 89 μL rehydration buffer) and used to dissolve a lyophilized enzyme mixture (TwistDx). Half of this enzyme mixture was used to make an activation solution (22.4 mM MgOAc), and the other half was used to make sample solutions containing either a reference dye (110 nM

Sulforhodamine 101) with no DNA template or templates of varying concentrations (160 fM or 16 pM). The template and reference solutions were kept on ice, mixed with activation solution in a 1:1 ratio, and then again cooled on ice to avoid premature reactions at room temperature. The aqueous inlets of the microfluidic device were filled with the template/amplification mixtures for RPA. Droplets for RPA were then generated by keeping the microfluidic chip on ice and pulling a vacuum of approximately 70 kPa. 1.8% w/w Krytox in HFE 7500 oil was used as the carrier phase, and a separate chip was used for each RPA solution. For end-point measurements, droplets were collected for 1 hour then transferred to individual tubes for incubation at 37 °C. Each emulsion was incubated for 30 min on a standard PCR thermal cycler (Eppendorf). The tubes were then placed on ice to stop the reaction. After incubation, sample droplets were mixed with reference droplets in a 1:1 ratio, and they were transferred to a single microfluidic channel for imaging via fluorescence microscopy. (**Figure 4D**)

Droplet Monolayer Imaging of RPA Efficiency in "Drop Cages." For stable and immobile droplet monolayer imaging during the RPA efficiency experiment, droplets were imaged inside "drop cages," in which a thin PDMS layer (density ≈ 0.97 g/mL, similar to aqueous droplets) was suspended between HFE 7500 oil (density ≈ 1.6 g/mL) and mineral oil (density ≈ 0.8 g/mL) as a simple method to trap and immobilize aqueous droplets into a monolayer between the two oils. These drop cages were prepared by spin-coating then curing PDMS onto a silicon wafer to a thickness of 150 μm. Once cured, an 8 mm diameter circular hole punch was applied vertically to define the periphery of the cages. Second, a 2 mm diameter circular hole punch was applied vertically within the 8 mm slice in several locations, and these severed circles of PDMS were removed to define droplet imaging regions. To hold cages during imaging, an 11 mm diameter circular reservoir was punched into a PDMS layer of approximately 6-mm height, and this PDMS was bonded by an air plasma treatment to a glass slide (1 mm thickness). The reservoir was filled to just less than half its volume with HFE 7500 oil containing 1.8% Krytox. Next, a drop cage (150 μm thick, 8 mm diameter, with multiple 2 mm holes) was placed on top of the more dense oil and pressed down gently with tweezers to release trapped air bubbles. Mineral oil (less dense) was then added until the drop cage was submerged. The full assembly on the glass slide was then

placed into the microscope stage-top incubator (Tokai Hit) and allowed to thermally equilibrate to 37 °C for 30 min. For imaging RPA in droplets, 0.3 μL of each microchip-generated emulsion was added into the 2 mm diameter cages by dispensing directly above the interface between the HFE 7500 and mineral oils. This approach generated a monolayer of droplets that were stable and immobile for fluorescence microscopy during RPA reactions (~10 min), serving as a measurement of reaction efficiency. (**Figure 5**)

Synthesis of Covalently-Modified Surfactant, KryJeffa. The synthetic procedure used to prepare KryJeffa follows previously reported methods.[16,18] Briefly, thionyl chloride (2.4 mL, 33.33 mmol) was added into a solution of Krytox 157 FSH (50 g, 6.67 mmol) in HFE 7100 oil (125 mL). After the mixture was refluxed overnight under nitrogen, the solvent and the excess $SOCl_2$ was removed under vacuum. The resulting pale yellow oil was dissolved in HFE 7100 oil (100 mL). Dimethylaminopyridine on polystyrene (2.2 g, 6.67 mmol) and a solution of Jeffamine ED 900 (2.83 mL, 3.33 mmol) in tetrahydrofuran (50 mL) was added into the solution. After stirring for 24 h, the reaction mixture was filtered through Celite and concentrated under vacuum. The residue was further dried in vacuum to yield KryJeffa (48 g, 92%) as a colorless oil. FT-IR Spectroscopy, ^{1}H NMR, and ^{19}F NMR were performed to confirm formation of the product. FT-IR (cm^{-1}): 1730 (CO). ^{1}H NMR (250 MHz, Acetone-d_6): δ 3.88(bs), 3.58 (bs), 2.22 (s), 1.41 (s). Peaks are referenced to acetone-d_6 at 2.05.

