

**Stimuli-Responsive Polyelectrolytes: Thermosensitive
Zwitterionic Polymers and Charged Shape-Changing Star
Molecular Bottlebrushes**

A Dissertation Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Evan Michael Lewoczko

August 2021

Acknowledgements

I express my sincere thanks to my advisor, Professor Bin Zhao. Thank you for your instruction, vision, and advice. I will remember the lessons I have learned from you throughout my life.

I extend my gratitude to my committee, Professor Alexei Sokolov, Professor Michael Best, and Professor Tao Wu. Thank you for sharing your precious time and advice with me.

Additionally, I thank the National Science Foundation for providing funding for my research.

I thank all the past and current members of the Zhao Group, especially Dr. Daniel Henn, Dr. Roger Wright, Dr. Bryan Seymour, Dr. Sisi Jiang, Dr. Wenxin Fu, Dr. Ethan Kent, Dr. Bo Liu, Dr. Ning Wang, Jessica Holmes, Andrew Chancellor, Caleb Bohannon, and Michael Kelly. You have been my friends, advisors, and encouragers.

Finally, to my family. I can offer but feeble words in comparison to the well of love I have for you all. To my parents, John and Dawn Lewoczko. You never cease to believe in, encourage, and love me. Ken, April, Mark, Jeffrey, Stephen, and Esther, you have been a constant source of rest, joy, and peace. You strengthen, encourage, and love me. Thank you.

Abstract

This dissertation work investigated two classes of stimuli-responsive polyelectrolytes: thermosensitive zwitterionic poly(sulfobetaine methacrylate)s (PSBMAs) and charged shape-changing star molecular bottlebrushes (SMBs). While zwitterionic polymers are explored for numerous applications, their structure-solution behavior relationship was poorly understood. The first part of this dissertation focused on the effects of *N*-substituents of PSBMAs on their behavior in water. A series of PSBMAs were synthesized, with systematically changed *N*-substituents, including symmetric *N*-*n*-alkyl substituents of various lengths and asymmetric *N*-substituents comprising one methyl and either one cyclohexyl, phenyl or 2-hydroxyethyl group. The behavior of PSBMAs with symmetric *N*-substituents in water varied from upper critical solution temperature (UCST) to water-soluble, lower critical solution temperature (LCST), and water-insoluble with increasing *N*-*n*-alkyl length due to two competing effects: weakening of zwitterionic attractive interactions and increasing of hydrophobic effect. For PSBMAs with asymmetric *N*-substituents, while substituting the *N*-*n*-hexyl group with cyclohexyl diminished the hydrophobic effect, making the LCST disappear, introducing an *N*-phenyl or *N*-2-hydroxyethyl substituent enhanced zwitterionic interactions, producing UCST-type thermoresponsive polymers. A collaborative study showed that LCST-type thermoresponsive PSBMA-based polymers were effective kinetic hydrate inhibitors for gas and oil transportation. The second part of this dissertation focused on star-globule shape transitions of three-arm SMBs composed of heterografted poly(ethylene oxide) (PEO) and either protonated or iodomethane-quaternized poly(*N,N*-dialkylaminoethyl methacrylate) side chains in aqueous solution. Super and moderate chaotropic anions (CAs) were shown to induce star-globule shape changes of protonated SMBs, caused by the ion pairing of CAs with ammonium cations and chaotropic effect. While super CA

(e.g., $[\text{Fe}(\text{CN})_6]^{3-}$)-collapsed SMBs remained in the globular state in acidic water upon heating to 70 °C, moderate CA (e.g., ClO_4^-)-collapsed brushes exhibited globule-to-star shape transitions. In contrast, weak chaotropic I^- and kosmotropic SO_4^{2-} had only small effects on the conformations of protonated SMBs in the studied salt concentration and temperature ranges. For SMBs with permanently quaternary ammonium groups in water, the size transitions induced by super and moderate CAs occurred at salt concentrations much lower than those of unquaternized SMBs. These findings not only improve our understanding of the behavior of polyelectrolytes but also provide guidelines for design of stimuli-responsive polyelectrolytes for potential applications.

Table of Contents

Chapter 1: Introduction	1
1. Introduction.....	2
1.1. Introduction to Stimuli-Responsive Polymers.....	2
1.2. Introduction to Molecular Bottlebrushes	9
1.3. Overview of Dissertation Research Work	23
References.....	27
Chapter 2: Effects of <i>N</i>-Substituents on Solution Behavior of Poly(sulfobetaine methacrylate)s in Water: UCST and LCST	35
Abstract.....	36
2.1. Introduction.....	37
2.2. Experimental Section.....	40
2.2.1. Materials	40
2.2.2. Characterization	41
2.2.3. Synthesis of Zwitterionic DPSMA Sulfobetaine Methacrylate Monomers with Various <i>N</i> -Substituents	42
2.2.4. Synthesis of Zwitterionic Homopolymers of M11, M22, M44, M55, M66, M16, M1C6, M1P6, and M12OSi by AIBN-Initiated Conventional Radical Polymerization	58
2.2.5. Reversible Addition-Fragmentation Chain Transfer (RAFT) Polymerization of M1P6	59

2.2.6. Synthesis of P(M1P6- <i>co</i> -M22) Copolymers with Various Molar Ratios of M1P6 to M22	62
2.2.7. Determination of Cloud Points of Thermoresponsive Zwitterionic Polymers in Water	64
2.2.8. Measurement of Intrinsic Viscosities of Zwitterionic Polymers in TFE	65
2.3. Results and Discussion	66
2.3.1. Synthesis and Characterization of PDPSMAs	66
2.3.2. Solution Behavior of PDPSMAs with Two Symmetric <i>N-n</i> -Alkyl Substituents in Water.....	68
2.3.3. Solution Behavior of P12OH with a Hydroxyethyl <i>N</i> -Substituent in Water.....	77
2.3.4. Solution Behavior of P1C6 and P1P6 in Water.....	79
2.3.5. Tuning of UCST Transitions by Copolymerization of M1P6 and M22	83
2.4. Conclusions.....	86
References.....	89
Appendix A.....	98
Chapter 3: Zwitterionic Poly(sulfobetaine methacrylates) as Kinetic Gas Hydrate Inhibitors	131
3.1. Introduction.....	133
3.2. Experimental Section.....	134
3.2.1. Materials	134
3.2.2. Instrumentation and Characterization Methods.....	136

3.2.3. Synthesis of Zwitterionic Polymers P14, P15, P16-1, P16-2, and P16-3	137
3.2.4. Synthesis of Zwitterionic Homopolymers P55 and P16-4 by Conventional Free Radical Polymerization Using AIBN as Initiator.....	138
3.2.5. Synthesis of Zwitterionic Copolymers PCO-1 and -2 by Conventional Free Radical Copolymerization of M6 and <i>N</i> -Isopropylmethacrylamide Using AIBN as Initiator	139
3.3. Results and Discussion	139
3.3.1. Synthesis of Zwitterionic PSBMA Homopolymers with Various <i>N</i> - <i>n</i> -Alkyl Substituents	139
3.3.2. Synthesis of Random Copolymers of M16 and <i>N</i> -Isopropyl Methacrylate	148
3.3.3. KHI Performance Testing of Zwitterionic Polymers.....	148
3.4. Conclusions.....	151
References.....	153
Chapter 4: Effects of Temperature on Chaotropic Anion-Induced Shape Transitions of Star Molecular Bottlebrushes with Heterografted Poly(ethylene oxide) and Poly(<i>N,N</i>-dialkylaminoethyl methacrylate) Side Chains in Acidic Aqueous Solutions.....	157
Abstract.	158
4.1. Introduction.....	160
4.2. Experimental Section.....	165
4.2.1. Materials	165
4.2.2. General Characterization	166
4.2.3. Synthesis of Alkyne End-Functionalized Poly(ethylene oxide) (PEO).....	167

4.2.4. Synthesis of Alkyne End-Functionalized Poly(2-(<i>N,N</i> -dimethylamino)ethyl methacrylate) (PDMAEMA) by Atom Transfer Radical Polymerization	168
4.2.5. Synthesis of Alkyne End-Functionalized Poly(2-(<i>N,N</i> -diethylamino)ethyl methacrylate) (PDEAEMA) by Atom Transfer Radical Polymerization	169
4.2.6. Synthesis of Star Molecular Bottlebrushes Composed of Heterografted PEO and PDMAEMA Side Chains (SMB-11)	170
4.2.7. Synthesis of Star Molecular Bottlebrushes Composed of Heterografted PEO and PDEAEMA Side Chains (SMB-22).....	171
4.2.8. Dynamic Light Scattering (DLS) Study of SMB-11 and -22 in Acidic Aqueous Solutions	172
4.2.9. Atomic Force Microscopy (AFM) Study of SMB-11 and -22 Under Various Conditions	173
4.3. Results and Discussion	174
4.3.1. Synthesis and Characterization of SMBs with Heterografted PEO and Poly(2-(<i>N,N</i> -dialkylamino)ethyl methacrylate) Side Chains: SMB-11 and SMB-22	174
4.3.2. Dynamic Light Scattering Study of the Effects of Various Salts on Conformations of SMB-11 and -22 in pH 4.50 Acidic Water at 25 °C	180
4.3.3. Effects of Temperature on Super Chaotropic Anion-Induced Collapse of Star Brushes SMB-11 and -22.....	184
4.3.4. Effects of Temperature on Moderate Chaotropic Anion-Induced Collapse of Star Brushes SMB-11 and -22.....	188

4.3.5. Temperature Effects on Conformations of Star Brushes SMB-11 and -22 in the Presence of Weak Chaotropic Anion I^- and Kosmotropic Anion SO_4^{2-}	194
4.4. Conclusions.....	200
References.....	202
Appendix B.....	209
B.1. Calculation of Grafting Density of SMB-11 and -22.....	210
B.2. Calculation of Weight-Average Molecular Weights of SMB-11 and -22	211
Chapter 5: Chaotropic Anion-Induced Conformational Changes of Three-Arm Star Molecular Bottlebrushes with Heterografted Poly(ethylene oxide) and Poly((2-(methacryloyloxy)ethyl)trimethylammonium iodide) Side Chains in Water	249
Abstract.....	250
5.1. Introduction.....	251
5.2. Experimental Section.....	252
5.2.1. Materials	252
5.2.2. General Characterization	255
5.2.3. Synthesis of Star Molecular Bottlebrushes Composed of Heterografted PEO and PDMAEMA Side Chains (SMB-M).....	255
5.2.4. Synthesis of Star Molecular Bottlebrushes Composed of Heterografted PEO and PDMAEMA-Q Side Chains (SMB-Q)	256
5.2.5. Synthesis of Poly((2-(methacryloyloxy)ethyl)trimethylammonium iodide) (PDMAEMA-Q)	256

5.2.6. Dynamic Light Scattering Study of SMB-Q in Aqueous Solutions With Salts	257
5.2.7. Behavior of PDMAEMA-Q in Water in the Presence of NaClO ₄ Upon Heating	258
5.3. Results and Discussion	258
5.3.1. Synthesis and Characterization of SMB-Q with Heterografted PEO and Poly((2-(methacryloyloxy)ethyl)trimethylammonium iodide) Side Chains	258
5.3.2. Dynamic Light Scattering Study of the Effects of Various Salts on the Conformation of SMB-Q in Milli-Q Water at 25 °C	263
5.4. Conclusions.....	269
References.....	271
Chapter 6: Conclusions and Future Work.....	275
References.....	280
Vita	27581

List of Tables

2.1. Characterization Data and Solution Behavior in Water of Zwitterionic PDPSMA Homopolymers.....	67
2.2. Synthesis and Characterization of Zwitterionic Copolymers of M1P6 and M22	84
3.1. Summary of Characterization Data for Zwitterionic Polymers	140
4.1. Characterization Data for Azide-Functionalized Star Backbone Polymer PHEMA-N ₃ , Alkyne End-Functionalized PEO, PDMAEMA and PDEAEMA, and Three-Arm Star-Shaped Bottlebrush Polymers (SMB-11 and -22).	177
5.1. Characterization Data for PHEMA-N ₃ , Alkyne End-Functionalized PEO, PDMAEMA, SMB-M, SMB-Q, and PDMAEMA-Q.	262

List of Figures

1.1. As the length of one <i>N</i> -alkyl substituent was increased in the poly(sulfobetaine), the solution behavior changed from UCST, to soluble, to LCST, and insoluble.	10
1.2. AFM height images obtained from spin cast 0.05 mg/g aqueous solutions at 0 °C of cross-linked BMB-5k onto freshly cleaved mica. The samples were diluted from the UV irradiated samples at 25 mg/g solution (A and B) and 100 mg/g (C and D).....	19
1.3. (A) DLS of 0.1 mg/g aqueous solution of SMB-1 at pH 4.00 at 25 °C for (A) D_h values for a series of salts. (B) representative size distributions $[\text{Fe}(\text{CN})_6]^{3-}$ at 0.01, 0.14, and 0.50 mM.	22
1.4. Deuterated solvent normalized ^1H NMR spectra with different concentrations of $[\text{Fe}(\text{CN})_6]^{3-}$ in 4.0 mg/g solutions of A) SMB-1 and B) SMB-2 in D_2O at pH 4.00.....	24
2.1. Optical photos of 1.0 wt% aqueous solutions of P11 (A), P22-1 (B), P44-2 (C), P55-1 (D), P12OH-1 (E), P16-2 (F), P1C6-2 (G), and P1P6-4 (H) at 10, 65, and 90 °C.....	70
2.2. Plots of transmittance at the wavelength of 500 nm versus temperature for (A) 1.0 wt% P11 in Milli-Q water from a cooling-heating cycle and (B) 1.0 wt% P55-1 in Milli-Q water from a heating-cooling cycle. (C) Plots of transmittance versus temperature for aqueous solutions of P55-1 with various concentrations from the heating processes, and (D) plot of cloud point versus polymer concentration obtained from (C).	71
2.3. ^1H NMR spectra of thermoresponsive zwitterionic PDPSMA homopolymers with different <i>N</i> -substituents in D_2O at various temperatures: (A) 10 mg/g P55-1 (CP = 39 °C by visual inspection), (B) 4 mg/g P12OH-2 (CP = 23 °C by visual inspection), (C) 10 mg/g P16-2 (CP = 11 °C by visual inspection), and (D) 2 mg/g P1P6-4 (CP = 41 °C by visual inspection) at	

various temperatures from variable temperature ^1H NMR spectroscopy analysis studies. The HDO peak at 4.79 ppm was used to normalize all spectra for each polymer.	73
2.4. Plots of transmittance at the wavelength of 500 nm versus temperature for P12OH-1 (A) and P12OH-2 (B) in water at a concentration of 1.0 wt% from a cooling-heating cycle. (C) Plots of transmittance versus temperature for P12OH-1 in water at different concentrations from cooling processes and (D) plot of cloud point versus concentration for P12OH-1 determined from (C).	78
2.5. (A) Plot of transmittance at the wavelength of 500 nm versus temperature for 1.0 wt% P16-1 in Milli-Q water from a heating-cooling cycle. (B) Plot of transmittance versus temperature for P1P6-4 at various concentrations upon cooling, and (C) plot of cloud point versus polymer concentration for P1P6-4 from (B).	80
2.6. (A) Optical photos of five copolymers of M1P6 and M22 (PCO-1, -2, -3, -4, and -5) in Milli-Q water at a concentration of 1.0 wt% at 10, 65, and 90 °C. (B) Plot of transmittance at the wavelength of 500 nm versus temperature for a 1.0 wt% aqueous solution of PCO-2, PCO-3, and PCO-4 upon cooling, and (C) Plot of cloud point versus molar content of M1P6 in the copolymer.	85
A1. ^1H (A) and ^{13}C (B) NMR spectra of zwitterionic monomer M22 in D_2O	99
A2. ^1H (A) and ^{13}C (B) NMR spectra of zwitterionic monomer M44 in D_2O	100
A3. ^1H (A) and ^{13}C (B) NMR spectra of M55 in D_2O	101
A4. ^1H (A) and ^{13}C (B) NMR spectra of zwitterionic monomer M66 in D_2O	102
A5. ^1H (A) and ^{13}C (B) NMR spectra of zwitterionic monomer M1C6 in D_2O	103
A6. ^1H (A) and ^{13}C (B) NMR spectrum of zwitterionic monomer M1P6 in D_2O	104

A7. ^1H (A) and ^{13}C (B) NMR spectra of 3-((2-((<i>tert</i> -butyldimethylsilyl)oxy)ethyl)(2-(methacryloyloxy)ethyl)(methyl)ammonio)propane-1-sulfonate (M12OSi) in CDCl_3	105
A8. ^1H NMR spectrum of P11 in D_2O with 0.5 M NaCl.....	106
A9. ^1H NMR spectra of P22-1 (A) and P22-2 (B) in D_2O with 0.5 M NaCl.....	107
A10. ^1H NMR spectra of P44-1 (A) and P44-2 (B) in D_2O with 0.5 M NaCl.....	108
A11. ^1H NMR spectra of P55-1 (A) and P55-2 (B) in D_2O with 0.5 M NaCl.....	109
A12. ^1H NMR spectra of P66-1 (A) and P66-2 (B) in CD_3OD	110
A13. ^1H NMR spectra of P16-1 (A) and P16-2 (B) in D_2O with 0.5 M NaCl.....	111
A14. ^1H NMR spectra of (A) P1C6-1 and P1C6-2 (B) in D_2O with 0.5 M NaCl.	112
A15. ^1H NMR spectra of P1P6-1 (A), P1P6-2 (B), P1P6-3 (C), and P1P6-4 (D) in D_2O with 0.5 M NaCl.	113
A16. ^1H NMR spectra of P12OH-1 (A) and P12OH-2 (B) in D_2O with 0.5 M NaCl.	114
A17. Plots of $\ln\eta_{\text{rel}}/c$ (Kraemer plot) and η_{sp}/c (Huggins plot) versus polymer concentration for (A) P22-1 and (B) P1C6-2. $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for polymer solution and pure solvent, respectively, at 25.0 °C, and $\eta_{\text{sp}} = \eta_{\text{rel}} - 1$	115
A18. Plot of $\ln\eta_{\text{rel}}$ versus polymer concentration for P11. $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for polymer solution and pure solvent, respectively, at 25.0 °C. Using Wolf's method, the slope is taken as the intrinsic viscosity of the zwitterionic polymer.....	116
A19. Plots of $\ln\eta_{\text{rel}}$ versus concentration for P22-1 (A) and P22-2 (B). $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.	117

A20. Plots of $\ln\eta_{\text{rel}}$ versus concentration for P44-1 (A) and P44-2 (B). $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.	118
A21. Plots of $\ln\eta_{\text{rel}}$ versus concentration for P55-1 (A) and P55-2 (B). $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.	119
A22. Plots of $\ln\eta_{\text{rel}}$ versus concentration for P66-1 (A) and P66-2 (B). $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.	120
A23. Plots of $\ln\eta_{\text{rel}}$ versus concentration for P16-1 (A) and P16-2 (B). $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.	121
A24. Plots of $\ln\eta_{\text{rel}}$ versus concentration for P1C6-1 (A) and P1C6-2 (B). $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.	122
A25. Plot of $\ln\eta_{\text{rel}}$ versus concentration for (A) P1P6-1, (B) P1P6-2, (C) P1P6-3, and (D) P1P6-4. $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.	123
A26. Plot of $\ln\eta_{\text{rel}}$ versus concentration for (A) P12OH-1 and (B) P12OH-2. $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.	124

A27. Size exclusion chromatography (SEC) curves of zwitterionic P1P6-2, P1P6-3, and P1P6-4 synthesized by reversible addition-fragmentation chain transfer polymerization using 0.2 M NaNO ₃ aqueous solution as eluent.....	125
A28. Plot of transmittance at the wavelength of 500 nm versus temperature for P55-2 in Milli-Q water at a concentration of 1.0 wt% in a heating-cooling cycle.	126
A29. Plot of transmittance at the wavelength of 500 nm versus temperature for P16-2 in Milli-Q water at a concentration of 1.0 wt% from a heating-cooling cycle.....	127
A30. (A) Plots of transmittance at the wavelength of 500 nm versus temperature for P1P6-3 in Milli-Q water at concentrations of 0.04, 0.06, 0.08, 0.15, and 0.2 wt% upon cooling. (B) Plot of cloud point as a function of P1P6-3 concentration in water obtained from (A).....	128
A31. ¹ H NMR spectra of (A) PCO-1, (B) PCO-2, (C) PCO-3, (D) PCO-4, and (E) PCO-5 (zwitterionic copolymers of M22 and M1P6 with different compositions) in D ₂ O with 0.5 M NaCl.	129
A32. Plot of $\ln\eta_{\text{rel}}$ versus concentration for (A) PCO-1, (B) PCO-2, (C) PCO-3, (D) PCO-4, and (E) PCO-5. $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.	130
3.1. (A) ¹ H NMR spectrum of P14 in D ₂ O and (B) SEC curve of P14 from an aqueous SEC system using a 0.2 M aqueous solution of NaNO ₃ as eluent.	141
3.2. (A) ¹ H NMR spectrum of P15 in D ₂ O and (B) SEC curve of P15 from an aqueous SEC system using a 0.2 M aqueous solution of NaNO ₃ as eluent.	142
3.3. (A) ¹ H NMR spectrum of P16-1 in D ₂ O and (B) SEC curve of P16-1 from an aqueous SEC system using a 0.2 M aqueous solution of NaNO ₃ as eluent.	143

3.4. Plots of $\ln\eta_{\text{rel}}$ versus polymer concentration for P16-2, P16-3, P16-4, and P55. From the slope determined from linear fit for each plot, the $[\eta]$ value was 0.116 dL/g for P16-2, 0.166 dL/g for P16-3, 0.442 dL/g for P16-4, and 0.490 dL/g for P55.	145
3.5. ^1H NMR spectra of P16-2, P16-3, and P16-4 in D_2O with 0.5 M NaCl.....	146
3.6. (A) ^1H NMR spectrum of P55 in D_2O and (B) SEC curve of P55 from an DMF SEC system using DMF with 50 mM LiBr as eluent.	147
3.7. ^1H NMR spectra of PCO-1 and PCO-2 in D_2O	149
3.8. Plots of $\ln\eta_{\text{rel}}$ versus polymer concentration for PCO-1 and PCO-2. The $[\eta]$ was 0.350 dL/g for PCO-1 and 0.286 dL/g for PCO-2.....	150
3.9. T_o and T_a values obtained from slow constant cooling tests for KHI performance at a polymer concentration of 0.25 wt%.	152
4.1. AFM height images ($1 \times 1 \mu\text{m}$) of SMB-11 (A) and SMB-22 (B) drop cast on glass disks from 0.050 mg/g brush polymer solutions in pH 4.50 acidic water at room temperature, with a cross-sectional profile along the dashed line in each image.	179
4.2. Apparent hydrodynamic diameter (D_h) at 25 °C of 0.2 mg/g SMB-11 (A) and SMB-22 (B) in pH 4.50 water as a function of increasing concentration of a salt from dynamic light scattering (DLS) measurements for $\text{Na}_4[\text{Fe}(\text{CN})_6]$ (black square), $\text{K}_3[\text{Fe}(\text{CN})_6]$ (red circle), NaPF_6 (navy up-pointing triangle), NaClO_4 (blue down-pointing triangle), NaI (magenta rhombus), and Na_2SO_4 (dark cyan left-pointing triangle). (C) and (D) Hydrodynamic size distributions of SMB-11 in the presence of 0.01 and 6.5 mM PF_6^- and SMB-22 in the presence of 0.01 and 50 mM ClO_4^-	182
4.3. (A) Apparent hydrodynamic size (D_h) of 0.2 mg/g SMB-11 in pH 4.50 acidic water in the presence of 0.25 mM (black square), 0.50 mM (red circle), and 0.80 mM (blue triangle)	

- $K_3[Fe(CN)_6]$ as a function of temperature. (B) D_h of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 0.12 mM $K_3[Fe(CN)_6]$ as a function of temperature. 185
- 4.4.** Atomic force microscopy height images ($1 \times 1 \mu m$) of SMB-11 drop cast on glass disks at 20 °C (A) and 70 °C (B) from a 0.050 mg/g SMB-11 solution in pH 4.50 acidic water in the presence of 0.075 mM $K_3[Fe(CN)_6]$, with a cross-sectional profile along the dashed line in each image. The solution used to prepare AFM samples was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 0.30 mM $K_3[Fe(CN)_6]$ using pH 4.50 acidic water. 187
- 4.5.** Dynamic light scattering studies of SMB-11 and SMB-22 in pH 4.50 acidic water in the presence of $NaPF_6$. (A) Apparent hydrodynamic size (D_h) of 0.2 mg/g SMB-11 in pH 4.50 water with 4.0 mM $NaPF_6$ as a function of temperature. (B) Hydrodynamic size distributions of 0.2 mg/g SMB-11 in pH 4.50 water with 4.0 mM $NaPF_6$ at 5 and 70 °C. (C) Plots of D_h of 0.2 mg/g SMB-22 in pH 4.50 water in the presence of 2.5, 3.0, 3.5, and 4.0 mM $NaPF_6$ versus temperature upon heating. (D) Plot of D_h of 0.2 mg/g SMB-22 in pH 4.50 water in the presence of 3.0 mM $NaPF_6$ versus temperature from heating and cooling processes. All data points correspond to a single-size distribution (Figure B21). 189
- 4.6.** (A) Plots of apparent hydrodynamic size (D_h) of 0.2 mg/g SMB-11 in pH 4.50 water in the presence of 125 mM (black square), 150 mM (red circle), and 200 mM (blue triangle) $NaClO_4$ versus temperature. (B) Plot of D_h of 0.2 mg/g SMB-11 in pH 4.50 water with 125 mM $NaClO_4$ versus from a heating and cooling cycle. (C) Plot of D_h of 0.2 mg/g SMB-22 in pH 4.50 water with 50 mM $NaClO_4$ versus temperature. All data points correspond to a single-size distribution (Figures B22 and B23). 191
- 4.7.** AFM height images ($1 \times 1 \mu m$) of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water with 1.5 mM $NaPF_6$ at 20 °C (A) and 70 °C (B), with a cross-

sectional profile along the dashed lin in each image. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-11 with 6 mM NaPF ₆ using pH 4.50 water.....	193
4.8. AFM height images of SMB-22 drop cast on a glass disk from a 0.050 mg/g solution of SMB-22 in pH 4.50 water with 1.0 mM NaPF ₆ at 0 °C (A) and 70 °C (B), with a cross-sectional profile along the dashed line in each image. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-22 with 4 mM NaPF ₆ using pH 4.50 water.....	195
4.9. (A) Plot of apparent hydrodynamic size (D_h) of 0.2 mg/g SMB-11 in pH 4.50 water in the presence of 50 mM NaI versus temperature. (B) Plot of D_h of 0.2 mg/g SMB-22 in pH 4.50 water in the presence of 10 mM NaI versus temperature. All data points correspond to a single hydrodynamic size distribution (Figures B30 and B31). (Note that in (A) at 45 °C large species were observed and the data was not included.)	196
4.10. (A) Plot of apparent hydrodynamic size (D_h) of 0.2 mg/g SMB-11 in pH 4.50 water with 5 mM Na ₂ SO ₄ versus temperature. (B) Plot of D_h of 0.2 mg/g SMB-22 in pH 4.50 acidic water with 50 mM Na ₂ SO ₄ versus temperature. All the data points are single size distributions.	198
4.11. AFM height images of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-22 in pH 4.50 water with 2.5 mM Na ₂ SO ₄ at 20 °C (A) and 70 °C (B), with a cross-sectional profile along the dashed line in each image. The solution was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 10 mM Na ₂ SO ₄ using pH 4.50 water.	199
B1. (A) Size exclusion chromatography (SEC) trace and (B) ¹ H NMR spectrum in CDCl ₃ of alkyne-end-functionalized poly(<i>N,N</i> -dimethylaminoethyl methacrylate) (PDMAEMA) with a degree of polymerization of 56. The SEC chromatogram was obtained from a SEC system equipped with GRAL columns using DMF with 50 mM LiBr as eluent.....	212

B2. Size exclusion chromatograph (SEC) trace (A) and ^1H NMR spectrum in CDCl_3 (B) of alkyne-end-functionalized PDEAEMA with a degree of polymerization (DP) of 53. The SEC chromatogram was obtained from a SEC system equipped with GRAL columns using DMF with 50 mM LiBr as eluent.	213
B3. Size exclusion chromatography (SEC) curves of the reaction mixture at the end of the reaction for the synthesis of SMB-11 (red) and the purified brushes, SMB-11 (blue), obtained using a SEC system equipped with GRAL columns and <i>N,N</i> -dimethylformamide containing 50 mM LiBr as the eluent.	214
B4. Size exclusion chromatography (SEC) curves of the final reaction mixture for the synthesis of SMB-22 (red) and the purified brushes, SMB-22 (blue), obtained using a SEC system equipped with GRAL columns and <i>N,N</i> -dimethylformamide containing 50 mM LiBr as the eluent.	215
B5. (A) ^1H NMR spectrum of purified SMB-11 in CDCl_3 . (B) SEC trace of purified SMB-11 from a GPC-MALS system using DMF with 0.10 M LiBr as the eluent.	216
B6. (A) ^1H NMR spectrum of purified SMB-22 in CDCl_3 and (B) SEC trace of purified SMB-22 obtained from a GPC-MALS system using DMF with 0.10 M LiBr as the eluent.	217
B7. Hydrodynamic size distribution of SMB-11 in an acidic water with a pH of 4.50 at a concentration of 0.2 mg/g obtained from dynamic light scattering (DLS) measurements at 25 °C.	218
B8. Hydrodynamic size distribution of SMB-22 in pH 4.50 acidic water at a concentration of 0.2 mg/g obtained from dynamic light scattering (DLS) measurements at 25 °C.	219
B9. Atomic force microscopy (AFM) height images ($3 \times 3 \mu\text{m}$) of SMB-11 spin cast onto bare mica from THF at a concentration of 0.05 mg/g at room temperature ($\sim 20^\circ\text{C}$).....	220

B10. Atomic force microscopy (AFM) height images ($1 \times 1 \mu\text{m}$) of SMB-11 spin cast onto bare mica from THF at a brush polymer concentration of 0.05 mg/g at room temperature ($\sim 20^\circ\text{C}$), with a cross-sectional height profile corresponding to the dashed line in each image.	221
B11. Atomic force microscopy (AFM) height images of SMB-11 drop cast on glass disks from a 0.050 mg/g SMB-11 solution in pH 4.50 acidic water at room temperature ($\sim 20^\circ\text{C}$), with a cross-sectional profile along the dashed line of the $1 \times 1 \mu\text{m}$ image.....	222
B12. Atomic force microscopy (AFM) height images of SMB-22 drop cast on glass disks from a 0.050 mg/g SMB-22 solution in pH 4.50 acidic water at room temperature ($\sim 20^\circ\text{C}$), with a cross-sectional profile along the dashed line in each of the bottom $1 \times 1 \mu\text{m}$ images..	223
B13. Hydrodynamic size distributions of 0.2 mg/g SMB-11 in pH 4.50 acidic water in the presence of $\text{Na}_4[\text{Fe}(\text{CN})_6]$ (A), $\text{K}_3[\text{Fe}(\text{CN})_6]$ (B), NaClO_4 (C), NaI (D), and Na_2SO_4 (E) at one low and one high concentration, obtained from DLS measurements at 25°C . These size distributions are for the corresponding data points in Figure 4.2A.	224
B14. Hydrodynamic size distributions of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of (A) $\text{Na}_4[\text{Fe}(\text{CN})_6]$, (B) $\text{K}_3[\text{Fe}(\text{CN})_6]$, (C) NaPF_6 , (D) NaI , and (E) Na_2SO_4 at one low and one high concentration, obtained from dynamic light scattering measurements at 25°C . These size distributions are for the corresponding data points in Figure 4.2B.	225
B15. (A) Apparent hydrodynamic size (D_h) of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 0.2 mM $\text{Na}_4[\text{Fe}(\text{CN})_6]$ as a function of temperature. (B) Hydrodynamic size distributions of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 0.2 mM $\text{Na}_4[\text{Fe}(\text{CN})_6]$ at 25 and 70°C	226

B16. Hydrodynamic size distributions of 0.2 mg/g SMB-11 in pH 4.50 acidic water in the presence of 0.25 mM (A), 0.50 mM (B), and 0.80 mM $K_3[Fe(CN)_6]$ (C) at one low and one high temperature. These size distributions are for the corresponding data point in Figure 4.3A.	227
B17. Hydrodynamic size distributions of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 0.12 mM $K_3[Fe(CN)_6]$ at 20 and 60 °C shown in Figure 4.3B in the main text.....	228
B18. Atomic force microscopy height images ($3 \times 3 \mu m$) of SMB-11 drop cast on glass disks at 20 °C from a 0.050 mg/g SMB-11 in pH 4.50 acidic water in the presence of 0.075 mM $K_3[Fe(CN)_6]$. The solution used to prepare AFM samples was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 0.30 mM $K_3[Fe(CN)_6]$ using pH 4.50 acidic water.	229
B19. Atomic force microscopy height images ($1 \times 1 \mu m$) of SMB-11 drop cast on glass disks at 20 °C from a 0.050 mg/g SMB-11 in pH 4.50 acidic water in the presence of 0.075 mM $K_3[Fe(CN)_6]$, with a cross-sectional profile along the dashed line in each image. The solution used to prepare AFM samples was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 0.30 mM $K_3[Fe(CN)_6]$ using pH 4.50 acidic water.....	230
B20. Atomic force microscopy height images of SMB-11 drop cast on glass disks at 70 °C from a 0.050 mg/g SMB-11 in pH 4.50 acidic water in the presence of 0.075 mM $K_3[Fe(CN)_6]$, with cross-sectional profiles along the dashed lines in the $1 \times 1 \mu m$ images. The solution used to prepare AFM samples was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 0.30 mM $K_3[Fe(CN)_6]$ using pH 4.50 acidic water.	231
B21. Hydrodynamic size distributions of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of (A) 2.5 mM, (B) 3.0 mM (heating), (C) 3.0 mM (cooling), (D) 3.5 mM, and (E) 4.0 mM	

NaPF ₆ at 5 and 70 °C, obtained from DLS measurements. These size distributions are for the corresponding data points in Figure 4.5C and D.	232
B22. Hydrodynamic size distributions of 0.2 mg/g SMB-11 in pH 4.50 acidic water in the presence of NaClO ₄ with various concentrations at one low and one high temperature, corresponding to the data points in Figure 4.6A and B.	233
B23. Hydrodynamic size distributions of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 50 mM NaClO ₄ at 20 and 70 °C for the corresponding data points in Figure 4.6C.	234
B24. AFM height images (3 × 3 μm) of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water in the presence of 1.5 mM NaPF ₆ at 20 °C. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-11 with 6 mM NaPF ₆ using pH 4.50 water.	235
B25. AFM height images (1 × 1 μm) of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water in the presence of 1.5 mM NaPF ₆ at 20 °C, with cross-sectional profiles along the dashed lines in the bottom two 1 × 1 μm images. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-11 with 6 mM NaPF ₆ using pH 4.50 water.	236
B26. AFM height images (1 × 1 μm) of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water in the presence of 1.5 mM NaPF ₆ at 70 °C, with cross-sectional profiles along the dashed lines in the bottom two images. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-11 with 6 mM NaPF ₆ using pH 4.50 water. ...	237
B27. AFM height images of SMB-22 drop cast on glass disks from a 0.050 mg/g solution of SMB-22 in pH 4.50 water in the presence of 1.0 mM NaPF ₆ at 0 °C. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-22 with 4 mM NaPF ₆ using pH 4.50 water.	238

B28. AFM height images of SMB-22 drop cast on glass disks from a 0.050 mg/g solution of SMB-22 in pH 4.50 water in the presence of 1.0 mM NaPF ₆ at 0 °C, with cross-sectional profiles along the dashed lines in the bottom two images. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-22 with 4 mM NaPF ₆ using pH 4.50 water.....	239
B29. Atomic force microscopy (AFM) height images of SMB-22 drop cast on glass disks from a solution of 0.050 mg/g SMB-22 in pH 4.50 acidic water in the presence of 1.0 mM NaPF ₆ at 70 °C, with cross-sectional profiles along the dashed lines in the bottom two images. The solution was diluted with pH 4.50 water from a 0.2 mg/g solution of SMB-22 with a pH of 4.50 in the presence of 4 mM NaPF ₆	240
B30. Hydrodynamic size distributions of 0.2 mg/g SMB-11 in pH 4.50 acidic water in the presence of 50 mM NaI at 5 and 70 °C for the corresponding data points in Figure 4.9A.	241
B31. Hydrodynamic size distribution of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 10 mM NaI at 20 and 65 °C for the corresponding data points in Figure 4.9B.....	242
B32. Hydrodynamic size distributions of 0.2 mg/g SMB-11 in pH 4.50 acidic water in the presence of 5 mM NaSO ₄ at one low (15 °C) and one high temperature (65 °C) for the corresponding data points in Figure 4.10A.....	243
B33. Hydrodynamic size distribution of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 50 mM Na ₂ SO ₄ at 5 and 65 °C for the corresponding data points in Figure 4.10B.	244
B34. AFM height images (3 × 3 μm) of SMB-11 drop cast on a glass disk from a solution of 0.050 mg/g SMB-11 in pH 4.50 water with 2.5 mM Na ₂ SO ₄ at 20 °C. The solution was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 10 mM Na ₂ SO ₄ using pH 4.50 water.	245

B35. AFM height images ($1 \times 1 \mu\text{m}$) of SMB-11 drop cast on a glass disk from a solution of 0.050 mg/g SMB-11 in pH 4.50 water with 2.5 mM Na_2SO_4 at 20 °C, with cross-sectional profiles along the dashed lines in the bottom two $1 \times 1 \mu\text{m}$ images. The solution was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 10 mM Na_2SO_4 using pH 4.50 water.	246
B36. AFM height images ($3 \times 3 \mu\text{m}$) of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water with 2.5 mM Na_2SO_4 at 70 °C. The solution was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 10 mM Na_2SO_4 using pH 4.50 water.	247
B37. AFM height images of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water with 2.5 mM Na_2SO_4 at 70 °C, with a cross-sectional profile along the dashed line in each image. The solution was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 10 mM Na_2SO_4 using pH 4.50 water.	248
5.1. Size exclusion chromatography (SEC) curves of the reaction mixture at the end of the click reaction (red) for the synthesis of SMB-M and the purified brushes (blue). The SEC chromatograms were obtained on a SEC system equipped with GRAL columns and DMF containing 50 mM LiBr as the eluent at 50 °C.	260
5.2. ^1H NMR spectra of (A) purified SMB-M in CDCl_3 and (B) SMB-Q in D_2O	261
5.3. Apparent hydrodynamic diameter (D_h) of SMB-Q in Milli-Q water at 25 °C as a function of increasing salt concentration for sodium persulfate (black square), sodium perchlorate (blue circle), sodium thiocyanate (dark cyan up-pointing triangle), sodium salicylate (violet down-pointing triangle), sodium thiosalicylate (magenta diamond), and sodium sulfate (orange	

star). All data points shown here are single size distributions except the concentration of 10 mM for NaClO ₄	264
5.4. Hydrodynamic size distributions obtained from DLS measurements at 25 °C of 0.1 mg/g SMB-Q in Milli-Q water in the presence of Na ₂ S ₂ O ₈ (A), NaClO ₄ (B), NaSCN (C), Na ₂ SO ₄ (D), sodium salicylate (E), and sodium thiosalicylate (F) at various concentrations. Large species were observed at 10 mM NaClO ₄ in (B).	266
5.5. ¹ H NMR spectra of alkyne-end-functionalized PDMAEMA in CDCl ₃ (A) and PDMAEMA-Q in D ₂ O.	268
5.6. Optical photographs of quaternized alkyne end-functionalized PDMAEMA homopolymer, PDMAEMA-Q, at various concentrations of NaClO ₄ and temperatures in Milli-Q water.	270

List of Schemes

1.1. Sketches of Phase Diagrams for Polymer Solutions with (a) Lower Critical Solution Temperature (LCST) Behavior and (b) Upper Critical Solution Temperature (UCST) Behavior.....	4
1.2. Illustration of the Hydrophobic effect for the LCST of PNIPAAm..	6
1.3. Three Strategies for the Preparation of Molecular Bottlebrushes, Grafting-To, Grafting-Through, and Grafting-From.	12
1.4. Illustration of the Grafting-To Preparation of Binary Heterografted Molecular Bottlebrushes via CuAAC Click Chemistry.	16
1.5. Illustration of (A) Moderately Concentrated Shape Changing Behavior from Worm-to-Globule and (B) Grafting-To Preparation of Thermoresponsive Binary Heterografted Linear Molecular Bottlebrushes Containing UV-Cross-Linkable Groups.....	17
1.6. Synthetic Scheme of Heterografted Three-Arm Star Molecular Bottlebrushes.	20
1.7. Illustration of the Synthesis of Heterografted Star Molecular Bottlebrushes SMB-1 and SMB-2 using the Grafting-To Method using CuAAC Click Chemistry.	21
2.1. Molecular Structures of Zwitterionic Homopolymers of DPSMA Sulfobetaine Methacrylates with Various <i>N</i> -Substituents, Monomer M12OSi, and Copolymers of Two Monomers with Various Molar Contents.	38
2.2. Synthesis of M11, M22, and M44	43
2.3. Synthesis of Zwitterionic Monomer M55 (A) and M66 (B).....	45
2.4. Synthesis of Sulfobetaine Methacrylate Monomer M1C6.....	49
2.5. Synthesis of Sulfobetaine Methacrylate Monomer M1P6	54
2.6. Synthesis of Sulfobetaine Methacrylate Monomer M12OSi	56

2.7. Synthesis of Zwitterionic Homopolymers of Sulfobetaine Methacrylate by AIBN-Initiated Conventional Free Radical Polymerization	60
2.8. Synthesis of Zwitterionic Homopolymers by Reversible Addition-Fragmentation Chain Transfer Polymerization of M1P6.	61
2.9. Synthesis of Zwitterionic Copolymers of M1P6 and M22 by AIBN-Initiated Conventional Free Radical Polymerization	63
2.10. Hypothetical phase diagrams of zwitterionic PDPSMAs with various symmetric <i>N-n</i> -alkyl substituents in H ₂ O.	74
3.1. Molecular Structures of <i>n</i> -Butyl (2-Cyano-2-propyl) Trithiocarbonate (the Chain Transfer Agent), Zwitterionic Monomers, and Polymers.	135
4.1. Synthesis and Behavior of Three-Arm Star Molecular Bottlebrushes (SMBs) Composed of Poly(ethylene oxide) (PEO) and Poly(<i>N,N</i> -dimethylaminoethyl methacrylate) (PDMAEMA) (SMB-11) or Poly(<i>N,N</i> -diethylaminoethyl methacrylate) (PDEAEMA) Side Chains (SMB-22)	164
5.1. Synthesis of Polyelectrolyte Three-Arm Star Brushes SMB-Q.....	253

Chapter 1: Introduction

1. Introduction

This dissertation work is focused on the synthesis and responsive behavior of two classes of stimuli-responsive polyelectrolytes: thermoresponsive zwitterionic polymers, specifically poly(sulfobetaine methacrylate)s, and charged shape-changing molecular bottlebrushes (MBBs). Polyelectrolytes are charged polymers and can exhibit large conformational changes in aqueous solution in response to a variety of environmental stimuli, including temperature changes, surfactants, specific ions, and oppositely charged polyelectrolytes. Section 1.1 provides a general introduction to stimuli-responsive polymers first and then focus on thermoresponsive noncharged water-soluble polymers (Section 1.1.1) and thermoresponsive zwitterionic polymers (Section 1.1.2). Section 1.2 concentrates on molecular bottlebrushes (MBBs) and consists of two subsections; Section 1.2.1 gives a general description of MBBs, including main synthetic strategies, and Section 1.2.2. is devoted to stimuli-responsive shape-changing MBBs, with an emphasis on MBBs with bicomponent side chains. Finally, Section 1.3 presents an overview of this dissertation research work described in Chapters 2 to 5.

1.1. Introduction to Stimuli-Responsive Polymers

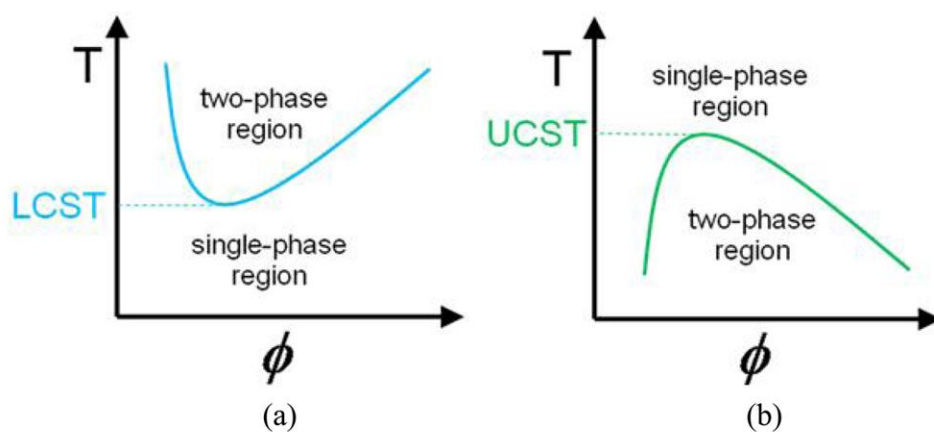
Stimuli-responsive polymers are a subcategory of polymers that exhibit large and abrupt physical or chemical changes in response to relatively small changes in their environment.¹ Stimuli fall into two categories: chemical stimuli (e.g., pH,² salts,³ small molecules,⁴ and solvent⁵) and physical stimuli (e.g., temperature,⁶ light,⁷ electric fields,⁸ magnetic fields,⁹ and mechanical stress¹⁰). These stimuli can cause changes in chain conformation, surface free energy, permeation rate, and optic, mechanical, and electrical property of polymers.^{1,11} Of these stimuli, pH and temperature are the most widely used and studied because they can be easily applied to polymer systems and are relevant to the biomedical field. Stimuli-responsive polymers are valuable for

fundamental studies and they have been used for applications such as sensors,¹² triggered drug delivery,¹³ environmental remediation,¹⁴ chemical actuators,¹⁵ etc.¹⁶

1.1.1. Thermoresponsive Noncharged Water-Soluble Polymers

Thermoresponsive polymers are among the most studied and understood stimuli-responsive polymers.^{1,16} Although thermoresponsive polymers have been reported in aqueous and organic solutions, water-soluble thermosensitive polymers are the more studied and especially show promise in biomedical applications (e.g., drug delivery, gene delivery, and tissue engineering).¹⁷ These polymers exhibit either upper critical solution temperature (UCST) or lower critical solution temperature (LCST) type behavior in water.¹⁸

LCST-type polymers undergo a distinct soluble-to-insoluble phase transition when the temperature is adjusted above a critical value. The LCST is the minimum of the isobaric temperature vs polymer concentration phase diagram (Scheme 1.1.a).¹⁸ Below the LCST, the polymer is miscible with the solvent at all concentrations. Cloud points for a specific polymer in water depend on several factors including concentration, molecular weight, and the presence of other solutes. Common examples of LCST-type polymers include poly(*N*-isopropylacrylamide) (PNIPAAm), poly(oligo(ethylene glycol) methacrylate)s, and poly(2-isopropyl-2-oxazoline).¹⁹ Of these, PNIPAAm is often used as the model LCST-type polymer likely due to the bio-relevant LCST of 32 °C across a broad concentration range. The LCST-type behavior of PNIPAAm arises from the interactions of the hydrophilic amide moieties, hydrophobic backbone, and isopropyl groups with water. While water interacts favorably with the amide moieties through hydrogen bonding, water does not hydrogen bond with the backbone and iso-propyl groups. Enthalpically, water interacts more favorably with itself than with the hydrophobic portions of the polymer. However, at cold enough temperatures, the thermal energy of the water molecules decreases



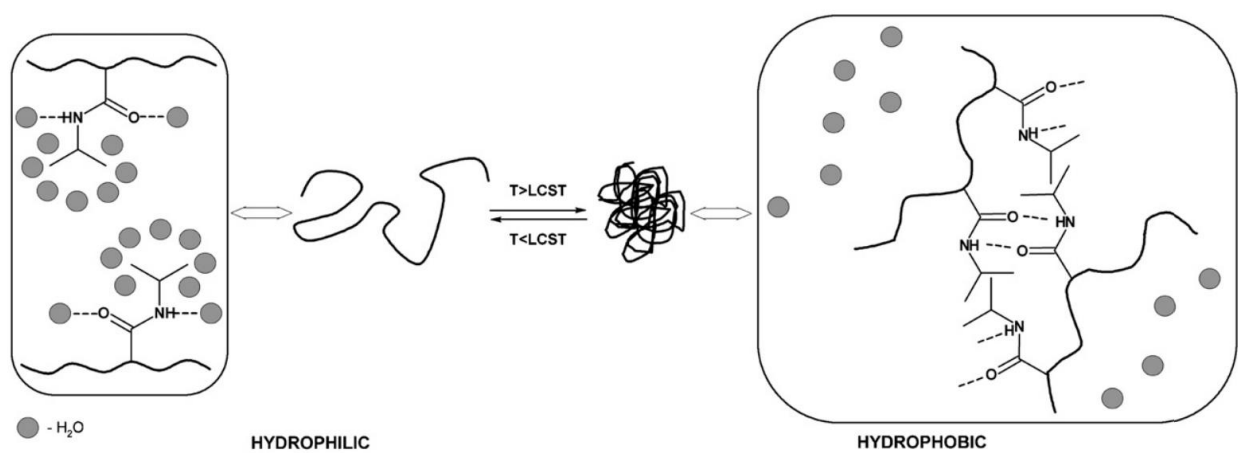
Scheme 1.1. Sketches of Phase Diagrams for Polymer Solutions with (a) Lower Critical Solution Temperature (LCST) Behavior and (b) Upper Critical Solution Temperature (UCST) Behavior.¹⁷ Reproduced from Ref. 17 with Permission from MDPI.

allowing the formation of ice-like water structures around the hydrophobic portions of PNIPAAm. The dissolution of the PNIPAAm is favorable at lower temperatures because the entropic penalty of forming the ice-like structures required to dissolve the polymer is small. As the temperature is raised across the LCST, the thermal energy of water molecules increases causing more vibration and rotation. This increased thermal energy breaks (or melts) the ice-like water structure around the hydrophobic groups, releasing the water molecules into bulk solution, causing phase separation (Scheme 1.2). Polymers with LCST-type solution behavior form the majority of thermoresponsive polymers.¹⁸

In contrast to LCST-type polymers, UCST-type polymers display the opposite trend with temperature (Scheme 1.1.b). In the isobaric phase diagram for UCST-type polymers, the water-soluble UCST-type polymers show a soluble-to-insoluble transition when decreasing the temperature across the curve. The maximum value of this phase diagram is the UCST. Common non-charged examples of UCST-type polymers primarily involve hydrogen bonding groups as in poly(*N*-acryloylglycinamide).²⁰ For UCST-type polymers, polymer-polymer interactions, such as hydrogen bonding, are favored at lower temperatures. But, as the temperature is raised across the UCST, increased thermal energy of water molecules breaks apart the polymer-polymer interactions in favor of water-polymer interactions.

1.1.2. Thermoresponsive Zwitterionic Polymers

Zwitterionic polymers are a special class of polyelectrolytes containing oppositely charged groups of equal magnitude in the repeat unit.²¹⁻²² Even though zwitterionic polymers possess a high concentration of charged groups, they are neutral both locally and globally. The high charge density grants common zwitterionic polymers high hydrophilicity,²¹⁻²² antipolyelectrolyte behavior,²³⁻²⁴ and stimuli-responsive behavior.²⁵⁻²⁷ Zwitterionic polymers have potential



Scheme 1.2. Illustration of the Hydrophobic effect for the LCST of PNIPAAm.²⁸ Reproduced from Ref 28 with Permission from Elsevier.

applications in anti(bio)fouling,²⁹⁻³⁰ lubrication,³¹⁻³² cryopreservation,³³ etc. Zwitterionic polymers can be structurally diverse.²¹⁻²² Of particular interest are UCST-type thermoresponsive zwitterionic polymers, such as poly(3-((2-methacryloyloxyethyl)dimethylammonio)propane-1-sulfonate) (PDMAPS) and poly(3-(*N*-(3-methacrylamidopropyl)dimethylammonio)propane-1-sulfonate) (PMAMPS).^{18, 22, 34-35} Due to the closely positioned oppositely charged groups on each repeat unit, zwitterionic polymers can display strong polymer-polymer attractive forces through intra-group, intra-chain, and inter-chain electrostatic interactions.¹⁸ Presumably, the competition between the electrostatic attractive interactions and the thermal energy causes UCST-type behavior for some zwitterionic polymers. In water, electrically charged groups can interact with either themselves or water molecules. In the case of PDMAPS, the positively charged ammonium and the negatively charged sulfonate can interact with each other. As temperature increases, the thermal energy overcomes the ionic attractive forces and the zwitterionic polymer dissolves into solution.

In 1958, Hart and Timmerman reported the first series of synthetic polysulfobetaines, and one of their polymers, poly(2-vinylpyridine *N*-butylsulfobetaine), was completely soluble at room temperature in pure water.³⁶ Later, Monroy Soto and Galin reported that PDMAPS was also soluble in water above 20 °C.²³ Then in 1986, shortly after the monomer of PDMAPS became commercially available, Garner and coworkers reported that PDMAPS displayed UCST-type behavior in water.²⁴ PDMAPS was synthesized by conventional free radical polymerization in water at 50 °C using ammonium sulfate as the initiator. Stock solutions of the polymer were prepared, diluted to appropriate concentrations, and equilibrated for 10 min at room temperature. Cloud points were determined by heating the solution well above the transition temperature and cooling in an ice/water bath. The temperature at which the solution first turned cloudy was taken

as the cloud point. The cloud point measurements were repeated by heating from well below the transition temperature to above. The cloud point was recorded as the temperature when the solution just turned transparent. The differences between the heating and cooling were within 4 °C.

Despite a wide variety of structures available in zwitterionic systems,²¹⁻²² most work on zwitterionic polymers has dealt with poly(sulfobetaine)s and some on poly(phosphorylcholine) and poly(3-(*N*-(3-methacrylamidopropyl)dimethylammonio)propane-1-sulfonate).^{18, 22, 34-35} Despite their potential importance in various applications, very little systematic work has been done to try to understand the structure-solution behavior relationship.

A series of poly(sulfobetaine) polymers were made by varying the number of carbon atoms between the ammonium and sulfonate groups from three to four, changing the *N,N*-substituents from dimethyl to piperidinyl to morpholinyl, varying the number of carbon atoms from 2 to 3 between the ammonium and ester group in the poly(sulfobetaine methacrylate) with two methyl groups at the nitrogen, and introducing a hydroxy group into the spacer between the ammonium and sulfonate group.³⁴⁻³⁵ The polymers were made by reversible addition fragmentation transfer (RAFT) polymerization using initiator V-501 in 2,2,2-trifluoroethanol at 75 °C. The number-average molecular weights were determined by comparing the UV-Vis absorbance of the chromophore end group of the RAFT chain transfer agent (CTA) to that of the polymer. The cloud points of the polymers were determined in 50 mg/g solutions in Milli-Q water by UV-Vis spectrometer. The cloud points were defined as the temperature at which the % transmittance decreased by 5% or 2% of the total difference between the maximum and minimum transmittances. In their series, incorporating a hydrophilic hydroxy group into the spacer between the ammonium and sulfonate group increased the UCST of the zwitterionic polymer in water (i.e., the solubility decreased).³⁵ However, increasing the spacer length from 3 to 4 carbon atoms also increased the

UCST and the solubility decreased. When the ammonium group was changed from the dimethylammonio moiety to the piperidinio ring, the polymer became completely soluble from 0-100 °C, while substituting the dimethylammonio group with a morpholino group maintained the UCST. The authors suggested that the observed differences might have to do with substituent sizes and various interactions.

Recently, Zhao and coworkers systematically studied a series of poly(sulfopropylbetaine methacrylate)s and observed an array of solution behavior (Figure 1.1).³⁷ The zwitterionic polymers were made by RAFT using AIBN as the initiator in 2,2,2-trifluoroethanol at 70 °C. The series was created by changing one of the two methyl substituents of PDMAAPS (P1 in Figure 1.1) to ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, *n*-hexyl, and *n*-heptyl groups. The solution behavior of the polymers changed from UCST for P1, to soluble for P2-P4, to LCST for P5 and P6, and insoluble for P7. This suggested that the solution behavior corresponded to the steric disruption of the zwitterionic electrostatic interactions. Further study was needed to establish more clearly a relationship between the structure and solution behavior of these poly(sulfobetaine)s.

1.2. Introduction to Molecular Bottlebrushes

1.2.1. Molecular Bottlebrushes: Concept, Synthesis, and Unique Characteristics

Molecular bottlebrushes (MBB)s are a subclass of graft copolymers in which side chain polymers are densely grafted onto a backbone polymer.³⁸⁻⁴² At high aspect ratios (brush contour length to diameter), MBBs adopt worm-like or cylindrical conformations in good solvents due to the steric interactions among the side chains. The densely grafted side chains lend unique characteristics such as adjustable persistence length, low chain entanglement, and large stimuli-responsive conformational changes. These characteristics have given increased attention to MBBs and given them potential in applications such as lubrication,³¹ supersoft elastomers,⁴³ and

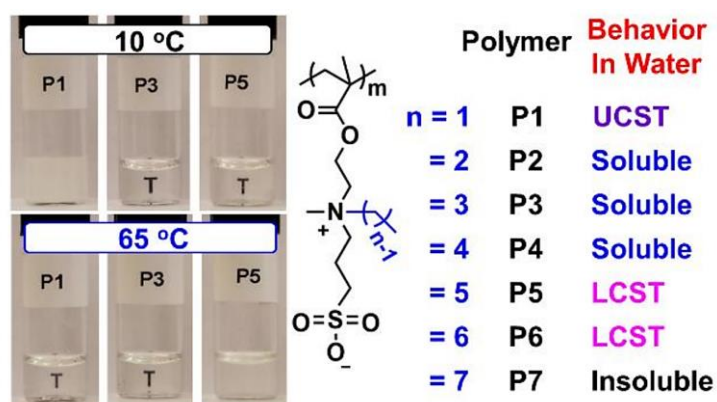


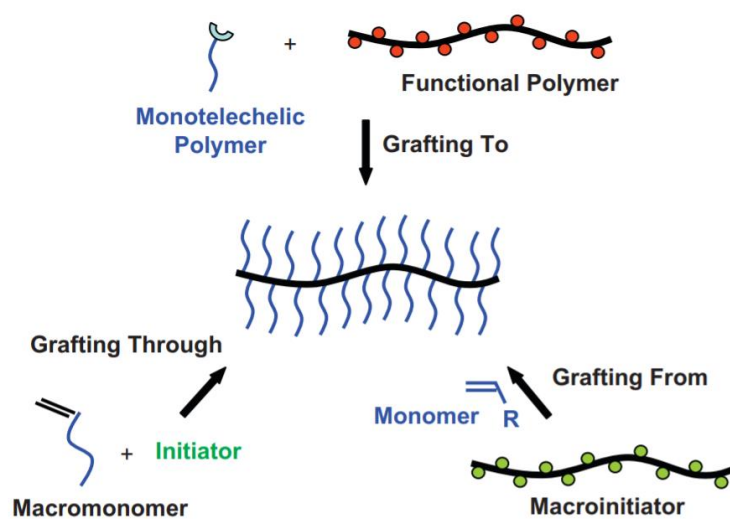
Figure 1.1. As the length of one *N*-alkyl substituent was increased in the poly(sulfobetaine), the solution behavior changed from UCST, to soluble, to LCST, and insoluble.³⁷ Reproduced from Ref 37 with permission from RSC.

nanomaterial templating.⁴⁴

With the advent of controlled/“living” polymerization techniques, development of well-defined MBBs with precise molecular weights, narrow dispersities, and varied architectures allowed the field to grow and receive more attention. Multiple structures have been prepared for molecular bottlebrushes with varied side chain composition, side chain distribution along the backbone, and backbone structure.⁴¹ These molecular bottlebrushes are prepared by one of three strategies: grafting-through, grafting-from, and grafting-to (Scheme 1.3).³⁸ For grafting-through, macromonomers are polymerized directly into MBBs. This method ensures a 100% grafting density for the brushes. However, owing to the relatively slow diffusion of macromonomers to ever increasing sterically bulky growing molecular bottlebrushes, achieving MBBs with high aspect ratios was challenging. This disadvantage has been somewhat mitigated by using ring-opening metathesis polymerization (ROMP) to give high molecular weight and well-defined molecular bottlebrushes.⁴⁵⁻⁴⁶ However, the norbornene monomer used in ROMP means a larger distance in the backbone between side chains compared with MBBs with a vinyl polymer backbone.

In the grafting-from technique, an initiator-functionalized backbone polymer is synthesized first and then the side chains are polymerized from the pendant initiating groups. This method generally gives high grafting densities and is well-suited for making homografted MBBs with only one type of side chain polymers.⁴⁷⁻⁴⁹ However, well-defined side chain polymers can be difficult to attain due to the increased steric hindrance around the side chains as they grow, which may lead to increased variability in the properties of the bottlebrushes. This method is especially difficult for growing different types of side chain polymers.

In the grafting-to preparation of MBBs, a backbone polymer is synthesized first with pendant



Scheme 1.3. Three Strategies for the Preparation of Molecular Bottlebrushes, Grafting-To, Grafting-Through, and Grafting-From.³⁸ Reproduced from Ref 38 with permission from Elsevier.

groups that can react with pre-formed side chain polymers containing complementary reactive groups. This method enables the synthesis of homografted and heterografted molecular bottlebrushes with well-defined backbone and side chain polymers and is compatible with a wide variety of side chains polymers. Obtaining high grafting densities by this method had been difficult due to the steric crowding at the backbone. However, with the use of click chemistry, such as the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, molecular bottlebrushes with excellent grafting densities have been prepared.⁵⁰⁻⁵³

1.2.2. Stimuli-Responsive Shape-Changing Molecular Bottlebrushes

When stimuli-responsive polymers are incorporated into molecular bottlebrushes as side chains, external stimuli can trigger abrupt changes to the size and shape of brushes. These stimuli-responsive side chain polymer-containing molecular bottlebrushes possess intriguing responsive properties, particularly unimolecular collapse and self-assembly into various morphologies.^{3,54-59} Earlier, Schmidt et al. reported the synthesis of thermoresponsive MBBs made by the grafting-from technique using ATRP to grow PNIPAAm side chains from the pendant α -bromoisobutyrate initiating sites on the backbone.⁵⁸ They observed by dynamic light scattering (DLS) and atomic force microscopy (AFM) that the brushes underwent unimolecular collapse from the worm-like to globular conformations under dilute conditions (0.03 mg/g) and in a limited temperature window. At temperatures greater than 38 °C, above the PNIPAAm transition temperature (32 °C), the brushes aggregated and precipitated. Later, thermoresponsive MBBs composed of a random copolymer of *N,N*-dimethylacrylamide and *n*-butyl acrylate as side chains grown by ATRP from the backbone were prepared.⁶⁰ From DLS, aqueous solutions of these MBBs under dilute conditions (0.1 mg/g) showed a decrease in the hydrodynamic diameter upon heating, indicating

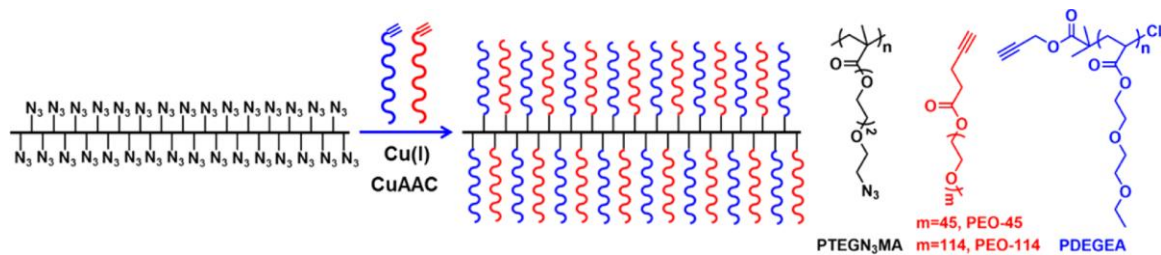
unimolecular collapse. But at higher concentrations (e.g., 1.0 mg/g), DLS showed an increase in size at temperatures above the LCST, indicating intermolecular aggregation.

Müller and coworkers showed that salt-responsive polyelectrolyte MBBs underwent aggregation except under highly dilute conditions.⁵⁹ A homografted linear MBB polymer composed of PDMAEMA side chains was made by grafting-from with ATRP, and then the PDMAEMA was quaternized using iodomethane. The salt-responsive behavior of the polyelectrolyte MBBs was tested at 0.2 mg/g with three salts, NaBr, $K_2[Ni(CN)_4]$, and $K_3[Co(CN)_6]$. For NaBr, DLS measurements showed an 8 nm decrease in D_h at 0.5 M NaBr. For the other salts, DLS measurements of the brushes at 0.2 mg/g showed an increase from 44 nm to 58 nm for $K_2[Ni(CN)_6]$ and to 68 nm for $K_3[Co(CN)_6]$, which indicated aggregation. Under very dilute conditions (0.02 mg/g), the authors observed unimolecular collapse by AFM. They suggested that the higher valency of the anions induced the aggregation of MBBs.

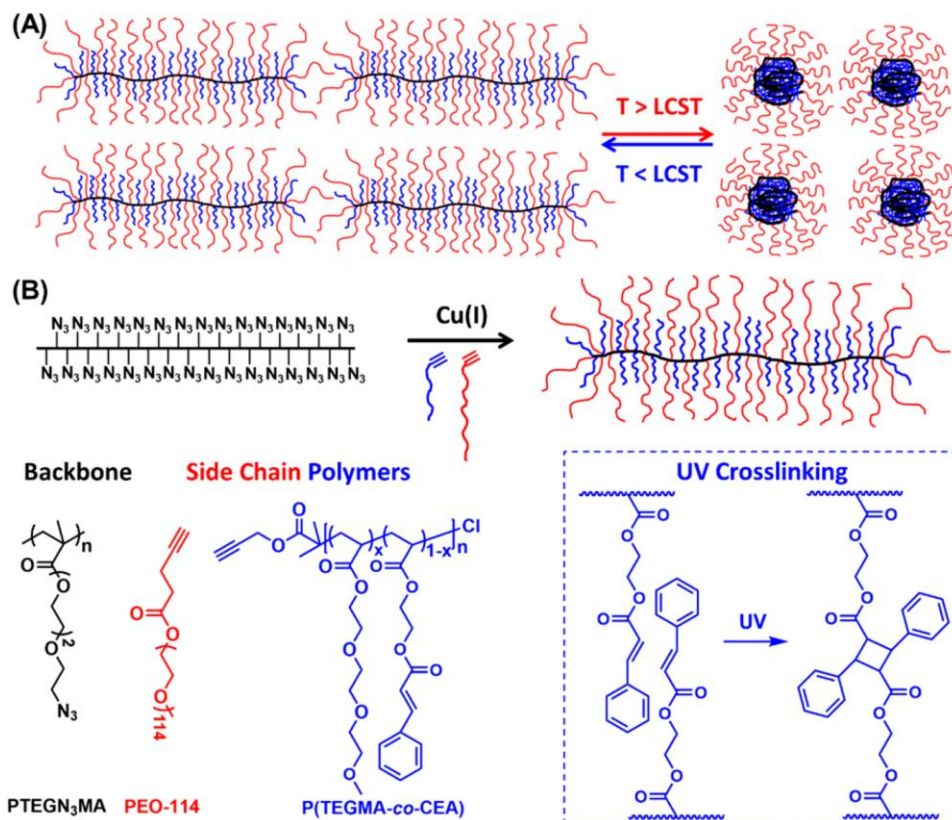
Other stimuli-responsive MBBs reported in the literature also showed aggregation even at low concentrations, which made the development of MBBs capable of worm-to-stable globule collapse imperative. Recently, our lab developed a strategy for the synthesis of stimuli-responsive MBBs that are capable of undergoing unimolecular collapse and forming stable globules. Henn et al. synthesized binary heterografted linear MBBs composed of LCST-type thermoresponsive poly(ethoxydi(ethylene glycol) acrylate) (PDEGEA, LCST = $\sim 9^\circ\text{C}$) and stabilizing water-soluble poly(ethylene oxide) (PEO) as side chains.⁵⁴ This bottlebrush showed stable unimolecular reversible collapse in aqueous solution. To further test the ability of PEO to stabilize MBB collapsed states, homografted MBBs with only PDEGEA side chains (HMB-1) and heterografted MBBs with PDEGEA and PEO (BMB-4) were prepared via the grafting-to method by attaching alkyne end-functionalized side chain polymers to the azide-bearing backbone polymer through

CuAAC click chemistry (Scheme 1.4). The 1.0 mg/g aqueous solutions of both brush polymers at 0 °C were clear, but at 22 °C, above the LCST for PDEGEA, the solution of the homografted PDEGEA brushes was cloudy, while the heterografted PDEGEA/PEO MBB stayed clear. This supports that the incorporation of water-soluble side chains, such as PEO, stabilizes the collapsed state of MBBs. Additionally, from DLS measurements, the heterografted PDEGEA/PEO MBB showed overlapping size distributions at concentrations of 0.2 mg/g and 1.0 mg/g, indicating unimolecular collapse at the higher concentration. AFM height images confirmed the thermally-induced shape transition of the PDEGEA/PEO brushes.

Henn et al. further showed that the unimolecular worm-to-globule shape transition for linear MBBs was possible in moderately concentrated solutions by incorporating stabilizing side chains significantly longer than the stimuli-responsive side chain polymer (Scheme 1.5). The MBBs were composed of poly(methoxytri(ethylene glycol) acrylate-*co*-2-cinnamoyl ethyl acrylate) (P(TEGMA-*co*-CEA), LCST $\approx$ 27 °C) with a degree of polymerization (DP) of 43 and PEO with a DP of 17 (BMB-750), 45 (BMB-2k), or 114 (BMB-5k) as side chains⁵⁵. The brushes were prepared by the grafting-to method using CuAAC chemistry to click the alkyne end-functionalized side chain polymers to the azide-bearing backbone. Aqueous solutions of three MBB polymers with a 10 mg/g concentration were made and their stability at different temperatures was tested. At 0 °C, all three solutions were clear. At 60 °C, only BMB-5k was clear, while BMB-2k had a blue tinge and BMB-750 was cloudy. The thermoresponsive behavior of BMB-5k was further studied by making solutions at 10, 25, and 100 mg/g in water, collapsing the brushes at elevated temperatures, crosslinking the UV-sensitive cinnamoyl moieties, and diluting the solution to appropriate concentrations for DLS (0.2 mg/g) and AFM (0.05 mg/g) studies. DLS of BMB-5k at 10 mg/g and 25 mg/g indicated unimolecular collapse, while at 100 mg/g. The brushes showed



Scheme 1.4. Illustration of the Grafting-To Preparation of Binary Heterografted Molecular Bottlebrushes via CuAAC Click Chemistry.⁵⁴ Reproduced from Ref. 54 with Permission from ACS.



Scheme 1.5. Illustration of (A) Moderately Concentrated Shape Changing Behavior from Worm-to-Globule and (B) Grafting-To Preparation of Thermoresponsive Binary Heterografted Linear Molecular Bottlebrushes Containing UV-Cross-Linkable Groups.⁵⁵ Reproduced from Ref 55 with Permission from ACS.

mostly unimolecular collapse, though large species were observed. AFM analysis supported the DLS finding that at 100 mg/g the vast majority of the brushes collapsed unimolecularly (Figure 1.2.).

