SUPPORTING INFORMATION

Contact Line Pinning is Not Required for Nanobubble Stability on Copolymer Brushes

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EXPERIMENTAL METHODS.

Solvent Purification. Prior to usage, water (dd-H2O) was deionized using Milli-Q Advantage A-10 water purification system (Millipore, USA).

Contact Angle Measurements. Contact angles were measured using the sessile drop technique. A camera (ARTCAM-130MI-BW) was used to image deionized water droplets placed on sample surfaces and illuminated by a backlight. Images were analyzed in an image analysis software (First Ten Angstroms Drop Analysis Software) that calculated contact angles of water droplets with respect to the surface. For comparisons to dye solutions, sessile contact angle measurements were performed with a 5 μM solution of AlexaFluor488-COOH (Invitrogen) in deionized water. Advancing and receding contact angles for hysteresis measurements were done by injecting water at ~200 µL/min on the surface to observe advancing contact angle and removing water from the droplet formed on the surface to observe receding contact angle at the same flow rate.

General Procedure for Cleaning of Glass Coverslips. Prior to any functionalization procedures, #0 glass coverslips (Electron Microscopy Sciences) were submerged in a piranha acid solution (70% sulfuric acid/30% hydrogen peroxide by volume) for 1 h, then thoroughly rinsed with deionized water. These coverslips were then cleaned by UV/Ozone for 1 h (PSD Series Digital UV Ozone Cleaner, Novascan), and immediately used for silane functionalization as described below.[1] This same procedure was also used for cleaning glass slides and silicon wafers, where indicated below.

Surface Methylation (HMDS Deposition). Clean coverslips were placed in a sealed chamber with an open vial containing 200 µL hexamethyldisilane (HMDS) (Oakwood Chemical and left for 48 h at room temperature. The HMDS reacted with the clean glass cover slip to coat the surface with trimethylsilane functional groups via vapor deposition. The HMDS-functionalized coverslips were then washed by sequential rinsing with water, ethanol, and isopropanol, then dried via a stream of compressed air filtered through a 0.20 µm filter.

Preparation of Surfaces with APTES-BIBB (ATRP initiator). Immediately following the general cleaning procedure, a coverslip was placed into a 1 v/v% solution of aminopropyltriethoxysilane (APTES) (Acros) in toluene. After 3 h, the slip was removed, then rinsed and dried. Next, 5 mL tetrahydrofuran (Fisher), 50 uL of bromoisobutyryl bromide (BIBB, Alfa Aesar), and 36 uL triethylamine (Fisher) were mixed in a glass petri dish. The coverslip was submerged in the solution for 16 h, retrieved, and rinsed sequentially with water, ethanol, and isopropanol, followed by drying by a stream of compressed air filtered through a 0.20 μm filter.

General Procedure for Surface-Initiated ATRP. The following chemicals were used in some or all of the procedures outlined below. Monomers: methacrylic acid (MAA, Sigma-Aldrich), methyl methacrylate (MMA) (Alfa Aesar), [2-(Methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide (SBMA, Sigma Aldrich), and 2-(dimethylamino)-ethylmethacrylate (DMAEMA, Alfa Aesar). Catalysts: bipyridine (bpy, Chem Impex Int'l Inc.), CuBr (Strem Chemicals, Inc.), $CuBr_2$ (Beantown Chemical).

APTES-BIBB coverslips were placed in a glass petri dish and introduced to an inert environment glove box (Unilab MBraun) along with argon-degassed solvents. Inside the glove box, one solution was prepared containing monomers, and a second solution was prepared with polymerization catalysts (see below). The two solutions were mixed together and immediately added to the glass petri dish, which was sealed with Parafilm to prevent evaporation. The reaction was run overnight.[2–4] Following polymerization, the coverslip was removed from the glove box and bath sonicated for 30 s in water. The coverslip was rinsed sequentially with water, ethanol, and isopropanol, followed by drying under a stream of compressed air flown through a 0.20 μm filter.

Synthesis of MAA-*co*-MMA Surfaces. Monomer Solution: 10 mL of a 1:9 v/v ratio of argon-degassed water:isopropanol and 1 g total mass of varying ratios of MAA and MMA. Catalyst Solution: 150 mg bpy, 58 mg CuBr, and 18 mg $CuBr_2$ were dissolved in 3 mL of the same cosolvent.

