Supporting Information

Anti-Fouling Properties of Pluronic and Tetronic Surfactants in Digital Microfluidics

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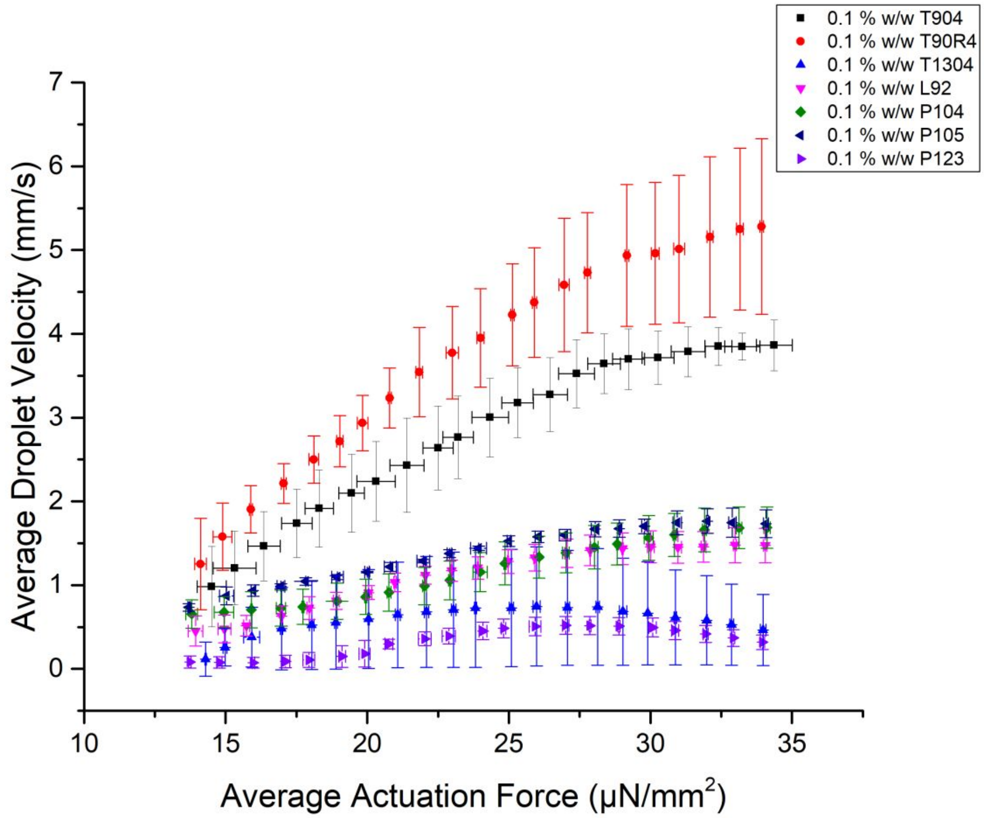

Figure S1. Plots of measured velocity as a function of average width-normalized driving force for DPBS containing 0.1% w/v T904 (black squares), T90R4 (red circles), T1304 (blue triangles), L92 (pink inverted triangles), P104 (green diamonds), P105 (sideways dark blue triangles), and P123 (sideways purple triangles). Error bars represent ± 1 std. deviation of velocity (vertical) and width-normalized force (horizontal) for three replicate droplets per condition. As shown, saturation forces were observed at around 25-29 μN/mm^2. After establishing the saturation forces for each liquid, care was taken to use driving forces below those levels for all subsequent experiments.