Kent and Zhao synthesized binary heterografted three-arm star brushes (Scheme 1.6) composed of two different stimuli-responsive polymers as side chains and demonstrated the attainment of two distinct stable globular states.⁵⁷ In addition, they synthesized pH-responsive three-arm star MBBs composed of PEO (DP = 114) and either poly(2-(*N,N*-dimethylamino)ethyl methacrylate) (PDMAEMA, $pK_a \approx 7.4$, DP = 45), called SMB-1, or poly(2-(*N,N*-diethylamino)ethyl methacrylate) (PDEAEMA, $pK_a \approx 7.4$, DP = 42), called SMB-2 (Scheme 1.7).³ The pH-responsiveness of each brush polymer was tested at 0.2 mg/g in a 10 mM phosphate buffer at 25 °C from pH 4-9.5. From DLS measurements, SMB-1 showed a small decrease in hydrodynamic size (D_h) around the pK_a of PDMAEMA and SMB-2 exhibited an abrupt and large decrease in D_h near the pK_a for PDEAEMA. For AFM study, the brushes were spin cast onto glass from 0.05 mg/g aqueous solutions with pH of 4.0 and 9.5 at room temperature. SMB-1 showed contraction in the arm length, while SMB-2 underwent worm-to-globule shape transition. They further tested the responsiveness of SMB-1 and SMB-2 in acidic aqueous solutions with a pH of 4.0 to a series of anions, $[\text{Fe}(\text{CN})_6]^{3-}$, $\text{S}_2\text{O}_8^{2-}$, ClO_4^- , SCN^- , Br^- , and Cl^- . DLS of SMB-1 showed abrupt and strong decreases in D_h with increasing anion concentration for $[\text{Fe}(\text{CN})_6]^{3-}$, $\text{S}_2\text{O}_8^{2-}$, and ClO_4^- , and small size decreases for SCN^- , Br^- , and Cl^- (Figure 1.3). SMB-2 exhibited abrupt and large size transitions with increasing anion concentration for $[\text{Fe}(\text{CN})_6]^{3-}$, $\text{S}_2\text{O}_8^{2-}$, ClO_4^- , and SCN^- , and only small size decreases for Br^- and Cl^- . These measurements are believed to be a result of ion-pairing and the chaotropic effect. ^1H NMR spectroscopy and AFM were used to support this claim. ^1H NMR spectroscopy of 4.0 mg/g solutions in D_2O of SMB-1 and SMB-2 at pH of 4.0 was

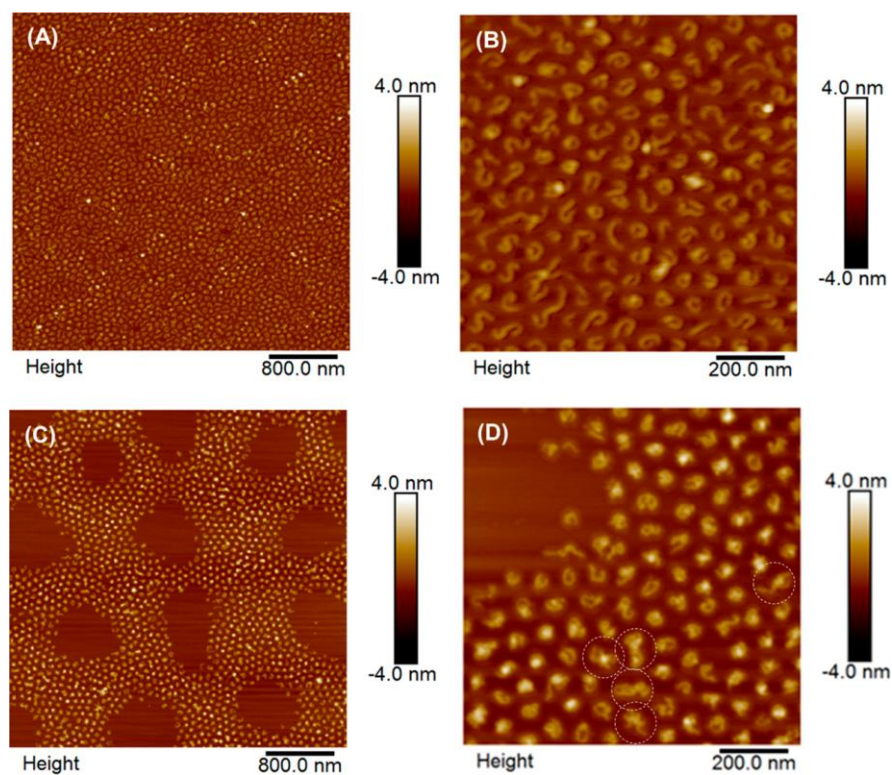
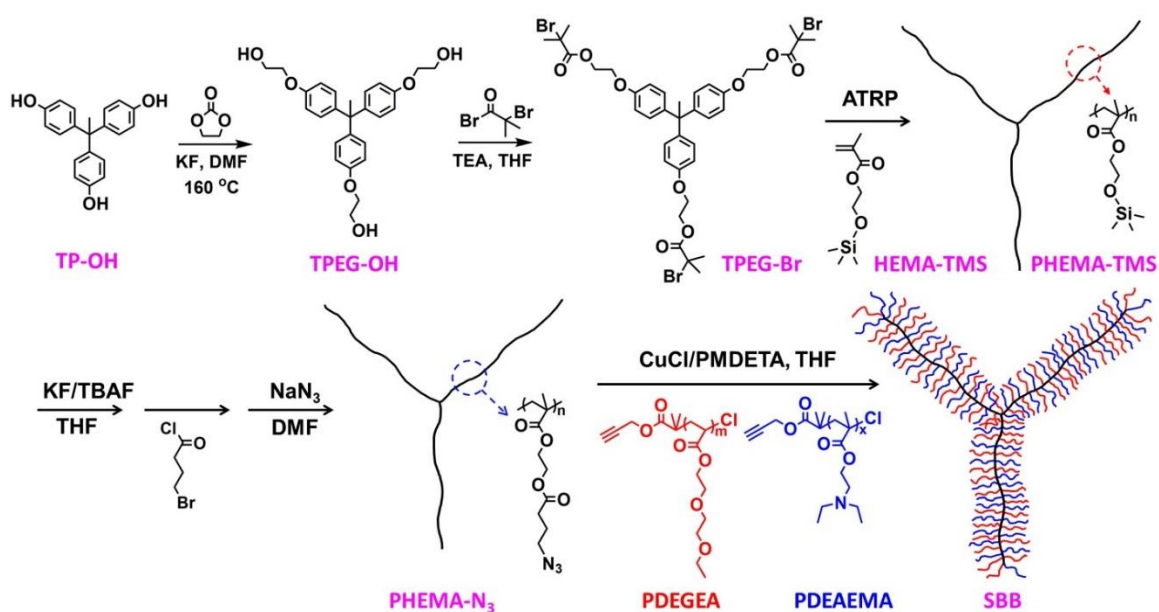
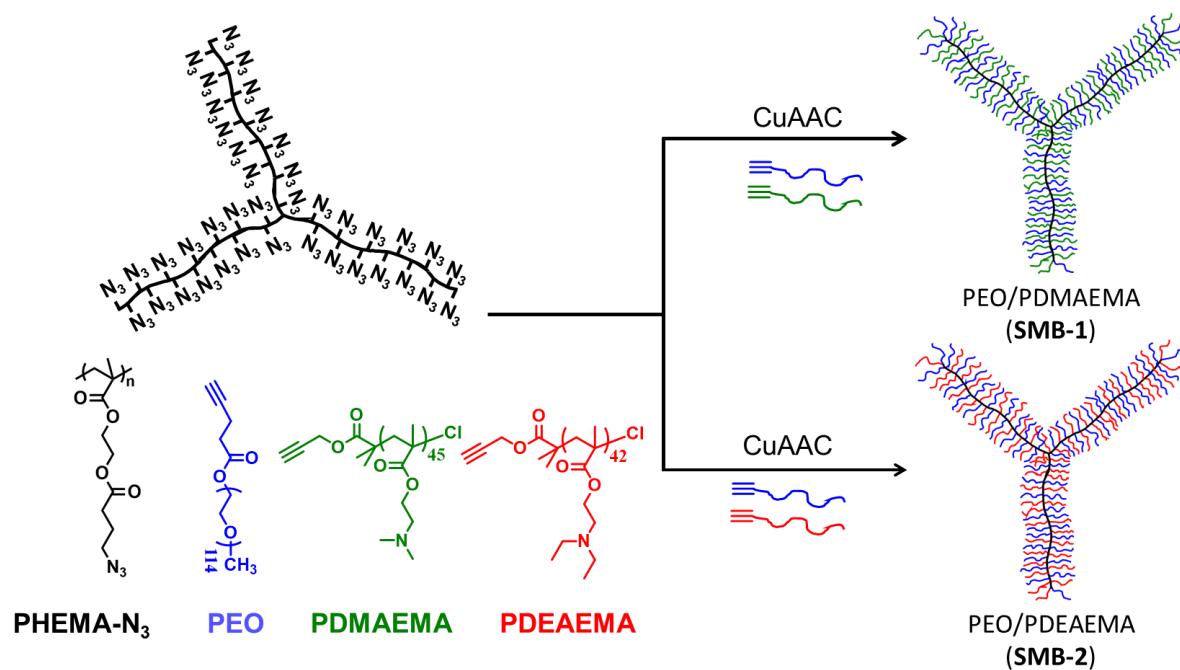


Figure 1.2. AFM height images obtained from spin cast 0.05 mg/g aqueous solutions at 0 °C of cross-linked BMB-5k onto freshly cleaved mica. The samples were diluted from the UV irradiated samples at 25 mg/g solution (A and B) and 100 mg/g (C and D).⁵⁵ Reproduced from Ref. 55 with permission from ACS.



Scheme 1.6. Synthetic Scheme of Heterografted Three-Arm Star Molecular Bottlebrushes.⁵⁷
Reproduced from Ref 57 with Permission from ACS



Scheme 1.7. Illustration of the Synthesis of Heterografted Star Molecular Bottlebrushes SMB-1 and SMB-2 using the Grafting-To Method using CuAAC Click Chemistry.³ Reproduced from Ref 3 with Permission from RSC.

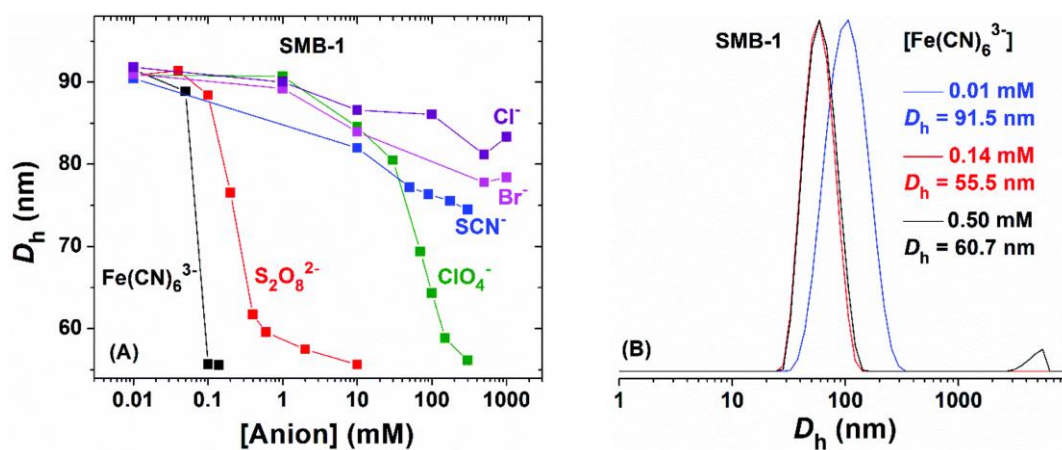


Figure 1.3. (A) DLS of 0.1 mg/g aqueous solution of SMB-1 at pH 4.00 at 25 °C for (A) D_h values for a series of salts. (B) representative size distributions $[\text{Fe(CN)}_6]^{3-}$ at 0.01, 0.14, and 0.50 mM.³ Reproduced from Ref 3 with permission from RSC.

performed at different concentrations of $K_3[Fe(CN)_6]$. The characteristic peaks for PDMAEMA and PDEAEMA side chains were monitored as the concentration of $K_3[Fe(CN)_6]$ in the solutions was increased (Figure 1.4). For SMB-1, the peaks at 4.58-4.16 ppm $[-OCH_2CH_2N^+H(CH_3)_2]$, 3.67–3.41 ppm $[-OCH_2CH_2N^+H(CH_3)_2]$, and 3.20–2.83 ppm $[-OCH_2CH_2N^+H(CH_3)_2]$ decreased significantly in intensity and became broader as the $[Fe(CN)_6]^{3-}$ was increased to 1.0 mM. Similarly, the characteristic peaks for SMB-2 also broadened and significantly decreased in intensity at 1.0 mM. Aqueous solutions of SMB-1 and SMB-2 with a brush concentration of 0.05 mg/g at pH 4.0 in the presence of 0.14 mM $[Fe(CN)_6]^{3-}$ were drop cast onto glass and AFM showed unimolecular collapsed states.

1.3. Overview of Dissertation Research Work

This dissertation work presents the synthesis and responsive behavior of two types of stimuli-responsive charged polymers: thermoresponsive zwitterionic polymer(sulfobetaine methacrylate)s (PSBMAs) and shape-changing polyelectrolyte molecular bottlebrushes. In the first part, a series of zwitterionic PSBMAs with differing *N*-substituents are synthesized with goals of elucidating the poorly understood structure-solution behavior relationship and investigating the effectiveness of PSBMAs as kinetic hydrate inhibitors (KHIs) in gas and oil transportation. In the second part, the effects of temperature on chaotropic anion-induced star-to-globule shape transitions of three-arm star polyelectrolyte MBBs are studied.

Chapter 2 provides a systematic study of *N*-substituent effects on the solution behavior of PSBMAs in water. Specifically, we examined the effects of symmetric *N*-*n*-alkyl substituents and various other *N*-substituents such as cyclohexyl, phenyl, and 2-hydroxyethyl groups. Zwitterionic sulfobetaine methacrylate monomers with a variety of *N*-substituents were synthesized through multi-step procedures and polymerized. The behavior of the obtained polymers in water was

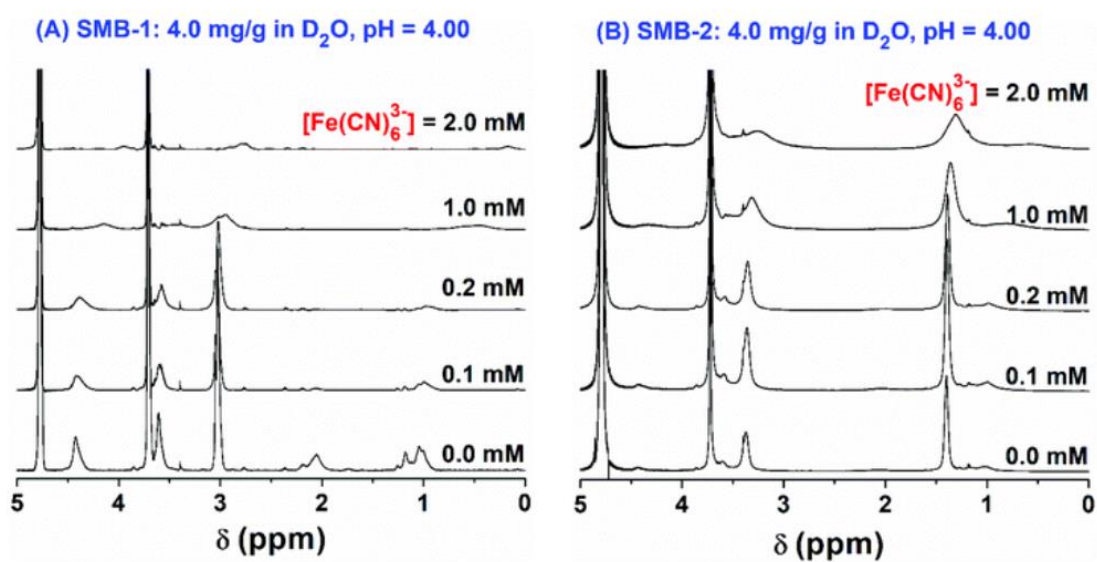


Figure 1.4. Deuterated solvent normalized ^1H NMR spectra with different concentrations of $[\text{Fe}(\text{CN})_6]^{3-}$ in 4.0 mg/g solutions of A) SMB-1 and B) SMB-2 in D_2O at pH 4.00.³ Reproduced from Ref 3 with permission from RSC.

inspected visually first and the cloud points of thermoresponsive zwitterionic PSBMAs were further investigated by UV-Vis spectrophotometry. The PSBMAs display an array of solution behavior from UCST to soluble, to LCST, and insoluble in water. This array of behavior is believed to come from the competition between electrostatic charge-charge interactions and the hydrophobic effect. As the length of the *N-n*-alkyl substituents increases, the zwitterionic electrostatic interactions decrease and the hydrophobic effect increases. Changing the *N-n*-hexyl group to cyclohexyl decreases the hydrophobic effect, while replacing the *N-n*-hexyl with a phenyl group caused a switch from LCST- to UCST-type polymer. Introducing 2-hydroxyethyl group as an *N*-substituent enhanced attractive zwitterionic interactions, creating a UCST-type polymer. Finally, by synthesizing a series of copolymers from a SBMA with an *N*-methyl and an *N*-phenyl substituent and a SBMA with diethyl *N*-substituents, we show that the cloud point could be adjusted by changing the monomer composition in the copolymer.

Chapter 3 presents the synthesis of a series of zwitterionic polymers for application as kinetic hydrate inhibitors (KHIs) for gas and oil transportation pipelines. The polymers include PSBMA homopolymers with 4-6 carbon atoms in the *N-n*-alkyl substituents and copolymers of a zwitterionic sulfobetaine methacrylate monomer with one methyl and one *n*-hexyl at the nitrogen atom and isopropyl methacrylate. The KHI tests showed some of our zwitterionic polymers performed similarly to the commercially used poly(*N*-vinylcaprolactam).

In Chapter 4, we investigated the thermoresponsive behavior of chaotropic anion (CA)-collapsed three-arm star brush polymers in acidic water. Two heterografted three-arm star MBBs composed of PEO and either PDMAEMA or PDEAEMA were synthesized by a click grafting to method. We first studied the effects of six salts on two brush polymers in aqueous solutions at pH 4.5, well-below the pK_a values, followed by the effects of temperature on collapsed brushes. DLS

and AFM studies showed that while super CA-collapsed brushes remained in the globular state in the temperature range of ~ 0 to $70\text{ }^{\circ}\text{C}$, moderately CA-collapsed brushes were thermoresponsive and underwent a UCST-type globule-to-extended star shape transition. For weak CAs and kosmotropic anions, temperature had little effect on the sizes of the brushes in the studied temperature range.

Chapter 5 presents a study on the CA-induced conformational changes in Milli-Q water for heterografted three-arm star MBBs composed of PEO and poly((2-(methacryloyloxy)ethyl)trimethylammonium iodide) (PDMAEMA-Q). The permanently charged bottlebrushes were synthesized by quaternizing a PEO/PDMAEMA star brush polymer with iodomethane. The effects of six anions ($\text{S}_2\text{O}_8^{2-}$, ClO_4^- , SCN^- , SO_4^{2-} , salicylate, and thiosalicylate) on the conformation of the brushes were studied by DLS. The size transitions occurred at much lower concentrations for CAs such as $\text{S}_2\text{O}_8^{2-}$ and ClO_4^- than the PEO/PDMAEMA star MBBs in acidic water. The observations indicated that quaternary ammonium groups in this brush system are stronger chaotropes than protonated tertiary amine groups. Additionally, we found that two drug relevant anions, salicylate and thiosalicylate, had an effect on the quaternized star brushes similar to the moderate CAs. Finally, we confirmed that the size transitions exhibited by the permanently charged brushes were a result of ion pairing of quaternary ammonium groups with the CAs by examining the behavior a PDMAEMA-Q homopolymer in water in the presence of a moderate chaotrope, ClO_4^- ; This moderate CA can induce phase separation of PDMAEMA-Q in water.

Finally, Chapter 6 provides a summary of this dissertation research and prospects in thermoresponsive zwitterionic PBSMAs and stimuli-responsive MBBs.

References:

1. Gil, E. S.; Hudson, S. M., Stimuli-reponsive polymers and their bioconjugates. *Progress in Polymer Science* **2004**, 29 (12), 1173-1222.
2. Dai, S.; Ravi, P.; Tam, K. C., pH-Responsive polymers: synthesis, properties and applications. *Soft Matter* **2008**, 4 (3), 435-449.
3. Kent, E. W.; Lewoczko, E. M.; Zhao, B., pH- and chaotropic anion-induced conformational changes of tertiary amine-containing binary heterografted star molecular bottlebrushes in aqueous solution. *Polymer Chemistry* **2021**, 12 (2), 265-276.
4. Ulijn, R. V., Enzyme-responsive materials: a new class of smart biomaterials. *Journal of Materials Chemistry* **2006**, 16 (23), 2217-2225.
5. Kim, E.; Lee, J.; Kim, D.; Lee, K. E.; Han, S. S.; Lim, N.; Kang, J.; Park, C. G.; Kim, K., Solvent-responsive polymernanocapsules with controlled permeability: encapsulation and release of a fluorescent dye by swelling and deswelling. *Chemical Communications* **2009**, (12), 1472-1474.
6. Heskins, M.; Guillet, J. E., Solution Properties of Poly(N-isopropylacrylamide). *Journal of Macromolecular Science: Part A - Chemistry* **1968**, 2 (8), 1441-1455.
7. Henn, D. M.; Lau, C. M.; Li, C. Y.; Zhao, B., Light-triggered unfolding of single linear molecular bottlebrushes from compact globular to wormlike nano-objects in water. *Polymer Chemistry* **2017**, 8 (17), 2702-2712.
8. TANAKA, T.; NISHIO, I.; SUN, S.-T.; UENO-NISHIO, S., Collapse of Gels in an Electric Field. *Science* **1982**, 218 (4571), 467-469.
9. Thévenot, J.; Oliveira, H.; Sandre, O.; Lecommandoux, S., Magnetic responsive polymer composite materials. *Chemical Society Reviews* **2013**, 42 (17), 7099-7116.

10. Davis, D. A.; Hamilton, A.; Yang, J.; Cremer, L. D.; Van Gough, D.; Potisek, S. L.; Ong, M. T.; Braun, P. V.; Martínez, T. J.; White, S. R.; Moore, J. S.; Sottos, N. R., Force-induced activation of covalent bonds in mechanoresponsive polymeric materials. *Nature* **2009**, 459 (7243), 68-72.
11. Hoffman, A. S., “intelligent” polymers in medicine and biotechnology. *Macromolecular Symposia* **1995**, 98 (1), 645-664.
12. Hu, J.; Liu, S., Responsive Polymers for Detection and Sensing Applications: Current Status and Future Developments. *Macromolecules* **2010**, 43 (20), 8315-8330.
13. Bajpai, A. K.; Shukla, S. K.; Bhanu, S.; Kankane, S., Responsive polymers in controlled drug delivery. *Progress in Polymer Science* **2008**, 33 (11), 1088-1118.
14. Parasuraman, D.; Serpe, M. J., Poly (N-Isopropylacrylamide) Microgels for Organic Dye Removal from Water. *ACS Applied Materials & Interfaces* **2011**, 3 (7), 2732-2737.
15. Ma, M.; Guo, L.; Anderson, D. G.; Langer, R., Bio-Inspired Polymer Composite Actuator and Generator Driven by Water Gradients. *Science* **2013**, 339 (6116), 186-189.
16. Wei, M.; Gao, Y.; Li, X.; Serpe, M. J., Stimuli-responsive polymers and their applications. *Polymer Chemistry* **2017**, 8 (1), 127-143.
17. Ward, M. A.; Georgiou, T. K., Thermoresponsive Polymers for Biomedical Applications. *Polymers* **2011**, 3 (3), 1215.
18. Seuring, J.; Agarwal, S., Polymers with Upper Critical Solution Temperature in Aqueous Solution. *Macromolecular Rapid Communications* **2012**, 33 (22), 1898-1920.
19. Mirvakili, S. M.; Hunter, I. W., Multidirectional Artificial Muscles from Nylon. *Advanced Materials* **2017**, 29 (4), 1604734.

20. Seuring, J.; Agarwal, S., Polymers with Upper Critical Solution Temperature in Aqueous Solution: Unexpected Properties from Known Building Blocks. *ACS Macro Letters* **2013**, 2 (7), 597-600.
21. Shao, Q.; Jiang, S., Molecular Understanding and Design of Zwitterionic Materials. *Advanced Materials* **2015**, 27 (1), 15-26.
22. Laschewsky, A.; Rosenhahn, A., Molecular Design of Zwitterionic Polymer Interfaces: Searching for the Difference. *Langmuir* **2019**, 35 (5), 1056-1071.
23. Monroy Soto, V. M.; Galin, J. C., Poly(sulphopropylbetaines): 2. Dilute solution properties. *Polymer* **1984**, 25 (2), 254-262.
24. Schulz, D. N.; Peiffer, D. G.; Agarwal, P. K.; Larabee, J.; Kaladas, J. J.; Soni, L.; Handwerker, B.; Garner, R. T., Phase behaviour and solution properties of sulphobetaine polymers. *Polymer* **1986**, 27 (11), 1734-1742.
25. Niskanen, J.; Tenhu, H., How to manipulate the upper critical solution temperature (UCST)? *Polymer Chemistry* **2017**, 8 (1), 220-232.
26. Biswas, Y.; Mandal, T. K., Structural Variation in Homopolymers Bearing Zwitterionic and Ionic Liquid Pendants for Achieving Tunable Multi-Stimuli Responsiveness and Hierarchical Nanoaggregates. *Macromolecules* **2017**, 50 (24), 9807-9820.
27. Vasantha, V. A.; Jana, S.; Parthiban, A.; Vancso, J. G., Water swelling, brine soluble imidazole based zwitterionic polymers – synthesis and study of reversible UCST behaviour and gel–sol transitions. *Chemical Communications* **2014**, 50 (1), 46-48.
28. Dimitrov, I.; Trzebicka, B.; Müller, A. H. E.; Dworak, A.; Tsvetanov, C. B., Thermosensitive water-soluble copolymers with doubly responsive reversibly interacting entities. *Progress in Polymer Science* **2007**, 32 (11), 1275-1343.

29. Mi, L.; Jiang, S., Integrated Antimicrobial and Nonfouling Zwitterionic Polymers. *Angewandte Chemie International Edition* **2014**, 53 (7), 1746-1754.
30. Jiang, S.; Cao, Z., Ultralow-Fouling, Functionalizable, and Hydrolyzable Zwitterionic Materials and Their Derivatives for Biological Applications. *Advanced Materials* **2010**, 22 (9), 920-932.
31. Banquy, X.; Burdyńska, J.; Lee, D. W.; Matyjaszewski, K.; Israelachvili, J., Bioinspired Bottle-Brush Polymer Exhibits Low Friction and Amontons-like Behavior. *Journal of the American Chemical Society* **2014**, 136 (17), 6199-6202.
32. Chen, M.; Briscoe, W. H.; Armes, S. P.; Klein, J., Lubrication at Physiological Pressures by Polyzwitterionic Brushes. *Science* **2009**, 323 (5922), 1698-1701.
33. Rajan, R.; Hayashi, F.; Nagashima, T.; Matsumura, K., Toward a Molecular Understanding of the Mechanism of Cryopreservation by Polyampholytes: Cell Membrane Interactions and Hydrophobicity. *Biomacromolecules* **2016**, 17 (5), 1882-1893.
34. Hildebrand, V.; Laschewsky, A.; Päch, M.; Müller-Buschbaum, P.; Papadakis, C. M., Effect of the zwitterion structure on the thermo-responsive behaviour of poly(sulfobetaine methacrylates). *Polymer Chemistry* **2017**, 8 (1), 310-322.
35. Hildebrand, V.; Laschewsky, A.; Wischerhoff, E., Modulating the solubility of zwitterionic poly((3-methacrylamidopropyl)ammonioalkane sulfonate)s in water and aqueous salt solutions via the spacer group separating the cationic and the anionic moieties. *Polymer Chemistry* **2016**, 7 (3), 731-740.
36. Hart, R.; Timmerman, D., New polyampholytes: The polysulfobetaines. *Journal of Polymer Science* **1958**, 28 (118), 638-640.

37. Wang, N.; Seymour, B. T.; Lewoczko, E. M.; Kent, E. W.; Chen, M.-L.; Wang, J.-H.; Zhao, B., Zwitterionic poly(sulfobetaine methacrylate)s in water: from upper critical solution temperature (UCST) to lower critical solution temperature (LCST) with increasing length of one alkyl substituent on the nitrogen atom. *Polymer Chemistry* **2018**, 9 (43), 5257-5261.
38. Sheiko, S. S.; Sumerlin, B. S.; Matyjaszewski, K., Cylindrical molecular brushes: Synthesis, characterization, and properties. *Progress in Polymer Science* **2008**, 33 (7), 759-785.
39. Zhang, M.; Müller, A. H. E., Cylindrical polymer brushes. *Journal of Polymer Science Part A: Polymer Chemistry* **2005**, 43 (16), 3461-3481.
40. Xie, G.; Martinez, M. R.; Olszewski, M.; Sheiko, S. S.; Matyjaszewski, K., Molecular Bottlebrushes as Novel Materials. *Biomacromolecules* **2019**, 20 (1), 27-54.
41. Lee, H.-i.; Pietrasik, J.; Sheiko, S. S.; Matyjaszewski, K., Stimuli-responsive molecular brushes. *Progress in Polymer Science* **2010**, 35 (1), 24-44.
42. Verduzco, R.; Li, X.; Pesek, S. L.; Stein, G. E., Structure, function, self-assembly, and applications of bottlebrush copolymers. *Chemical Society Reviews* **2015**, 44 (8), 2405-2420.
43. Daniel, W. F. M.; Burdyńska, J.; Vatankhah-Varnoosfaderani, M.; Matyjaszewski, K.; Paturej, J.; Rubinstein, M.; Dobrynin, A. V.; Sheiko, S. S., Solvent-free, supersoft and superelastic bottlebrush melts and networks. *Nature Materials* **2016**, 15 (2), 183-189.
44. Zhang, M.; Drechsler, M.; Müller, A. H. E., Template-Controlled Synthesis of Wire-Like Cadmium Sulfide Nanoparticle Assemblies within Core–Shell Cylindrical Polymer Brushes. *Chemistry of Materials* **2004**, 16 (3), 537-543.
45. Xia, Y.; Kornfield, J. A.; Grubbs, R. H., Efficient Synthesis of Narrowly Dispersed Brush Polymers via Living Ring-Opening Metathesis Polymerization of Macromonomers. *Macromolecules* **2009**, 42 (11), 3761-3766.

46. Xia, Y.; Olsen, B. D.; Kornfield, J. A.; Grubbs, R. H., Efficient Synthesis of Narrowly Dispersed Brush Copolymers and Study of Their Assemblies: The Importance of Side Chain Arrangement. *Journal of the American Chemical Society* **2009**, *131* (51), 18525-18532.
47. Cheng, G.; Böker, A.; Zhang, M.; Krausch, G.; Müller, A. H. E., Amphiphilic Cylindrical Core–Shell Brushes via a “Grafting From” Process Using ATRP. *Macromolecules* **2001**, *34* (20), 6883-6888.
48. Matyjaszewski, K.; Qin, S.; Boyce, J. R.; Shirvanyants, D.; Sheiko, S. S., Effect of Initiation Conditions on the Uniformity of Three-Arm Star Molecular Brushes. *Macromolecules* **2003**, *36* (6), 1843-1849.
49. Beers, K. L.; Gaynor, S. G.; Matyjaszewski, K.; Sheiko, S. S.; Möller, M., The Synthesis of Densely Grafted Copolymers by Atom Transfer Radical Polymerization. *Macromolecules* **1998**, *31* (26), 9413-9415.
50. Tang, H.; Li, Y.; Lahasky, S. H.; Sheiko, S. S.; Zhang, D., Core–Shell Molecular Bottlebrushes with Helical Polypeptide Backbone: Synthesis, Characterization, and Solution Conformations. *Macromolecules* **2011**, *44* (6), 1491-1499.
51. Tsarevsky, N. V.; Bencherif, S. A.; Matyjaszewski, K., Graft Copolymers by a Combination of ATRP and Two Different Consecutive Click Reactions. *Macromolecules* **2007**, *40* (13), 4439-4445.
52. Gao, H.; Matyjaszewski, K., Synthesis of Molecular Brushes by “Grafting onto” Method: Combination of ATRP and Click Reactions. *Journal of the American Chemical Society* **2007**, *129* (20), 6633-6639.

53. Yan, Y.; Shi, Y.; Zhu, W.; Chen, Y., Highly efficient synthesis of cylindrical polymer brushes with various side chains via click grafting-onto approach. *Polymer* **2013**, *54* (21), 5634-5642.
54. Henn, D. M.; Fu, W.; Mei, S.; Li, C. Y.; Zhao, B., Temperature-Induced Shape Changing of Thermosensitive Binary Heterografted Linear Molecular Brushes between Extended Wormlike and Stable Globular Conformations. *Macromolecules* **2017**, *50* (4), 1645-1656.
55. Henn, D. M.; Holmes, J. A.; Kent, E. W.; Zhao, B., Worm-to-Sphere Shape Transition of Thermoresponsive Linear Molecular Bottlebrushes in Moderately Concentrated Aqueous Solution. *The Journal of Physical Chemistry B* **2018**, *122* (27), 7015-7025.
56. Kent, E. W.; Lewoczko, E. M.; Zhao, B., Effect of Buffer Anions on Pearl-Necklace Morphology of Tertiary Amine-Containing Binary Heterografted Linear Molecular Bottlebrushes in Acidic Aqueous Buffers. *Langmuir* **2020**, *36* (44), 13320-13330.
57. 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* (17), 6714-6724.
58. Li, C.; Gunari, N.; Fischer, K.; Janshoff, A.; Schmidt, M., New Perspectives for the Design of Molecular Actuators: Thermally Induced Collapse of Single Macromolecules from Cylindrical Brushes to Spheres. *Angewandte Chemie International Edition* **2004**, *43* (9), 1101-1104.
59. Xu, Y.; Bolisetty, S.; Drechsler, M.; Fang, B.; Yuan, J.; Ballauff, M.; Müller, A. H. E., pH and salt responsive poly(N,N-dimethylaminoethyl methacrylate) cylindrical brushes and their quaternized derivatives. *Polymer* **2008**, *49* (18), 3957-3964.

60. Pietrasik, J.; Sumerlin, B. S.; Lee, R. Y.; Matyjaszewski, K., Solution Behavior of Temperature-Responsive Molecular Brushes Prepared by ATRP. *Macromolecular Chemistry and Physics* **2007**, *208* (1), 30-36.

**Chapter 2: Effects of *N*-Substituents on Solution Behavior of
Poly(sulfobetaine methacrylate)s in Water: UCST and LCST**

The work presented in this chapter has been published in *ACS Applied Polymer Materials* **2021**, 3, 867-878. Dr. Ning Wang synthesized the monomer M1P6, P1P6 homopolymers by RAFT polymerization, determined the molecular weights and dispersities of P1P6 polymers by SEC, recorded NMR spectra of P1P6 polymers, measured the cloud points of P1P6-3 and -4 in water by transmittance, and also developed synthetic methods for M22 and M44. I did the majority of the rest of the work presented in this Chapter and in the publication. Prof. Bin Zhao advised this work.

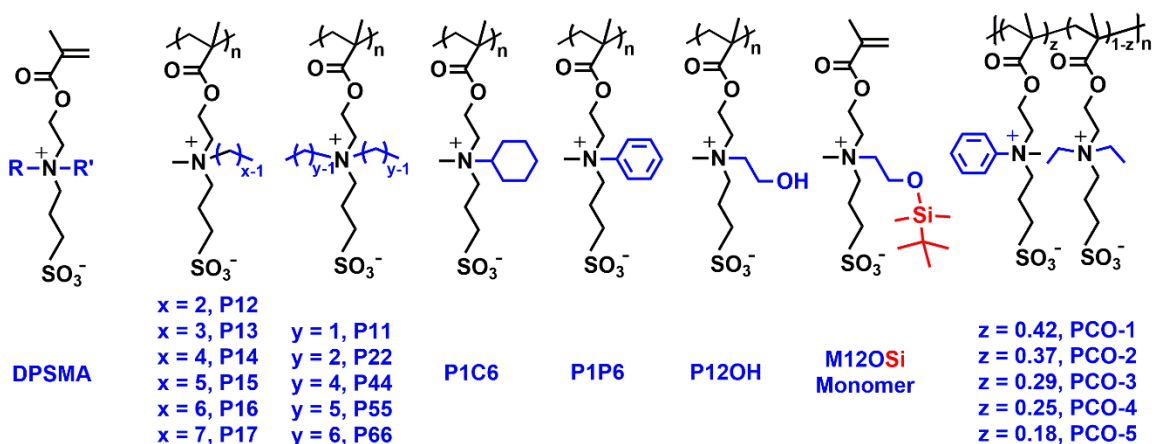
Abstract

A series of zwitterionic poly(sulfobetaine methacrylate)s (PSBMAs) with various *N*-substituents, including *n*-alkyl, cyclohexyl, 2-hydroxyethyl, and phenyl, was synthesized in order to elucidate the effects of *N*-substituents on solution behavior of PSBMAs in water. Upon increasing the lengths of two symmetric *N*-*n*-alkyl substituents simultaneously, PSBMAs exhibited an array of behavior in water, from UCST to soluble, to LCST, and insoluble, which presumably stemmed from the competition between the decrease in electrostatic zwitterionic interactions and the increase in hydrophobic effect with increasing alkyl length. Replacing *N*-*n*-hexyl with cyclohexyl while keeping the *N*-methyl substituent diminished the hydrophobic effect and made the LCST vanish. Introducing a 2-hydroxyethyl or a phenyl *N*-substituent enhanced the attractive interactions between zwitterionic groups, resulting in UCST behavior. The insight gained from this work can facilitate the design of UCST- and LCST-type thermoresponsive zwitterionic PSBMAs by varying *N*-substituents and will open up more opportunities for potential applications of already widely used zwitterionic materials (e.g., thermoresponsive zwitterionic polymer brushes and nanogels for use in bioseparation and drug delivery).

2.1. Introduction

Zwitterionic polymers are a special class of polyelectrolytes in which each repeat unit contains two oppositely charged moieties of equal magnitude that are most frequently both located on the pendant group.^{1,2} Although highly charged, zwitterionic polymers maintain charge neutrality globally and locally and exhibit many intriguing properties, including exceptional hydrophilicity (for common polyzwitterions),^{1,2} antipolyelectrolyte effect,^{3,4} and stimuli-responsive property.^{2,5-11} These unique characteristics have been exploited intensively for numerous applications, such as anti(bio)fouling,^{1,2,12-17} lubrication,^{18,19} anti-icing and anti-freezing,^{20,21} oil-water separation,²² cryopreservation,²³ etc. Among common zwitterionic homopolymers, poly(3-((2-methacryloyloxyethyl)dimethylammonio)propane-1-sulfonate) (P11 in Scheme 2.1), a representative of poly(sulfobetaine methacrylate)s (PSBMAs), and poly(3-(*N*-(3-methacrylamidopropyl)dimethylammonio)propane-1-sulfonate) (PMAMPS) are known to exhibit an upper critical solution temperature (UCST) in water.^{2,5,9,24,25} The UCST behavior is likely a result of the competition between electrostatic attractive interactions of zwitterionic groups and thermal energy; presumably, heating overcomes the electrostatic inter-locking of zwitterionic groups, causing the polymer to dissolve in water and thus exhibit a UCST.

Despite the fact that zwitterionic polymers are structurally versatile,^{1-7,24-33} the vast majority of the systems reported in the literature for potential applications are built from the polymers of a small number of monomers.² Varying the structure of zwitterionic materials will change their solution behavior in water, and studies have shown that the effect is subtle, often counterintuitive, and hard to predict. For example, if a hydrophilic hydroxy group is introduced onto the middle carbon atom in the spacer between the ammonium and the sulfonate group of PMAMPS, the cloud point (CP) of the resultant polymethacrylamide in water is found to be higher (i.e., the solubility



Scheme 2.1. Molecular Structures of Zwitterionic Homopolymers of DPSMA Sulfobetaine Methacrylates with Various *N*-Substituents, Monomer M12OSi, and Copolymers of Two Monomers with Various Molar Contents.

becomes poor).²⁴ When the dimethylammonio group in P11 is replaced with a six-membered piperidinio ring, the UCST disappears and the polymer becomes soluble in water in the temperature range of 0 – 100 °C, although the alkyl ring is larger and more hydrophobic than the two methyl groups.²⁵ The improved solubility of zwitterionic polymers in water with increasing alkyl size is attributed to the weakening of the electrostatic attractive forces between zwitterions caused by the steric hindrance from larger alkyl groups. On the other hand, larger hydrophobic groups introduce a larger hydrophobic effect, which is the origin of the lower critical solution temperature (LCST) transition of polymers in water.³³⁻³⁵ By keeping one methyl group on the nitrogen atom and systematically increasing the length of the other *N-n*-alkyl substituent, our group previously prepared a series of PSBMA homopolymers (P11 – P17 in Scheme 2.1) from the corresponding zwitterionic DPSMA monomers (Scheme 2.1) and observed a range of solution behavior in water.³³ While P11 was a UCST thermoresponsive polymer as reported, P12, P13, and P14 were soluble in water in the temperature range of 0-100 °C likely due to the steric disruption of zwitterionic attractive interactions. Intriguingly, P15 and P16 exhibited LCST behavior with CPs at ~ 50 and 22 °C, respectively. On the other hand, P17 was found to be insoluble in water, apparently caused by the high hydrophobicity of the *N*-heptyl group.

To further understand the effects of *N*-substituents on solution behavior of DPSMA-based PSBMA (PDPSMA, with two CH₂ groups between the ester and the ammonium, three CH₂ groups between the ammonium and the sulfonate group, and two separate substituents, R and R', at the nitrogen atom) in water for facilitating the design of UCST- and LCST-type thermoresponsive zwitterionic polymers, we synthesized and investigated three subgroups of such PSBMA with various *N*-substituents (Scheme 2.1). (i) P11 – P66 are prepared for studying the effects of the number and length of *N-n*-alkyl substituents. (ii) A polar 2-hydroxyethyl group,

capable of hydrogen bonding and dipole-ion interactions, is introduced as an *N*-substituent to examine its effect on the solution behavior of P12OH in water in comparison to the corresponding P12, which is soluble in water. (iii) P16 with a *n*-hexyl, P1C6 with a cyclohexyl, and P1P6 with a phenyl substituent at the nitrogen atom are prepared for investigating the effects of molecular shape and aromaticity of *N*-substituents. Compared with the *n*-hexyl group, cyclohexyl is more compact and has a smaller contact area with water; the *N*-phenyl substituent can interact with cations and anions. The influences of these structural variations are revealed, and the results from this study not only improve our understanding of the structure-solution-behavior relationship of PDPSMAs in water but also provide an insight into the design of UCST- and LCST-type thermoresponsive as well as water-soluble zwitterionic PSBMAs.

2.2. Experimental Section

2.2.1. Materials

2-(Methylamino)ethanol (99%), *N*-methylcyclohexylamine (98%), 2-aminoethanol (99%), 1,3-propanesultone (99%), 1-bromobutane (99%), 1-bromopentane (99%), and 1-bromohexane (98%) were purchased from Alfa Aesar and used directly. 2-(*N*-Methylanilino)ethanol (97.0+%) and hydroquinone (99%) were obtained from TCI America and Acros Organics, respectively. 2,2,2-Trifluoroethanol (TFE, >99% from Alfa Aesar or 99.9% from Oakwood Chemical), 2-bromoethanol (95%, TCI America), 4-dimethylaminopyridine (99.0+%, TCI America), *tert*-butyldimethylsilyl chloride (99%, Oakwood Products) were used as received. Methacryloyl chloride (97%, Sigma-Aldrich) was distilled under vacuum and stored in a refrigerator. Triethylamine (99%, Alfa Aesar) was stirred over CaH₂ and distilled under vacuum prior to use. α,α' -Azobisisobutyronitrile (AIBN, 98%, Sigma-Aldrich) was recrystallized from ethanol, dried under vacuum, and stored in a refrigerator. Tetrahydrofuran (THF) was dried by refluxing over

sodium/benzophenone and used immediately. 2-(*N,N*-Dimethylamino)ethyl methacrylate (98%) and 2-(*N,N*-diethylamino)ethyl methacrylate (99%) were purchased from Aldrich and used as received. *N,N*-Di(*n*-butyl)aminoethyl methacrylate was prepared by reacting methacryloyl chloride with *N,N*-di(*n*-butyl)aminoethanol (99%, Aldrich) in the presence of triethylamine;¹ the crude product was purified by silica gel column chromatography and vacuum distillation, and the molecular structure was verified by ¹H and ¹³C NMR spectroscopy analysis. *n*-Butyl (2-cyano-2-propyl) trithiocarbonate was synthesized according to the procedures from the literature.^{2,3} Acetonitrile (99%, Aldrich) was dried with 4 Å molecular sieves (Aldrich). Water purified from a Milli-Q Plus (Millipore) system was used for the preparation of aqueous solutions of zwitterionic polymers. All other chemical reagents were obtained from either Aldrich or Fisher Scientific and used without further purification.

2.2.2. Characterization

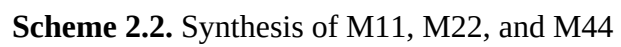
A Varian VNMRS 600 or 500 MHz or a Mercury 300 MHz spectrometer was used for ¹H and ¹³C NMR spectroscopy analysis and the residual solvent proton signal was used as the reference. The mass spectrometry (ESI MS) was performed using a Waters SYNAPT G2-Si quadrupole mass spectrometer or an Exactive Plus orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA). The samples for mass spectroscopy experiments were prepared by dissolving zwitterionic monomers in Milli-Q water at a concentration of 10 µg/g. Size exclusion chromatography (SEC) of zwitterionic P1P6-2, -3, and -4 was performed using a SEC system with a 515 HPLC pump from Waters equipped with a refractive index detector from Knauer (RI detector K-2301), one PSS Suprema 10 µm guard column (50 × 8 mm) and three PSS Suprema 10 µm columns (each 300 × 18 mm, 100 Å, 3000 Å, and 10000 Å). A 0.2 M sodium nitrate aqueous solution was used as the eluent; the flow rate was set at 1.0 mL/min in the analysis. This aqueous

SEC system was calibrated with narrow disperse poly(ethylene oxide) (PEO) standards with a molecular weight range from 238 to 222k Da. The SEC data were processed using Cirrus GPC/SEC software from Polymer Laboratories, Inc. Note that P55 and P66 cannot be eluted out from this aqueous SEC system. The zwitterionic monomers and polymers were freeze-dried using a LABCONCO 76705 Series Freeze Dryer.

2.2.3. Synthesis of Zwitterionic DPSMA Sulfobetaine Methacrylate Monomers with Various *N*-Substituents

The detailed synthetic procedures and characterization data for the corresponding sulfobetaine methacrylate monomers of P11, P22, P44, P55, P66, P16, P1C6, and P1P6 in Scheme 2.1 (i.e., monomer M11, M22, M44, M55, M66, M16, M1C6, and M1P6, respectively) as well as monomer M12OSi (Scheme 2.1) are shown below. M11, M22, and M44 were prepared by reacting 2-(*N,N*-dimethylamino)ethyl methacrylate, 2-(*N,N*-diethylamino)ethyl methacrylate, and 2-(*N,N*-di(*n*-butyl)amino)ethyl methacrylate with 1,3-propanesultone (Scheme 2.2), respectively, using a similar procedure previously reported for M11.³³ The monomers were purified and freeze-dried. The characterization data for M11 can be found in a previous publication from our group.³³

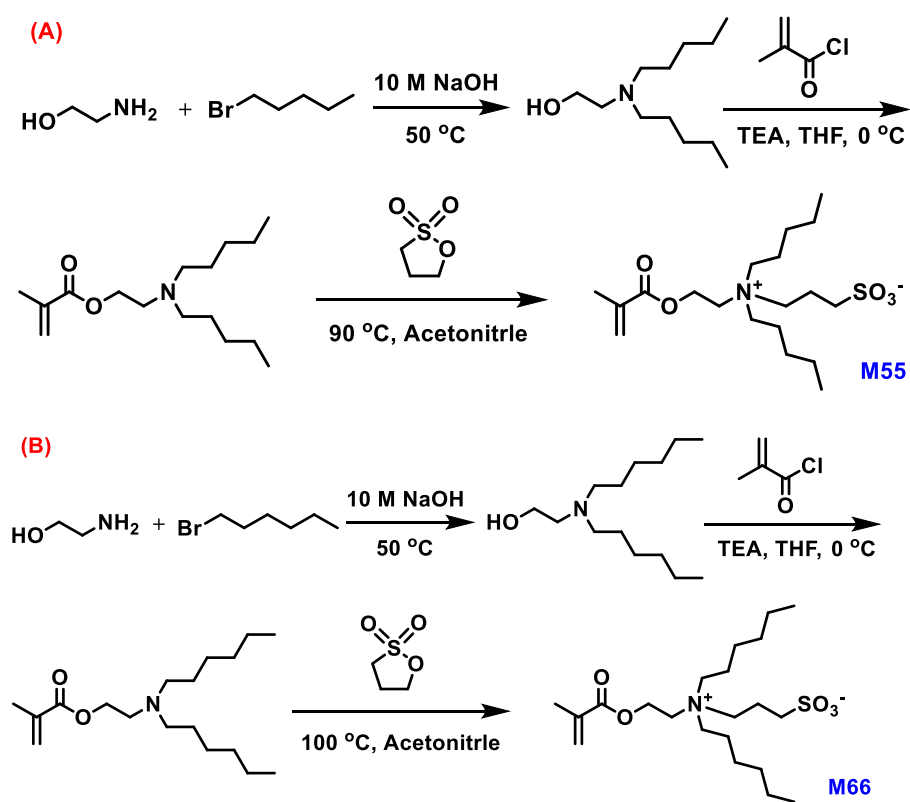
Monomer M22: ¹H NMR (D₂O, 500 MHz), δ (ppm) = 6.26 (s, CHH=C(CH₃), 1H), 5.91 (s, CHH=C(CH₃), 1H), 4.71 (t, -OCH₂CH₂N(CH₂CH₃)₂-, 2H), 3.87 (t, -OCH₂CH₂N(CH₂CH₃)₂-, 2H), 3.68-3.51 (m, -OCH₂CH₂N(CH₂CH₃)₂-, -NCH₂CH₂CH₂SO₃⁻, 6H), 3.08 (t, -CH₂CH₂SO₃⁻, 2H), 2.38-2.21 (m, -NCH₂CH₂CH₂SO₃⁻, 2H), 2.06 (s, CH₂=C(CH₃), 3H), 1.47 (t, -OCH₂CH₂N(CH₂CH₃)₂-, 6H). ¹³C NMR (D₂O, 125 MHz), δ (ppm) = 168.24, 135.17, 127.85, 58.08, 56.23, 55.40, 54.36, 47.25, 17.49, 6.96. MS: *m/z* cal [M+H]⁺: 308.1487; found: 308.1526; mass error: 12.66 ppm. The ¹H and ¹³C NMR spectra of M22 are included in Appendix A (Figure A1).



Monomer M44: ^1H NMR (D_2O , 300 MHz), δ (ppm) = 6.19 (s, $\text{CHH}=\text{C}(\text{CH}_3)$, 1H), 5.83 (s, $\text{CHH}=\text{C}(\text{CH}_3)$, 1H), 4.63 (t, $-\text{COOCH}_2\text{CH}_2\text{N}-$, 2H), 3.80 (t, $-\text{COOCH}_2\text{CH}_2\text{N}-$, 2H), 3.63-3.48 (m, $-\text{N}^+\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$, 2H), 3.41-3.38 (m, $-\text{N}^+(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_2-$, 4H), 2.97 (t, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$, 2H), 2.30-2.12 (m, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$, 2H), 1.98 (s, $\text{CH}_2=\text{C}(\text{CH}_3)$, 3H), 1.86-1.61 (m, $-\text{N}(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_2$, 4H), 1.51-1.27 (m, $-\text{N}(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_2$, 4H), 0.99 (t, $-\text{N}(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_2$, 6H). ^{13}C NMR (D_2O , 75 MHz), δ (ppm) = 168.30, 135.07, 127.75, 59.63, 59.36, 59.26, 58.01, 47.09, 23.13, 19.02, 17.27, 12.78. MS: m/z cal $[\text{M}+\text{H}]^+$: 364.2113; found: 364.2153; mass error: 10.98 ppm. The ^1H and ^{13}C NMR spectra of M44 are included in Appendix A (Figure A2).

2.2.3.1. Synthesis of Zwitterionic Monomer M55

M55 was prepared according to Scheme 2.3A via a multi-step process. 2-Aminoethanol (5.275 g, 86.36 mmol) was added to a 250 mL 3-necked round-bottom flask equipped with a magnetic stir bar and an addition funnel. The reaction flask was then placed in a 50 °C oil bath. 1-Bromopentane (30.00 g, 198.6 mmol) was added dropwise from the addition funnel while stirring. After 3 h, a 10 M aqueous solution of NaOH (25.9 mL, 0.259 mol) was added dropwise from the addition funnel. After the mixture was stirred at 50 °C overnight, the flask was removed from the oil and cooled to room temperature. The reaction mixture was transferred to a separatory funnel and the product was extracted with methylene chloride (50 mL $\times$ 3). The organic layers were combined, washed with brine (30 mL), and dried over anhydrous sodium sulfate. After filtering off the solid, the solution was concentrated by a rotary evaporator and further dried under high vacuum. The crude product was purified by silica gel column chromatography using a mixture of ethyl acetate and hexanes (2 : 1, v/v) as the eluent. The product was thoroughly dried under high vacuum, yielding a colorless liquid (7.312 g, 42.1 %). ^1H NMR (CDCl_3 , 500 MHz), δ (ppm) =



Scheme 2.3. Synthesis of Zwitterionic Monomer M55 (A) and M66 (B).

3.52 (t, HOCH₂CH₂-, 2H), 3.05 (br s, HOCH₂CH₂-, 1H), 2.56 (t, HOCH₂CH₂-, 2H), 2.43 (t, -N(CH₂CH₂CH₂CH₂CH₃)₂, 4H), 1.50 – 1.14 (m, -N(CH₂CH₂CH₂CH₂CH₃)₂, 12H), and 0.89 (t, -N(CH₂CH₂CH₂CH₂CH₃)₂, 6H). ¹³C NMR (CDCl₃, 126 MHz), δ (ppm) = 58.31, 55.46, 53.78, 29.61, 26.86, 22.59, 14.05.

2-(Di(*n*-pentyl)amino)ethan-1-ol (6.912 g, 34.33 mmol), triethylamine (4.518 g, 44.68 mmol), and dry THF (15 mL) were added to a 250 mL 3-necked round-bottom flask equipped with a magnetic stir bar and an addition funnel. After the mixture was stirred in an ice/water bath for 5 min, a solution of methacryloyl chloride (4.669 g, 44.66 mmol) in dry THF (20 mL) was added dropwise by the addition funnel. The reaction mixture became cloudy and slightly brown, and was allowed to warm naturally to room temperature. After stirring overnight, the precipitate was filtered off, and the volatiles were removed by rotary evaporation. The crude product was redissolved in methylene chloride and washed with an aqueous solution of 1 M NaOH (25 mL × 3). The organic phase was dried over anhydrous sodium sulfate. After removal of the solid and the solvents, the product was purified by silica gel column chromatography using a mixture of ethyl acetate and hexanes (1 : 10, v/v) as the eluent, yielding a colorless liquid (5.968 g, 64.6 %). ¹H NMR (CDCl₃, 500 MHz), δ (ppm) = 6.10 and 5.55 (s, CH₂=C(CH₃)COO-, 2H), 4.20 (t, -OCH₂CH₂N-, 2H), 2.74 (t, -OCH₂CH₂N-, 2H), 2.45 (-N(CH₂CH₂CH₂CH₂CH₃)₂, 4H), 1.94 (s, CH₂=C(CH₃)COO-, 3H), 1.50 – 1.15 (m, -N(CH₂CH₂CH₂CH₂CH₃)₂, 12H), 0.89 (t, -N(CH₂CH₂CH₂CH₂CH₃)₂, 6H). ¹³C NMR (CDCl₃, 126 MHz), δ (ppm) = 167.41, 136.40, 125.27, 63.15, 54.82, 52.13, 29.62, 27.08, 22.63, 18.30, 14.07.

2-(Di(*n*-pentyl)amino)ethyl methacrylate (2.009 g, 7.461 mmol), 1,3-propanesultone (1.018 g, 8.335 mmol), hydroquinone (16.4 mg), and acetonitrile (10 mL) were added to a 50 mL two-necked round-bottom flask equipped with a magnetic stir bar and a reflux condenser. The mixture

was stirred at room temperature until homogeneous, and the flask was then placed into an oil bath with a preset temperature of 90 °C. After stirring for 42 h, the flask was removed from the oil bath and placed in a freezer. A small amount of a white precipitate was observed after storing in the freezer for 4 h. The volatiles were removed using a rotavapor, and Milli-Q water was added to dissolve the crude product. The aqueous solution of the crude product was thoroughly washed with stabilizer-free diethyl ether (25 mL $\times$ 5) to remove the inhibitor. The resultant monomer solution was concentrated in an ice/water bath using a nitrogen gas stream. The concentrated solution was stored in the freezer, and the monomer concentration was determined to be 56.4 wt% by gravimetric analysis of a small portion after drying under high vacuum. Yield: 1.200 g (41.1 %). ^1H NMR (D_2O , 300 MHz), δ (ppm) = 6.17 and 5.82 (s, $\text{CH}_2=\text{C}(\text{CH}_3)\text{COO}^-$, 2H), 4.62 (t, $-\text{OCH}_2\text{CH}_2\text{N}^+$, 2H), 3.79 (t, $-\text{OCH}_2\text{CH}_2\text{N}^+$, 2H), 3.52 (t, $-\text{N}^+\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$, 2H), 3.39 (t, $-\text{N}^+(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_2$, 4H), 2.98 (t, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$, 2H), 2.25-2.15 (m, $-\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$, 2H), 1.96 (s, $\text{CH}_2=\text{C}(\text{CH}_3)\text{COO}^-$, 3H), 1.80-1.70 (m, $-\text{N}^+(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_2$, 4H), 1.42-1.32 (m, $-\text{N}^+(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_2$, 8H), 0.91 (t, $-\text{N}^+(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_2$, 6H). ^{13}C NMR (D_2O , 126 MHz), δ (ppm) = 168.46, 135.06, 127.75, 59.41, 58.01, 57.14, 56.48, 47.08, 27.56, 21.50, 20.82, 17.45, 17.26, and 13.00. MS: m/z cal $\text{C}_{19}\text{H}_{37}\text{NO}_5\text{S}$ $[\text{M}+\text{H}]^+$: 392.2465; found: 392.2562; mass error: 24.73 ppm. The ^1H and ^{13}C NMR of M55 are included in Appendix A (Figure A3).

2.2.3.2. Synthesis of Zwitterionic Monomer M66

M66 was prepared using a procedure similar to that for M55 (Scheme 2.3B). The yield of 2-(di(*n*-hexyl)amino)ethanol: 64.6 %. ^1H NMR (CDCl_3 , 500 MHz), δ (ppm) = 3.52 (t, $\text{HOCH}_2\text{CH}_2-$, 2H), 3.03 (s, $\text{HOCH}_2\text{CH}_2-$, 1H), 2.56 (t, $\text{HOCH}_2\text{CH}_2-$, 2H), 2.43 (t, $-\text{N}(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_2$, 4H), 1.51-1.38 (m, $-\text{N}(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_2$, 4H), 1.32-1.25

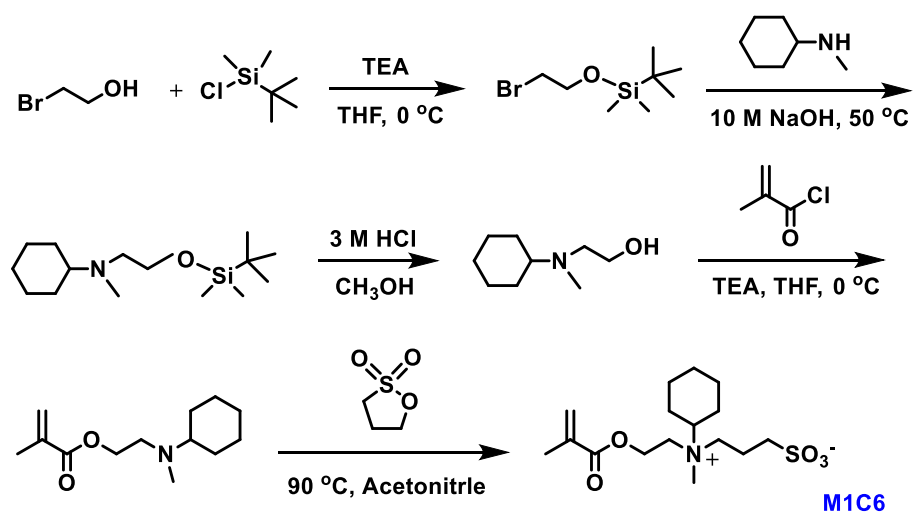
(m, -N(CH₂CH₂CH₂CH₂CH₂CH₃)₂, 12H), 0.88 (t, -N(CH₂CH₂CH₂CH₂CH₂CH₃)₂, 6H). ¹³C NMR (CDCl₃, 125 MHz), δ (ppm) = 58.32, 55.48, 53.83, 31.78, 27.16, 27.09, 22.63, 14.03.

The yield of 2-(di(*n*-hexyl)amino)ethyl methacrylate: 80.9 %. ¹H NMR (CDCl₃, 500 MHz), δ (ppm) = 6.10 and 5.55 (s, CH₂=C(CH₃)COO-, 2H), 4.20 (t, -OCH₂CH₂N-, 2H), 2.74 (t, -OCH₂CH₂N-, 2H), 2.46 (t, -N(CH₂CH₂CH₂CH₂CH₂CH₃)₂, 4H), 1.94 (s, CH₂=C(CH₃)COO-, 3H), 1.48-1.38 (m, -N(CH₂CH₂CH₂CH₂CH₂CH₃)₂, 4H), 1.32-1.23 (m, -N(CH₂CH₂CH₂CH₂CH₂CH₃)₂, 12H), 0.88 (t, -N(CH₂CH₂CH₂CH₂CH₂CH₃)₂, 6H). ¹³C NMR (CDCl₃, 125 MHz), δ (ppm) = 167.43, 136.41, 125.30, 63.17, 54.87, 52.14, 31.83, 27.11, 22.67, 18.32, 14.05.

The yield of 3-(di-*n*-hexyl(2-(methacryloyloxy)ethyl)ammonio)propane-1-sulfonate (M66) from the reaction of 2-(di(*n*-hexyl)amino)ethyl methacrylate with 1,3-propanesultone: 19.7 %. ¹H NMR (D₂O, 300 MHz), δ (ppm) = 6.18 and 5.82 (s, CH₂=C(CH₃)COO-, 2H), 4.63 (t, -OCH₂CH₂N⁺-, 2H), 3.78 (t, -OCH₂CH₂N⁺-, 2H), 3.53 (t, -N⁺CH₂CH₂CH₂SO₃⁻, 2H), 3.38 (t, -N⁺(CH₂CH₂CH₂CH₂CH₂CH₃)₂-, 4H), 2.98 (t, -N⁺CH₂CH₂CH₂SO₃⁻, 2H), 2.30-2.06 (m, -N⁺CH₂CH₂CH₂SO₄⁻, 2H), 1.95 (s, CH₂=C(CH₃)COO-, 3H), 1.80-1.67 (m, -N⁺(CH₂CH₂CH₂CH₂CH₂CH₃)₂, 4H), 1.44-1.25 (m, -N⁺(CH₂CH₂CH₂CH₂CH₂CH₃)₂, 12H), 0.89 (t, -N⁺(CH₂CH₂CH₂CH₂CH₂CH₃)₂, 6H). ¹³C NMR (D₂O, 151 MHz), δ (ppm) = 168.46, 135.05, 127.75, 59.39, 58.01, 57.09, 56.48, 47.07, 30.38, 25.05, 21.68, 21.04, 17.43, 17.26, and 13.11. MS: m/z cal C₂₁H₄₁NO₅S [M+H]⁺: 420.2778; found: 420.2895; mass error: 27.84 ppm. The ¹H and ¹³C NMR of M66 are included in Appendix A (Figure A4).

2.2.3.3. Synthesis of Zwitterionic Monomer M1C6

M1C6 was synthesized via a multi-step process as shown in Scheme 2.4. Synthesis of (2-bromoethoxy)(*tert*-butyl)dimethylsilane. 2-Bromoethanol (10.468 g, 83.77 mmol), 4-dimethylaminopyridine (1.034 g, 8.464 mmol), triethylamine (10.175 g, 100.55 mmol, distilled



Scheme 2.4. Synthesis of Sulfobetaine Methacrylate Monomer M1C6

from CaH_2), and dry THF (50 mL) were added to a 250 mL 3-necked round-bottom flask equipped with an addition funnel. The flask was placed in an ice/water bath, and the mixture was stirred with a magnetic stir bar. A solution of *tert*-butyldimethylsilyl chloride (15.127 g, 100.36 mmol) in dry THF (20 mL) was added dropwise from the addition funnel into the reaction flask. After stirring overnight, the reaction mixture was filtered to remove the precipitate and the solvent was evaporated by a rotavapor. The crude product was dissolved in dichloromethane (75 mL) and washed with 0.5 M HCl (40 mL $\times$ 2), an aqueous NaHCO_3 solution (sat. $\text{NaHCO}_3(\text{aq})/\text{H}_2\text{O}$, 50/50, v/v, 40 mL $\times$ 3), and brine (40 mL). The organic phase was then dried over anhydrous sodium sulfate. After the solid was removed by gravity filtration, the solution was concentrated by rotary evaporation; the product was dried under vacuum and collected as a clear liquid (yield: 16.090 g, 80.3 %). ^1H NMR (CDCl_3 , 600 MHz), δ (ppm) = 3.89 (t, $\text{BrCH}_2\text{CH}_2\text{O}$ -, 2H), 3.39 (t, $\text{BrCH}_2\text{CH}_2\text{O}$ -, 2H), 0.90 (s, $-\text{OSi}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$, 9H), 0.09 (s, $-\text{OSi}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$, 6H). ^{13}C NMR (CDCl_3 , 151 MHz), δ (ppm) = 63.51, 33.27, 25.82, 18.32, and 15.27.

N-Cyclohexyl-*N*-methylaniline (9.465 g, 83.61 mmol) was added to a 100 mL 3-necked round-bottom flask equipped with a magnetic stir bar and an addition funnel. The flask was placed in an oil bath with a preset temperature of 75 °C, and (2-bromoethyl)(*tert*-butyl)dimethylsilane (10.009 g, 41.84 mmol) was added dropwise to the flask under the stirring conditions. After the mixture was stirred vigorously for 1 h, a solution of NaOH (10 M, 5.43 mL, 54.3 mmol) was added through the addition funnel. After 12 h, ^1H NMR analysis showed that the reaction was complete. The flask was removed from the oil bath and cooled to room temperature. The mixture was transferred to a separatory funnel, and the product was extracted with dichloromethane (30 mL $\times$ 3). The organic layers were combined and washed with H_2O (40 mL $\times$ 4) and brine (40 mL); the product solution was dried over anhydrous sodium sulfate overnight. After gravity filtration to remove the

solid, the solution was concentrated, and the crude product was vacuum distilled using a short path distillation apparatus, yielding a colorless liquid (yield: 7.298 g, 73.0 %). ^1H NMR (CDCl_3 , 600 MHz), δ (ppm) = 3.67 (t, $-\text{NCH}_2\text{CH}_2\text{O}-$, 2H), 2.56 (t, $-\text{NCH}_2\text{CH}_2\text{O}-$, 2H), 2.36-2.32 (m, $-\text{N}(\text{CH}_3)\text{CH}(\text{CH}_2)_2$, 1H), 2.29 (s, $-\text{N}(\text{CH}_3)-$, 3H), 1.80-1.04 (m, $-\text{N}(\text{CH}_3)(\text{CH}(\text{CH}_2\text{CH}_2)_2\text{CH}_2)$, 10H), 0.89 (s, $-\text{OSi}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$, 9H), 0.05 (s, $-\text{OSi}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$, 6H). ^{13}C NMR (CDCl_3 , 151 MHz), δ (ppm) = 63.26, 62.56, 55.59, 39.06, 28.72, 26.36, 25.97, 18.36, 5.28.

(*N*-(2-((*tert*-Butyldimethylsilyl)oxy)ethyl)-*N*-methylcyclohexanamine (7.203 g, 26.53 mmol) and methanol (30.0 mL) were added to a 250 mL two-necked round-bottom flask equipped with an addition funnel. After the flask was placed in an ice/water bath, a 3 M HCl solution (22.1 mL, 66.3 mmol) was added dropwise. ^1H NMR analysis showed that the reaction was complete after 5 h. The solution was concentrated and washed with 50 wt% NaOH(aq) (8 mL) followed by washing the aqueous layer with methylene chloride (75 mL). This was repeated once. The product was purified by vacuum distillation using a short path distillation apparatus and collected as a clear colorless liquid (yield: 4.154 g, 99.6 %). ^1H NMR (CDCl_3 , 600 MHz), δ (ppm) = 3.51 (t, $\text{HOCH}_2\text{CH}_2\text{N}-$, 2H), 3.20 (br s, $\text{HOCH}_2\text{CH}_2\text{N}-$, 1H), 2.57 (t, $\text{HOCH}_2\text{CH}_2\text{N}-$, 2H), 2.40-2.34 (m, $-\text{N}(\text{CH}_3)(\text{CH}(\text{CH}_2\text{CH}_2)_2\text{CH}_2)$, 1H), 2.22 (s, $-\text{N}(\text{CH}_3)-$, 3H), 1.78-1.02 (m, $-\text{N}(\text{CH}_3)\text{CH}(\text{CH}_2\text{CH}_2)_2\text{CH}_2$, 10H). ^{13}C NMR (CDCl_3 , 151 MHz), δ (ppm) = 63.19, 57.99, 54.39, 36.64, 28.71, 26.26, and 25.97.

2-(Cyclohexyl(methyl)amino)ethan-1-ol (5.218g, 33.18 mmol), triethylamine (4.267 g, 42.17 mmol, distilled from CaH_2), and dry THF (15 mL) were added to a 100 mL 3-necked round-bottom flask equipped with a magnetic stir bar and an addition funnel. While the mixture was stirred in an ice/water bath, a solution of methacryloyl chloride (4.00 mL, 40.94 mmol) in THF (10 mL) was added dropwise via the addition funnel. After stirring overnight, the mixture was filtered to remove

the precipitate, concentrated by rotary evaporation, dissolved in dichloromethane (75 mL), and washed with in water (25 mL $\times$ 3). The product solution was dried by stirring the organic solution over anhydrous sodium sulfate. After concentration via rotary evaporation, the crude product was purified by silica gel column chromatography using a mixture of ethyl acetate and hexanes (1:1, v/v) as eluent. The pure product was collected as a clear, colorless liquid (2.660 g, 35.6 %). ^1H NMR (CDCl_3 , 600 MHz), δ (ppm) = 6.10 and 5.54 (s, $\text{CH}_2=\text{C}(\text{CH}_3)\text{CO}_2^-$, 2H), 4.20 (t, - $\text{OCH}_2\text{CH}_2\text{N}^-$, 2H), 2.73 (t, - $\text{OCH}_2\text{CH}_2\text{N}^-$, 2H), 2.38-2.35 (m, - $\text{N}(\text{CH}_3)(\text{CH}(\text{CH}_2\text{CH}_2)_2\text{CH}_2)$, 1H), 2.33 (s, - $\text{N}(\text{CH}_3)-$, 3H), 1.93 (s, $\text{CH}_2=\text{C}(\text{CH}_3)\text{CO}_2^-$, 3H), 1.78-1.04 (m, - $\text{N}(\text{CH}_3)(\text{CH}(\text{CH}_2\text{CH}_2)_2\text{CH}_2)$, 10H). ^{13}C NMR (CDCl_3 , 151 MHz), δ (ppm) = 167.44, 136.36, 125.35, 63.65, 63.14, 51.60, 38.93, 28.78, 26.27, 25.91, 18.32.