Synthesis of SBMA-*co*-MMA Surfaces. Monomer Solution 10 mL of a 1:1 v/v ratio of argon-degassed water:methanol and 1 g total mass of varying ratios of SBMA and MMA. Catalyst Solution: 75 mg CuBr and 125 mg bpy were dissolved in 2 mL of the same cosolvent.

Synthesis of SBMA-*co*-DMAEMA Surfaces. Monomer Solution: 10 mL of a 1:1 v/v ratio of argon-degassed water:methanol and 1 g total mass of varying ratios of SBMA and DMAEMA. Catalyst Solution: 75 mg CuBr and 125 mg bpy were dissolved in 2 mL of the same cosolvent.

Synthesis of MAA-*co*-DMAEMA Surfaces. Monomer Solution: 10 mL of argon-degassed water adjusted to pH 8 with sodium bicarbonate (Macron Fine Chemicals) and 1 g total mass of varying ratios of MAA and DMAEMA. Catalyst Solution: 75 mg CuBr and 125 mg bpy were dissolved in 2 mL of the same solvent.

Preparation of Microfluidic Molds. First, #0 glass cover slips (Electron Microscopy Sciences) were cleaned with piranha acid (1 h), thoroughly rinsed with deionized water, and exposed to UV/Ozone (1 h). Next, the slips were immersed in a solution of methacryloxypropyltrimethoxysilane (Chem Impex International Inc.) at 10 mM in toluene (Fisher) for 3 h. The functionalized slips were cleaned by rinsing with water, ethanol, and isopropanol sequentially, then dried using compressed air passed through a 0.2 µm syringe filter. Photomasks printed on Nylon (FineLine Imaging) were placed between two slips with 102 µm thick polyester spacers (Precision Brand Shim Stock Sheet). A photopolymerizable monomer mix comprised of pentaerythritol tetrakis(3-mercaptopropionate) (PETMP) (Sigma Aldrich) and triallyl-1,3,5-triazine-2,4,6-trione (TATATO) in a 1:1 stoichiometric thiol:ene ratio with initiator Irgacure 184 (0.15 mol% of thiol and ene combined) and inhibitor Q-1301 n-nitorosophenylhydroxylamine aluminum salt (0.15 mol% of thiol and ene combined) was placed between the mask and functionalized slide, clamped, and irradiated for 50 s using a Model J500 Mask Aligner with intensity peaks at 367, 407 and 438 nm and an intensity of 15 mW/cm^2. (Optical Associates Inc). Unreacted monomer was washed away using ethyl acetate leaving polymerized features. Molds were examined by light microscopy under 4X magnification to check for defects on a Nikon Ci-L upright microscope. The molds were covered in polydimethylsiloxane (PDMS) (Sylgard 184), for which the base and curing agent were mixed in a 10:1 ratio (Dow Corning Corporation), and air was removed via house vacuum to create a homogeneous PDMS cast. This cast was cured in a 70^{o}C oven overnight, then exposed to UV/Ozone for 15 min to create a hydrophilic surface. Finally, a 0.5 mm biopsy pen was used to punch two inlet flow channels to connect the Microbore PTFE tubing (Cole-Parmer 0.012"ID x 0.030"OD) from the syringes to the microfluidic device with an outlet flow channel built in via the mold.

Nanobubble Generation and TIRF Imaging. Images were captured on an inverted Nikon Eclipse TI Microscope with a Plan-Apo 100x 1.45 NA oil immersion objective (Nikon, Melville, NY) using a Hamamatsu CMOS camera at 10 frames per second (100ms exposure time). Excitation light was passed through a motorized total internal reflection (TIR) illuminator (Nikon TI-TIRF-EM) to adjust the angle of incidence on the sample to generate an evanescent field at the SLB-water interface. Excitation light for the nanobubble experiments was generated by a 100 mW 491 nm solid state laser (Cobolt Calypso 100, Solna, Sweden) and calculated to have an intensity incident on the surface of ~0.75 kW/cm^2.[5]

In a general procedure, a PDMS mold was attached to a functionalized coverslip, then placed over the objective of the TIRF microscope setup. Two silicon oil free syringes were attached to the inlet flow channels. One syringe contained a 5 μM solution of AlexaFluor488-COOH

(Invitrogen) in deionized water, and the other contained a 5 µM solution of AF488-COOH in 100% ethanol. The syringes were attached to Microbore PTFE tubing and inserted into inlet flow channels built with a biopsy punch. The liquid flowed through the inlet flow channel into the mold as seen in **Figure S1**. The liquid would pass through the mold and out the exit channel allowing solvent exchange to take place in the exit channel of the mold. The microscope was focused on the exit channel of the mold where solvent exchange took place to witness the formation and removal of nanobubbles.

