

Supporting Information for:

Stimuli-Induced Star-Globule Shape Transitions of Dually Responsive Binary Heterografted Three-Arm Star Molecular Brushes in Aqueous Solution

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Experimental Section

Materials. CuCl (99%, Sigma-Aldrich) was stirred in glacial acetic acid overnight, collected by vacuum filtration, washed thoroughly with absolute ethanol and diethyl ether, and then dried under high vacuum. 2-(*N,N*-Diethylamino)ethyl methacrylate (DEAEMA, 98.5%, TCI) and 2-(2-ethoxyethoxy)ethyl acrylate (DEGEA, 98%, TCI) were passed through a basic alumina (top)/silica gel (bottom) column to remove the inhibitor prior to polymerization. 2-Bromoisobutyryl bromide (97%, Alfa Aesar), 4-bromobutyryl chloride (95%, Acros), and *N,N,N',N'',N''*-pentamethyldiethylenetriamine (PMDETA, 99%, Acros) were purified by vacuum distillation. 2-Hydroxyethyl methacrylate (HEMA, 97%, Aldrich), chlorotrimethylsilane (TMS-Cl, 98%, Acros), triethylamine (TEA, 99%, Alfa Aesar), 1,1,1-tris(4-hydroxyphenyl)ethane (TP-OH, 99%, Sigma-Aldrich), ethylene carbonate (99%, Acros), tetrabutylammonium fluoride (1 M solution in THF, Acros), potassium fluoride (99%, Fisher), 4,4'-dinonyl-2,2'-dipyridyl (97%, Sigma-Aldrich), sodium azide (99%, Acros), *N,N*-dimethylformamide (Spectranalyzed, Fisher), and 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 97%, Sigma-Aldrich) were used as received. 2-(Trimethylsilyloxy)ethyl methacrylate (HEMA-TMS) was synthesized by the reaction of 2-

hydroxyethyl methacrylate with chlorotrimethylsilane, and the molecular structure was confirmed by ^1H NMR spectroscopy analysis. Propargyl 2-bromoisobutyrate (PBiB) was synthesized according to a procedure described in a previous publication.¹ All other chemicals were purchased from either Sigma-Aldrich or Fisher and used as received.

General Characterization. ^1H and ^{13}C NMR spectra were recorded on either a Varian Mercury 300 NMR, a Varian VNMRs 500 NMR, or a Varian VNMRs 600 spectrometer, using the residual solvent proton signal as the internal standard. Variable temperature ^1H NMR spectroscopy was employed to study the responsive properties of the binary heterografted 3-arm star brushes, SBB, in aqueous solution using a Varian VNMRs 600 MHz NMR spectrometer equipped with a temperature controller (FTS Systems, Inc.). Aqueous solutions of SBB with a concentration of 4.0 mg/g were prepared using a 10 mM phosphate buffer made with D_2O . The pH was measured with a pH meter (AB-15, calibrated using pH = 4.01, 7.00, and 10.01 standard buffer solutions at 0 °C) and adjusted to the desired value using 0.1 M HCl and 0.1 M NaOH solutions prepared in D_2O . For variable temperature experiments, the samples were equilibrated for 5 min at each selected temperature in the spectrometer before spectrum acquisition was started. Each spectrum reported in this work was the result of 64 scans. The high resolution mass spectrometry (HRMS) experiment was performed using a JEOL Model JMS-T100LC (AccuTOF) orthogonal time-of-flight (TOF) mass spectrometer (Peabody, MA) with an IonSense (Danvers, MA) DART source. The HRMS sample of the trifunctional ATRP initiator, TPEG-Br, was prepared in ethyl acetate with a concentration of 1.0 mg/g.

Size exclusion chromatography (SEC) of alkyne end-functionalized side chain polymers, PDEGEA and PDEAEMA, was performed at ambient temperature using a PL-GPC 20 (an integrated GPC/SEC system from Polymer Laboratories, Inc.) with a differential refractive index

(RI) detector, one PLgel 5 μm guard column (50×7.5 mm, Agilent Technologies), and two PLgel 5 μm mixed-C columns (each 300×7.5 mm, linear range of molecular weights from 200 to 2 000 000 Da, Agilent Technologies). The mobile phase was tetrahydrofuran (THF) set at a flow rate of 1.0 mL/min, and the system was calibrated with a set of narrow disperse linear polystyrene standards (Scientific Polymer Products, Inc.). SEC of the backbone precursors, PHEMA-TMS and PHEMA-Br, the azide-functionalized backbone polymer, PHEMA-N₃, as well as the 3-arm star molecular brushes was performed at 50 °C using a PL-GPC 50 Plus system (an integrated GPC/SEC system from Polymer Laboratories, Inc.) with a differential refractive index detector, one PSS GRAL 10 μm guard column (50×8 mm, Polymer Standards Service-USA, Inc.) and two PSS GRAL 10 μm linear columns (each 300×8 mm, linear range of molecular weight from 500 to 1 000 000 Da, Polymer Standards Service-USA, Inc.). *N,N*-Dimethylformamide (DMF), containing 50 mM LiBr, was used as the eluent at a flow rate of 1.0 mL/min. The absolute molecular weight of the purified star brushes, SBB, was determined by a GPC-MALS system comprising one PSS GRAM 10 μm guard column (8×50 mm, Polymer Standards Service-USA, Inc.), three PSS GRAM 10 μm linear columns (8×300 mm; 100, 1000, and 3000 Å, Polymer Standards Service-USA, Inc.), an Agilent model 1260 Infinity pump, a Rheodyne model 7725 manual injector with a 200 μL loop, and a Varian 390 LC detector system consisting of an RI detector and a two angle light scattering detector (15° and 90°). The mobile phase was DMF with 50 mM LiBr, and the analysis was carried out at 50 °C.

Dynamic Light Scattering (DLS) Study and Visual Inspection of 3-Arm Star Brushes in Aqueous Solution. DLS of SBB was performed using a Malvern Zetasizer Nano ZS instrument equipped with a He-Ne 633 nm laser and a temperature controller at a scattering angle of 173°. Each DLS sample was prepared by a wet-transfer method from a stock solution of SBB in THF.

The stock solution of the star brushes in THF with a known concentration was weighed into a flask and diluted with a calculated amount of a 10 mM phosphate buffer. The solution was concentrated under high vacuum to remove THF, which was confirmed by ^1H NMR spectroscopy analysis. Finally, the solution was reconstituted to the desired concentration with Milli-Q water. To study the pH-responsive property of SBB, the pH of the solution was gradually adjusted using 0.1 M NaOH and 0.1 M HCl in an ice/water bath, monitored by an Accumet AB-15 pH meter (calibrated at 0 °C with pH = 4.01, 7.00, and 10.01 standard buffer solutions) and allowed to equilibrate for 15 min. A portion of the solution was transferred into a DLS tube without filtering and loaded into the DLS instrument with a pre-set temperature of 1 °C. The DLS sample was equilibrated for an additional 5 min before measurements were taken. Temperature ramps were performed at various pH values, with equilibration for 5 min at each selected temperature. Each reported DLS value was an average of 3 measurements, each of which was comprised of 10 runs; z-average hydrodynamic diameters were used. For visual inspection, the 1.0 mg/g aqueous solutions of SBB in a 10 mM phosphate buffer with various pH values were prepared similarly in an ice/water bath, and the photos were taken at the selected temperatures after equilibration for 10 min using a Nikon Coolpix B500 camera.

