Electronic Supplementary Information for:

pH- and Chaotropic Anion-Induced Conformational Changes of Tertiary Amine-Containing Binary Heterografted Star Molecular Bottlebrushes in Aqueous Solution

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Experimental Section

Materials. 2-(*N,N*-Dimethylamino)ethyl methacrylate (DMAEMA, 98%, TCI) and 2-(*N,N*-diethylamino)ethyl methacrylate (DEAEMA, 98.5%, TCI) were passed through a basic alumina/silica gel column to remove the inhibitor prior to use. Alkyne end-functionalized poly(ethylene oxide) (PEO) was synthesized by reacting poly(ethylene glycol) methyl ether (5 kDa, Sigma-Aldrich) with 4-pentynoic acid in methylene chloride using *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (98+%, Acros Organic) and 4-(dimethylamino)pyridine (99.0+%, TCI America) as catalysts. SEC analysis showed a $M_{n,SEC}$ of 8300 Da and a *Đ* value of 1.04 for the alkyne end-functionalized PEO relative to polystyrene standards (Figure S1A). Copper(I) chloride (CuCl, 99%, Sigma-Aldrich) was stirred in glacial acetic acid overnight, collected by vacuum filtration, washed thoroughly on the filter with absolute ethanol followed by diethyl ether, and then dried under high vacuum. *N,N,N′,N″,N″*-Pentamethyldiethylenetriamine (PMDETA, 99%, Sigma-Aldrich) and 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA,

97%, Sigma-Aldrich) were purified by vacuum distillation. The preparation of the azide-functionalized star backbone polymer with a DP of 434 per arm (PHEMA-N$_3$) was reported in a previous publication.[1] Propargyl 2-bromoisobutyrate (PBiB) was synthesized according to a procedure described in the literature.[2] Potassium ferricyanide (K$_3$[Fe(CN)$_6$], 98%, Acros Organics), potassium persulfate (K$_2$S$_2$O$_8$, > 99%, Fisher Scientific), sodium perchlorate (NaClO$_4$, 98%, Alfa Aesar), sodium thiocyanate (NaSCN, 95%, Fisher Scientific), sodium chloride (NaCl, 99.6%, Fisher Scientific), sodium bromide (NaBr, Acros Organics 99.5 %) were used as received. All other chemicals were purchased from either Sigma-Aldrich or Fisher and used directly.

General Characterization and Instrumentation. The molecular weights and dispersities of the azide-bearing backbone polymer, PHEMA-N$_3$, and the alkyne end-functionalized tertiary amine-containing side chain polymers, PDMAEMA and PDEAEMA, were determined by size exclusion chromatography (SEC) relative to polystyrene standards, performed at 50 °C using a PL-GPC 50 Plus system from Polymer Laboratories, Inc. The integrated GPC/SEC system consisted of 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 SEC of the alkyne end-functionalized PEO was carried out 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, Inc.), and two PLgel 5µm mixed-C columns (each 300 × 7.5 mm, linear range of molecular weights from 200 to 2 000 000 Da, Agilent Technologies, Inc.). 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.).

The absolute molecular weights of the purified star brushes, SMB-1 and SMB-2, were determined by a SEC-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 *N,N*-dimethylformamide (DMF) with 50 mM LiBr, and the SEC analysis was carried out at 50 °C.

^{1}H NMR spectra were recorded on either a Varian VNMRS 500 NMR or a Varian VNMRS 600 NMR spectrometer using the residual solvent proton signal as the internal standard. ^{1}H NMR spectroscopy was also employed to study the responsive properties of SMB-1 and -2 in aqueous solution with the addition of various amounts of either potassium ferricyanide or sodium chloride. Aqueous solutions of the molecular bottlebrushes with a concentration of 4.0 mg/g were prepared using D_2O as the solvent. The pH values of the solutions were measured with a pH meter (AB-15, calibrated using pH = 4.01, 7.00, and 10.01 standard buffer solutions at room temperature) and adjusted to a pH of 4.00 using 0.1 M HCl and 0.1 M NaOH solutions prepared in D_2O. For the experiments involving the addition of NaCl, after each spectrum was recorded (64 scans), the solution in the NMR tube was poured into a vial, and an appropriate amount of solid NaCl was added to achieve the desired concentration of NaCl. The solution was then stirred at room temperature for 5 min before it was transferred to the NMR tube for recording a new ^{1}H NMR spectrum. For the experiments involving the addition of potassium ferricyanide, a similar

procedure was followed, except that the salt was added via an adjustable pipet from a 0.1 M solution prepared in D_2O.

Synthesis of Alkyne End-Functionalized PDMAEMA. Propargyl 2-bromoisobutyrate (48.8 mg, 2.38×10^{-4} mol, delivered via 1.204 g of a 40.5 mg/g stock solution in anisole), DMAEMA (3.009 g, 1.914×10^{-2} mol), HMTETA (59.6 mg, 2.59×10^{-4} mol), CuCl (22.3 mg, 2.25×10^{-4} mol), and anisole (3.893 g) were added to a 50 mL two-necked round bottom flask equipped with a magnetic stir bar, a rubber septum, and a gas flow adapter. The mixture was degassed by three freeze-pump-thaw cycles and then immersed in an oil bath with the temperature preset at 50 °C. The polymerization was stopped after 2.5 h by removing the flask from the oil bath and opening it to air; a sample was taken for ^{1}H NMR analysis and the mixture was diluted immediately with methylene chloride. The copper catalyst was removed by passing the mixture through a basic alumina (top)/silica gel (bottom) column with excess methylene chloride as eluent. The crude solution was concentrated by rotary evaporation and the polymer was purified by precipitation from methylene chloride into hexanes in an ice/water bath three times. The purified polymer was collected and dried under high vacuum, yielding a glassy, white polymer (1.502 g, 49.9%). The monomer conversion was 56.0%, determined by ^{1}H NMR spectroscopy analysis of the final polymerization mixture using the integrals of the peaks at 4.32-4.23 ppm (-COOCH_2- of the monomer) and at 4.18-4.01 ppm (-COOCH_2- of the polymer). The degree of polymerization (DP) of PDMAEMA was calculated to be 45 by using the monomer conversion and the monomer-to-initiator feed ratio. SEC analysis relative to polystyrene standards using PSS GRAL columns and DMF with 50 mM LiBr as eluent at 50 °C: $M_{n,SEC} = 11.2$ kDa and $Đ = 1.17$ (Figure S2A).

Synthesis of Alkyne End-Functionalized PDEAEMA. Propargyl 2-bromoisobutyrate (134.3 mg, 6.55×10^{-4} mol), DEAEMA (6.113 g, 3.29×10^{-2} mol), HMTETA (153.2 mg, 6.65×10^{-4}

mol), CuCl (64.3 mg, 6.5×10^{-4} mol), and anisole (9.043 g) were added to a 50 mL two-necked round bottom flask and degassed by three freeze-pump-thaw cycles. The flask was immersed in an oil bath with a preset temperature of 50 °C, and the polymerization proceeded under nitrogen while stirring. After 3.75 h, the flask was removed from the oil bath and opened to air; an aliquot was withdrawn for ^{1}H NMR spectroscopy analysis and the polymerization mixture was diluted with methylene chloride. The copper catalyst was removed by passing the mixture through a column of basic alumina (top)/silica gel (bottom). The polymer was purified by precipitating the concentrated polymer solution into hexanes from methylene chloride in a dry ice/acetone bath (-78 °C) four times followed by drying under high vacuum to yield a slightly yellow, rubbery polymer (4.390 g, 71.8%). The monomer conversion was determined to be 82.7% via ^{1}H NMR spectroscopy analysis of the final polymerization mixture by comparing the integrals of the peaks at 4.32-4.17 ppm (-COOCH_2- of the monomer) and at 4.17-3.98 ppm (-COOCH_2- of the polymer). Using the monomer conversion and the initial monomer-to-initiator ratio, the DP of the polymer was calculated to be 42. SEC results relative to polystyrene standards using PSS GRAL columns with DMF containing 50 mM LiBr as eluent: $M_{n,SEC}$ = 10.7 kDa and $Đ$ = 1.18 (Figure S3A).

Synthesis of Binary Heterografted Star Molecular Brushes with PEO and PDMAEMA Side Chains (SMB-1). PHEMA-N$_3$ (8.01 mg, 3.32×10^{-5} mol of -N$_3$ groups assuming quantitative functionalization, delivered via 0.706 g of 11.34 mg/g stock solution in THF), alkyne end-functionalized PEO (90.9 mg, 1.78×10^{-5} mol), alkyne end-functionalized PDMAEMA (131.8 mg, 1.82×10^{-5} mol), CuCl (3.5 mg, 3.5×10^{-5} mol), and THF (7 mL) were added to a 20 mL scintillation vial equipped with a magnetic stir bar. The vial was sealed with a rubber septum and purged with nitrogen through needles for 15 min. PMDETA (10 µL, 4.79×10^{-5} mol) was injected via a microsyringe. After the purging with nitrogen was continued for 15 min, the needles were

removed, and the reaction mixture was stirred under nitrogen for 23 h at ambient temperature. Propargyl benzyl ether (25 µL) was then injected to cap unreacted azide groups on the backbone and the reaction continued for 3.5 h. After a sample was withdrawn for SEC analysis, the reaction mixture was diluted with methylene chloride and passed through a basic alumina (top)/silica gel (bottom) column to remove the catalyst. Rotary evaporation was used to concentrate the brush solution to ~ 10 mL; ethanol (50 mL) was added and the solution was concentrated by rotary evaporation again. This process was repeated three times to remove methylene chloride and THF. The brush solution in ethanol was then diluted with Milli-Q water, and the unreacted side chain polymers were removed by 7 rounds of centrifugal filtration at 5000 rpm using a 50 kDa MWCO filter and a mixture of water and ethanol as solvent (50/50, v/v). SEC analysis (PSS GRAL columns with DMF containing 50 mM LiBr as eluent) confirmed the complete removal of unreacted side chain polymer (Figure S4A).