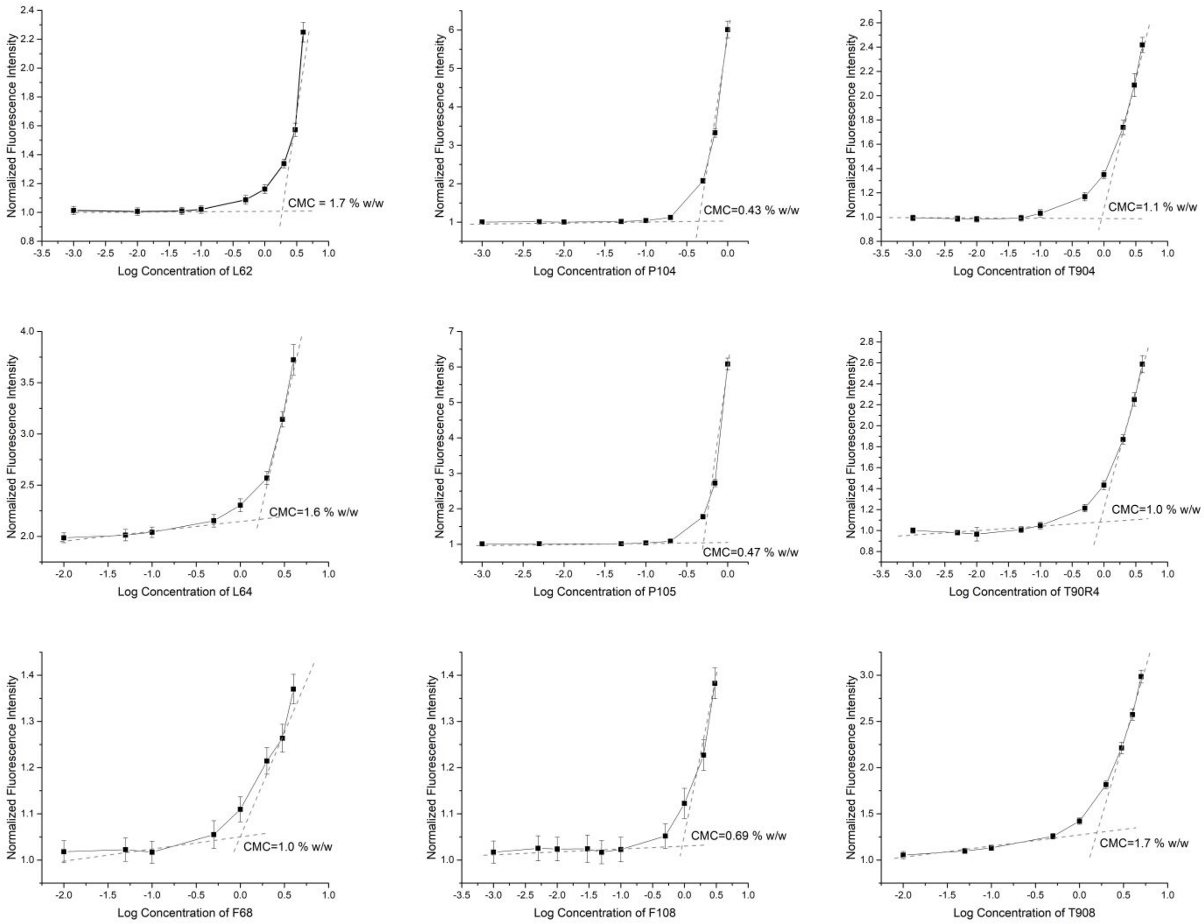

Figure S2. Experimental determination of critical micelle concentration (CMC). Plots of normalized pyrene fluorescence intensity as a function of log concentration in % wt/wt of Pluronics (L62, L64, F68, P104, P105, F108) and Tetronics (T904, T90R4, T908) in DPBS. Error bars represent $\pm$ std. deviation for three replicates at each condition and concentrations.