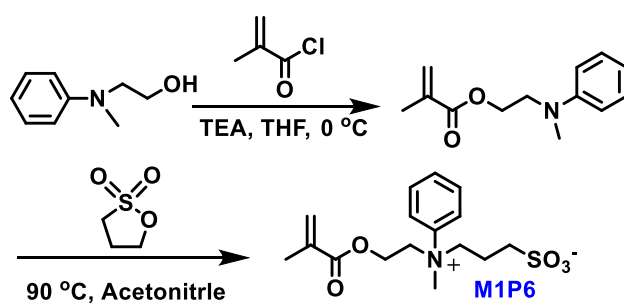
2-(Cyclohexyl(methyl)amino)ethyl methacrylate (2.388 g, 10.60 mmol), 1,3-propanesultone (1.361 g, 11.14 mmol), hydroquinone (21.3 mg), and dry acetonitrile (5 mL) were added to a 100 mL three-necked round-bottom flask equipped with a magnetic stir bar and a reflux condenser. After the mixture was stirred at room temperature to become homogeneous, the flask was placed in an oil bath with a preset temperature of 90 °C. From ^1H NMR spectroscopy analysis, the reaction reached completion after 18 h; the flask was removed from the oil bath and placed in an ice bath while stirring. The product was precipitated into diethyl ether three times (150 mL $\times$ 3). The precipitate was dissolved in Milli-Q water (3 mL) and washed with purified diethyl ether (20 mL $\times$ 4). The product was concentrated using a filtered nitrogen gas stream and then freeze-dried. The product was collected as a white solid (yield: 2.376 g, 64.5 %). ^1H NMR (D_2O , 500 MHz), δ (ppm) = 6.18 and 5.81 (s, $\text{CH}_2=\text{C}(\text{CH}_3)\text{COO}^-$, 2H), 4.64 (t, - $\text{OCH}_2\text{CH}_2\text{N}^+$, 2H), 3.88-3.77 (m, - $\text{OCH}_2\text{CH}_2\text{N}^+$, 2H), 3.65-3.53 (m, - $\text{N}^+(\text{CH}_3)(\text{CH}(\text{CH}_2\text{CH}_2)_2\text{CH}_2)\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_4^-$, 3H), 3.13 (s, - $\text{N}^+(\text{CH}_3)-$, 3H), 2.97 (t, - $\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_4^-$, 2H), 2.26-2.19 (m, - $\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3^-$, 2H), 2.26-1.16

(m, $-N^+(CH_3)(CH(CH_2CH_2)_2CH_2)$, 10H), 1.96 (s, $CH_2=C(CH_3)CO_2^-$, 3H). ^{13}C NMR ($CDCl_3$, 151 MHz), δ (ppm) = 168.45, 135.07, 127.70, 72.77, 58.18, 57.94, 57.05, 47.23, 45.82, 25.50, 25.42, 25.01, 24.28, 17.81, and 17.26. MS: m/z cal $C_{16}H_{29}NO_5S$ $[M+H]^+$: 348.1839; found: 348.1889; mass error: 14.36 ppm. The 1H and ^{13}C NMR of M1C6 are included in Appendix A (Figure A5).

2.2.3.4. Synthesis of Zwitterionic Monomer M1P6

M1P6 was synthesized according to Scheme 2.5. 2-(Methyl(phenyl)amino)ethanol (6.111 g, 40.4 mmol) and triethylamine (5.728 g, 56.6 mmol) were dissolved in chloroform (15.9 g) in a 250 mL three-necked flask and cooled in an ice/water bath for 10 min under an N_2 environment. Methacryloyl chloride (4.835 g, 46.3 mmol) dissolved in CH_2Cl_2 (15.9 g) was added dropwise from an addition funnel into the mixture over a period of 20 min at $\sim 0^\circ C$. The reaction mixture was allowed to warm naturally to room temperature and stirred overnight. Afterwards, the mixture was transferred to a separatory funnel and washed with a saturated $NaHCO_3$ aqueous solution (50 mL $\times$ 3) and saturated $NaCl$ aqueous solution (50 mL $\times$ 2). The organic phase was dried with anhydrous sodium sulfate and concentrated using a rotary evaporator. The product was purified by column chromatography using ethyl acetate/hexane (v/v, 1/40) as eluent, and a clear liquid was obtained after drying under high vacuum (3.817 g, 39.1% yield). 1H NMR ($CDCl_3$, 500 MHz), δ (ppm) = 7.26 (t, aromatic, 2H), 6.78 (d, aromatic, 2H), 6.74 (t, aromatic, 1H), 6.09 (s, $CHH=C(CH_3)-$, 1H), 5.57 (s, $CHH=C(CH_3)-$, 1H), 4.35 (t, $-OCH_2CH_2N(CH_3)-$, 2H), 3.68 (t, $-OCH_2CH_2N(CH_3)-$, 2H), 3.02 (s, $-OCH_2CH_2N(CH_3)C_6H_5$, 3H), 1.94 (s, $CHH=C(CH_3)-$, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz), δ (ppm) = 167.31, 148.77, 136.01, 129.25, 125.86, 116.72, 112.17, 62.02, 51.16, 38.58, 18.34.

2-(Methyl(phenyl)amino)ethyl methacrylate (1.556 g, 7.10 mmol), 1,3-propanesultone (0.0874 g, 7.16 mmol), and hydroquinone (32.5 mg) were dissolved in dry acetonitrile (7 mL) in

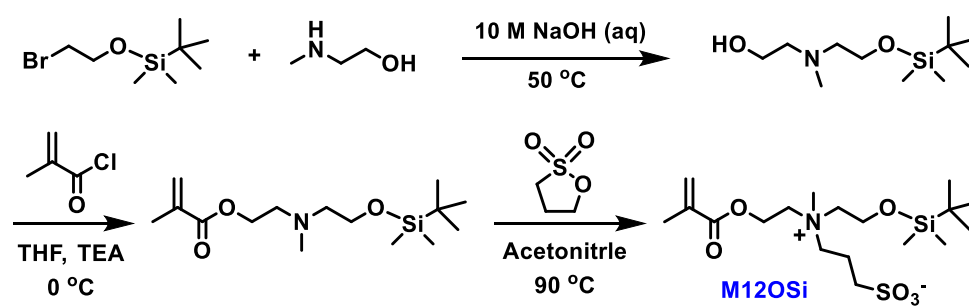


Scheme 2.5. Synthesis of Sulfobetaine Methacrylate Monomer M1P6

a 100 mL three-necked flask equipped with a magnetic stir bar. The mixture was stirred and refluxed at 90 °C for 60 h; a white solid was observed at the bottom of the flask. After the reaction mixture was cooled to room temperature, acetone (50 mL) was added into the flask to precipitate as much the zwitterionic product as possible. The white solid was collected and dissolved with DI water, and the solution was washed extensively with diethyl ether to remove the inhibitor and other possible impurities. The product was freeze-dried using a LABCONCO 76705 Series Freeze Dryer and obtained as a white solid (0.781 g, 32.1 % yield). ^1H NMR (D_2O , 500 MHz), δ (ppm) = 7.84 (d, aromatic, 2H), 7.74 (t, aromatic, 2H), 7.68 (t, aromatic, 1H), 5.92 (s, $\text{CHH}=\text{C}(\text{CH}_3)-$, 1H), 5.69 (s, $\text{CHH}=\text{C}(\text{CH}_3)-$, 1H), 4.75-4.52 (m, $-\text{OCH}_2\text{CH}_2\text{N}^+(\text{CH}_3)(\text{C}_6\text{H}_5)-$, 2H), 4.43-4.27 (m, $-\text{OCH}_2\text{CH}_2\text{N}^+(\text{CH}_3)(\text{C}_6\text{H}_5)-$, $-\text{CH}_2\text{N}^+(\text{CH}_3)(\text{C}_6\text{H}_5)\text{CHHCH}_2\text{CH}_2-$, 3H), 4.15-4.05 (m, $-\text{CH}_2\text{N}^+(\text{CH}_3)(\text{C}_6\text{H}_5)\text{CHHCH}_2\text{CH}_2-$, 1H), 3.80 (s, $-\text{OCH}_2\text{CH}_2\text{N}^+(\text{CH}_3)(\text{C}_6\text{H}_5)-$, 3H), 3.04-2.87 (m, $-\text{CH}_2\text{CH}_2\text{SO}_3^-$, 2H), 2.30-2.13 (m, $-\text{N}^+(\text{CH}_3)(\text{C}_6\text{H}_5)\text{CH}_2\text{CHHCH}_2\text{SO}_3^-$, 1H), 1.81 (s, $\text{CH}_2=\text{C}(\text{CH}_3)-$, 3H), 1.79-1.69 (m, $-\text{NCH}_2\text{CHHCH}_2\text{SO}_3^-$, 1H). ^{13}C NMR (D_2O , 126 MHz), δ (ppm) = 168.20, 140.98, 134.64, 130.99, 128.13, 128.06, 121.50, 67.90, 67.78, 58.49, 47.76, 47.23, 18.53, 17.33. MS: m/z cal $[\text{M}+\text{H}]^+$: 342.1330; found: 342.1362; error: 9.35 ppm. The ^1H and ^{13}C NMR of M1P6 are included in Appendix A (Figure A6).

2.2.3.5. Synthesis of Sulfobetaine Methacrylate Monomer M12OSi

M12OSi was prepared according to Scheme 2.6. 2-(Methylamino)ethanol (6.284 g, 83.66 mmol) was added to a 100 mL two-necked round-bottom flask equipped with an addition funnel and stirred with a magnetic stir bar at 50 °C. 2-Bromoethyl)(*tert*-butyl)dimethylsilane (10.013 g, 41.855 mmol) was added dropwise from the addition funnel, and a colorless, heterogeneous mixture was formed. After stirring for 1 h, an aqueous solution of 10 M NaOH (5.45 mL, 54.5 mmol) was added dropwise from the addition funnel. After the reaction proceeded for 2 days, the



Scheme 2.6. Synthesis of Sulfobetaine Methacrylate Monomer M12OSi

flask was removed from the oil bath and the reaction mixture was cooled to room temperature. The product was extracted with dichloromethane (40 mL $\times$ 3) using a separatory funnel. The organic fractions were combined, washed with a NaHCO₃ aqueous solution (40 mL $\times$ 3) and brine (30 mL), and dried with anhydrous sodium sulfate while stirring. After removal of the solid, the solution was concentrated by rotary evaporation, and the product, 2-((2-((*tert*-butyldimethylsilyl)oxy)ethyl)(methyl)amino)ethan-1-ol, was further dried under vacuum and obtained as a clear liquid (yield: 5.920 g, 60.7 %). ¹H NMR (CDCl₃, 600 MHz), δ (ppm) = 3.69 (t, -CH₂CH₂OSi-, 2H), 3.56 (t, HOCH₂CH₂-, 2H), 3.07 (br, HOCH₂CH₂-, 1H), 2.59-2.57 (m, HOCH₂CH₂N(CH₃)CH₂CH₂OSi-, 4H), 2.31 (s, -N(CH₃)-, 3H), 0.89 (s, -Si(CH₃)₂C(CH₃)₃, 9H), and 0.06 (s, -Si(CH₃)₂C(CH₃)₃, 6H). ¹³C NMR (CDCl₃, 126 MHz), δ (ppm) = 61.36, 59.22, 58.98, 58.51, 42.46, 25.89, 18.28, and -5.38.

2-((2-((*tert*-Butyldimethylsilyl)oxy)ethyl)(methyl)amnio)ethan-1-ol (2.040 g, 8.738 mmol), triethylamine (1.154 g, 11.41 mmol), and dry THF (5 mL) were added to a 50 mL two-necked round-bottom flask equipped with a magnetic stir bar and an addition funnel. The flask was then placed in an ice/water bath, and a solution of freshly distilled methacryloyl chloride (1.10 mL, 11.2 mmol) in dry THF (3 mL) was added dropwise through the addition funnel. A white precipitate formed as the methacryloyl chloride solution was added. After 2.5 h, ¹H NMR spectroscopy analysis showed that the reaction was complete. After 3 h, the reaction mixture was filtered to remove the precipitate, concentrated using a rotary evaporator, dissolved in methylene chloride and washed with deionized water (10 mL $\times$ 3), followed by washing with 1 M NaOH (aq) (7 mL $\times$ 2). After drying over sodium sulfate overnight, the product was concentrated, dried under high vacuum, and collected as a clear, colorless oil (yield: 2.042 g, 77.5 %). ¹H NMR (CDCl₃, 600 MHz), δ (ppm) = 6.11 and 5.55 (2s, CH₂=C(CH₃)COO-, 2H), 4.24 (t, -COOCH₂CH₂N-, 2H), 3.71

(t,-NCH₂CH₂OSi-), 2.78 (t, -COOCH₂CH₂N-, 2H), 2.61 (t, -NCH₂CH₂OSi-, 2H), 2.36 (s, -N(CH₃)-, 3H), 1.94 (s, CH₂=C(CH₃)COO-, 3H), 0.89 (s, -OSi(CH₃)₂C(CH₃)₃, 9H), 0.05 (s, -OSi(CH₃)₂C(CH₃)₃, 6H). ¹³C NMR (CDCl₃, 151 MHz), δ (ppm) = 167.35, 136.28, 125.45, 62.80, 61.66, 59.76, 56.13, 48.39, 25.90, 18.31, and -5.36.

2-((2-((*tert*-Butyldimethylsilyl)oxy)ethyl)(methyl)amino)ethyl methacrylate (1.938 g, 6.427 mmol), 1,3-propanesultone (0.825 g, 6.753 mmol), hydroquinone (19.3 mg), and acetonitrile (4.0 mL) were added to a 50 mL two-necked flask equipped with a magnetic stir bar and a reflux condenser. After the reaction mixture was stirred in a 90 °C oil bath for 24 h, the flask was removed from the oil bath and cooled to room temperature. The volatiles were removed, and the crude product was dissolved in a mixture of Milli-Q water and acetonitrile (4 : 1, v/v) and washed with stabilizer-free diethyl ether to remove the inhibitor (40 mL × 5). The aqueous solution of the product was then concentrated with a stream of filtered air flow, freeze-dried using a LABCONCO 76705 Series Freeze Dryer, and further dried under high vacuum at room temperature. The product was collected as a white, fluffy solid (yield: 2.213 g, 81.3 %). ¹H NMR (CDCl₃, 600 MHz), δ (ppm) = 6.10 and 5.64 (s, CH₂=C(CH₃)COO-, 2H), 4.66 (t, -OCH₂CH₂N-, 2H), 4.10 (t, -CH₂CH₂OSi-, 2H), 4.00-3.65 (m, -OCH₂CH₂N⁺(CH₃)(CH₂CH₂OSi(CH₃)₂C(CH₃)₃)CH₂CH₂-CH₂SO₃⁻, 6H), 3.30 (s, -N⁺(CH₃)-, 3H), 2.89 (t, -CH₂CH₂CH₂SO₃⁻, 2H), 2.35-2.23 (m, -CH₂CH₂CH₂SO₃⁻, 2H), 1.92 (s, CH₂=C(CH₃)COO-, 3H), 0.87 (s, -OSi(CH₃)₂C(CH₃)₃, 9H), 0.09 (s, -OSi(CH₃)₂C(CH₃)₃, 6H). ¹³C NMR (CDCl₃, 126 MHz), δ (ppm) = 166.09, 135.01, 126.76, 63.62, 62.66, 60.81, 57.95, 57.36, 49.27, 47.32, 25.49, 18.91, 17.98, 17.68, and -5.89. MS: m/z cal C₁₈H₃₅NO₆SSi [M+H]⁺: 424.2184; found 424.2281; mass error 22.86 ppm. The ¹H and ¹³C NMR of M12OSi are included in Appendix A (Figure A7).

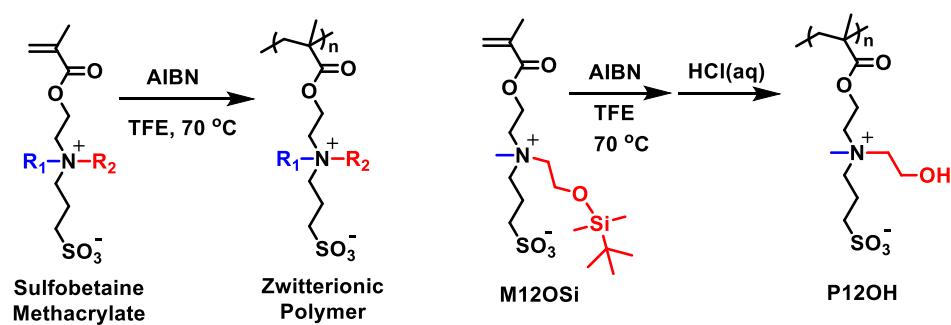
2.2.4. Synthesis of Zwitterionic Homopolymers of M11, M22, M44, M55, M66, M16, M1C6,

M1P6, and M12OSi by AIBN-Initiated Conventional Radical Polymerization

M11, M22, M44, M55, M66, M16, M1C6, M1P6, and M12OSi were polymerized directly by conventional free radical polymerization using AIBN as initiator (Scheme 2.7). The following shows the detailed procedure for the synthesis of P55 (P55-2) from M55 as an example. M55 (0.300 g, 0.767 mmol), AIBN (6.1 mg, 0.0037 mmol), TFE (0.951 g), and *N,N*-dimethylformamide (DMF, 69.2 mg) were added to a 25 mL Schlenk tube and stirred with a magnetic stir bar to form a clear and colorless solution. The reaction mixture was degassed by three cycles of freeze-pump-thaw. The polymerization was started by placing the Schlenk tube into an oil bath with a preset temperature of 70 °C. After 19 h, the polymerization was stopped by removing the tube from the oil bath and cooling it in an ice/water bath. The mixture was diluted with Milli-Q water, and the polymer was purified by dialysis (MWCO: 3500 Da, regenerated cellulose) against Milli-Q water, which was frequently changed, in an ice/water bath for 2 days. The aqueous solution of the polymer was then concentrated under a nitrogen gas stream and then freeze dried using a LABCONCO 76705 Series Freeze Dryer. The polymer was obtained as a white solid (yield: 0.145 g, 48.3%). For the deprotection of the homopolymers of M12OSi, a 1.0 M HCl solution (7.4 mL, 0.74 mmol) was added dropwise into the aqueous solution of the zwitterionic polymer after the dialysis. After the mixture was stirred at room temperature overnight, the volatiles were removed under high vacuum and the deprotected polymer was re-dissolved in Milli-Q water, freeze-dried, and obtained as a white solid (64.1 % yield).

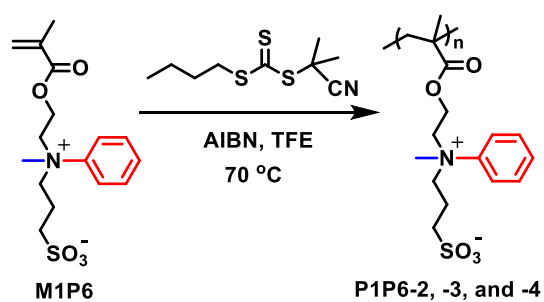
2.2.5. Reversible Addition-Fragmentation Chain Transfer (RAFT) Polymerization of M1P6

M1P6 was also polymerized by RAFT polymerization using *n*-butyl (2-cyano-2-propyl) trithiocarbonate as chain transfer agent (CTA) and AIBN as initiator (Scheme 2.8). The following



R_1	R_2	Monomer	Polymer
-CH ₃	-CH ₃	M11	P11
-CH ₃	-(CH ₂) ₅ CH ₃	M16	P16
-CH ₂ CH ₃	-CH ₂ CH ₃	M22	P22
-(CH ₂) ₃ CH ₃	-(CH ₂) ₃ CH ₃	M44	P44
-(CH ₂) ₄ CH ₃	-(CH ₂) ₄ CH ₃	M55	P55
-(CH ₂) ₆ CH ₃	-(CH ₂) ₆ CH ₃	M66	P66
-CH ₃	-(CH ₂) ₆ (cyclohexyl)	M1C6	P1C6
-CH ₃	-C ₆ H ₅ (phenyl)	M1P6	P1P6
-CH ₃	-CH ₂ CH ₂ O-tBDMSi	M12OSi	P12OH

Scheme 2.7. Synthesis of Zwitterionic Homopolymers of Sulfobetaine Methacrylate by AIBN-Initiated Conventional Free Radical Polymerization

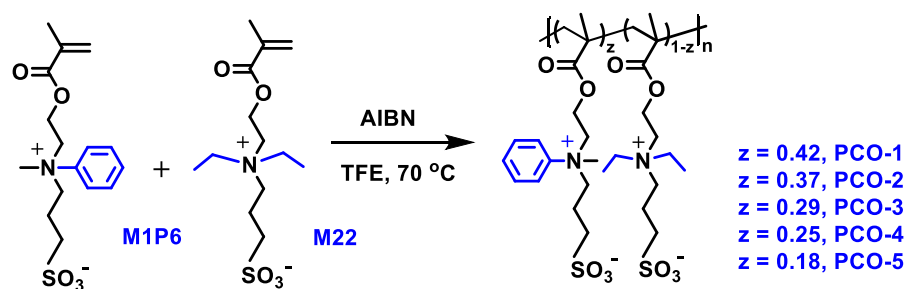


Scheme 2.8. Synthesis of Zwitterionic Homopolymers by Reversible Addition-Fragmentation Chain Transfer Polymerization of M1P6.

shows the synthesis of P1P6-4 from M1P6 by RAFT polymerization. M1P6 (0.308 g, 0.902 mmol), CTA (0.447 g of a stock solution of CTA in TFE with a concentration of 22.80 mg/g, 0.0437 mmol), AIBN (0.111 g of a stock solution of AIBN in TFE with a concentration of 20.38 mg/g, 0.0138 mmol), TFE (0.999 g), and DMF (51 mg) were added into a 25 mL 2-necked round bottom flask. After the mixture was degassed by three cycles of freeze-pump-thaw, the flask was placed in a 70 °C oil bath. The polymerization was stopped after 24 h by cooling the reaction flask in an ice/water bath. The polymer was purified by dialysis against Milli-Q water using Fisherbrand dialysis tubing (MWCO = 3500) for 3 days, and the water was changed frequently. The aqueous polymer mixture was concentrated with a filtered stream of air flow and then freeze-dried (yield: 0.158 g, 52.3%). The obtained polymer, P1P6-4, was characterized by aqueous SEC analysis. The SEC sample was prepared by dissolving an appropriate amount of P1P6-4 in a 0.2 M NaNO₃ aqueous solution to obtain a concentration of 2.5 mg/mL. SEC results: $M_{n,SEC}$ = 2.0 kDa and $\bar{D}$ = 2.13, relative to PEO standards. Two additional polymers of M1P6, P1P6-2 and P1P6-3, were synthesized and purified using similar procedures. SEC results (PEO standards): for P1P6-2, $M_{n,SEC}$ = 2.6 kDa and $\bar{D}$ = 2.13; for P1P6-3, $M_{n,SEC}$ = 2.3 kDa and $\bar{D}$ = 2.04. These three RAFT polymers were slightly pink in the bulk state, and their intrinsic viscosity values in 2,2,2-trifluoroethanol (TFE) at 25.0 °C were also measured.

2.2.6. Synthesis of P(M1P6-co-M22) Copolymers with Various Molar Ratios of M1P6 to M22

Five random copolymers of M1P6 and M22 (PCO-1, -2, -3, -4 and -5) were synthesized by AIBN-initiated conventional free radical polymerization using various molar ratios of M1P6 to M22 in the feeds (Scheme 2.9). Shown below is the detailed synthesis of PCO-1; other copolymers were prepared using similar procedures. M22 (0.685 g of a stock solution in TFE, 20.9 wt%, 0.466 mmol), M1P6 (0.159 g, 0.466 mmol), AIBN (3.0 mg, 18 μ mol), TFE (1.095 g), and DMF (56.2



Scheme 2.9. Synthesis of Zwitterionic Copolymers of M1P6 and M22 by AIBN-Initiated Conventional Free Radical Polymerization.

mg) were added to a 25 mL Schlenk tube equipped with a magnetic stir bar. The solution was degassed by three cycles of freeze-pump-thaw and placed in a preheated oil bath (70 °C). After 24 h, the polymerization was stopped by removing the flask from the oil bath, cooling it to room temperature and opening it to air. The polymer was purified by dialysis (regenerated cellulose, MWCO – 3500 Da) against Milli-Q Water for 2 days. The resultant mixture was concentrated under a N₂ gas stream and freeze dried. The copolymer, PCO-1, was collected as a white powder (85.2 mg, 28.2% yield). The composition of PCO-1 was determined by ¹H NMR spectroscopy analysis using the integrals of the peaks located in the range of 7.52-8.19 ppm, which corresponded to the five hydrogen atoms in the phenyl group of M1P6, and the peaks from 2.72 – 3.19 ppm, which corresponded to -CH₂SO₃⁻ of M22 and M1P6 units. Calculations showed that the molar content of M1P6 units in PCO-1 was 42.1%, which was close to that of M1P6 in the feed (50.0 %).

2.2.7. Determination of Cloud Points of Thermoresponsive Zwitterionic Polymers in Water

The cloud points (CPs) of thermoresponsive zwitterionic polymers at various concentrations were determined from the plots of transmittance versus temperature. For each thermoresponsive zwitterionic polymer, the solution with the highest concentration studied was prepared first by dissolving the polymer in a certain amount of Milli-Q water at either an elevated temperature (for UCST-type thermoresponsive polymers) or in an ice/water bath (for LCST-type thermoresponsive polymers). The aqueous polymer solution with a lower concentration was prepared by diluting the more concentrated solution with Milli-Q water. The optical transmittance of an aqueous polymer solution in a quartz cuvette (3.5 mL) at a wavelength of 500 nm was measured as a function of temperature upon cooling or heating using a T6U UV-visible spectrophotometer (Persee Analytics, Inc.) equipped with a Peltier temperature controller (PTC-2). The polymer solution was equilibrated at each selected temperature for 1 min with a heating/cooling rate of about 1 °C/min

before the transmittance was measured. The temperature at which a 50% transmittance change occurred was taken as the CP in this work.³⁶ The hysteresis of thermoresponsive behavior of a zwitterionic polymer in water was investigated at a selected concentration in a cooling-heating or a heating-cooling cycle following the same procedure detailed above.

2.2.8. Measurement of Intrinsic Viscosities of Zwitterionic Polymers in TFE

The intrinsic viscosities of zwitterionic polymers in TFE were determined according to a method by Wolf.^{37,38} Below is a procedure for the determination of the intrinsic viscosity for P55-1. A similar procedure was used for all of other zwitterionic polymers and copolymers. TFE (8 mL) was filtered through a 0.45 μm PTFE filter into a clean, dry Ubbelohde viscometer (Cannon-Ubbelohde Dilution Viscometer, 9722-L53 (150 D429), Size 50) in a water bath with the temperature preset at 25.0 $^{\circ}\text{C}$ using a Lauda Alpha immersion thermostat and thermally equilibrated for 5 min. The flow time was measured by pulling the TFE past the upper meniscus mark and using a stopwatch to record the time for the solvent to travel from the upper mark to the lower mark. The measurement was repeated 4 times with a maximum allowable variation of 0.1 sec. The average of the five runs was taken as t_0 . After the solvent was poured out, the viscometer was dried by a $\text{N}_2(\text{g})$ flow. A solution of P55-1 in TFE (11.0 mg/mL) was then filtered into the Ubbelohde viscometer and equilibrated at 25.0 $^{\circ}\text{C}$. The time for the solution's meniscus to travel from the upper to the lower mark was measured five times, and the average was recorded as t_1 for this concentration. The concentration of the P55-1 solution was then diluted to 10.0 mg/mL by adding an appropriate amount of filtered TFE to the Ubbelohde viscometer; the solution was mixed thoroughly and equilibrated at 25.0 $^{\circ}\text{C}$ for at least 5 min, followed by the measurement of flow time for a total of 5 times. This process was repeated for the concentrations of 8.0, 6.0, 5.0, 4.0, and 2.4 mg/mL. The 2.4 mg/mL polymer solution was then poured out; the viscometer was

thoroughly rinsed with TFE (8 mL $\times$ 3), dried under a N₂(g) stream, and mounted in the 25.0 °C water bath. Subsequently, 4.0 mL of the 2.4 mg/mL solution was added and diluted to 1.0 mg/mL. Five measurements of flow time were taken and the average was used. A plot of the natural logarithm of relative viscosity ($\eta_{\text{rel}} = t_1/t_0$) versus concentration was made and a linear regression was performed. The slope was taken as the intrinsic viscosity of the polymer in TFE at 25.0 °C according to the Wolf's method.^{37,38}

2.3. Results and Discussion

2.3.1. Synthesis and Characterization of PDPSMAs

A total of seven PDPSMAs with various *N*-substituents, including symmetric di(*n*-alkyl) (P22, P44, P55, and P66), methyl and 2-hydroxyethyl (P12OH), methyl and cyclohexyl (P1C6), and methyl and phenyl (P1P6) on the nitrogen atom (Scheme 2.1), were synthesized by conventional free radical polymerization in 2,2,2-trifluoroethanol (TFE) using 2,2'-azobis(2-methylpropionitrile) (AIBN) as initiator. We also made P11 for comparison with the PDPSMAs with symmetric *N,N*-di(*n*-alkyl) substituents and P16 for comparison with P1C6 and P1P6. All of the homopolymers were synthesized directly from the corresponding DPSMA sulfobetaine methacrylate monomers except P12OH, which was prepared by polymerization of M12OSi (Scheme 2.1) and subsequent removal of *tert*-butyldimethylsilyl protective groups under acidic conditions. In addition, three lower molecular weight P1P6 homopolymers were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization.^{36,39,40} All of the polymer samples were purified by dialysis against Milli-Q water and freeze-dried. ¹H NMR analysis showed that the spectra agreed with the molecular structures of the zwitterionic polymers (Figure A8-A16). The characterization data for the polymers are summarized in Table 2.1.

Table 2.1. Characterization Data and Solution Behavior in Water of Zwitterionic PDPSMA Homopolymers

PDPSMA Homopolymer	DPSMA Monomer	$[\eta]$ (dL/g) ^a	Solution Behavior in H ₂ O	Cloud Point ^c
P11	M11	0.284	UCST	47 °C
P22-1	M22	0.478	Soluble	N/A
P22-2	M22	0.684	Soluble	N/A
P44-1	M44	0.376	Soluble	N/A
P44-2	M44	0.309	Soluble	N/A
P55-1	M55	0.129	LCST	42 °C
P55-2	M55	0.288	LCST	40 °C
P66-1	M66	0.116	Insoluble	N/A
P66-2	M66	0.098	Insoluble	N/A
P16-1	M16	0.248	LCST	11 °C
P16-2	M16	0.227	LCST	12 °C
P1C6-1	M1C6	0.176	Soluble	N/A
P1C6-2	M1C6	0.520	Soluble	N/A
P1P6-1	M1P6	0.155	Insoluble	N/A
P1P6-2	M1P6	0.075; ^a ($M_{n,SEC} = 2.6$ kDa; $\bar{D} = 2.13$) ^b	Insoluble	N/A
P1P6-3	M1P6	0.056; ^a ($M_{n,SEC} = 2.3$ kDa; $\bar{D} = 2.04$) ^b	UCST	56 °C ^d
P1P6-4	M1P6	0.051; ^a ($M_{n,SEC} = 2.0$ kDa; $\bar{D} = 2.13$) ^b	UCST	37 °C ^d
P12OH-1	M12OSi	0.358	UCST	33 °C
P12OH-2	M12OSi	0.202	UCST	23 °C

^a $[\eta]$ values in 2,2,2-trifluoroethanol (TFE) at 25.0 °C were determined using the Wolf's method.^{37,38} ^b Number average molecular weight ($M_{n,SEC}$) and dispersity ($\bar{D}$) were measured by aqueous SEC analysis relative to PEO standards using a 0.2 M NaNO₃ solution as eluent. Note that P1P6-2, -3, and -4 are soluble in the 0.2 M NaNO₃ aqueous solution and also in D₂O with 0.5 M NaCl despite the observation that they are not soluble at room temperature in pure water. ^c Cloud points (CPs) were the temperatures at which a 50% transmittance change occurred. The CPs of UCST and LCST polymers were determined from cooling and heating curves, respectively, at 1.0 wt%. ^d The CPs are for 0.2 wt% polymer solutions. Note that the CP of P1P6-4 at 1.0 wt% was 66.5 °C.

The PDPSMAs shown in Table 2.1 represent a diverse set of zwitterionic materials with varying intra- and intermolecular interactions. We found that it was extremely challenging to characterize the molecular weights and dispersities of these samples by size exclusion chromatography (SEC) using one set of columns and one eluent. Therefore, we elected to measure the intrinsic viscosities of these zwitterionic polymers in TFE at 25.0 °C using a Ubbelohde viscometer. While conventional Huggins and Kraemer plots were attempted, we noticed that the curves often deviated significantly from linear relationships (see Figure A17 in Appendix A). This pronounced non-linearity of η_{sp}/c (or $\ln\eta_{rel}/c$) versus c likely resulted from the complex electrostatic interactions between charged groups at dilute conditions as discussed by Wolf.³⁷ Therefore, we followed Wolf's method^{37,38} to determine the intrinsic viscosities of our zwitterionic polymers in TFE from the initial slope of the dependence of $\ln\eta_{rel}$ on polymer concentration c (Figure A18-A26 in Appendix A) and the obtained $[\eta]$ values were in the range from 0.051 to 0.684 dL/g (Table 2.1). Note that the molecular weights and dispersities of lower molecular weight P1P6 samples made by RAFT polymerization (i.e., P1P6-2, -3, and -4) were measured by aqueous SEC analysis relative to PEO standards using a 0.2 M NaNO₃ aqueous solution as eluent (Table 2.1), and the SEC traces can be found in Appendix A (Figure A27).

2.3.2. Solution Behavior of PDPSMAs with Two Symmetric *N-n*-Alkyl Substituents in Water.

The solution behavior of P11, P22, P44, P55, and P66 in water at various temperatures was first examined by visual inspection at a concentration of 1.0 wt% to study the effect of the lengths of two symmetric *N-n*-alkyl substituents. Those exhibiting thermoresponsive properties were further examined using a UV-vis spectrometer to record the transmittance at 500 nm as a function of temperature. As expected, P11 displayed a UCST transition in water; the polymer solution was clear at elevated temperatures but turned cloudy upon cooling as can be seen from the photos in

Figure 2.1A. From the plot of transmittance versus temperature (Figure 2.2A), the CP of P11, which was determined as the temperature at which a 50% transmittance change occurred, was 47 °C. This value was higher than our previously reported CP (31 °C) of a P11 homopolymer synthesized by RAFT polymerization.³³ The CPs of UCST polymers in liquid media are known to be very sensitive to the molecular weight change, end group identity, and the presence of a small amount of structural defects,^{2,5,9,25,41-43} usually more than LCST-type thermosensitive polymers.^{5,34} For instance, Hildebrand et al. reported that the CP of P11 in H₂O increased from 41 to 71 °C with increasing the degree of polymerization from 85 to 585.²⁵ In contrast, P22 was completely soluble in water from 0 – 100 °C (Figure 2.1B), similar to the solution behavior of P12, P13, and P14.³³ Note that P22-2 has a $[\eta]$ value of 0.684 dL/g, much higher than that of P11 ($[\eta]$ = 0.284 dL/g). Interestingly, even with a total of 8 carbon atoms in the two *n*-alkyl substituents, P44 was completely soluble in water (Figure 2.1C), which is different from P17 that also has 8 carbon atoms in the two *N*-alkyl substituents but is insoluble in water.³³ This indicates that the effects of two alkyl substituents are not additive.

More interestingly, P55 with a total of 10 carbon atoms in the two substituents exhibited an LCST in water; the solution was clear at 10 °C but cloudy at 65 and 90 °C (Figure 2.1D). At a concentration of 1.0 wt% in Milli-Q water, the CPs of P55-1 ($[\eta]$ = 0.129 dL/g) and P55-2 ($[\eta]$ = 0.288 dL/g) were 42 °C (Figure 2.2B) and 40 °C (Figure A28 in Appendix A), respectively, consistent with the common observation that a higher molecular weight LCST polymer exhibits a lower CP.^{44,45} The thermal hysteresis from heating and cooling processes was minimal for both samples (Figures 2.2B and A28). The CP increased with decreasing concentration of the polymer (Figure 2.2C), showing a concave curve characteristic of LCST polymers (Figure 2.2D). The heating-induced LCST transition of P55-1 was further investigated by variable temperature ¹H

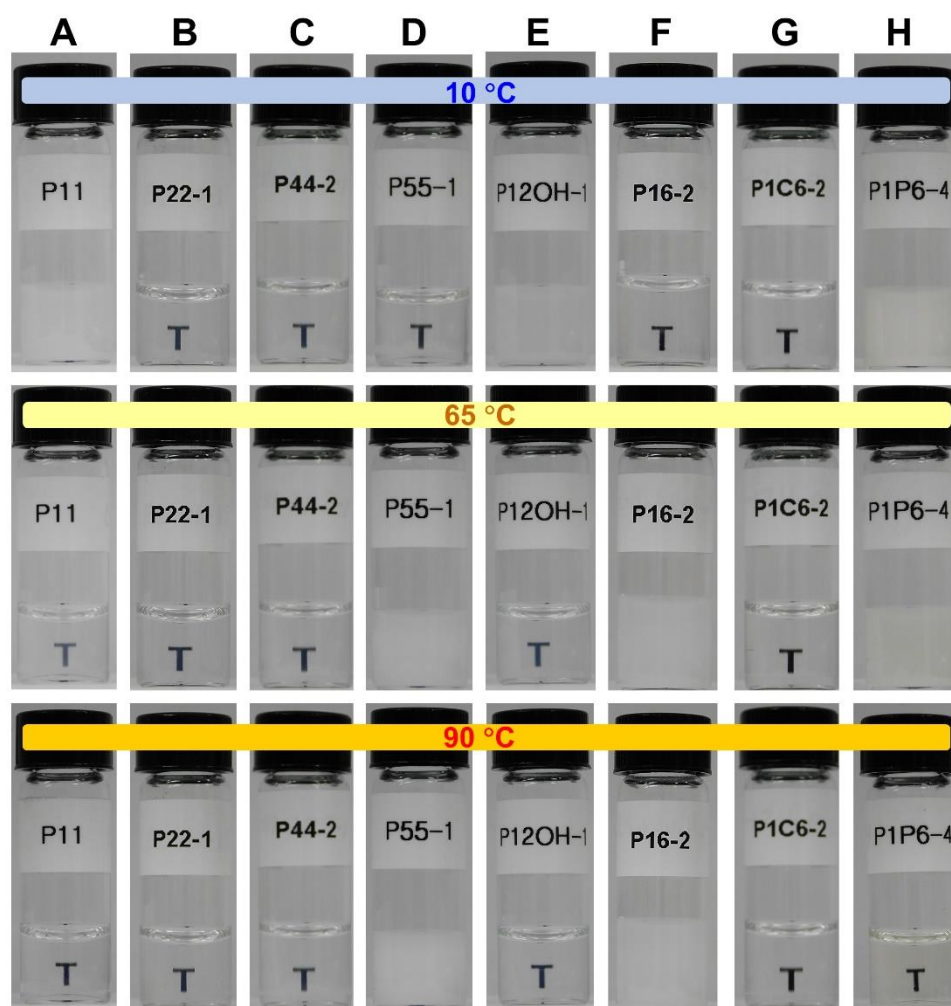


Figure 2.1. Optical photos of 1.0 wt% aqueous solutions of P11 (A), P22-1 (B), P44-2 (C), P55-1 (D), P12OH-1 (E), P16-2 (F), P1C6-2 (G), and P1P6-4 (H) at 10, 65, and 90 °C.

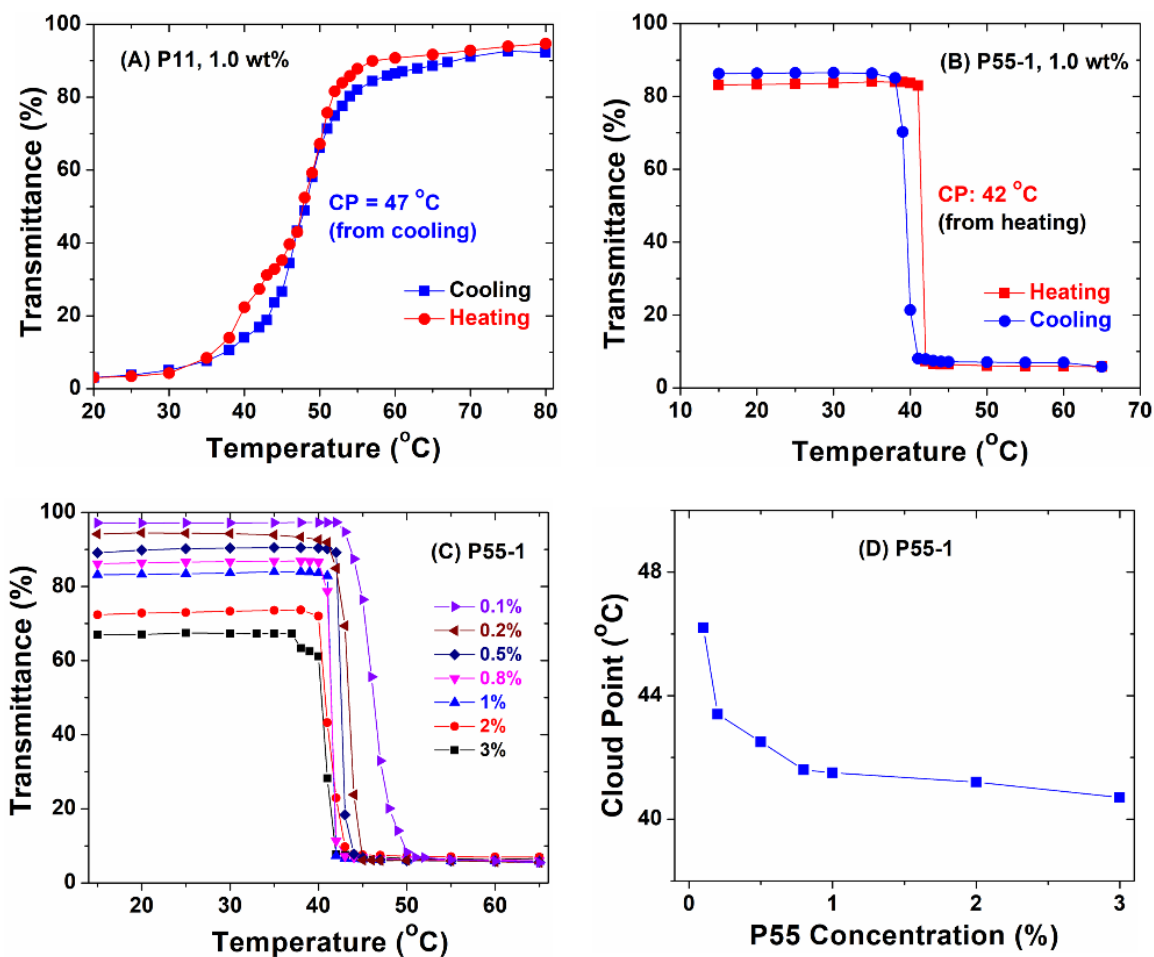


Figure 2.2. Plots of transmittance at the wavelength of 500 nm versus temperature for (A) 1.0 wt% P11 in Milli-Q water from a cooling-heating cycle and (B) 1.0 wt% P55-1 in Milli-Q water from a heating-cooling cycle. (C) Plots of transmittance versus temperature for aqueous solutions of P55-1 with various concentrations from the heating processes, and (D) plot of cloud point versus polymer concentration obtained from (C).

NMR analysis at a concentration of 1 wt% in D₂O (Figure 2.3A); the peaks marked by the arrows decreased noticeably in intensity when the temperature was raised from 40 °C to 43 °C and above, consistent with the CP of the same D₂O solution (39 °C) observed by visual inspection. Further increasing the lengths of two *n*-alkyl substituents by just one carbon atom resulted in a water-insoluble polymer, P66, despite the relatively low intrinsic viscosities (0.116 and 0.098 dL/g) of the two samples. We previously observed that the CPs of P15 and P16 in H₂O were ~ 50 and ~ 22 °C,³³ showing that adding one CH₂ unit into the longer alkyl while keeping the *N*-methyl constant decreases the CP substantially. The overall trend for both polymer series is qualitatively similar to the decrease of CP for LCST-type thermoresponsive poly(oligo(ethylene glycol) (meth)acrylate)s caused by the addition of one CH₂ group to the end of the side chain in each repeat unit.⁴⁶⁻⁴⁸

The observed evolution of solution behavior of PDPSMAs with two symmetric *N*-*n*-alkyl substituents in water from UCST to water-soluble, LCST and water-insoluble may be attributed to two competing effects that resulted from increasing alkyl length: steric weakening of electrostatic attractive interactions between zwitterionic groups and increase of the hydrophobic effect associated with *n*-alkyl groups. When the two *N*-substituents are as small as methyl, the electrostatic inter-locking of zwitterionic groups causes P11 to be insoluble in cold water. Upon heating, the thermal motions presumably overcome the electrostatic forces, resulting in the dissolution of P11 in water and a UCST transition (convex black curve in Scheme 2.10). Upon increasing the lengths of two symmetric *N*-substituents to 2 and 4 carbon atoms, the zwitterionic interactions are likely sterically disrupted by two larger symmetric *N*-*n*-alkyl groups, making the polymers soluble in water and shifting the UCST curve below 0 °C (convex red dashed line in Scheme 2.10). Meanwhile, the entropic penalty from the *N*-*n*-alkyl is still small compared with the enthalpic gain from the hydration of zwitterionic groups and the LCST curve is still above 100 °C

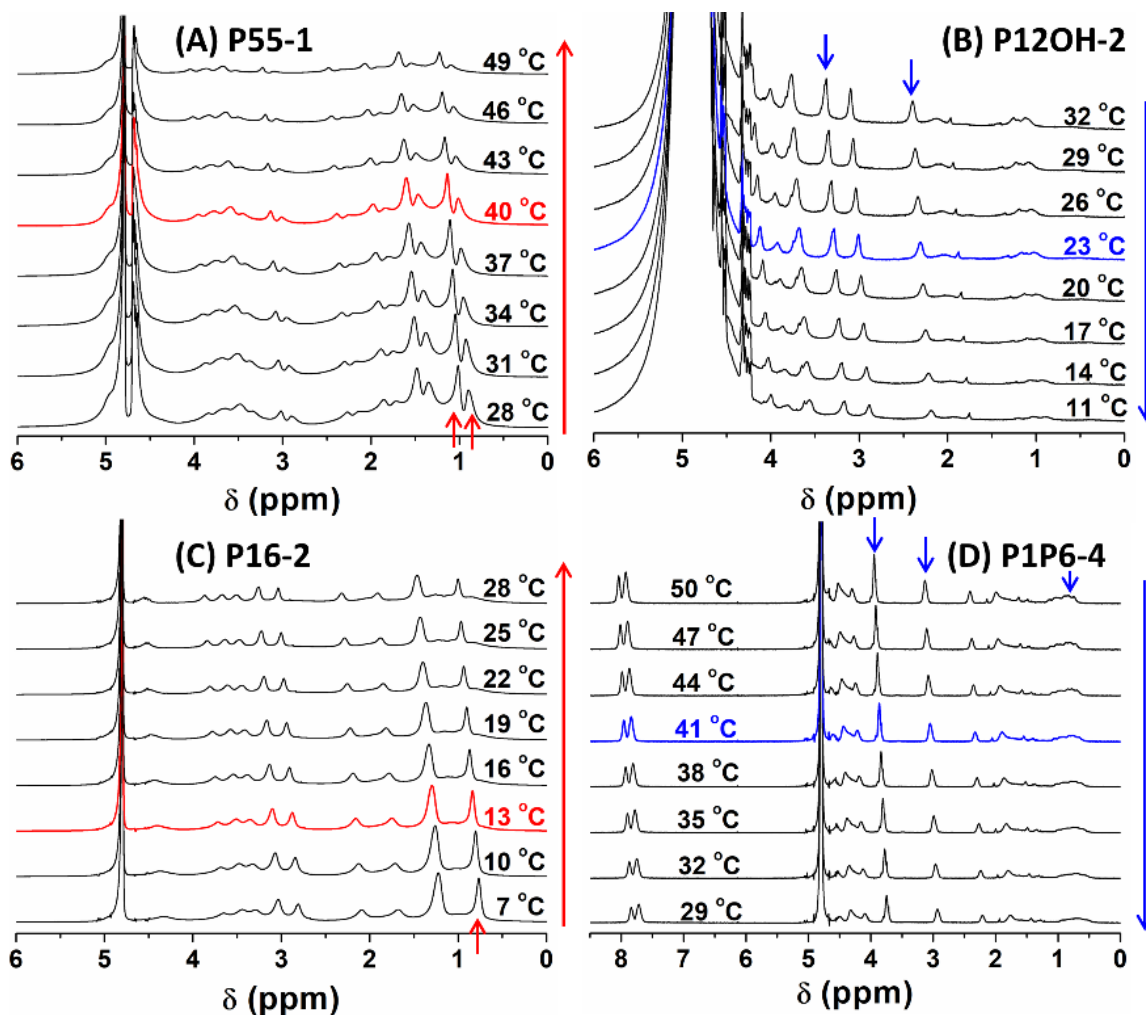
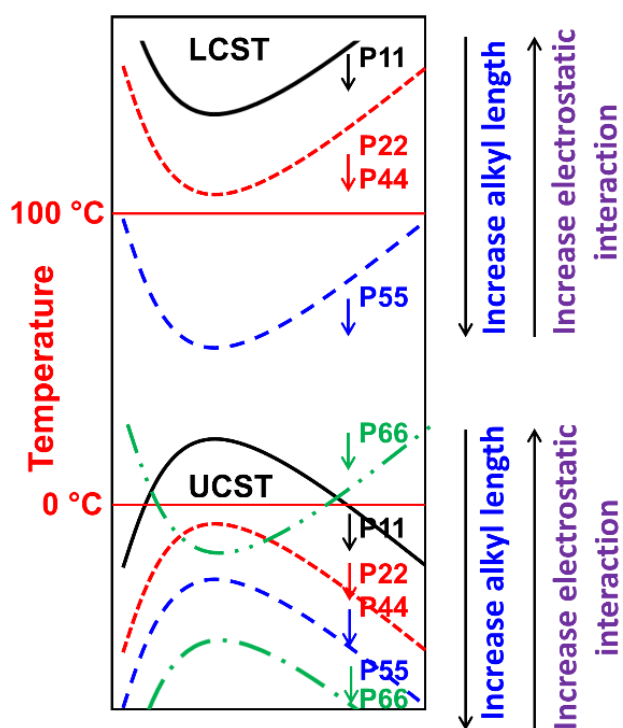


Figure 2.3. ^1H NMR spectra of thermoresponsive zwitterionic PDPSMA homopolymers with different *N*-substituents in D_2O at various temperatures: (A) 10 mg/g P55-1 (CP = 39 °C by visual inspection), (B) 4 mg/g P12OH-2 (CP = 23 °C by visual inspection), (C) 10 mg/g P16-2 (CP = 11 °C by visual inspection), and (D) 2 mg/g P1P6-4 (CP = 41 °C by visual inspection) at various temperatures from variable temperature ^1H NMR spectroscopy analysis studies. The HDO peak at 4.79 ppm was used to normalize all spectra for each polymer.



Scheme 2.10. Hypothetical phase diagrams of zwitterionic PDPSMAs with various symmetric *N*-*n*-alkyl substituents in H₂O.

(concave red dashed line in Scheme 2.10). Consequently, the polymers are soluble from 0 – 100 °C. Note that the steric weakening of electrostatic zwitterionic interactions by larger *N*-alkyl groups would lead to a lower glass transition temperature in the bulk state. Köberle and Laschewsky reported that the glass transition temperature of a zwitterionic polymethacrylate with a molecular structure similar to P22 (the only difference is the spacer length between the ester and the ammonium moiety: eleven CH₂ groups vs. two CH₂ group in P22) was lower by more than 100 °C than that of the corresponding polymer with two *N*-methyl groups,⁴⁹ suggesting weaker interactions between zwitterionic groups for larger *N*-alkyls. Further increasing the lengths to 5 carbon atoms introduces a larger entropy penalty. While the polymer is soluble at lower temperatures, it is not at higher temperatures and hence exhibits an LCST in water (concave blue dashed line in Scheme 2.10). For P66, the entropic penalty is too high and the polymer becomes insoluble in water (concave green curve in Scheme 2.10). We emphasize here that our explanations are focused on PDPSMAs with a general molecular structure shown in Scheme 2.1 (i.e., two CH₂ groups between the ester and the ammonium, three CH₂ groups between the ammonium and sulfonate groups, and two separate substituents, R and R', at the nitrogen atom). It is not our intention in this work to extend our explanations to other zwitterionic polymers, e.g., with different spacers or spacer lengths between the ester or amide and the ammonium and between the ammonium and the sulfonate group.

As mentioned earlier, P44 with a total 8 carbon atoms in the two *N*-substituents is soluble in water from 0 to 100 °C, while P15 and P16 with 6 and 7 carbon atoms, respectively, exhibit LCST transitions in water.³³ This difference is likely caused by the disruption of structured water on the portion of the hydrophobic alkyl groups adjacent to the positive charge center. The hydration of positively charged ammonium species occurs at the expense of nearby hydrophobic hydration⁵⁰

and thus diminishes the hydrophobic effect of two *n*-butyl substituents. Since the LCST is determined by the amount of water of hydrophobic hydration, the remaining hydrophobic hydration for the *n*-butyl groups is not sufficient for P44 to exhibit LCST behavior in water. On examining the behavior of P44 (soluble), P55 (LCST, CP = ~ 40 °C), P14 (soluble), P15 (LCST, CP = ~ 50 °C), and P16 (LCST, CP = ~ 22 °C) in water, it appears that a longer *n*-alkyl group is more important than the total number of carbon atoms in the two *N*-*n*-alkyl substituents for PDPSMAs to display an LCST in water. Moreover, since both P15 and P55 exhibit LCST behavior and their CPs are quite close to each other (~ 50 and ~ 40 °C, respectively), P25 with one ethyl and one *n*-pentyl, P35 with one *n*-propyl and one *n*-pentyl, and P45 with one *n*-butyl and one *n*-pentyl substituent at the nitrogen atom would be expected to all exhibit LCST transitions in water likely with CPs in the range of 40 to 50 °C at similar molecular weights. Thus, increasing the lengths of two symmetric and asymmetric *N*-*n*-alkyl substituents of PDPSMAs presumably weakens the electrostatic attractive interactions between zwitterionic groups (Scheme 2.2), favoring the dissolution of the polymers in water, and simultaneously increases the hydrophobic effect. As a result, the behavior of zwitterionic PDPSMAs in water changes from UCST to soluble, LCST, and finally insoluble. We note here that for DPSMAs with two different *N*-substituents the nitrogen atom is a chiral center and we used racemic monomer mixtures in the polymerizations. It is unclear at this point how the chirality and the extent of symmetry affect the electrostatic interactions between zwitterionic groups and contribute to the solution behavior in water observed for the two polymer series, P11 – P17 and P11 – P66. This needs a further investigation probably through computer simulations.

2.3.3. Solution Behavior of P12OH with a Hydroxyethyl *N*-Substituent in Water.

Our group previously reported that P12 with one methyl and one ethyl group at the nitrogen atom was soluble in water in the temperature range of 0 to 100 °C.³³ To study how the presence of a polar, hydrogen bonding-capable hydroxyl group at the end of the *N*-ethyl substituent affects the polymer's behavior in water, we prepared two P12OH samples (P12OH-1 with $[\eta] = 0.358$ dL/g and P12OH-2 with $[\eta] = 0.202$ dL/g) and found that both P12OH-1 and -2 exhibited UCST behavior in water. As shown in Figure 2.1E, a 1.0 wt% P12OH-1 solution was clear and homogeneous at 90 and 65 °C but cloudy at 10 °C. From the transmittance data (Figure 2.4A and B), the CPs of P12OH-1 and -2 at a concentration of 1.0 wt% from the cooling curves were 33 and 23 °C, respectively, with negligible thermal hysteresis in the heating and cooling cycle. The CP of the high molecular weight P12OH sample is higher, as expected for UCST polymers.⁵ Contrary to LCST thermoresponsive P55, the CP of P12OH decreased with decreasing concentration (Figure 2.4C) and the plot of CP versus concentration showed a convex shape (Figure 2.4D), which is a characteristic of UCST thermoresponsive polymers. The UCST behavior of P12OH-2 was also studied by variable temperature ¹H NMR spectroscopy (Figure 2.3B). In contrast to LCST polymer P55, the peaks decreased in intensity with decreasing temperature from 32 to 11 °C. However, the transition was not as sharp as P55, likely because of the retention of a large amount of water by zwitterionic groups after the collapse.

Thus, replacing the *N*-ethyl substituent of P12 with a polar 2-hydroxyethyl group turned a water-soluble polymer into a UCST thermosensitive polymer. Instead of improving the polymer's solubility in water, the polar hydroxy group enhanced the intra- and intermolecular attractions between zwitterionic groups, likely through the hydrogen bonding between each other and the dipole-ion interactions with zwitterionic groups, resulting in a poorer solubility in water. Note that

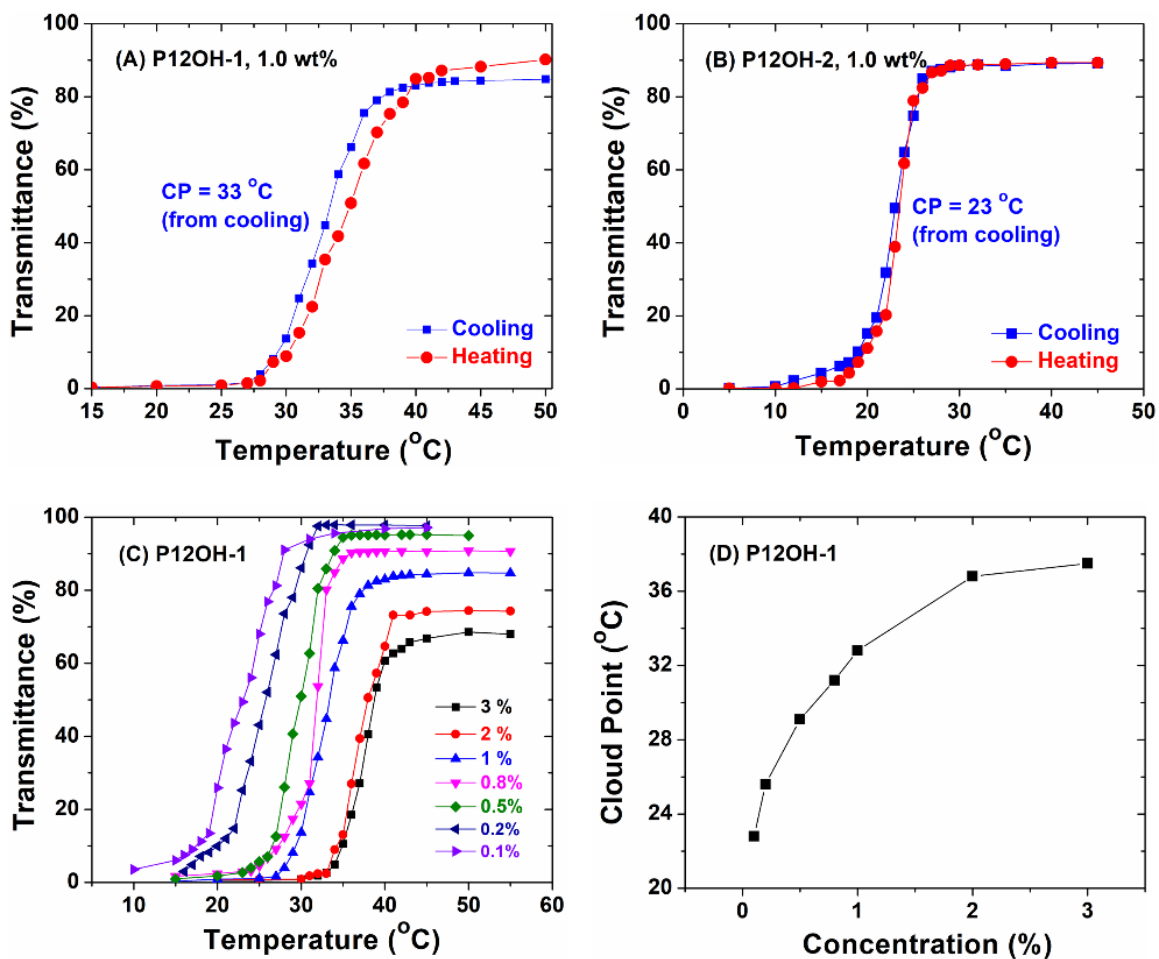


Figure 2.4. Plots of transmittance at the wavelength of 500 nm versus temperature for P12OH-1 (A) and P12OH-2 (B) in water at a concentration of 1.0 wt% from a cooling-heating cycle. (C) Plots of transmittance versus temperature for P12OH-1 in water at different concentrations from cooling processes and (D) plot of cloud point versus concentration for P12OH-1 determined from (C).

the $[\eta]$ of P12OH-1 (0.358 dL/g) is higher than that of P11 (0.284 dL/g), but the CP (33 °C) was lower by 14 °C than P11, which means that everything else being equal the solubility of P12OH in water is better than P11. This is likely caused by the larger size of -CH₂CH₂OH, compared with the *N*-methyl of P11, which presents a higher steric hindrance than the methyl group and weakens to some degree the interactions between zwitterionic groups, as well as the hydrogen bonding between -CH₂CH₂OH and water. Based on this reasoning, it is possible that increasing the length of the *N*-(CH₂)_nOH substituent in a certain range while keeping the *N*-methyl substituent could result in the disappearance of the UCST transition in water without introducing LCST for corresponding PDPSMAs.

2.3.4. Solution Behavior of P1C6 and P1P6 in Water

Next, we investigated the effect of molecular shape of one *N*-alkyl substituent and the effect of aromaticity of one *N*-substituent, where the aromatic moiety does not comprise and is directly attached to the nitrogen atom as a substituent, on solution behavior of PDPSMAs in water. P16 with a *n*-hexyl and a methyl group at the nitrogen atom is a LCST-type thermoresponsive zwitterionic polymer with a CP of ~ 22 °C in water.³³ Compared with *n*-hexyl, cyclohexyl has a compact structure and a smaller surface area. Unlike saturated alkyls, aromatic *N*-substituents without containing the ammonium nitrogen can interact with charges. Therefore, to gain an understanding of the structure-solution-property relationship, it would be beneficial to examine how the molecular shape and the aromaticity of *N*-substituents of PDPSMAs affect the polymer's behavior in water. For comparison, two P16 samples were made by conventional free radical polymerization in this work and as expected both exhibited a LCST transition in water (Figure 2.1F) with CPs of 11 °C (for P16-1 with $[\eta]$ of 0.248 dL/g) and 12 °C (for P16-2 with $[\eta]$ of 0.227 dL/g) from the heating process (Figures 2.5A and A29 in Appendix A). These CPs were lower

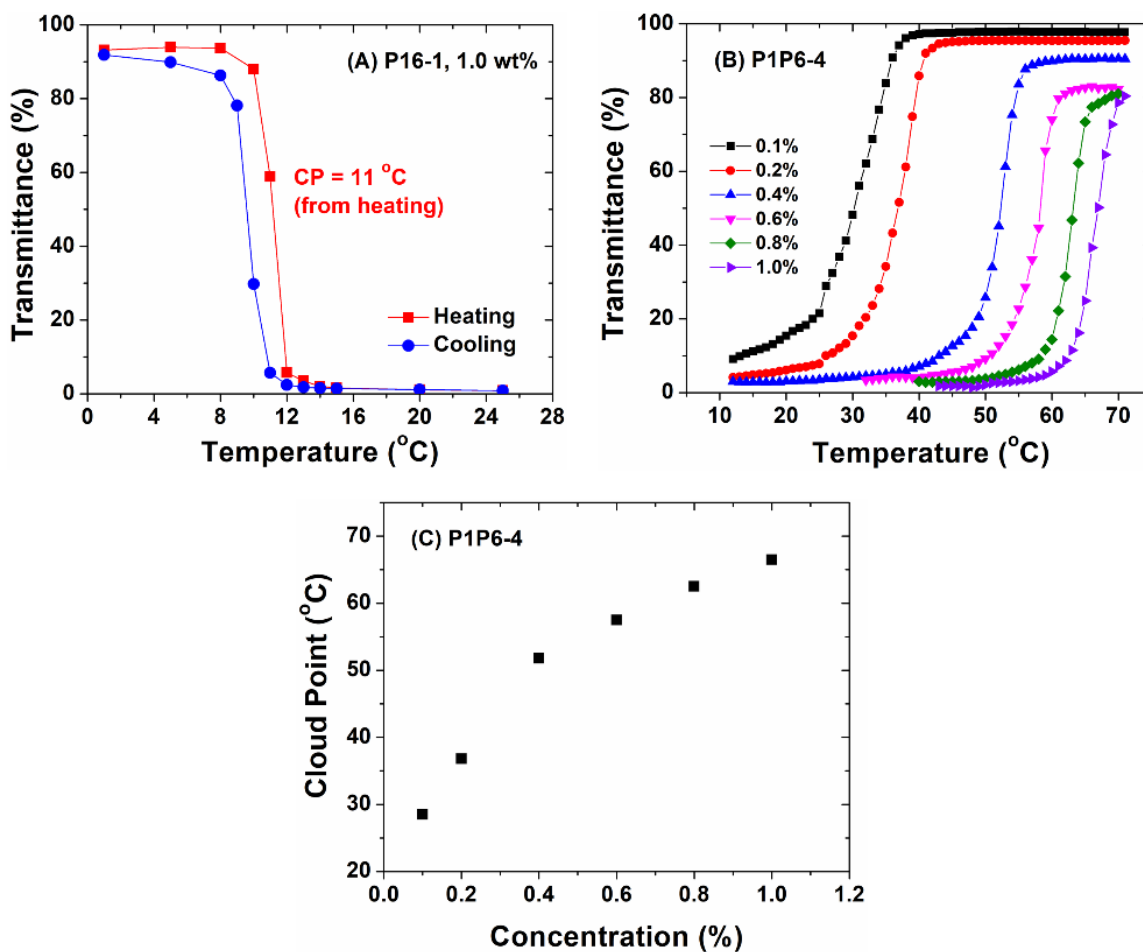


Figure 2.5. (A) Plot of transmittance at the wavelength of 500 nm versus temperature for 1.0 wt% P16-1 in Milli-Q water from a heating-cooling cycle. (B) Plot of transmittance versus temperature for P1P6-4 at various concentrations upon cooling, and (C) plot of cloud point versus polymer concentration for P1P6-4 from (B).

than that of the P16 we previously made by RAFT polymerization ($\sim 22\text{ }^{\circ}\text{C}$),³³ likely because the molecular weights of P16-1 and -2 are much higher. Similar to P55-1 in Figure 2.3A, variable temperature ^1H NMR spectroscopy analysis showed that the peaks of P16-2 decreased in intensity around the CP upon heating (Figure 2.3C). Replacing the linear *N*-hexyl substituent with cyclohexyl, we found, produced a soluble polymer (P1C6) with no LCST or UCST transition in water in the temperature range of 0 – 100 $^{\circ}\text{C}$ (Figure 2.1G). Note that P1C6-2 had a $[\eta]$ of 0.520 dL/g, much higher than those of P16-1 and -2. This could be attributed to three factors. (i) A compact, bulkier cyclohexyl group is likely more effective in sterically weakening the electrostatic attractive interactions between zwitterionic groups, making the polymer more soluble in water. (ii) Hydrophobic effect depends on the surface contact area of the hydrophobic moiety with water; a more compact molecular geometry of cyclohexyl means less structured water and thus less entropic penalty.^{51,52} Studies have shown that it costs 5.2 kJ mol⁻¹ to transfer cyclohexane into water from the vapor phase at 298 K, whereas it costs 10.7 kJ mol⁻¹ for hexane.⁵² (iii) A more compact molecular shape of the cyclohexyl group as an *N*-substituent means more carbon atoms closer to the positive charge center, resulting in more disruption of hydrophobic hydration and thus less water of hydrophobic hydration.⁵⁰

On the other hand, replacing the *N*-cyclohexyl with a phenyl ring gave a water-insoluble polymer by conventional free radical polymerization (P1P6-1 in Table 2.1). Suspecting that the molecular weight from the AIBN-initiated radical polymerization was too high, we made three lower molecular weight P1P6 samples by RAFT polymerization: P1P6-2 ($[\eta] = 0.075\text{ dL/g}$; $M_{n,\text{SEC}} = 2.6\text{ kDa}$), P1P6-3 ($[\eta] = 0.056\text{ dL/g}$; $M_{n,\text{SEC}} = 2.3\text{ kDa}$), and P1P6-4 ($[\eta] = 0.051\text{ dL/g}$; $M_{n,\text{SEC}} = 2.0\text{ kDa}$) (Table 2.1). Even with a $[\eta]$ value of 0.075 dL/g and a $M_{n,\text{SEC}}$ of 2.6 kDa, P1P6-2 was still insoluble in water; intriguingly, P1P6-3 and P1P6-4 displayed UCST behavior in pure water

at low concentrations, and P1P6-4 with the lowest molecular weight had a better solubility. As can be seen from Figure 2.1H, the 1 wt% P1P6-4 in water is clear at 90 °C but cloudy at 65 and 10 °C. Figure 2.5B shows the plots of transmittance versus temperature for P1P6-4 at concentrations from 0.1 wt% to 1.0 wt%. Similar to P12OH-1 in Figures 2.4C and D, the CP decreased with decreasing concentration, a characteristic feature for UCST polymers (Figure 2.5C). The transmittance data for P1P6-3 in the concentration range of 0.04 wt% to 0.2 wt% can be found in Appendix A (Figure A30). (It was difficult to obtain homogeneous solutions at higher concentrations for P1P6-3.) At the concentration of 0.2 wt%, the CP of P1P6-3 was 56 °C, much higher than that of P1P6-4 (37 °C). Evidently, the solubility and the UCST transition of P1P6 in water are very sensitive to the molecular weight. Figure 2.3D shows the ^1H NMR spectra of 0.2 wt% P1P6-4 in D_2O at various temperatures; similar to P12OH-2 (Figure 2.3B), the peaks' intensities decreased with decreasing temperature across the CP, although no sharp transition was discerned. Our observation that high molecular weight P1P6 homopolymers are insoluble in water is consistent with many reports in the literature that incorporating aromatic rings, particularly N-containing heterocycles, into other zwitterionic polymers often produces polymers insoluble in pure water,^{3,6,26-28} although there also exist examples in the literature that the polymers are soluble in water.^{4,53-55} Vasantha et al. reported that imidazole-based zwitterionic polystyrenics were insoluble in pure water but exhibited UCST behavior in brine.⁶

The results presented above showed that varying one *N*-substituent from *n*-hexyl to cyclohexyl and phenyl led to dramatic changes in the solution behavior and thermoresponsive properties of PDPSMAs in water. The introduction of an *N*-phenyl substituent significantly increased the attractive interactions between zwitterionic groups, making the polymer much less soluble in water. The enhanced overall intra- and intermolecular attractive forces between the pendant

zwitterions likely resulted from the following effects and interactions. (i) The π - π interactions⁵⁶ between neighboring *N*-phenyl substituents will increase the attractive force between zwitterionic groups. (ii) There are strong intra- and interchain cation- π interactions between the positively charged ammonium cation and the electro-rich phenyl ring. The interaction between benzene and methylammonium in water could amount to 5.5 kcal/mol,⁵⁷ which is comparable to water-water hydrogen bonding. (iii) There are also anion- π interactions⁵⁸ between sulfonate anions and phenyl rings, which could also contribute to the overall electrostatic interactions between zwitterionic groups. Note that for aromatic rings, the π system above and below the ring results in a quadrupole charge distribution in the ring, making it possible for aromatic rings to interact with both cations and anions. Despite the enhanced interactions, when the molecular weight of P1P6 is low, the electrostatic inter-locking can still be disrupted by thermal motions as shown above for P1P6-3 and -4, rendering a UCST transition to the polymers in water.

2.3.5. Tuning of UCST Transitions by Copolymerization of M1P6 and M22

Copolymerization of two or more monomers is widely used for tuning the LCST and UCST transitions of thermoresponsive polymers in water.⁵⁹⁻⁶² Because P1P6 is a UCST polymer only at rather low molecular weights and P22 is completely soluble in water from 0 to 100 °C, we copolymerized M1P6 and M22 with various molar ratios via AIBN-initiated radical polymerization in order to increase the solubility of the polymers in water and modify their UCST transition temperatures. Five copolymers with the molar contents of M1P6 units from 42.1 to 18.1% were synthesized and the characterization data are summarized in Table 2.2 (see Figures A31 and A32 in Appendix A). All five copolymers were found to display a UCST transition in water. Figure 2.6A shows 1.0 wt% aqueous solutions of PCO-1, -2, -3, -4, and -5 at 10, 65, and 90 °C. The CPs of PCO-1 and -5 at a concentration of 1.0 wt% were 88 and 1 °C, respectively,

Table 2.2. Synthesis and Characterization of Zwitterionic Copolymers of M1P6 and M22

Copolymer	Mole % of M1P6 in Feed ^a	Mole % of M1P6 in Copolymer ^b	[η] (dL/g)	Cloud Point (°C)
PCO-1	50.0	42.1	0.299	88 °C
PCO-2	41.2	37.0	0.291	46 °C
PCO-3	33.3	29.3	0.415	36 °C
PCO-4	27.0	24.7	0.372	11 °C
PCO-5	16.7	18.1	0.439	1 °C

^a Molar content of M1P6 in the feed. ^b The molar content of M1P6 units in the copolymer was determined from the ¹H NMR spectrum using the integrals of the peaks in the range of 7.52 – 8.19 ppm, which corresponded to the five hydrogen atoms in the phenyl ring of M1P6 units, and the peaks from 2.72 – 3.19 ppm, which corresponded to -CH₂SO₃⁻ of M1P6 and M22 monomer units.