Atomic Force Microscopy (AFM) Study of SBB. The star brushes were studied by AFM using a Digital Instruments Multimode IIIa Scanning Probe Microscope in tapping mode under ambient conditions. Reflective Al-coated Si probes (Budget Sensors) with a resonant frequency of 300 kHz and a force constant of 40 Nm^{-1} were used. PMMA-coated mica substrates were prepared by cleaving a fresh layer of mica disk (Ted Pella, Inc.) with Scotch tape, placing three drops of a 2.5 wt% solution of PMMA ($M_n = 54.9\text{ kDa}$) in CHCl_3 onto the disk, and immediately spinning the solution off at 3000 rpm (P-6000 Spin Coater, Specialty Coating Systems, Inc.) to create a thin

PMMA film. Aqueous solutions of SBB with a concentration of 0.05 mg/g were prepared by diluting a brush solution with a concentration of 0.20 mg/g using a 5 mM phosphate buffer and were spin cast onto the PMMA-coated mica substrates (3000 rpm). The pH values of the aqueous solutions of SBB used for preparing AFM samples were measured with a pH meter (Accument AB-15) in an ice/water bath and adjusted to the desired values using 0.1 M HCl and 0.1 M NaOH solutions. For the AFM samples prepared at 0 °C, the spin casting stage and the PMMA-coated mica disk were cooled in the refrigerator (4 °C), taken out and used immediately.

Synthesis of Trifunctional Initiator ((Ethane-1,1,1-triyltris(benzene-4,1-diyl))tris(oxy))tris(ethane-2,1-diyl)tris(2-bromo-2-methylpropanoate) (TPEG-Br). The trifunctional initiator, ((ethane-1,1,1-triyltris(benzene-4,1-diyl))tris(oxy))tris(ethane-2,1-diyl)tris(2-bromo-2-methylpropanoate) (TPEG-Br), was prepared according to Scheme 2. TPEG-OH was synthesized from 1,1,1-tris(4-hydroxyphenyl)ethane (TP-OH) and ethylene carbonate according to a procedure modified from the literature for a similar reaction.² 1,1,1-Tris(4-hydroxyphenyl)ethane (2.005 g, 6.54 mmol), ethylene carbonate (2.114 g, 24.0 mmol), KF (17.0 mg, 0.29 mmol), and DMF (5 mL) were added to a 50 mL two-necked flask equipped with a reflux condenser. After the reaction mixture was refluxed and stirred in a 160 °C oil bath for 20 h, DMF was removed under high vacuum, and the crude product was purified by column chromatography using CH₂Cl₂/MeOH (10/1, v/v) as eluent. After being dried under high vacuum, the product was obtained as a white solid. ¹H NMR spectroscopy analysis showed four extra sets of small peaks in the range of 3.6 – 4.2 ppm (Figure S1), likely due to the fluoride-involved side reaction leading to the formation of -OCH₂CH₂F; washing with saturate NaHCO₃ and a second round of column chromatography did not remove the extra peaks.

To remove the impurity, the crude product was reacted with trimethylchlorosilane and purified by column chromatography; subsequent cleavage of the silyl ether protective groups yielded the desired pure product, TPEG-OH. The detailed procedures are presented below. The crude product from the reaction described in the preceding paragraph (2.105 g, 4.800 mmol), triethylamine (2.676 g, 26.45 mmol), and dichloromethane (25 mL) were added to a 100 mL three-necked flask equipped with a pressure-equalizing addition funnel and a magnetic stir bar. The mixture was cooled and stirred in an ice/water bath for 15 min before chlorotrimethylsilane (2.156 g, 19.85 mmol) in dichloromethane (10 mL) was added dropwise. The reaction was allowed to proceed under nitrogen at ambient temperature for 16 h. The precipitate was removed by vacuum filtration, and the filtrate was diluted with dichloromethane (100 mL) and washed three times with DI water (25 mL). The pure product, (((((ethane-1,1,1-triyltris(benzene-4,1-diyl))tris(oxy))tris(ethane-2,1-diyl))tris(oxy))tris(trimethylsilane) (TPEG-TMS), was isolated by column chromatography using a mixture of ethyl acetate and hexanes (1:5, v:v) as the eluent, yielding a colorless viscous oil after drying under high vacuum (1.215 g, 28.0 % with respect to 1,1,1-tris(4-hydroxyphenyl)ethane)). ¹H NMR (CDCl₃, 500 MHz), δ (ppm) = 6.97 (d, aromatic -OC(CH)₂(CH)₂C-, 6H), 6.79 (d, aromatic, -OC(CH)₂(CH)₂C-, 6H), 4.01 (t, -OCH₂CH₂OSi-, 6H), 3.93 (t, -OCH₂CH₂OSi, 6H), 2.10 (s, -C(CH₃), 3H), 0.15 (s, -Si(CH₃)₃, 27H). ¹³C NMR (CDCl₃, 126 MHz), δ (ppm) = 156.80 (-OC(CH)₂(CH)₂C-), 141.88 (-OC(CH)₂(CH)₂C-), 129.57 (-OC(CH)₂(CH)₂C-), 113.67 (-OC(CH)₂(CH)₂C-), 69.02 (-OCH₂CH₂O-), 61.41 (-OCH₂CH₂O-), 50.58 (-C(CH₃)), 30.75 (-C(CH₃)), 0.36 (-Si(CH₃)₃). The ¹H and ¹³C NMR spectra of TPEG-TMS are shown in Figure S2.

TPEG-TMS (0.629 g, 0.960 mmol) was dissolved in ethanol (10 mL) in a 50 mL two-necked flask, and 1 M aqueous HCl solution (2.98 mL, 2.98 mmol) was added via a syringe. After the reaction mixture was stirred under nitrogen at room temperature overnight, the volatile

components were removed by rotary evaporation and subsequently under high vacuum. The product, 2,2',2''-((ethane-1,1,1-triyltris(benzene-4,1-diyl))tris(oxy))tris(ethan-1-ol) (TPEG-OH), was obtained as an off-white solid (0.405 g, 96.2 %). ^1H NMR (CDCl_3 , 500 MHz), δ (ppm) = 6.99 (d, aromatic, $-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}-$, 6H), 6.81 (d, aromatic, $-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}-$, 6H), 4.07 (t, $-\text{OCH}_2\text{CH}_2\text{OH}$, 6H), 3.95 (t, $-\text{OCH}_2\text{CH}_2\text{OH}$, 6H), 2.11 (s, $-\text{C}(\text{CH}_3)$, 3H), 2.05-1.70 (br s, $-\text{CH}_2\text{OH}$). ^{13}C NMR (CDCl_3 , 126 MHz), δ (ppm) = 156.52 (aromatic, $-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}-$), 142.01 (aromatic, $-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}-$), 129.54 (aromatic, $-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}-$), 113.66 (aromatic, $-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}-$), 69.09 ($-\text{OCH}_2\text{CH}_2\text{OH}$), 61.27 ($-\text{OCH}_2\text{CH}_2\text{O}-$), 50.52 ($-\text{C}(\text{CH}_3)$), 30.67 ($-\text{C}(\text{CH}_3)$). The ^1H and ^{13}C NMR spectra of TPEG-OH are shown in Figure S3.

TPEG-OH (0.235 g, 0.536 mmol, 1.61 mmol -OH), TEA (0.200 g, 1.97 mmol), and dry THF (20 mL) were added to a 50 mL two-necked flask and stirred under nitrogen in an ice/water bath, followed by dropwise addition of α -bromoisobutyryl bromide (0.410 g, 1.78 mmol). After 16 h, the precipitate was filtered off, and the filtrate was concentrated by rotary evaporation to remove the volatiles. The crude product was dissolved in CH_2Cl_2 (100 mL) and washed two times with saturated sodium bicarbonate aqueous solution (25 mL) and once with 50% saturated aqueous NaCl solution (25 mL). The organic phase was dried over anhydrous sodium sulfate; the solid was filtered off and the solvents were removed by rotary evaporation. The crude product was purified by column chromatography using ethyl acetate/hexanes (1 : 2, v : v) as the eluent. The isolated product was a white, crystalline solid upon drying by rotary evaporation. This white solid was further purified by recrystallization using a mixture of ethyl acetate and hexanes, yielding the pure product, TPEG-Br, as a white, needle-like crystal (0.297 g, 62.5 %). ^1H NMR (CDCl_3 , 500 MHz), δ (ppm) = 6.99 (d, aromatic, $-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}-$, 6H), 6.81 (d, aromatic, $-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}-$, 6H), 4.50 (t, $-\text{OCH}_2\text{CH}_2\text{OOC}-$, 6H), 4.20 (t, $-\text{OCH}_2\text{CH}_2\text{OOC}-$, 6H), 2.10 (s, $-\text{C}(\text{CH}_3)$, 3H), 1.93 (s, -