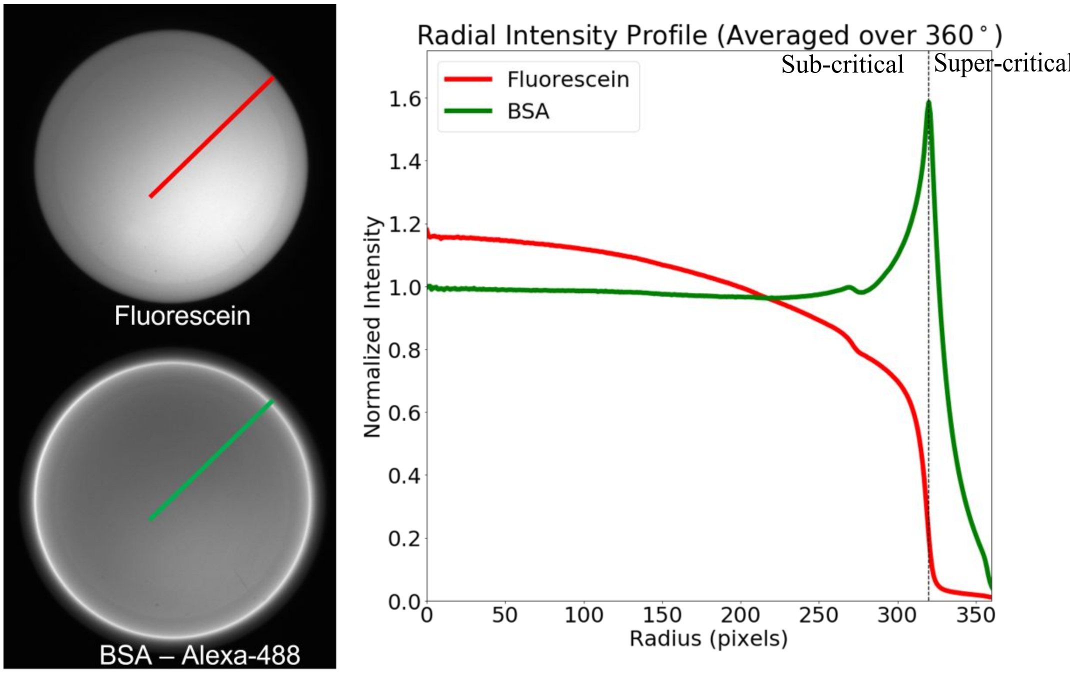

Figure S3. Supercritical angle fluorescence (SAF) microscopy. Left: images of droplets of 1 µM Fluorescein (top) and 0.01 mg/mL Alexa®-488 BSA (bottom) on a Teflon® AF surface. Right: plots of radial fluorescence intensity for the fluorescein (red) and Alexa®-488 BSA (green) solutions, illustrated by the corresponding lines in the images. As shown, the labeled dye exhibits a stronger SAF signal, which appears in the images as a bright ring. SAF signals were quantified by averaging the intensity (over 360˚) observed from 325 to 351 pixels in each image.

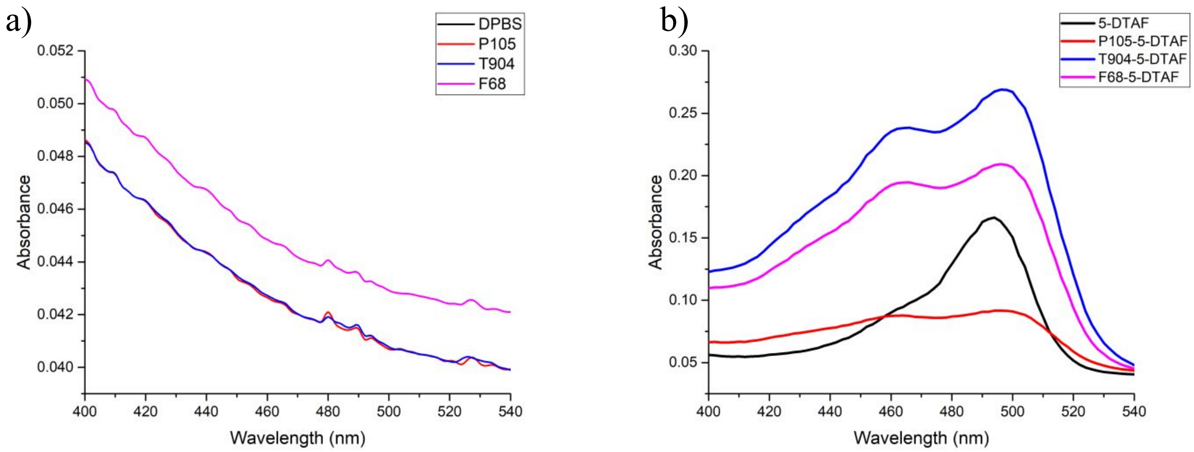

Figure S4. Surfactant derivatization. (a) Absorbance spectra of DPBS (black, hidden behind F68-pink), P105 (0.021 % wt/wt, red), T904 (0.09 % wt/wt, blue), and F68 (0.08 % wt/wt, pink) in DPBS. (b) Absorbance spectra of free fluorescent label (5-DTAF, black) in DPBS (0.01 mM) and 5-DTAF conjugated F68, P105, and T904 after purification. As shown, the conjugated surfactants had a second peak at ~460 nm.