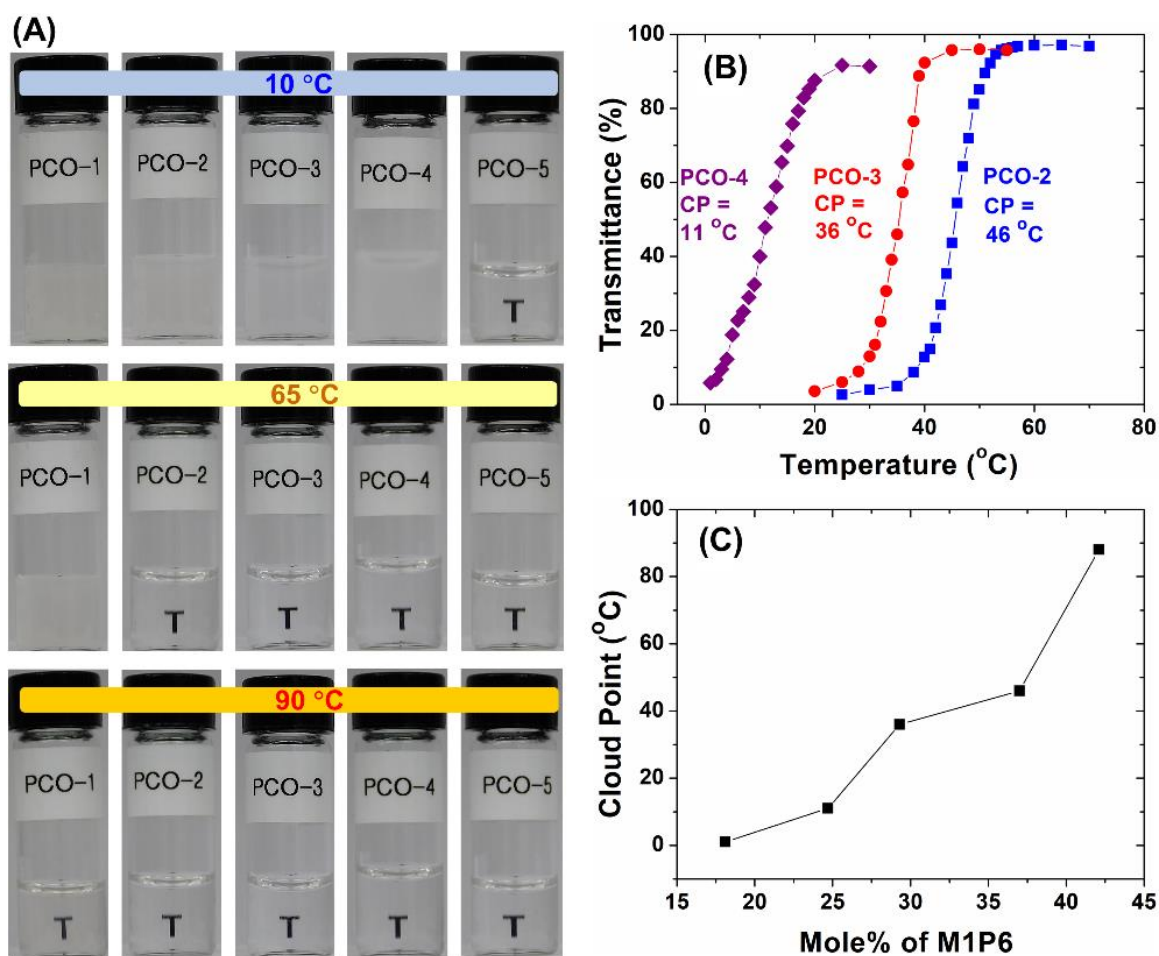


Figure 2.6. (A) Optical photos of five copolymers of M1P6 and M22 (PCO-1, -2, -3, -4, and -5) in Milli-Q water at a concentration of 1.0 wt% at 10, 65, and 90 °C. (B) Plot of transmittance at the wavelength of 500 nm versus temperature for a 1.0 wt% aqueous solution of PCO-2, PCO-3, and PCO-4 upon cooling, and (C) Plot of cloud point versus molar content of M1P6 in the copolymer.

determined by visual inspection using a thermostated oil bath and an Isotemp water bath, respectively. The CPs of the other three copolymers (PCO-2, -3 and -4) were measured from the plots of transmittance versus temperature presented in Figure 2.6B, and they were 46, 36, and 11 °C, respectively. Figure 2.6C shows the plot of CP versus molar content of M1P6 monomer units in the copolymer. Although there is a clear trend that the CP increased with increasing molar content of M1P6 monomer units, the correlation is not perfectly linear. This could be caused by the different $[\eta]$ values of these copolymers, dispersities, distributions of two types of monomer units, etc. In particular, the CPs of UCST polymers are known to be heavily affected by molecular weight,^{5,25} as can also be seen from our P1P6 homopolymers. Nevertheless, this study showed that the CPs of the UCST zwitterionic copolymer of M1P6 and M22 can be tuned by varying the molar ratio of two zwitterionic monomer units.

2.4. Conclusions

In summary, we synthesized a series of DPSMA-based zwitterionic PSBMAs with various *N*-substituents, including two symmetric *n*-alkyls with varying lengths, one methyl and one *n*-hexyl, one methyl and one cyclohexyl, as well as one methyl and one phenyl group at the nitrogen atom, to elucidate the effects of *N*-substituents on solution behavior of these PDPSMAs in water.⁶⁸ The main results are summarized below. (i) With increasing the lengths of two symmetric *N*-*n*-alkyl substituents simultaneously, the behavior of PDPSMAs in water changed from UCST to soluble, LCST, and insoluble, which likely is a result of the competition between the weakening of zwitterionic interactions and the increase of hydrophobic effect with increasing alkyl length. (ii) Installing a hydroxy group at the end of the *N*-ethyl substituent of P12 enhanced the attractive interactions between zwitterion groups, turning a water-soluble polymer to a UCST polymer. (iii)

Replacing the *N*-*n*-hexyl substituent of P16 with cyclohexyl changed the polymer from LCST-type thermoresponsive to water-soluble due to the decreased hydrophobic effect. Replacing the *N*-cyclohexyl group with a phenyl ring led to a water-insoluble polymer when the molecular weights were high, which was attributed to the increased zwitterionic inter-locking, while lower molecular weight species exhibited UCST behavior in water. In addition, we showed that the CP of the UCST thermoresponsive copolymers of M1P6 and M22 can be readily modified by varying the molar ratio of two monomer units.

This study provides an insight into the structure-solution-behavior relationship of zwitterionic PDPSMAs with a general molecular structure shown in Scheme 2.1 (i.e., two CH₂ groups between the ester and the ammonium, three CH₂ groups between the ammonium and the sulfonate group, and two separate substituents, R and R', at the nitrogen atom) in water, which may facilitate the design of UCST- and LCST-type thermoresponsive as well as water-soluble zwitterionic polymers by varying *N*-substituents. (i) LCST-type thermoresponsive PDPSMAs may be produced by introducing *n*-alkyls of appropriate lengths (five or six carbon atoms) as *N*-substituents. (ii) UCST-type thermoresponsive PDPSMAs may be obtained by increasing zwitterionic interactions through the introduction of an aromatic or a small, hydroxyl-containing *N*-substituent. (iii) Water-soluble PDPSMAs may be obtained by using *n*-alkyls of intermediate lengths ($1 < \text{number of carbon atoms in each } N\text{-}n\text{-alkyl substituent} < 5$) or cycloalkyl or other compact (e.g., branched) structures as *N*-substituents. It is possible to achieve zwitterionic PDPSMAs that show UCST and LCST transitions in water in the temperature of 0 – 100 °C^{31,63} by using one substituent from (i) and another substituent from (ii). The knowledge generated from this study about PDPSMAs may be useful for understanding the structure-solution-behavior relationships of other types of zwitterionic polymers in water. Given the widespread use of zwitterionic materials, the insight gained from this

work could open new research opportunities for both fundamental studies and potential applications. Moreover, the zwitterionic polymers reported here could be used in the design of functional and responsive surfaces^{1,2,11-22} and in the synthesis of zwitterionic nanogels,⁹ zwitterionic schizophrenic block copolymer micelles,^{64,65} and shape-changing molecular bottlebrushes^{66,67} for possible applications such as drug delivery.

References:

1. Shao, Q.; Jiang S. Y. Molecular Understanding and Design of Zwitterionic Materials. *Adv. Mater.* **2015**, *27*, 15-26.
2. Laschewsky, A.; Rosenhahn, A. Molecular Design of Zwitterionic Polymer Interfaces: Searching for the Difference. *Langmuir*, **2019**, *35*, 1056-1071.
3. Monroy Soto, V. M.; Galin, J. C. Poly(sulphopropylbetaines): 2. Dilute Solution Properties. *Polymer* **1984**, *25*, 254-262.
4. Schulz, D. N.; Peiffer, D. G.; Agarwal, P. K.; Larabee, J.; Kaladas, J. J.; Soni, L.; Handwerker, B.; Garner, R. T. Phase Behaviour and Solution Properties of Sulphobetaine Polymers. *Polymer* **1986**, *27*, 1734-1742.
5. Seuring, J.; Agarwal, S. Polymers with Upper Critical Solution Temperature in Aqueous Solution. *Macromol. Rapid Commun.* **2012**, *33*, 1898–1920.
6. Vasantha, V. A.; Jana, S.; Parthiban, A.; Vancso. J. G. Water Swelling, Brine Soluble Imidazole Based Zwitterionic Polymers – Synthesis and Study of Reversible UCST Behaviour and Gel–Sol Transitions. *Chem. Commun.* **2014**, *50*, 46-48.
7. Woodfield, P. A.; Zhu, Y.; Pan, Y.; Roth, P. J. Hydrophobically Modified Sulfobetaine Copolymers with Tunable Aqueous UCST through Postpolymerization Modification of Poly(pentafluorophenyl acrylate). *Macromolecules* **2014**, *47*, 750–762.
8. Niskanen, J.; Tenhu, H. How to Manipulate the Upper Critical Solution Temperature (UCST)? *Polym. Chem.* **2017**, *8*, 220-232.
9. Fu, W. X.; Luo, C. H.; Morin, E. A.; He, W.; Li, Z. B.; Zhao, B. UCST-Type Thermosensitive Hairy Nanogels Synthesized by RAFT Polymerization-Induced Self-Assembly. *ACS Macro Lett.* **2017**, *6*, 127-133.

10. Biswas, Y.; Mandal, T. K. Structural Variation in Homopolymers Bearing Zwitterionic and Ionic Liquid Pendants for Achieving Tunable Multi-Stimuli Responsiveness and Hierarchical Nanoaggregates. *Macromolecules* **2017**, *50*, 9807–9820.
11. Letteri, R. A.; Santa Chalarca, C. F.; Bai, Y.; Hayward, R. C.; Emrick, T. Forming Sticky Droplets from Slippery Polymer Zwitterions. *Adv. Mater.* **2017**, *29*, 1702921.
12. Zhang, Z.; Chen, S. F.; Chang, Y.; Jiang, S. Y. Surface Grafted Sulfobetaine Polymers via Atom Transfer Radical Polymerization as Superlow Fouling Coatings. *J. Phys. Chem. B* **2006**, *110*, 10799-10804.
13. Jiang, S. Y.; Cao, Z. Q. Ultralow-Fouling, Functionalizable, and Hydrolyzable Zwitterionic Materials and Their Derivatives for Biological Applications. *Adv. Mater.* **2010**, *22*, 920-932.
14. Mi, L.; Jiang, S. Y. Integrated Antimicrobial and Nonfouling Zwitterionic Polymers. *Angew. Chem. Int. Ed.* **2014**, *53*, 1746-1754.
15. Sae-ung, P.; Kolewe, K. W.; Bai, Y.; Rice, E. W.; Schiffman, J. D.; Emrick, T.; Hoven, V. P. Antifouling Stripes Prepared from Clickable Zwitterionic Copolymers *Langmuir* **2017**, *33*, 7028–7035.
16. Baggerman, J.; Smulders, M. M. J.; Zuilhof, H. Romantic Surfaces: A Systematic Overview of Stable, Biospecific, and Antifouling Zwitterionic Surfaces. *Langmuir* **2019**, *35*, 1072-1084.
17. Xia, Y. Q.; Adibnia, V.; Huang, R. L.; Murschel, F.; Faivre, J.; Xie, G. J.; Olszewski, M.; Crescenzo, G. D.; Qi, W.; He, Z. M.; Su, R. X.; Matyjaszewski, K.; Banquy, X. Biomimetic Bottlebrush Polymer Coatings for Fabrication of Ultralow Fouling Surfaces. *Angew. Chem. Int. Ed.* **2019**, *58*, 1308–1314.
18. Chen, M.; Briscoe, W. H.; Armes, S. P.; Klein, J. Lubrication at Physiological Pressures by Polyzwitterionic Brushes. *Science* **2009**, *323*, 1698-1701.

19. Banquy, X.; Burdyńska, J.; Lee, D. W.; Matyjaszewski, K.; Israelachvili, J. Bioinspired Bottle-Brush Polymer Exhibits Low Friction and Amontons-like Behavior. *J. Am. Chem. Soc.* **2014**, 136, 6199–6202.
20. Liang, B.; Zhang, G. Y.; Zhong, Z. X.; Huang, Y.; Su, Z. H. Superhydrophilic Anti-Icing Coatings Based on Polyzwitterion Brushes. *Langmuir* **2019**, 35, 1294–1301.
21. Ma, M.-Q.; Zhang, C.; Chen, T.-T.; Yang, J.; Wang, J.-J.; Ji, J.; Xu, Z.-K. Bioinspired Polydopamine/Polyzwitterion Coatings for Underwater Anti-Oil and -Freezing Surfaces. *Langmuir* **2019**, 35, 1895–1901.
22. Liang, B.; Zhang, G. Y.; Zhong, Z. X.; Sato, T.; Hozumi, A.; Su, Z. H. Substrate-Independent Polyzwitterionic Coating for Oil/Water Separation Membranes. *Chem. Eng. J.* **2019**, 362, 126–135.
23. Rajan, R.; Hayashi, F.; Nagashima, T.; Matsumura, K. Toward a Molecular Understanding of the Mechanism of Cryopreservation by Polyampholytes: Cell Membrane Interactions and Hydrophobicity. *Biomacromolecules* **2016**, 17, 1882–1893.
24. Hildebrand, V.; Laschewsky, A.; Wischerhoff, E. Modulating the Solubility of Zwitterionic Poly((3-methacrylamidopropyl)ammonioalkane sulfonate)s in Water and Aqueous Salt Solutions via the Spacer Group Separating the Cationic and the Anionic Moieties. *Polym. Chem.* **2016**, 7, 731–740.
25. Hildebrand, V.; Laschewsky, A.; Päch, M.; Müller-Buschbaum, P.; Papadakis, C. M. Effect of the Zwitterion Structure on the Thermo-Responsive Behaviour of Poly(sulfobetaine methacrylates). *Polym. Chem.* **2017**, 8, 310–322.
26. Itoh, Y.; Abe, K.; Senoh, S. Solution and Membrane Properties of Zwitterionic Polymers. *Makromol. Chem.* **1986**, 187, 1691–1697.

27. Wielema, T. A.; Engberts, J. B. F. N. Zwitterionic Polymers—I. Synthesis of a Novel Series of Poly(vinylsulphobetaines). Effect of Structure of Polymer on Solubility in Water. *Eur. Polym. J.* **1987**, 23, 947-950.
28. Laschewsky, A.; Zerbe, I. Polymerizable and Polymeric Zwitterionic Surfactants: 1. Synthesis and Bulk Properties. *Polymer* **1991**, 32, 2070-2080.
29. Laschewsky, A. Oligoethyleneoxide Spacer Groups in Polymerizable Surfactants. *Colloid Polym. Sci.* **1991**, 269, 785-794.
30. Laschewsky, A. Structures and Synthesis of Zwitterionic Polymers. *Polymers*, **2014**, 6, 1544-1601.
31. Zhu, Y. C.; Batchelor, R.; Lowe, A. B.; Roth, P. J. Design of Thermoresponsive Polymers with Aqueous LCST, UCST, or Both: Modification of a Reactive Poly(2-vinyl-4,4-dimethylazlactone) Scaffold. *Macromolecules* **2016**, 49, 672–680.
32. Schönemann, E.; Koc, J.; Aldred, N.; Clare, A. S.; Laschewsky, A.; Rosenhahn, A.; Wischerhoff, E. Synthesis of Novel Sulfobetaine Polymers with Differing Dipole Orientations in Their Side Chains, and Their Effects on the Antifouling Properties *Macromol. Rapid Commun.* **2020**, 41, 1900447.
33. Wang, N.; Seymour, B. T.; Lewoczko, E. M.; Kent, E. W.; Chen, M.-L.; Wang, J.-H.; Zhao, B. Zwitterionic Poly(sulfobetaine methacrylate)s in Water: From Upper Critical Solution Temperature (UCST) to Lower Critical Solution Temperature (LCST) with Increasing Length of One Alkyl Substituent on the Nitrogen Atom. *Polym. Chem.* **2018**, 9, 5257-5261.
34. Schild, H. G. Poly(N-isopropylacrylamide): Experiment, Theory and Application. *Prog. Polym. Sci.* **1992**, 17, 163-249.

35. Jin, N. X.; Woodcock, J. W.; Xue, C. M.; O'Lenick, T. G.; Jiang, X. G.; Jin, S.; Dadmun, M. D.; Zhao, B. Tuning of Thermo-Triggered Gel-to-Sol Transition of Aqueous Solution of Multi-Responsive Diblock Copolymer Poly(methoxytri(ethylene glycol) acrylate-*co*-acrylic acid)-*b*-poly(ethoxydi(ethylene glycol) acrylate). *Macromolecules* **2011**, *44*, 3556-3566.
36. Fu, W. X.; Bai, W.; Jiang, S. S.; Seymour, B. T.; Zhao, B. UCST-Type Thermoresponsive Polymers in Synthetic Lubricating Oil Polyalphaolefin (PAO). *Macromolecules* **2018**, *51*, 1674-1680.
37. Wolf, B. A. Polyelectrolytes Revisited: Reliable Determination of Intrinsic Viscosities. *Macromol. Rapid Commun.* **2007**, *28*, 164-170.
38. Eckelt, J.; Knopf, A.; Wolf, B. A. Polyelectrolytes: Intrinsic Viscosities in the Absence and in the Presence of Salt. *Macromolecules* **2008**, *41*, 912-918.
39. Moad, G.; Rizzardo, E.; Thang, S. H. Toward Living Radical Polymerization. *Acc. Chem. Res.* **2008**, *41*, 1133-1142.
40. Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. Living Free-Radical Polymerization by Reversible Addition–Fragmentation Chain Transfer: The RAFT Process. *Macromolecules* **1998**, *31*, 5559-5562.
41. Seuring, J.; Frank, F.; Huber, M. K.; Agarwal, S. Upper Critical Solution Temperature of Poly(*N*-acryloyl glycinamide) in Water: A Concealed Property. *Macromolecules* **2012**, *45*, 374-384.
42. Fu, W. X.; Zhao, B. Thermoreversible Physically Crosslinked Hydrogels from UCST-type Thermosensitive ABA Linear Triblock Copolymers. *Polym. Chem.* **2016**, *7*, 6980-6991.

43. Zhang, H.; Guo, S. W.; Fan, W. Z.; Zhao, Y. Ultrasensitive pH-Induced Water Solubility Switch Using UCST Polymers. *Macromolecules* **2016**, *49*, 1424–1433.
44. Hua, F. J.; Jiang, X. G.; Li, D. J.; Zhao, B. Well-Defined Thermosensitive, Water-Soluble Polyacrylates and Polystyrenics with Short Pendant Oligo(ethylene glycol) Groups Synthesized by Nitroxide-Mediated Radical Polymerization. *J. Polym. Sci. Part A: Polym. Chem.* **2006**, *44*, 2454-2467.
45. Jiang, X. G.; Zhao, B. End Group Effect on the Thermo-sensitive Properties of Well-Defined Water-Soluble Polystyrenics with Short Pendant Oligo(ethylene glycol) Groups Synthesized by Nitroxide-Mediated Radical Polymerization. *J. Polym. Sci. Part A: Polym. Chem.* **2007**, *45*, 3707-3721.
46. Han, S.; Hagiwara, M.; Ishizone, T. Synthesis of Thermally Sensitive Water-Soluble Polymethacrylates by Living Anionic Polymerizations of Oligo(ethylene glycol) Methyl Ether Methacrylates. *Macromolecules* **2003**, *36*, 8312–8319.
47. Ishizone, T.; Seki, A.; Hagiwara, M.; Han, S.; Yokoyama, H.; Oyane, A.; Deffieux, A.; Carlotti, A. Anionic Polymerizations of Oligo(ethylene glycol) Alkyl Ether Methacrylates: Effect of Side Chain Length and ω -Alkyl Group of Side Chain on Cloud Point in Water. *Macromolecules* **2008**, *41*, 2963–2967.
48. Horton, J. M.; Bai, Z.; Jiang, X. M.; Li, D. J.; Lodge, T. P.; Zhao, B. Spontaneous Phase Transfer of Thermosensitive Hairy Particles between Water and an Ionic Liquid. *Langmuir* **2011**, *27*, 2019-2027.
49. Köberle, P.; Laschewsky, A. Hydrophobically Modified Zwitterionic Polymers: Synthesis, Bulk Properties, and Miscibility with Inorganic Salts. *Macromolecules* **1994**, *27*, 2165-2173.

50. Urry, D. W. Physical Chemistry of Biological Free Energy Transduction As Demonstrated by Elastic Protein-Based Polymers. *J. Phys. Chem. B* **1997**, *101*, 11007-11028.
51. Hillyer, M. B.; Gibb, B. C. Molecular Shape and the Hydrophobic Effect. *Annu. Rev. Phys. Chem.* **2016**, *67*, 307-329.
52. Southall, N. T.; Dill, K. A.; Haymet, A. D. J. A View of the Hydrophobic Effect. *J. Phys. Chem. B* **2002**, *106*, 521-533.
53. Xue, W.; Huglin, M. B.; Khoshdel, E. Behaviour of Crosslinked and Linear Poly[1-(3-sulphopropyl)-2-vinyl-pyridiniumbetaine] in Aqueous Salt Solutions. *Polym. Int.* **1999**, *48*, 8-14.
54. Billing, M.; Elter, J. K.; Schacher, F. H. Sulfo-and Carboxybetaine-containing Polyampholytes Based on Poly(2-vinyl pyridine)s: Synthesis and Solution Behavior. *Polymer* **2016**, *104*, 40-48.
55. Liaw, D.-J.; Huang, C.-C.; Sang, H.-C.; Kang, E.-T. Aqueous Solution and Photophysical Properties of Cationic Poly(trimethyl methacrylamidophenyl ammonium methylsulfate) and Zwitterionic Poly(*N,N*-dimethylmethacrylamidophenyl ammonium propane sultone). *Polymer* **2001**, *42*, 209-216.
56. Sinnokrot, M. O.; Valeev, E. F.; Sherrill, C. D. Estimates of the Ab Initio Limit for π - π Interactions: The Benzene Dimer. *J. Am. Chem. Soc.* **2002**, *124*, 10887-10893.
57. Gallivan, J. P.; Dougherty, D. A. A Computational Study of Cation- π Interactions vs Salt Bridges in Aqueous Media: Implications for Protein Engineering. *J. Am. Chem. Soc.* **2000**, *122*, 870-874.
58. Wang, D.-X.; Wang, M.-X. Anion- π Interactions: Generality, Binding Strength, and Structure. *J. Am. Chem. Soc.* **2013**, *135*, 892-897.

59. Lutz, J. F.; Hoth, A. Preparation of Ideal PEG Analogues with a Tunable Thermosensitivity by Controlled Radical Copolymerization of 2-(2-Methoxyethoxy)ethyl Methacrylate and Oligo(ethylene glycol) Methacrylate. *Macromolecules* **2006**, *39*, 893-896.
60. Woodcock, J. W.; Jiang, X. G.; Wright, R. A. E.; Zhao, B. Enzyme-Induced Formation of Thermoreversible Micellar Gels from Aqueous Solutions of Multiresponsive Hydrophilic ABA Triblock Copolymers. *Macromolecules* **2011**, *44*, 5764-5775.
61. Zhu, Y.; Noy, J.-M.; Lowe, A. B.; Roth, P. J. The Synthesis and Aqueous Solution Properties of Sulfobutylbetaine (Co)polymers: Comparison of Synthetic Routes and Tuneable Upper Critical Solution Temperatures. *Polym. Chem.* **2015**, *6*, 5705-5718.
62. Nizardo, N. M.; Schanzenbach, D.; Schönemann, E.; Laschewsky, A. Exploring Poly(ethylene glycol)-Polyzwitterion Diblock Copolymers as Biocompatible Smart Macrosurfactants Featuring UCST-Phase Behavior in Normal Saline Solution. *Polymers* **2018**, *10*, 325.
63. Zhang, H.; Zhang, J.; Dai, W.; Zhao, Y. Facile Synthesis of Thermo-, pH-, CO₂- and Oxidation-Responsive Poly(amido thioether)s with Tunable LCST and UCST Behaviors. *Polym. Chem.* **2017**, *8*, 5749-5760.
64. Arotçaréna, M.; Heise, B.; Ishaya, S.; Laschewsky, A. Switching the Inside and the Outside of Aggregates of Water-Soluble Block Copolymers with Double Thermoresponsivity. *J. Am. Chem. Soc.* **2002**, *124*, 3787-3793.
65. Papadakis, C. M.; Muller-Buschbaum, P.; Laschewsky, A. Switch It Inside-Out: “Schizophrenic” Behavior of All Thermoresponsive UCST–LCST Diblock Copolymers. *Langmuir* **2019**, *35*, 9660-9676.

66. 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 Wormlike and Stable Globular Conformations. *Macromolecules* **2017**, *50*, 1645-1656.
67. 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.
68. The work described in this Chapter has been published in *ACS Applied Polymer Materials*. Dr. Ning Wang synthesized monomer M1P6, P1P6 homopolymers by RAFT polymerization, determined the molecular weights and dispersities of P1P6 polymers by SEC, recorded NMR spectra of P1P6 polymers, measured the cloud points of P1P6-3 and -4 in water by transmittance, and also developed synthetic methods for M22 and M44. I did the majority of the rest of the work presented in this Chapter and in the following publication. Lewoczko, E. M.;[†] Wang, N.;[†] Lundberg, C. E.; Kelly, M. T.; Kent, E. W.; Wu, T.; Chen, M.-L.; Wang, J.-H.; Zhao, B. Effects of *N*-Substituents on Solution Behavior of Poly(sulfobetaine methacrylate)s in Water: UCST and LCST. *ACS Applied Polymer Materials* **2021**, *3*, 867-878.

[†] These authors contributed equally to this work.

DOI: [10.1021/acsapm.0c01191](https://pubs.acs.org/doi/10.1021/acsapm.0c01191) (<https://pubs.acs.org/doi/10.1021/acsapm.0c01191>)

Appendix A

For

Chapter 2: Effects of *N*-Substituents on Solution Behavior of Poly(sulfobetaine methacrylate)s in Water: UCST and LCST

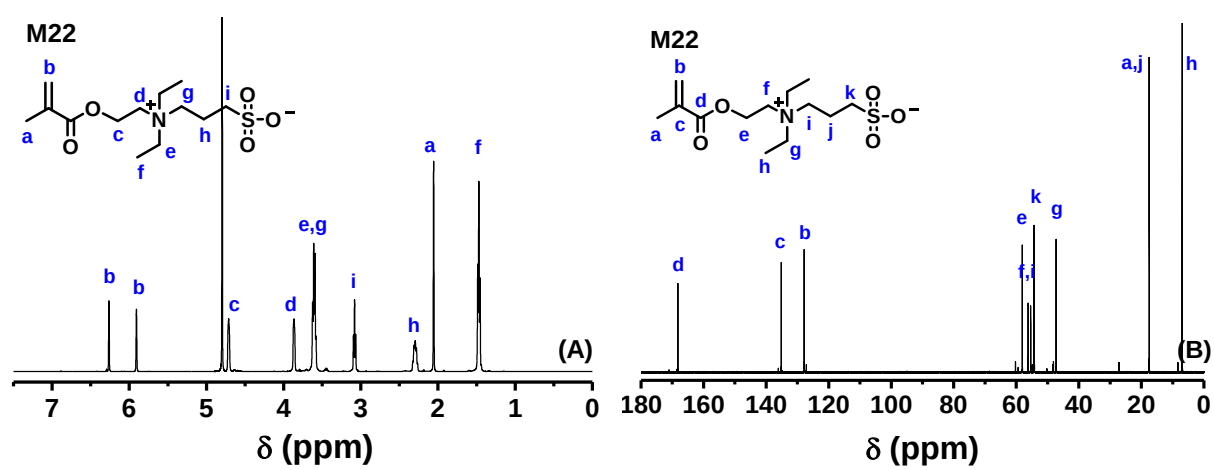


Figure A1. ^1H (A) and ^{13}C (B) NMR spectra of zwitterionic monomer M22 in D_2O .

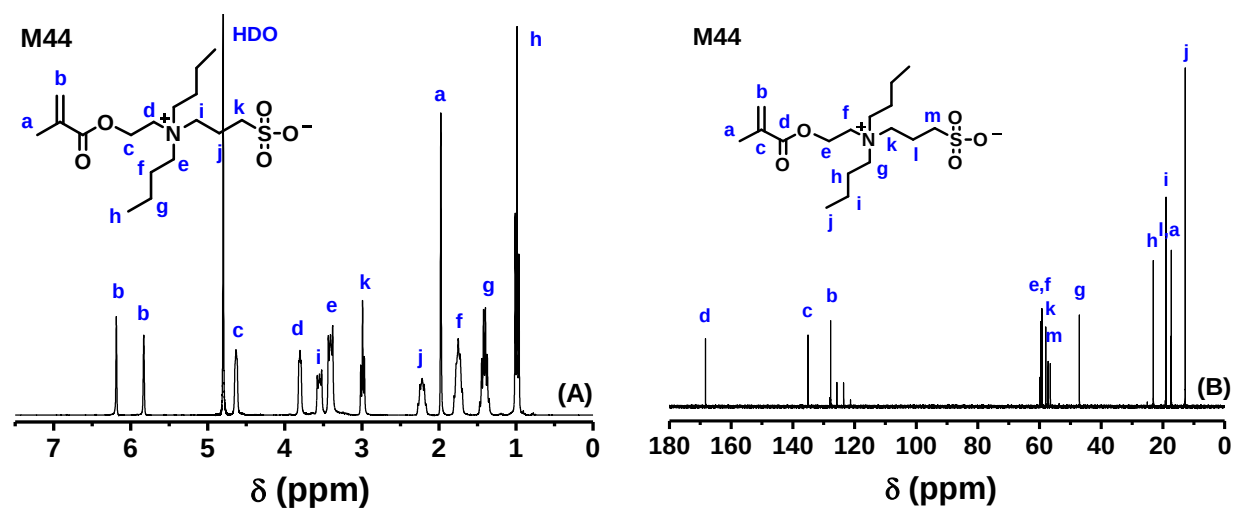


Figure A2. ^1H (A) and ^{13}C (B) NMR spectra of zwitterionic monomer M44 in D_2O .

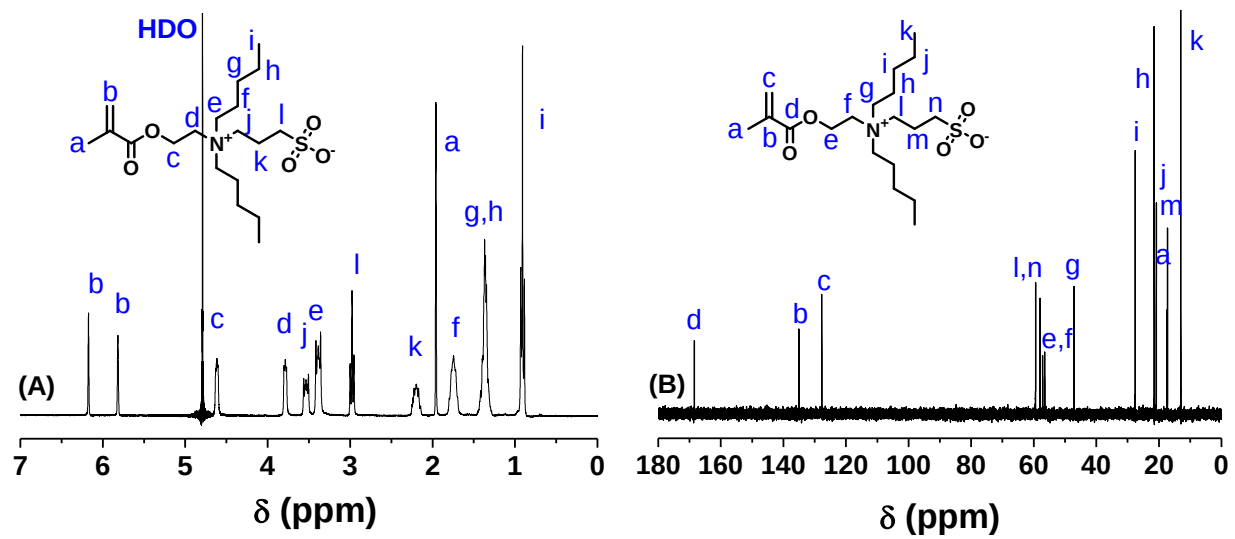


Figure A3. ^1H (A) and ^{13}C (B) NMR spectra of M55 in D_2O .

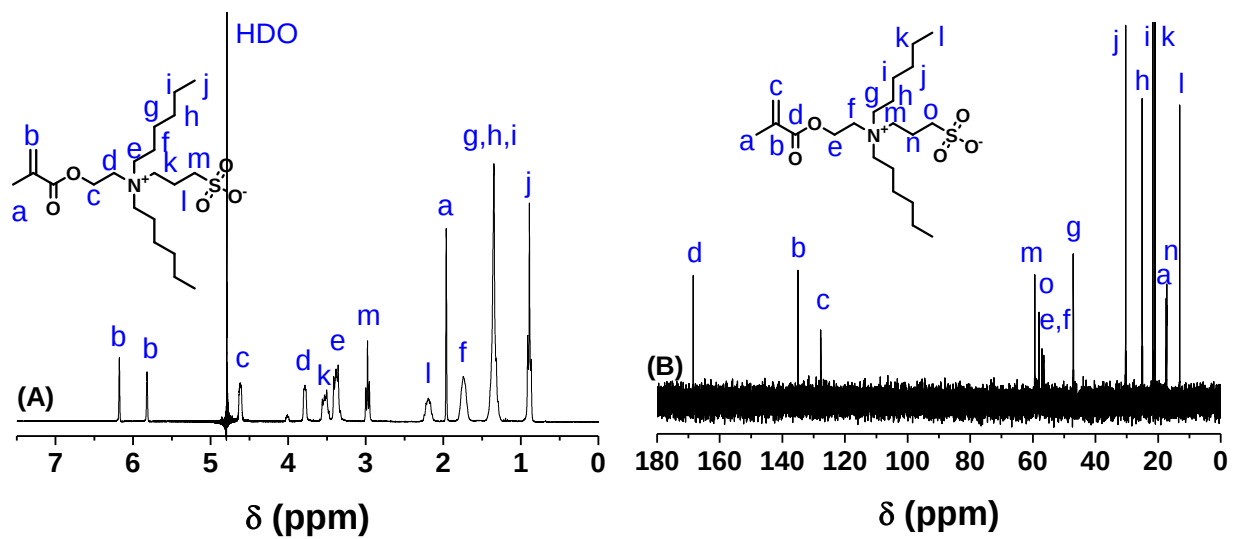


Figure A4. ^1H (A) and ^{13}C (B) NMR spectra of zwitterionic monomer M66 in D_2O .

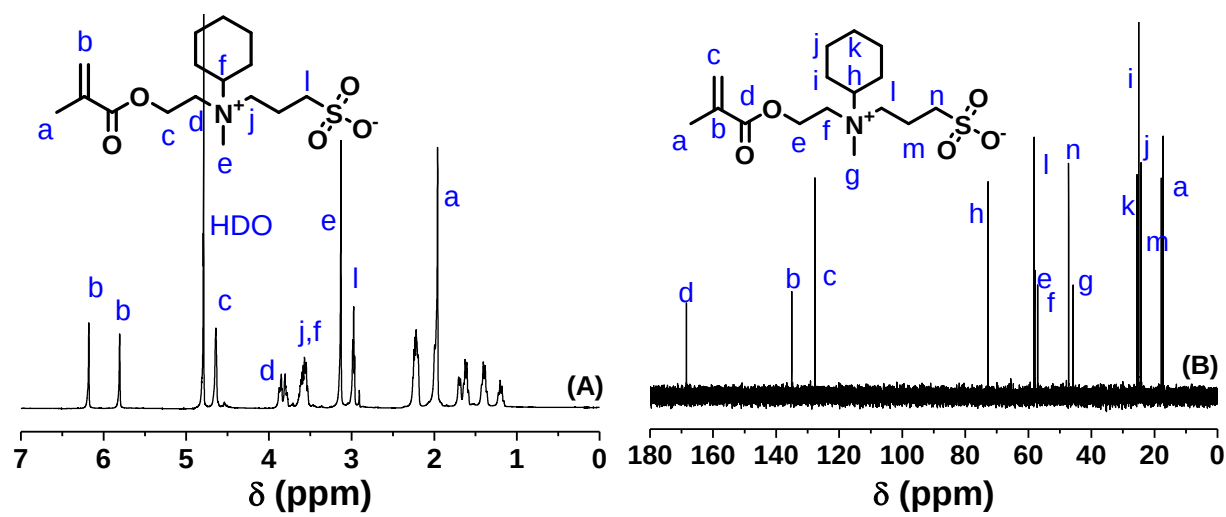


Figure A5. ^1H (A) and ^{13}C (B) NMR spectra of zwitterionic monomer M1C6 in D_2O .

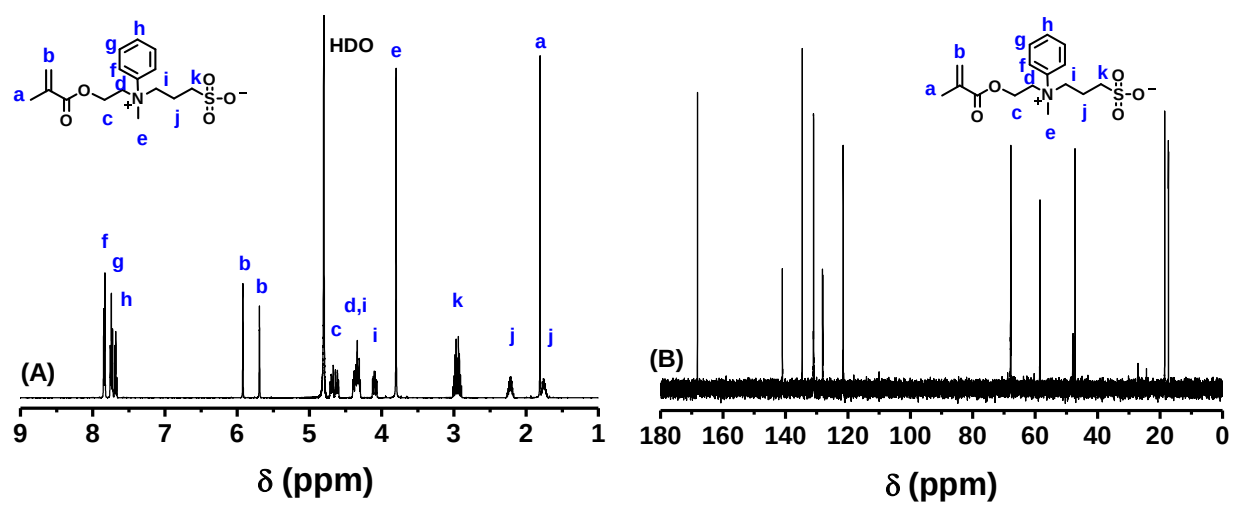


Figure A6. ^1H (A) and ^{13}C (B) NMR spectrum of zwitterionic monomer M1P6 in D_2O .

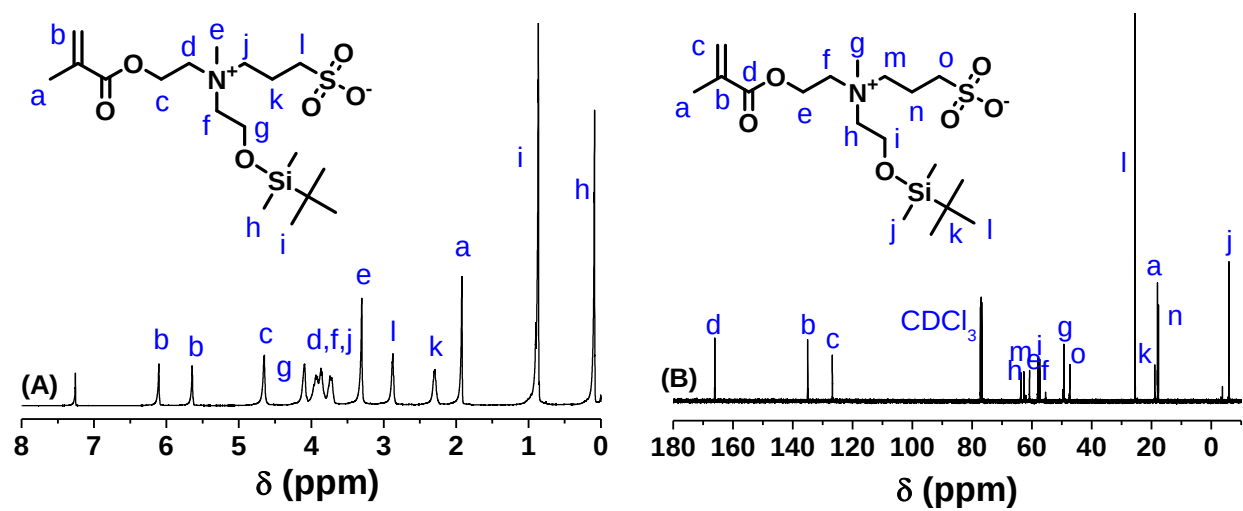


Figure A7. ^1H (A) and ^{13}C (B) NMR spectra of 3-((2-((*tert*-butyldimethylsilyl)oxy)ethyl)(2-(methacryloyloxy)ethyl)(methyl)ammonio)propane-1-sulfonate (M12OSi) in CDCl_3 .

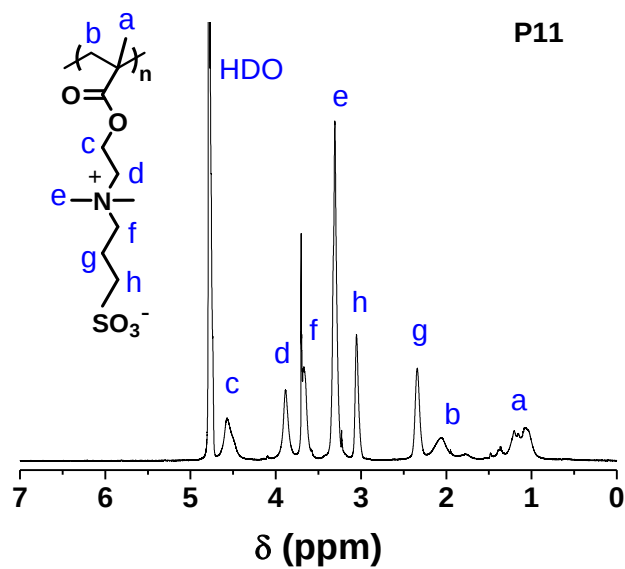


Figure A8. ^1H NMR spectrum of P11 in D_2O with 0.5 M NaCl.

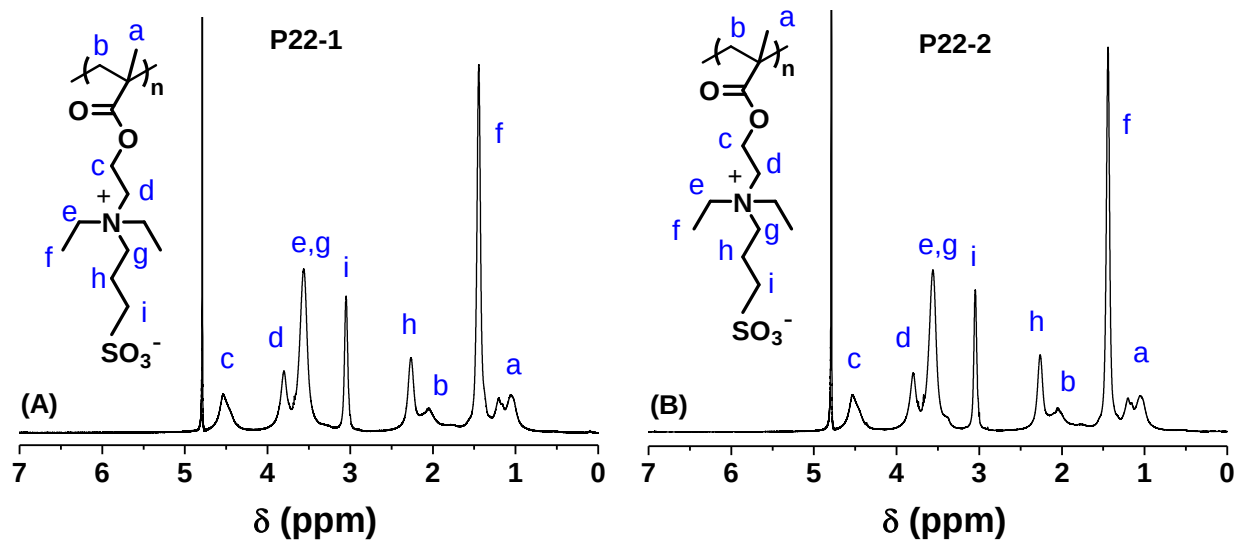


Figure A9. ^1H NMR spectra of P22-1 (A) and P22-2 (B) in D_2O with 0.5 M NaCl.

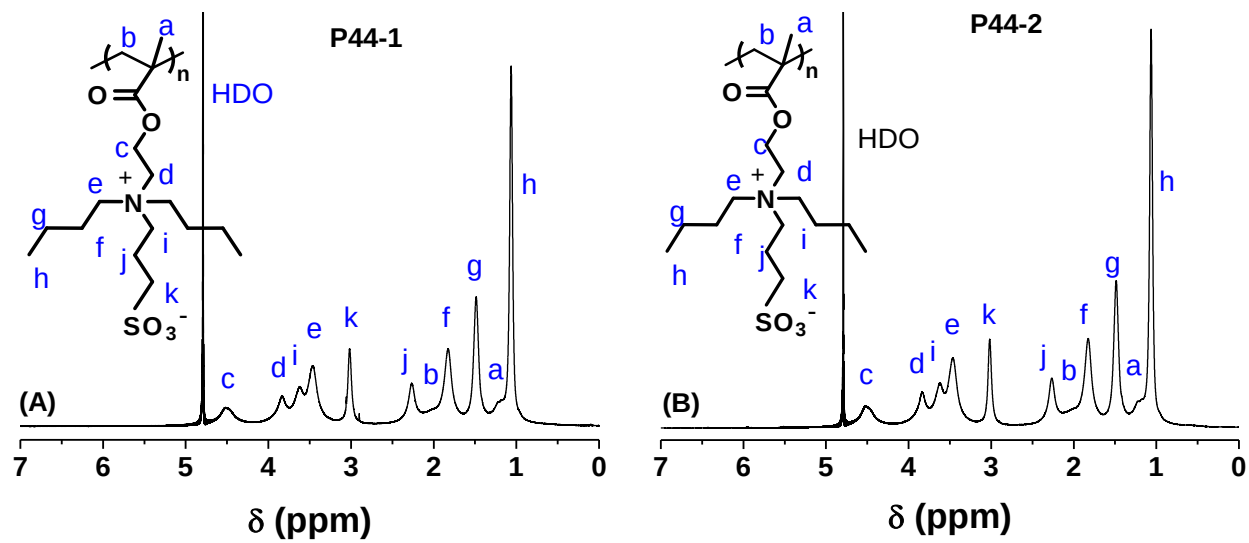


Figure A10. ^1H NMR spectra of P44-1 (A) and P44-2 (B) in D_2O with 0.5 M NaCl.

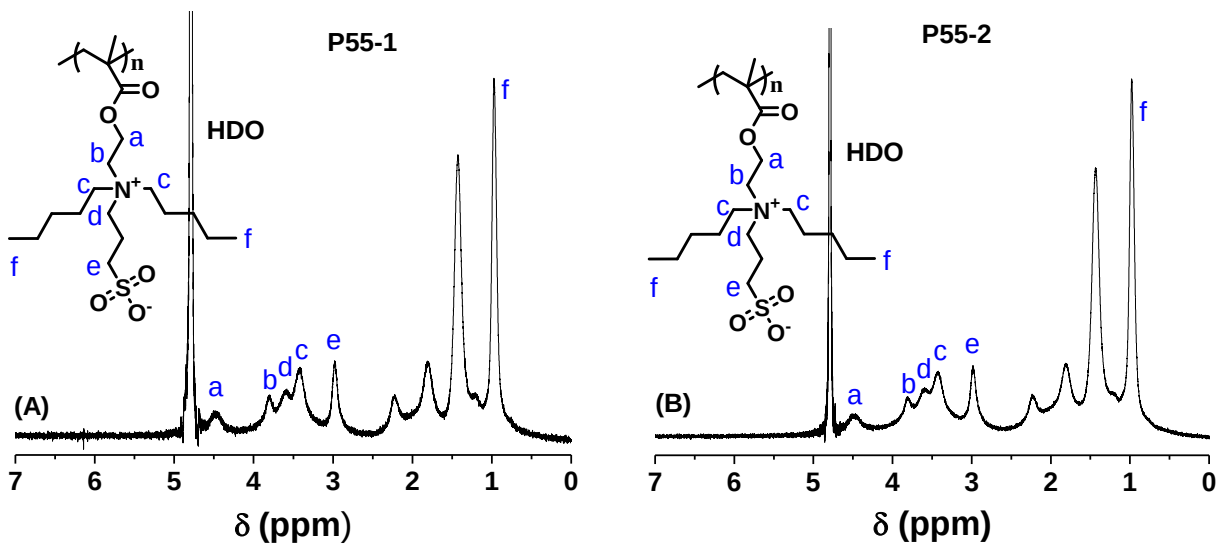


Figure A11. ^1H NMR spectra of P55-1 (A) and P55-2 (B) in D_2O with 0.5 M NaCl.

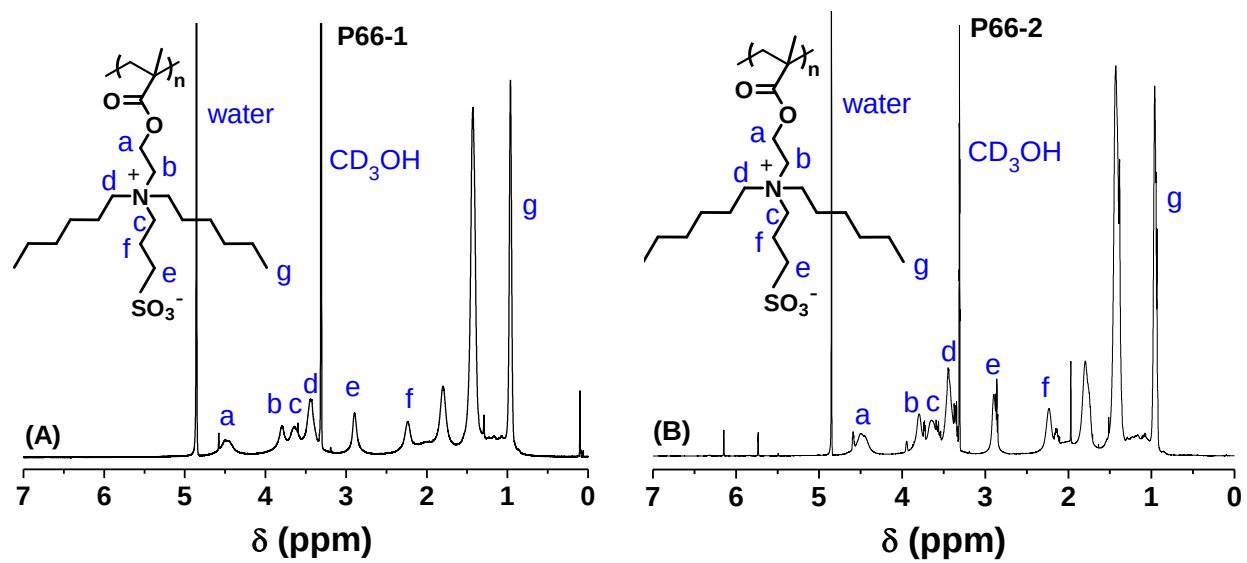


Figure A12. ^1H NMR spectra of P66-1 (A) and P66-2 (B) in CD_3OD .

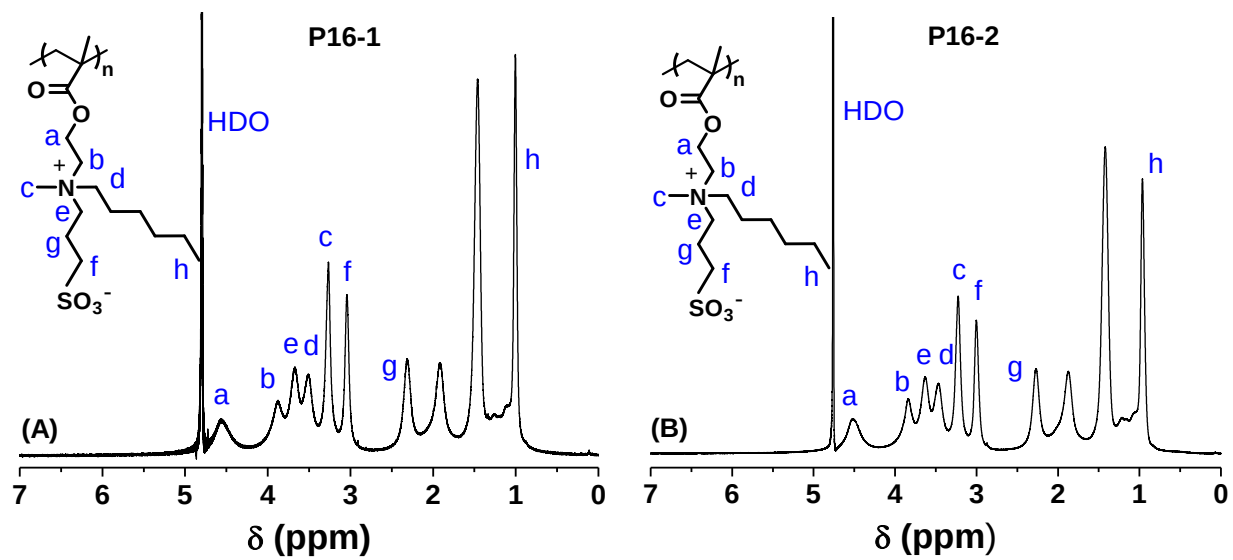


Figure A13. ^1H NMR spectra of P16-1 (A) and P16-2 (B) in D_2O with 0.5 M NaCl.

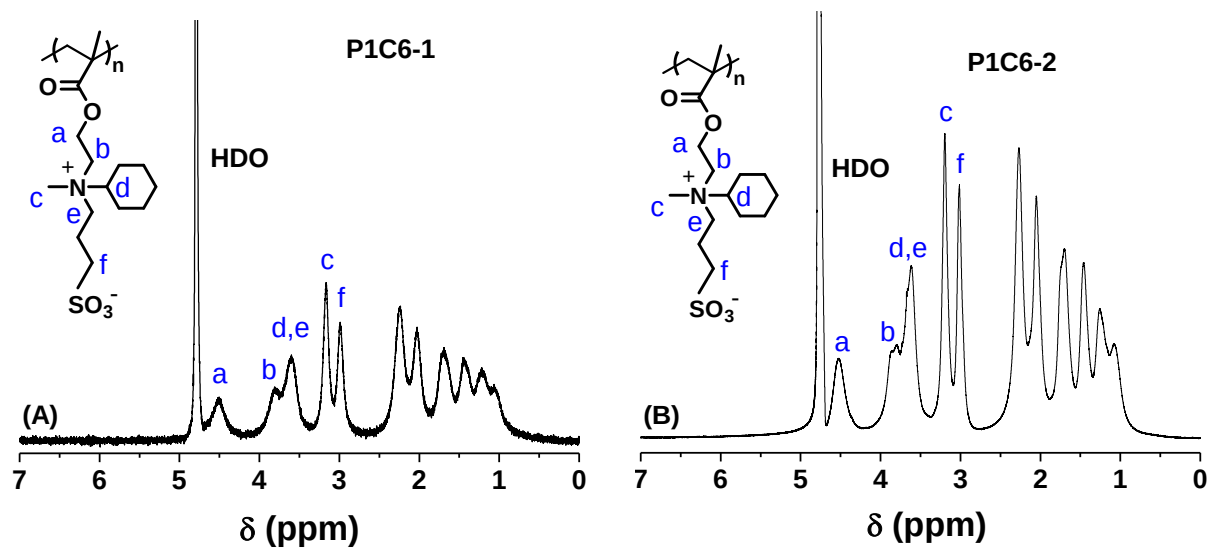


Figure A14. ^1H NMR spectra of (A) P1C6-1 and P1C6-2 (B) in D_2O with 0.5 M NaCl.

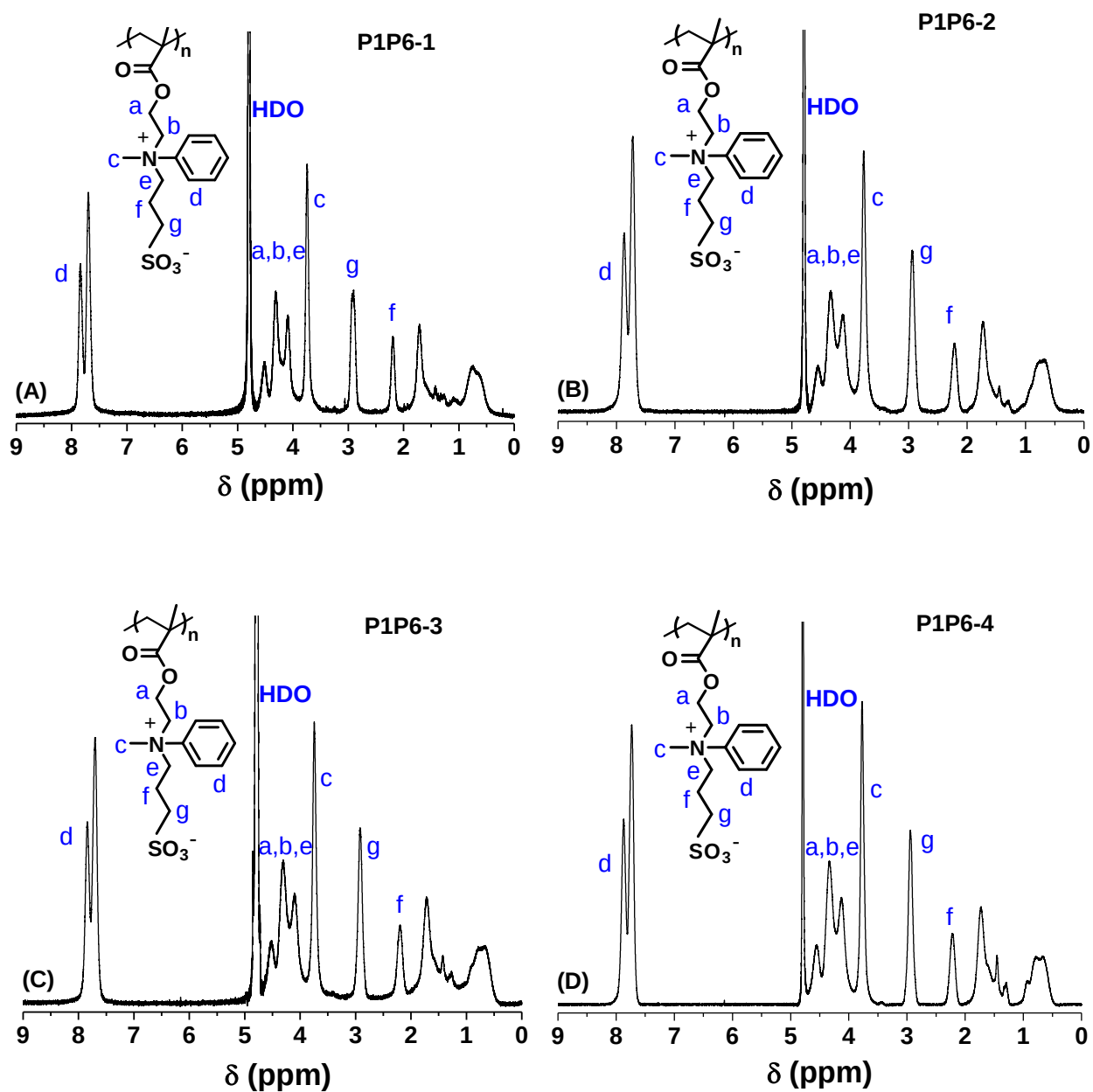


Figure A15. ^1H NMR spectra of P1P6-1 (A), P1P6-2 (B), P1P6-3 (C), and P1P6-4 (D) in D_2O with 0.5 M NaCl.

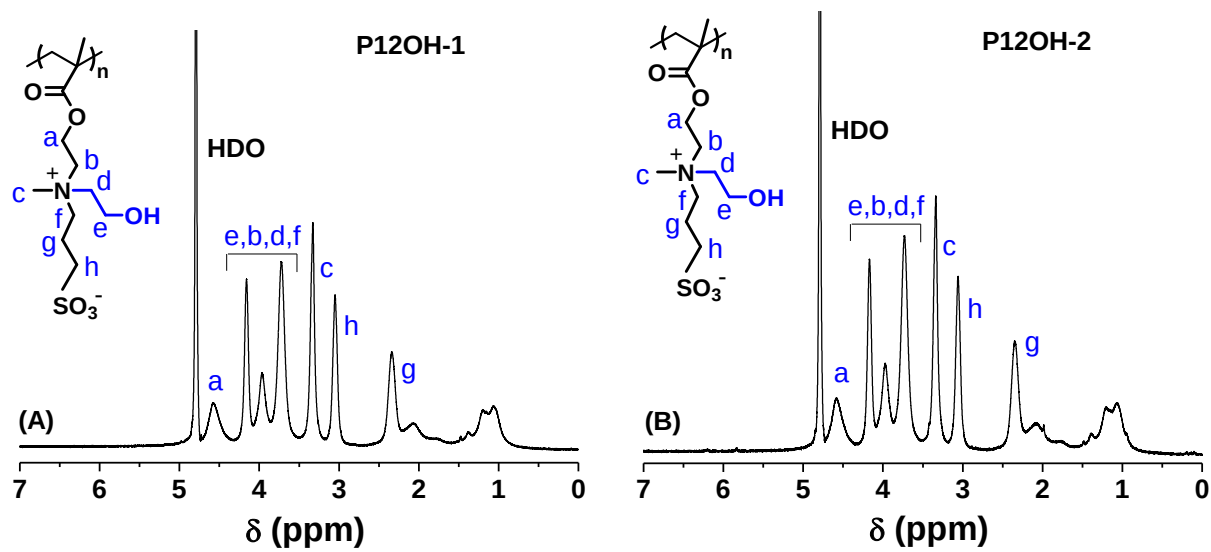


Figure A16. ^1H NMR spectra of P12OH-1 (A) and P12OH-2 (B) in D_2O with 0.5 M NaCl.

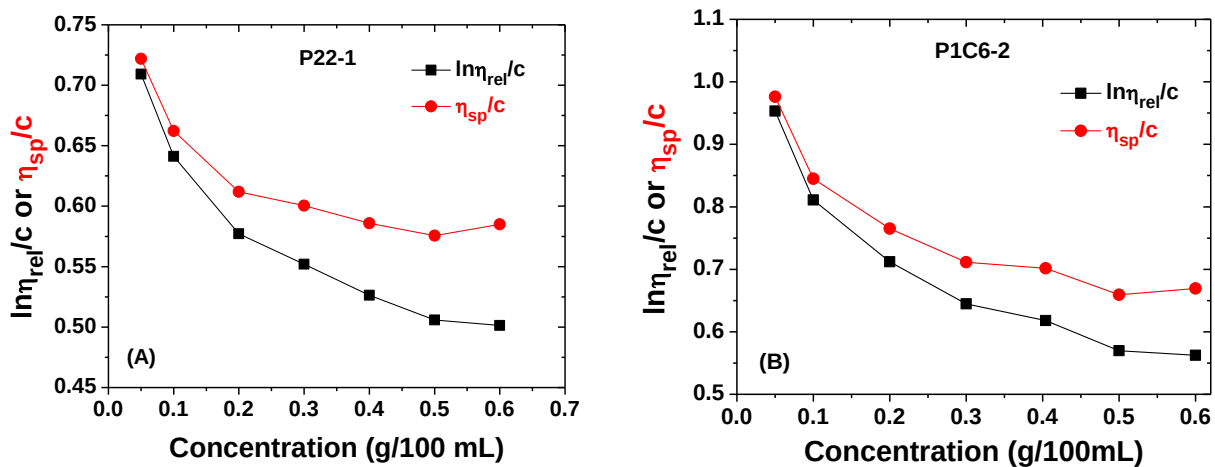


Figure A17. Plots of $\ln\eta_{\text{rel}}/c$ (Kraemer plot) and η_{sp}/c (Huggins plot) versus polymer concentration for (A) P22-1 and (B) P1C6-2. $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for polymer solution and pure solvent, respectively, at 25.0 °C, and $\eta_{\text{sp}} = \eta_{\text{rel}} - 1$.

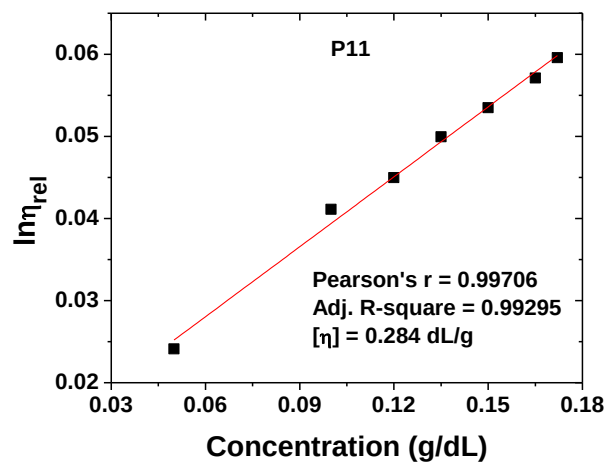


Figure A18. Plot of $\ln \eta_{\text{rel}}$ versus polymer concentration for P11. $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for polymer solution and pure solvent, respectively, at 25.0 °C. Using Wolf's method, the slope is taken as the intrinsic viscosity of the zwitterionic polymer.

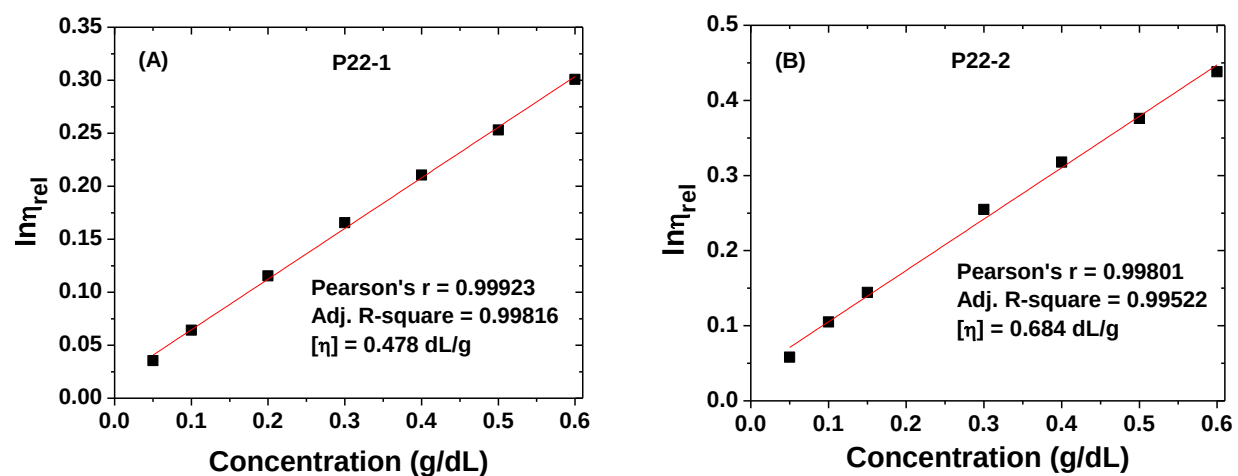


Figure A19. Plots of $\ln \eta_{rel}$ versus concentration for P22-1 (A) and P22-2 (B). $\eta_{rel} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.

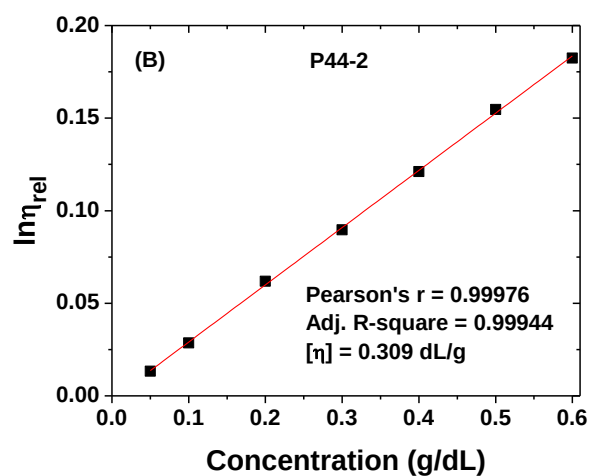
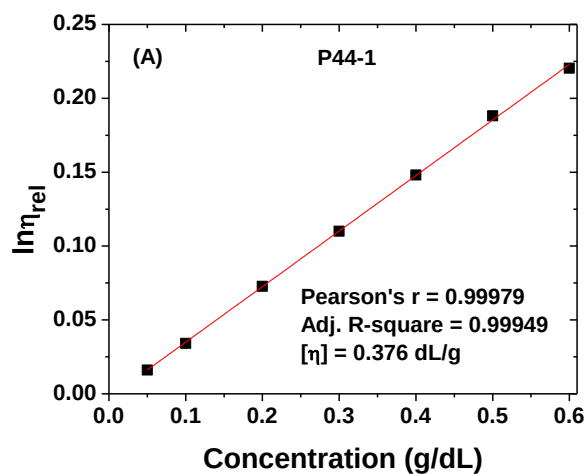


Figure A20. Plots of $\ln \eta_{rel}$ versus concentration for P44-1 (A) and P44-2 (B). $\eta_{rel} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.