Synthesis of Binary Heterografted Star Molecular Brushes with PEO and PDEAEMA Side Chains (SMB-2). PHEMA-N$_3$ (8.25 mg, 3.42×10^{-5} mol of azide groups assuming quantitative functionalization, delivered via 0.7275 g of 11.34 mg/g stock solution in THF), alkyne end-functionalized PEO (100.7 mg, 1.97×10^{-5} mol), alkyne end-functionalized PDEAEMA (174.8 mg, 2.20×10^{-5} mol), CuCl (3.1 mg, 3.1×10^{-5} mol), and THF (6 mL) were added to a 20 mL scintillation vial that contained a magnetic stir bar. The vial was sealed with a rubber septum and the headspace was purged with nitrogen through needles for 15 min, followed by the injection of PMDETA (9.0 µL, 4.31×10^{-5} mol) via a microsyringe. The purging with nitrogen was continued for 15 min. The needles were then removed, and the reaction proceeded under nitrogen at ambient temperature. After 22 h, propargyl benzyl ether (25 µL) was injected in an attempt to cap unreacted azide moieties on the backbone and the reaction continued for another 3 h. The

reaction vial was opened to air, and a sample was withdrawn for SEC analysis. The mixture was diluted with methylene chloride and passed through a column of basic alumina (top)/silica gel (bottom) to remove the catalyst. The crude brush solution was concentrated by rotary evaporation to approximately 10 mL. Ethanol (50 mL) was then added, and the solution was concentrated by rotary evaporation again to remove volatile organic solvents without allowing the brushes to become dry. This process was repeated three times to ensure the removal of methylene chloride and THF. The ethanol solution of the star brushes was diluted with Milli-Q water for purification by centrifugal filtration. Excess side chain polymers were removed by 9 rounds of centrifugal filtration at 5000 rpm using a 50 kDa MWCO centrifugal filter with a mixture of ethanol and water (50/50, v/v) as the solvent. The complete removal of excess side chain polymers was confirmed by SEC analysis using PSS GRAL columns with DMF containing 50 mM LiBr as eluent (Figure S4B).

Dynamic Light Scattering (DLS) Study of SMB-1 and SMB-2 in Aqueous Solutions. DLS of aqueous solutions of SMB-1 and SMB-2 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 transferring a known amount of a stock solution of the molecular brushes in water to a vial and diluting it to the desired concentration (0.1 or 0.2 mg/g) with Milli-Q water or a 10 mM phosphate buffer. The pH was adjusted to 4.00 and the solution was stirred at room temperature overnight to ensure complete dissolution of the brush molecules. To study the pH-responsive properties of the molecular brushes, 0.2 mg/g solutions were prepared using a 10 mM phosphate buffer; the solution pH was gradually adjusted using 0.1 M NaOH and 0.1 M HCl, monitored by an Accumet AB-15 pH meter (calibrated at room temperature using standard buffer solutions with pH = 4.01, 7.00, and 10.01), and allowed to equilibrate for 15 min.

A portion of the solution was then transferred to a DLS cuvette without filtering and loaded into the instrument with a preset temperature of 25 °C. The DLS sample was equilibrated for an additional 5 min before measurements were taken.

To study the effects of various salts on the hydrodynamic size of the star brushes, aqueous solutions with a concentration of 0.1 mg/g were prepared with Milli-Q water and the pH was adjusted to 4.00. In total, six salts were used in the study: potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium persulfate ($K_2S_2O_8$), sodium perchlorate ($NaClO_4$), sodium thiocyanate (NaSCN), sodium bromide (NaBr), and sodium chloride (NaCl). Potassium ferricyanide and potassium persulfate were added via adjustable pipets from 0.1 M solutions prepared in Milli-Q water; the dilution of the brush solution was accounted for when determining the concentration of the salt in the DLS sample. The other salts were added via 0.1 M solutions prepared in Milli-Q water up to a concentration of 10 mM in the brush solution, and then the solid salt was added to achieve higher concentrations to avoid excessive dilution of the brush solution. Each DLS data point presented was the average result of three measurements, where each measurement was comprised of 10 runs.

Atomic Force Microscopy (AFM) Study of SMB-1 and SMB-2. The star molecular 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. Mica substrates were prepared by cleaving a fresh layer of mica disk (Ted Pella, Inc.) with Scotch tape. Solutions of molecular brushes prepared in THF at a concentration of 0.01 mg/g were spin cast onto the freshly cleaved mica surface at a spin rate of 3000 rpm. Glass substrates were prepared by thoroughly cleaning glass disks (Ted Pella, Inc.) with a piranha solution followed by rinsing with Milli-Q water and finally storing the disks in isopropanol. To study the pH-responsive behavior of SMB-

1 and SMB-2, aqueous solutions of the star brushes in Milli-Q water at a concentration of 0.05 mg/g with a pH of 4.00 or 9.50 were spin cast onto the glass substrates at 3000 rpm. To study the chaotropic anion-induced shape transitions, aqueous solutions of SMB-1 and SBM-2 with a concentration of 0.05 mg/g were prepared in Milli-Q water, and the pH was adjusted to 4.00 using 0.1 M NaOH and 0.1 M HCl. Then, an appropriate amount of a potassium ferricyanide stock solution in water with a concentration of 0.1 M was added to make the final ferricyanide concentration of 0.14 mM in the aqueous solutions of SMB-1 and -2. The brush solutions were stirred for 10 min. Two drops of each brush solution were then placed onto a glass disk and a steady stream of nitrogen was used to push the solution off from the glass disk; the nitrogen was continuously flowed over the disk for an additional 30 s until the disk was dry.

References:

1. Kent, E. W.; Zhao, B. Stimuli-Induced Star-Globule Shape Transitions of Dually Responsive Binary Heterografted Three-Arm Star Molecular Brushes in Aqueous Solution. *Macromolecules* **2019**, *52*, 6714-6724.
2. 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.

Calculation of Grafting Density of Star Molecular Bottlebrushes. The grafting density of SMB-1 was calculated to be 85.3 % by comparing the SEC peak areas of the molecular brushes (78.0 %) and the remaining side chain polymers (22.0 %) in the final reaction mixture as well as considering the composition of the two side chain polymers in the purified molecular brushes. ^{1}H NMR spectroscopy analysis revealed that SMB-1 was composed of 45.7 mol % PDMAEMA and 54.3 mol % PEO side chains using the integrals of the peaks at 4.25 – 3.94 ppm (-COOCH_2- of PDMAEMA) and at 3.37 ppm (-OCH_3 of PEO). Using the molar mass of PDMAEMA from the