Image Processing and Analysis. Image processing was performed via a custom Mathematica (Wolfram) code. First, a Gaussian blur was performed to reduce background noise, followed by manual thresholding of pixel intensity. Finally, the number, sizes, locations, and intensities of each nanobubble was calculated and marked in red (**Figure S4**). To quantify colocalization, two images from bubble deposition and re-deposition were manually aligned to a defect site to account for sample drift. Next, the Mathematica script was run to highlight nanobubbles in red. Finally, the red color splits were input into ImageJ (NIH) with a Coloc2 plugin to calculate Pearson correlation coefficients.

Zeta Potential Measurements. Zeta potential measurements were performed on a Surpass 3 (Anton Paar). A 1 mM potassium iodide (KI) (Sigma Aldrich) solution of water was passed through a 100±3 µm gap between a polypropylene film and the sample being measured. The sample being measured was grown on a select plain microscope slide (Fisher Scientific) in parallel with the TIRF samples being produced. The samples were rinsed and dried using the general procedure. Each sample was loaded onto a SurPASS3 Clamping Cell (Anton Paar), and sequential zeta potential readings were taken in 1 mM KCl until a stable value was obtained and recorded. This process was performed three times for each sample.

Ellipsometry Measurements. Variable angle, spectroscopic ellipsometry measurements were performed on polymer brushes grown on silicon wafers (University Wafer) that were made in parallel with glass surfaces. The wafers were rinsed, dried, and functionalized using the general procedures described above. Ellipsometry measurements were performed on a J.A. Woollam VASE-VB 250 Ellipsometer. A Cauchy model was applied for each layer of synthesis (i.e. APTES, BIBB, Polymer) before additional layers were added. Polymers were measured for thickness at a refractive index of 1.45.

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (FTIR-ATR). FTIR-ATR was performed using a Nicolet 6700 FTIR from Thermo Scientific with a Harrick VariGATR ATR. Polymer brushes were grown on select plain microscope slides (Fisher Scientific) then rinsed and dried as described above. A background spectrum was taken of the environment without a slide, followed by a copolymer-functionalized slide.

SUPPORTING FIGURES.

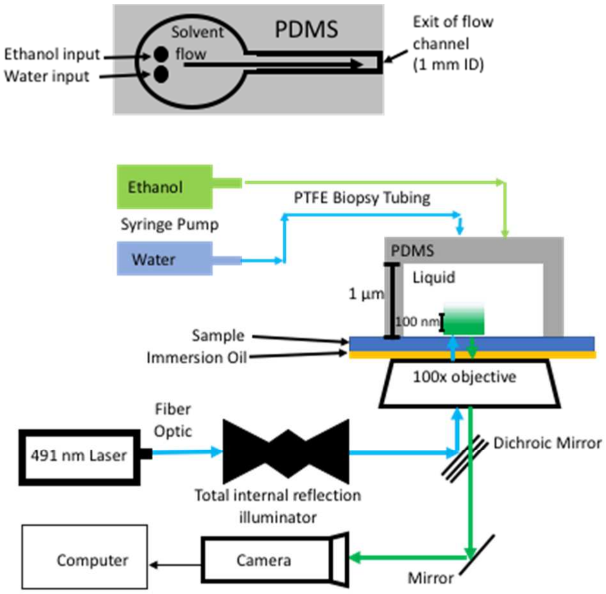


Figure S1. Top: Schematic of flow chamber for TIRF microscope setup. Bottom: Schematic of TIRF microscope setup.