Optimal Jeffamine Additive for Biocompatibility (**Table S-1**). Several additional in-house-developed tests were performed in order to optimize conditions for binding of polyetherdiamine (Jeffamine) to a carboxlyated, perfluorocarbon surfactant (Krytox). Different sizes and geometries of Jeffamine were chosen to determine which would provide the most assay biocompatibility after bonding to Krytox. Jeffamine M600 (9 PEG/4 PPG units), ED600 (9 PEG/4 PPG units), ED900 (12 PEG/5 PPG units), and ED2003 (26 PEG/11 PPG units) were investigated. A prefix of 'M' distinguishes a monoamine from a diamine, which is labeled with the prefix 'ED'. These choices allowed us to test the effects of size and geometry on droplet formation and stability. A monoamine, having a single binding group, should bind

in a 1:1 ratio with surfactants. A diamine, having the ability to bind two surfactants, could form either 1:1 binding or 2:1 (Krytox:Jeffamine) binding. All of the Jeffamine groups were reacted using the same standard protocol mentioned previously. qPCR and emulsion heat stability tests were used to determine which of the polyetheramines provided both droplet stability and biocompatibility. From this series of tests, Jeffamine ED 900 was determined to be the best candidate.

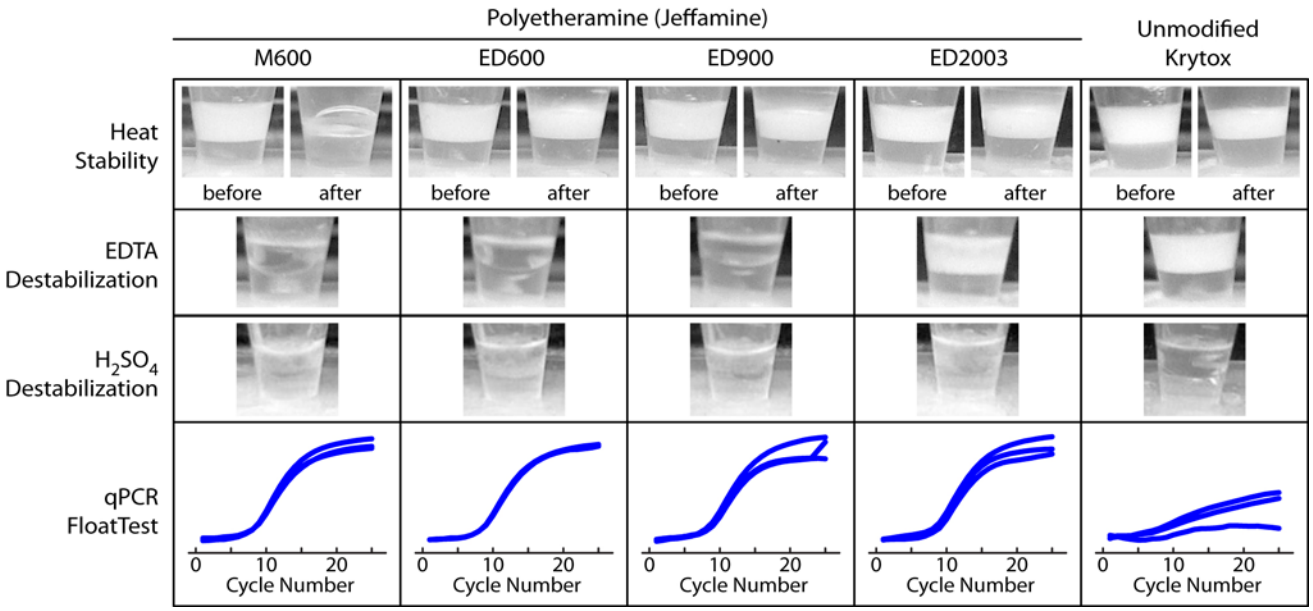

Table S-1: Qualitative testing of various polyetheramines (PEA). Several sizes and geometries of PEAs were used to bind to Krytox 157 FSH and then compared to the unmodified surfactant. Jeffamines M600, ED600, ED900, and ED2003 contained 9, 9, 12, and 26 PEG units, respectively. Jeffamine M600 was the only monoamine tested. Heat stability tests were performed at 95 °C for 10 min to mimic PCR conditions. 0.5 M EDTA and 1 M H_2SO_4 destabilization tests were used as qualitative determinants of binding. A real-time PCR (qPCR) "float test" was used to determine surfactant biocompatibility for future applications shown in the manuscript. Jeffamine ED 900 was determined to be the most suitable PEA group, based on its heat stability and biocompatibility. Qualitative binding tests (acid and EDTA) also agreed with proposed binding that is further supported by spectroscopic results in the manuscript.

Table S-1 is a summary of this information. A heat stability test was performed to assess each surfactant's ability to withstand PCR conditions. All of the surfactants withstood 95 °C for 10 min except for Jeffamine M600, which was slightly destabilized. This provided evidence that the polyetherdiamines participate in multivalent binding (2:1, Krytox:Jeffamine). Next, a test of emulsion destabilization using EDTA was developed. The negative effect of EDTA on PEG aqueous solubilty is a well-studied process[27], thus in the presence of EDTA, Jeffamine-bound surfactant should not form an emulsion. Using 0.5 M EDTA, we found that Jeffamine was completely destabilized, while other PEA groups were only slightly destabilized. The same solution with