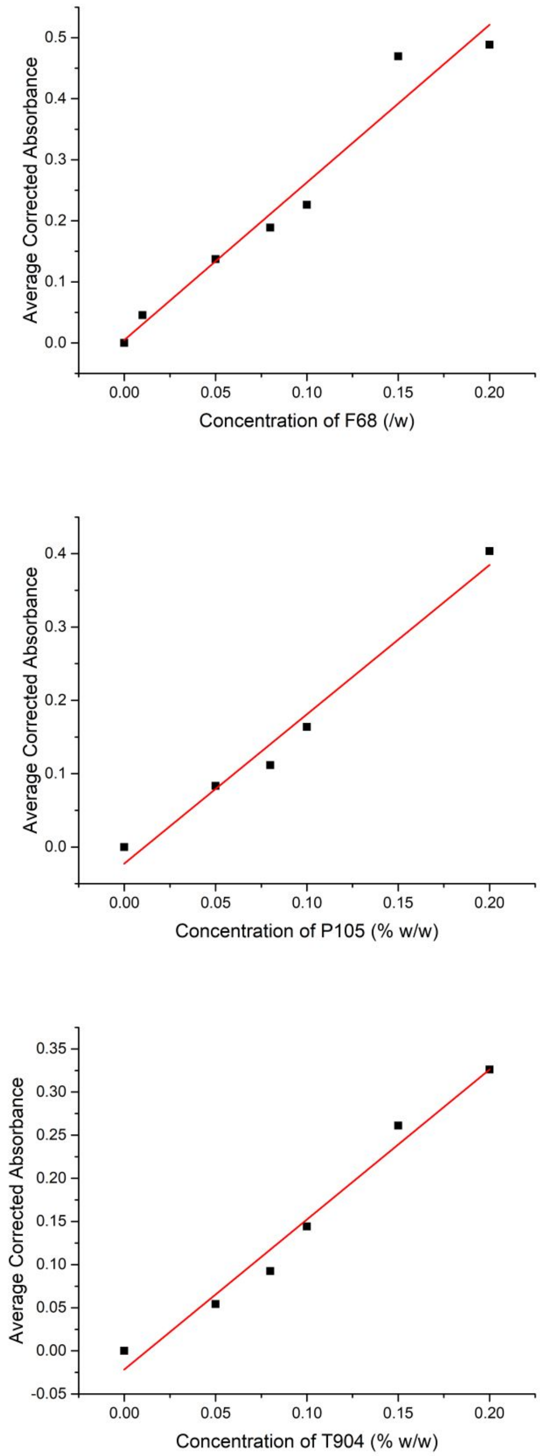

Figure S5. Quantitation of labeled surfactants. Plots of corrected absorbance at 623 nm as a function of concentration of standard solutions of F68, P105, and T904 in DPBS after processing for the colorimetric assay. Each marker (black square) represents the average of three replicates, and data were fit with a linear regression (red lines).