C(CH₃)₂Br), 18H). ¹³C NMR (CDCl₃, 126 MHz), δ (ppm) = 171.66 (-OOC(CH₃)₂Br), 156.48 (-OC(CH₂)₂(CH₂)₂C-), 142.23 (-OC(CH₂)₂(CH₂)₂C-), 129.64 (-OC(CH₂)₂(CH₂)₂C-), 113.95 (-OC(CH₂)₂(CH₂)₂C-), 65.55 (-COOCH₂CH₂O-), 64.29 (-COOCH₂CH₂O-), 55.50 (-C(CH₃)₂Br), 50.66 (-C(CH₃)), 30.77 (-C(CH₃)) and 30.72 (-OOC(CH₃)₂Br). The ¹H and ¹³C NMR spectra of TPEG-Br are shown in Figure S4. MS: m/z calc C₃₈H₄₅O₉Br₃ [M+H]⁺: 885.06660; found: 885.06796; mass error: 1.54 ppm.

Synthesis of the Azide-Bearing 3-Arm Star Backbone Polymer (PHEMA-N₃).

Trifunctional initiator TPEG-Br (5.12 mg, 5.78×10^{-3} mmol, delivered from 0.324 g of a 15.8 mg/g stock solution in anisole), HEMA-TMS (3.042 g, 15.03 mmol), CuCl (2.5 mg, 2.5×10^{-2} mmol), 4,4'-dinonyl-2,2'-dipyridyl (15.0 mg, 3.67×10^{-2} mmol), and anisole (0.833 g) were added to a 25 mL two-necked flask and degassed by three freeze-pump-thaw cycles. The flask was then placed into an oil bath with a pre-set temperature of 75 °C, and the reaction mixture was stirred with a magnetic stir bar. After 26 h, the flask was taken out from the oil bath and opened to air. An aliquot was immediately withdrawn for ¹H NMR spectroscopy analysis, and the polymerization mixture was quickly diluted with chloroform. The copper catalyst was removed by passing through a column of neutral alumina (top)/silica gel (bottom) with chloroform as the eluent. The solution was concentrated by rotary evaporation and then dried under high vacuum for 6 h at 50 °C to remove the unreacted monomer, yielding the 3-arm star polymer of HEMA-TMS (PHEMA-TMS) as a highly viscous polymer (1.484 g). The monomer conversion was 50.1%, calculated from the ¹H NMR spectrum of the final reaction mixture by using the integrals of the peaks at 4.26 – 4.18 ppm (-COOCH₂- of the monomer) and 4.12 – 3.89 ppm (-COOCH₂- of the polymer). Thus, the average degree of polymerization (DP) per arm was 434. SEC analysis results (PSS GRAL

columns with DMF containing 50 mM LiBr as the eluent) relative to polystyrene standards: $M_{n,SEC}$ = 179.8 kDa, dispersity (\mathcal{D}) = 1.13 (Figure S5).

PHEMA-TMS (1.039 g, 5.14 mmol of -OTMS groups), KF (0.386 g, 6.65 mmol), and THF (25 mL) were added to a 50 mL two-necked flask and stirred under nitrogen for 15 min in an ice/water bath. TBAF (105 μ L of a 1 M solution in THF, 0.105 mmol) was added using a microsyringe, followed immediately by the addition of 4-bromobutyryl chloride (2.207 g, 11.90 mmol). The mixture was removed from the ice/water bath and stirred under nitrogen at ambient conditions for 3.5 h. The polymer solution was concentrated to about 10 mL and precipitated in methanol (100 mL) three times from THF (10 mL). The purified polymer was dried under high vacuum to yield an off-white rubbery polymer (PHEMA-Br, 1.023 g, 71.4%). SEC analysis results (PSS GRAL columns with DMF containing 50 mM LiBr as the eluent) relative to polystyrene standards: $M_{n,SEC}$ = 211.4 kDa, dispersity (\mathcal{D}) = 1.17 (Figure S5).

PHEMA-Br (0.922 g, 3.30 mmol of -CH₂Br assuming a 100% conversion), NaN₃ (1.113 g, 17.12 mmol), and DMF (20 mL) were added to a 50 mL two-necked flask and stirred at room temperature for 20 h. DMF was removed under high vacuum and the crude mixture was precipitated twice in MeOH/H₂O (80/20, v/v), once in methanol, and once in hexanes using THF as the good solvent. The purified polymer was dried under high vacuum for 3 h, yielding PHEMA-N₃ as a slightly yellow, rubbery polymer (0.605 g, 80.8%) and stored as a stock solution in THF in the freezer. SEC analysis results relative to polystyrene standards (PSS GRAL columns with DMF containing 50mM LiBr as the eluent): $M_{n,SEC}$ = 224.5 kDa, dispersity (\mathcal{D}) = 1.16 (Figure S5).

The degree of azide functionalization of the 3-arm star backbone polymer was 88.3%, determined by ¹H NMR spectroscopy analysis of PHEMA-N₃ with PHEMA-TMS and PHEMA-Br as references (Figure S6). The deprotection of TMS groups using KF/TBAF as the catalyst was

highly efficient, as can be seen from the nearly complete disappearance of the silyl ether peak at 0.13 ppm ($-\text{OSi}(\text{CH}_3)_3$) with only a tiny amount of protective TMS groups remaining. After the subsequent esterification of the pendant $-\text{OH}$ with 4-bromobutyryl chloride, three new peaks appeared at 3.59 – 3.45 ppm ($-\text{OOCCH}_2\text{CH}_2\text{CH}_2\text{Br}$), 2.68 – 2.48 ppm ($-\text{OOCCH}_2\text{CH}_2\text{CH}_2\text{Br}$), and 2.32 – 2.11 ppm ($-\text{OC}(\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{Br}$), and the peaks at 4.14 – 3.86 ppm ($-\text{COOCH}_2\text{CH}_2\text{O}-$) and 3.85 – 3.66 ppm ($-\text{COOCH}_2\text{CH}_2\text{O}-$) shifted to 4.46 – 4.25 ppm and 4.25 – 4.02 ppm, respectively. Further modification to the azide groups resulted in shifting of the three new peaks to 3.45 – 3.34 ppm ($-\text{OOCCH}_2\text{CH}_2\text{CH}_2\text{N}_3$), 2.52 – 2.42 ppm ($-\text{OOCCH}_2\text{CH}_2\text{CH}_2\text{N}_3$), and 1.99 – 1.88 ppm ($-\text{OOCCH}_2\text{CH}_2\text{CH}_2\text{N}_3$). A set of three peaks next to each of the new peaks, located at 3.65, 2.57, and 2.12 ppm, was believed to be from $-\text{OOCCH}_2\text{CH}_2\text{CH}_2\text{Cl}$, which was formed by the reaction of deprotected PHEMA with the impurity of $\text{ClCH}_2\text{CH}_2\text{CH}_2\text{COCl}$ in the reactant $\text{BrCH}_2\text{CH}_2\text{CH}_2\text{COCl}$ and did not participate in the azide substitution reaction. This accounted for 5.8% of repeat units from the ^1H NMR analysis. The peak at 3.52 ppm was believed to be from the unreacted bromide moieties, which accounted for 4.0 % of repeat units. Lastly, the peak at 3.82 was believed to be from the unreacted PHEMA units (1.5 %). The small amount of the remaining TMS groups at 0.06 ppm corresponded to 0.4 % of monomer units. Taking into consideration the repeat units that do not bear an azide functionality, the degree of azide functionalization of the backbone polymer, PHEMA- N_3 , was calculated to be 88.3%.