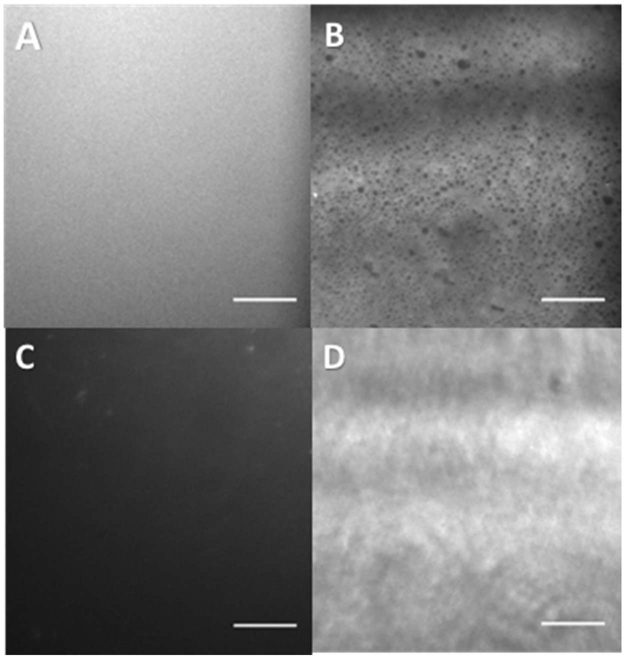


Figure S2. TIRF micrographs of various glass surfaces with AF488 contrast: (A) clean glass; (B) MMA ($\theta \sim 75^o$); (C) SBMA ($\theta \sim 10^o$); (D) MAA ($\theta \sim 35^o$). Nanobubbles are only observed on MMA. All scale bars = 10 µm.

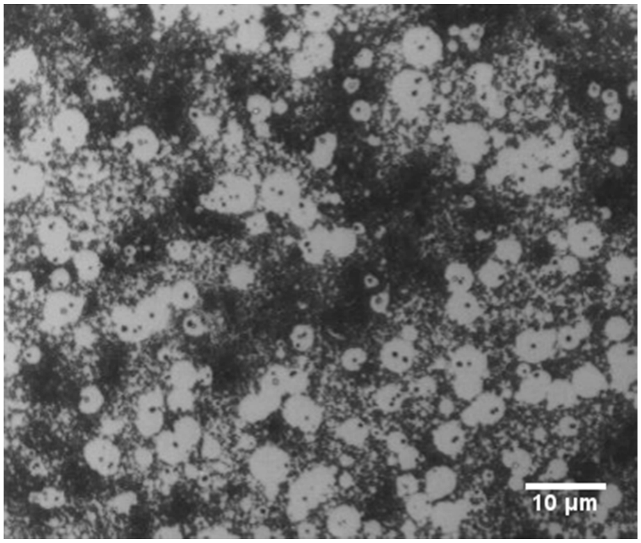


Figure S3. Nanobubbles (small black spots) appear to form on islands of adsorbed Rhodamine 6G (bright circles).

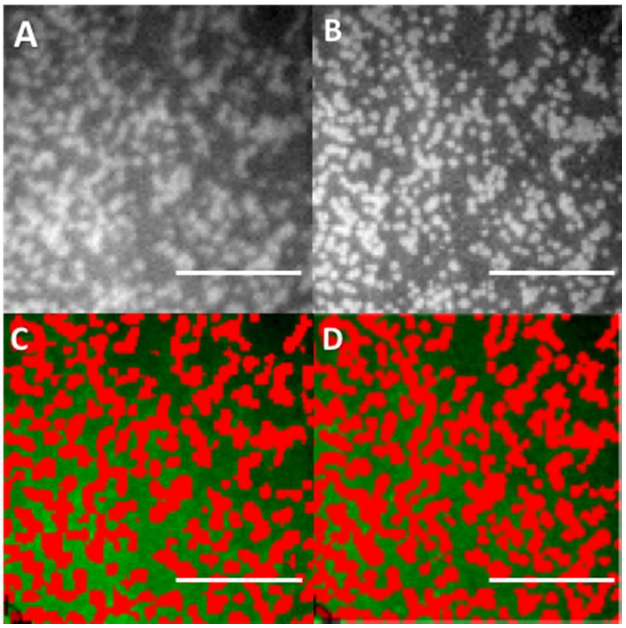


Figure S4. TIRF micrographs of nanobubbles on a methylated glass surface with AF488 contrast showing consistent stability in same locations. Nanobubbles formed on methylated glass (A), removed by ethanol wash, and re-deposited from water (B). (C, D) Images (A) and (B) run through Mathematica processing, where red regions indicate selected nanobubbles. Scale bar = 5 µm for all images.