unmodified surfactant was not destabilized. Next, a test of emulsion destabilization using H_2SO_4 was developed. In this case, the opposite effect should occur. Since the unmodified surfactant is a carboxylate, upon contact with aqueous solution, it is considered an anionic surfactant. If this group is re-protonated back to its carboxylic acid form (at very low pH), the molecule should be pushed away from the oil-water interface and lose its emulsion-forming capabilities. When using 1 M H_2SO_4 as the aqueous phase for an emulsion, this trend was observed. Unmodified surfactant, which the acid will re-protonate to some extent, should not form as stable of an emulsion. Conversely, Jeffamine-bound surfactant may still maintain some stability. Indeed, in **Table S-1** we see that the unmodified surfactant is completely destabilized by the acid, while all of the modified surfactants are only partially destabilized, suggesting that Jeffamine-bound Krytox is somewhat protected from acid destabilization.

Finally, a real-time PCR float test was conducted to determine if amplification was dependent on the type of polyetheramine (PEA) group. The results demonstrate that all types of Jeffamine-bound surfactants allow for successful PCR amplification, while the unmodified surfactant showed much lower reaction efficiency. This results is likely due to the negative charge of the carboxylate group on the unmodified surfactant binding to important PCR reagents (likely enzymes) and inhibiting the reaction. Based on the results of the qualitative tests of biocompatibility, destabilization, and heat stability, we determined that the optimal PEA group for surfactant modification was Jeffamine ED 900.

Optimal Surfactant Concentration for Droplet Stability (**Figure S-1**). Solutions of 2%, 1%, 0.1%, 0.01%, and 0.001% w/w Krytox in HFE 7500 oil were made by simply dissolving surfactant in oil. At each concentration, two separate mechanical emulsions were produced by vortexing, containing the following aqueous phases: 31 μM $CaCl_2$ and 1 μM EDTA in one emulsion, and 300 nM Fluo-4 Ca^{2+} binding dye in the other emulsion. At each surfactant concentration, the two emulsions were combined, and aqueous solutions of the two were combined as a positive control. Two emulsions, made with HEPES buffer and 2% w/w oil/surfactant, were combined and used as the negative control. All emulsion combinations and controls were heated to 95 ºC for 30 min on an analog heating block (VWR), and fluorescence emission of Fluo-4 was measured at excitation/emission wavelengths of 494/506 nm on a Filter Max F5 Multimode Microplate Reader (Beckman Coulter). Any increase in fluorescence above the negative control is consistent with droplet coalescence, or emulsion destabilization, since this brought the Ca^{2+} ions into contact with their fluorescent indicator (Fluo-4). The concentration of 1.8% w/w was determined to be optimal based on these results and on prior work.[16]