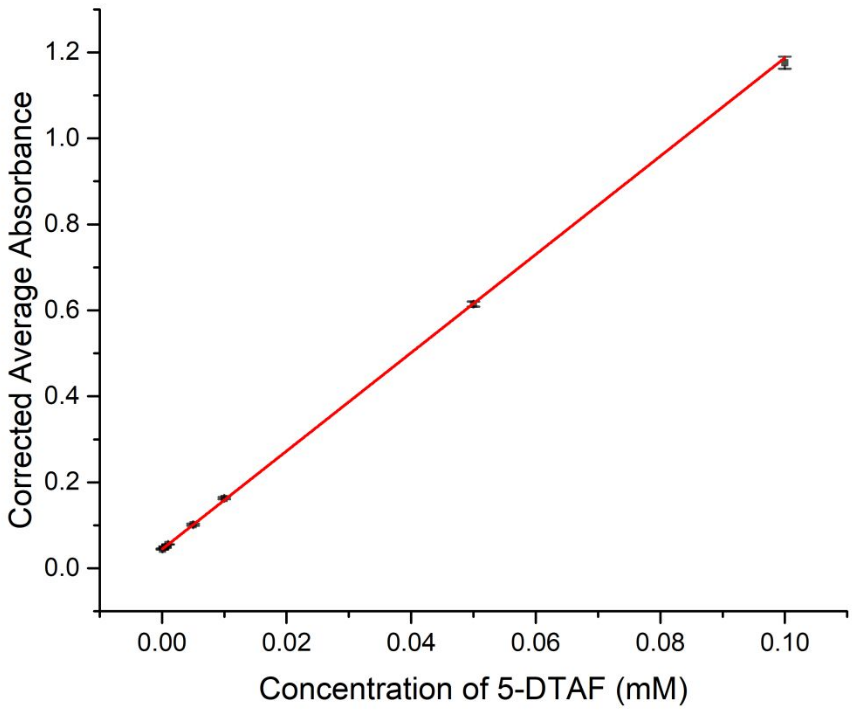

Figure S6. Plot of corrected absorbance (Black markers) at 498 nm as a function of concentration of standard solutions of 5-DTAF in DPBS. Error bars represent $\pm$ 1 std. deviation for three replicates at each concentration. Data were fit with a linear regression (red line).

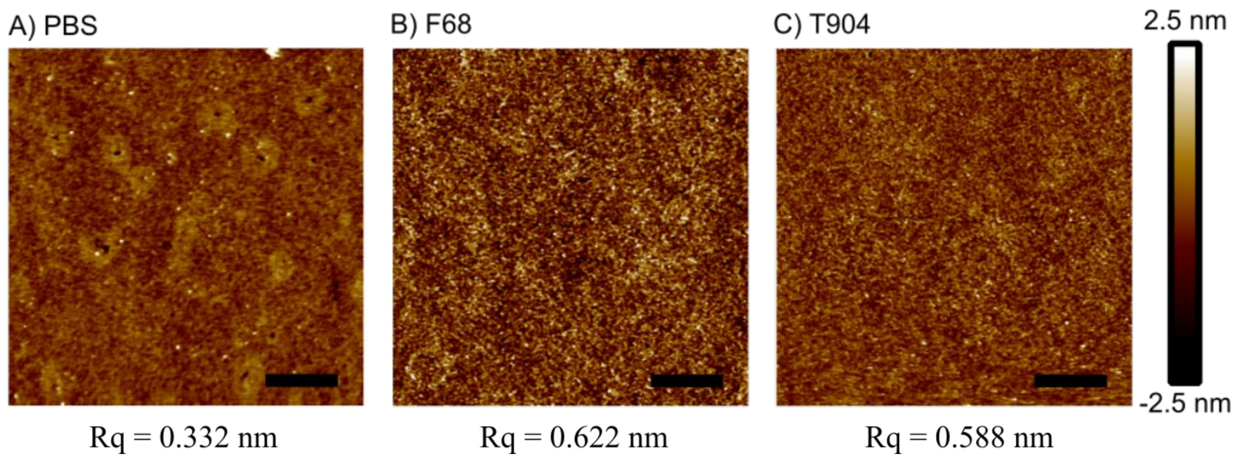

Figure S7. AFM height images with root mean square surface roughness (R_q) of Teflon-AF coated micro-cover glass exposed to PBS and surfactant solutions. A) Teflon AF surface exposed to PBS only, B) Teflon AF surface exposed to 0.1 % wt/wt F68, and C) Teflon AF surface exposed to 0.1 % wt/wt T904.