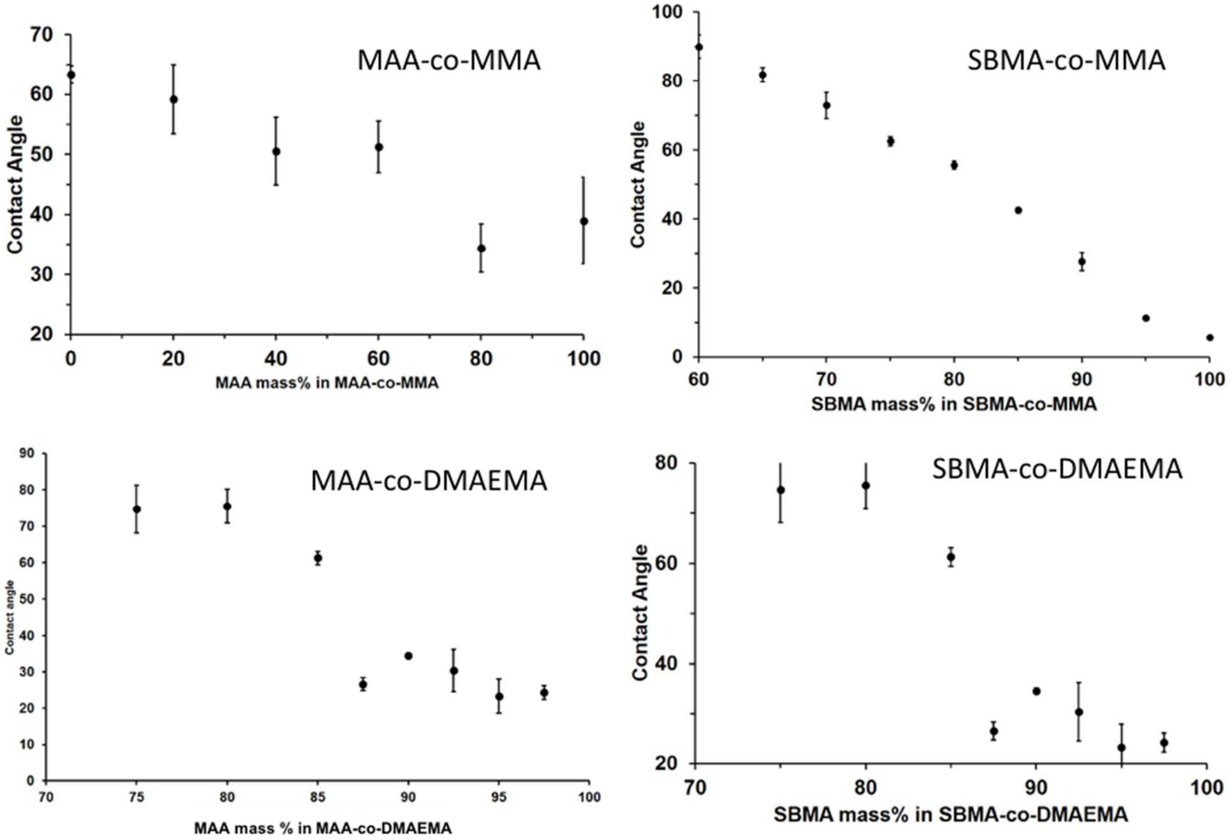


Figure S5. Contact angles vs. copolymer composition for various copolymer mixtures grown on glass coverslips: (A) MAA-co-MMA; (B) SBMA-co-MMA; (C) MAA-co-DMAEMA; (D) SBMA-co-DMAEMA.

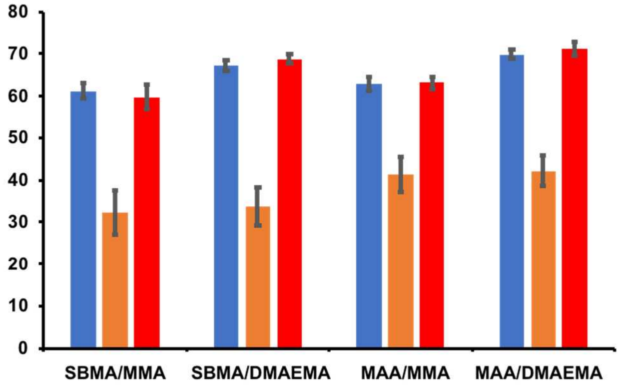


Figure S6. Advancing (left bar), receding (middle bar), and sessile (right bar) contact angle measurements for different surface polymer compositions (all ratios are weight percent): 50:50 SBMA-co-MMA; 50:50 SBMA-co-DMAEMA; 50:50 MAA-co-MMA; and 60:40 MAA-co-DMAEMA. Error bars represent standard deviation from triplicate measurements for advancing and receding, six measurements for static.