DP (7230 g/mol) and PEO (5100 g/mol), the mass percentage was calculated to be 54.4 % for PDMAEMA and 45.6 % for PEO. SEC analysis showed that the relative peak areas of the molecular brushes and side chains were 78.0 % and 22.0 %, respectively. The total mass of reacted side chains was 171.9 mg, determined by multiplying the brush peak area by the feed masses of the side chain polymers and the backbone polymer and then subtracting the mass of the backbone. The masses of reacted PDMAEMA and PEO were found to be 93.5 mg and 78.4 mg, respectively. Using the molar mass of each side chain polymer, the total number of moles of side chain polymers reacted was 2.83×10^{-5} mol. The overall grafting density was determined to be 85.3 % by dividing the number of moles of reacted side chains by the number of moles of available backbone repeat units (assuming complete azide functionalization). Using the same method, the grafting density of SMB-2 was calculated to be 84.8%.
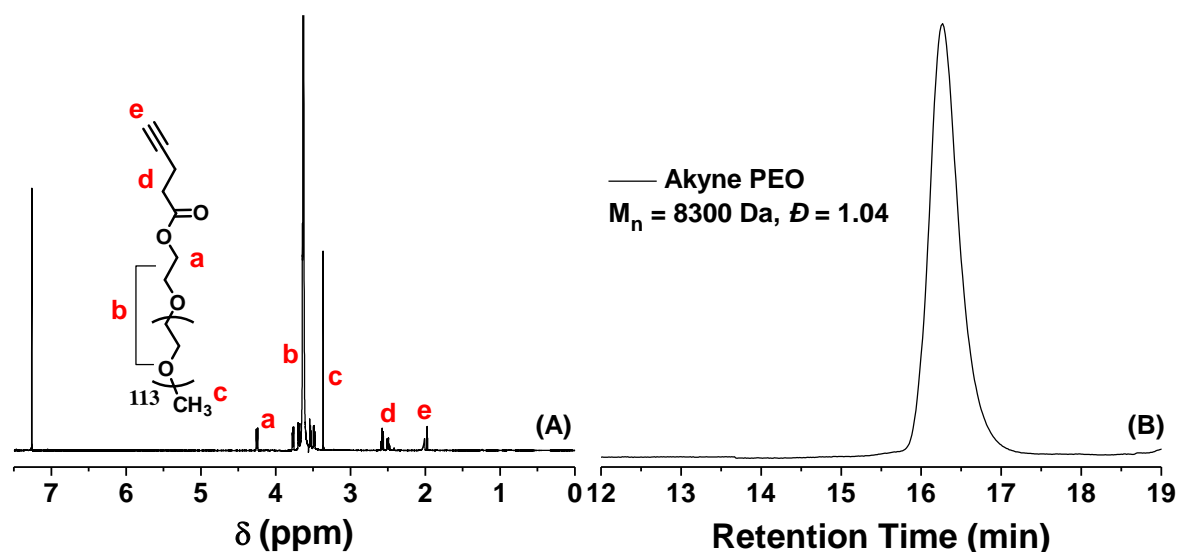
Figure S1. ^{1}H NMR spectrum (A) in CDCl$_3$ and SEC trace (B) of alkyne end-functionalized PEO.
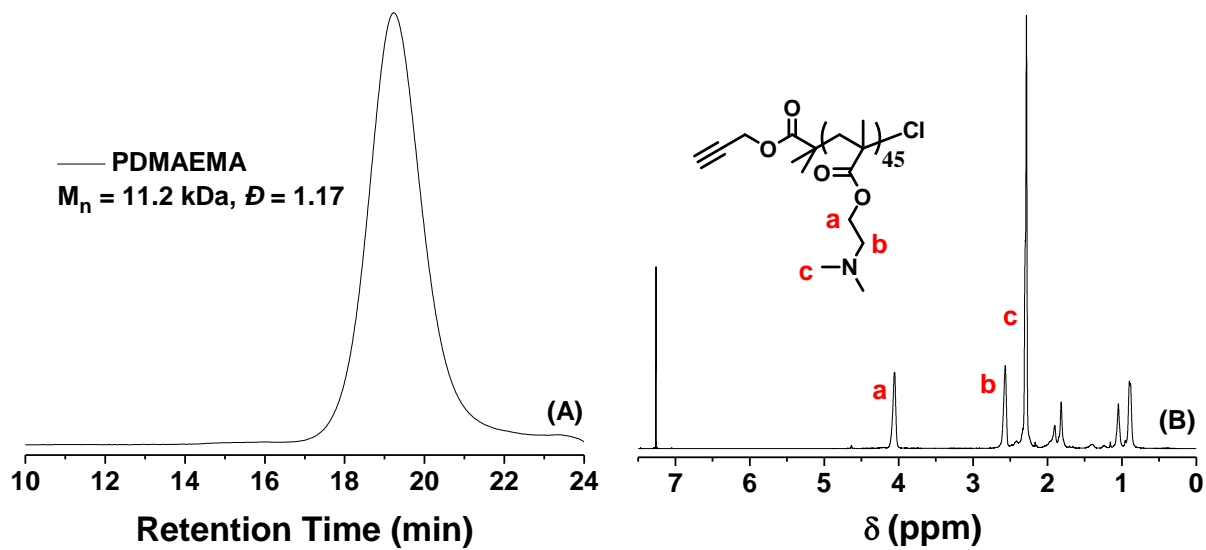
Figure S2. SEC trace (A) and ^{1}H NMR spectrum (B) in CDCl₃ of alkyne end-functionalized PDMAEMA.
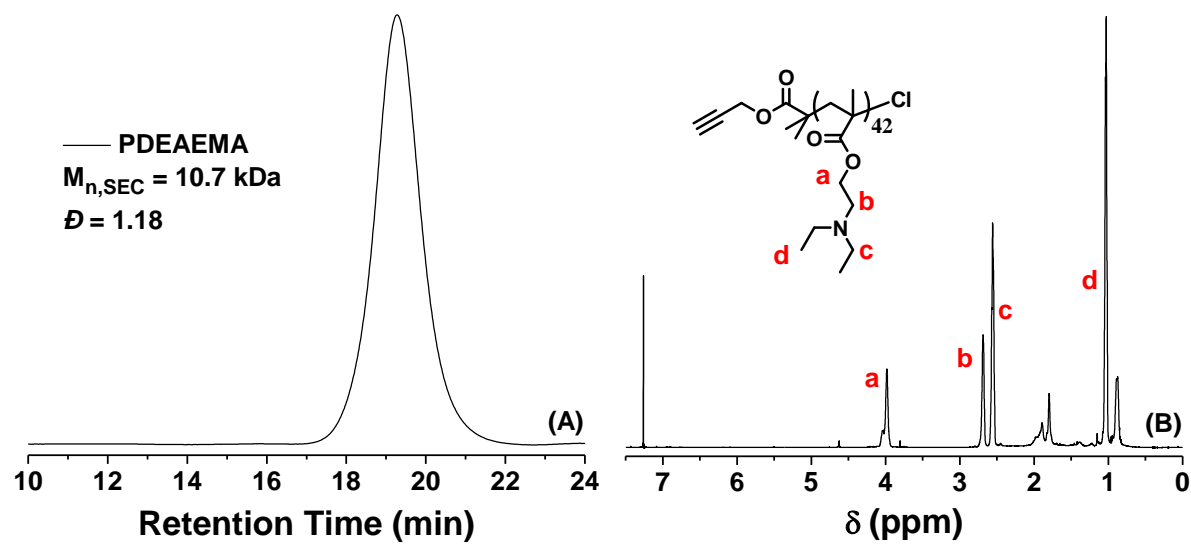
Figure S3. SEC trace (A) and ^{1}H NMR spectrum (B) in CDCl₃ of alkyne end-functionalized PDEAEMA.
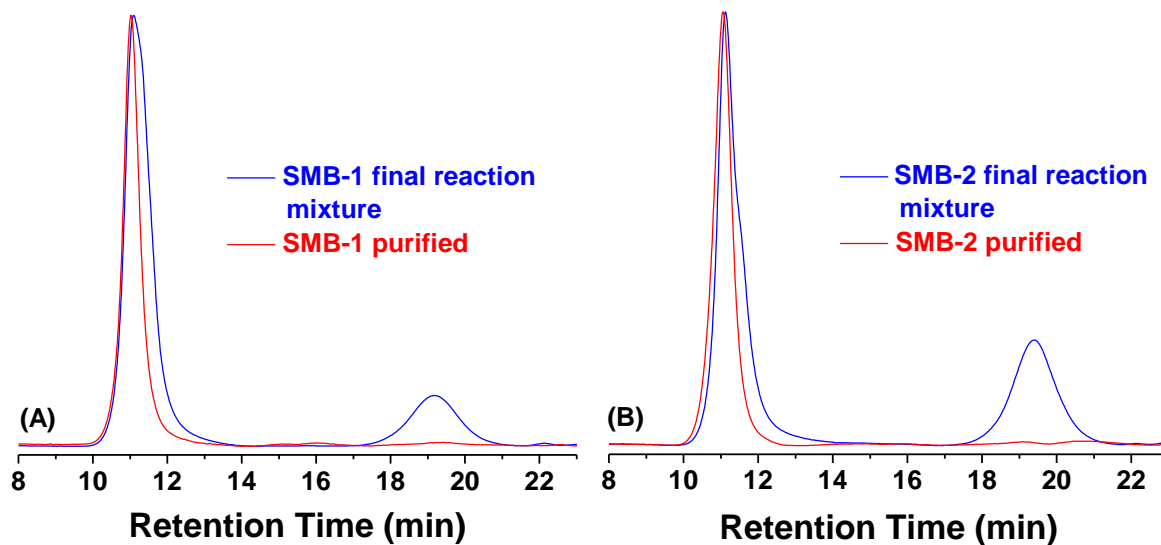
Figure S4. SEC traces of crude and purified star molecular brushes SMB-1 (A) and SMB-2 (B).
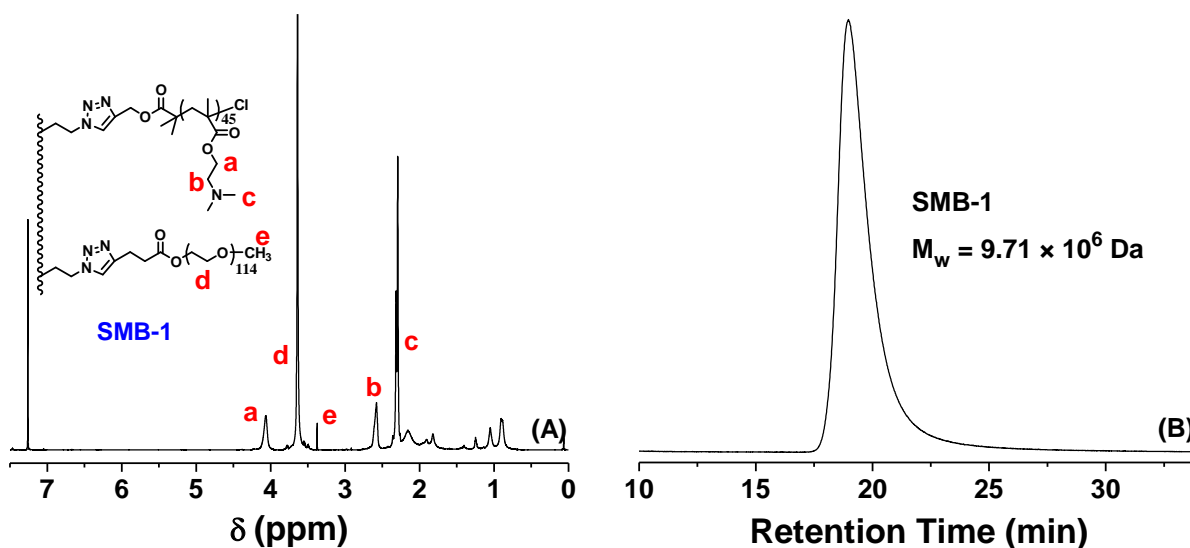
Figure S5. (A) ^{1}H NMR spectrum of the purified star molecular brushes, SMB-1, in CDCl$_3$ and (B) SEC trace of SMB-1 from a SEC-MALS system.
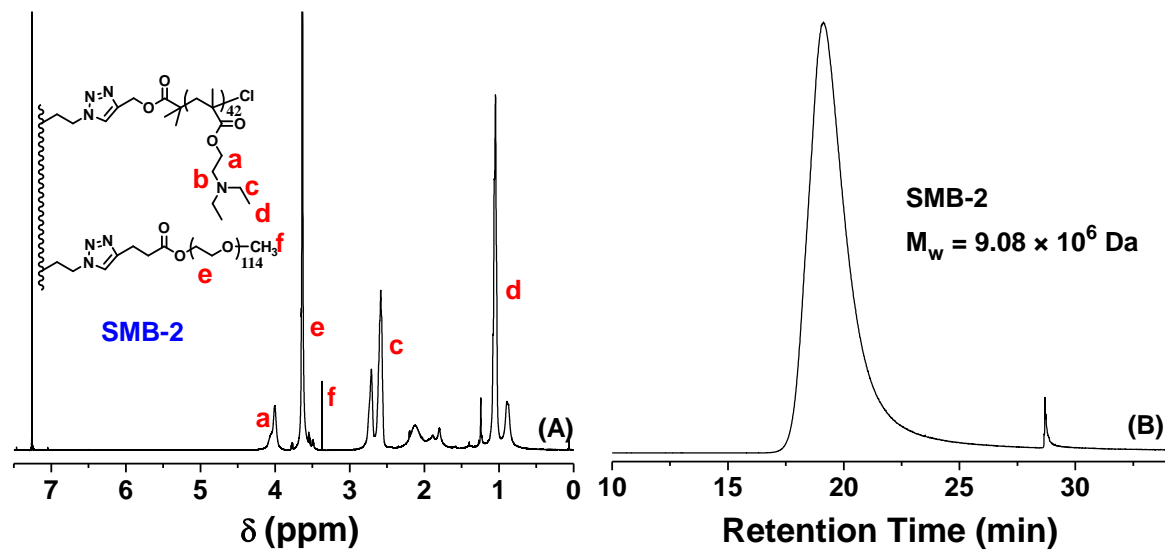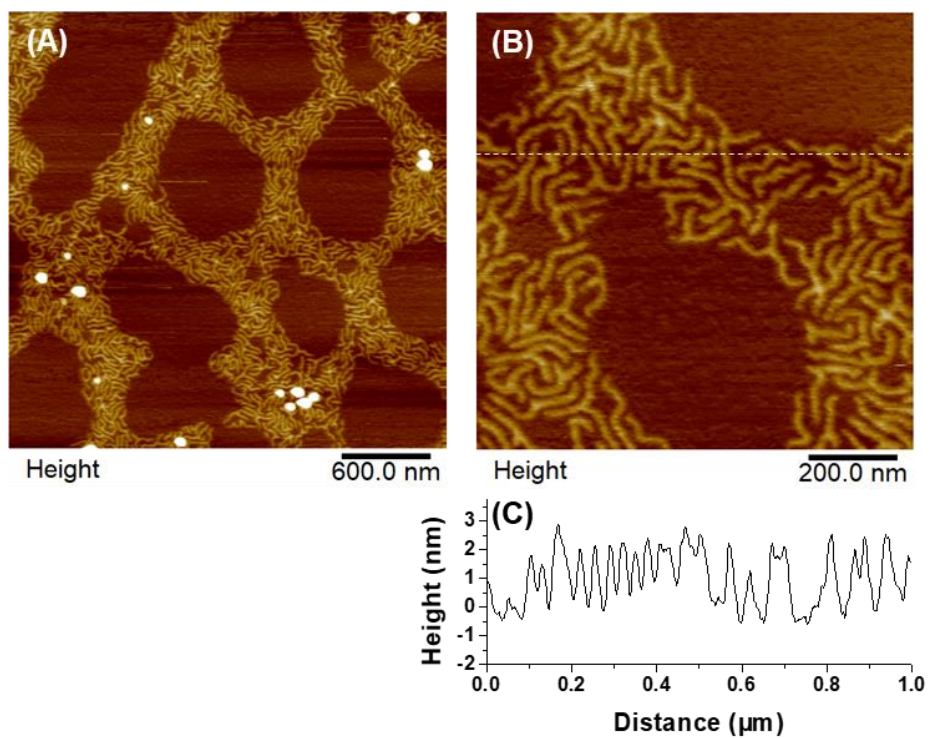
Figure S6. (A) ^{1}H NMR spectrum of the purified star molecular brushes, SMB-2, in CDCl$_3$ and (B) SEC trace of SMB-2 from a SEC-MALS system.