Synthesis of Alkyne End-Functionalized Side Chain Polymer PDEGEA. PBiB (54.8 mg, 0.267 mmol, delivered via 0.454 g of a 120.8 mg/g stock solution in anisole), DEGEA (5.028 g, 26.71 mmol), PMDETA (47.8 mg, 0.276 mmol), CuCl (26.4 mg, 0.267 mmol), and anisole (5.202 g) were added to a 50 mL two-necked flask equipped with a magnetic stir bar, a rubber septum, and a gas flow control adapter. The mixture was degassed by three freeze-pump-thaw cycles and

immersed in an 80 °C oil bath. The polymerization was stopped after 17 h by opening the flask to air. After a sample was taken for ^1H NMR spectroscopy analysis, the reaction mixture was diluted immediately with dichloromethane. The copper catalyst was removed by passing the mixture through a column of basic alumina (top)/silica gel (bottom). The polymer solution was concentrated to about 10 mL by rotary evaporation and precipitated into hexanes (100 mL) three times. The purified polymer was dried under high vacuum for 2 h, yielding a slightly yellow, viscous liquid (2.685 g). The monomer conversion was 57.4%, calculated from the ^1H NMR spectrum of the final reaction mixture by comparing the monomer ester peak at 4.36-4.31 ppm (-COOCH₂-) to the polymer ester peak at 4.28-4.16 ppm (-COOCH₂-). From the monomer conversion and the monomer-to-initiator molar ratio, the DP of the polymer was determined to be 57. SEC analysis results relative to polystyrene standards using mixed-C columns and THF as eluent: $M_{n,SEC} = 10.3$ kDa and $\bar{D} = 1.17$ (Figure S7A).

Synthesis of Alkyne End-Functionalized Side Chain Polymer PDEAEMA. PBiB (55.7 mg, 0.272 mmol, delivered via 0.255 g of 218.4 mg/g stock solution in anisole), DEAEMA (6.007 g, 32.42 mmol), 1,1,4,7,10,10-hexamethyltriethylenetetramine (72.8 mg, 0.316 mmol), CuCl (27.0 mg, 0.273 mmol) and anisole (9.006 g) were added to a 50 mL two-necked round bottom flask and degassed by three freeze-pump-thaw cycles. The polymerization was started by immersing the flask in an oil bath at 50 °C. After 5.5 h, the polymerization was stopped by removing the flask from the oil bath and opening it to air, and an aliquot was withdrawn for ^1H NMR spectroscopy analysis. The reaction mixture was diluted with THF and passed through a column of basic alumina (top)/silica gel (bottom) to remove the copper catalyst. The polymer was purified by precipitation of its THF solution in hexanes three times in a dry ice/acetone bath (-78 °C) and then dried under high vacuum. A white, glassy polymer was obtained (2.850 g, 47.4%). The monomer conversion

was 50.3%, determined by ^1H NMR analysis of the final polymerization mixture by comparing the integrals of the peaks at 4.32-4.17 ppm ($-\text{COOCH}_2-$ of monomer) and at 4.17-3.98 ppm ($-\text{COOCH}_2-$ of the polymer). The degree of polymerization of PDEAEMA was calculated to be 60. SEC analysis relative to polystyrene standards using mixed-C columns and THF as eluent: $M_{n,\text{SEC}} = 12.4$ kDa and $\text{Đ} = 1.14$ (Figure S7B).

Synthesis of 3-Arm Star Binary Heterografted PDEGEA/PDEAEMA Molecular Brushes and Calculation of Overall Grafting Density of SBB. PHEMA- N_3 (10.54 mg, 4.37×10^{-2} mmol $-\text{N}_3$ assuming 100% functionalization, delivered via 0.2850 g of a 36.98 mg/g stock solution in THF), alkyne end-functionalized PDEGEA (286.8 mg, 2.63×10^{-2} mmol, delivered via 1.986 g of a 144.4 mg/g stock solution in THF), alkyne end-functionalized PDEAEMA (295.0 mg, 2.62×10^{-2} mmol, delivered via 3.105 g of a 95.0 mg/g stock solution in THF), CuCl (4.3 mg, 4.3×10^{-2} mmol), and THF (5 mL) were added to a vial equipped with a magnetic stir bar. The vial was sealed with a rubber septum and purged with nitrogen via needles for 15 min. PMDETA (10 μL , 4.83×10^{-2} mmol) was then injected. Purging of the vial with nitrogen continued for an additional 15 min, and the reaction was conducted under a slightly positive pressure of nitrogen. After 21 h, propargyl benzyl ether (25 μL) was injected to attempt to cap possible unreacted azide groups. After an additional 2 h, the vial was opened to the air, and a final sample was taken for SEC analysis to determine the grafting density. The reaction mixture was diluted with THF and then passed through a basic alumina (top)/silica gel (bottom) column to remove the copper catalyst. The solution was concentrated to ~ 3 mL via rotary evaporation and then fractionated at -12°C (in a freezer) with 10 mL of hexanes. The fractionation ceased removing excess side chain polymers after three attempts. The remaining excess side chain polymers were removed by 4 rounds of centrifugal filtration using 50 kDa MWCO filters and a mixture of ethanol and water (50/50, v/v)

at 5000 rpm (Eppendorf 5804 Centrifuge). The complete removal of excess side chain polymers was confirmed by SEC analysis using PSS GRAL columns with DMF (containing 50 mM LiBr) as the mobile phase (Figure S8). The absolute weight-average molecular weight (M_w) was determined by a GPC-MALS system equipped with one PSS GRAM 10 μ m guard column, three PSS GRAM 10 μ m linear columns, and a Varian 390 LC-detector system consisting of an RI detector and a multi-angle light scattering detector (15° and 90°), and it was 1.49×10^7 Da.

The overall grafting density of the 3-arm star brushes was 84.7 %, calculated from the peak areas of the brushes and the side chains along with the feed ratio of the backbone azide groups and the alkyne end-functionalized side chain polymers as well as the consideration of the molar ratio of two side chain polymers in the purified star brushes. The details are presented in the following. ^1H NMR spectroscopy analysis showed that the brushes were composed of 48.9 mol % PDEGEA monomer units and 51.1 mol % PDEAEMA monomer units using the integrals of the peaks at 4.33-4.11 ppm ($-\text{COOCH}_2-$ of PDEGEA) and 4.11-3.91 ppm ($-\text{COOCH}_2-$ of PDEAEMA). From the DP of each side chain polymer ($\text{DP}_{\text{PDEGEA}} = 57$, $\text{DP}_{\text{PDEAEMA}} = 60$), the relative brush composition is calculated to be 50.2 mol % PDEGEA and 49.8 mol % PDEAEMA side chains. Using the molar mass of PDEGEA (10889 g/mol) and PDEAEMA (11277 g/mol), the relative mass percent of each side chain was found to be 49.3 % PDEGEA and 50.7 % PDEAEMA. The peak areas for the star brushes and the side chain polymers by SEC analysis were 71.0 % and 29.0 %, respectively (Figure S8). Multiplying the area percentage for the brushes by the total feed mass of the backbone polymer PHEMA- N_3 and two side chain polymers yielded 420.32 mg of the brushes. By subtracting the amount of backbone polymer, we found that 409.78 mg of side chain polymers had reacted. Using the mass percentages of two side chain polymers calculated earlier, we found the mass of the reacted PDEGEA (202.0 mg) and PDEAEMA (207.8 mg). Using the

molar mass of each side chain polymer, the number of moles of the reacted side chain polymers was 3.70×10^{-5} mol. Therefore, the overall grafting density was calculated to be 84.7 % by dividing the total moles of side chains that reacted by the moles of repeat units in the backbone polymer (assuming 100% azide functionalization).