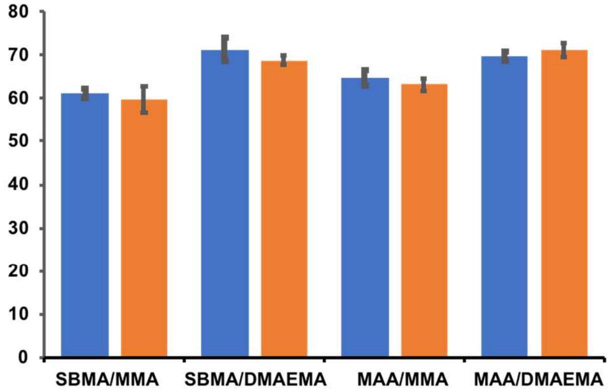


Figure S7. Contact angle measurements for deionized water (left bar) and 5 µM AF488 (right bar) for different surface polymer compositions (all ratios are weight percent): 50:50 SBMA-co-MMA; 50:50 SBMA-co-DMAEMA; 50:50 MAA-co-MMA; and 60:40 MAA-co-DMAEMA. Error bars represent standard deviation from triplicate measurements.

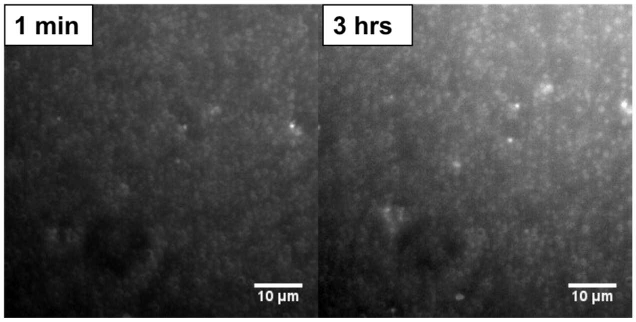


Figure S8. Nanobubbles imaged on a 50:50 mass% MAA-co-MMA surface at 1 min (left) and 3 h (right) after deposition. The laser was turned off between measurements to prevent dye photobleaching. Note the small sample drift to the right.

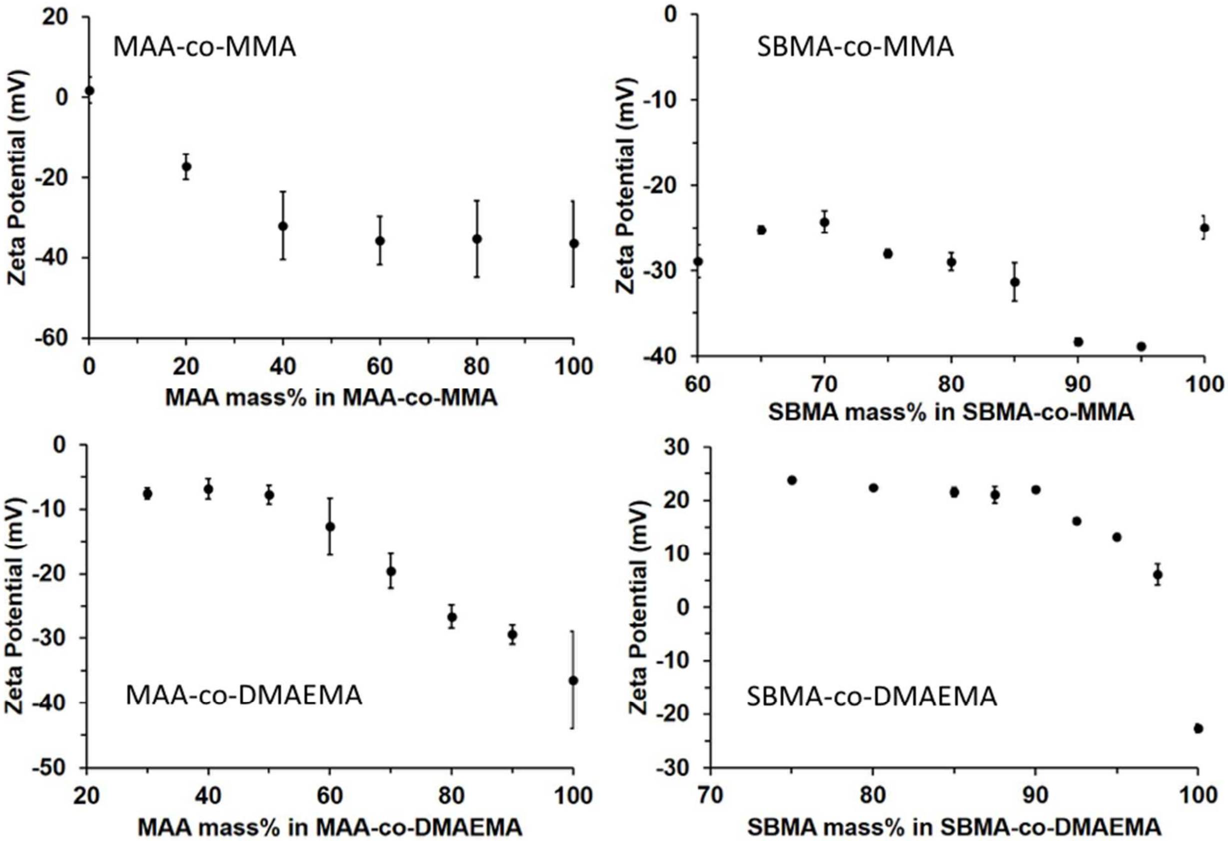
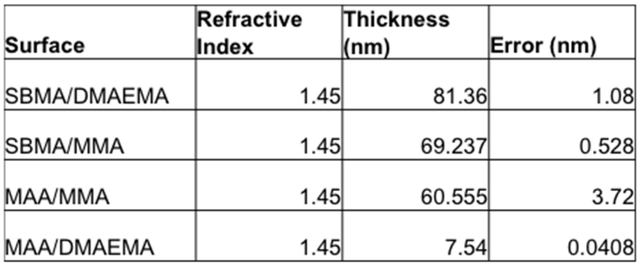