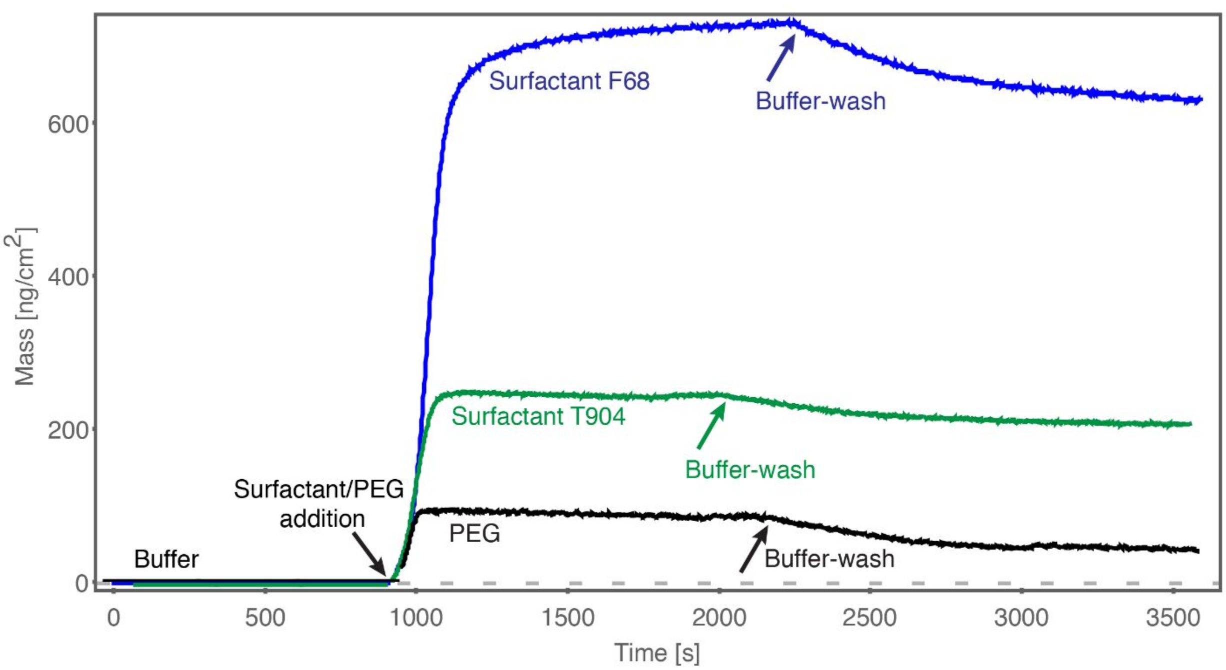

Figure S8. QCM-D plots of total mass of adlayers formed upon exposure of Teflon-AF coated sensors to solutions of F68 (blue), T904 (green), or PEG 8000 (black), all at 0.001 % wt/wt in PBS (buffer). The sensors were pre-equilibrated in buffer, with the solutions exchanged to surfactant or PEG at around 1,000 s, and then exchanged again with buffer at around 2,000 s.

Table S1. Initial DMF droplet movement longevity assay results (with 150 programmed steps) for Pluronic® and Tetronic® surfactants in 90% v/v FBS.

Surfactants	Concentration (% wt/wt)	Successful actuation steps ± 1 S.D. for n = 3 replicates per condition
L35	0.1	0
	0.5 (0.5x CMC in water)	0
	1.0 (CMC in water)	0
	2.0 (2x CMC in water)	0
F38	0.1	0
	0.5	0
	1.0	0
	2.0	0
L44	0.1	0
	0.40 (0.5x CMC in water)	0
	0.79 (CMC in water)	0
	1.6 (2x CMC in water)	8 ± 6
L62	0.05 (0.5x CMC in water)	0
	0.1 (CMC in water)	26 ± 9
	0.2(2x CMC in water)	33 ± 7
L64	0.07 (0.5x CMC in water)	7 ± 4
	0.1	0
	0.14 (CMC in water)	25 ± 14
	0.28 (2x CMC in water)	114 ± 16
F68	0.1	0
	0.2(0.5x CMC in water)	0
	0.4 (CMC in water)	0
	0.8 (2x CMC in water)	0
F88	0.10	0
	0.14 (0.5x CMC in water)	0
	0.29 (CMC in water)	0
	0.60 (2x CMC in water)	0
L92	0.016 (0.5x CMC in water)	8 ± 5
	0.032 (CMC in water)	48 ± 22
	0.064 (2x CMC in water)	150 ± 7
	0.1	150 ± 0
P104	0.0005 (0.5x CMC in water)	0
	0.001 (CMC in water)	0
	0.002 (2x CMC in water)	0
	0.1	150 ± 0
P105	0.002 (0.5x CMC in water)	0
	0.004 (CMC in water)	0
	0.008 (2x CMC in water)	0
	0.1	150 ± 0