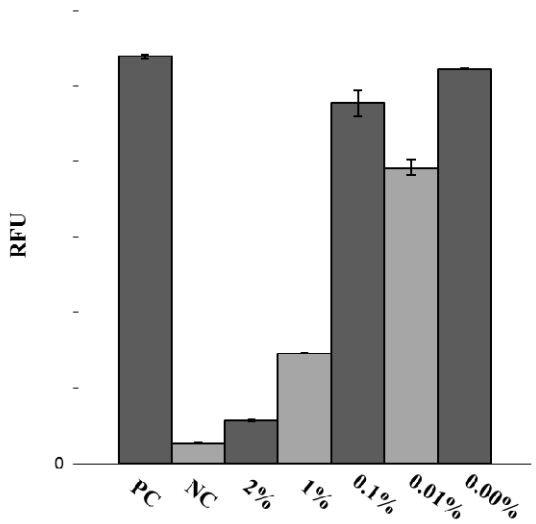

Figure S-1: The optimal concentration of surfactant in oil for maximum droplet stability was determined to be ~2 % w/w. After creating mechanical emulsions containing either 31 μM $CaCl_2$ and 1 μM EDTA , or 300 nM Ca^{2+} at each concentration, the two emulsions were mixed and heated to 95 °C for 30 min. Any increase in fluorescence above the negative control is consistent with droplet coalescence, or emulsion destabilization.

Microchip Fabrication and Droplet Formation (**Figure S-2**). PDMS microchips for emulsion generation were fabricated using standard soft lithography. PDMS elastomer base and curing agent were mixed in a 10:1 ratio, poured over the master defined by photoresist (SU-8, Microchem) on a silicon wafer, and cured overnight at 65 °C. Patterned PDMS was removed from the silicon master, and holes were punched for channel access. The device was then cleaned with methanol, air dried with a nitrogen stream, then bonded immediately to a glass slide after exposure to an air plasma for 45 s (Harrick Plasma). Microchannels were then treated with Aquapel (Pittsburgh Glass Works), rinsed with methanol, and placed in an oven at 65 °C overnight prior to use.

Flow control in the microfluidic emulsion generating devices (**Fig. S-2**) was achieved passively using a handheld, 100-mL glass syringe (SGE Analytical Science) connected to 0.02-in I.D. Tygon tubing (Small Parts). The tubing was interfaced to the device via a PDMS plug. This plug was constructed by simply punching out a large hole from a blank piece of PDMS. This hole was approximately 1 mm larger than the size of the outlet. A blunt needle was used to punch a hole for the tubing in the center of the plug. When the device was ready for use, the plug was inserted into the outlet opening on the device, and a vacuum was applied using the handheld syringe.

Our PDMS/Glass microfluidic emulsion generator (**Figure S-2**) has several features that make it ideal for different applications. First, the large outlet reservoir (~350 μL) eases droplet collection and transfer. This is particularly important when generating emulsions for PCR. A pipette can easily fit into this reservoir, and droplets can be transferred directly to a PCR tube. Next, the three aqueous inlets provide flexibility. Different reagents can be added to each inlet, allowing for on-chip mixing immediately prior to droplet formation. Finally, the device is passively controlled. Droplets were generated using a handheld, glass syringe. Eliminating the need for mechanically-driven pumps and switches makes this device ideal for use outside of a laboratory setting. DIC images of droplets packed into the outlet region of the chip are shown in **Figure S-2** (bottom), and are the result of pulling a vacuum of ~80 kPa. Jeffamine-bound surfactant was used as the carrier phase, and droplets exhibited stable packing and did not coalesce.