Figure S7. (A) and (B) AFM height images of SMB-1 spin cast onto freshly cleaved bare mica from a 0.01 mg/g solution in THF. (C) Cross-sectional height profile along the dashed line in (B). Note that the AFM image shown in (B) is the same as the one in Figure 1A in the main text.
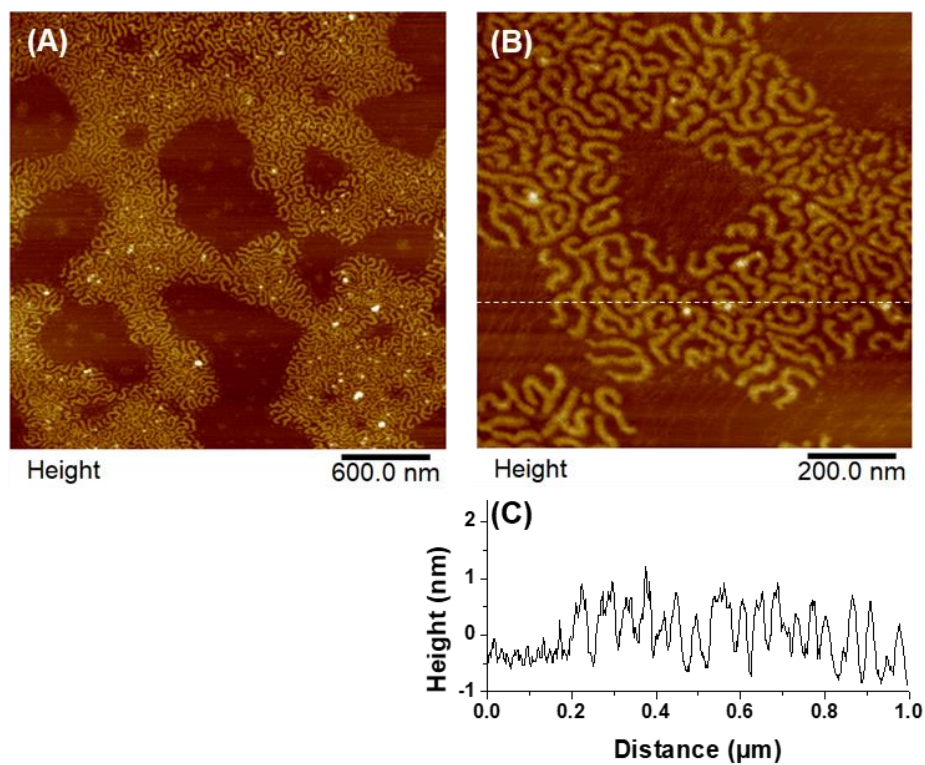
Figure S8. (A) and (B) AFM height images of SMB-2 spin cast onto freshly cleaved bare mica from a 0.01 mg/g solution in THF. (C) Cross-sectional height profile along the dashed line in (B). Note that the AFM image shown in (B) here is the same one in Figure 1B in the main text.