Table S1(Continued). Initial DMF droplet movement longevity assay results (with 150 programmed steps) for Pluronic® and Tetronic® surfactants in 90% v/v FBS

Surfactants	Concentration (% wt/wt)	Successful actuation steps ± 1 S.D. for n = 3 replicates per condition
F108	0.016 (0.5x CMC in water)	0
	0.032 (CMC in water)	0
	0.064 (2x CMC in water)	0
	0.1	0
P123	0.0012	0
	0.0025	0
	0.0050	0
	0.1	79 ± 44
F127	0.0017 (0.5x CMC in water)	0
	0.0035 (CMC in water)	0
	0.0070 (2x CMC in water)	0
	0.1	0
T304	0.1	0
	0.5 (0.5x CMC in water)	0
	1 (CMC in water)	0
	2 (2x CMC in water)	0
T904	0.1	150 ± 0
	0.35 (0.5x CMC in water)	150 ± 0
	0.70 (CMC in water)	150 ± 0
	1.40 (2x CMC in water)	115 ± 94
T90R4	0.007 (0.5x CMC in water)	0
	0.014 (CMC in water)	0
	0.028 (2x CMC in water)	0
	0.1	150 ± 0
T908	0.25 (0.5x CMC in water)	0
	0.50 (CMC in water)	0
	1.0 (2x CMC in water)	0
	2.0	0
T1107	0.1	0
	0.35	2 ± 2
	0.70	4 ± 2
	1.4	34 ± 32
T1304	0.1	150 ± 0
	0.5	0
	1.0	0
	2.0	0

Table S2. Concentrations and conjugation efficiencies (± st. dev. for three replicates per condition) for 5-DTAF-labeling of F68, P105, and T904.

Surfactant	Concentration of conjugated surfactant after purification (mM)	Concentration of fluorophore (5-DTAF) after purification (mM)	Conjugation efficiency (%)	Concentration of fluorophore in 0.1 % wt/wt labeled Surfactant (mM)
F68	1.0 ± 0.1	0.11 ± 0.01	11 ± 1	0.013
P105	0.33 ± 0.20	0.04 ± 0.01	12 ± 7	0.013
T904	1.1 ± 0.2	0.24 ± 0.01	22 ± 4	0.034

Table S3. Mean supercritical intensities (± st. dev. for five replicates per condition) of 0.1 % wt/wt fluorescently labeled surfactant and 30 μM fluorescein in 0.1 % wt/wt unlabeled surfactant solutions on Teflon-AF coated glass substrates.

Surfactants	Mean supercritical intensity			
	DPBS		40 mg/mL BSA in DPBS	
	Labeled surfactant	Fluorescein in unlabeled surfactant	Labeled surfactant	Fluorescein in unlabeled surfactant
F68	729 ± 155	30 ± 1	128 ± 15	41 ± 10
P105	3166 ± 867	138 ± 5	603 ± 312	53 ± 5
T904	6601 ± 938	556 ± 20	1776 ± 300	196 ± 6

Table S4. AFM surface roughness values for PBS, F68 (0.1 % wt/wt) in PBS, and T904 (0.1 % wt/wt) in PBS on Teflon-AF coated glass substrates (one replicate, extracted from the data in Figure S7).

Solution on Teflon-AF	R_a, arithmetic surface roughness (nm)	$R_{q,}$ root mean square surface roughness (nm)
PBS	0.444	0.332
0.1 % wt/wt F68 in PBS	0.807	0.622
0.1 % wt/wt T904 in PBS	0.771	0.588