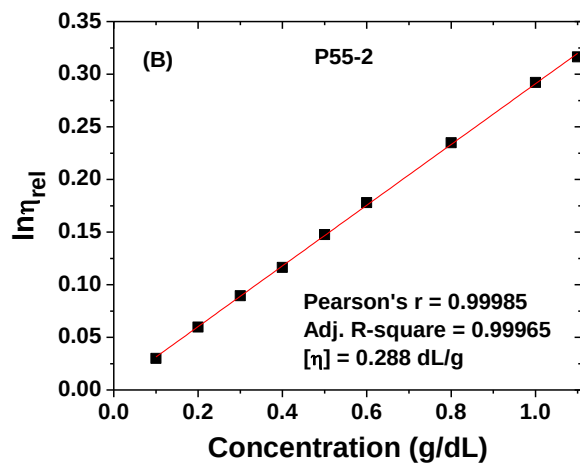
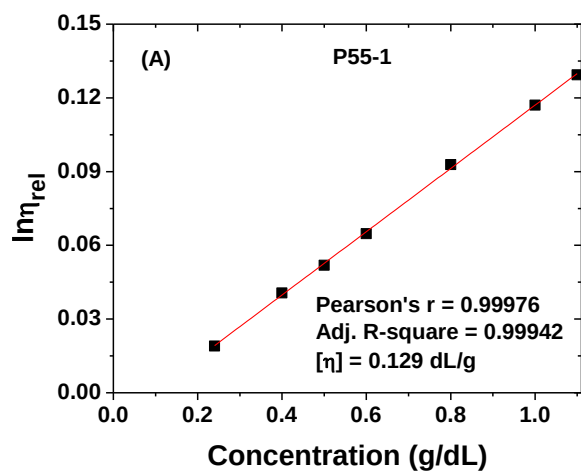


Figure A21. Plots of $\ln \eta_{\text{rel}}$ versus concentration for P55-1 (A) and P55-2 (B). $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.

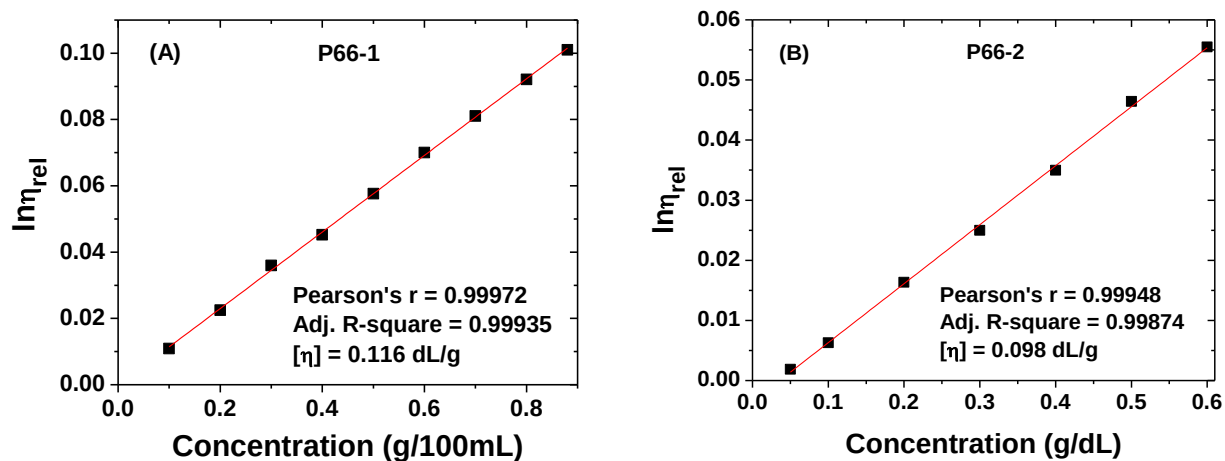


Figure A22. Plots of $\ln \eta_{rel}$ versus concentration for P66-1 (A) and P66-2 (B). $\eta_{rel} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.

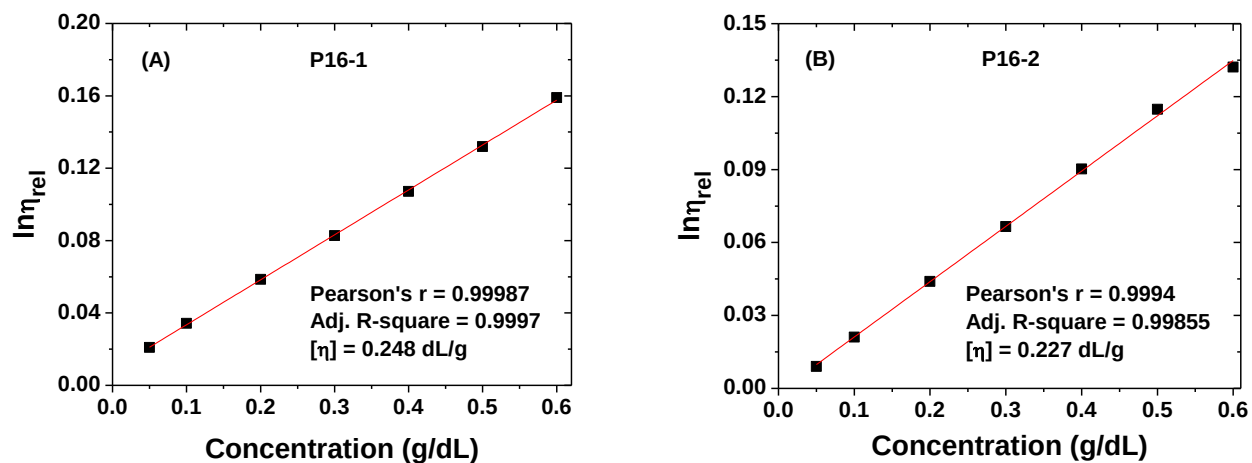


Figure A23. Plots of $\ln \eta_{rel}$ versus concentration for P16-1 (A) and P16-2 (B). $\eta_{rel} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.

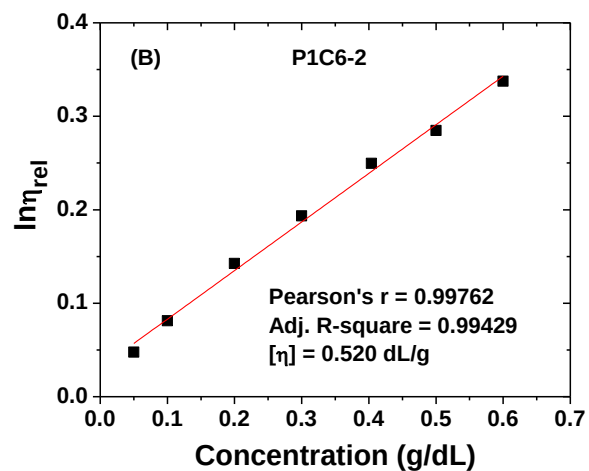
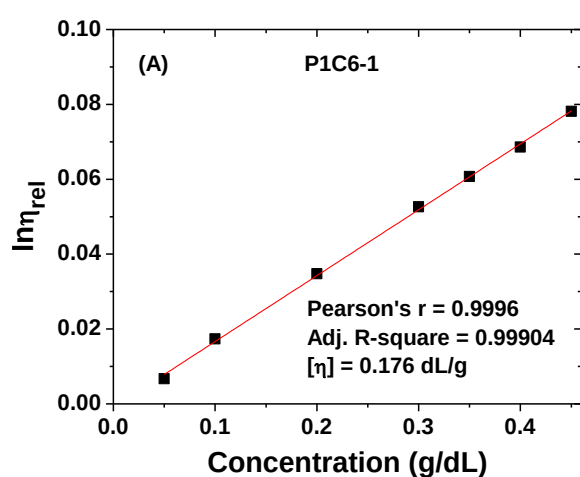


Figure A24. Plots of $\ln \eta_{\text{rel}}$ versus concentration for P1C6-1 (A) and P1C6-2 (B). $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.

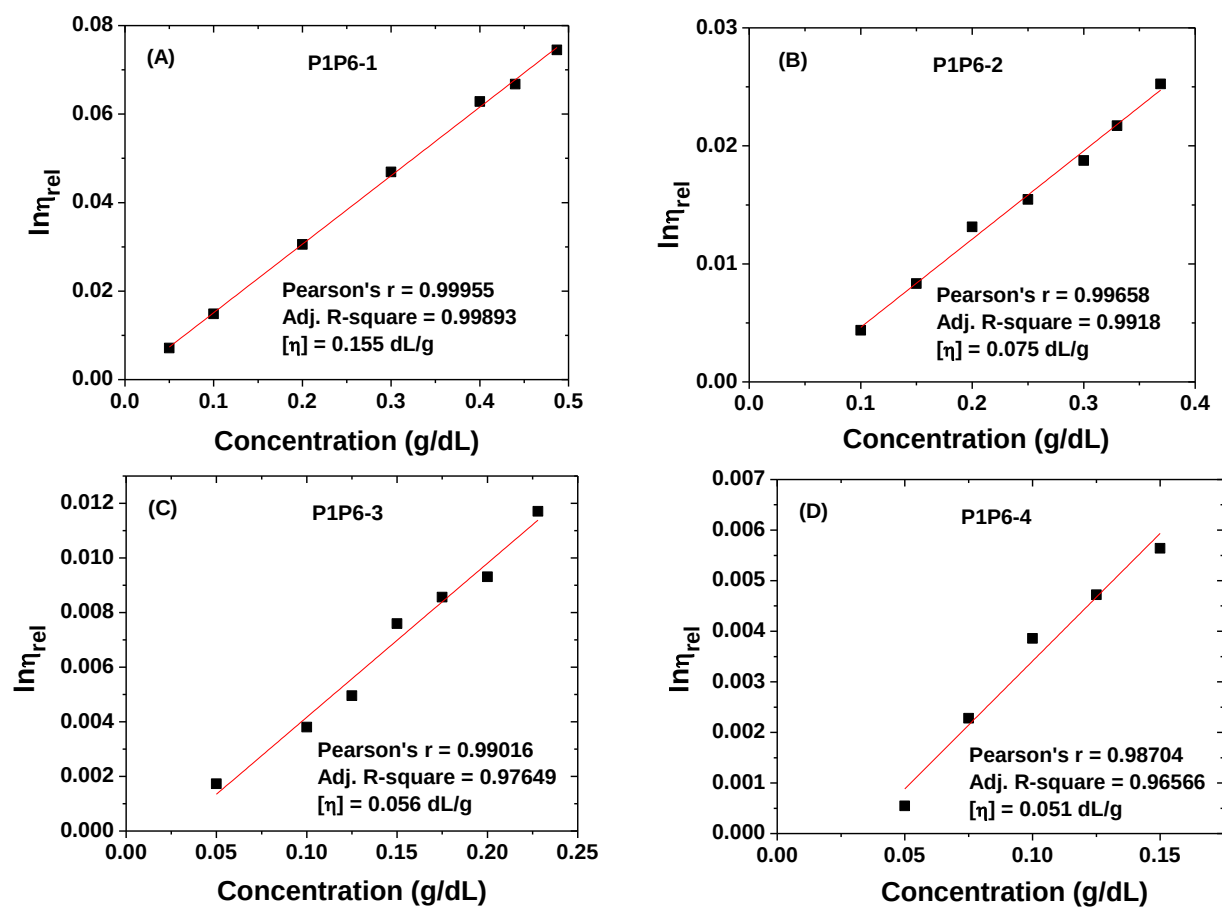


Figure A25. Plot of $\ln\eta_{rel}$ versus concentration for (A) P1P6-1, (B) P1P6-2, (C) P1P6-3, and (D) P1P6-4. $\eta_{rel} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.

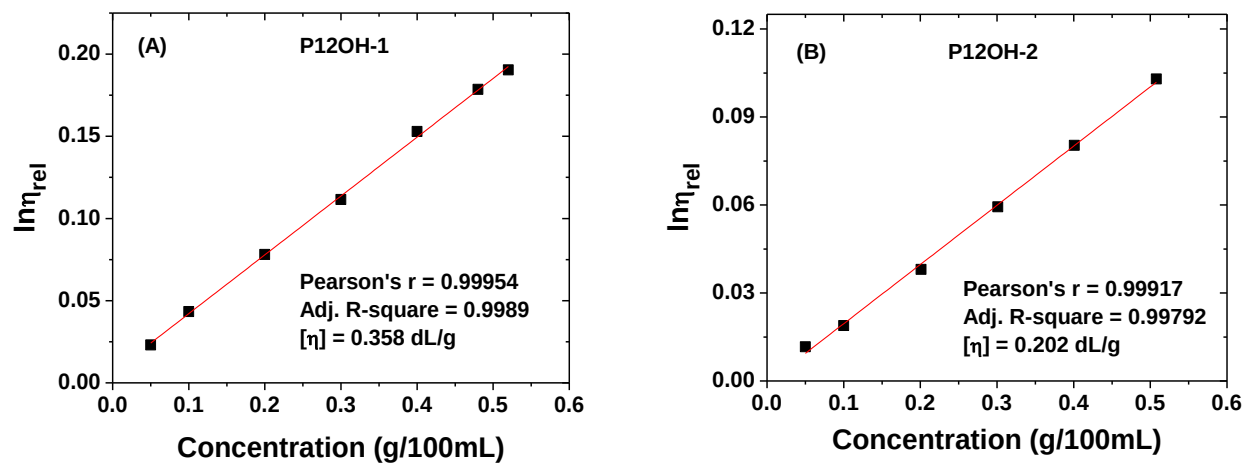


Figure A26. Plot of $\ln \eta_{\text{rel}}$ versus concentration for (A) P12OH-1 and (B) P12OH-2. $\eta_{\text{rel}} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.

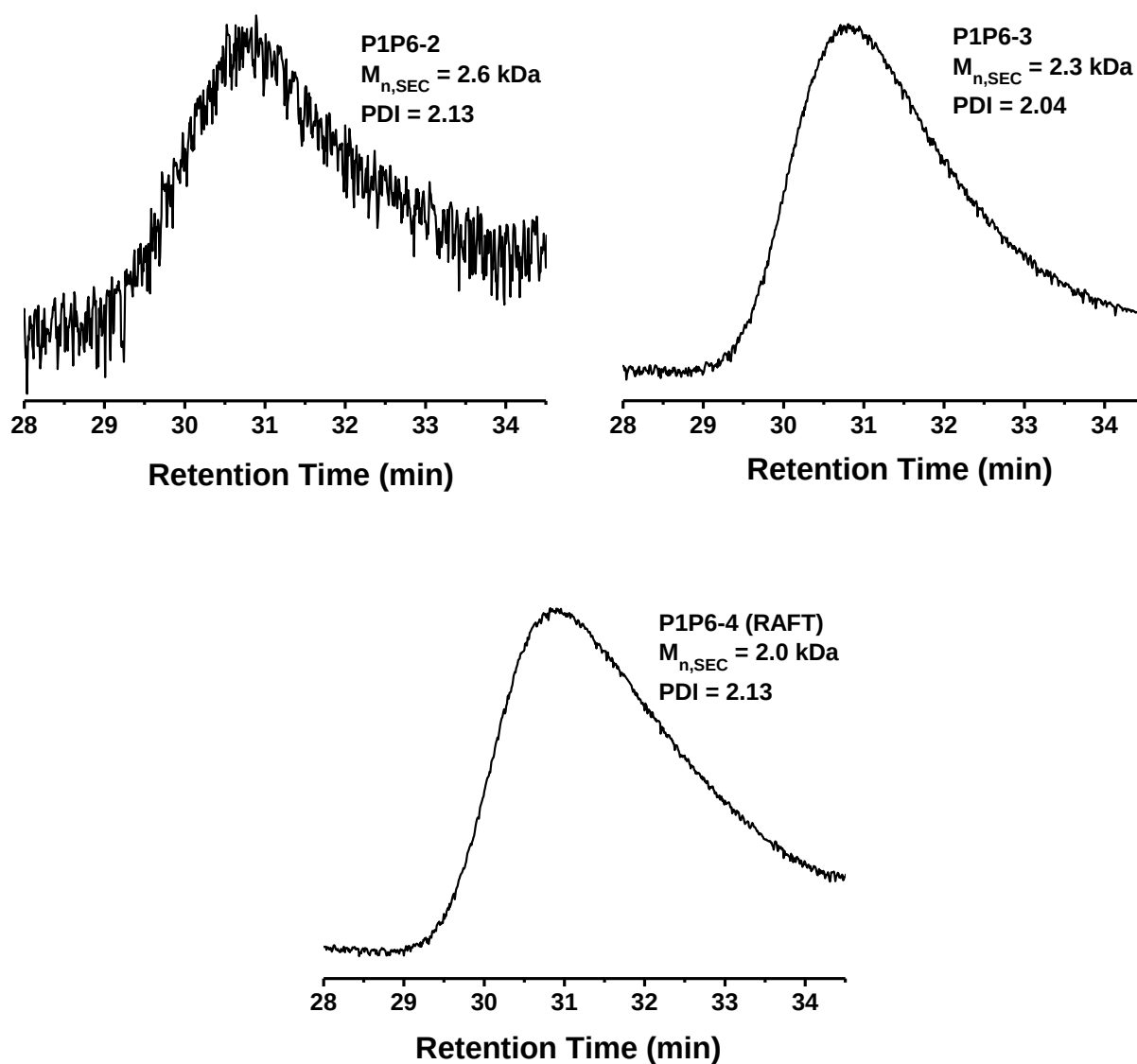


Figure A27. Size exclusion chromatography (SEC) curves of zwitterionic P1P6-2, P1P6-3, and P1P6-4 synthesized by reversible addition-fragmentation chain transfer polymerization using 0.2 M NaNO_3 aqueous solution as eluent.

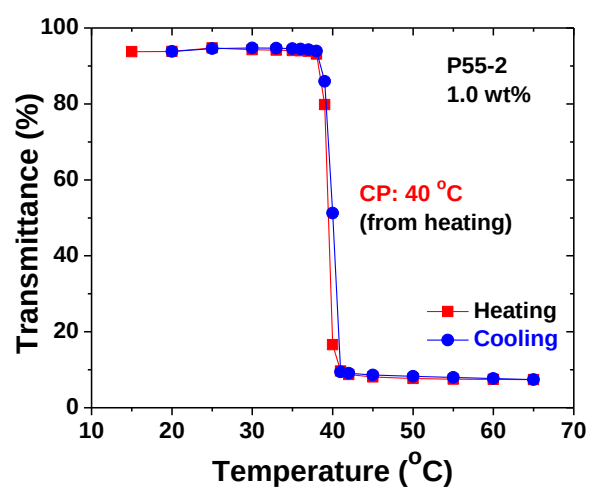


Figure A28. Plot of transmittance at the wavelength of 500 nm versus temperature for P55-2 in Milli-Q water at a concentration of 1.0 wt% in a heating-cooling cycle.

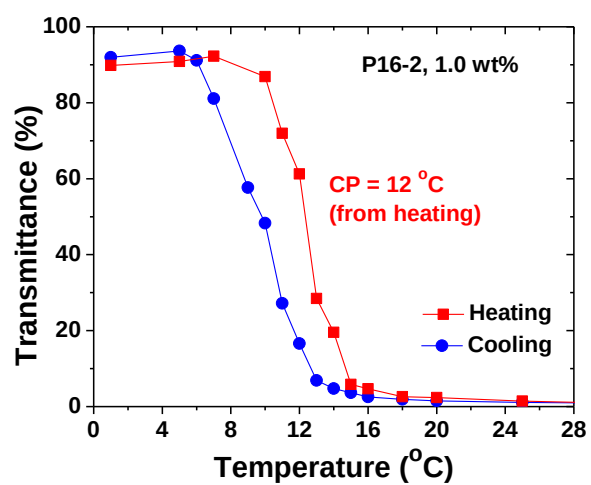


Figure A29. Plot of transmittance at the wavelength of 500 nm versus temperature for P16-2 in Milli-Q water at a concentration of 1.0 wt% from a heating-cooling cycle.

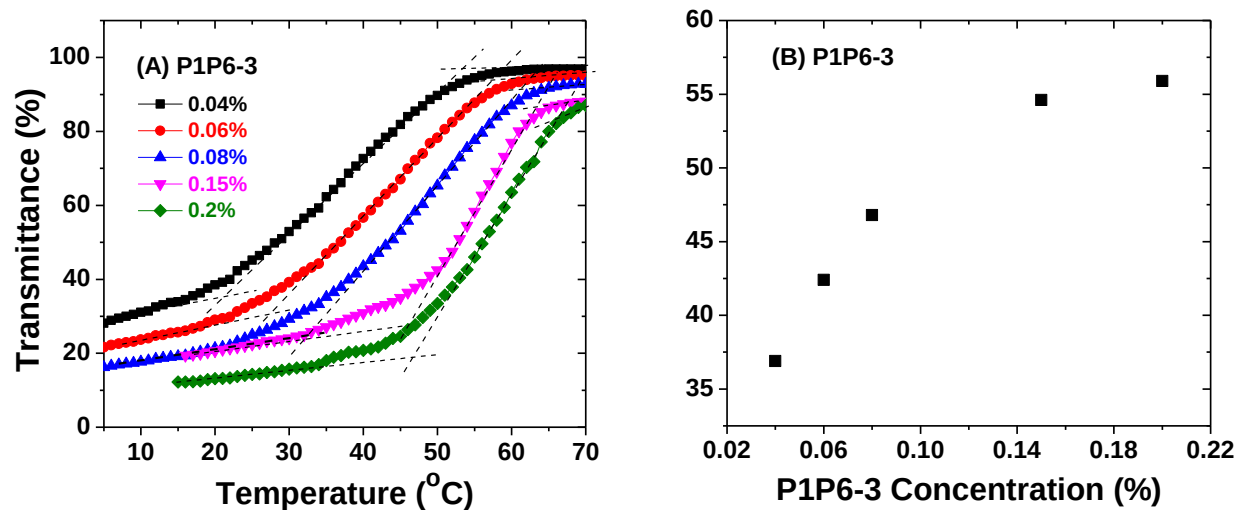


Figure A30. (A) Plots of transmittance at the wavelength of 500 nm versus temperature for P1P6-3 in Milli-Q water at concentrations of 0.04, 0.06, 0.08, 0.15, and 0.2 wt% upon cooling. (B) Plot of cloud point as a function of P1P6-3 concentration in water obtained from (A).

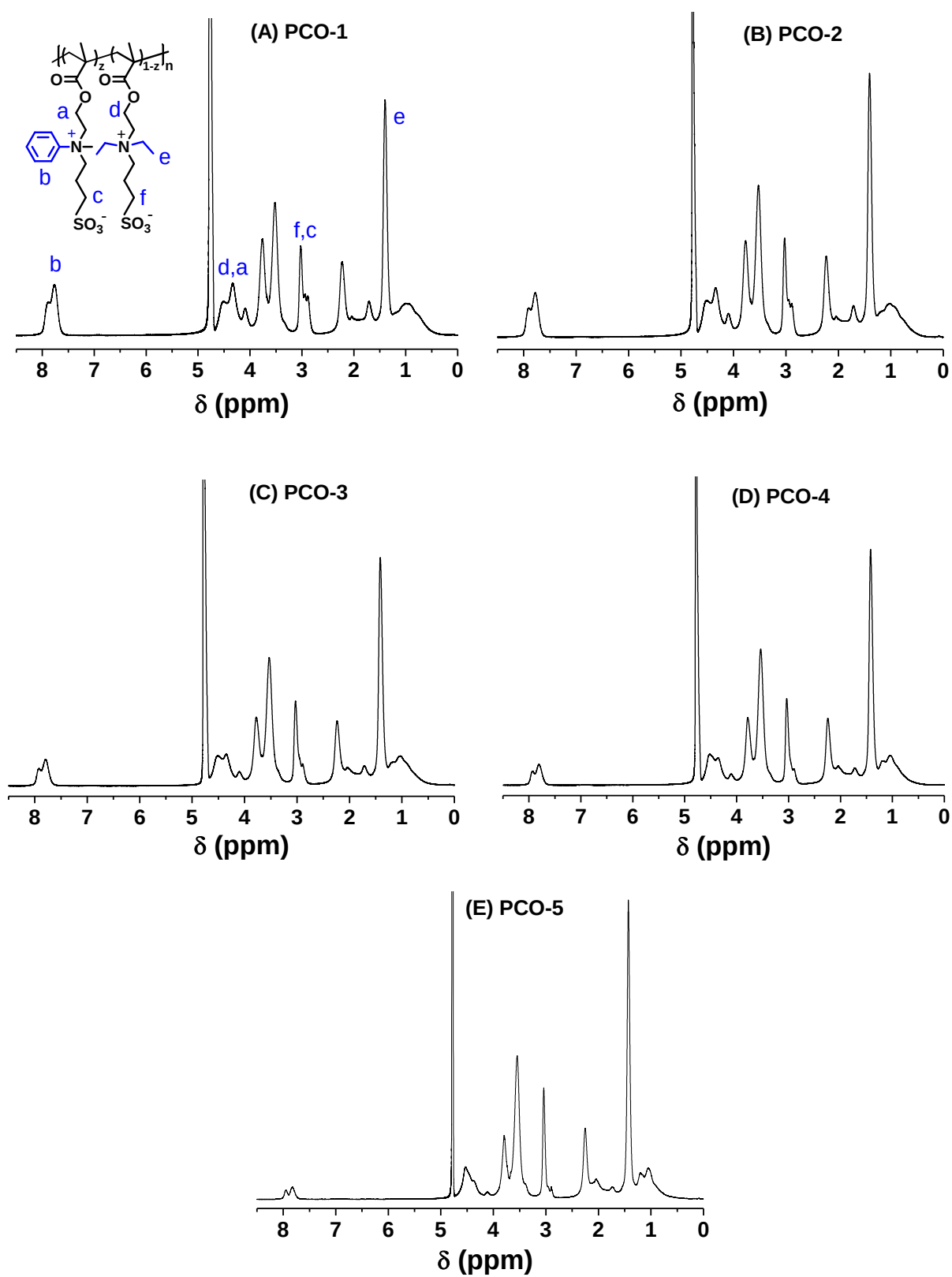


Figure A31. ^1H NMR spectra of (A) PCO-1, (B) PCO-2, (C) PCO-3, (D) PCO-4, and (E) PCO-5 (zwitterionic copolymers of M22 and M1P6 with different compositions) in D_2O with 0.5 M NaCl.

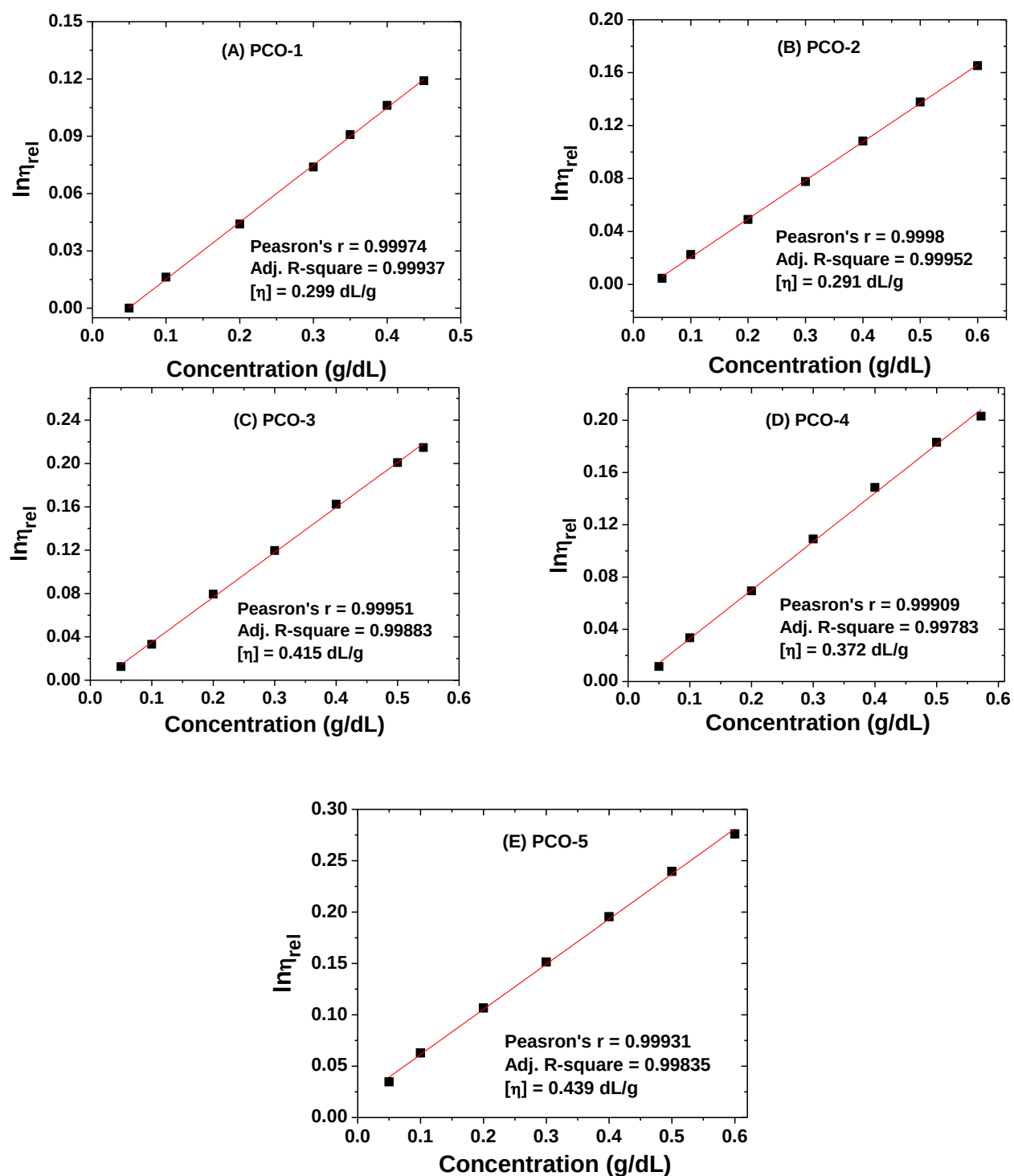


Figure A32. Plot of $\ln \eta_{rel}$ versus concentration for (A) PCO-1, (B) PCO-2, (C) PCO-3, (D) PCO-4, and (E) PCO-5. $\eta_{rel} = t_1/t_0$, where t_1 and t_0 are the flow times for a polymer solution with a certain concentration and 2,2,2-trifluoroethanol, respectively, at 25.0 °C.

Chapter 3: Zwitterionic Poly(sulfobetaine methacrylates) as Kinetic Gas Hydrate Inhibitors

The work presented in this Chapter has been published in *Chemical Engineering Science* **2021**, 229, 116031. I synthesized and characterized P14, P15, P16-1, and P55 and Caleb A. Bohannon synthesized and characterized P6-2, P6-3, P6-4, PCO-1 and PCO-2 except for viscometry (done by me). The KHI tests were done by our collaborators Qian Zhang and Prof. Malcolm A. Kelland at the University of Stavanger in Norway. Prof. Bin Zhao advised the synthesis and characterization part of the work at the University of Tennessee, Knoxville, Tennessee.

Abstract

Kinetic hydrate inhibitors (KHIs) are commercially used to prevent the formation of gas hydrates in gas and oil transportation pipelines, and continued effort is being made to seek more efficient KHIs. To test the potential of zwitterionic PSBMAs as KHIs, a series of zwitterionic PSBMA homopolymers with *N-n*-alkyl substituents of 4-6 carbon atoms and copolymers of a zwitterionic PSBMA with one *N*-methyl and one *N-n*-hexyl substituent and isopropyl methacrylate were synthesized by either reversible addition-fragmentation chain transfer polymerization or AIBN-initiated conventional free radical polymerization and thoroughly characterized. KHI tests showed that PSBMAs with one *N*-methyl and one *N-n*-hexyl substituent performed similarly or even slightly better than the commercially used poly(*N*-vinylcaprolactam), which is likely because of its water solubility and the large hydrophobic effect associated with the *n*-hexyl group. This study demonstrates the potential use of zwitterionic polymers as kinetic gas hydrate inhibitors.

3.1. Introduction

Zwitterionic polymers, such as poly(sulfobetaine methacrylate)s (PSBMAs), contain two oppositely charged groups most often in the same repeat units.¹ Two well-known and widely used examples of zwitterionic homopolymers are poly(3-((2-methacryloyloxyethyl)dimethylammonio)propane-1-sulfonate), a PSBMA, and poly(3-(*N*-(3-methacrylamidopropyl)dimethylammonio)propane-1-sulfonate) (PMAMPS).² These polyelectrolytes display many interesting properties, such as high hydrophilicity (for common zwitterionic polymers),¹ stimuli-responsive property,^{2,3} and antipolyelectrolyte effect.^{4,5} They have been evaluated and have shown promise for a variety of applications, including anti(bio)fouling,^{1,6,7} anti-icing and anti-freezing,^{8,9} lubrication,¹⁰ oil-water separation,¹¹ and cryopreservation.¹² This chapter describes a new potential application of zwitterionic polymers – kinetic gas hydrate inhibitors.

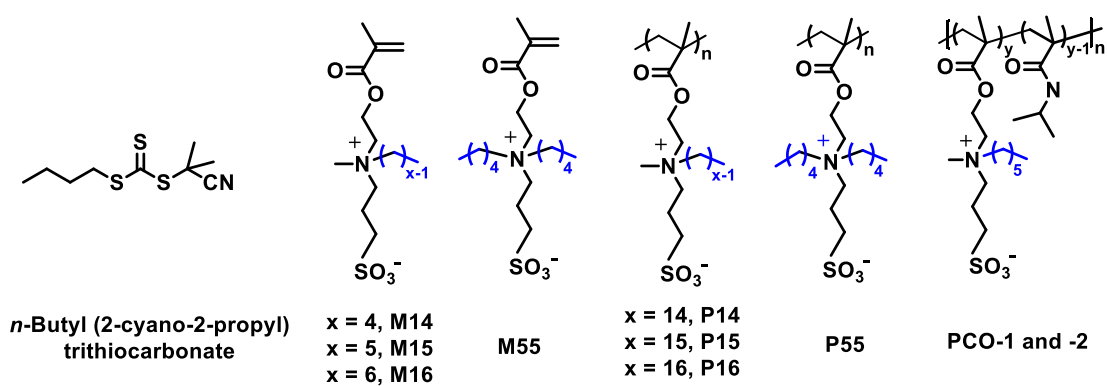
Gas hydrates are clathrate crystalline solids that are formed from low molar mass hydrocarbon compounds (e.g., methane and ethane) and water under low temperature and high pressure conditions, where water molecules form a cage around a gas molecule.¹³ Gas hydrates are also known as combustible ice, which could be exploited as a fuel and has attracted tremendous interest. However, when gas hydrates are formed in the gas and oil transportation pipelines, they could cause blockage, which may lead to a disaster. Adding kinetic hydrate inhibitors (KHIs) can prevent gas hydrate formation in gas and oil field production lines. KHIs are water-soluble polymers used at the low concentration range of 0.1 to 2.0 wt%.¹⁴ Commercially used KHIs are mostly amide-containing water-soluble polymers,^{14,15} such as poly(*N*-vinylcaprolactam) (PVCap) and poly(*N*-isopropyl methacrylamide). To seek more efficient KHIs, various water-soluble polymers have been investigated. For example, poly(hydroxyl-*N*-alkylcarbamate)s were studied, and it was found that increasing the alkyl pendant group increased the KHI performance,¹⁶ likely due to a nucleation

inhibition effect exerted by the alkyl pendant group in the formation of water cage-like structures around gas molecules. However, if the alkyl pendant group is too long, the polymer becomes insoluble in water. Thus, PSBMAs with suitable *N*-alkyl substituents could be efficient KHIs, which led us to collaborate with Professor Malcolm Kelland's group at the University of Stavanger in Norway to investigate our zwitterionic PSBMAs as KHIs. A series of PSBMAs homopolymers and copolymers with *N*-*n*-alkyl substituents of 4-6 carbon atoms were synthesized (Scheme 3.1). KHI testing showed that these polymers were efficient KHIs and the best performer was P16. Since the KHI testing was conducted in Norway, this chapter mainly presents the synthesis and characterization of zwitterionic polymers and briefly discusses the testing results.

3.2. Experimental Section

3.2.1. Materials

2-(Methylamino)ethanol (99%, Alfa Aesar), 2-aminoethanol (99%, Alfa Aesar), 1,3-propanesultone (99%, Alfa Aesar), 1-bromobutane (99%, Alfa Aesar), 1-bromopentane (99%, Alfa Aesar), 1-bromohexane (98%, Alfa Aesar), hydroquinone (99%, Acros Organics), 2,2,2-trifluoroethanol (>99% from Alfa Aesar or 99.9% from Oakwood Chemical), and *N,N*-dimethylacetamide (99.5%, extra dry, Acros Organics) were used as received. Methacryloyl chloride (97%, Sigma-Aldrich) was distilled under vacuum and stored in a freezer. Triethylamine (99%, Alfa Aesar) was stirred over CaH₂ and distilled prior to use. α,α' -Azobisisobutyronitrile (AIBN, 98%, Sigma-Aldrich) was recrystallized from ethanol, dried under vacuum, and stored in a refrigerator. *N*-Isopropyl methacrylamide (97%) was purchased from Sigma-Aldrich and was recrystallized from *n*-hexane twice. *n*-Butyl (2-cyano-2-propyl) trithiocarbonate (Scheme 3.1), the chain transfer agent used for reversible addition-fragmentation chain transfer polymerization in



Scheme 3.1. Molecular Structures of n -Butyl (2-Cyano-2-propyl) Trithiocarbonate (the Chain Transfer Agent), Zwitterionic Monomers, and Polymers.

this work, was prepared according to the procedures from the literature,¹⁷⁻¹⁹ and the molecular structure was confirmed by NMR spectroscopy analysis. Zwitterionic monomers M14, M15, and M16 (Scheme 3.1) were synthesized using the same procedures reported previously.²⁰ The synthesis of monomer M55 for zwitterionic homopolymer P55 (Scheme 3.1) was described in Chapter 2, and the molecular structure was verified by ¹H and ¹³C NMR spectroscopy. All other chemical reagents were obtained from either Fisher Scientific or Aldrich and used without further purification. The dialysis tubing with a molecular weight cutoff of 3500 Da used for the purification of zwitterionic polymers was purchased from Fisher Scientific and made from regenerated cellulose.

3.2.2. Instrumentation and Characterization Methods

¹H NMR spectroscopy analysis of zwitterionic polymers was performed on a Mercury 300 MHz or a Varian VNMRs 500 or a Varian VNMRs 600 MHz spectrometer using D₂O with 0.5 M NaCl or pure D₂O. Size exclusion chromatography (SEC) of P14, P15, and P16-1 was performed using a SEC system composed of a refractive index detector (RI detector K-2301 from Knauer), one PSS Suprema 10 µm guard column (50 × 8 mm) and three PSS Suprema 10 µm columns (300 × 18 mm each, 100 Å, 3000 Å, and 10000 Å). A 0.2 M aqueous solution of NaNO₃ was used as the eluent, and the flow rate was 1.0 mL/min during the analysis. The SEC system was calibrated with narrow disperse PEO standards. The molecular weight and dispersity of P55 were measured, relative to polystyrene standards, at 50 °C using a PL-GPC 50 Plus system (Polymer Laboratories, Inc.) consisting of one PSS GRAL 10 µm guard column (50 × 8 mm, PSS-USA, Inc.), two PSS GRAL 10 µm linear columns (each 300 × 8 mm, linear range of molecular weight from 500 to 1 000 000 Da, PSS-USA, Inc.), and a differential refractive index detector. *N,N*-Dimethylformamide (DMF) with 50 mM LiBr was used as the mobile phase at a flow rate of

1.0 mL/min. Narrow disperse linear polystyrene standards (Scientific Polymer Products, Inc.) were used for calibration. The SEC data were processed using Cirrus GPC/SEC software from Polymer Laboratories, Inc. The purified zwitterionic monomers and polymers were freeze-dried using a LABCONCO 76705 Series Freeze Dryer.

The intrinsic viscosity ($[\eta]$) values of zwitterionic polymers P16-2, P16-3, P16-4, P55, PCO-1, and PCO-2 in 2,2,2-trifluoroethanol (TFE) at 25 °C were measured using a Ubbelohde viscometer (Cannon-Ubbelohde Dilution Viscometer 150 D429) according to a method proposed by Wolf, et al. for polyelectrolytes.^{21,22} The initial slope of the plot of $\ln\eta_{\text{rel}}$ versus polymer concentration was determined by linear fitting, yielding the $[\eta]$ value as described by Wolf, et al. It should be noted here that the traditional method for determining $[\eta]$ by linear extrapolation of η_{sp}/c toward $c \rightarrow 0$ fails for many polyelectrolytes in solution.^{21,22} The cloud points of thermoresponsive zwitterionic polymers in Milli-Q water at a concentration of 3 wt% or 1 wt% were measured by visual inspection using an Isotemp water bath (Fisher Scientific, model 3006). The temperature of the water bath increased in a stepwise fashion and at each selected temperature, the polymer solution was equilibrated for 1 min. The temperature at which the solution turned cloudy was recorded as the cloud point.

3.2.3. Synthesis of Zwitterionic Polymers P14, P15, P16-1, P16-2, and P16-3

Zwitterionic homopolymers P14, P15, P16-1, P16-2, and P16-3 were synthesized from the corresponding monomers by reversible addition fragmentation chain transfer (RAFT) polymerization using *n*-butyl (2-cyano-2-propyl) trithiocarbonate as the chain transfer agent and AIBN as initiator, as described in a publication from our group.²⁰ For P14: $M_{n,\text{SEC}} = 3.4$ kDa, $\bar{D} = 3.80$ (relative to PEO standards). P14 was soluble in water and no cloud point was observed. For P15: $M_{n,\text{SEC}} = 2.3$ kDa, $\bar{D} = 4.69$ (relative to PEO standards). The cloud point of P15 in water at a

concentration of 3 wt% was about 51 °C. For P16-1: $M_{n,SEC} = 7.5$ kDa, $\bar{D} = 3.51$ (relative to PEO standards). The cloud point of P16-1 in water at a concentration of 3 wt% was about 18 °C. The $[\eta]$ of P6-2 in 2,2,2-trifluoroethanol at 25 °C was 0.116 dL/g. The cloud points of P16-2 in water at concentration of 3 wt% and 1 wt% were about 14 and 18 °C, respectively. The $[\eta]$ of P16-3 in 2,2,2-trifluoroethanol at 25 °C was 0.166 dL/g. The cloud points of P16-3 in water at concentration of 3 wt% and 1 wt% were about 15 and 21 °C, respectively. Note that P16-3 exhibited a rather broad LCST transition in water upon heating, likely because of a broad molecular weight distribution.

3.2.4. Synthesis of Zwitterionic Homopolymers P55 and P16-4 by Conventional Free Radical Polymerization Using AIBN as Initiator

3-((2-(Methacryloyloxy)ethyl)dipentylammonio)propane-1-sulfonate (M55, 0.603 g, 2.03 mmol), AIBN (7.1 mg, 0.043 mmol), 2,2,2-trifluoroethanol (2.039 g), and *N,N*-dimethylformamide (90.5 mg) were added to a 25 mL two-necked flask equipped with a magnetic stir bar. The mixture was degassed by freeze-pump-thaw and placed in a preheated oil bath with a temperature of 70 °C. After 17 h, the polymerization was stopped, and the polymer was purified by dialysis against MilliQ water using regenerated cellulose dialysis tubing (MWCO: 3500 Da). The purified polymer was concentrated and freeze dried to give a white powder (yield: 0.508 g, 84.2%). $M_{n,SEC} = 64.9$ kDa and $\bar{D} = 2.36$ (relative to polystyrene standards using DMF with 50 mM LiBr as the mobile phase). The $[\eta]$ of P55 in 2,2,2-trifluoroethanol at 25 °C was determined to be 0.490 dL/g. The cloud point of P55 in MilliQ water at 1 wt% was 37 °C. P16-4 was synthesized in a similar manner, and the $[\eta]$ in 2,2,2-trifluoroethanol at 25 °C was determined to be 0.442 dL/g. The cloud points of P6-4 in water at concentration of 3 wt% and 1 wt% were about 10 and 11 °C, respectively.

3.2.5. Synthesis of Zwitterionic Copolymers PCO-1 and -2 by Conventional Free Radical Copolymerization of M16 and *N*-Isopropylmethacrylamide Using AIBN as Initiator

PCO-1 and -2 (Scheme 3.1) were prepared using similar procedures, and the following shows the synthesis of PCO-1. M16 (0.611 g, 1.73 mmol), *N*-isopropylmethacrylamide (0.219 g, 1.75 mmol), AIBN (11.6 mg, 7.06×10^{-2} mol), and 2,2,2-trifluoroethanol (3.0 g) were weighed into a 25 mL two-necked flask with a magnetic stirrer and were degassed by three freeze-pump-thaw cycles. The flask was then placed into a 70 °C oil bath to start the polymerization. After 26 h, the flask was removed from the oil bath, and the mixture was transferred into a regenerated cellulose dialysis tubing with a molecular weight cut-off of 3500 Da and dialyzed against Milli-Q water. The aqueous solution of the copolymer, PCO-1, was then concentrated and freeze-dried using a LABCONCO 76705 Series Freeze Dryer.

3.3. Results and Discussion

3.3.1. Synthesis of Zwitterionic PSBMA Homopolymers with Various *N*-*n*-Alkyl Substituents

A total of seven PSBMA homopolymers were synthesized from M14, M15, M16, and M55 by reversible addition fragmentation chain transfer (RAFT) polymerization using the CTA shown in Scheme 3.1 or conventional radical polymerization, as summarized in Table 3.1. P14, P15, and P16-1 were made by RAFT polymerization, and their molecular weights and dispersities were measured using an aqueous size exclusion chromatography (SEC) system with PEO standards for calibration. The ^1H NMR spectra and SEC curves for these three polymers are shown in Figures 3.1 – 3.3. The rather high dispersities indicated that the polymerizations were not controlled, a problem encountered for zwitterionic monomers that have not been solved. A possible reason is that there might be some impurities in the monomers that interfered the RAFT polymerization,

Table 3.1. Summary of Characterization Data for Zwitterionic Polymers

Polymer	Monomer(s)	Synthesis Method ^b	Polymer's Molecular Characteristics	CP in H ₂ O (3 wt%) ^e	CP in H ₂ O (1 wt%) ^e
P14	M14	RAFT	$M_{n,SEC} = 3.4 \text{ kDa}$; $\bar{D} = 3.80$ ^c	Soluble	Soluble
P15	M15	RAFT	$M_{n,SEC} = 2.3 \text{ kDa}$; $\bar{D} = 4.69$ ^c	51 °C	NA
P16-1	M16	RAFT	$M_{n,SEC} = 7.5 \text{ kDa}$; $\bar{D} = 3.51$ ^c	18 °C	NA
P16-2	M16	RAFT	$[\eta] = 0.116 \text{ dL/g}$ ^d	14 °C	18 °C
P16-3	M16	RAFT	$[\eta] = 0.166 \text{ dL/g}$ ^d	15 °C ^f	21 °C ^f
P16-4	M16	AIBN	$[\eta] = 0.442 \text{ dL/g}$ ^d	10 °C	11 °C
P55	M55	AIBN	$M_{n,SEC} = 64.9 \text{ kDa}$; $\bar{D} = 2.36$ ^e $[\eta] = 0.490 \text{ dL/g}$ ^d	NA	37 °C
PCO-1	M16 + NIPMAM ^a	AIBN	$[\eta] = 0.350 \text{ dL/g}$ ^d	NA	27 °C
PCO-2	M16 + NIPMAM ^a	AIBN	$[\eta] = 0.286 \text{ dL/g}$ ^d	NA	30 °C

^a NIPMAM: *N*-isopropyl methacrylamide. ^b RAFT: reversible addition-fragmentation chain transfer polymerization; AIBN: α,α' -azobisisobutyronitrile (AIBN)-initiated conventional free radical polymerization. ^c $M_{n,SEC}$ and $\bar{D}$ were determined by an aqueous SEC system relative to PEO standards. ^d Intrinsic viscosity in 2,2,2-trifluoroethanol at 25 °C determined using the Wolf method.^{21,22} ^e $M_{n,SEC}$ and $\bar{D}$ were determined by a DMF SEC system relative to polystyrene standards. ^e CP: cloud point in Milli-Q water determined by visual inspection. ^f P6-3 exhibited a broad LCST transition in water upon heating.

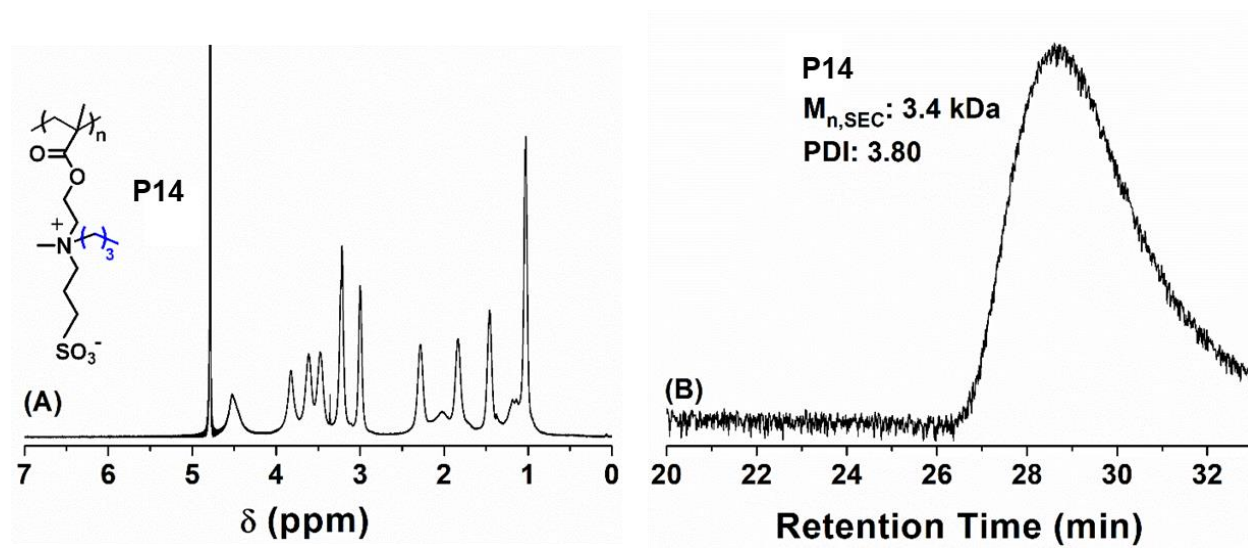


Figure 3.1. (A) ^1H NMR spectrum of P14 in D_2O and (B) SEC curve of P14 from an aqueous SEC system using a 0.2 M aqueous solution of NaNO_3 as eluent.

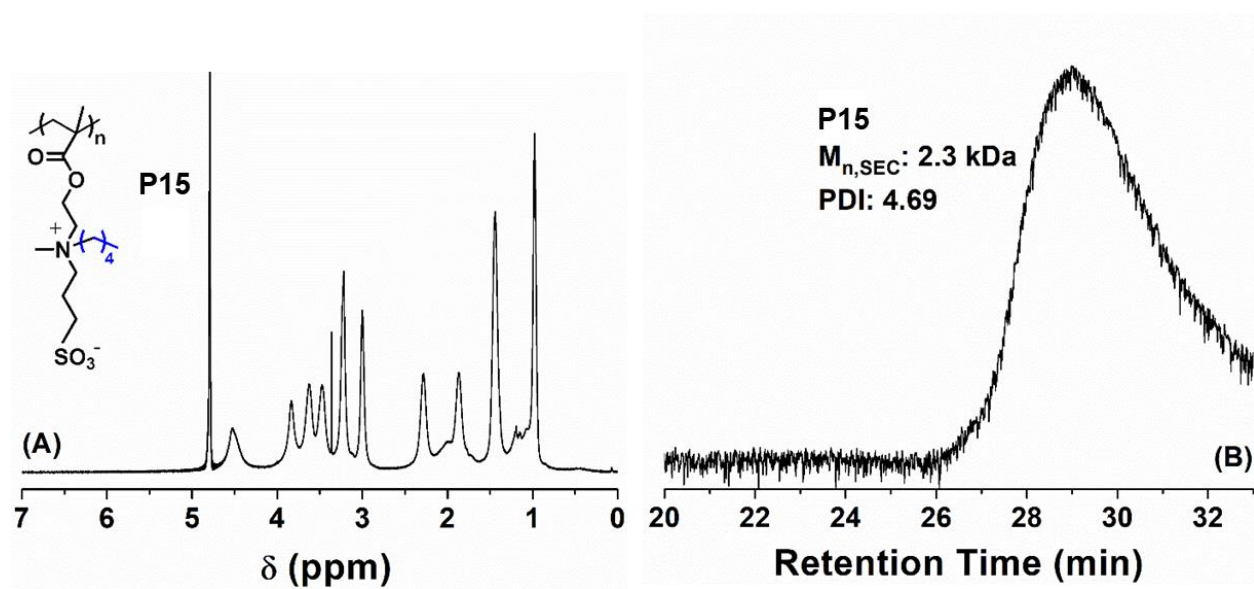


Figure 3.2. (A) ^1H NMR spectrum of P15 in D_2O and (B) SEC curve of P15 from an aqueous SEC system using a 0.2 M aqueous solution of NaNO_3 as eluent.

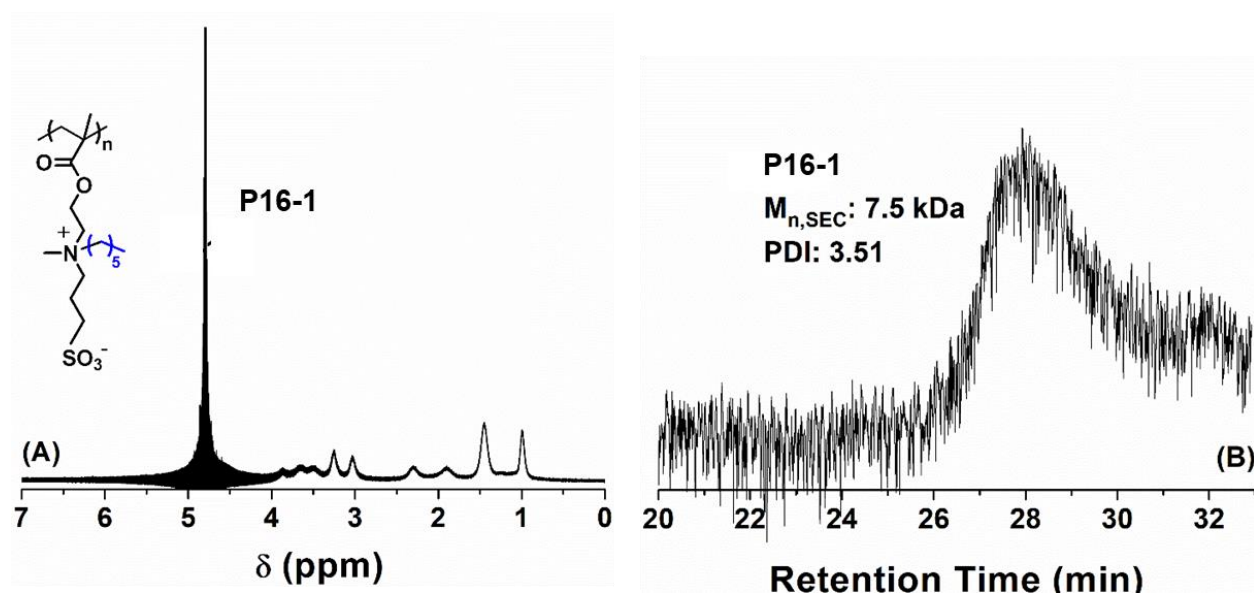


Figure 3.3. (A) ^1H NMR spectrum of P16-1 in D_2O and (B) SEC curve of P16-1 from an aqueous SEC system using a 0.2 M aqueous solution of NaNO_3 as eluent.

even if these impurities were not visible in ^1H NMR spectra. Washing was the only means for purifying zwitterionic monomers, and likely washing alone could not remove all the impurities. P14 was soluble in water, while 3 wt% P15 and P16-1 exhibited a cloud point (CP) at 51 and 18 °C, respectively. These CPs were very close to the values observed before,²⁰ 47 °C for 3.0 wt% P15 and 21 °C for P16.

For P16-2, P16-3, P16-4, and P55, we measured their intrinsic viscosity $[\eta]$ values in 2,2,2-trifluoroethanol at 25 °C. Instead of plotting of η_{sp}/c (or $\ln\eta_{\text{rel}}/c$) versus concentration c , we used the method reported by Wolf to make a plot of $\ln\eta_{\text{rel}}$ versus polymer concentration c .^{21,22} The initial slope of the plot is determined by linear fitting, yielding the $[\eta]$. The plots are shown in Figure 3.4, and the $[\eta]$ values are summarized in Table 3.1. The ^1H NMR spectra of P16-2, P16-3 and P16-4 are shown in Figure 3.5, which agreed with their molecular structures. Interestingly, we found that P55 is soluble in DMF, allowing us to measure its molecular weight and dispersity by a DMF GPC system. Figure 3.6 shows the ^1H NMR spectrum in D_2O and the SEC trace of P55; the $M_{n,\text{SEC}}$ was 64.9 kDa and the dispersity was 2.36, relative to polystyrene standards. The cloud points of P16-2 and P16-3 were 14 and 15 °C, respectively, slightly lower than that of P16-1, whereas the cloud point of P16-4 was significantly lower, 10 °C, presumably because of its much higher molecular weight as can be seen from its much higher $[\eta]$ value (0.442 dL/g) compared with that of P16-3 (0.166 dL/g). P55 is also an LCST-type thermosensitive polymer as described in Chapter 2. Here, the cloud point was found to be 37 °C at an $[\eta]$ value of 0.490 dL/g, slightly lower than that of P55 with an $[\eta]$ of 0.288 dL/g in Chapter 2. This is reasonable because for LCST-type polymers the cloud point decreases with increasing molecular weight or intrinsic viscosity.²⁰

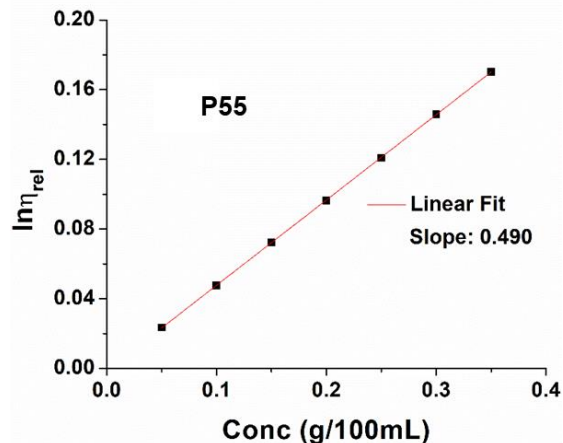
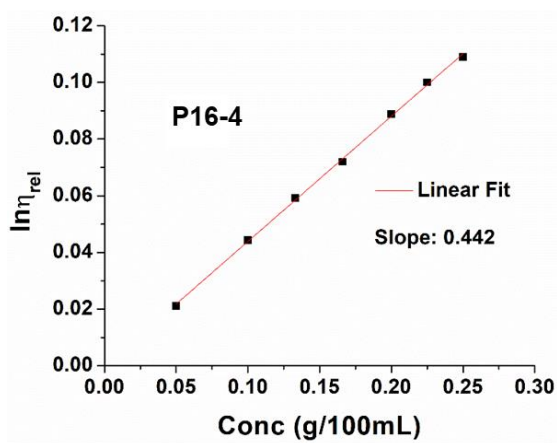
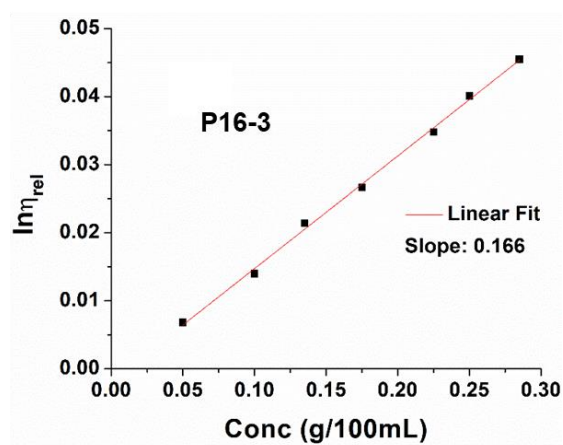
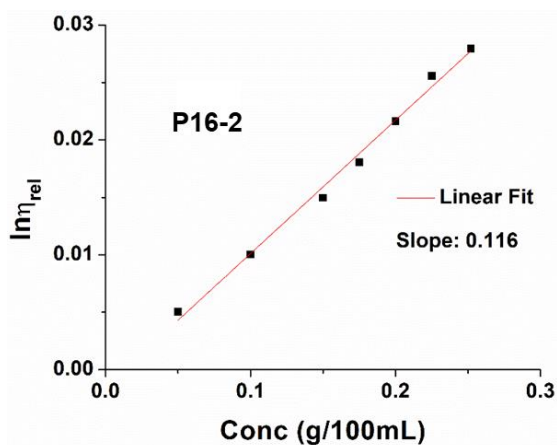


Figure 3.4. Plots of $\ln \eta_{rel}$ versus polymer concentration for P16-2, P16-3, P16-4, and P55. From the slope determined from linear fit for each plot, the $[\eta]$ value was 0.116 dL/g for P16-2, 0.166 dL/g for P16-3, 0.442 dL/g for P16-4, and 0.490 dL/g for P55.

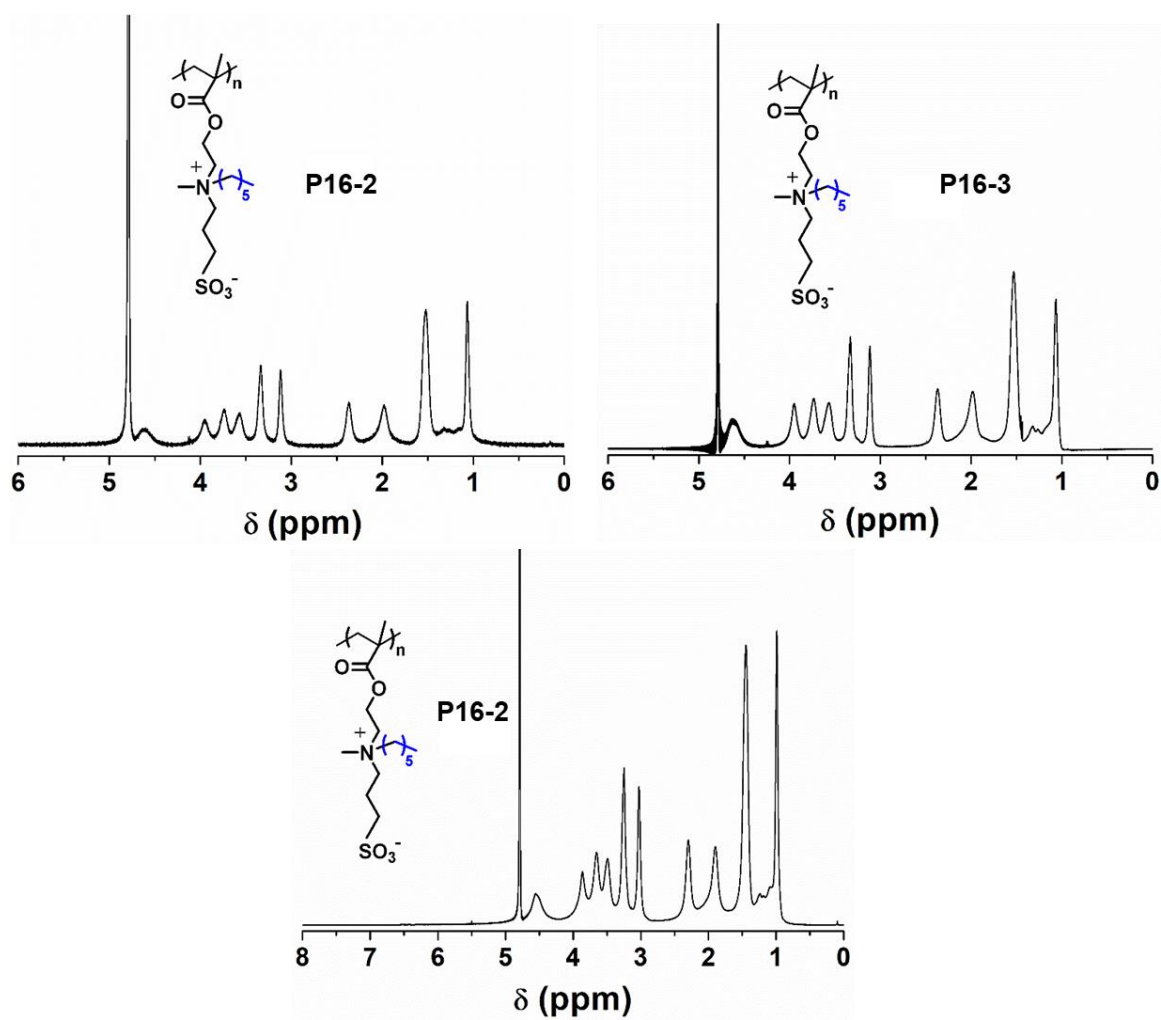


Figure 3.5. ^1H NMR spectra of P16-2, P16-3, and P16-4 in D_2O with 0.5 M NaCl.

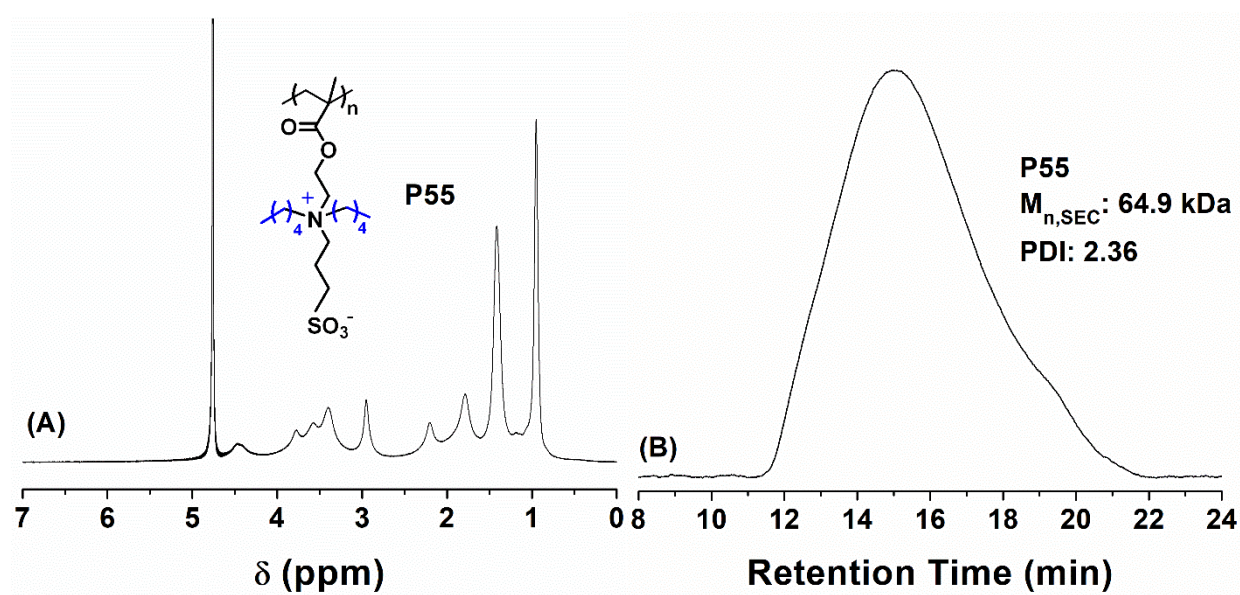


Figure 3.6. (A) ^1H NMR spectrum of P55 in D_2O and (B) SEC curve of P55 from a DMF SEC system using DMF with 50 mM LiBr as eluent.

3.3.2. Synthesis of Random Copolymers of M16 and N-Isopropyl Methacrylate

Poly(*N*-isopropyl methacrylate) is an efficient KHI.¹⁴ We further synthesized two random copolymers of M16 and *N*-isopropyl methacrylate, PCO-1 and PCO-2, by AIBN-initiated conventional free radical polymerization using similar procedure. Figure 3.7 shows the ¹H NMR spectra of the two copolymers. The molar content of M16 monomer units in copolymer PCO-1 was determined to be 58% from ¹H NMR spectroscopy analysis using the integrals of the peak from 7.00 to 7.74 ppm (-CONH(CH₃)₂ of *N*-isopropylmethacrylamide monomer units) and the peak from 4.45 – 4.72 (-COOCH₂CH₂ of M16 monomer units). Similarly, the molar content of M16 monomer units in PCO-2 was found to be 56% from ¹H NMR analysis. The [η] values of PCO-1 and PCO-2 in 2,2,2-trifluoroethanol at 25 °C were 0.350 dL/g and 0.286 dL/g, respectively (Figure 3.8). The cloud point of PCO-1 in water at a concentration of 1 wt% was 27 °C, while PCO-2 exhibited a cloud point of 30 °C in water when the concentration was 1 wt%.

3.3.3. KHI Performance Testing of Zwitterionic Polymers

The slow constant cooling (SCC) method was employed to test the KHI performances of nine zwitterionic polymers shown in Table 3.1. These tests were conducted in high-pressure rocker rig RC5 (PSL Systemtechnik, Germany) using a synthetic natural gas (SNG) mixture, and the details can be found in the literature.^{16,23} From the graphs of pressure-time and temperature-time acquired from SCC tests, one can determine the gas hydrate onset temperature (*T*_o), at which the pressure deviated from the decreasing line because some gas molecules form gas hydrates. The rapid hydrate formation temperature (*T*_a) is the temperature at which the fastest decrease in the pressure is observed. The fast drop of the pressure is caused by the rapid growth of gas hydrates involving a large amount of gas. From the SCC tests, one obtains *T*_o and *T*_a values. At the same starting temperature and pressure of the SCC tests, lower *T*_o and *T*_a values indicate better KHI

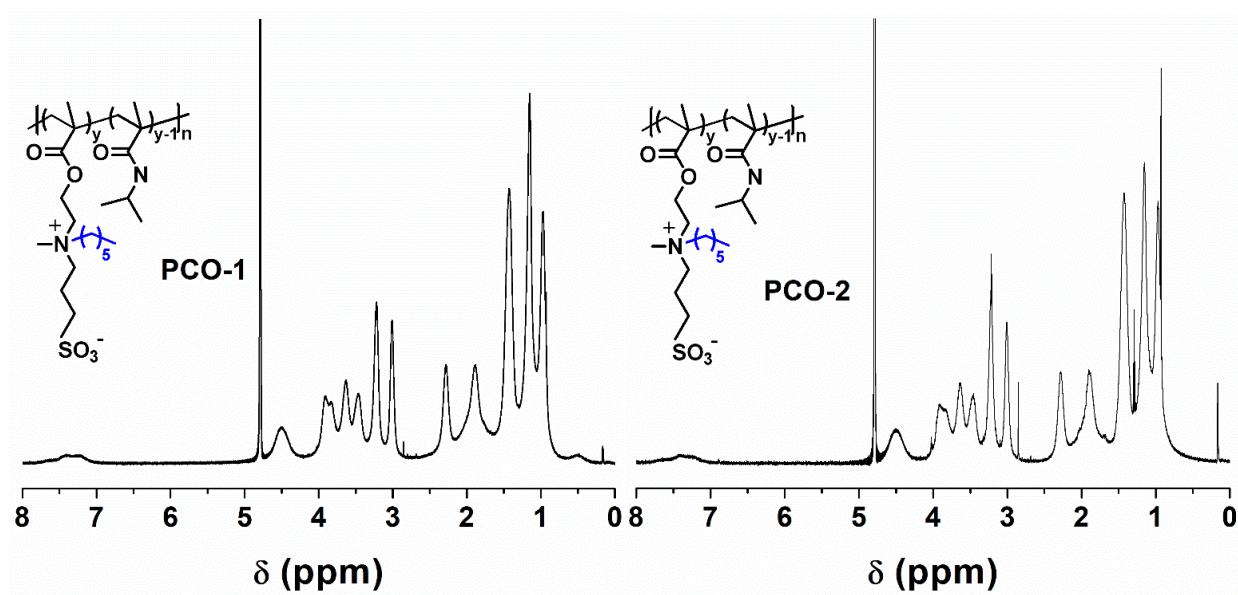


Figure 3.7. ^1H NMR spectra of PCO-1 and PCO-2 in D_2O .