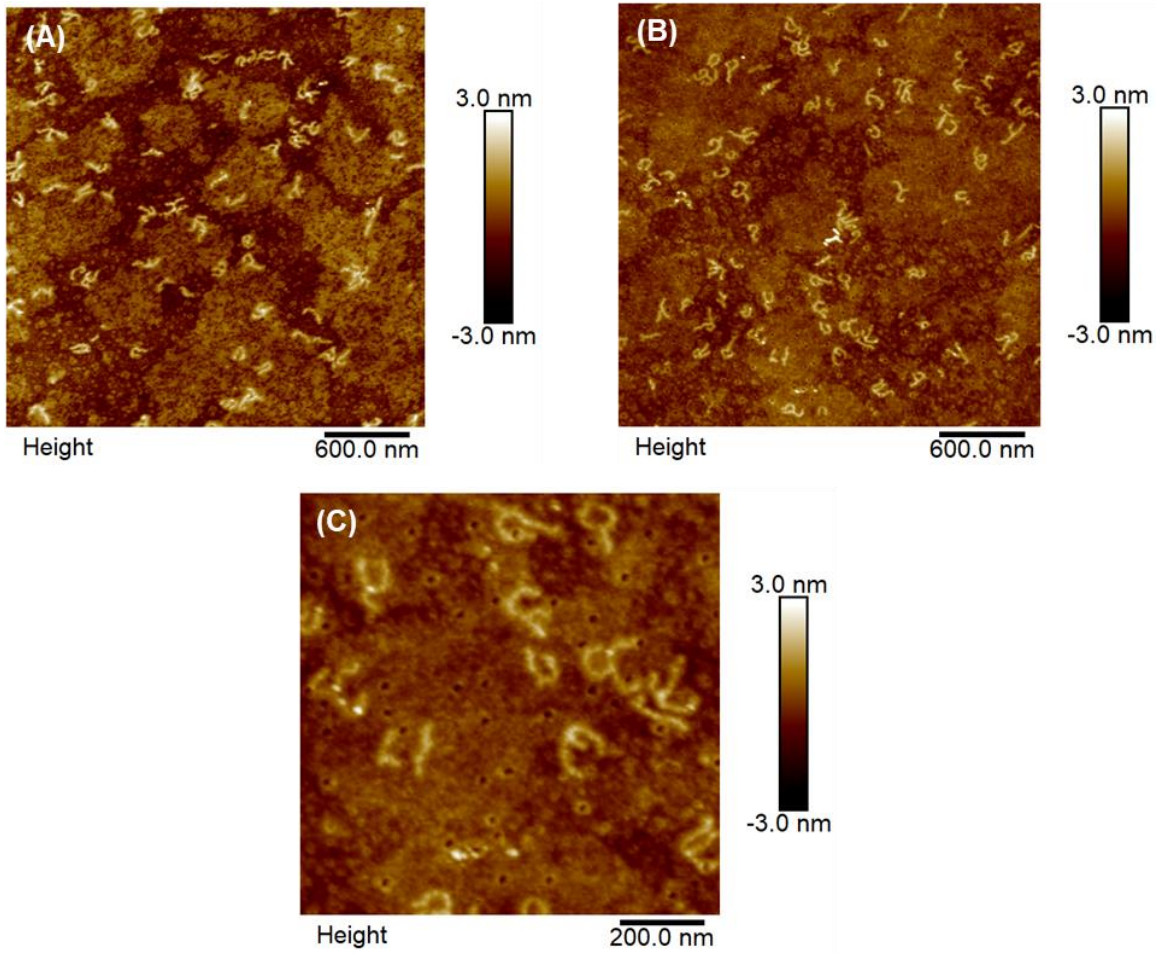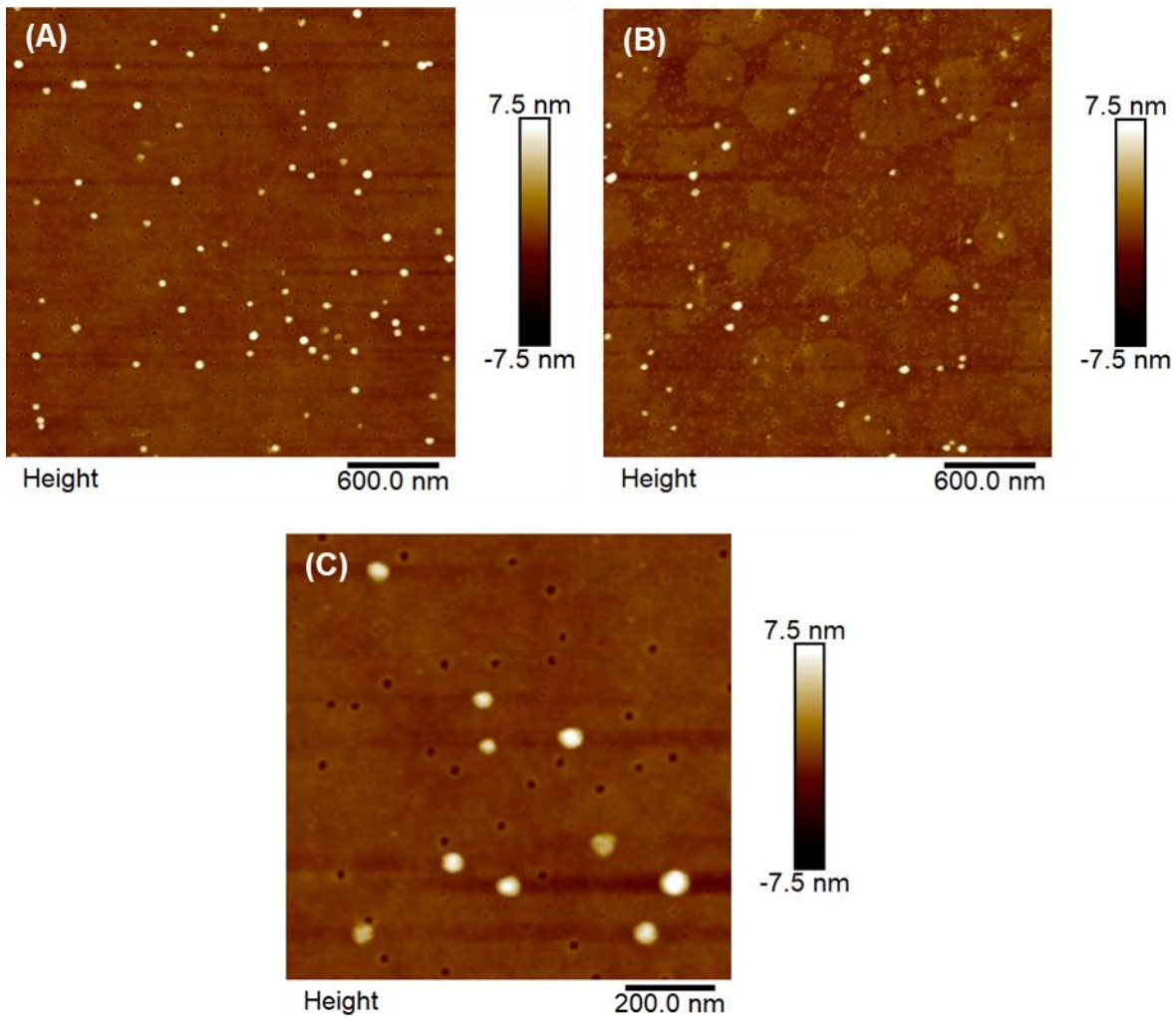
Figure S9. Additional AFM height images of SMB-1 spin cast onto a glass disk from a 0.05 mg/g solution with a pH of 4.00.
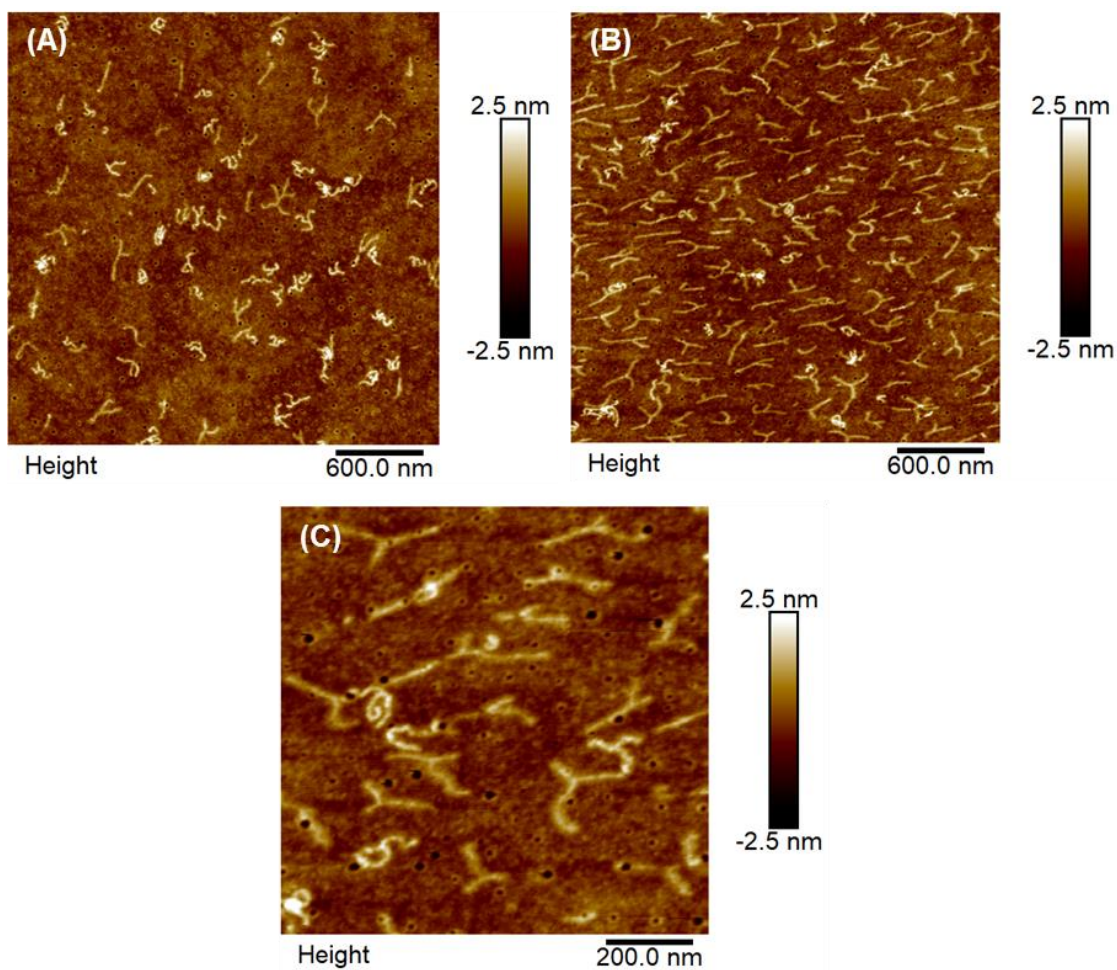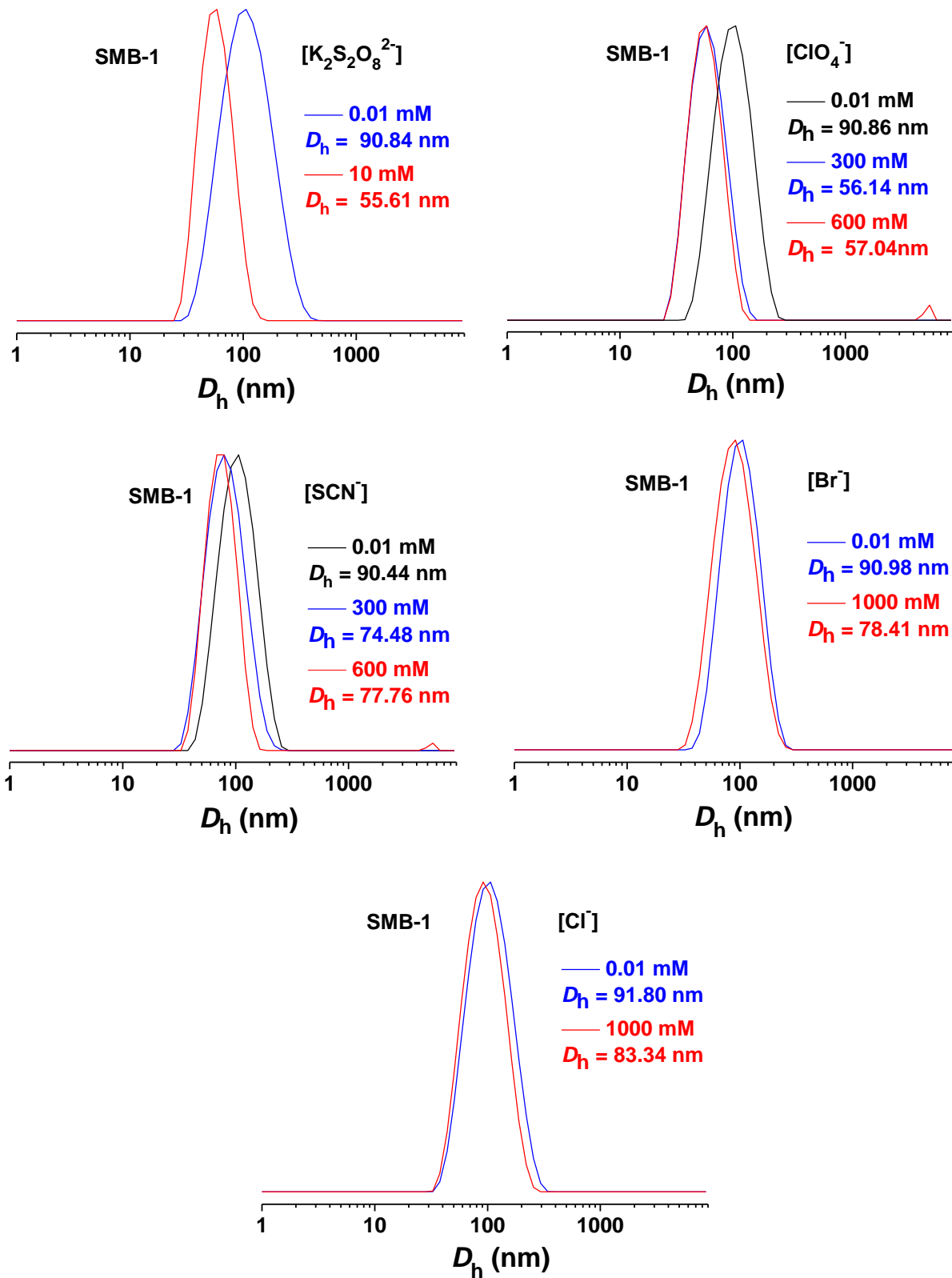
Figure S10. Additional AFM height images of SMB-1 spin cast onto a glass disk from a 0.05 mg/g solution with a pH of 9.50.
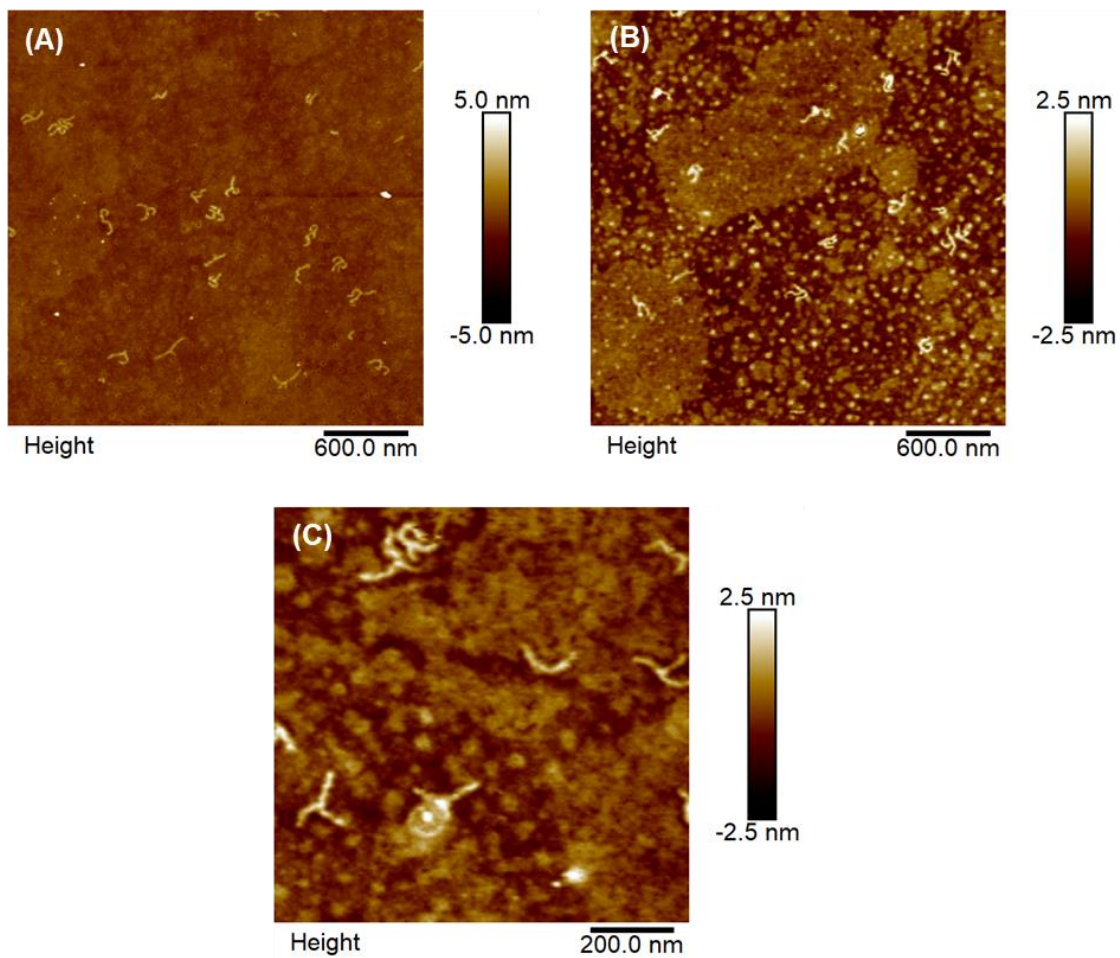
Figure S11. Additional AFM height images of SMB-2 spin cast onto a glass disk from a 0.05 mg/g solution with a pH of 4.00.