Cloud Points of Alkyne End-Functionalized PDEGEA and PDEAEMA in H₂O and D₂O at a Concentration of 0.5 wt%. The cloud points of alkyne end-functionalized PDEGEA and PDEAEMA in both H₂O and D₂O solutions were determined by visual examination. For PDEGEA, a calculated amount of a stock solution of the polymer in THF with a known concentration was weighed into each of two flasks and dried under high vacuum to remove THF. Milli-Q water or D₂O (Cambridge Isotope Laboratories, Inc.) was added to dissolve the polymer in an ice/water bath to give a concentration of 0.5 wt%. For PDEAEMA, similarly, a calculated amount of the polymer was transferred into each of two flasks from a THF stock solution; the aqueous solutions were prepared at a concentration of 0.5 wt% using a 10 mM phosphate buffer in either Milli-Q water or D₂O. The pH values of the PDEAEMA samples were adjusted in an ice/water bath using 0.1 M NaOH and 0.1 M HCl (prepared in Milli-Q water or D₂O for the respective samples) and monitored with an AB-15 pH meter calibrated at 0 °C using three standard buffer solutions with pH values of 4.01, 7.00, and 10.01. Cloud points for all solutions were determined by visual inspection upon gradually heating the samples from 4 °C in an Isotemp water bath (Fisher Scientific). The cloud points of PDEGEA in H₂O and D₂O at a concentration of 0.5 wt% were 12.0 and 11.5 °C, respectively. The cloud points for PDEAEMA in H₂O and D₂O buffers are summarized in Figure S14. Interestingly, the cloud point of PDEAEMA in the D₂O buffer (red solid circle) was slightly higher than that in H₂O (black squares).

References:

1. Henn, D. M.; Fu, W. X.; Mei, S.; Li, C. Y.; Zhao, B. Temperature-Induced Shape Changing of Thermosensitive Binary Heterografted Linear Molecular Brushes between Extended Worm-Like and Stable Globular Conformations. *Macromolecules* **2017**, *50*, 1645-1656.
2. Stregé, P.E. Inorganic Halide Salt Catalysts for Hydroxyalkylation of Phenols or Thiophenols. U.S. Patent 4,341,905, 1982.

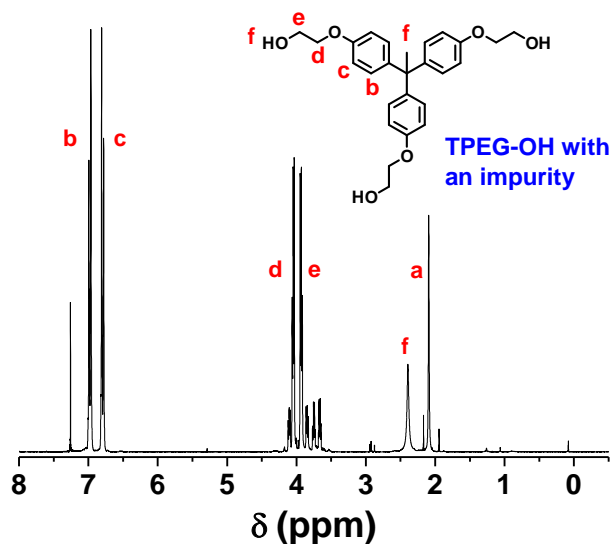


Figure S1. ^1H NMR spectrum of impure TPEG-OH in CDCl_3 .

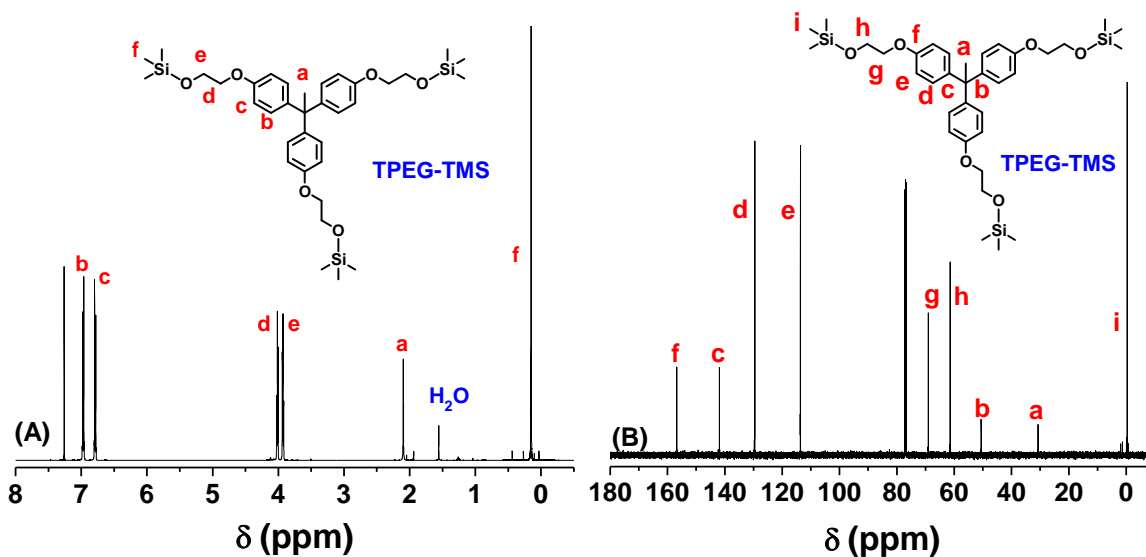


Figure S2. (A) ^1H and (B) ^{13}C NMR spectra of the TMS-protected TPEG-OH, TPEG-TMS, in CDCl_3 .

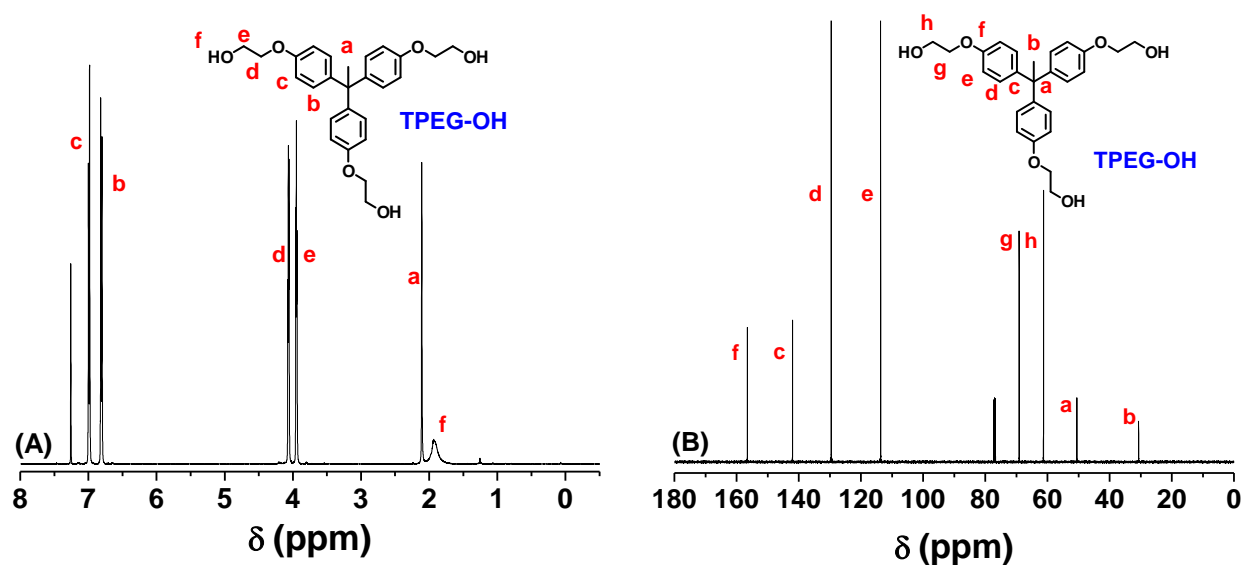


Figure S3. (A) ^1H and (B) ^{13}C NMR spectra of TPEG-OH in CDCl_3 .