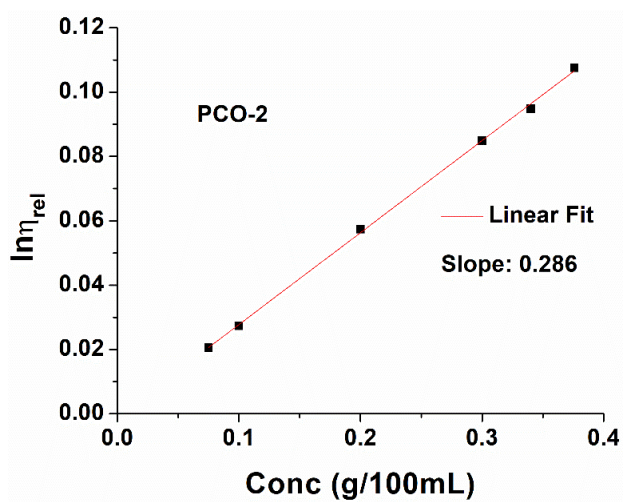
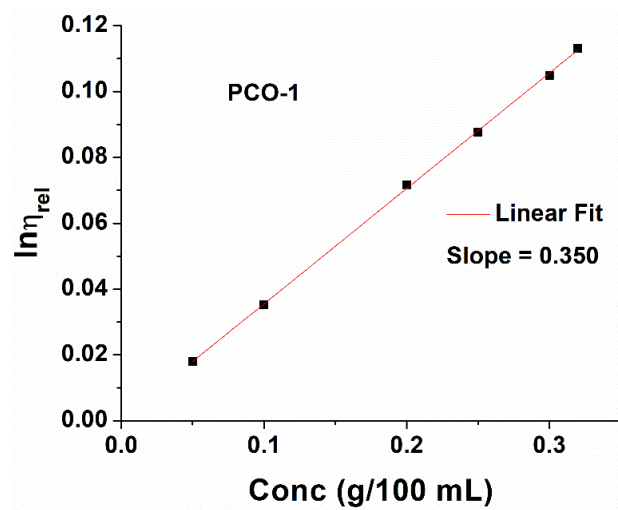


Figure 3.8. Plots of $\ln \eta_{rel}$ versus polymer concentration for PCO-1 and PCO-2. The $[\eta]$ was 0.350 dL/g for PCO-1 and 0.286 dL/g for PCO-2.

performances. Figure 3.9 shows the results for the seven zwitterionic homopolymers and two copolymers obtained from SCC tests at a polymer concentration of 0.25 wt%. For comparison, DI water (DIW) and poly(*N*-vinylcaprolactam) (PVCap) were also tested under the same conditions. All of the zwitterionic polymers showed significant decreases in T_o and T_a compared with DIW, and among all zwitterionic homopolymers, P16-3 performed slightly better than the commercially used PVCap, demonstrating the potential of zwitterionic PSBMA as KHIs. PCO-1 was the best KHI among all of the polymers in this study. Interestingly, T_o and T_a decreased from P14 to P15 and P16, indicating that increasing hydrophobicity can improve the KHI performance. Note that P17 with one *N*-methyl and *N*-*n*-heptyl substituent is insoluble in water²⁰ and thus cannot be tested. Another interesting finding is that P55, with two *n*-pentyl groups at the nitrogen atom, performed essentially the same as P15 with one *N*-*n*-pentyl substituent, suggesting that the effects of two long alkyls, however, are not additive. The longer *N*-*n*-alkyl side chain in the PSBMA give a better KHI performance, likely because it exerts a nucleation inhibition effect on the formation of gas hydrates. A longer linear alkyl means a larger hydrophobic effect, which competes more effectively with gas molecules for hydration and consequently inhibits the formation of gas hydrates.

3.4. Conclusions

A series of zwitterionic PSBMA homopolymers with *N*-*n*-alkyl substituents of 4-6 carbon atoms and copolymers were synthesized by either RAFT polymerization or AIBN-initiated conventional free radical polymerization for testing as KHIs.²³ The polymers were thoroughly characterized by ¹H NMR spectroscopy, SEC, viscometry, and cloud point measurement. KHI tests showed that the best homopolymer performer was P16-3, which performed slightly better

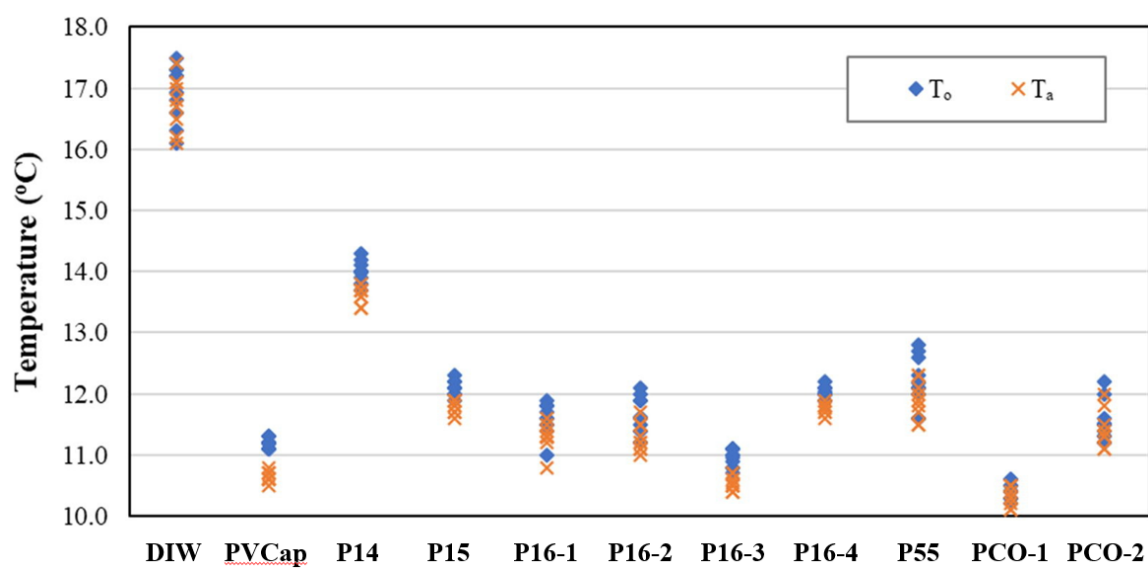


Figure 3.9. T_o and T_a values obtained from slow constant cooling tests for KHI performance at a polymer concentration of 0.25 wt%.

than the commercially used PVCap. This is likely because of its water solubility and the large hydrophobic effect associated with the *N*-*n*-hexyl substituent.

References:

1. Shao, Q.; Jiang S. Y. Molecular Understanding and Design of Zwitterionic Materials. *Adv. Mater.* **2015**, 27, 15-26.
2. Seuring, J.; Agarwal, S. Polymers with Upper Critical Solution Temperature in Aqueous Solution. *Macromol. Rapid Commun.* **2012**, 33, 1898–1920.
3. Fu, W. X.; Luo, C. H.; Morin, E. A.; He, W.; Li, Z. B.; Zhao, B. UCST-Type Thermosensitive Hairy Nanogels Synthesized by RAFT Polymerization-Induced Self-Assembly. *ACS Macro Lett.* **2017**, 6, 127-133.
4. Monroy Soto, V. M.; Galin, J. C. Poly(sulphopropylbetaines): 2. Dilute Solution Properties. *Polymer* **1984**, 25, 254-262.
5. Schulz, D. N.; Peiffer, D. G.; Agarwal, P. K.; Larabee, J.; Kaladas, J. J.; Soni, L.; Handwerker, B.; Garner, R. T. Phase Behaviour and Solution Properties of Sulphobetaine Polymers. *Polymer* **1986**, 27, 1734-1742.
6. Jiang, S. Y.; Cao, Z. Q. Ultralow-Fouling, Functionalizable, and Hydrolyzable Zwitterionic Materials and Their Derivatives for Biological Applications. *Adv. Mater.* **2010**, 22, 920-932.
7. Mi, L.; Jiang, S. Y. Integrated Antimicrobial and Nonfouling Zwitterionic Polymers. *Angew. Chem. Int. Ed.* **2014**, 53, 1746-1754.
8. Liang, B.; Zhang, G. Y.; Zhong, Z. X.; Huang, Y.; Su, Z. H. Superhydrophilic Anti-Icing Coatings Based on Polyzwitterion Brushes. *Langmuir* **2019**, 35, 1294–1301.
9. Ma, M.-Q.; Zhang, C.; Chen, T.-T.; Yang, J.; Wang, J.-J.; Ji, J.; Xu, Z.-K. Bioinspired Polydopamine/Polyzwitterion Coatings for Underwater Anti-Oil and -Freezing Surfaces. *Langmuir* **2019**, 35, 1895–1901.

10. Chen, M.; Briscoe, W. H.; Armes, S. P.; Klein, J. Lubrication at Physiological Pressures by Polyzwitterionic Brushes. *Science* **2009**, 323, 1698-1701.
11. Liang, B.; Zhang, G. Y.; Zhong, Z. X.; Sato, T.; Hozumi, A.; Su, Z. H. Substrate-Independent Polyzwitterionic Coating for Oil/Water Separation Membranes. *Chem. Eng. J.* **2019**, 362, 126-135.
12. Rajan, R.; Hayashi, F.; Nagashima, T.; Matsumura, K. Toward a Molecular Understanding of the Mechanism of Cryopreservation by Polyampholytes: Cell Membrane Interactions and Hydrophobicity. *Biomacromolecules* **2016**, 17, 1882-1893.
13. Sloan, E.D.; Koh, C.A., 2008. Clathrate Hydrates of Natural Gases. CRC Press.
14. Ke, W.; Kelland, M. A. Kinetic Hydrate Inhibitor Studies for Gas Hydrate Systems: A Review of Experimental Equipment and Test Methods. *Energy Fuels* **2016**, 30, 10015–10028.
15. Ajiro, H.; Takemoto, Y.; Akashi, M.; Chua, P. C.; Kelland, M. A. Study of the Kinetic Hydrate Inhibitor Performance of a Series of Poly(*N*-alkyl-*N*-vinylacetamide)s. *Energy Fuels* **2010**, 24, 6400–6410.
16. Abrahamsen, E.; Kelland, M. A. Carbamate Polymers as Kinetic Hydrate Inhibitors. *Energy Fuels* **2016**, 30, 8134–8140.
17. Moad, G.; Chong, Y. K.; Postma, A.; Rizzardo, E.; Thang, S. H. Advances in RAFT Polymerization: the Synthesis of Polymers with Defined End-Groups. *Polymer* **2005**, 46, 8458-8468.
18. Seymour, B. T.; Wright, R. A. E.; Parrott, A. C.; Gao, H. Y.; Martini, A.; Qu, J.; Dai, S.; Zhao, B. Poly(alkyl methacrylate) Brush-Grafted Silica Nanoparticles as Oil Lubricant Additives: Effects of Alkyl Pendant Groups on Oil Dispersibility, Stability, and Lubrication Property. *ACS Appl. Mater. Interfaces* **2017**, 9, 25038–25048.

19. Fu, W. X.; Bai, W.; Jiang, S. S.; Seymour, B. T.; Zhao, B. UCST-Type Thermoresponsive Polymers in Synthetic Lubricating Oil Polyalphaolefin (PAO). *Macromolecules* **2018**, *51*, 1674–1680.
20. Wang, N.; Seymour, B. T.; Lewoczko, E. M.; Kent, E. W.; Chen, M.-L.; Wang, J.-H.; Zhao, B. Zwitterionic Poly(sulfobetaine methacrylate)s in Water: From Upper Critical Solution Temperature (UCST) to Lower Critical Solution Temperature (LCST) with Increasing Length of One Alkyl Substituent on the Nitrogen Atom. *Polym. Chem.* **2018**, *9*, 5257-5261.
21. Wolf, B. A. Polyelectrolytes Revisited: Reliable Determination of Intrinsic Viscosities. *Macromol. Rapid Commun.* **2007**, *28*, 164-170.
22. Eckelt, J.; Knopf, A.; Wolf, B. A. Polyelectrolytes: Intrinsic Viscosities in the Absence and in the Presence of Salt. *Macromolecules* **2008**, *41*, 912-918.
23. The work presented in this Chapter has been published in *Chemical Engineering Science*. I synthesized and characterized P14, P15, P16-1, and P55 and Caleb A. Bohannon synthesized and characterized P6-2, P6-3, P6-4, PCO-1 and PCO-2 except for viscometry data (done by me). The KHI tests were done by our collaborators at the University of Stavanger in Norway. Zhang, Q.; Kelland, M. A.; Lewoczko, E. M.; Bohannon, C. A.; Zhao, B. Non-amide Based Zwitterionic Poly(sulfobetaine methacrylate)s as Kinetic Hydrate Inhibitors. *Chemical Engineering Science* **2021**, *229*, 116031. DOI: 10.1016/j.ces.2020.116031 (<https://www.sciencedirect.com/science/article/pii/S0009250920305637>)

**Chapter 4: Effects of Temperature on Chaotropic Anion-Induced Shape
Transitions of Star Molecular Bottlebrushes with Heterografted Poly(ethylene
oxide) and Poly(*N,N*-dialkylaminoethyl methacrylate) Side Chains in Acidic
Aqueous Solutions**

The work presented in this chapter has been submitted for publication. Ethan W. Kent synthesized the azide-functionalized star backbone polymer. Michael T. Kelly obtained the AFM images of SMB-22 in the presence of NaPF₆. I synthesized and characterized the remainder of the polymers and systems. Prof. Bin Zhao advised this work.

Abstract.

This Chapter presents a study of the effects of temperature on chaotropic anion (CA)-induced star-globule shape transitions of two three-arm star bottlebrush polymers with heterografted poly(ethylene oxide) (PEO) and either poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA) or poly(*N,N*-diethylaminoethyl methacrylate) (PDEAEMA) (the brushes denoted as SMB-11 and -22, respectively) in acidic water. The brush polymers were synthesized by grafting alkyne-end-functionalized PEO and PDMAEMA or PDEAEMA onto an azide-bearing three-arm star backbone polymer using the highly efficient copper(I)-catalyzed alkyne-azide cycloaddition reaction. PEO side chains acted as a stabilizer for the brushes when the protonated tertiary amine-containing polymer side chains became insoluble upon addition of chaotropic anions (CAs) in acidic water. Six anions were studied for their effects on the conformations of SMB-11 and -22: super chaotropic [Fe(CN)₆]⁴⁻ and [Fe(CN)₆]³⁻, moderate chaotropic PF₆⁻ and ClO₄⁻, weak chaotropic I⁻, and, for comparison, kosmotropic anion SO₄²⁻. At 25 °C, the addition of super and moderate CAs induced shape changes of SMB-11 and -22 from a starlike to a collapsed yet stable globular state in pH 4.50 acidic water, driven by the ion pairing of protonated tertiary amine groups with chaotropic CAs and the chaotropic effect. The shape transitions occurred at much lower salt concentrations, by at least one order in magnitude, for super CAs than moderate CAs. Upon heating from (near) room temperature to 70 °C, the super CA-collapsed SMB-11 and -22 brush molecules

remained in the globular state, whereas the moderate CA-collapsed brushes underwent reversible globule-to-star shape transitions. The transition temperature increased with increasing salt concentration and was found to be higher for SMB-22 at the same salt concentration, presumably caused by the chaotropic effect. In contrast, weak CA Γ^- and kosmotropic anion SO_4^{2-} had small effects on the hydrodynamic sizes of SMB-11 and -22 at 25 °C, and only small size variations were observed upon heating to 70 °C. AFM showed that SMB-11 in the presence of Na_2SO_4 remained in the star shape at both 20 and 70 °C. The results described here may have potential uses in the design of stimuli-responsive polymeric systems from molecular bottlebrushes for encapsulation and release of substances.

4.1. Introduction

Molecular bottlebrushes (MBBs), also called brush polymers or cylindrical brushes, are composed of one or more types of relatively short polymeric side chains covalently grafted on a backbone polymer with a sufficiently high grafting density.¹⁻⁴ These architecturally complex polymers have received tremendous interest in recent years owing to their unique structural characteristics,¹⁻⁶ intriguing behavior, and potential applications in a wide variety of areas including drug delivery,⁷ aqueous lubrication,⁸ and fabrication of photonic crystals and supersonic elastomers.^{6,9} The unique characteristics and behavior of MBBs, such as high density of functional groups, large and tunable persistence length,^{2,3} lack of main chain entanglement,⁶ and unusual crystal habit,¹⁰ originate mainly from the high grafting density of macromolecular side chains on the backbone. At a sufficiently high aspect ratio, linear MBBs take on an extended wormlike morphology in good solvents due to the excluded volume interactions,¹⁻⁴ which can be easily visualized by atomic force microscopy (AFM) and transmission electron microscopy. When the environmental conditions change, MBBs can exhibit marked shape transitions,^{2,3} e.g., from wormlike or starlike to globular.

Although the intriguing worm-globule shape transitions of MBBs have been shown at air-water interfaces by lateral compression and on solid substrates by exposure to different solvent vapors,¹¹⁻¹³ stimuli-responsive shape-changing MBBs in solution are more relevant to potential applications such as encapsulation and release of substances.^{3,14} These shape-changing brush polymers are commonly synthesized with the use of stimuli-responsive polymers, which exhibit large and abrupt conformational transitions, often accompanied with noticeable solubility changes in solvents, in response to external stimuli, such as pH, temperature, and solvent variations, specific ions, and light.¹⁴⁻²⁷ Schmidt and coworkers synthesized the first thermoresponsive linear MBBs using a

“grafting from” method via atom transfer radical polymerization (ATRP) to grow poly(*N*-isopropylacrylamide) (PNIPAm) side chains from a long macroinitiator with pendant ATRP initiating moieties.¹⁴ The PNIPAM brush polymer was observed by AFM to exhibit a pronounced worm-to-globule shape transition in water upon heating. However, above the lower critical solution temperature (LCST), they found that the collapsed globular brushes were unstable and underwent aggregation and precipitation in the solution. Matyjaszewski et al. synthesized loosely grafted poly(acrylic acid) MBBs and found that the brushes spin cast on mica from aqueous solutions of different pH values exhibited a gradual transition from a compact globular to a more extended wormlike conformation upon increasing the pH from ~ 4 to ~ 9 .¹⁸ Polyelectrolyte MBBs have been shown to undergo conformational transitions in aqueous solutions in response to the addition of a variety of species, including ionic surfactants, oppositely charged linear polyelectrolytes, and multivalent ions.²²⁻²⁷ For example, Müller et al. reported that a cationic brush polymer underwent worm-to-globule-worm shape transitions in dilute aqueous solutions with the sequential addition of sodium dodecyl sulfate (SDS) and β -cyclodextrin (β -CD) due to the complexation of the brushes with SDS and the formation of an inclusion complex between β -CD and SDS.²³ However, even at a rather low concentration, the charged brushes precipitated out immediately from the solution after the addition of SDS. In addition, interesting helical structures of individual polyelectrolyte bottlebrushes were observed in highly dilute aqueous solutions by Müller and Schmidt after the addition of multivalent ions and surfactants, respectively.²⁵⁻²⁷

In the above examples of stimuli-responsive shape-changing MBBs, the collapsed brushes are unstable in the solutions, which have prevented their use in potential applications. To solve this problem, our research group designed and synthesized shape-changing MBBs with bicomponent side chains,²⁸⁻³³ either binary heterografted MBBs composed of two types of side chains or

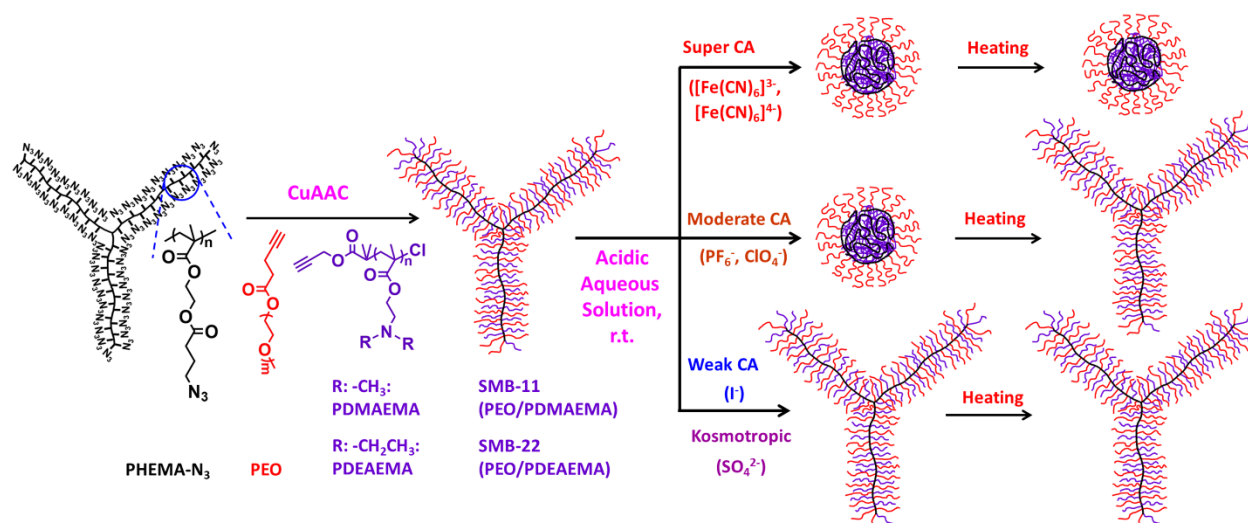
homografted bottlebrushes with diblock copolymer side chains. One of the side chain polymers in heterografted brushes and the outer block in homografted MBBs serve as a stabilizer for the collapsed MBBs when the other polymer in the side chains become insoluble in solution upon application of external stimuli. By using two different stimuli-responsive polymers as side chains in binary heterografted MBBs, two distinct collapsed yet stable globular states were attained from the same brush polymers under different conditions.³² Very recently, our group reported chaotropic anion (CA)-induced star-globule shape changes of three-arm star MBBs (SMBs) with poly(ethylene oxide) (PEO) and either poly(2-(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA) or poly(2-(*N,N*-diethylaminoethyl methacrylate) (PDEAEMA) as side chains in acidic aqueous solutions at ambient conditions.³³ CAs, such as ClO_4^- and SCN^- , typically have a large size and a relatively low charge density.³⁴⁻³⁸ These anions are water-structure breakers, that is, they cause the water molecules surrounding them to be less hydrogen bonded and have a higher entropy than water molecules in the bulk. CAs tend to form tight ion pairs with chaotropic cations in water.^{36,37} They also display a high tendency to associate with hydrophobic surfaces and groups in aqueous solution, which is termed chaotropic effect.³⁷ Both chaotropic ion pairing and chaotropic effect stem from the release of less hydrogen bonded water molecules from the hydration shells of CAs into bulk water to form stronger water-water interactions. Thus, they are mainly caused by an enthalpic effect, in contrast to the hydrophobic effect, which is an entropic effect. It was found that the addition of super CAs (e.g., $[\text{Fe}(\text{CN})_6]^{3-}$ and $\text{S}_2\text{O}_8^{2-}$) and moderate CAs such as ClO_4^- induced star-to-globule shape transitions of SMBs in acidic aqueous solutions, while weak CAs such as Br^- and Cl^- only caused small decreases in hydrodynamic sizes of SMBs.³³

Since chaotropic ion pairing and chaotropic effect are mainly an enthalpic effect, it is possible to break apart the association by heating. Plamper et al. and Zhang et al. reported that adding small

amounts of large multivalent anions such as $[\text{Co}(\text{CN})_6]^{3-}$, $[\text{Fe}(\text{CN})_6]^{4-}$, $[\text{Fe}(\text{CN})_6]^{3-}$, and $[\text{Cr}(\text{CN})_6]^{3-}$ into acidic or near-neutral aqueous solutions of PDMAEMA induced upper critical solution temperature (UCST) behavior in the presence of 0.1 M NaCl.^{39,40} Using in situ ATR-FTIR spectroscopy, Fleming et al. observed the wavenumber shifts of the $\text{C}\equiv\text{N}$ stretching vibration band of $[\text{Fe}(\text{CN})_6]^{3-}$ when paired with protonated PDMAEMA brushes grafted on silicon wafers and the intensity changes upon heating,⁴¹ confirming the ion pairing mechanism for the UCST behavior. Moreover, quaternized PDMAEMA polymers have been reported to exhibit UCST behavior in the presence of specific ions, such as BF_4^- or bis(trifluoromethane)sulfonamide and CF_3SO_3^- .^{42,43}

On the basis of these reports, we hypothesized that heating might be able to unfold the CA-collapsed globular SMBs with tertiary amine-containing side chains in acidic aqueous solution to an extended star shape. In the present work, we examined the effects of temperature on CA-collapsed SMB-11, with PEO and PDMAEMA side chains, and SMB-22, with PEO and PDEAEMA side chains, in acidic water (Scheme 4.1). Both brush polymers were made by grafting two alkyne-end-functionalized side chain polymers onto an azide-bearing three-arm star backbone polymer using the highly efficient copper(I)-catalyzed alkyne-azide cycloaddition reaction.^{28-33,44-}

⁴⁸ Six anions were investigated for their effects on the conformations of SMB-11 and -22: super chaotropic $[\text{Fe}(\text{CN})_6]^{4-}$ and $[\text{Fe}(\text{CN})_6]^{3-}$, moderate chaotropic PF_6^- and ClO_4^- , weak chaotropic I^- , and kosmotropic anion SO_4^{2-} for comparison.³⁷ We found that while super CA-collapsed SMB-11 and -22 remained in the globule state upon heating to 70 °C, moderate CA-collapsed bottlebrushes underwent thermally-triggered globule-to-star shape changes. On the other hand, I^- and SO_4^{2-} had little effects on the conformations of both brush polymers in the studied salt concentration range. These findings enrich the field of stimuli-responsive shape-changing MBBs and may be used in the design of smart polymers for potential applications such as triggered release of payloads.



Scheme 4.1. Synthesis and Behavior of Three-Arm Star Molecular Bottlebrushes (SMBs) Composed of Poly(ethylene oxide) (PEO) and Poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA) (SMB-11) or Poly(*N,N*-diethylaminoethyl methacrylate) (PDEAEMA) Side Chains (SMB-22)

4.2. Experimental Section

4.2.1. Materials

Copper(I) chloride (CuCl, 97%, Alfa-Aesar) was purified by stirring in glacial acetic acid overnight, collected by vacuum filtration, rinsed sequentially with absolute ethanol and diethyl ether, and dried under high vacuum. 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA, 97%, Alfa-Aesar) was purified by vacuum distillation. 2-(*N,N*-Dimethylamino)ethyl methacrylate (DMAEMA, 98.5%, TCI) and 2-(*N,N*-diethylamino)ethyl methacrylate (DEAEMA, 98.5%, TCI) were purified by passing through a basic alumina column with a silica gel plug in the bottom to remove the inhibitor prior to use. The azide-functionalized three-arm star backbone polymer with a DP of 434 per arm (PHEMA-N₃) was provided by Dr. Ethan W. Kent, and the detailed synthesis can be found in the literature.³² The degree of azide functionalization was 88.3%, determined from the ¹H NMR spectroscopy analysis. SEC analysis using PSS GRAL columns with DMF containing 50 mM LiBr as the eluent showed that the $M_{n,SEC}$ of PHEMA-N₃ was 224.5 kDa and the dispersity ($\bar{D}$) was 1.16, with respect to polystyrene standards. Propargyl 2-bromoisobutyrate was synthesized according to a procedure from the literature²⁹ and the molecular structure was verified by NMR spectroscopy analysis. Sodium ferrocyanide decahydrate (Na₄[Fe(CN)₆]•10H₂O, 99%, Acros Organics), potassium ferricyanide (K₃[Fe(CN)₆], 98%, Acros Organics), sodium hexafluorophosphate (NaPF₆, 98.5%, Acros Organics), sodium perchlorate (NaClO₄, 98 %, Alfa Aesar), sodium iodide (NaI, 99%, Alfa Aesar), and sodium sulfate (Na₂SO₄, 99%, Fisher Scientific) were used as received. Aqueous solutions of each salt with various concentrations were prepared by dissolving calculated amounts of the salt in Milli-Q and then filtered through 0.45 μ m hydrophilic PTFE filters. Poly(ethylene glycol) methyl ether (5 kDa, Sigma-Aldrich), 4-pentynoic acid (95%, Acros Organics), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride

(EDC, 98%, Acros Organics), and 4-(dimethylamino)pyridine (DMAP, 99%, TCI America) were used as received. All other chemicals were purchased from either Sigma-Aldrich, Fisher Scientific, TCI, Acros Organics, or Alfa-Aesar and used as received.

4.2.2. General Characterization

The molecular weights and dispersities of alkyne end-functionalized tertiary amine-containing side chain polymers, PDMAEMA and PDEAEMA, were determined by size exclusion chromatography (SEC) using a PL-GPC 50 Plus system, an integrated GPC/SEC system from Polymer Laboratories, Inc. This SEC instrument was equipped with a differential refractive index (RI) detector, one PSS GRAL guard column (10 micron particles, 50 × 8 mm, Polymer Standards Service-USA, Inc.), and two PSS GRAL linear columns (10 micron particles, 300 × 8 mm, molecular weight range of 500 to 1 000 000 g/mol, Polymer Standards Service-USA, Inc.). The analysis was performed at 50 °C using *N,N*-dimethylformamide (DMF) containing 50 mM LiBr as the eluent at a flow rate of 1.0 mL/min. The system was calibrated with narrow dispersity linear polystyrene standards (Scientific Polymer Products, Inc.). The molecular weight and dispersity of the alkyne end-functionalized PEO was measured at room temperature using a PL-GPC 20 (Polymer Laboratories, Inc.) comprising a RI detector, one PLgel guard column (5 micron particles, 50 × 7.5 mm, Agilent Technologies, Inc.) and two PLgel mixed-C columns (each 5 micron particles, 300 × 7.5 mm, linear range of molecular weights from 200 to 2 000 000 g/mol, Agilent Technologies, Inc.). The eluent was tetrahydrofuran (THF) and the flow rate was 1.0 mL/min. This GPC system was also calibrated with narrow dispersity linear polystyrene standards (Scientific Polymer Products, Inc.). The SEC data were processed using the Cirrus SEC software (Polymer Laboratories, Inc.).

The absolute weight-average molecular weights (M_w) of the purified star molecular bottlebrushes, SMB-11 and SMB-22, were measured at 50 °C by a GPC-MALS system, equipped with one PSS GRAM guard column (10 micron particles, 8 × 50 mm, Polymer Standards Service-USA, Inc.), three PSS GRAM linear columns (10 micron particles, 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 composed of a RI detector and a two-angle light scattering detector (15° and 90°). The mobile phase was DMF with 0.10 M LiBr. ^1H NMR spectra were obtained from either a Varian VNMRs 500 NMR or a Varian VNMRs 600 NMR spectrometer.

4.2.3. Synthesis of Alkyne End-Functionalized Poly(ethylene oxide) (PEO)

Poly(ethylene glycol) methyl ether with a molecular weight of 5000 g/mol ($\text{CH}_3\text{O-PEO-OH}$) (5.007 g, 1.001×10^{-3} mol) was added to a two-necked round-bottom flask equipped with a magnetic stir bar and dried at 75 °C under high vacuum for 5 h. The PEO was then cooled to room temperature and dissolved in methylene chloride (10 mL, dried by reflux over calcium hydride). 4-Pentynoic acid (0.119 g, 1.21×10^{-3} mol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, 0.219 g, 2.82×10^{-3} mol), and 4-(dimethylamino)pyridine (DMAP, 12.8 mg, 1.05×10^{-4} mol) were dissolved in methylene chloride (5 mL, dried by reflux over calcium hydride) in a vial to form a homogeneous mixture, which was added dropwise to the PEO solution through an addition funnel under nitrogen while stirring in an ice water bath. The reaction mixture was warmed naturally to room temperature and stirred overnight. The mixture was then diluted with methylene chloride (100 mL) and washed sequentially with 0.5 M HCl (20 mL × 2), a mixture of 1 M NaOH and a saturated NaCl aqueous solution (50/50, v/v, 20 mL × 2), and a saturated aqueous NaCl solution (20 mL). The organic phase was then separated, dried over anhydrous sodium

sulfate, and concentrated via rotary evaporation to about 10 mL. The polymer was further purified by precipitation into diethyl ether (120 mL) three times. After drying in a vacuum oven overnight, the polymer was collected as a white powder (3.399 g, 66.6 %). ^1H NMR δ (ppm, CDCl_3): 4.26 (t, $\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{COOCH}_2$ -, 2H), 3.84-3.43 (m, $-\text{OCH}_2\text{CH}_2$ - of PEO repeat units), 3.38 (s, $-\text{OCH}_3$ of the methyl end group, 3H), 2.62-2.46 (m, $\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{COOCH}_2$ -, 4H), 1.94 (s, $\text{HC}\equiv\text{CCH}_2\text{CH}_2\text{COOCH}_2$ -, 1H). SEC analysis using the PL-GPC 20 system: $M_{n,\text{SEC}} = 8.4$ kDa and $D = 1.04$.

4.2.4. Synthesis of Alkyne End-Functionalized Poly(2-(*N,N*-dimethylamino)ethyl methacrylate) (PDMAEMA) by Atom Transfer Radical Polymerization

Propargyl 2-bromoisobutyrate (48.8 mg, 2.38×10^{-4} mol), DMAEMA (3.016 g, 1.918×10^{-2} mol), HMTETA (65.1 mg, 2.83×10^{-4} mol), and anisole (5.233 g) were added to a 25 mL two-necked round-bottom flask equipped with a magnetic stir bar, a rubber septum, and a gas-flow adapter. After the mixture was degassed by one round of freeze-pump-thaw, CuCl (23.8 mg, 2.40×10^{-4} mol) was added under a slightly positive pressure of nitrogen. The flask was then sealed with a rubber septum, and three more freeze-pump-thaw cycles were performed. The degassed flask was placed in a preheated oil bath with a temperature of 50 °C under nitrogen gas, and the mixture was stirred by a magnetic stirrer. After 3.5 h, the polymerization was stopped by quickly cooling and exposing to air. An aliquot was taken for ^1H NMR spectroscopy analysis for the determination of the final monomer conversion, and the polymerization mixture was quickly diluted with methylene chloride and passed through a basic alumina (top)/silica gel (bottom) plug to remove the copper catalyst using methylene chloride as the eluent. The polymer solution was concentrated using a rotary evaporator, followed by precipitation into cold hexanes (0 °C, cooled in an ice/water bath) three times. The purified product was dried under high vacuum, yielding a

white rubbery polymer (yield: 0.931 g, 30.9 %). The monomer conversion was determined to be 70.0% by comparing the integrals of the peaks at 4.30-4.13 ppm (-COOCH₂- of the monomer and at 4.13-4.01 ppm (-COOCH₂ of the polymer) in the ¹H NMR spectrum of the final polymerization mixture. The DP of the polymer was calculated to be 56 using the monomer conversion and the initial monomer-to-initiator ratio. SEC analysis using the DMF SEC system with GRAL columns gave a M_{n,SEC} value of 8.6 kDa and a *D* of 1.25, relative to polystyrene standards.

4.2.5. Synthesis of Alkyne End-Functionalized Poly(2-(*N,N*-diethylamino)ethyl methacrylate) (PDEAEMA) by Atom Transfer Radical Polymerization

Propargyl 2-bromoisobutyrate (41.8 mg, 2.04 × 10⁻⁴ mol), DEAEMA (3.007 g, 1.623 × 10⁻² mol), HMTETA (51.8 mg, 2.25 × 10⁻⁴ mol), CuCl (20.2 mg, 2.04 × 10⁻⁴ mol), and anisole (4.506 g) were added to a 25 mL round-bottom flask equipped with a stir bar, a rubber septum, and a gas flow adapter. The polymerization mixture was degassed by three freeze-pump-thaw cycles, placed in an oil bath with a preset temperature of 50 °C under nitrogen, and stirred. After 4 h, the polymerization was stopped by removing the flask from the oil bath, cooling it to room temperature, and opening it to air. An aliquot was taken for ¹H NMR spectroscopy analysis to determine the monomer conversion, and the reaction mixture was quickly diluted with methylene chloride and passed through a basic alumina (top)/silica gel (bottom) column to remove the copper catalyst. The polymer was purified by precipitation of the concentrated polymer mixture into cold hexanes (-78 °C, cooled with an acetone/dry ice bath) five times. The purified polymer was dried under high vacuum, yielding a white tacky polymer (1.828 g, 60.8 %). From the integrals of the peaks at 4.29-4.15 ppm (-COOCH₂- of the monomer and at 4.15-3.91 ppm (-COOCH₂- of the polymer) in the ¹H NMR spectrum of the final reaction mixture, the monomer conversion was found to be 66.0 %. The DP of the polymer was calculated to be 53 using this conversion and the

initial monomer-to-initiator ratio. SEC analysis using the DMF SEC system with GRAL columns showed that the $M_{n,SEC}$ was 9.4 kDa and the $\bar{D}$ was 1.19, relative to polystyrene standards.

4.2.6. Synthesis of Star Molecular Bottlebrushes Composed of Heterografted PEO and PDMAEMA Side Chains (SMB-11)

PHEMA-N3 (6.00 mg, 2.49×10^{-5} mol azide moieties assuming quantitative functionalization, delivered through the addition of 0.853 g of a 7.03 mg/g stock solution in THF), alkyne end-functionalized PEO (76.7 mg, 1.50×10^{-5} mol), alkyne end-functionalized PDMAEMA (109.0 mg, 1.21×10^{-5} mol), copper(I) chloride (2.5 mg, 2.5×10^{-5} mol), and dry THF (7 mL) were added to a 20 mL scintillation vial equipped with a magnetic stir bar and sealed with a rubber septum and needles for purging. After flushing the headspace with nitrogen gas, PMDETA (5.8 μ L, 2.8×10^{-5} mol) was injected by microsyringe. The headspace was purged for an additional 15 min; the needles were removed, and the reaction mixture was stirred at room temperature. After 6 h, propargyl benzyl ether (10 μ L) was injected by microsyringe to react with unreacted azide groups. After 2 h, a second round of capping of unreacted azide groups with propargyl benzyl ether was performed as follows. The reaction mixture was diluted with THF, passed through a basic alumina (top)/silica gel (bottom) plug to remove the copper catalyst, and concentrated to about 7 mL. Additional copper(I) chloride (3.0 mg, 3.0×10^{-5} mol) and PMDETA (6.0 μ L, 2.9×10^{-5} mol) were added. The headspace was purged with nitrogen gas for 5 min, and propargyl benzyl ether (10 μ L) was added and stirred overnight. The reaction mixture was passed through a basic alumina (top)/silica gel (bottom) column to remove the copper catalyst using methylene chloride as the eluent. The solution was slowly concentrated by rotary evaporation in a warm water bath (~ 35 °C) to avoid the formation of de-solvated polymer on the wall of the flask. The concentrated polymer solution was diluted with 200 proof ethanol (30 mL) and carefully concentrated again to ~ 8 mL.

This process was repeated three times to remove as much THF and methylene chloride as possible. The concentrated solution in ethanol was then diluted with an equal volume of Milli-Q water. The obtained molecular bottlebrush polymer was purified by seven rounds of centrifugal filtration at 5000 rpm using a 50 kDa MWCO ultracentrifugal filter (Millipore™ Amicon™) to remove unreacted side chains. The complete removal of the remaining side chain polymers was confirmed by SEC analysis using a PL-GPC 50 Plus system comprising GRAL columns with DMF containing 50 mM LiBr as the mobile phase. The polymer solution was slowly concentrated and freeze dried (LABCONCO 76705 Series Freeze Dryer), which yielded SMB-11 as a white powder (yield: 69.7 mg, 39.7 %). The absolute M_w from GPC-MALS analysis was 9.49×10^6 g/mol.

4.2.7. Synthesis of Star Molecular Bottlebrushes Composed of Heterografted PEO and PDEAEMA Side Chains (SMB-22)

PHEMA-N₃ (8.00 mg, 3.32×10^{-5} mol azide groups assuming 100% functionalization, added by 1.146 g of a stock solution in THF with a concentration of 6.98 mg/g), alkyne end-functionalized PEO (101.5 mg, 1.991×10^{-5} mol), alkyne end-functionalized PDEAEMA (199.4 mg, 1.990×10^{-5} mol), copper(I) chloride (3.4 mg, 3.4×10^{-5} mol), and THF (7 mL) were added to a 2 dram scintillation vial equipped with a magnetic stir bar, a rubber septum, and needles for purging. After the headspace of the vial was flushed with nitrogen for 10 min, PMDETA (7.8 μ L) was injected through the septum using a microsyringe under a positive pressure of nitrogen. The headspace was flushed for an additional 10 min. The needles were removed, and the mixture was stirred at room temperature for 16 h. Propargyl benzyl ether (15 μ L) was then injected using a microsyringe to cap unreacted azide groups. After 7 h, the reaction mixture was opened to air. To determine the grafting density of the brushes by SEC, a sample was withdrawn, diluted with methylene chloride, and passed through a basic alumina (top)/silica gel (bottom) plug to remove

the copper catalyst using methylene chloride as the eluent. The brush polymer solution was then concentrated by rotary evaporation using a warm water bath ($\sim 35\text{ }^{\circ}\text{C}$), while close attention was paid to avoid the formation of any polymer dried on the wall. The concentrated polymer solution was diluted with 200 proof ethanol (30 mL) and carefully concentrated again to $\sim 5\text{ mL}$. This process was repeated 3 times to remove THF and methylene chloride. The ethanol solution of the polymer was then diluted with an equal volume of Milli-Q water and transferred to a 50 kDa MWCO ultracentrifugal filter unit (MilliporeSigma™ Amicon™). The unreacted side chain polymers were removed by 8 rounds of centrifugal filtration at 5000 rpm, which was confirmed by SEC analysis using the SEC with GRAL columns and DMF containing 50 mM LiBr as the eluent. The polymer solution was slowly concentrated and freeze dried (LABCONCO 76705 Series Freeze Dryer), yielding a white powder (yield: 56.6 mg, 23.0 %). The absolute M_w from GPC-MALS was $9.77 \times 10^6\text{ g/mol}$.

4.2.8. Dynamic Light Scattering (DLS) Study of SMB-11 and -22 in Acidic Aqueous Solutions

Dynamic light scattering (DLS) measurements of SMB-11 and -22 in acidic aqueous solutions under various conditions were taken using a Malvern Zetasizer Nano ZS instrument with a He-Ne 644 nm laser and a temperature controller at a scattering angle of 173° . A stock solution of each brush polymer in pH 4.50 water with a certain concentration (for SMB-11: 3.00 mg/g; for SMB-22: 3.67 mg/g) was prepared first and then diluted to a concentration of 0.2 mg/g using a pH 4.50 aqueous solution for DLS experiments. The pH values of the acidic water and the brush polymer solution were monitored by an Accumet AB-15 pH meter or a Denver Instrument UltraBasic pH meter (calibrated using standard buffer solutions with pH 4.01, 7.00, and 10.01 at room temperature) and were adjusted to 4.50, if needed, using 0.1 M HCl or 0.1 M NaOH aqueous solution. The 0.2 mg/g solution of each brush polymer with a pH value of 4.50 was slowly filtered

through a 0.45 μm hydrophilic PTFE filter. To investigate the effects of salts on the conformation of SMB-11 and -22 in a pH 4.50 solution, aqueous stock solutions of six salts ($\text{Na}_4[\text{Fe}(\text{CN})_6]$, $\text{K}_3[\text{Fe}(\text{CN})_6]$, NaPF_6 , NaClO_4 , NaI , and Na_2SO_4) with concentrations of 0.010, 0.10, and 1.0 M were made using Milli-Q water, filtered through 0.45 μm hydrophilic PTFE filters, and added in a small aliquot each time ($< 10\ \mu\text{L}$) into a 1.0 mL solution of 0.2 mg/g SMB-11 or SMB-22 to obtain an appropriate salt concentration using a pipette (Gilson, Pipetman Classic P20). The DLS measurements were performed at 25 $^\circ\text{C}$ first to study the effects of salt concentration on the hydrodynamic size of the brush polymer. The temperature effects of the salts were examined at selected salt concentrations in a typical temperature range of 5 to 70 $^\circ\text{C}$. The DLS samples were heated or cooled in the cell holder of the instrument, and at each selected temperature the samples were equilibrated for 2 min before the measurements were taken. Each data point in the DLS size plots is the average of three measurements and each measurement is the average of 10 runs. All data points in the DLS plots are single size distributions without any large aggregates, unless explicitly stated. Representative hydrodynamic size distributions are presented as examples.

4.2.9. Atomic Force Microscopy (AFM) Study of SMB-11 and -22 Under Various Conditions

AFM of SMB-11 and -22 was performed at ambient conditions using a Digital Instruments Multimode IIIa scanning probe microscope in the tapping mode. The probes were reflective Al-coated Si probes (Budget Sensors or Ted Pella, Inc., resonant frequency: 300 kHz, force constant: 40 Nm^{-1}). Glass disks (Ted Pella, Inc.) were used for the preparation of AFM samples. These substrates were thoroughly cleaned with a “Piranha” solution (97.2% sulfuric acid/30% H_2O_2 , 70/30, v/v), rinsed with Milli-Q water extensively, and stored in isopropanol (Honeywell, 99.9%, HPLC grade). For AFM study, aqueous solutions of SMB-11 and -22 with a concentration of 0.050 mg/g and a pH of 4.50 in the absence or presence of a salt were prepared from the 0.2 mg/g DLS

samples by diluting with a pH 4.50 aqueous solution. To prepare AFM samples at room temperature ($\sim 20\text{ }^{\circ}\text{C}$), two drops of a brush solution were added onto a glass disk under ambient conditions. After 2 s, the solution was removed from the glass substrate using a glass pipet and any residual solution was wicked away using a Kimwipe tissue (for solutions with a salt) or blown away from the glass disk with a gentle stream of a filtered nitrogen gas (for solutions without any salt). To prepare AFM samples at $70\text{ }^{\circ}\text{C}$ from pH 4.50 aqueous solutions of brush polymers with a polymer concentration of 0.050 mg/g in the presence of a salt (diluted from the 0.20 mg/g DLS samples using pH 4.50 water), the polymer solutions, glass disks, and glass pipets were placed in a $70\text{ }^{\circ}\text{C}$ oven and equilibrated for 15 min. The drop casting was performed inside the oven to maintain the temperature. For the preparation of AFM samples at $0\text{ }^{\circ}\text{C}$, the brush polymer solutions were equilibrated in an ice/water bath, while glass disks and pipets were placed in a fridge with a temperature of $\sim 4\text{ }^{\circ}\text{C}$. After 30 min, the glass disks and pipets were removed from the fridge, and the AFM samples were prepared quickly under the ambient conditions.

4.3. Results and Discussion

4.3.1. Synthesis and Characterization of SMBs with Heterografted PEO and Poly(2-(*N,N*-dialkylamino)ethyl methacrylate) Side Chains: SMB-11 and SMB-22

Three-arm star-shaped SMB-11, composed of PEO and PDMAEMA side chains, and SMB-22, comprising PEO and PDEAEMA side chains, were prepared using a method that our group reported in previous publications.^{28-33,48} Briefly, two alkyne-end-functionalized polymers, PEO with a molecular weight of 5 kDa and either PDMAEMA with a degree of polymerization (DP) of 56 (for SMB-11), or PDEAEMA with a DP of 53 (for SMB-22), were grafted concurrently onto an azide-bearing three-arm star backbone polymer, PHEMA- N_3 , using the highly efficient CuAAC

click reaction (Scheme 4.1). The star backbone PHEMA-N₃, with a DP of 434 per arm, was synthesized by Dr. Ethan W. Kent through a multi-step process that started from ATRP from a trifunctional initiator (Scheme 1.6) followed by a series of post-polymerization reactions to install azide functional groups as described before.³² Alkyne-end-functionalized PEO was prepared by an esterification reaction between the hydroxyl chain end of poly(ethylene oxide) monomethyl ether with a molecular weight of 5000 Da and 4-pentynoic acid using *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride and 4-(dimethylamino)pyridine as the catalysts. Alkyne end-functionalized PDMAEMA and PDEAEMA were made by ATRP of corresponding tertiary amine methacrylate monomers in anisole at 50 °C using propargyl 2-bromoisobutyrate as the initiator. From size exclusion chromatography (SEC) analysis, the dispersities of both alkyne-end-functionalized polymethacrylates were ≤ 1.25 (Figures B1A and B2A), indicating that the polymerizations were controlled. The DPs of PDMAEMA and PDEAEMA, 56 and 53, respectively, were calculated from the monomer conversions, determined by ¹H NMR spectroscopy, and the monomer-to-initiator feed ratios. The ¹H NMR spectra of PDMAEMA and PDEAEMA are shown in Appendix B (Figures B1B and B2B).

The click reactions for the preparation of SMB-11 and SMB-22 were performed in THF in 20 mL scintillation vials at ambient temperature under a nitrogen atmosphere. For both brush polymers, slightly excess side chain polymers were used with respect to the azide functional groups on star backbone polymer PHEMA-N₃ in order to achieve a high grafted density. Propargyl benzyl ether was added into the mixtures in the later stage of the click reactions to cap possible remaining unreacted azide moieties on the backbone. To purify the bottlebrush polymers, the copper catalyst was first removed from the reaction mixtures by passing through a basic alumina/silica gel column. The unreacted side chain polymers were removed by 7 – 8 rounds of centrifugal filtration of the

reaction mixtures at 5000 rpm using 50 kDa MWCO centrifugal filters with a mixture of water and ethanol (50/50, v/v) as the solvent. SEC analysis confirmed that the remaining unreacted side chain polymers in the final reaction mixtures were completely separated (Figures B3 and B4). ^1H NMR spectroscopy analysis of the purified brush polymers showed that SMB-11 was composed of 43.7% PDMAEMA and 56.3% PEO side chains and SMB-22 contained 41.8% PDEAEMA and 58.2% PEO side chains (Figure B5A and B6A). The calculated grafting densities of SMB-11 and -22 were 86.7% and 83.9%, obtained by using the SEC peak area fractions of the molecular bottlebrushes and the remaining side chain polymers in the SEC traces of the final reaction mixtures, the feed ratios, as well as the side chain composition of the purified bottlebrushes (Appendix B1). Using a SEC-MALS system equipped with a RI detector and a two-angle light scattering detector, the experimental absolute molecular weights of SMB-11 and -22 were measured to be 9.49×10^6 Da and 9.77×10^6 Da, respectively (Figures B5B and B6B). These values were close to the calculated weight-average molecular weights, 9.31×10^6 Da for SMB-11 and 9.17×10^6 Da for SMB-22, which were obtained by using the actual weight average molecular weights ($M_n \times D$) of the backbone and the two side chain polymers, the grafting densities, and the side chain compositions from ^1H NMR spectroscopy analysis (Appendix B2).

The characterization data for azide-functionalized star backbone polymer PHEMA- N_3 , alkyne-end-functionalized PEO, PDMAEMA, and PDEAEMA, and two purified bottlebrush polymers (SMB-11 and SMB-22) are summarized in Table 4.1. The calculated weight-average molecular weights and calculated grafting densities of SMB-11 and SMB-22 are also included in Table 4.1, as well as the side chain compositions of the two brush polymers determined from ^1H NMR spectroscopy analysis.

Table 4.1. Characterization Data for Azide-Functionalized Star Backbone Polymer PHEMA-N₃, Alkyne End-Functionalized PEO, PDMAEMA and PDEAEMA, and Three-Arm Star-Shaped Bottlebrush Polymers (SMB-11 and -22).

Polymer Sample	DP or Grafting Density (σ)	Molecular Weight and $\bar{D}$
PHEMA-N ₃	DP = 434 per arm ^a	$M_{n,SEC} = 2.25 \times 10^5$ Da, $\bar{D} = 1.16$ ^e
PEO	DP = 114 ^b	$M_{n,SEC} = 8.4 \times 10^3$ Da, $\bar{D} = 1.04$ ^f
PDMAEMA	DP = 56 ^a	$M_{n,SEC} = 8.6 \times 10^3$ Da, $\bar{D} = 1.25$ ^e
PDEAEMA	DP = 53 ^a	$M_{n,SEC} = 9.4 \times 10^3$ Da, $\bar{D} = 1.19$ ^e
SMB-11 (PEO/PDMAEMA)	$\sigma = 86.7\%$ ^c (molar ratio: 56.3% (PEO): 43.7%) ^d	$M_{w,exp} = 9.49 \times 10^6$ Da ^g $M_{w,cal} = 9.31 \times 10^6$ Da ^h
SMB-2 (PEO/PDEAEMA)	$\sigma = 83.9\%$ ^c (molar ratio: 58.2% (PEO): 41.8%) ^d	$M_{w,exp} = 9.77 \times 10^6$ Da ^g $M_{w,cal} = 9.17 \times 10^6$ Da ^h

^a Degree of polymerization (DP) was calculated from the monomer-to-initiator molar ratio and the monomer conversion (obtained from ¹H NMR analysis). ^b The DP of PEO was calculated from the molecular weight of 5000 g/mol provided by the vendor. ^c The value of grafting density (σ) was calculated using the ratio of SEC peak areas of the bottlebrush polymer and the unreacted side chain polymers remaining in the final reaction mixture, the feed ratios of the star backbone polymer to the side chain polymers, and the side chain composition of the purified bottlebrush polymer determined by ¹H NMR analysis. ^d The molar ratio of PEO to the tertiary amine-containing polymethacrylate was determined from the ¹H NMR spectrum of the brush polymer. ^e $M_{n,SEC}$ and $\bar{D}$ were determined by SEC relative to polystyrene standards using a SEC system with GRAL columns and DMF containing 50 mM LiBr as solvent. ^f $M_{n,SEC}$ and $\bar{D}$ were determined by SEC relative to polystyrene standards using a SEC system with Mixed C columns and THF as solvent. ^g The experimental absolute molecular weight ($M_{w,exp}$) was measured using a SEC-MALS system consisting of a RI detector and a two-angle light scattering detector. ^h The calculated absolute molecular weight ($M_{w,cal}$) was obtained using the weight-average molecular weight of PHEMA-N₃ and the side chain polymers calculated from the DP and the dispersity ($M_n \times \bar{D}$) and the grafting density.

The pK_a values of both PDMAEMA and PDEAEMA in water are around 7.4,^{49,50} and thus both polymers are protonated in acidic water. The apparent hydrodynamic sizes (D_h) of 0.2 mg/g SMB-11 and -22 in acidic water with a pH of 4.50 at 25 °C were found to be very similar to each other, 100.5 and 100.8 nm, respectively (Figures B7 and B8). The star shape of the brush polymers can be seen from the AFM height images of bottlebrush molecules drop cast on bare mica or “Piranha” solution-cleaned glass disks from a 0.050 mg/g solution of SMB-11 in THF and acidic aqueous solutions of SMB-11 and -22 with a polymer concentration of 0.050 mg/g and a pH value of 4.50 at room temperature (Figures 4.1 and B9-B12). For both SMB-11 and -22, most of the brush molecules shown in the images were three-arm stars, although some molecules appeared to miss one arm, which could be due to the defects in the synthesis of the azide-functionalized star backbone or the mechanical shearing forces during the purification of the brush polymers and the preparation of AFM samples. The average arm length and height of SMB-11 from THF were 88.1 ± 26.7 nm (from measurements of 288 arms of three-arm brushes) and 1.5 ± 0.3 nm (from 200 arms). In the pH 4.50 aqueous solution, the PDMAEMA side chains were protonated; image analysis showed that the average arm length of SMB-11 on glass disks from 207 arms was slightly larger, 98.1 ± 28.2 nm, which could be attributed to the electrostatic repulsive forces between the protonated PDMAEMA side chains, and the average height from the measurements of 200 arms was similar, 1.3 ± 0.3 nm. For SMB-22 from acidic water, the average arm length and height were 95.0 ± 29.0 nm (from 120 arms of three-arm star brushes) and 1.7 ± 0.4 nm (from 200 arms). The maximum length of an arm with a DP of 434 is 110.2 nm, which means that the brushes were highly stretched on the substrates, with degrees of stretching of 89.0% and 86.2% for SMB-11 and -22 on glass disks from pH 4.50 aqueous solutions and 80.0% for SMB-11 from THF. The high stretching could be partially caused by the strong electrostatic interactions with the substrates.

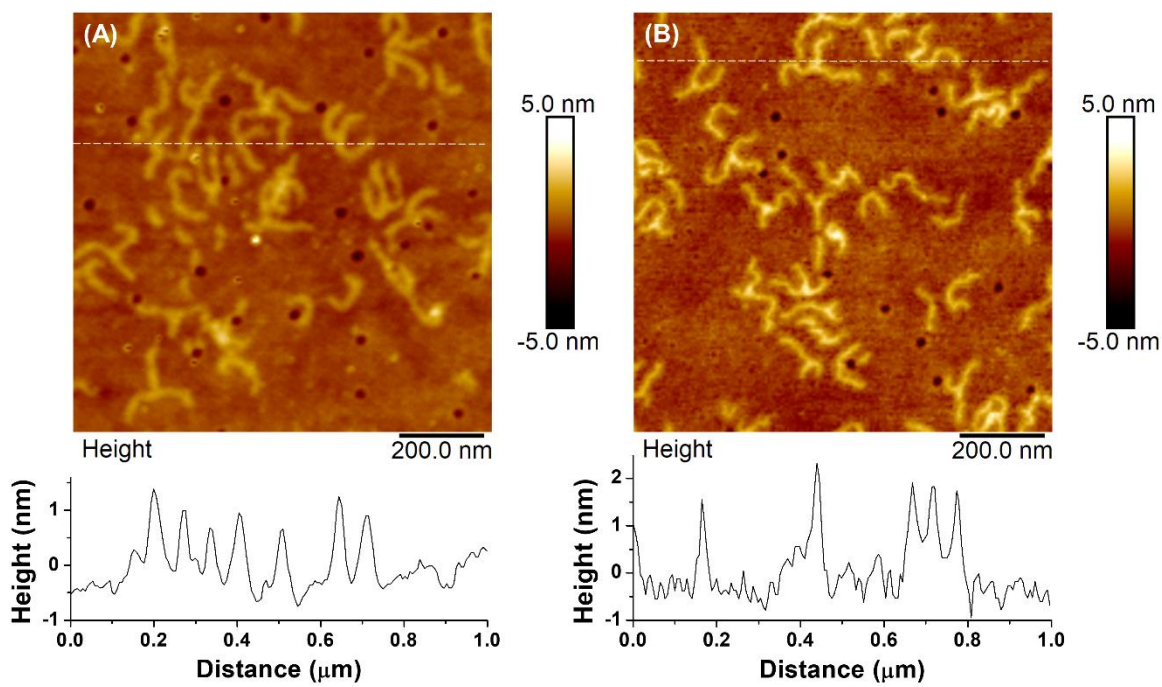


Figure 4.1. AFM height images ($1 \times 1 \mu\text{m}$) of SMB-11 (A) and SMB-22 (B) drop cast on glass disks from 0.050 mg/g brush polymer solutions in pH 4.50 acidic water at room temperature, with a cross-sectional profile along the dashed line in each image.

4.3.2. Dynamic Light Scattering Study of the Effects of Various Salts on Conformations of SMB-11 and -22 in pH 4.50 Acidic Water at 25 °C

Chaotropic ion pairing and chaotropic effect, which have recently emerged as a generic driving force for supramolecular assembly,^{37,51,52} provide new opportunities to control conformations and trigger shape transitions of polyelectrolyte MBBs in aqueous solutions. Our research group previously investigated the effects of six CAs (Cl^- , Br^- , SCN^- , ClO_4^- , $\text{Fe}(\text{CN})_6^{3-}$, and $\text{S}_2\text{O}_8^{2-}$) on the conformations of similar PEO/PDMAEMA and PEO/PDEAEMA star MBBs in acidic aqueous solutions at 25 °C with increasing anion concentration.³³ Among the six anions, Cl^- and Br^- are weak CAs, exerting negligible effects on the brush conformation in pH 4.00 acidic water, while $\text{Fe}(\text{CN})_6^{3-}$ and $\text{S}_2\text{O}_8^{2-}$ are super CAs,³⁷ which collapsed protonated star brushes at concentrations less than 1 mM for 0.1 mg/g SMBs. On the other hand, SCN^- and ClO_4^- are moderate CAs, which showed an ability to collapse PEO/PDEAEMA star brushes into globules at concentrations of tens to hundreds mM. These observations are consistent with the chaotropicity scale proposed by Marcus based on the ion hydration parameters: $\text{Cl}^- < \text{Br}^- < \text{SCN}^- < \text{ClO}_4^- \ll \text{S}_2\text{O}_8^{2-} \sim [\text{Fe}(\text{CN})_6]^{3-}$,^{33,37} where Cl^- , Br^- , SCN^- , and ClO_4^- CAs caused an effective loss of 1.01 – 1.27 hydrogen bonds in the solvation shell around the ion, and $\text{S}_2\text{O}_8^{2-}$ and $\text{Fe}(\text{CN})_6^{3-}$ brought about a loss of ~ 2 hydrogen bonds in the surrounding hydration layer.³⁷

In the present work, we first studied the effects of five CAs from the following salts, $\text{Na}_4[\text{Fe}(\text{CN})_6]$, $\text{K}_3[\text{Fe}(\text{CN})_6]$, NaPF_6 , NaClO_4 , and NaI , on the conformations of SMB-11 and -22 at 25 °C and then examined the effects of temperature. Aqueous solutions of 0.2 mg/g SMB-11 and -22 in pH 4.50 acidic water were prepared, and for each CA, the salt concentration was gradually increased. $[\text{Fe}(\text{CN})_6]^{3-}$ is known to be a super CA with an effective loss of 2.01 hydrogen bonds in the solvation shell around the anion in water.³⁷ From the literature, $[\text{Fe}(\text{CN})_6]^{4-}$ appears

to be a super CA with a water structure breaking ability similar to $[\text{Fe}(\text{CN})_6]^{3-}$.^{39,40} $\text{S}_2\text{O}_8^{2-}$ was not selected here because $\text{K}_2\text{S}_2\text{O}_8$ is a redox radical initiator.⁵³ PF_6^- and ClO_4^- are moderate CAs, causing a loss of 1.43 and 1.27 hydrogen bonds in the solvation shell of the anion, respectively.³⁷ According to Assaf and Nau, there is a loss of 1.37 hydrogen bonds in the hydration layer around I^- ,³⁷ which places I^- in between ClO_4^- and PF_6^- in the water-structure breaking ability. However, the experimental results from Gao et al. in the study of CA-induced micellization of PEO-*b*-PDEAEMA in acidic aqueous solutions showed that I^- was actually a weaker CA than ClO_4^- .⁵¹ On the other hand, SO_4^{2-} is a kosmotropic anion, which increases water structuring in the solvation shell with a gain of 0.78 hydrogen bonds,³⁷ and is used here for comparison with various CAs. K^+ is a weakly structure-breaking cation, while Na^+ is a borderline ion;³⁵ these two cations are often listed side by side in the Hoffmeister series, and thus we believe that their effects on the conformation of SMBs should be very similar to each other and the effects observed here should be mainly from the anions of the salts. According to Marcus, NH_4^+ , CH_3NH_3^+ , and $\text{N}^+(\text{CH}_3)_4$ are chaotropic cations, while $\text{N}^+(\text{CH}_2\text{CH}_3)_4$ is a borderline ion between water-structure making and breaking.³⁵ Thus, on the basis of this trend, we believe that it is very likely that the protonated monomer units of PDMAEMA and PDEAEMA in acidic water are chaotropic cations.

Figure 4.2A and B shows the plots of the hydrodynamic diameter (D_h) of 0.2 mg/g SMB-11 and -22 in pH 4.50 acidic water at 25 °C as a function of increasing salt concentration for the six salts, respectively, from dynamic light scattering (DLS) measurements. Note that as mentioned earlier PDMAEMA and PDEAEMA exhibit similar pK_a values of ~ 7.4 in water,⁴⁹ although the lengths of two alkyl substituents at the nitrogen atom are different. Thus, at pH = 4.50, the tertiary amine groups in both brush polymers were fully protonated. The D_h values of SMB-11 and -22 in the presence of a salt with a concentration of 0.01 mM were very similar, ~ 97 nm, regardless of

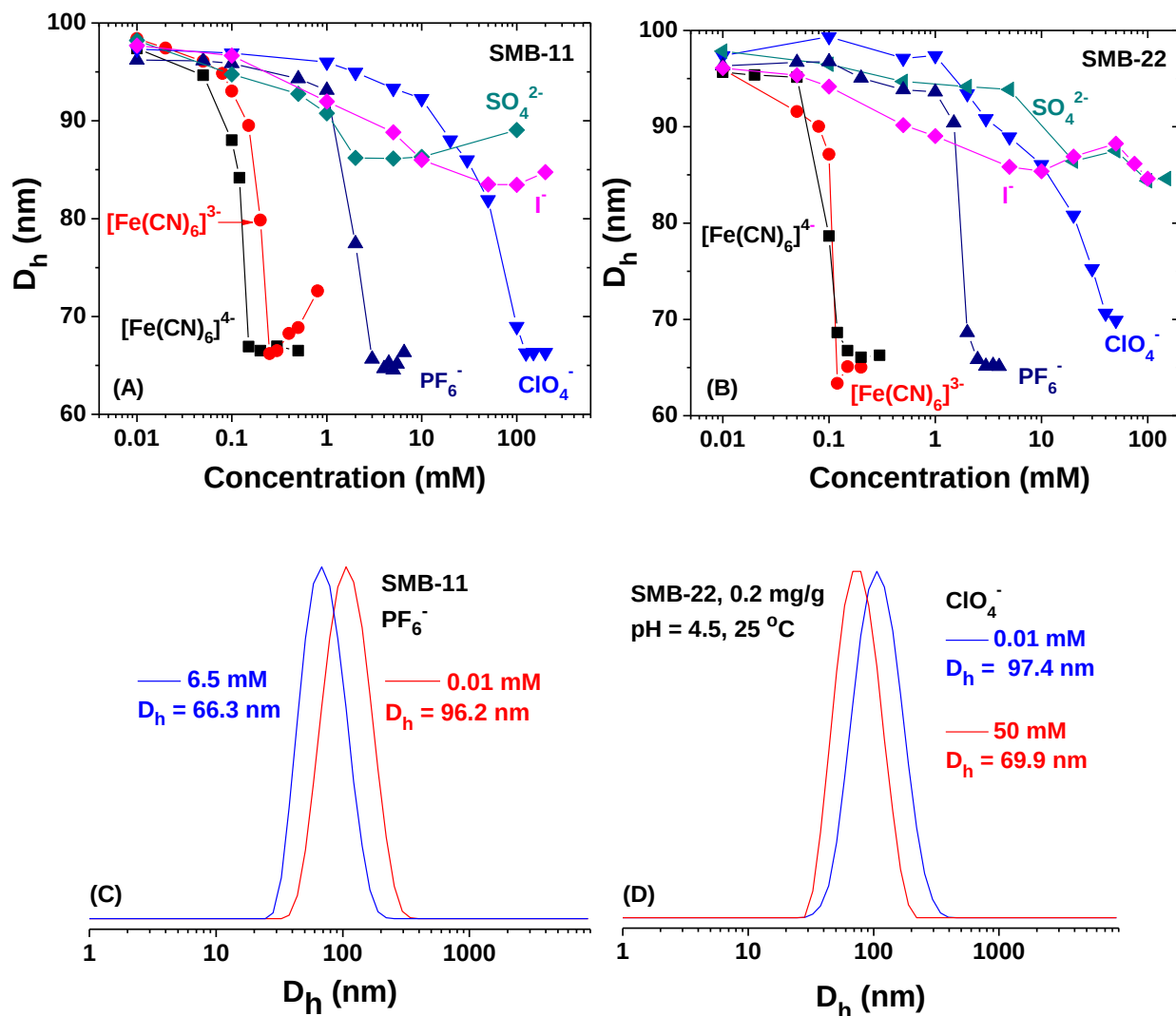


Figure 4.2. Apparent hydrodynamic diameter (D_h) at 25 °C of 0.2 mg/g SMB-11 (A) and SMB-22 (B) in pH 4.50 water as a function of increasing concentration of a salt from dynamic light scattering (DLS) measurements for $Na_4[Fe(CN)_6]$ (black square), $K_3[Fe(CN)_6]$ (red circle), $NaPF_6$ (navy up-pointing triangle), $NaClO_4$ (blue down-pointing triangle), NaI (magenta rhombus), and Na_2SO_4 (dark cyan left-pointing triangle). (C) and (D) Hydrodynamic size distributions of SMB-11 in the presence of 0.01 and 6.5 mM PF_6^- and SMB-22 in the presence of 0.01 and 50 mM ClO_4^- .

the types of the salts. Upon increasing the concentrations of the salts, the two brush polymers behaved similarly to each other in response to the same salt (i.e., the same anion). For $[\text{Fe}(\text{CN})_6]^{4-}$ and $[\text{Fe}(\text{CN})_6]^{3-}$, the hydrodynamic sizes of both SMB-11 and -22 decreased sharply to ~ 67 nm in the salt concentration range of 0.10 – 0.30 mM, while single, narrow hydrodynamic size distributions were maintained (Figures B13 and B14). Although $[\text{Fe}(\text{CN})_6]^{4-}$ appeared to be more efficient in collapsing SMB-11 than $[\text{Fe}(\text{CN})_6]^{3-}$, both anions performed very similarly in inducing the size changes of SMB-22. These observations indicated that $[\text{Fe}(\text{CN})_6]^{4-}$ is a super CA as expected, with a similar water-structure breaking ability to $[\text{Fe}(\text{CN})_6]^{3-}$. Although there appears to be no difference in the salt concentration for the collapse of both brush polymers by $[\text{Fe}(\text{CN})_6]^{4-}$, the full collapse for SMB-22 by $[\text{Fe}(\text{CN})_6]^{3-}$ occurred at a slightly lower concentration (0.12 mM for SMB-22 versus 0.25 mM for SMB-11). Interestingly, the D_h of SMB-11 exhibited a slight increase with the increase of the concentration of $[\text{Fe}(\text{CN})_6]^{3-}$, from 66.2 nm at 0.25 mM to 72.6 nm at 0.80 mM, for which the reason is unclear at this point.

For PF_6^- , the sharp size reduction transitions of both brush polymers occurred in the concentration range of 1 – 3 mM, which was about one order in magnitude higher than those for the two super CAs. For ClO_4^- , the size transition was somewhat broader and occurred at even higher concentrations than PF_6^- ; the full collapse was observed at 125 mM with a D_h of 66.3 nm for SMB-11 and at 50 mM with a D_h of 69.9 nm for SMB-22. Both PF_6^- and ClO_4^- appeared to be more efficient in collapsing SMB-22 than SMB-11 as evidenced by the lower concentrations for the full collapse of SMB-22. Note that all the data points in Figure 4.2A and B correspond to single size distributions with representative examples shown in Figure 4.2C and D (as well as Figures B13 and B14). In contrast, the addition of I^- and SO_4^{2-} did not produce sharp size decreases but small variations for both brush polymers, suggesting no significant conformation changes in the

studied concentration range. Our observations for the effects of ClO_4^- and I^- on the hydrodynamic diameters of SMB-11 and -22 were in line with their abilities in inducing the micellization of PEO-*b*-PDMAEMA and PEO-*b*-PDEAEMA reported by Gao et al.⁵¹ Clearly, super CAs caused larger and more abrupt decreases in the D_h values of SMB-11 and -22 at lower concentrations, while moderate CAs collapsed the brushes at higher concentrations and weak CA I^- and kosmotropic anion SO_4^{2-} produced small variations of the D_h for both brush polymers.