Figure S12. Additional AFM height images of SMB-2 spin cast onto a glass disk from a 0.05 mg/g aqueous solution with a pH of 9.50.

Figure S13. Hydrodynamic size distributions of 0.1 mg/g SMB-1 in water with a pH of 4.00 in the presence of various salts of different concentrations.
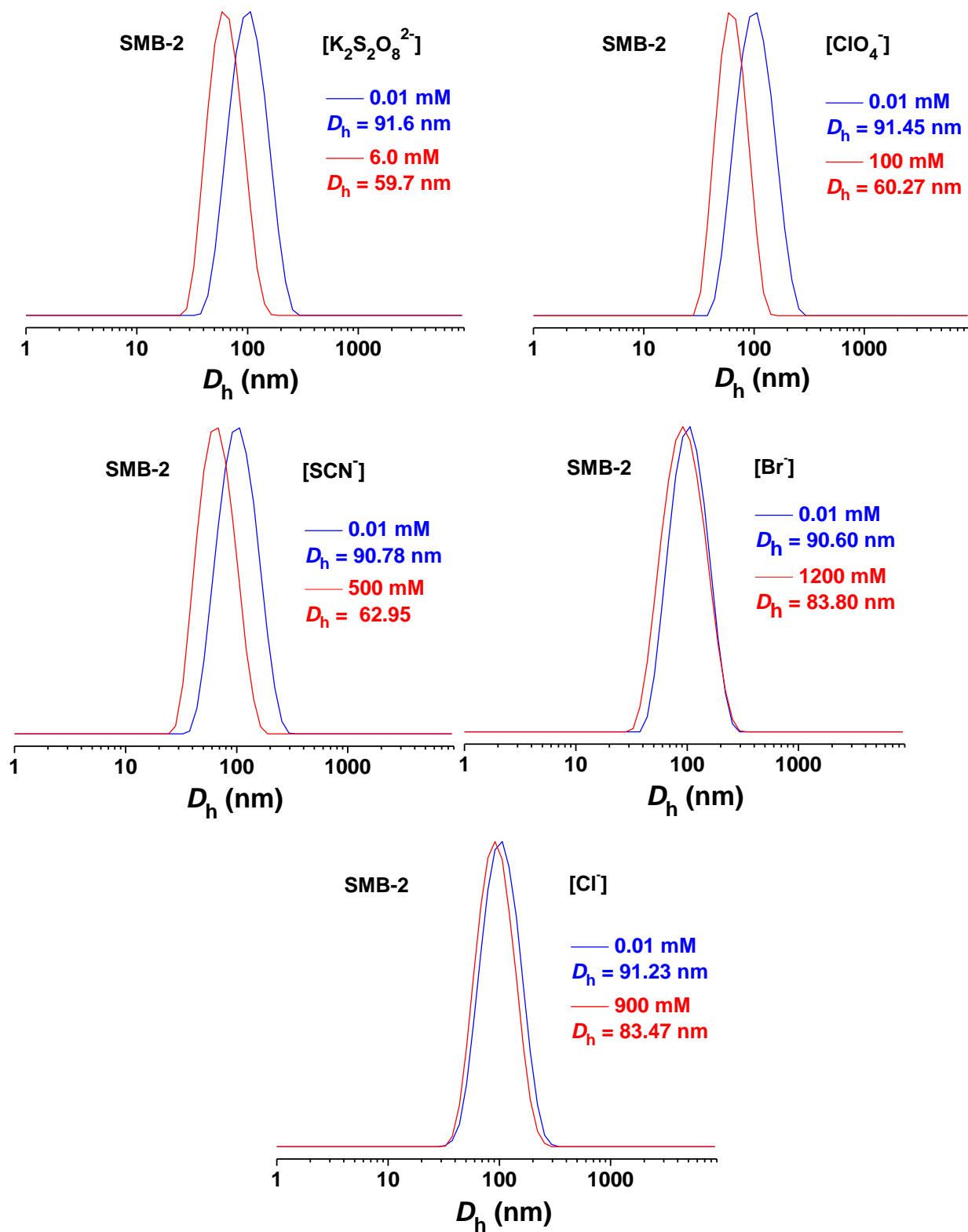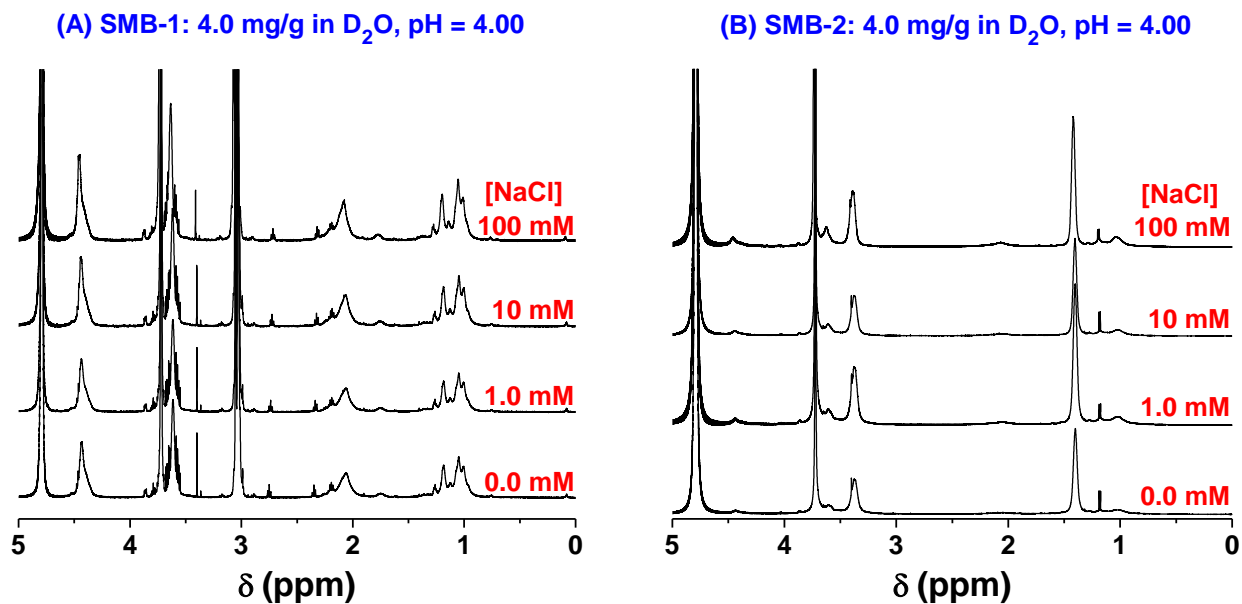
Figure S14. Hydrodynamic size distributions of 0.1 mg/g SMB-1 in water with a pH of 4.00 in the presence of various salts of different concentrations.