Figure S9. Zeta potential vs. copolymer composition for various copolymer mixtures grown on glass coverslips: (A) MAA-co-MMA; (B) SBMA-co-MMA; (C) MAA-co-DMAEMA; (D) SBMA-co-DMAEMA.



Surface	Refractive Index	Thickness (nm)	Error (nm)
SBMA/DMAEMA	1.45	81.36	1.08
SBMA/MMA	1.45	69.237	0.528
MAA/MMA	1.45	60.555	3.72
MAA/DMAEMA	1.45	7.54	0.0408

Table S1. Ellipsometry measurements of copolymers on silicon surfaces. Each copolymer contains 50:50 (by mass) of indicated monomers.

References.

(1) Faulón Marruecos, D.; Kastantin, M.; Schwartz, D. K.; Kaar, J. L. Dense Poly(Ethylene Glycol) Brushes Reduce Adsorption and Stabilize the Unfolded Conformation of Fibronectin. Biomacromolecules 2016, 17 (3), 1017–1025.

(2) Yang, W.; Chen, S.; Cheng, G.; Vaisocherová, H.; Xue, H.; Li, W.; Zhang, J.; Jiang, S. Film Thickness Dependence of Protein Adsorption from Blood Serum and Plasma onto Poly(Sulfobetaine)-Grafted Surfaces. Langmuir 2008, 24 (17), 9211–9214.

(3) Tugulu, S.; Barbey, R.; Harms, M.; Fricke, M.; Volkmer, D.; Rossi, A.; Klok, H.-A. Synthesis of Poly(Methacrylic Acid) Brushes via Surface-Initiated Atom Transfer Radical Polymerization of Sodium Methacrylate and Their Use as Substrates for the Mineralization of Calcium Carbonate. Macromolecules 2007, 40 (2), 168–177.

(4) Karanam, S.; Goossens; Klumperman, B.; Lemstra. "Controlled" Synthesis and Characterization of High Molecular Weight Methyl Methacrylate/Tert-Butyl Methacrylate Diblock Copolymers via ATRP. Macromolecules 2003, 36 (22), 8304–8311.

(5) Wang, D.; Hu, R.; Mabry, J. N.; Miao, B.; Wu, D. T.; Koynov, K.; Schwartz, D. K. Scaling of Polymer Dynamics at an Oil–Water Interface in Regimes Dominated by Viscous Drag and Desorption-Mediated Flights. J. Am. Chem. Soc. 2015, 137 (38), 12312–12320.