4.3.3. Effects of Temperature on Super Chaotropic Anion-Induced Collapse of Star Brushes SMB-11 and -22

The effects of temperature on $[\text{Fe}(\text{CN})_6]^{4-}$ - and $[\text{Fe}(\text{CN})_6]^{3-}$ -collapsed SMB-11 and -22 were investigated by DLS and AFM first. Figure 4.3A and B shows the plots of D_h of 0.2 mg/g SMB-11 in pH 4.50 acidic water in the presence of 0.25, 0.50, and 0.80 mM $[\text{Fe}(\text{CN})_6]^{3-}$ and 0.20 mg/g SMB-22 in the presence of 0.12 mM $[\text{Fe}(\text{CN})_6]^{3-}$ as a function of temperature in the typical temperature range of 20 to 70 °C. The plot of the D_h of 0.20 mg/g SMB-22 in pH 4.50 water with the addition of 0.20 mM $[\text{Fe}(\text{CN})_6]^{4-}$ versus temperature in the same temperature range is included in Appendix B (Figure B15). For all the five solutions, temperature had little effect on the hydrodynamic sizes of the brush polymers in the temperature range of 20 to 70 °C and no large and sharp size changes were observed while maintaining a single size distribution (Figures B16 and B17), suggesting that the bottlebrush molecules remained in the collapsed globular state even at 70 °C. Note that Plamper et al. reported a UCST of ~ 24 °C for 0.1 mg/mL PDMAEMA with a DP of 100 at pH = 5.0 in the presence of 1.2 mM $[\text{Co}(\text{CN})_6]^{3-}$ and 0.1 N NaCl and stated that $[\text{Fe}(\text{CN})_6]^{4-}$ showed a similar effect.³⁹ In contrast, Zhang et al. reported a UCST of ~ 60 °C for 0.5 mg/mL PDMAEMA with a DP of 64 at pH = 5.0 in the presence of 8 mM $[\text{Fe}(\text{CN})_6]^{3-}$ and 0.1 M NaCl.⁴⁰ Although we cannot compare the molecular brushes directly with the linear PDMAEMA

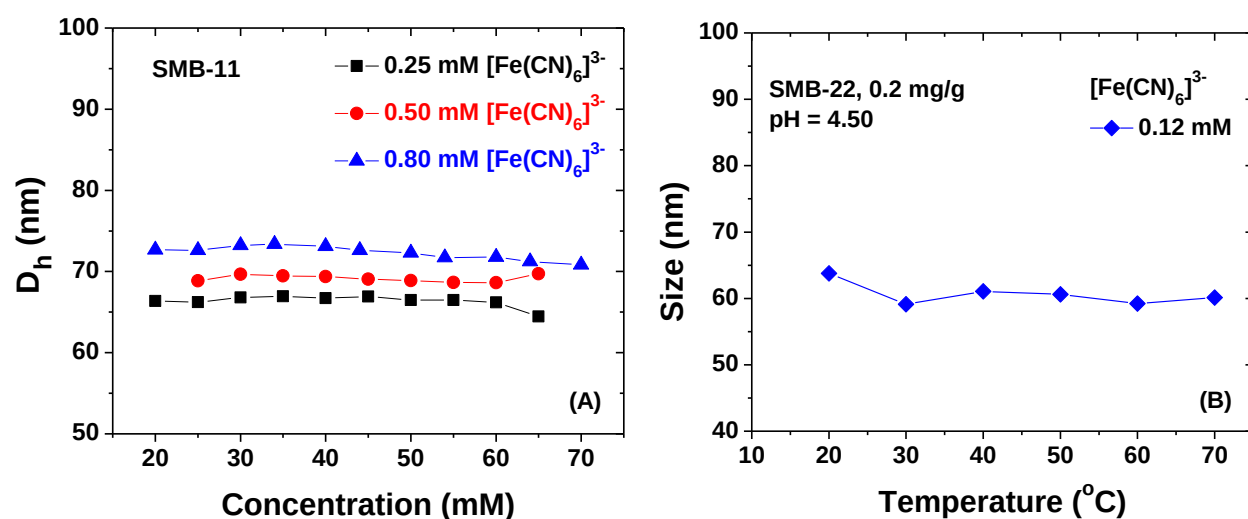


Figure 4.3. (A) Apparent hydrodynamic size (D_h) of 0.2 mg/g SMB-11 in pH 4.50 acidic water in the presence of 0.25 mM (black square), 0.50 mM (red circle), and 0.80 mM (blue triangle) $\text{K}_3[\text{Fe}(\text{CN})_6]$ as a function of temperature. (B) D_h of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 0.12 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ as a function of temperature.

polymer and it is possible that the UCST transition temperature for our systems could be higher than 70 °C, we note here that in those two reports 0.1 M NaCl was added to moderate the electrostatic interaction between the protonated tertiary amine groups and complex metal anions. Plamper et al. emphasized in their publication that the effects were only observed in buffers containing a considerable amount of NaCl (~ 0.1 M NaCl) and no UCST was found in the absence of a buffer.³⁹ Presumably, the chaotropic ion pairing of protonated PDMAEMA and PDEAEMA with super CAs was so strong that heating could not break apart the ion pairs. Adding a large amount of NaCl could modulate the electrostatic interactions and make the UCST transition appear in a convenient temperature range. Thus, it is not surprising that no shape transitions were observed for collapsed globular brushes upon heating under our experimental conditions, presumably because the interactions between protonated tertiary amine groups and super CAs were very strong.

AFM was then used to visualize the brush molecules at different temperatures. A portion of the DLS sample with 0.20 mg/g SMB-11 in the presence of 0.3 mM $K_3[Fe(CN)_6]$ shown in Figure 4.2A was diluted with pH 4.50 acidic water at ambient conditions to 0.050 mg/g SMB-11 with 0.075 mM $K_3[Fe(CN)_6]$ for the preparation of AFM samples. The diluted solution was drop cast on “Piranha”-treated glass disks at room temperature (20 °C) and in a 70 °C oven, which were imaged by AFM at ambient conditions. Figure 4.4A and B shows the AFM height images at 20 and 70 °C, respectively, and more images can be found in Appendix B (Figures B18-B20). Evidently, SMB-11 brush molecules were collapsed and overall globular at both 20 and 70 °C, unlike the star-shaped molecules shown in Figure 4.1A. In Figure 4.4A, one brush molecule with three tightly associated lobes from collapsed arms, as indicated by three arrows, can be clearly seen. Despite the observation of the collapsed globular state for SMB-11 in the presence of super

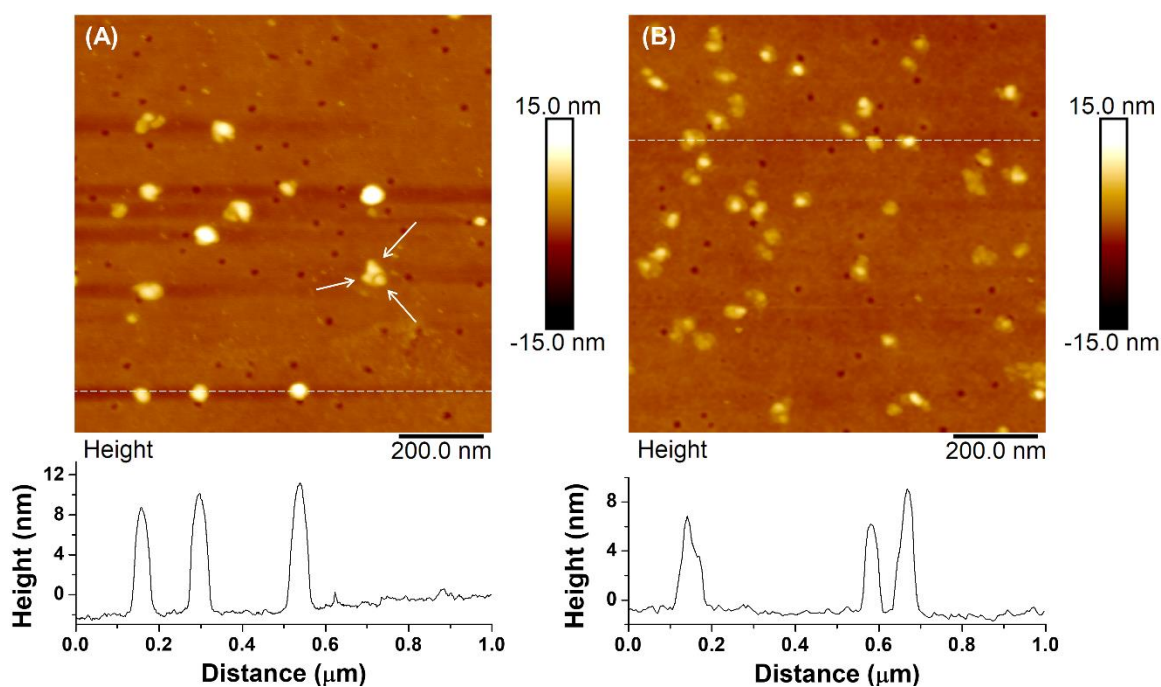


Figure 4.4. Atomic force microscopy height images ($1 \times 1 \mu\text{m}$) of SMB-11 drop cast on glass disks at 20 °C (A) and 70 °C (B) from a 0.050 mg/g SMB-11 solution in pH 4.50 acidic water in the presence of 0.075 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$, with a cross-sectional profile along the dashed line in each image. The solution used to prepare AFM samples was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 0.30 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ using pH 4.50 acidic water.

CA $[\text{Fe}(\text{CN})_6]^{3-}$ at both 20 and 70 °C, there are still some noticeable differences. From the cross-sectional profiles, the brush molecules in Figure 4.4A are generally taller than those in Figure 4.4B. Image analysis using Image J shows that the average height was 10.6 ± 2.4 nm (from measurements of 100 brushes) at 20 °C and 7.0 ± 1.9 nm at 70 °C (from 200 globules). In addition, the brush molecules in Figure 4.4B appear to be flattened to some degree, likely caused by the high temperature that might soften the collapsed globular brushes. The average size of the brushes was 46.3 ± 9.0 nm at 20 °C (from measurements of 109 globules) and 49.4 ± 9.2 nm at 70 °C (from measurements of 200 globular nanoobjects).

4.3.4. Effects of Temperature on Moderate Chaotropic Anion-Induced Collapse of Star Brushes SMB-11 and -22

This section focuses on moderate CAs, PF_6^- and ClO_4^- . Figure 4.5 shows the DLS data for SMB-11 and -22 in pH 4.50 acidic water with the addition of NaPF_6 at various temperatures. For SMB-11 in the presence of 4.0 mM NaPF_6 , the D_h was ~ 65 nm in the temperature range of 5 – 30 °C (Figure 4.5A). Upon further heating from 30 to 40 °C, the hydrodynamic diameter increased sharply from 67.2 nm to 93.6 nm, respectively, with a transition middle point at 35 °C. Above 40 °C, the D_h showed only small changes; at 70 °C the apparent size was 97.3 nm, which is almost the same as that observed at 0.01 mM NaPF_6 at 25 °C (96.2 nm), suggesting that the brushes underwent a thermally-induced globule-to-star transition. Note that throughout the heating process during the DLS measurements, a single size distribution was maintained; the distributions for 5 and 70 °C are shown in Figure 4.5B as examples.

Similar size transitions were observed for SMB-22 in pH 4.50 water in the presence of 2.5, 3.0, 3.5, and 4.0 mM NaPF_6 , with the D_h increasing from ~ 65 nm at 20 °C to ~ 95 nm at 70 °C (Figure 4.5C). There appears to be a general trend that the transition temperature increases with

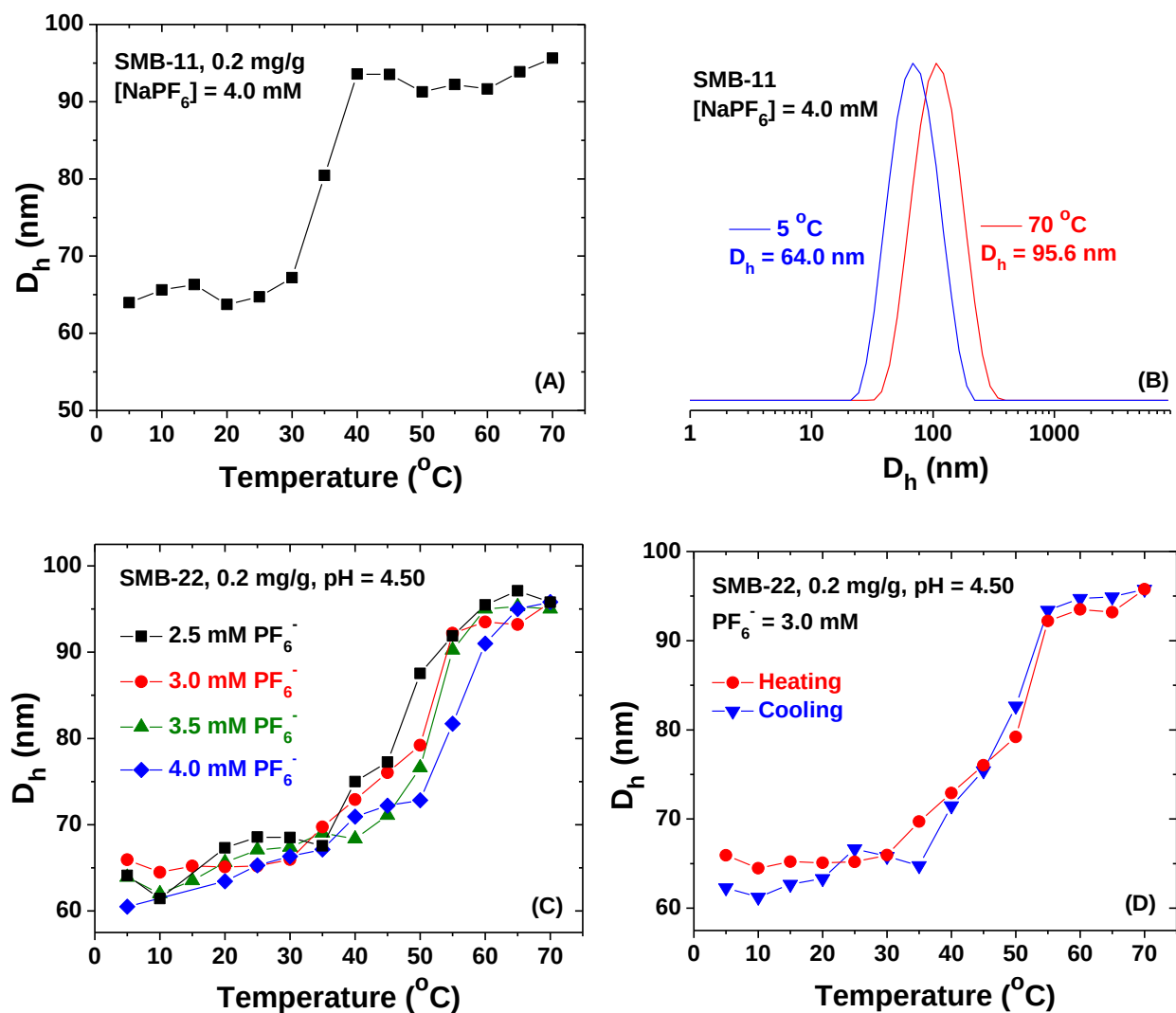


Figure 4.5. Dynamic light scattering studies of SMB-11 and SMB-22 in pH 4.50 acidic water in the presence of NaPF_6 . (A) Apparent hydrodynamic size (D_h) of 0.2 mg/g SMB-11 in pH 4.50 water with 4.0 mM NaPF_6 as a function of temperature. (B) Hydrodynamic size distributions of 0.2 mg/g SMB-11 in pH 4.50 water with 4.0 mM NaPF_6 at 5 and 70 $^{\circ}\text{C}$. (C) Plots of D_h of 0.2 mg/g SMB-22 in pH 4.50 water in the presence of 2.5, 3.0, 3.5, and 4.0 mM NaPF_6 versus temperature upon heating. (D) Plot of D_h of 0.2 mg/g SMB-22 in pH 4.50 water in the presence of 3.0 mM NaPF_6 versus temperature from heating and cooling processes. All data points correspond to a single-size distribution (Figure B21).

increasing the concentration of NaPF₆ from 2.5 to 4.0 mM. This is reasonable, considering that the ion pairing is a dynamic equilibrium between paired ions and free ions. A larger concentration of PF₆⁻ would suppress the dissociation and thus a higher temperature is needed to unfold the brushes to the starlike state. The size transition was reversible, as shown in Figure 4.5D by the plots of D_h of SMB-22 in the presence of 3.0 mM NaPF₆ versus temperature from a heating and cooling cycle.

Similar to the brushes collapsed by PF₆⁻, both ClO₄⁻-collapsed brush polymers in acidic water exhibited thermally-induced size transitions (Figure 4.6). Upon heating from 20 to 70 °C, the D_h of SMB-11 in the presence of 125, 150, and 200 mM NaClO₄ increased substantially, from ~ 65 nm at 20 °C to ~ 85 nm at 70 °C, with the transitions occurring in the temperature range of 30 to 45 °C (Figure 4.6A), and the size transitions were reversible as shown in Figure 4.6B for the brushes with 125 mM NaClO₄. Similar to the observation for the transitions of SMB-22 in the presence of different concentrations of NaPF₆, the transition temperature is higher for a higher NaClO₄ concentration. However, the apparent hydrodynamic sizes at 70 °C (~ 85 nm) were noticeably smaller than that of SMB-11 in the presence of 0.01 mM ClO₄⁻ at 25 °C (~ 97 nm in Figure 4.2A) and the D_h in the presence of 4.0 mM NaPF₆ at 70 °C (95.6 nm in Figure 4.5A), which could be caused by the higher NaClO₄ salt concentration that screened the electrostatic repulsive interactions at higher temperatures. SMB-22 in the presence of 50 mM NaClO₄ also underwent a size transition upon heating from ~ 70 nm in the range of 10 – 25 °C to 96 nm at 70 °C (Figure 4.6C). These observations indicate that the two brush polymers collapsed by moderate CAs can undergo temperature-induced reversible globule-to-star shape transitions.

Comparing the thermal behavior of SMB-11 and -22 at the same concentration of PF₆⁻ in Figure 4.5A and C (4.0 mM), the transition temperature for SMB-22 (~ 55 °C in Figure 4.5C) is

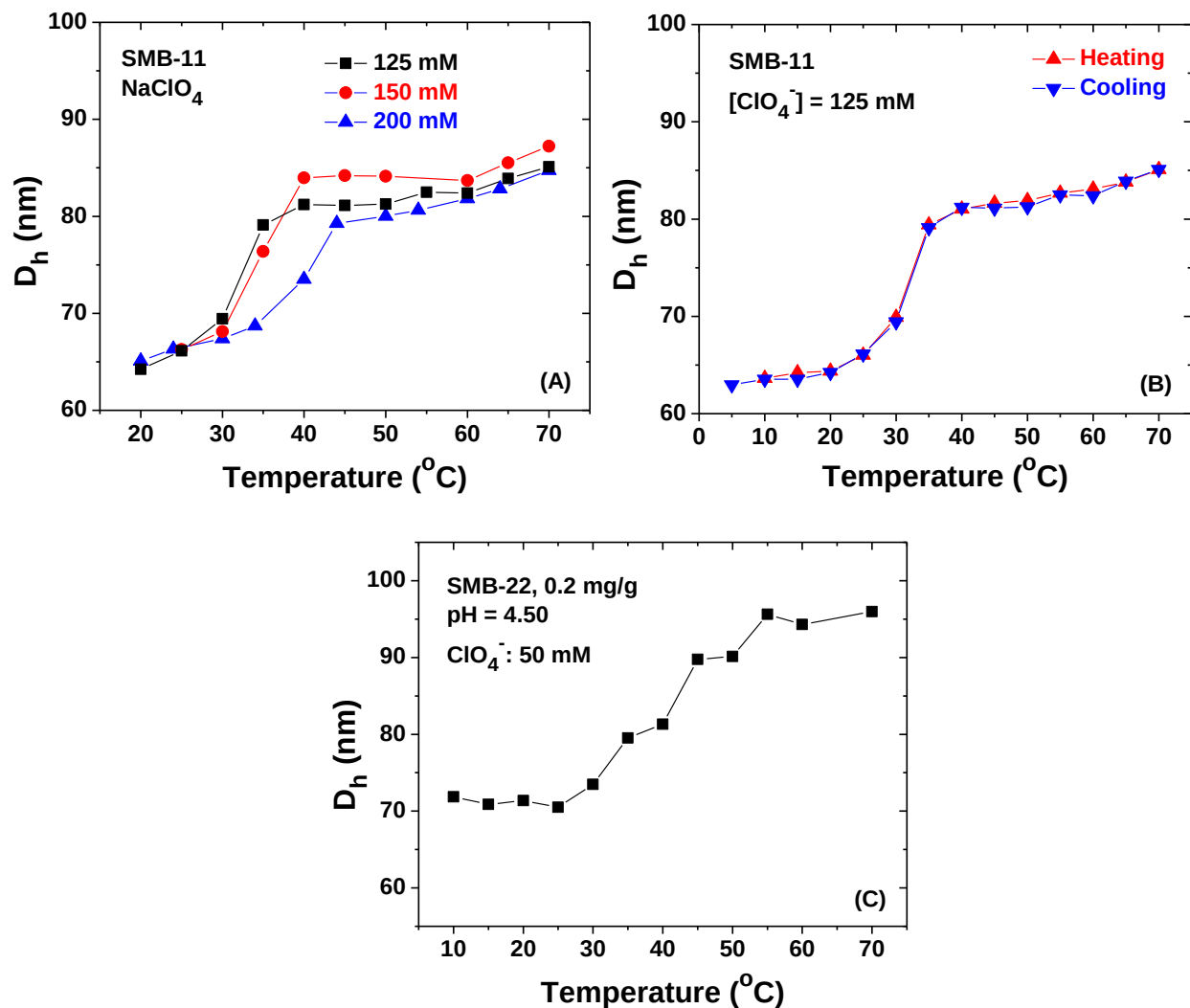


Figure 4.6. (A) Plots of apparent hydrodynamic size (D_h) of 0.2 mg/g SMB-11 in pH 4.50 water in the presence of 125 mM (black square), 150 mM (red circle), and 200 mM (blue triangle) NaClO_4 versus temperature. (B) Plot of D_h of 0.2 mg/g SMB-11 in pH 4.50 water with 125 mM NaClO_4 versus from a heating and cooling cycle. (C) Plot of D_h of 0.2 mg/g SMB-22 in pH 4.50 water with 50 mM NaClO_4 versus temperature. All data points correspond to a single-size distribution (Figures B22 and B23).

substantially higher than that for SMB-11 (35 °C in Figure 4.5A). Similarly, even the NaClO₄ concentration was only 50 mM for SMB-22 in Figure 4.6C, the transition temperature was higher than those of SMB-11 at higher salt concentrations (125 -200 mM in Figure 4.6A). These data indicate that the interactions of PF₆⁻ and ClO₄⁻ with protonated PDEAEMA are stronger than protonated PDMAEMA, consistent with the observation in Figure 4.2A that a full collapse of SMB-22 requires lower concentrations of PF₆⁻ and ClO₄⁻ than SMB-11. As discussed earlier, according to Marcus, (CH₃)₄N⁺ is chaotropic and (C₂H₅)₄N⁺ is on the borderline between chaotropic and kosmotropic cations. On the basis of this, we reasoned that under the same conditions the protonated tertiary amine groups of PDMAEMA, -CH₂CH₂N⁺H(CH₃)₂, should be stronger in terms of chaotropicity than those of PDEAEMA, -CH₂CH₂N⁺H(C₂H₅)₂, which means that the ion pair of -CH₂CH₂N⁺H(CH₃)₂ with a moderate CA (either PF₆⁻ or ClO₄⁻ in our case) should be stronger than that between -CH₂CH₂N⁺H(C₂H₅)₂ and the same moderate CA. However, this cannot explain what we observed here. The stronger effect of the same moderate CA for SMB-22 than SMB-1, as indicated by the full collapse of the brushes at lower concentrations and the higher transition temperature upon heating at the same or lower salt concentration, very likely comes from the chaotropic effect that chaotropic anions have a high tendency to be associated with the more hydrophobic ethyl groups in the tertiary amine pendant groups of PDEAEMA.

AFM was used to visualize the SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water with 1.5 mM NaPF₆ at 20 °C and 70 °C (Figures 4.7 and B24-B26), which was diluted from a 0.2 mg/g solution of the brush polymer in the presence of 6.0 mM NaPF₆. At 20 °C, the brush molecules are round in shape on the substrate, but the collapsed arms were not as tight as the globular brushes in Figure 4.4A. The three-arm star-shaped backbone can be seen for most of the molecules. The average size from the measurements of 121 molecules was 63.8 ±

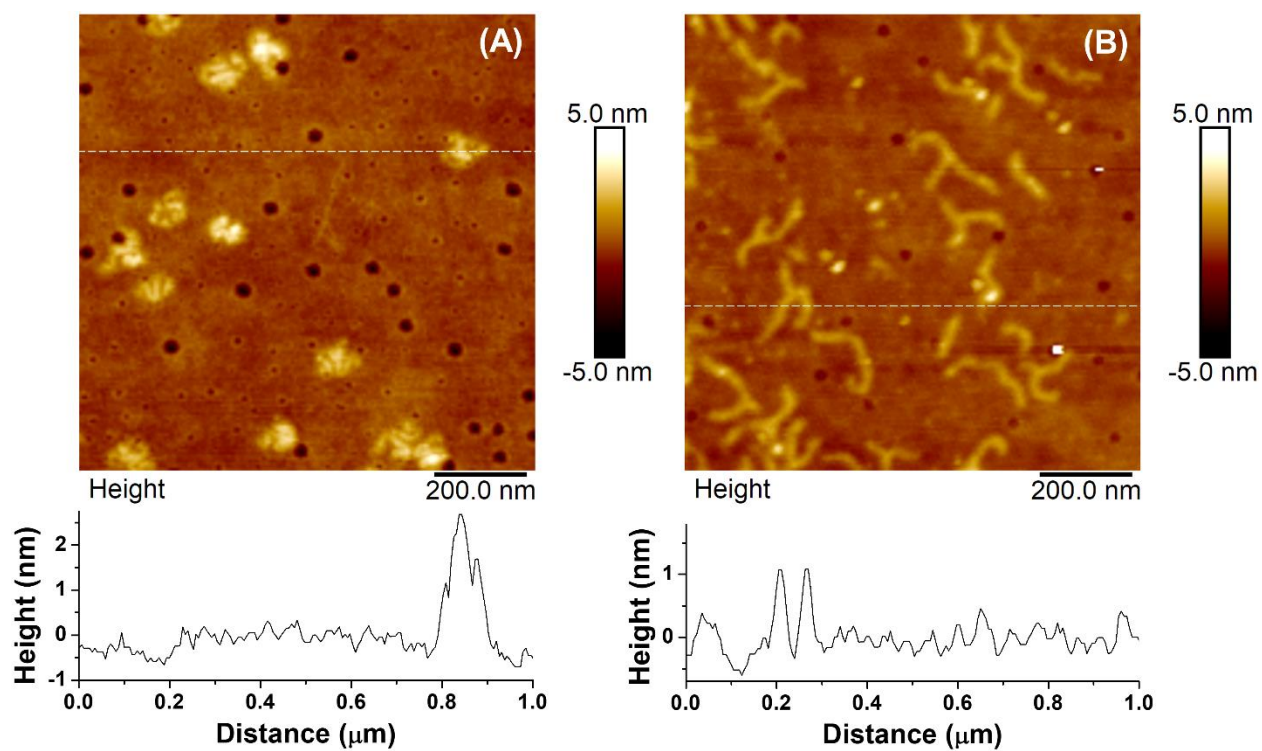


Figure 4.7. AFM height images ($1 \times 1 \mu\text{m}$) of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water with 1.5 mM NaPF_6 at 20 °C (A) and 70 °C (B), with a cross-sectional profile along the dashed line in each image. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-11 with 6 mM NaPF_6 using pH 4.50 water.

11.3 nm, and the average height from 200 globular molecules was found to be 2.9 ± 0.8 nm. In contrast, at 70 °C, extended three-arm star brushes were observed, although some molecules appeared to be broken, resulting in linear nanoobjects. The average height of 200 arms of the brushes on the substrate was 1.5 ± 0.3 nm. Measurements of 156 arms of star-shaped molecules showed an average arm length of 95.7 ± 33.4 nm, similar to the average arm length of SMB-11 from pH 4.50 water without a salt (98.4 ± 28.2 nm). We also conducted AFM of SMB-22 drop cast on glass from a 0.050 mg/g solution in pH 4.50 water with 1.0 mM NaPF₆ at 0 °C and 70 °C (Figures 4.8 and B27-B29), which was diluted from a 0.2 mg/g solution with 4 mM NaPF₆ using pH 4.50 water. Collapsed globular and ellipsoidal nanoobjects were seen, with an average height of 6.5 ± 1.4 nm (from 200 molecules). The average size of 300 nanoobjects was 40.9 ± 7.2 nm. Same as SMB-11 shown in Figure 4.7B, extended star-shaped molecules were observed (Figures 4.8B and B29), with an average height of 1.6 ± 0.3 nm. The average arm length from the measurements of 255 arms of star-shaped brushes was 92.6 ± 33.6 nm. These images confirmed the thermally induced globule-to-star shape transitions hinted from DLS studies (Figure 4.5).

4.3.5. Temperature Effects on Conformations of Star Brushes SMB-11 and -22 in the Presence of Weak Chaotropic Anion I⁻ and Kosmotropic Anion SO₄²⁻

Lastly, we investigated the effects of temperature on the conformations of SMB-11 and -22 in the presence of weak chaotropic anion I⁻ and kosmotropic anion SO₄²⁻. As shown in Figure 4.2, the addition of these two anions into 0.2 mg/g solutions of SMB-11 and -22 with a pH of 4.50 only caused small size variations, which suggested no star-to-globule shape transition. Figure 4.9A and B shows the D_h of 0.2 mg/g SMB-11 in pH 4.50 acidic water with 50 mM NaI and 0.2 mg/g SMB-22 in pH 4.50 acidic water with 10 mM NaI as a function of temperature from a heating process, respectively. For both SMB-11 and SMB-22, the apparent hydrodynamic size appears to increase

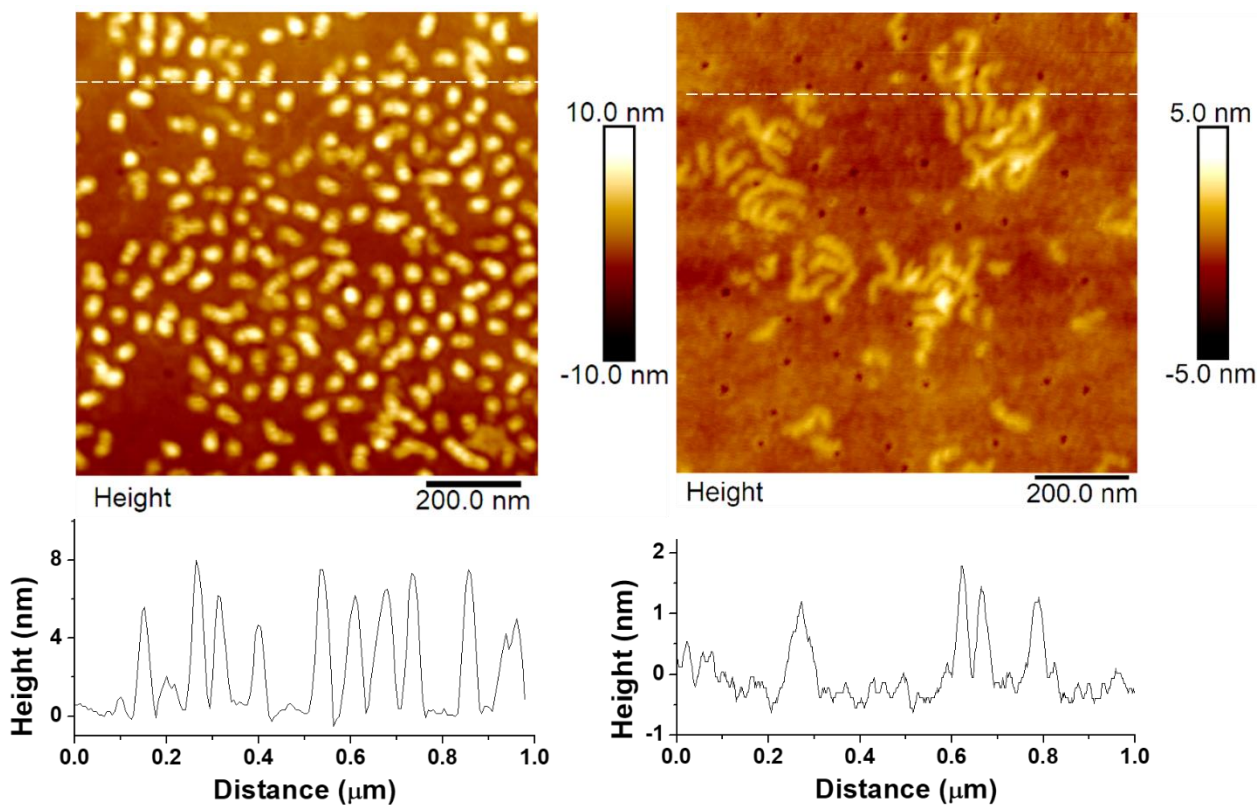


Figure 4.8. AFM height images of SMB-22 drop cast on a glass disk from a 0.050 mg/g solution of SMB-22 in pH 4.50 water with 1.0 mM NaPF_6 at 0 °C (A) and 70 °C (B), with a cross-sectional profile along the dashed line in each image. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-22 with 4 mM NaPF_6 using pH 4.50 water.

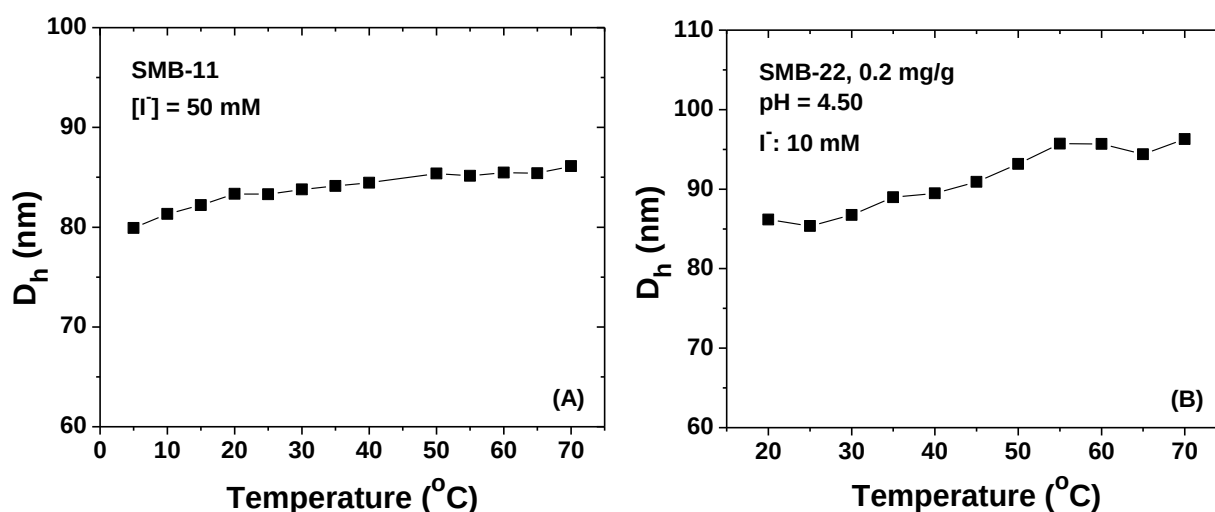


Figure 4.9. (A) Plot of apparent hydrodynamic size (D_h) of 0.2 mg/g SMB-11 in pH 4.50 water in the presence of 50 mM NaI versus temperature. (B) Plot of D_h of 0.2 mg/g SMB-22 in pH 4.50 water in the presence of 10 mM NaI versus temperature. All data points correspond to a single hydrodynamic size distribution (Figures B30 and B31). (Note that in (A) at 45 $^{\circ}\text{C}$ large species were observed and the data was not included.)

slightly with increasing temperature, but there is no sharp size transition as expected. At a lower salt concentration for SMB-22 (10 mM), the size increase was slightly larger than that for SMB-11 at a higher salt concentration (50 mM) when the temperature was raised from 20 to 70 °C. Figure 4.10A and B shows the plots of D_h of SMB-11 in the presence of 5 mM Na_2SO_4 and SMB-22 with 50 mM Na_2SO_4 versus temperature from a heating process. The hydrodynamic sizes of both brush polymers varied slightly with temperature; similar to the addition of NaI there was no abrupt size change. Here, however, the size of SMB-11 in the presence of 5 mM Na_2SO_4 showed a small decrease of ~ 3 nm from 15 to 65 °C, whereas the D_h of SMB-22 with 50 mM Na_2SO_4 increased slightly upon heating. Since each sulfate anion has a charge of 2^- and can form a bridge between two protonated tertiary amine groups, it is possible that the bridging interactions are weakened at higher temperatures, causing the size to increase slightly with temperature. However, we could not rationalize the small but noticeable decrease in size for SMB-11.

Figures 4.11 and B34-B37 show the AFM height images of SMB-11 drop cast on glass substrates from a 0.050 mg/g solution with a pH value of 4.50 in the presence 2.5 mM Na_2SO_4 at 20 and 70 °C. The solution for the preparation of AFM samples was obtained by diluting the 0.20 mg/g SMB-11 solution in the presence of 10 mM Na_2SO_4 using pH 4.50 acidic water as for the preparation of other AFM samples. At both temperatures, the brush molecules were in a three-arm star shape, as expected. The average arm length of SMB-11 molecules from the image analysis using Image J was 83.0 ± 31.1 nm at 20 °C (from 120 arms of three-arm star brushes) and 87.5 ± 30.0 nm at 70 °C from 75 arms. These two values are similar to each other but noticeably smaller than those of SMB-11 in the star state under other conditions (e.g., 95.7 ± 33.4 nm in the presence of NaPF_6 at 70 °C). The average arm height of SMB-11 was 1.6 ± 0.3 nm at 20 °C (from 200 arms) and 1.8 ± 0.5 nm at 70 °C (from 87 arms), which were also slightly larger than that of SMB-11

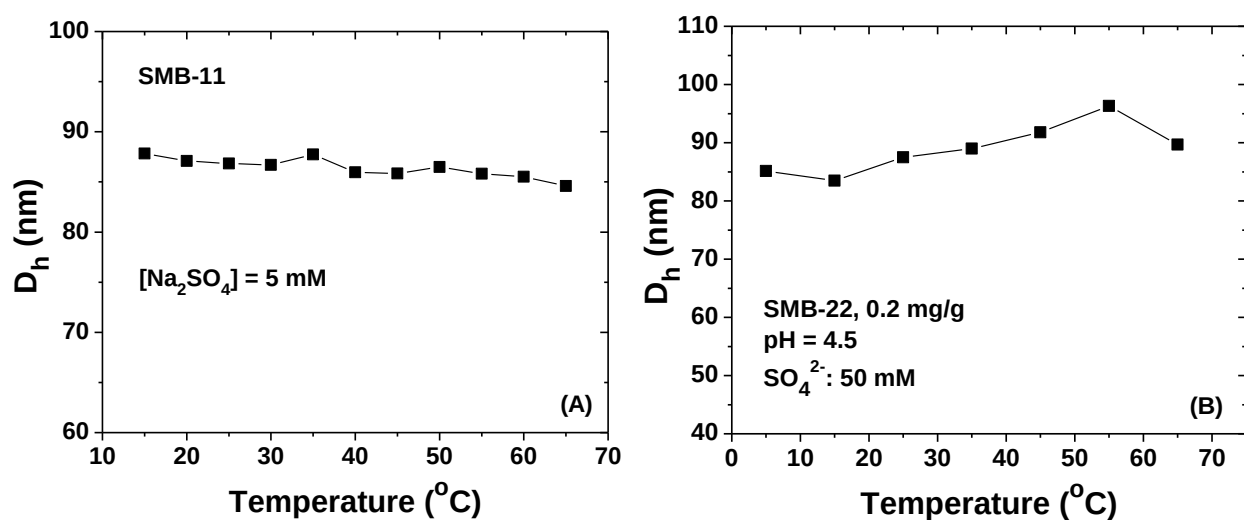


Figure 4.10. (A) Plot of apparent hydrodynamic size (D_h) of 0.2 mg/g SMB-11 in pH 4.50 water with 5 mM Na_2SO_4 versus temperature. (B) Plot of D_h of 0.2 mg/g SMB-22 in pH 4.50 acidic water with 50 mM Na_2SO_4 versus temperature. All the data points are single size distributions (Figures B32 and B33).

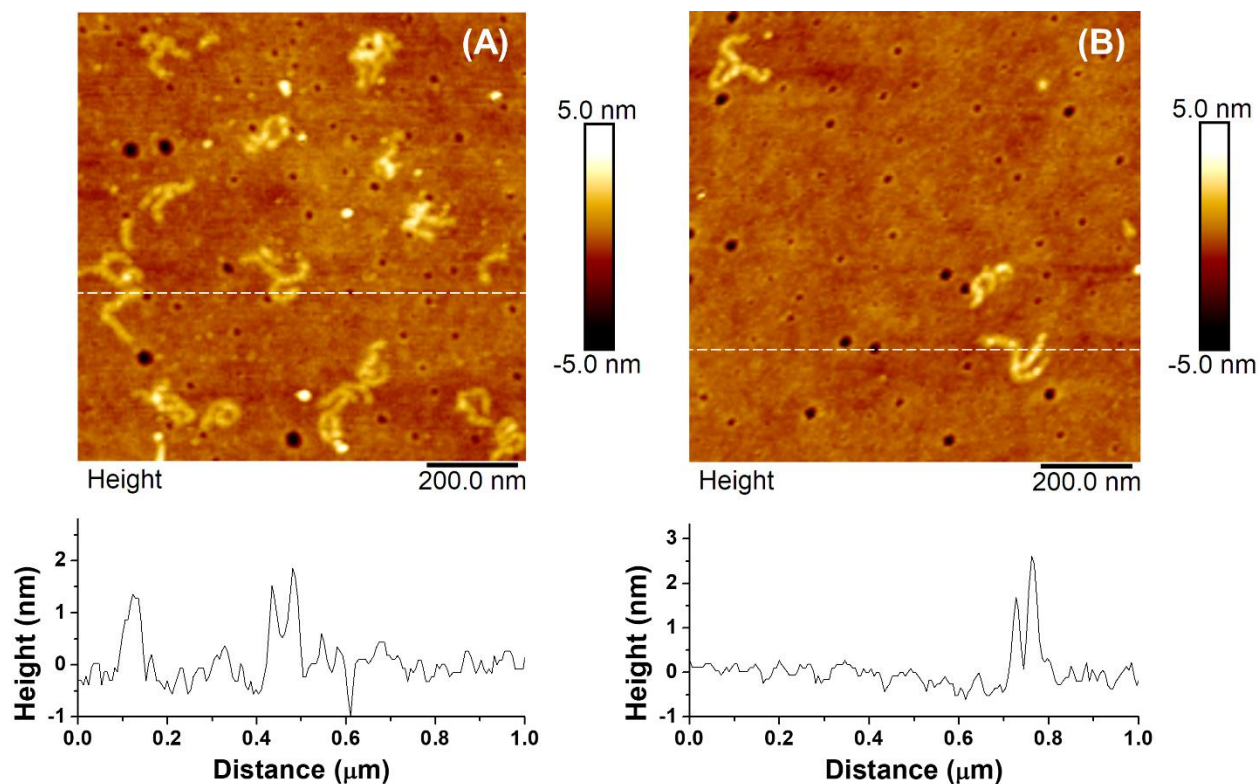


Figure 4.11. AFM height images of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water with 2.5 mM Na_2SO_4 at 20 °C (A) and 70 °C (B), with a cross-sectional profile along the dashed line in each image. The solution was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 10 mM Na_2SO_4 using pH 4.50 water.

(1.5 ± 0.3 nm) in the presence of PF_6^- at 70 °C. These observations are likely caused by the bridging interactions of divalent SO_4^{2-} anions with protonated PDMAEMA, resulting in brush shrinking.

4.4. Conclusions

In summary, we synthesized two three-arm star-shaped bottlebrush polymers heterografted with 5 kDa PEO and either PDMAEMA (for SMB-11) or PDEAEMA (for SMB-22) and investigated the effects of temperature on super and moderate CA-induced star-globule shape transitions of the two brush polymers in acidic water and the interactions of protonated bottlebrushes with weak CA I^- and kosmotropic anion SO_4^{2-} .⁵⁴ Consistent with the results previously reported from our group, the addition of super CAs, such as $[\text{Fe}(\text{CN})_6]^{3-}$ and $[\text{Fe}(\text{CN})_6]^{4-}$, and moderate CAs, such as PF_6^- and ClO_4^- , induced starlike-to-globular shape transitions of both SMB-11 and -22 at pH 4.50 at 25 °C due to the chaotropic ion pairing of protonated tertiary amine groups with CAs as well as the chaotropic effect. DLS and AFM studies showed that the globular brushes collapsed by super CAs remained in the globular state when the temperature was increased to 70 °C. In contrast, the collapsed bottlebrush molecules in the presence of moderate CAs underwent globule-to-star shape changes upon heating. On the other hand, weak CA I^- and kosmotropic anion SO_4^{2-} exerted rather small influences on the conformations of SMB-11 and -22 at 25 °C, and only small size variations were observed upon increasing temperature to 70 °C from the DLS measurements. AFM showed that SMB-11 in the presence of Na_2SO_4 remained in the star shape at both 20 and 70 °C. The findings described in this chapter might have implications in the design and fabrication of stimuli-responsive drug delivery systems. For example, many drugs are charged (e.g., thiosalicylate) and might be chaotropic ions because of their large sizes and low charge densities. Such drug molecules might be able to induce shape changes of molecular bottlebrushes and the collapsed globular brushes might be

thermoreponsive in aqueous solution, which could be utilized to develop stimuli-responsive delivery systems.

References:

1. Zhang, M. F.; Müller, A. H. E. Cylindrical Polymer Brushes. *J. Polym. Sci. Part A: Polym. Chem.* **2005**, *43*, 3461-3481.
2. Sheiko, S. S.; Sumerlin, B. S.; Matyjaszewski, K. Cylindrical Molecular Brushes: Synthesis, Characterization, and Properties. *Prog. Polym. Sci.* **2008**, *33*, 759-785.
3. Lee, H.-I.; Pietrasik, J.; Sheiko, S. S.; Matyjaszewski, K. Stimuli-Responsive Molecular Brushes. *Prog. Polym. Sci.* **2010**, *35*, 24-44.
4. Xie, G.; Martinez, M. R.; Olszewski, M.; Sheiko, S. S.; Matyjaszewski, K. Molecular Bottlebrushes as Novel Materials. *Biomacromolecules* **2019**, *20*, 27-54.
5. Sheiko, S. S.; Zhou, J.; Arnold, J.; Neugebauer, D.; Matyjaszewski, K.; Tsitsilianis, C.; Tsukruk, V. V.; Carrillo, J.-M. Y.; Dobrynin, A. V.; Rubinstein, M. Perfect Mixing of Immiscible Macromolecules at Fluid Interfaces. *Nature Mater.* **2013**, *12*, 735-740.
6. Daniel, W. F.; Burdyska, J.; Vatankhah-Varnoosfaderani, M.; Matyjaszewski, K.; Paturej, J.; Rubinstein, M.; Dobrynin, A. V.; Sheiko, S. S. Solvent-Free, Supersoft and Superelastic Bottlebrush Melts and Networks. *Nat. Mater.* **2016**, *15*, 183-189.
7. Johnson, J. A.; Lu, Y. Y.; Burts, A. O.; Xia, Y.; Durrell, A. C.; Tirrell, D. A.; Grubbs, R. H. Drug-Loaded, Bivalent-Bottle-Brush Polymers by Graft-through ROMP. *Macromolecules* **2010**, *43*, 10326–10335.
8. Banquy, X.; Burdyńska, J.; Lee, D. W.; Matyjaszewski, K.; Israelachvili, J. Bioinspired Bottle-Brush Polymer Exhibits Low Friction and Amontons-Like Behavior. *J. Am. Chem. Soc.* **2014**, *136*, 6199 -6202.
9. Liberman-Martin, A. L.; Chu, C. K.; Grubbs, R. H. Application of Bottlebrush Block Copolymers as Photonic Crystals. *Macromol. Rapid Commun.* **2017**, *38*, 1700058.

10. Qi, H.; Liu, X. T.; Henn, D. M.; Mei, S.; Staub, M. C.; Zhao, B.; Li, C. Y. Breaking Translational Symmetry via Polymer Chain Overcrowding in Molecular Bottlebrush Crystallization. *Nature Communications* **2020**, *11*, 2152.
11. Sheiko, S. S.; Prokhorova, S. A.; Beers, K. L.; Matyjaszewski, K.; Potemkin, I. I.; Khokhlov, A. R.; Möller, M. Single Molecule Rod–Globule Phase Transition for Brush Molecules at a Flat Interface. *Macromolecules* **2001**, *34*, 8354–8360.
12. Boyce, J. R.; Shirvanyants, D.; Sheiko, S. S.; Ivanov, D. A.; Qin, S. H.; Börner, H. G.; Matyjaszewski, K. Multiarm Molecular Brushes: Effect of the Number of Arms on the Molecular Weight Polydispersity and Surface Ordering. *Langmuir*, **2004**, *20*, 6005–6011.
13. Gallyamov, M. O.; Tartsch, B.; Khokhlov, A. R.; Sheiko, S. S.; Börner, H. G.; Matyjaszewski, K.; Möller, M. Reversible Collapse of Brushlike Macromolecules in Ethanol and Water Vapours as Revealed by Real-Time Scanning Force Microscopy. *Chem. Eur. J.*, **2004**, *10*, 4599–4605.
14. Li, C.; Gunari, N.; Fischer, K.; Janshoff, A.; Schmidt, M. New Perspectives for the Design of Molecular Actuators: Thermally Induced Collapse of Single Macromolecules from Cylindrical Brushes to Spheres. *Angew. Chem. Int. Ed.*, **2004**, *43*, 1101–1104.
15. Lee, H.-i.; Pietrasik, J.; Matyjaszewski, K. Phototunable Temperature-Responsive Molecular Brushes Prepared by ATRP. *Macromolecules*, **2006**, *39*, 3914–3920.
16. Yamamoto, S.-i.; Pietrasik, J.; Matyjaszewski, K. ATRP Synthesis of Thermally Responsive Molecular Brushes from Oligo(ethylene oxide) Methacrylates. *Macromolecules*, **2007**, *40*, 9348–9353.

17. Pietrasik, J.; Sumerlin, B. S.; Lee, R. Y.; Matyjaszewski, K. Solution Behavior of Temperature-Responsive Molecular Brushes Prepared by ATRP. *Macromol. Chem. Phys.*, **2007**, *208*, 30–36.
18. Lee, H.-i.; Boyce, J. R.; Nese, A.; Sheiko, S. S.; Matyjaszewski, K. pH-Induced Conformational Changes of Loosely Grafted Molecular Brushes Containing Poly(acrylic acid) Side Chains. *Polymer*, **2008**, *49*, 5490–5496.
19. Li, X.; ShamsiJazeyi, H.; Pesek, S. L.; Agrawal, A.; Hammouda, B.; Verduzco, R. Thermoresponsive PNIPAAAM Bottlebrush Polymers with Tailored Side-Chain Length and End-Group Structure. *Soft Matter*, **2014**, *10*, 2008–2015.
20. Kutnyanszky, E.; Hempenius, M. A.; Vancso, G. J. Polymer Bottlebrushes with a Redox Responsive Backbone Feel the Heat: Synthesis and Characterization of Dual Responsive Poly(ferrocenylsilane)s with PNIPAM Side Chains. *Polym. Chem.*, **2014**, *5*, 771–783.
21. Zhu, X. M.; Zhang, J.; Miao, C.; Li, S. Y.; Zhao, Y. L. Synthesis, Thermoresponsivity and Multi-Tunable Hierarchical Self-Assembly of Multi-Responsive (AB)_mC Miktobrush-Coil Terpolymers. *Polym. Chem.* **2020**, *11*, 3003–3017.
22. Xu, Y. Y.; Bolisetty, S.; Drechsler, M.; Fang, B.; Yuan, J.; Ballauff, M.; Müller, A. X. E. pH and Salt Responsive Poly(*N,N*-dimethylaminoethyl methacrylate) Cylindrical Brushes and Their Quaternized Derivatives. *Polymer*, **2008**, *49*, 3957–3964.
23. Xu, Y. Y.; Bolisetty, S.; Ballauff, M.; Müller, A. X. E. Switching the Morphologies of Cylindrical Polycation Brushes by Ionic and Supramolecular Inclusion Complexes *J. Am. Chem. Soc.*, **2009**, *131*, 1640–1641.
24. Xu, Y. Y.; Borisov, O. V.; Ballauff, M.; Müller, A. X. E. Manipulating the Morphologies of Cylindrical Polyelectrolyte Brushes by Forming Interpolyelectrolyte Complexes with

- Oppositely Charged Linear Polyelectrolytes: An AFM Study. *Langmuir* **2010**, *26*, 6919–6926.
25. Xu, Y. Y.; Bolisetty, S.; Drechsler, M.; Fang, B.; Yuan, J.; Harnau, L.; Ballauff, M.; Müller, A. H. E. Manipulating cylindrical polyelectrolyte brushes on the nanoscale by counterions: collapse transition to helical structures. *Soft Matter*, **2009**, *5*, 379–384.
26. Gunari, N.; Cong, Y.; Zhang, B.; Fischer, K.; Janshoff, A.; Schmidt, M. Surfactant-Induced Helix Formation of Cylindrical Brush Polymers with Poly(L-lysine) Side Chains. *Macromol. Rapid Commun.*, **2008**, *29*, 821–825.
27. Cong, Y.; Gunari, N.; Zhang, B.; Janshoff, A.; Schmidt, M. Hierarchical Structure Formation of Cylindrical Brush Polymer–Surfactant Complexes. *Langmuir*, **2009**, *25*, 6392–6397.
28. 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 Wormlike and Stable Globular Conformations. *Macromolecules*, **2017**, *50*, 1645–1656.
29. Henn, D. M.; Lau, C. M.; Li, C. Y.; Zhao, B. Light-Triggered Unfolding of Single Linear Molecular Bottlebrushes from Compact Globular to Wormlike Nano-objects in Water. *Polym. Chem.* **2017**, *8*, 2702–2712.
30. Henn, D. M.; Holmes, J. A.; Kent, E. W.; Zhao, B. Worm-to-Sphere Shape Transition of Thermoresponsive Linear Molecular Bottlebrushes in Moderately Concentrated Aqueous Solution. *J. Phys. Chem. B*, **2018**, *122*, 7015–7025.
31. Kent, E. W.; Henn, D. M.; Zhao, B. Shape-Changing Linear Molecular Bottlebrushes with Dually pH- and Thermo-Responsive Diblock Copolymer Side Chains. *Polym. Chem.*, **2018**, *9*, 5133–5144.

32. 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.
33. Kent, E. W.; Lewoczko, E. M.; Zhao, B. pH- and Chaotropic Anion-Induced Conformational Changes of Tertiary Amine-Containing Binary Heterografted Star Molecular Bottlebrushes in Aqueous Solution. *Polym. Chem.* **2021**, *12*, 265-276.
34. Collins, K. D. Charge Density-Dependent Strength of Hydration and Biological Structure. *Biophys. J.* **1997**, *72*, 65-76.
35. Marcus, Y. Effect of Ions on the Structure of Water: Structure Making and Breaking. *Chem. Rev.* **2009**, *109*, 1346–1370.
36. Marcus, Y.; Hefter, G. Ion Pairing. *Chem. Rev.*, **2006**, *106*, 4585-4621.
37. Assaf, K. I.; Nau, W. M. The Chaotropic Effect as an Assembly Motif in Chemistry. *Angew. Chem. Int. Ed.* **2018**, *57*, 13968–13981.
38. Liu, L. D.; Kou, R.; Liu, G. M. Ion Specificities of Artificial Macromolecules. *Soft Matter*, **2017**, *13*, 68-80.
39. Plamper, F. A.; Schmalz, A.; Ballauff, M.; Müller, A. H. E. Tuning the Thermoresponsiveness of Weak Polyelectrolytes by pH and Light: Lower and Upper Critical-Solution Temperature of Poly(*N,N*-dimethylaminoethyl methacrylate). *J. Am. Chem. Soc.* **2007**, *129*, 14538-14539.
40. Zhang, Q.; Tosi, F.; Ügdüler, Maji, S. S.; Hoogenboom, R. Tuning the LCST and UCST Thermoresponsive Behavior of Poly(*N,N*-dimethylaminoethyl methacrylate) by Electrostatic Interactions with Trivalent Metal Hexacyano Anions and Copolymerization. *Macromol. Rapid Commun.* **2015**, *36*, 633–639.

41. Flemming, P.; Müller, M.; Fery, A.; Münch, A. S.; Uhlmann, P. Mechanistic Investigation of the Counterion-Induced UCST Behavior of Poly(*N,N*-dimethylaminoethyl methacrylate) Polymer Brushes. *Macromolecules* **2020**, *53*, 1957-1966.
42. Karjalainen, E.; Aseyev, V.; Tenhu, H. Counterion-Induced UCST for Polycations. *Macromolecules* **2014**, *47*, 7581–7587.
43. Cao, X.; An, Z. RAFT Synthesis in Water of Cationic Polyelectrolytes with Tunable UCST. *Macromol. Rapid Commun.* **2015**, *36*, 2107–2110.
44. Han, D.; Tong, X.; Zhao, Y. One-Pot Synthesis of Brush Diblock Copolymers through Simultaneous ATRP and Click Coupling. *Macromolecules*, **2011**, *44*, 5531–5536.
45. Tang, H.; Li, Y.; Lahasky, S. H.; Sheiko, S. S.; Zhang, D. H. Core–Shell Molecular Bottlebrushes with Helical Polypeptide Backbone: Synthesis, Characterization, and Solution Conformations. *Macromolecules*, **2011**, *44*, 1491–1499.
46. Zhao, P.; Yan, Y.; Feng, X.; Liu, L.; Wang, C.; Chen, Y. Highly efficient synthesis of polymer brushes with PEO and PCL as side chains via click chemistry. *Polymer*, **2012**, *53*, 1992-2000.
47. Luo, H.; Szymusiak, M.; Garcia, E. A.; Lock, L. L.; Cui, H.; Liu, Y.; Herrera-Alonso, M. Solute-Triggered Morphological Transitions of an Amphiphilic Heterografted Brush Copolymer as a Single-Molecule Drug Carrier. *Macromolecules*, **2017**, *50*, 2201–2206.
48. Kent, E. W.; Lewoczko, E. M.; Zhao, B. Effect of Buffer Anions on Pearl-Necklace Morphology of Tertiary Amine-Containing Binary Heterografted Linear Molecular Bottlebrushes in Acidic Aqueous Buffers. *Langmuir*, **2020**, *36*, 13320-13330.

49. Zhou, K. J.; Wang, Y. G.; Huang, X. N.; Luby-Phelps, K.; Sumer, B. D.; Gao, J. M. Tunable, Ultrasensitive pH-Responsive Nanoparticles Targeting Specific Endocytic Organelles in Living Cells. *Angew. Chem. Int. Ed.*, **2011**, 50, 6109–6114.
50. Henn, D. M.; Wright, R. A. E.; Woodcock, J. W.; Hu, B.; Zhao, B. Tertiary-Amine-Containing Thermo- and pH-Sensitive Hydrophilic ABA Triblock Copolymers: Effect of Different Tertiary Amines on Thermally Induced Sol–Gel Transitions. *Langmuir*, **2014**, 30, 2541–2550.
51. Li, Y.; Wang, Y. G.; Huang, G.; Ma, X. P.; Zhou, K. J.; Gao, J. M. Chaotropic-Anion-Induced Supramolecular Self-Assembly of Ionic Polymeric Micelles. *Angew. Chem. Int. Ed.* **2014**, 53, 8074 –8078.
52. Wang, L.-H.; Zhang, Z.-D.; Hong, C.-Y.; He, X.-H.; You, W.; You, Y.-Z. Anion-Dipole Interactions Make the Homopolymers Self-Assemble into Multiple Nanostructures. *Adv. Mater.*, **2015**, 27, 3202–3207.
53. Li, D. J.; Dunlap, J. R.; Zhao, B. Thermosensitive Water-Dispersible Hairy Particle-Supported Pd Nanoparticles for Catalysis of Hydrogenation in an Aqueous/Organic Biphasic System. *Langmuir* **2008**, 24, 5911-5918.
54. The work presented in this chapter has been submitted for publication. Ethan W. Kent synthesized the azide-functionalized star backbone polymer. Michael T. Kelly obtained the AFM images of SMB-22 in the presence of NaPF₆. I synthesized and characterized the remainder of the polymers and systems.

Appendix B

for

**Chapter 4: Effects of Temperature on Chaotropic Anion-Induced Shape Transitions of
Star Molecular Bottlebrushes with Heterografted Poly(ethylene oxide) and Poly(*N,N*-
dialkylaminoethyl methacrylate) Side Chains in Acidic Aqueous Solutions**

B.1. Calculation of Grafting Density of SMB-11 and -22

The side chain composition of purified SMB-11 was determined by ^1H NMR spectroscopy analysis using the integrals of the peaks at 4.15 – 3.96 (-COOCH₂- of PDMAEMA) and at 3.38 ppm (-OCH₃ of PEO). Taking the DP of PDMAEMA (DP = 56) into the account, the number ratio (molar ratio) of PDEAEMA and PEO side chains in SMB-11 was determined to be 43.7% : 56.3%. For SMB-22, the integrals of the peaks at 4.16 – 3.87 ppm (-COOCH₂- of PDEAEMA) and at 3.38 ppm (-OCH₃ of PEO) were used along with the DP of PDEAEMA (DP = 53). The molar ratio of PDEAEMA and PEO side chains in SMB-22 was determined to be 41.8% : 58.2%.

The grafting density of SMB-11 was calculated to be 86.7% by using the SEC peak area fractions of the star brush polymer and the remaining unreacted side chains in the final reaction mixture, the feed masses, and the side chain composition of purified SMB-11 as detailed below. The peak area percentages of the brush polymer and the unreacted side chains from the SEC analysis of the final reaction mixture were 79.9% and 20.1%, respectively, which are related to the mass percentages of the brushes and the side chain polymers. The M_n value of PDMAEMA calculated from the DP, including end groups, was 9010 g/mol, while the M_n for PEO with end groups was 5100 g/mol. Thus, based on the side chain molar composition of SMB-11 from ^1H NMR spectroscopy analysis (43.7% for PDMAEMA and 56.3% for PEO), the mass percentages of PDMAEMA and PEO were calculated to be 57.8% and 42.2%, respectively. To determine the total mass of the reacted side chain polymers, the sum of the feed masses of backbone and side chain polymers was multiplied by the SEC area fraction of the brush polymer (79.9%), from which the mass of the backbone polymer was subtracted. This gave the total mass of 147.2 mg for the reacted side chain polymers. Therefore, the masses of reacted PDMAEMA and PEO were 85.1 mg and 62.1 mg, respectively. Using the aforementioned M_n values of PDMAEMA and PEO, the total

number of moles of the reacted side chain polymers was 2.16×10^{-5} mol. The number of moles of the azide groups on PHEMA-N₃ in the feed was 2.49×10^{-5} mol, assuming that the azide functionalization of PHEMA-N₃ was 100%. The grafting density of SMB-11 was calculated by dividing the number of moles of reacted side chain polymers by the number of moles of azide groups, which gave 86.7%. Using the same method, the grafting density of SMB-22 was calculated to be 83.9%.

B.2. Calculation of Weight-Average Molecular Weights of SMB-11 and -22

The calculated weight average molecular weight of SMB-11 was found to be 9.31×10^6 g/mol. The value was obtained by adding together the weight-average molecular weights of the backbone and all the grafted PDMAEMA and PEO side chains. The weight-average molecular weight of the backbone was calculated to be 3.65×10^5 g/mol by multiplying the M_n , calculated using the total DP of the backbone and including the molar mass of the trifunctional initiator, by the dispersity of azide backbone polymer PHEMA-N₃ ($\bar{D} = 1.16$). The calculated weight-average molecular weight of PDMAEMA from the DP and $\bar{D}$ was 1.13×10^4 g/mol ($M_n \times 1.25$), and the calculated weight average molecular weight of PEO was 5.3×10^3 g/mol). The grafting density of SMB-11 was 86.7%, and the brushes were composed of 43.7% for PDMAEMA and 56.3% for PEO as detailed earlier. Therefore, there were 569 PDMAEMA chains and 733 PEO chains grafted in each SMB-11 brush molecule, and the calculated weight average molecular weight of SMB-11 was 9.31×10^6 g/mol. Using the same method, the calculated average molecular weight of SMB-22 was 9.17×10^6 g/mol.

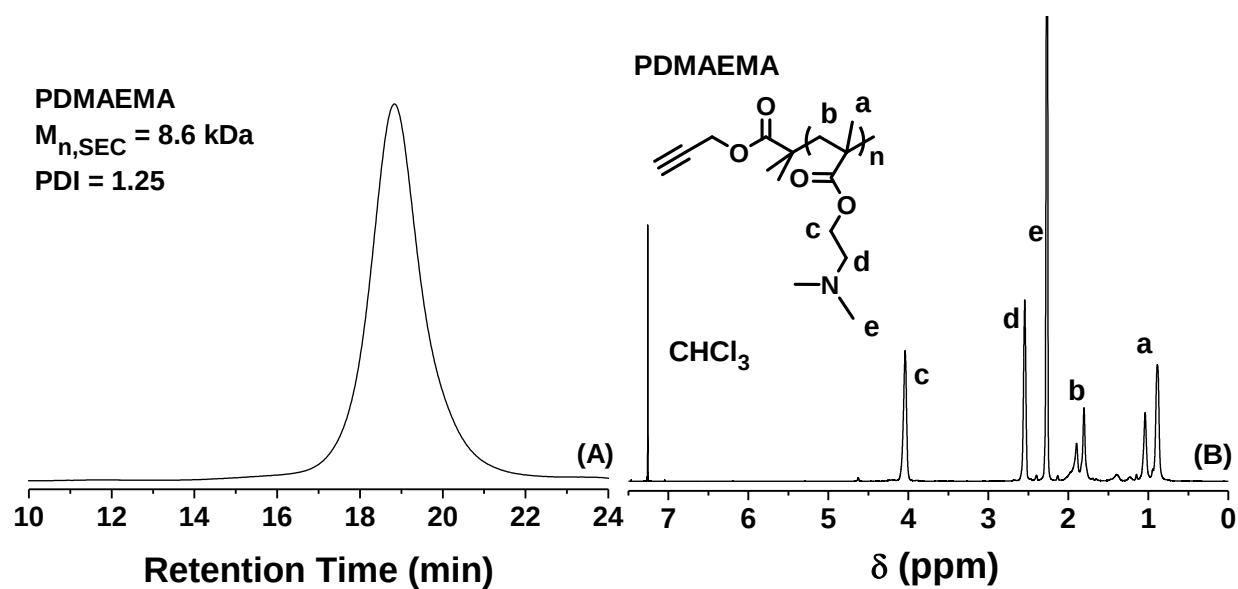


Figure B1. (A) Size exclusion chromatography (SEC) trace and (B) ^1H NMR spectrum in CDCl_3 of alkyne-end-functionalized poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA) with a degree of polymerization of 56. The SEC chromatogram was obtained from a SEC system equipped with GRAL columns using DMF with 50 mM LiBr as eluent.

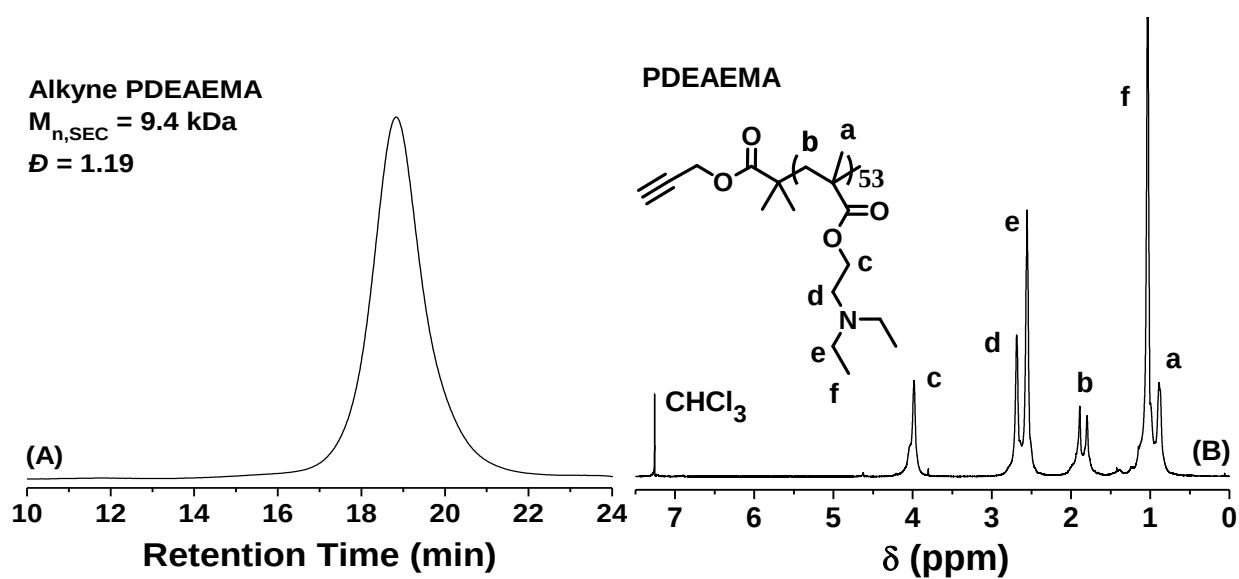


Figure B2. Size exclusion chromatograph (SEC) trace (A) and ^1H NMR spectrum in CDCl_3 (B) of alkyne-end-functionalized PDEAEMA with a degree of polymerization (DP) of 53. The SEC chromatogram was obtained from a SEC system equipped with GRAL columns using DMF with 50 mM LiBr as eluent.

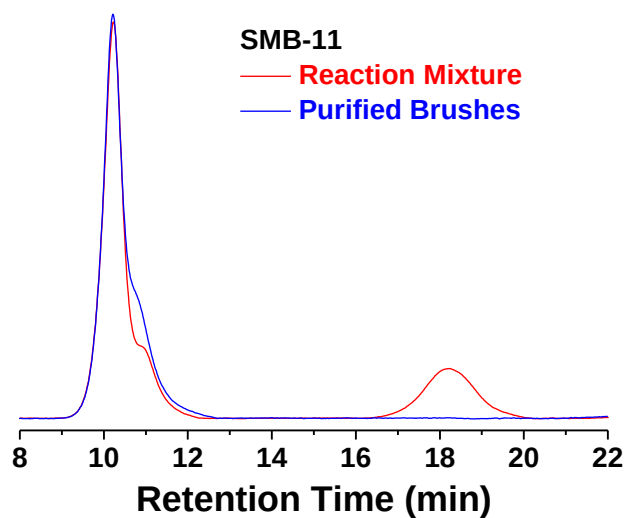


Figure B3. Size exclusion chromatography (SEC) curves of the reaction mixture at the end of the reaction for the synthesis of SMB-11 (red) and the purified brushes, SMB-11 (blue), obtained using a SEC system equipped with GRAL columns and *N,N*-dimethylformamide containing 50 mM LiBr as the eluent.

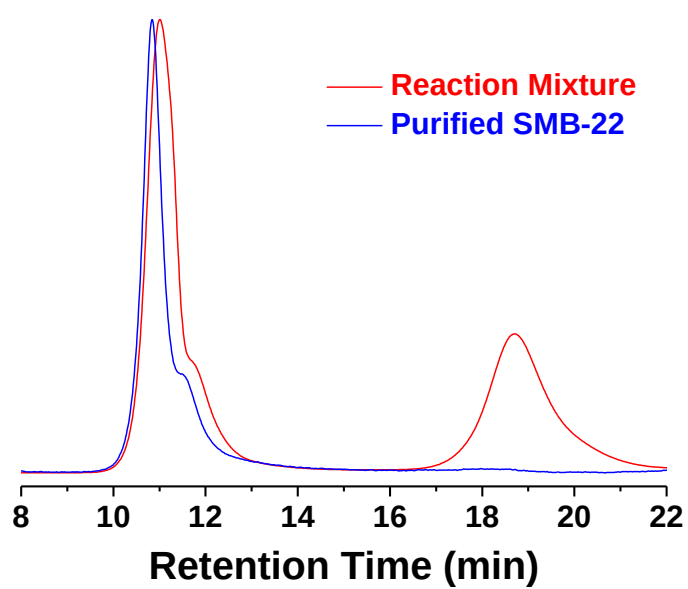


Figure B4. Size exclusion chromatography (SEC) curves of the final reaction mixture for the synthesis of SMB-22 (red) and the purified brushes, SMB-22 (blue), obtained using a SEC system equipped with GRAL columns and *N,N*-dimethylformamide containing 50 mM LiBr as the eluent.