(A) SMB-1: 4.0 mg/g in D_2O, pH = 4.00

(B) SMB-2: 4.0 mg/g in D_2O, pH = 4.00

Figure S15. ^1H NMR spectra of SMB-1 (A) and SMB-2 (B) in D_2O with a concentration of 4.0 mg/g at a pH of 4.00 in the presence of various concentrations of sodium chloride. All spectra are normalized to the residual HDO peak at 4.79 ppm.
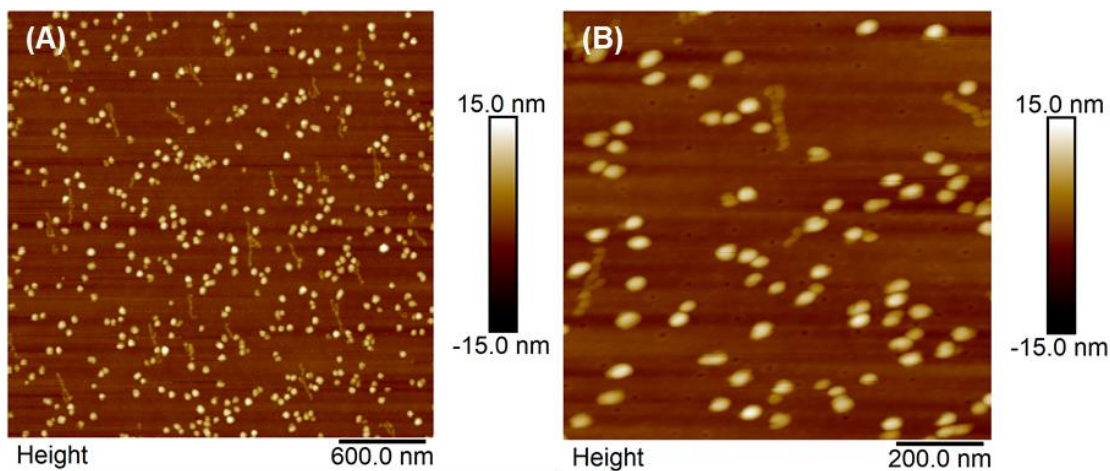