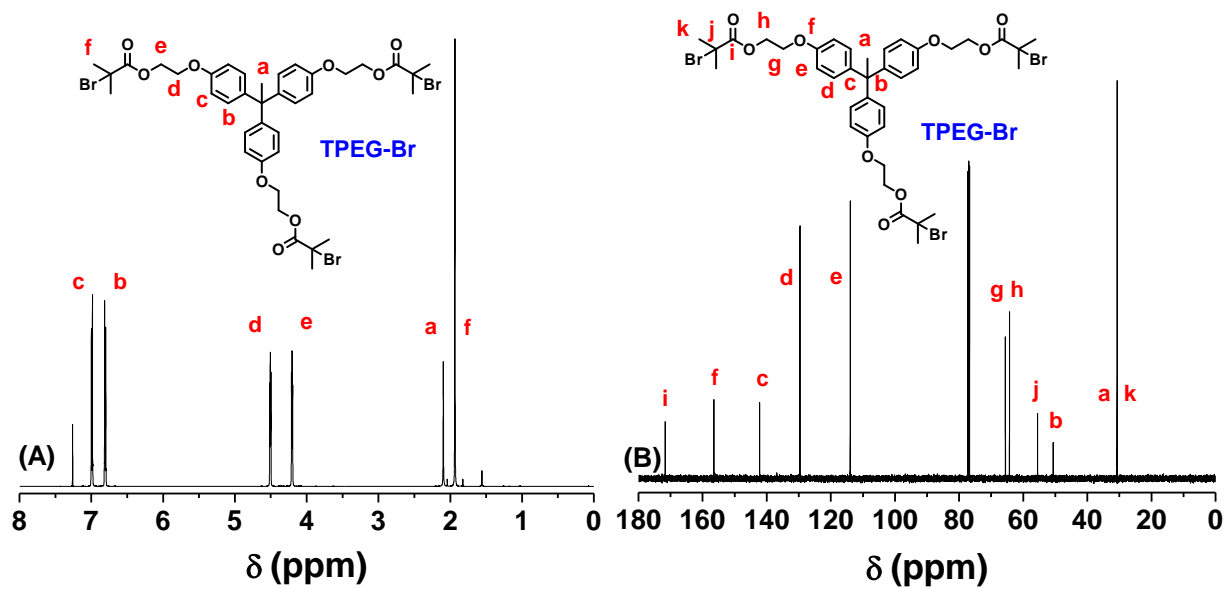


Figure S4. (A) ^1H and (B) ^{13}C NMR spectrum of trifunctional initiator (TPEG-Br) in CDCl_3 .

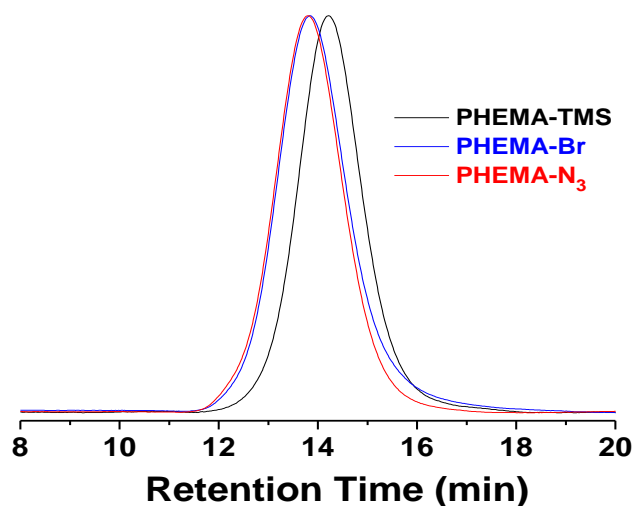


Figure S5. SEC traces of star polymer PHEMA-TMS ($M_{n,SEC} = 179.8$ kDa, $D = 1.13$), PHEMA-Br ($M_n = 211.4$ kDa, $D = 1.17$), and PHEMA-N₃ ($M_n = 224.5$ kDa, $D = 1.16$) obtained from a GPC system equipped with PSS GRAL columns using DMF + 50 mM LiBr as the mobile phase at 50 °C.

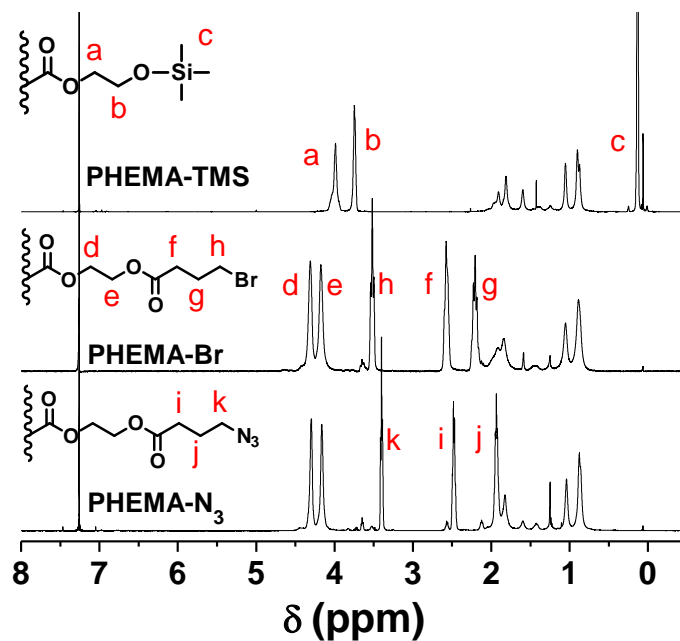


Figure S6. ¹H NMR spectra of PHEMA-TMS, PHEMA-Br, and PHEMA-N₃ in CDCl₃ (500 MHz).

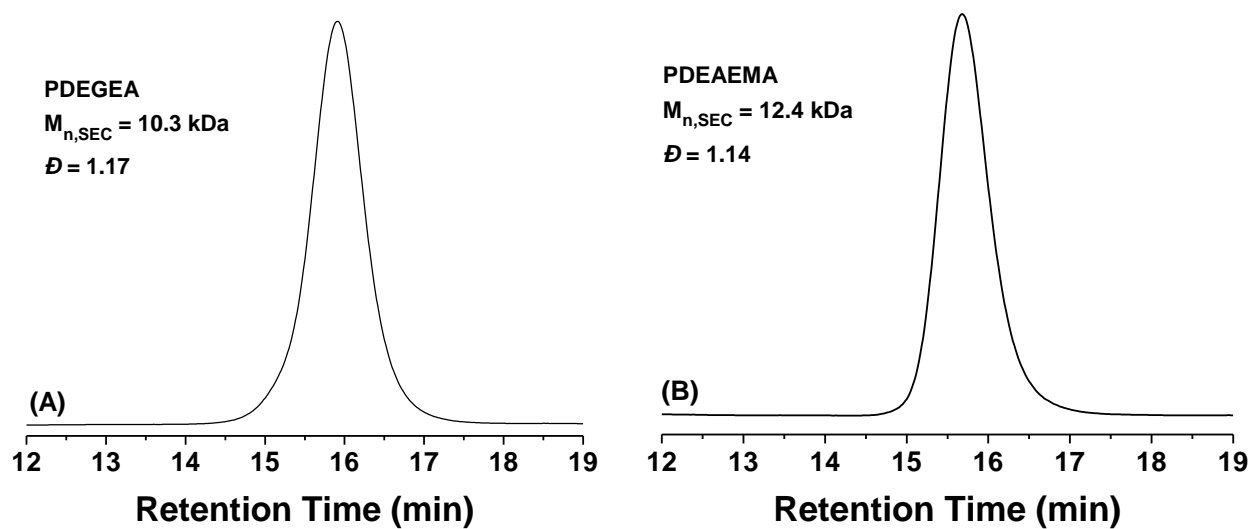


Figure S7. SEC traces of alkyne end-functionalized PDEGEA (A) and PDEAEMA (B).