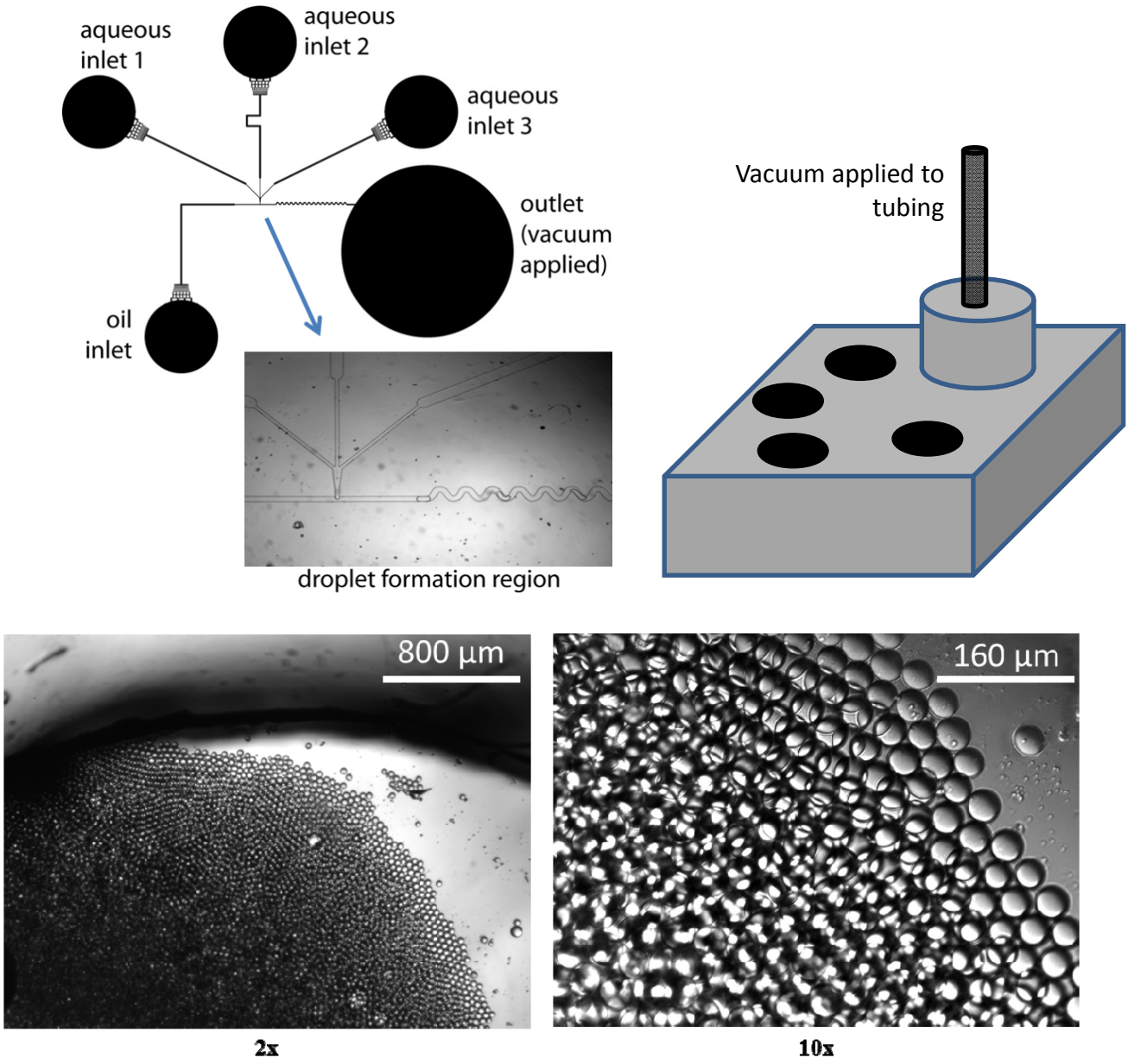

Figure S-2: Microfluidic droplet formation in an emulsion generating device. The passively-controlled microfluidic droplet generator with 16 µm channel depth was used to rapidly create aqueous-in-oil droplets, using Jeffamine-bound surfactants. The device has three aqueous inlets to allow for flexibility in reagent addition. The large outlet reservoir (~350 µL), interfaced to the vacuum (~ 80 kPa) via a PDMS plug, allows for ease of droplet collection and transfer. DIC images of droplets collected in the outlet reservoir of the device show uniform size, stable packing, and essentially no coalescence of droplets.