Figure S16. Additional AFM height images of SMB-1 drop cast onto a glass disk from a 0.05 mg/g aqueous solution with a pH of 4.00 in the presence of 0.14 mM potassium ferricyanide.
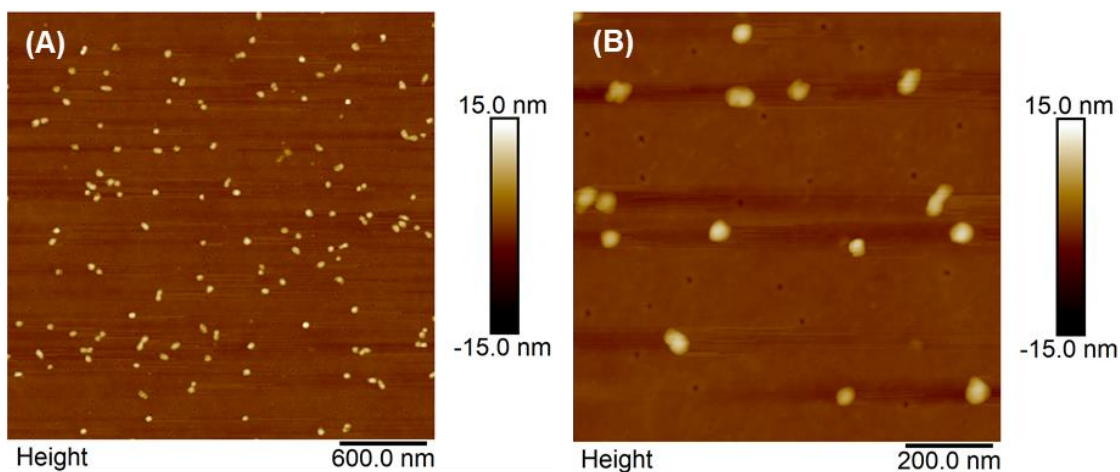
Figure S17. Additional AFM height images of SMB-2 drop cast onto a glass disk from a 0.05 mg/g aqueous solution with a pH of 4.00 in the presence of 0.14 mM potassium ferricyanide.
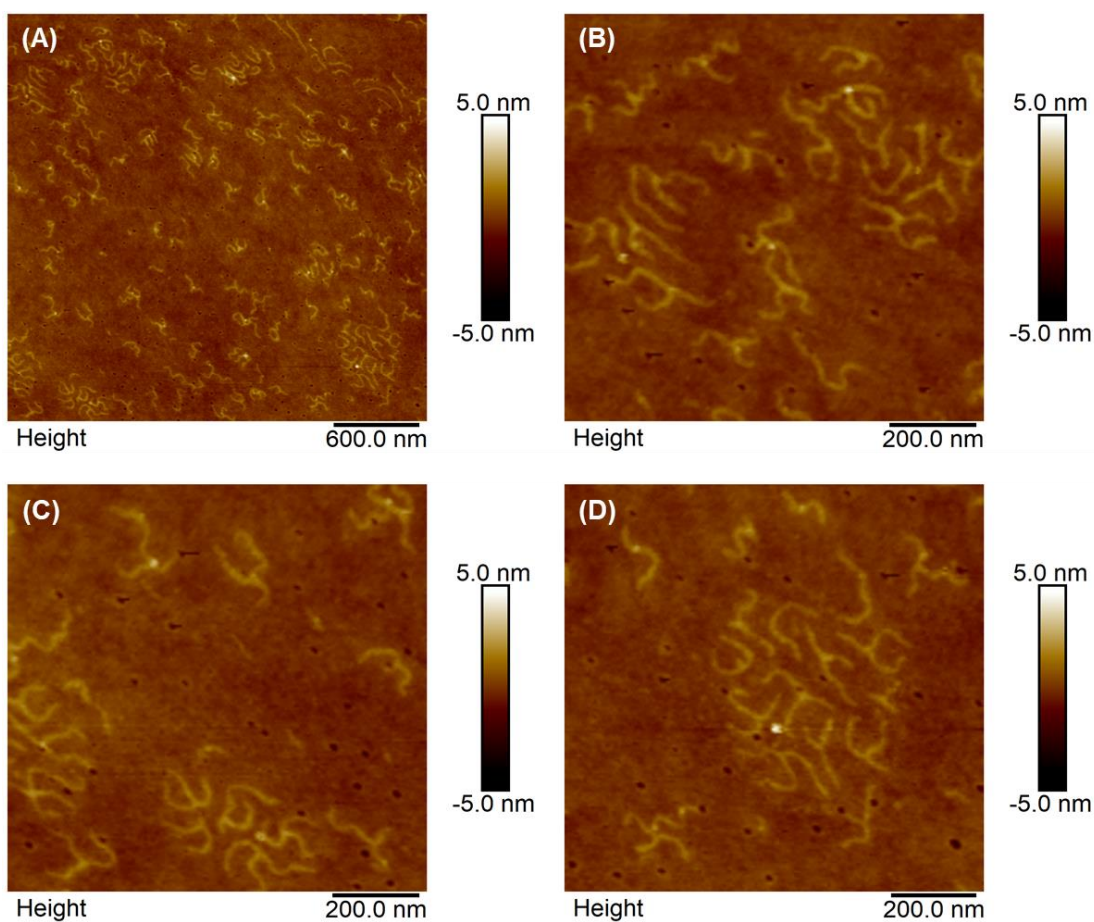

Figure S18. AFM height images of SMB-1 drop cast onto a glass disk from a 0.05 mg/g aqueous solution with a pH of 4.00 in the presence of 100 mM NaCl.
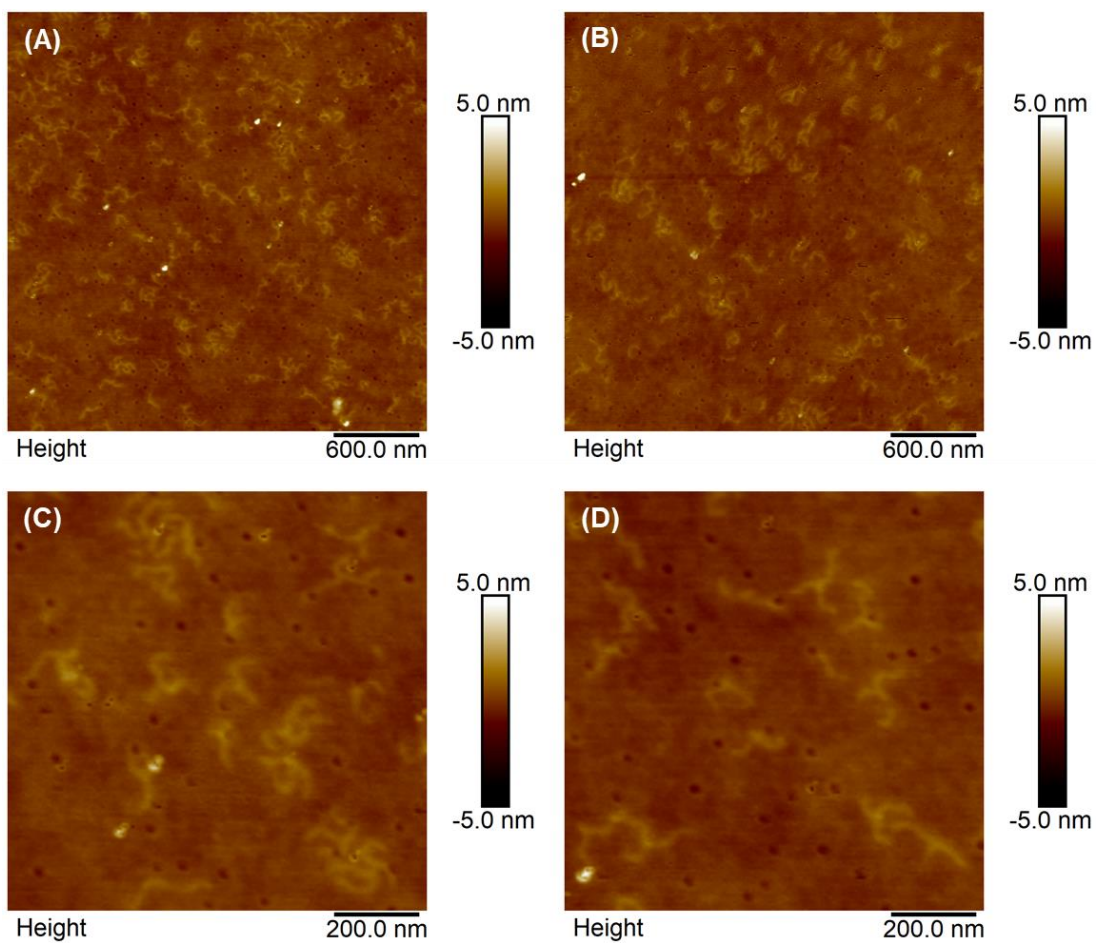
Figure S19. AFM height images of SMB-2 drop cast onto a glass disk from a 0.05 mg/g aqueous solution with a pH of 4.00 in the presence of 100 mM NaCl.