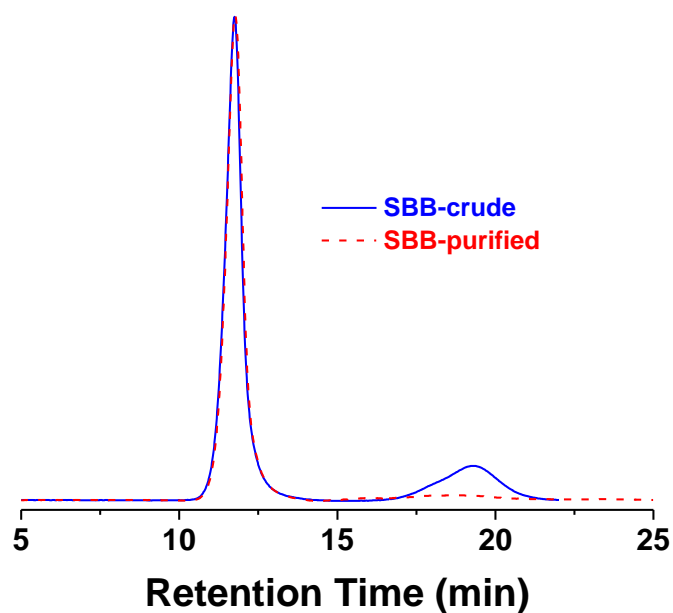


Figure S8. SEC traces of star brush polymer SBB before and after purification.

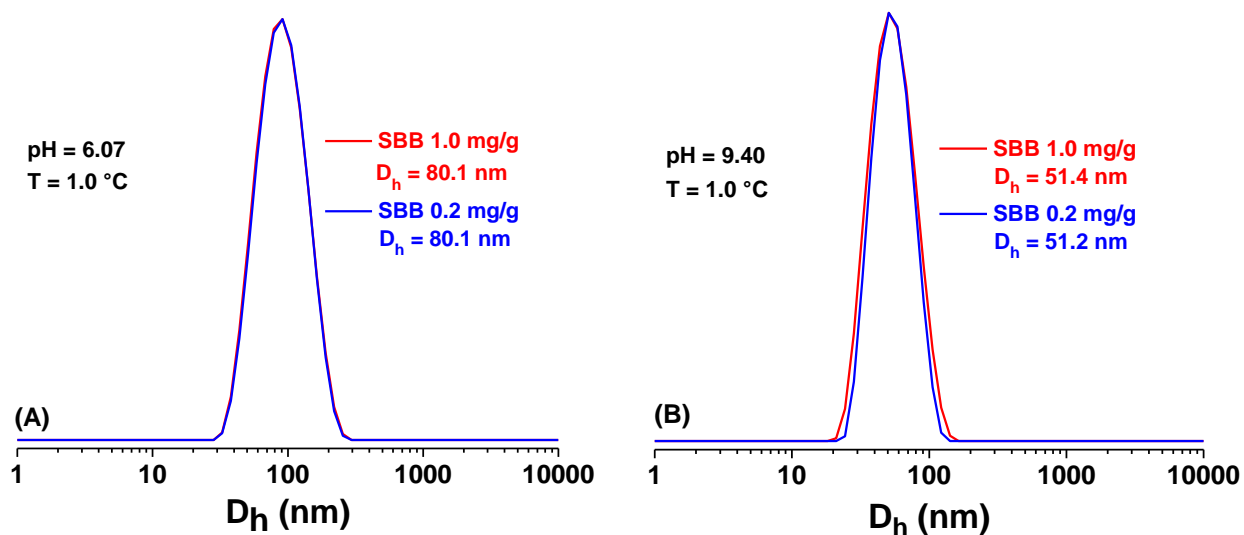


Figure S9. Apparent hydrodynamic size distributions for 0.2 mg/g and 1.0 mg/g solutions of SBB at $T = 1.0\text{ }^{\circ}\text{C}$ and $\text{pH} = 6.07$ (A) and $\text{pH} = 9.40$ (B).

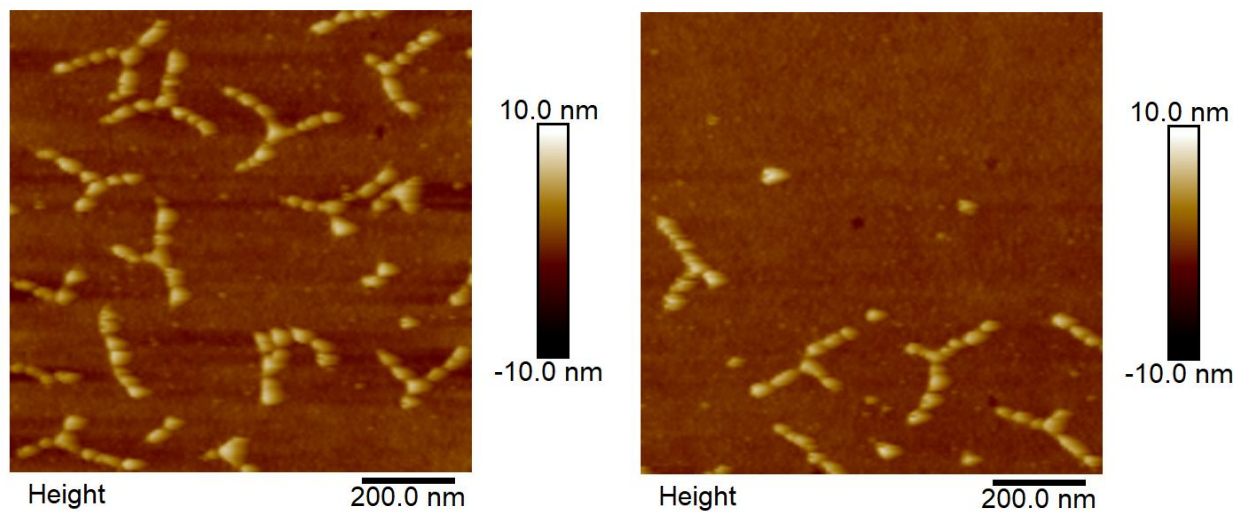


Figure S10. AFM height images of SBB spin cast onto PMMA-coated mica from a 0.05 mg/g solution in a 5 mM phosphate buffer with a pH of 6.60 at $0\text{ }^{\circ}\text{C}$.

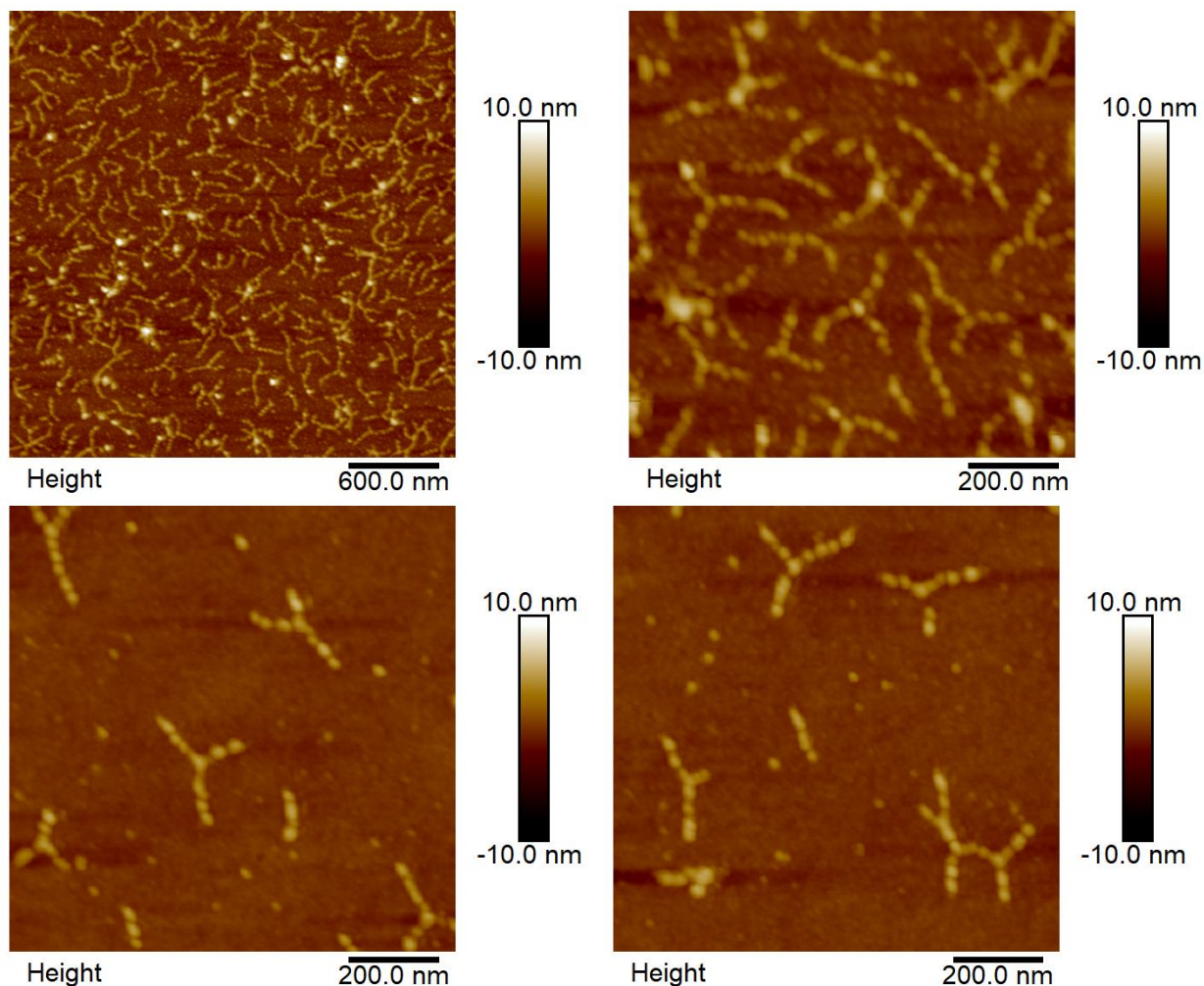


Figure S11. AFM height images of SBB spin cast onto PMMA-coated mica from a 0.05 mg/g solution in a 5 mM phosphate buffer with a pH of 7.43 at 0 °C.

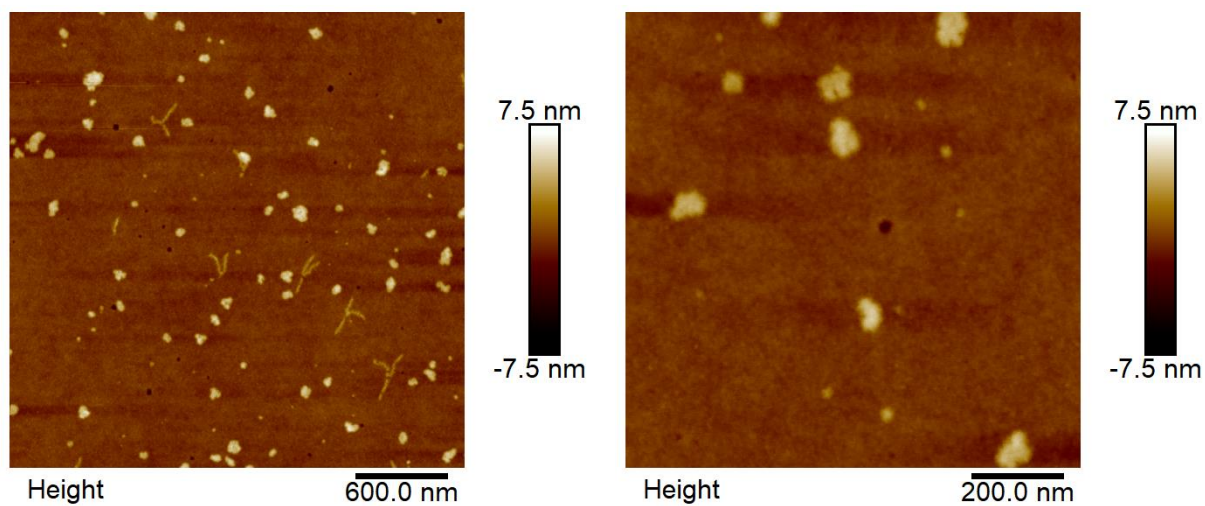


Figure S12. AFM height images of SBB spin cast onto PMMA-coated mica from a 0.05 mg/g solution in a 5 mM phosphate buffer solution with a pH of 9.50 at 0 °C.

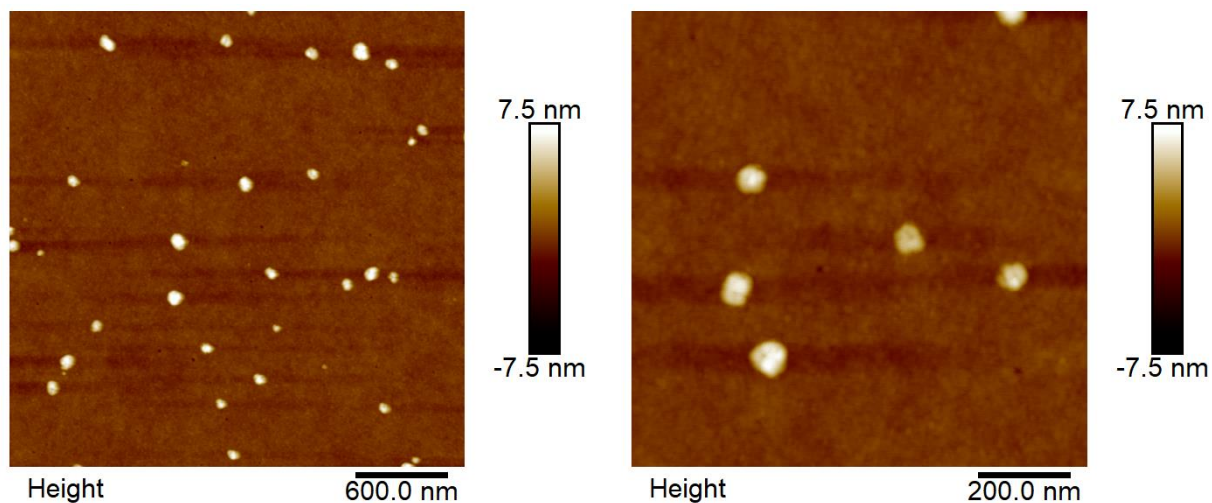


Figure S13. AFM height images of SBB spin cast onto PMMA-coated mica at 22 °C from a 0.05 mg/g solution in 5 mM phosphate buffer solution with a pH of 7.43.

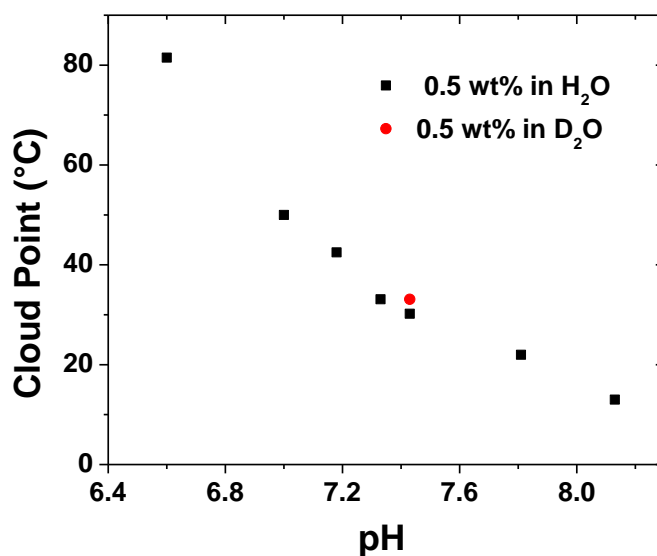


Figure S14. Cloud points for 0.5 wt% PDEAEMA at various pH values in 10 mM phosphate buffers prepared with H₂O (black squares) and D₂O (red circle).