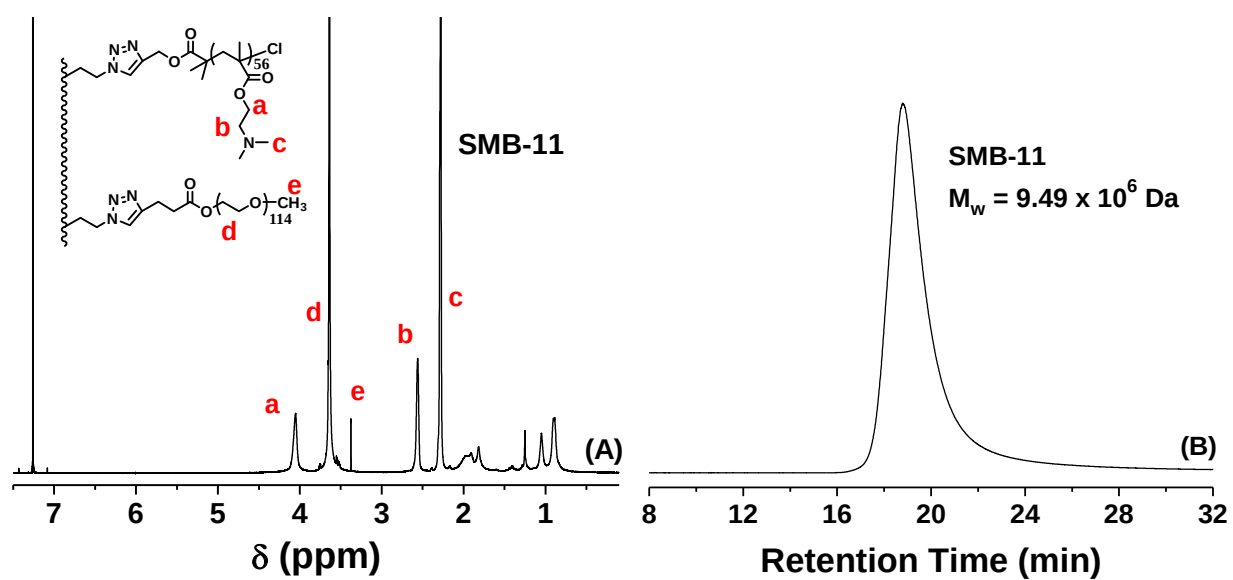


Figure B5. (A) ^1H NMR spectrum of purified SMB-11 in CDCl_3 . (B) SEC trace of purified SMB-11 from a GPC-MALS system using DMF with 0.10 M LiBr as the eluent.

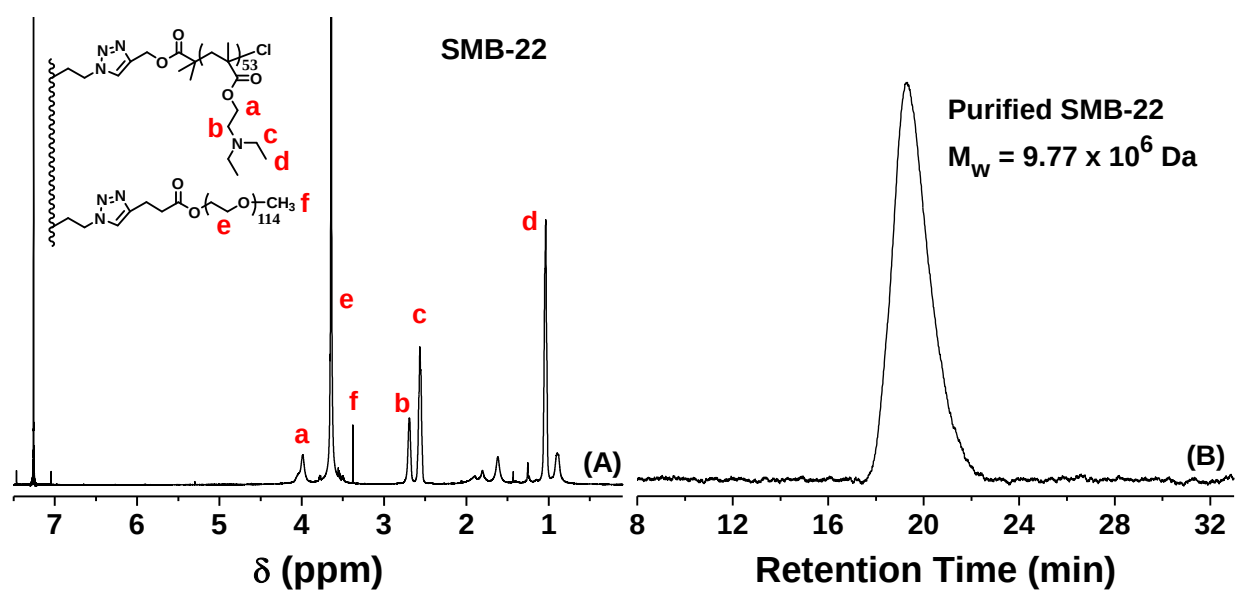


Figure B6. (A) ^1H NMR spectrum of purified SMB-22 in CDCl_3 and (B) SEC trace of purified SMB-22 obtained from a GPC-MALS system using DMF with 0.10 M LiBr as the eluent.

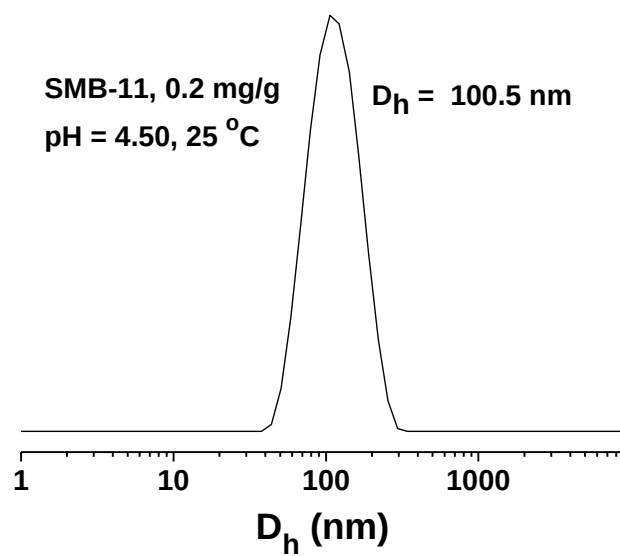


Figure B7. Hydrodynamic size distribution of SMB-11 in an acidic water with a pH of 4.50 at a concentration of 0.2 mg/g obtained from dynamic light scattering (DLS) measurements at 25 °C.

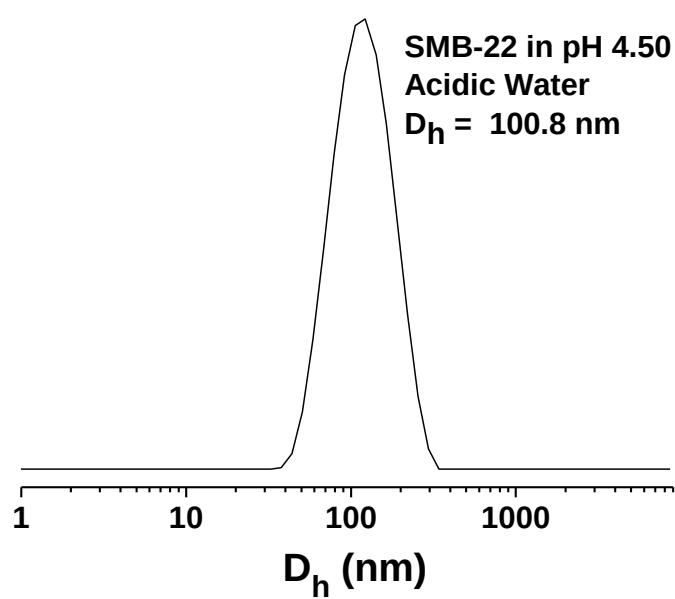


Figure B8. Hydrodynamic size distribution of SMB-22 in pH 4.50 acidic water at a concentration of 0.2 mg/g obtained from dynamic light scattering (DLS) measurements at 25 °C.

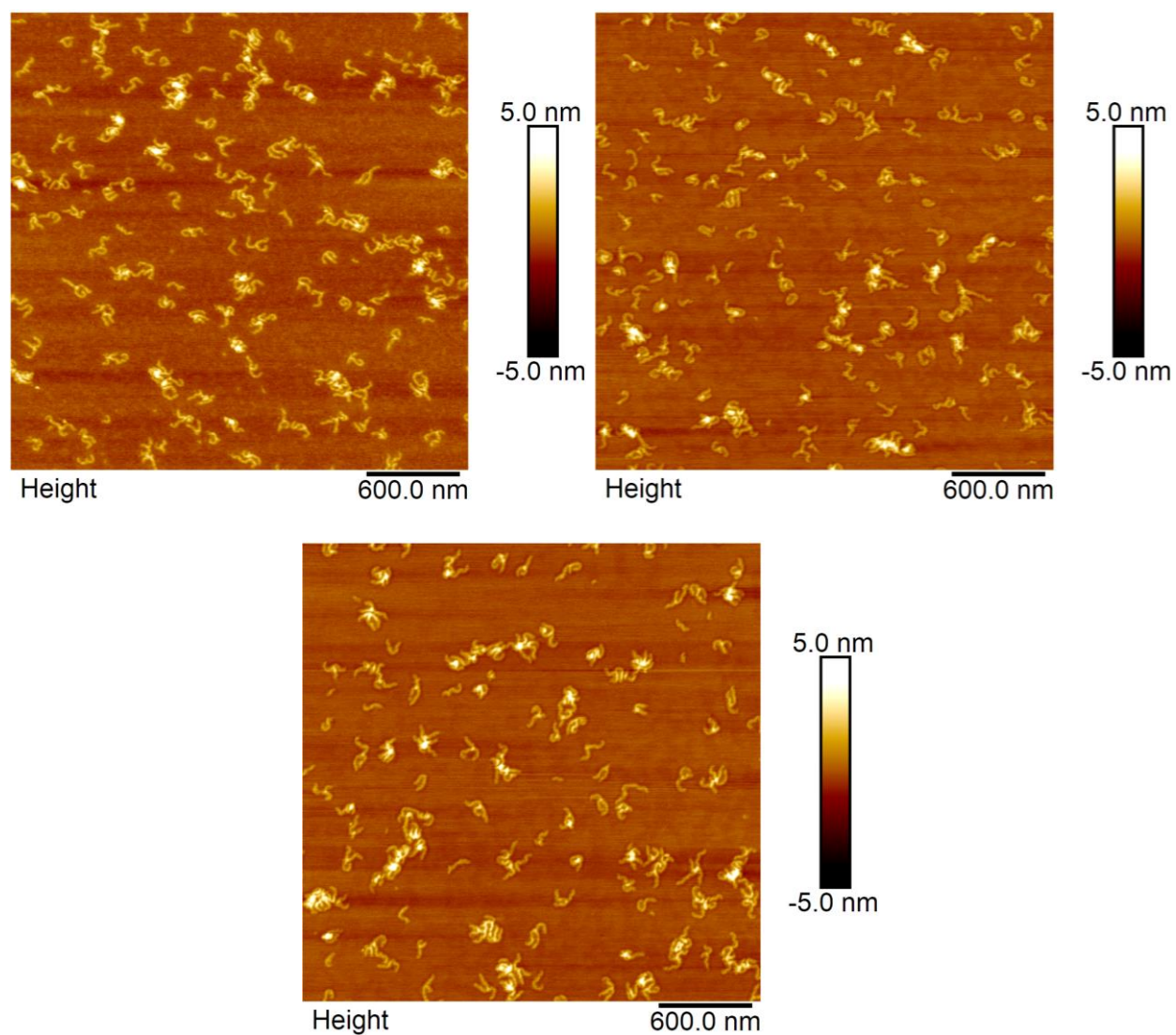


Figure B9. Atomic force microscopy (AFM) height images ($3 \times 3 \mu\text{m}$) of SMB-11 spin cast onto bare mica from THF at a concentration of 0.05 mg/g at room temperature ($\sim 20^\circ\text{C}$).

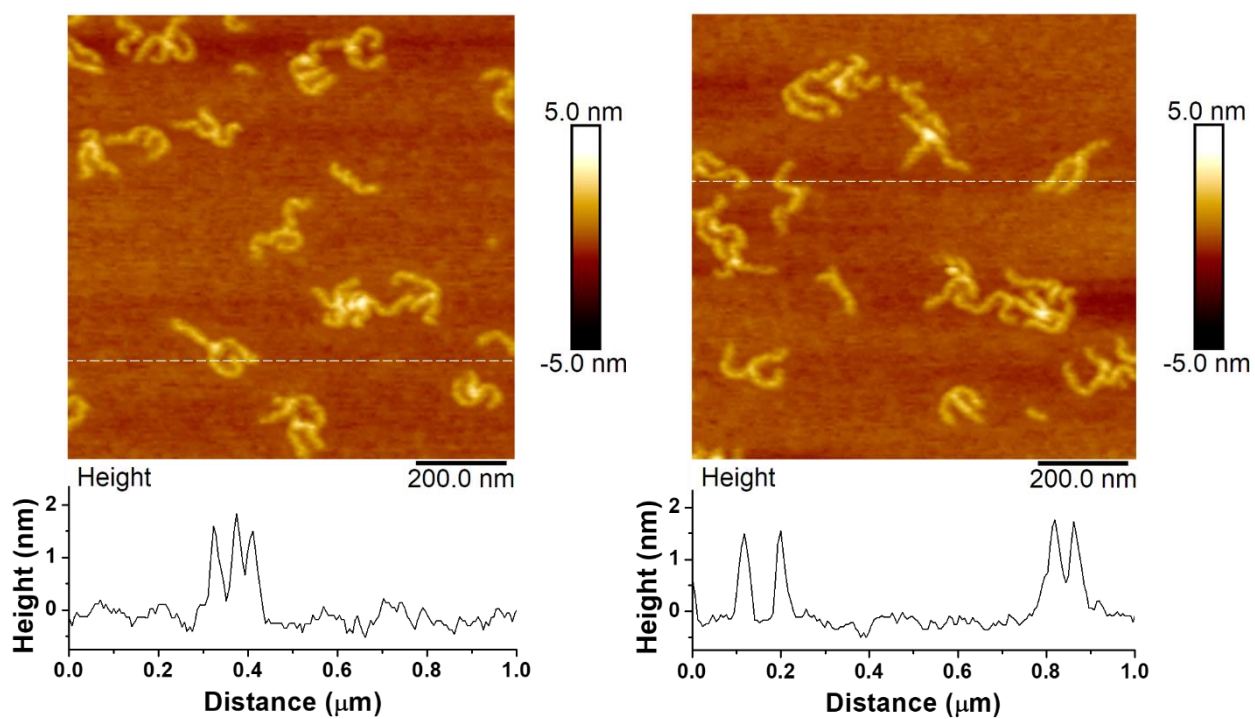


Figure B10. Atomic force microscopy (AFM) height images ($1 \times 1 \mu\text{m}$) of SMB-11 spin cast onto bare mica from THF at a brush polymer concentration of 0.05 mg/g at room temperature ($\sim 20^\circ\text{C}$), with a cross-sectional height profile corresponding to the dashed line in each image.

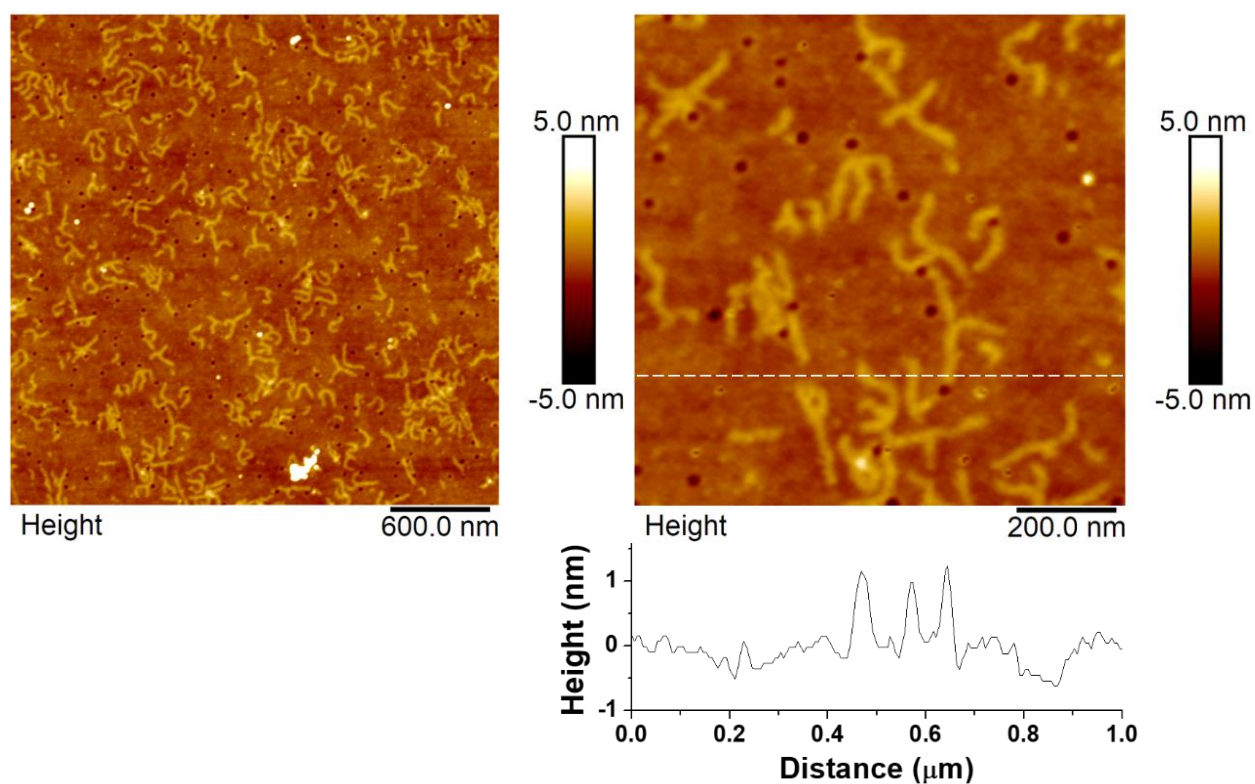


Figure B11. Atomic force microscopy (AFM) height images of SMB-11 drop cast on glass disks from a 0.050 mg/g SMB-11 solution in pH 4.50 acidic water at room temperature ($\sim 20\text{ }^{\circ}\text{C}$), with a cross-sectional profile along the dashed line of the $1 \times 1\text{ }\mu\text{m}$ image.

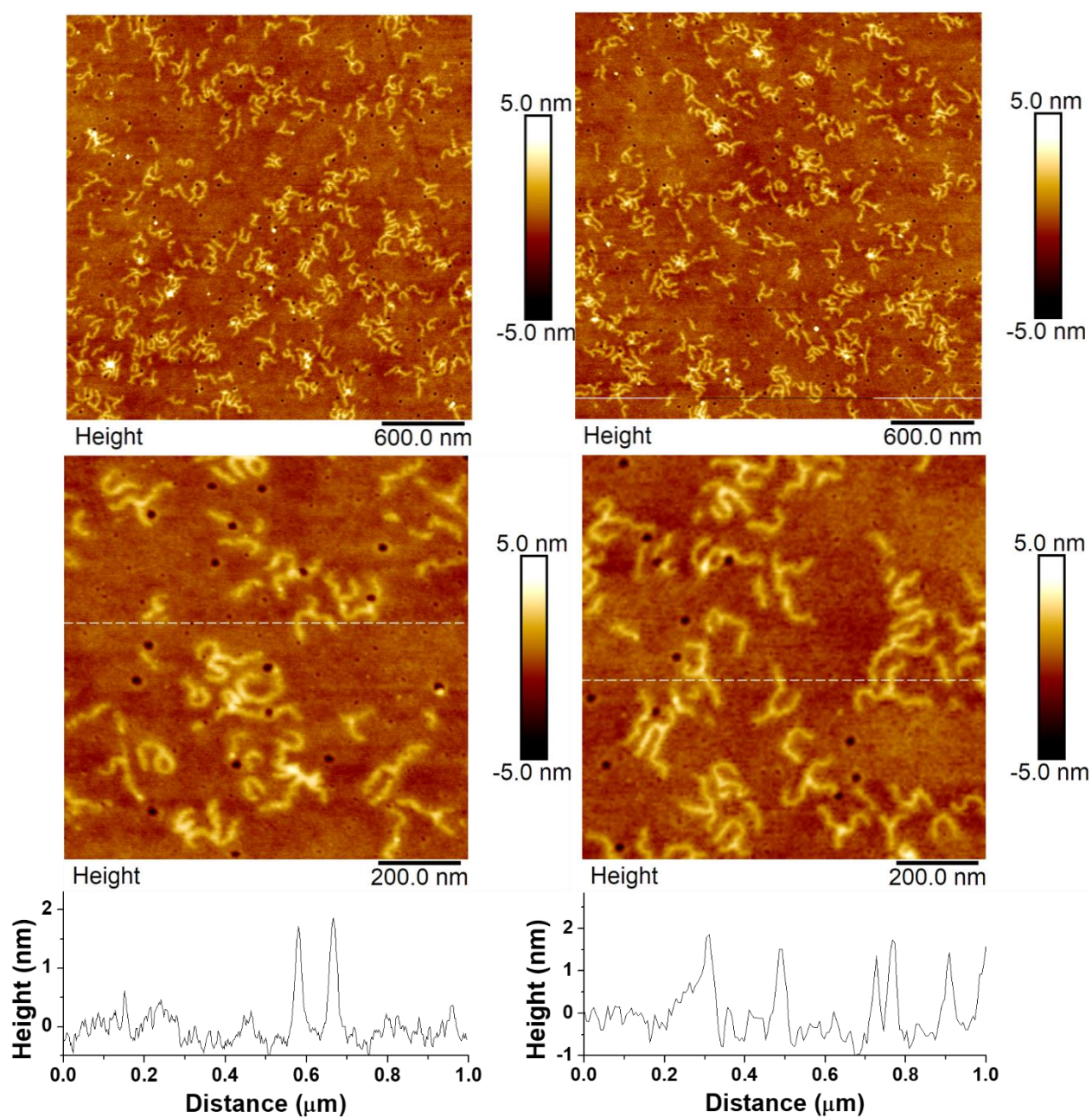


Figure B12. Atomic force microscopy (AFM) height images of SMB-22 drop cast on glass disks from a 0.050 mg/g SMB-22 solution in pH 4.50 acidic water at room temperature ($\sim 20^\circ\text{C}$), with a cross-sectional profile along the dashed line in each of the bottom $1 \times 1 \mu\text{m}$ images.

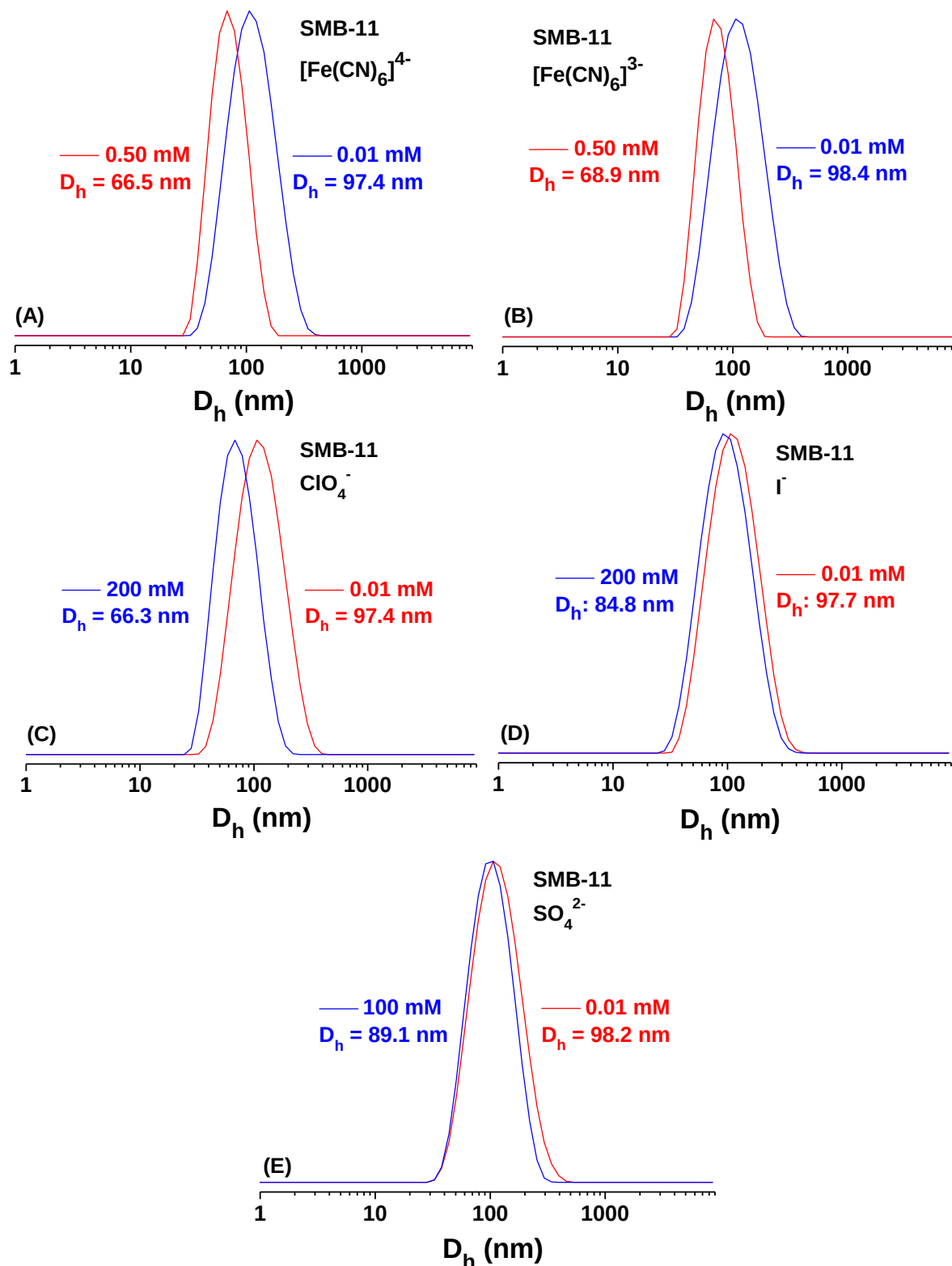


Figure B13. Hydrodynamic size distributions of 0.2 mg/g SMB-11 in pH 4.50 acidic water in the presence of $\text{Na}_4[\text{Fe}(\text{CN})_6]$ (A), $\text{K}_3[\text{Fe}(\text{CN})_6]$ (B), NaClO_4 (C), NaI (D), and Na_2SO_4 (E) at one low and one high concentration, obtained from DLS measurements at 25 °C. These size distributions are for the corresponding data points in Figure 4.2A.

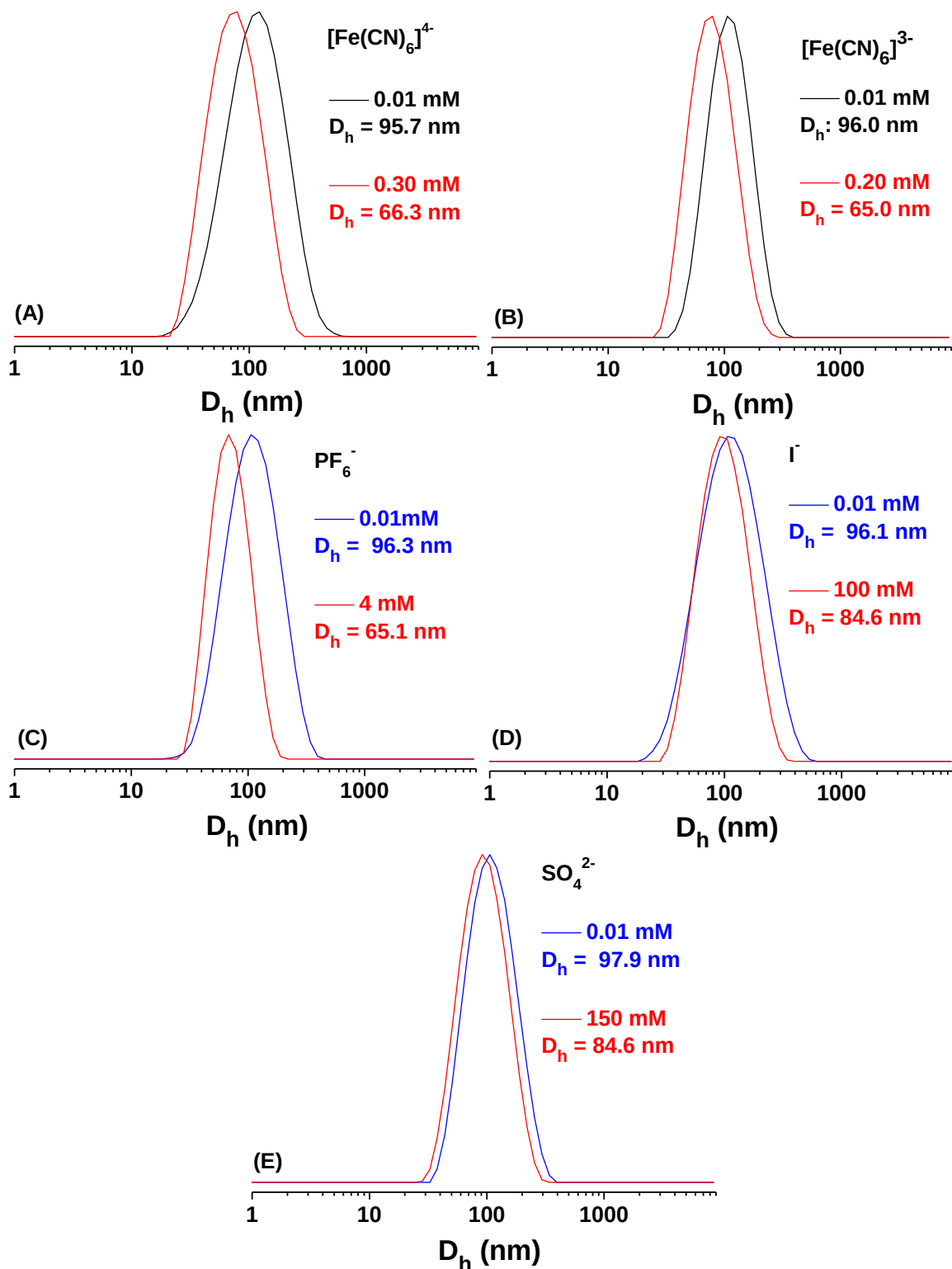


Figure B14. Hydrodynamic size distributions of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of (A) $\text{Na}_4[\text{Fe}(\text{CN})_6]$, (B) $\text{K}_3[\text{Fe}(\text{CN})_6]$, (C) NaPF_6 , (D) NaI , and (E) Na_2SO_4 at one low and one high concentration, obtained from dynamic light scattering measurements at 25 °C. These size distributions are for the corresponding data points in Figure 4.2B.

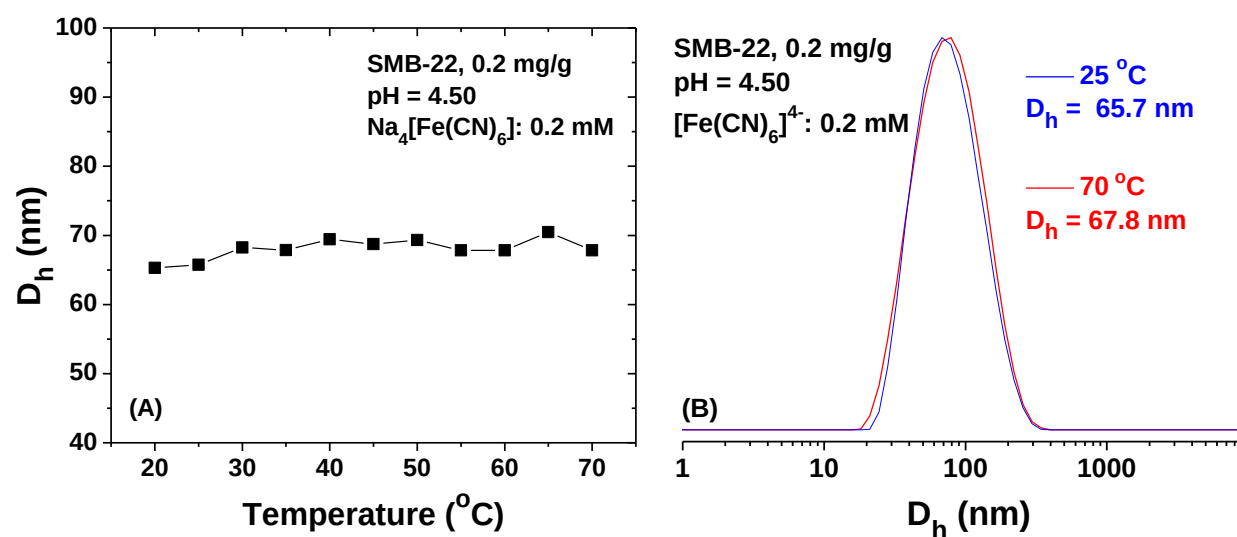


Figure B15. (A) Apparent hydrodynamic size (D_h) of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 0.2 mM $\text{Na}_4[\text{Fe}(\text{CN})_6]$ as a function of temperature. (B) Hydrodynamic size distributions of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 0.2 mM $\text{Na}_4[\text{Fe}(\text{CN})_6]$ at 25 and 70 $^{\circ}\text{C}$.

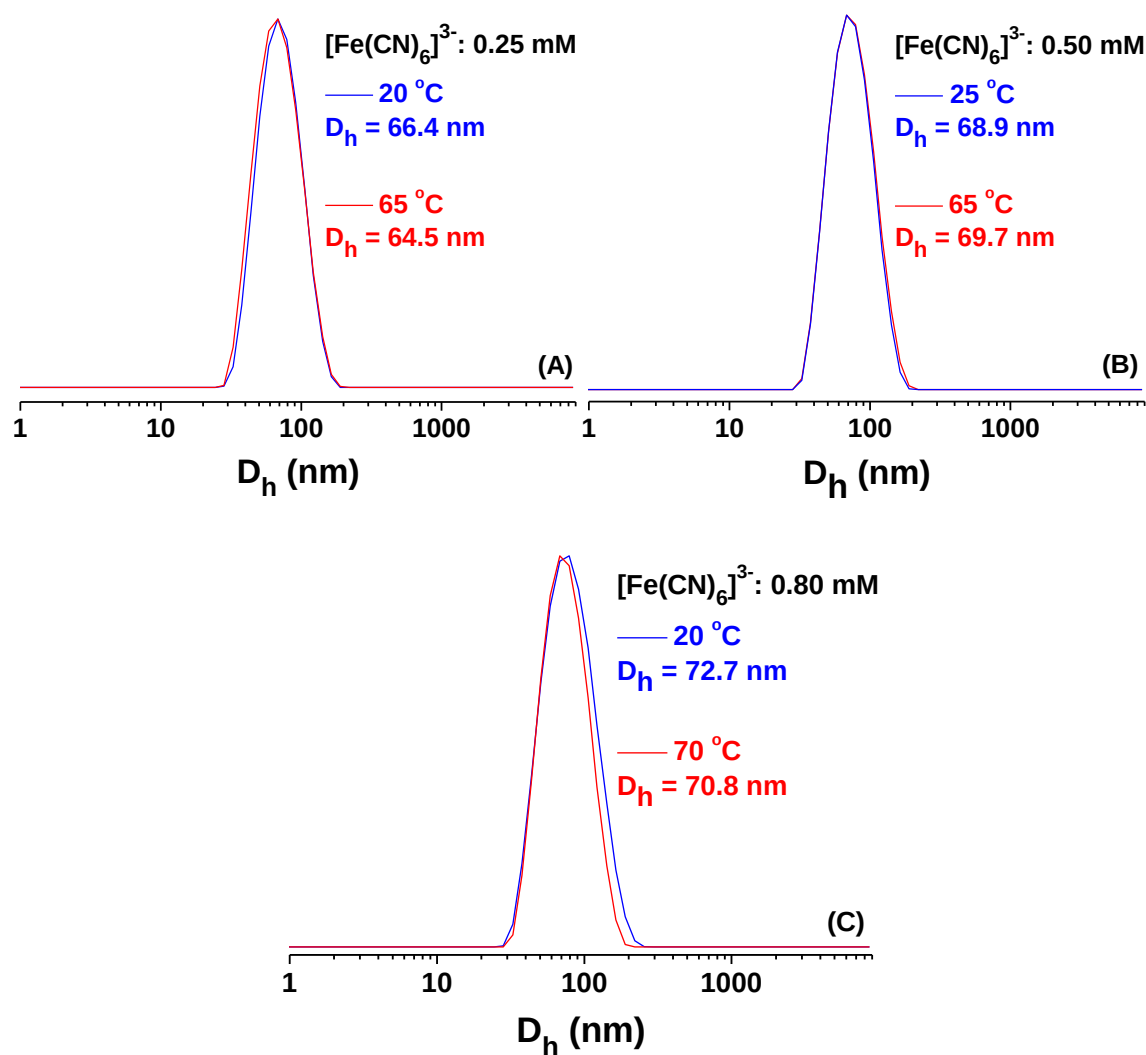


Figure B16. Hydrodynamic size distributions of 0.2 mg/g SMB-11 in pH 4.50 acidic water in the presence of 0.25 mM (A), 0.50 mM (B), and 0.80 mM $K_3[Fe(CN)_6]$ (C) at one low and one high temperature. These size distributions are for the corresponding data point in Figure 4.3A.

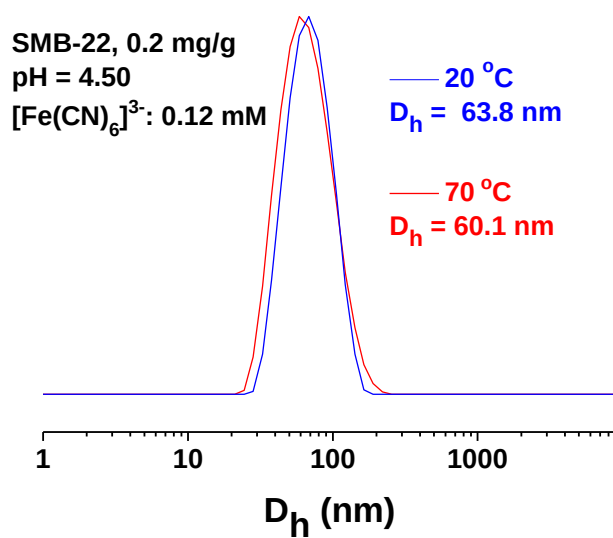


Figure B17. Hydrodynamic size distributions of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 0.12 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ at 20 and 60 °C shown in Figure 4.3B in the main text.

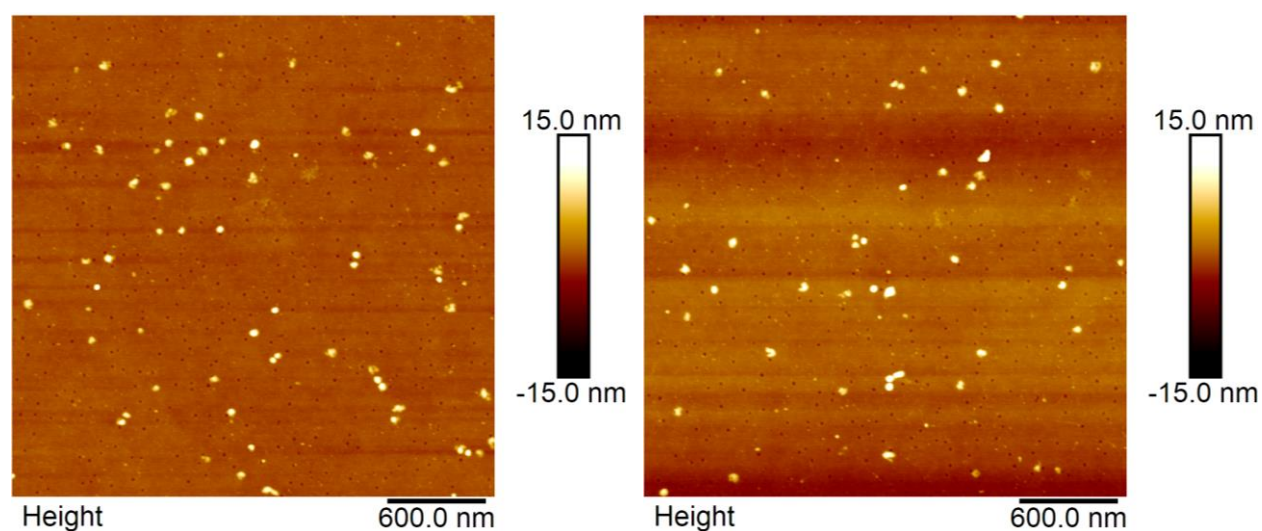


Figure B18. Atomic force microscopy height images ($3 \times 3 \mu\text{m}$) of SMB-11 drop cast on glass disks at 20°C from a 0.050 mg/g SMB-11 in pH 4.50 acidic water in the presence of 0.075 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$. The solution used to prepare AFM samples was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 0.30 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ using pH 4.50 acidic water.

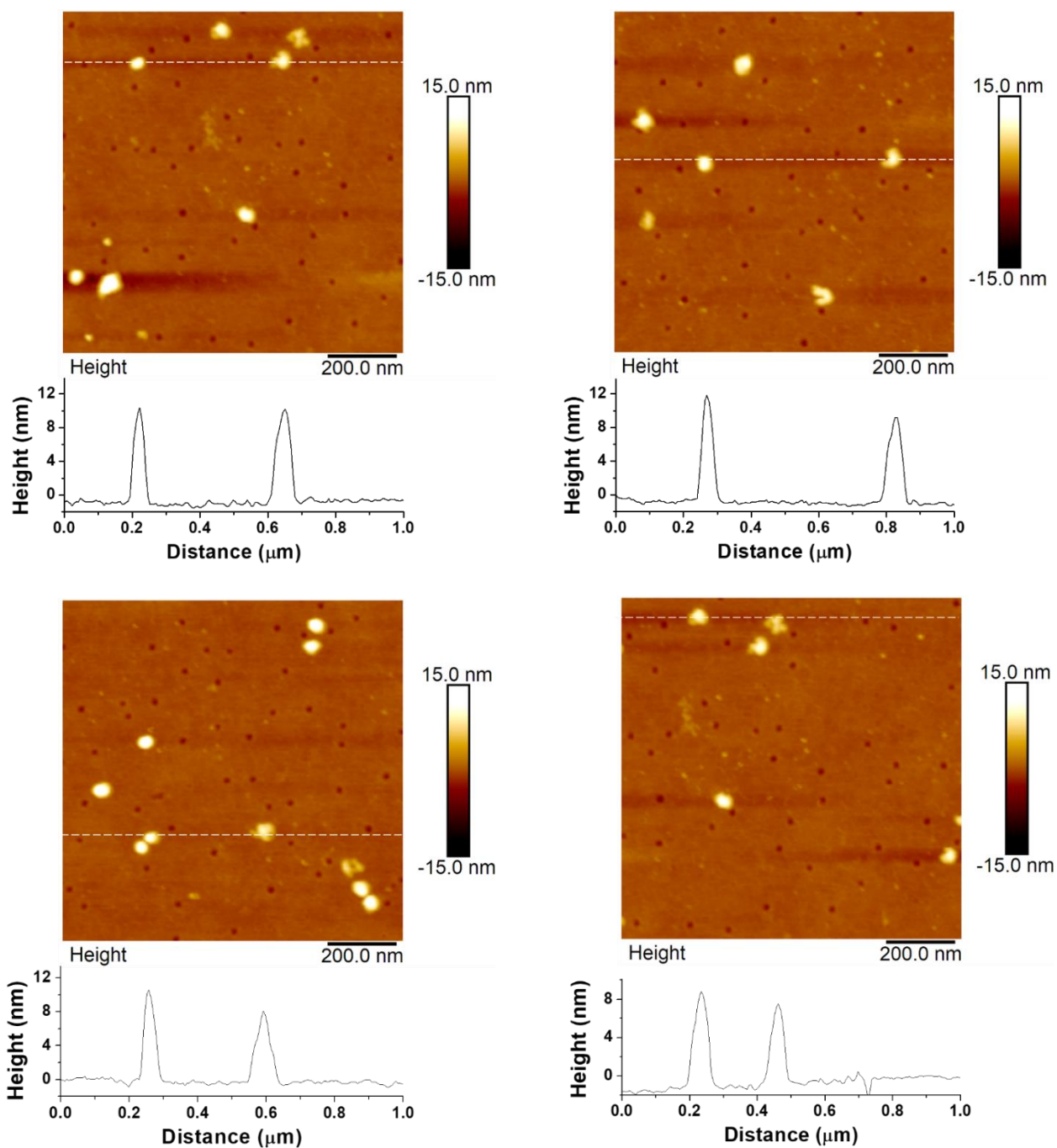


Figure B19. Atomic force microscopy height images ($1 \times 1 \mu\text{m}$) of SMB-11 drop cast on glass disks at 20°C from a 0.050 mg/g SMB-11 in pH 4.50 acidic water in the presence of 0.075 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$, with a cross-sectional profile along the dashed line in each image. The solution used to prepare AFM samples was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 0.30 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$ using pH 4.50 acidic water.

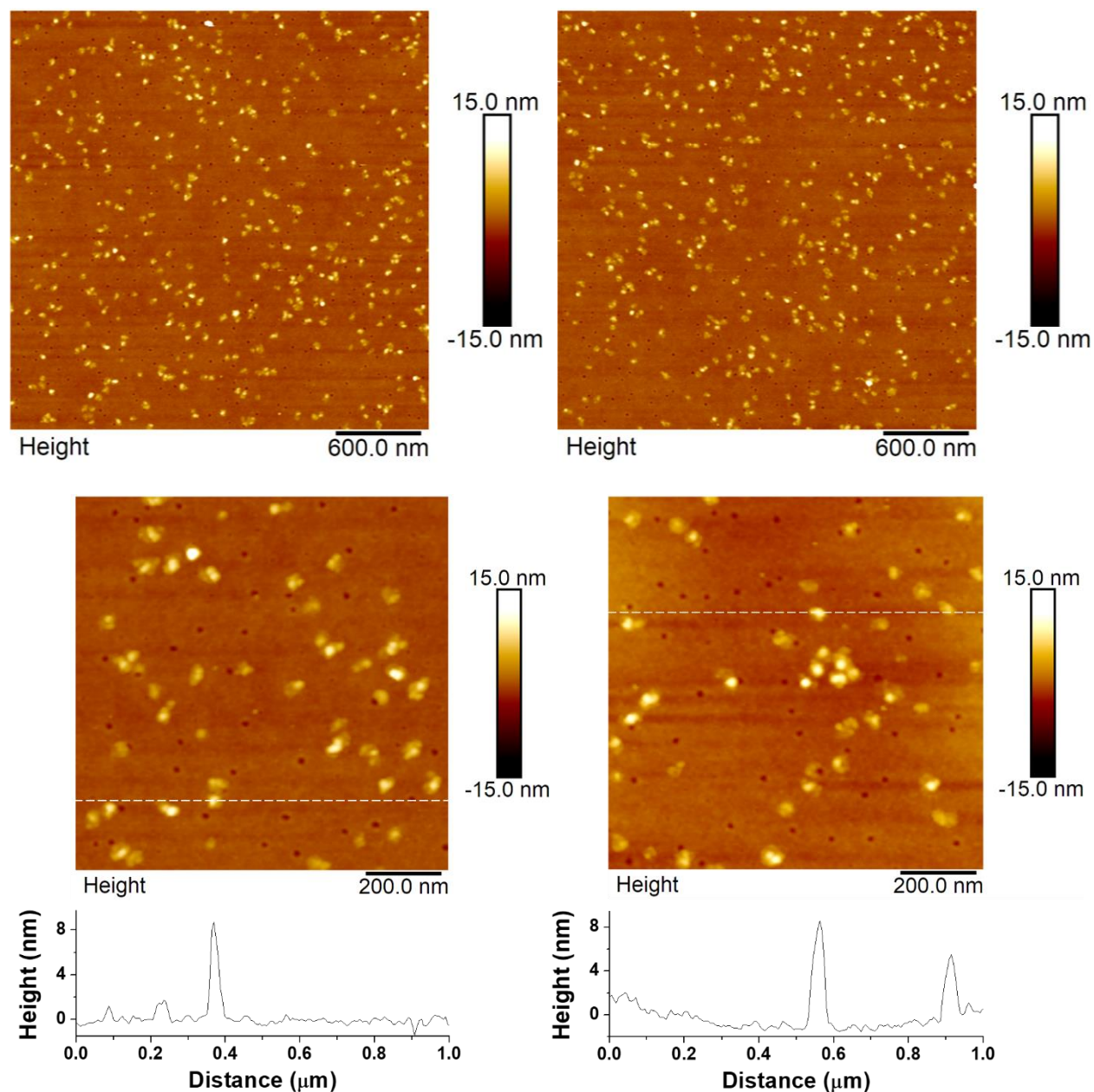


Figure B20. Atomic force microscopy height images of SMB-11 drop cast on glass disks at 70 °C from a 0.050 mg/g SMB-11 in pH 4.50 acidic water in the presence of 0.075 mM $K_3[Fe(CN)_6]$, with cross-sectional profiles along the dashed lines in the $1 \times 1 \mu\text{m}$ images. The solution used to prepare AFM samples was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 0.30 mM $K_3[Fe(CN)_6]$ using pH 4.50 acidic water.

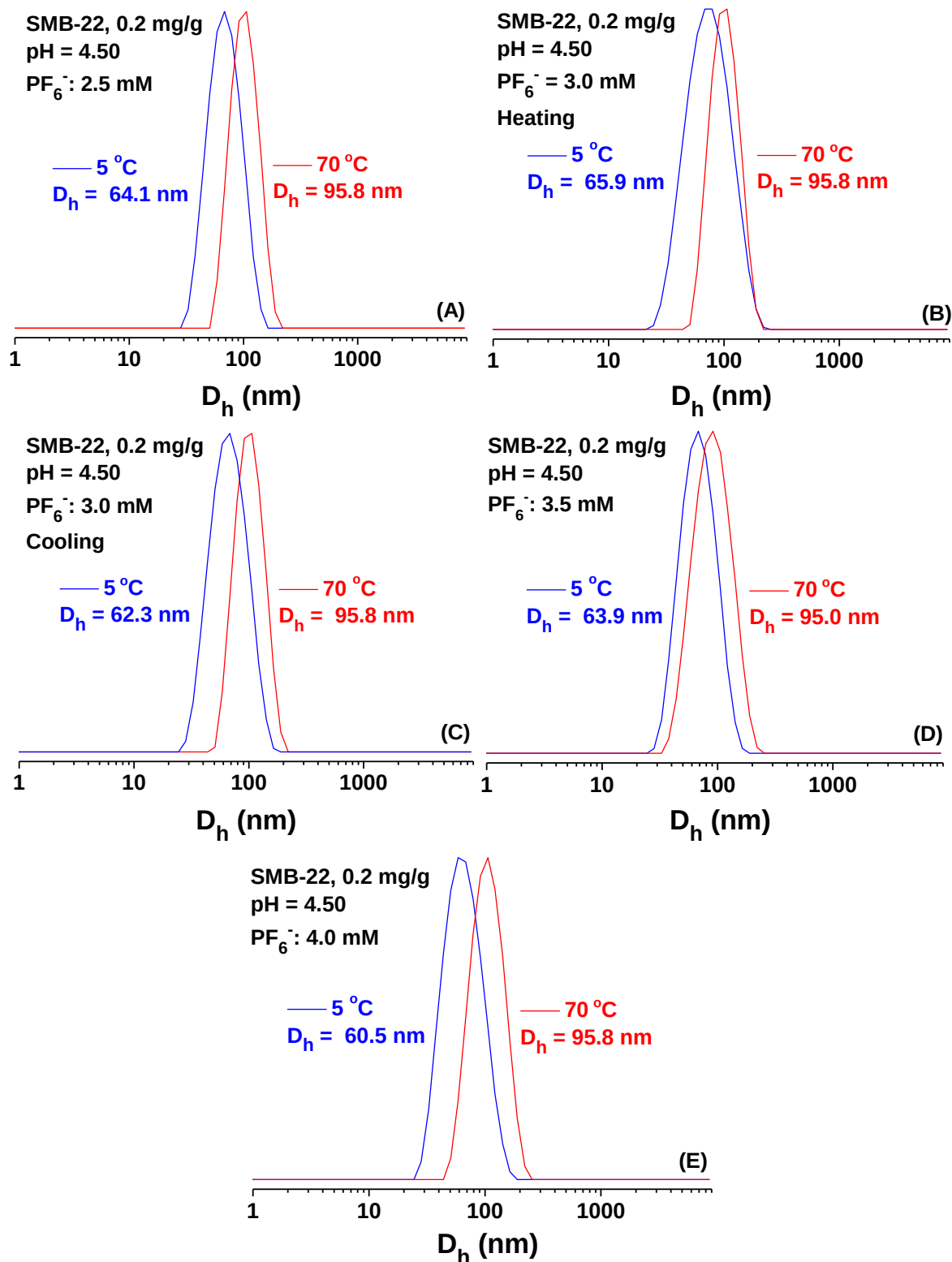


Figure B21. Hydrodynamic size distributions of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of (A) 2.5 mM, (B) 3.0 mM (heating), (C) 3.0 mM (cooling), (D) 3.5 mM, and (E) 4.0 mM NaPF_6 at 5 and 70 °C, obtained from DLS measurements. These size distributions are for the corresponding data points in Figure 4.5C and D.

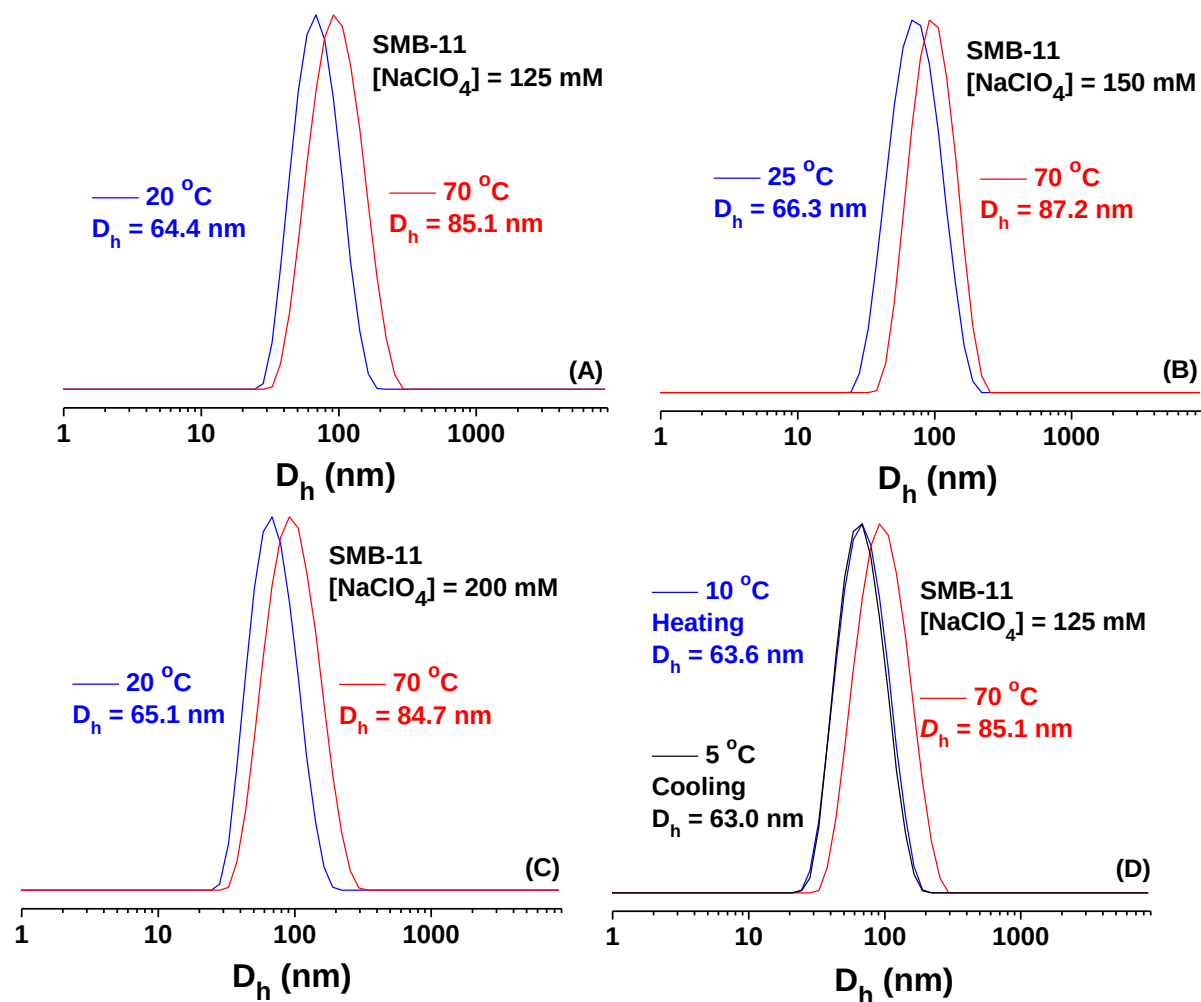


Figure B22. Hydrodynamic size distributions of 0.2 mg/g SMB-11 in pH 4.50 acidic water in the presence of NaClO₄ with various concentrations at one low and one high temperature, corresponding to the data points in Figure 4.6A and B.

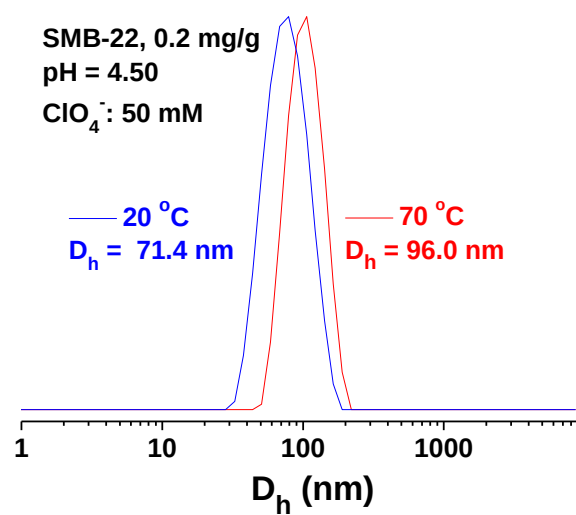


Figure B23. Hydrodynamic size distributions of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 50 mM NaClO₄ at 20 and 70 °C for the corresponding data points in Figure 4.6C.

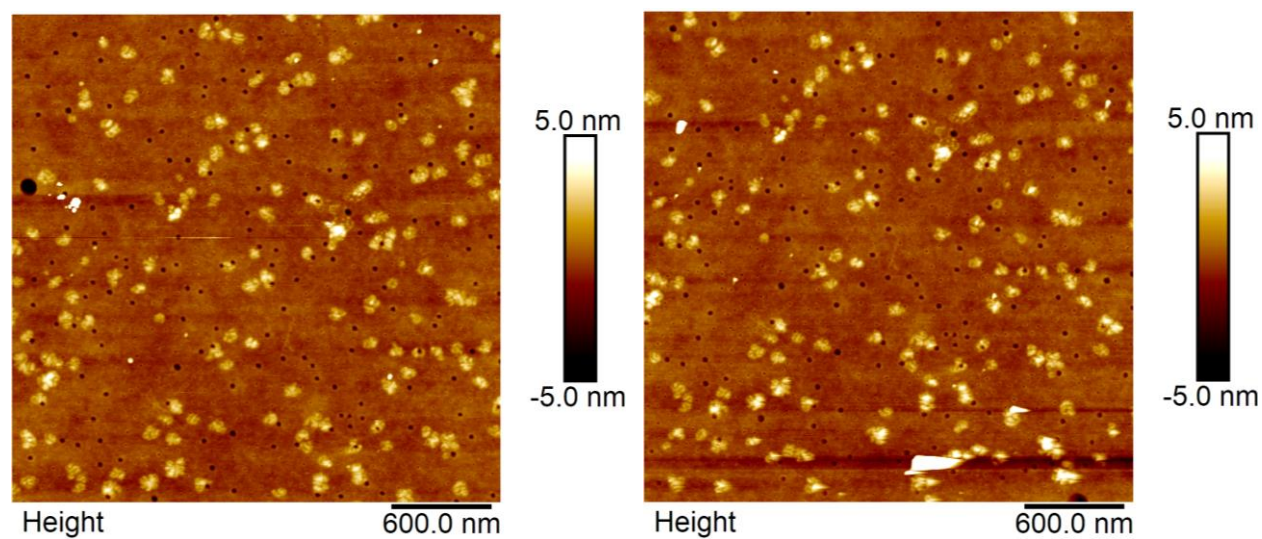


Figure B24. AFM height images ($3 \times 3 \mu\text{m}$) of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water in the presence of 1.5 mM NaPF_6 at 20 °C. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-11 with 6 mM NaPF_6 using pH 4.50 water.

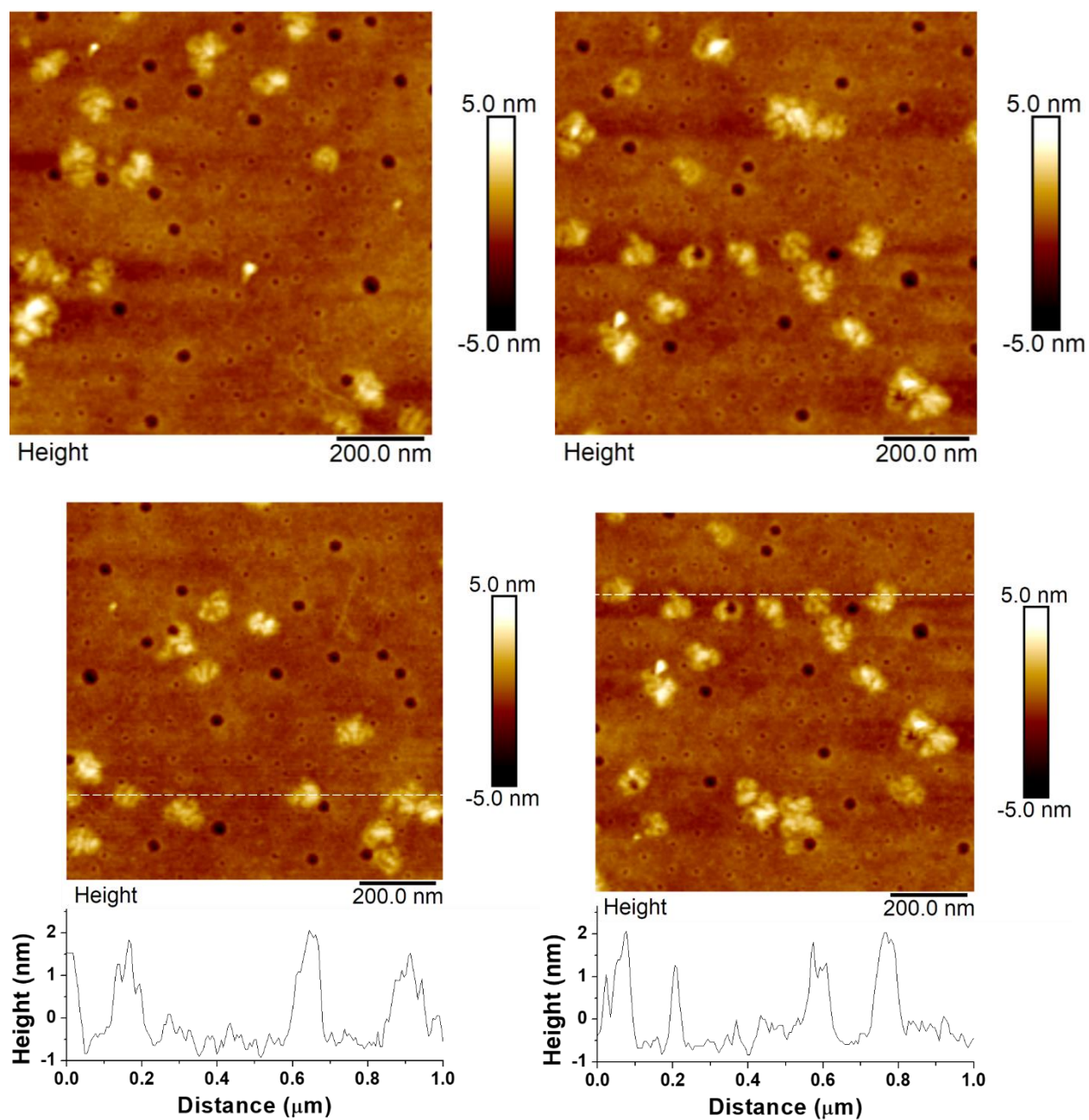


Figure B25. AFM height images ($1 \times 1 \mu\text{m}$) of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water in the presence of 1.5 mM NaPF_6 at 20 °C, with cross-sectional profiles along the dashed lines in the bottom two $1 \times 1 \mu\text{m}$ images. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-11 with 6 mM NaPF_6 using pH 4.50 water.

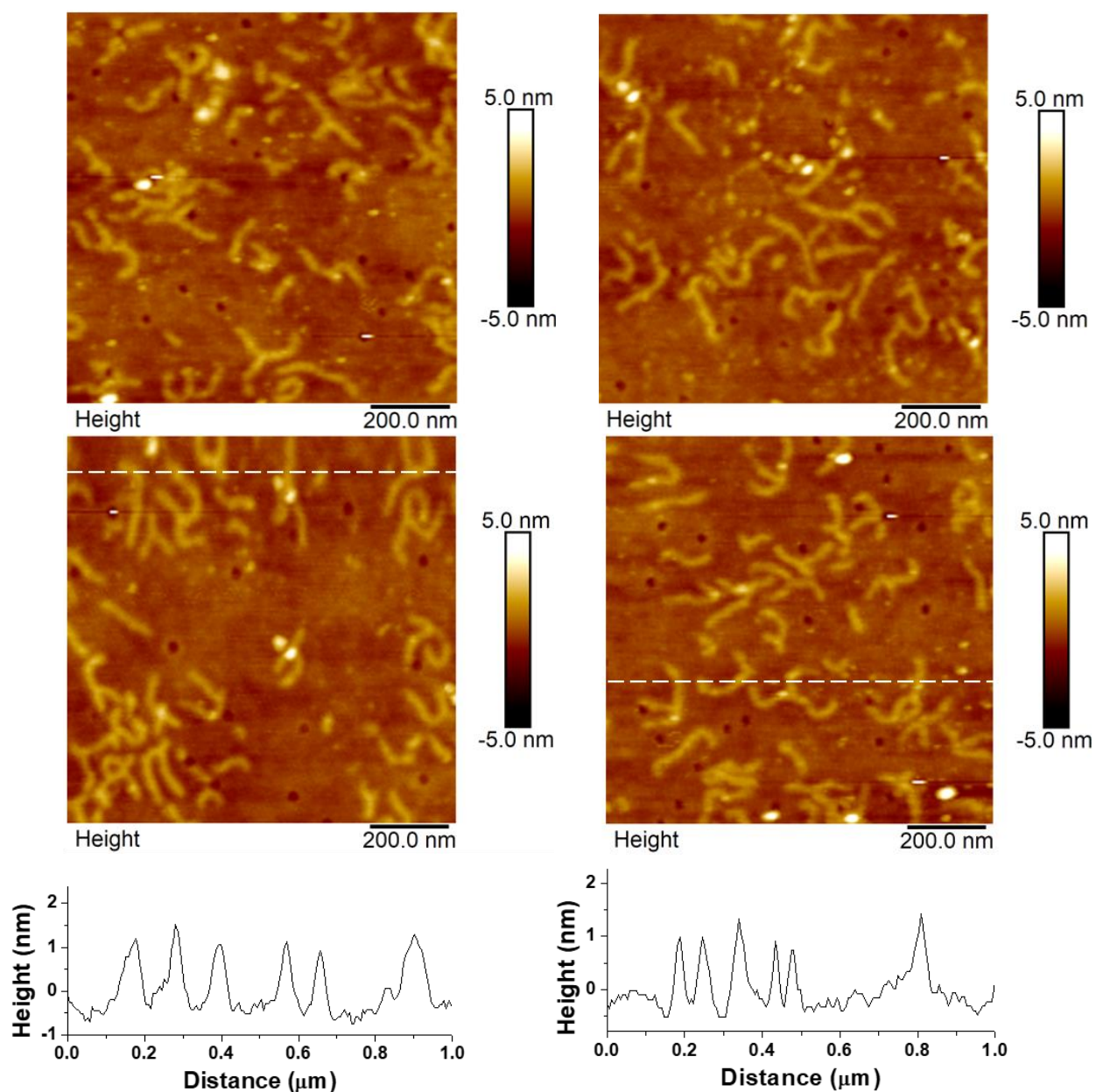


Figure B26. AFM height images ($1 \times 1 \mu\text{m}$) of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water in the presence of 1.5 mM NaPF_6 at 70°C , with cross-sectional profiles along the dashed lines in the bottom two images. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-11 with 6 mM NaPF_6 using pH 4.50 water.

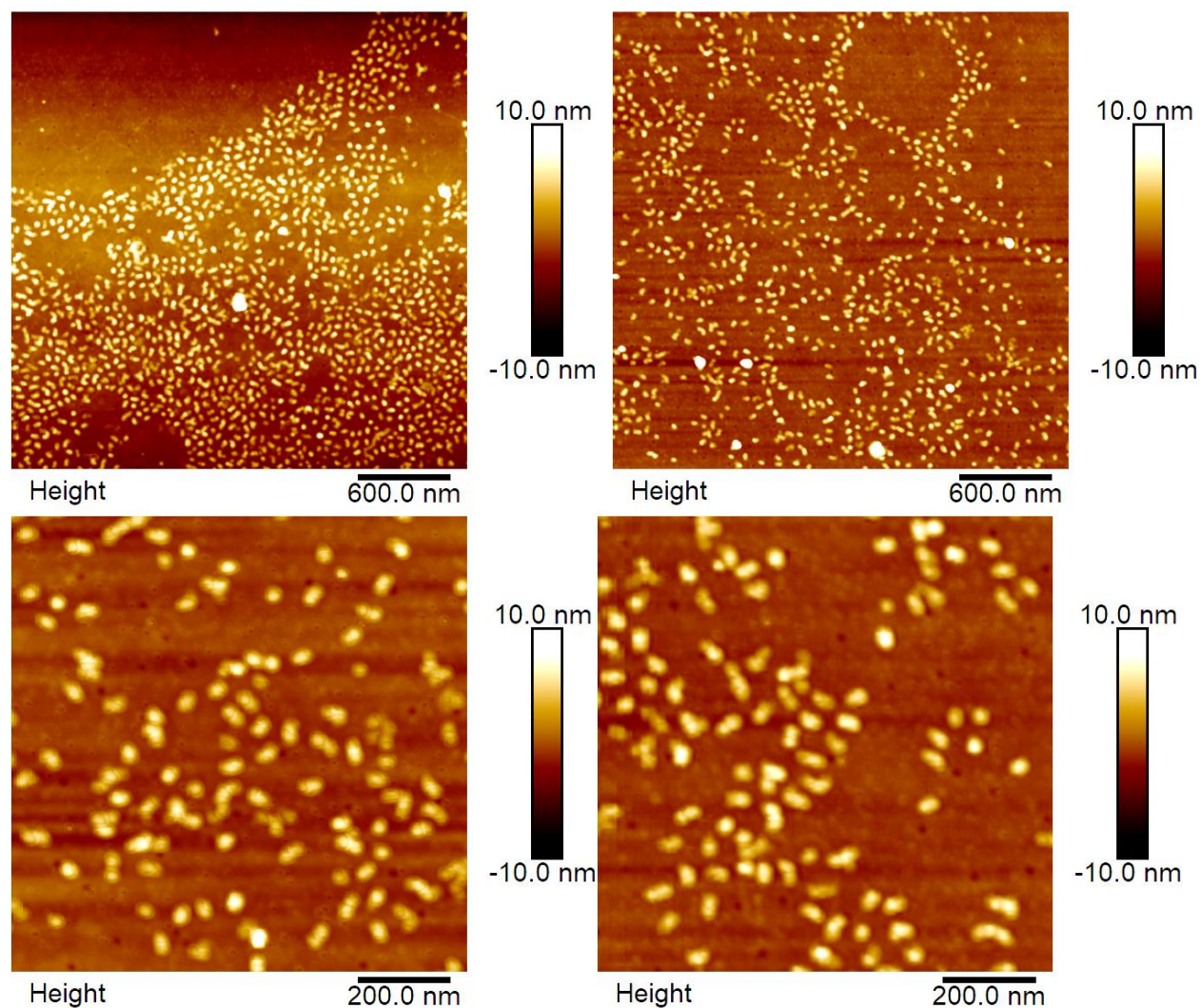


Figure B27. AFM height images of SMB-22 drop cast on glass disks from a 0.050 mg/g solution of SMB-22 in pH 4.50 water in the presence of 1.0 mM NaPF₆ at 0 °C. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-22 with 4 mM NaPF₆ using pH 4.50 water.

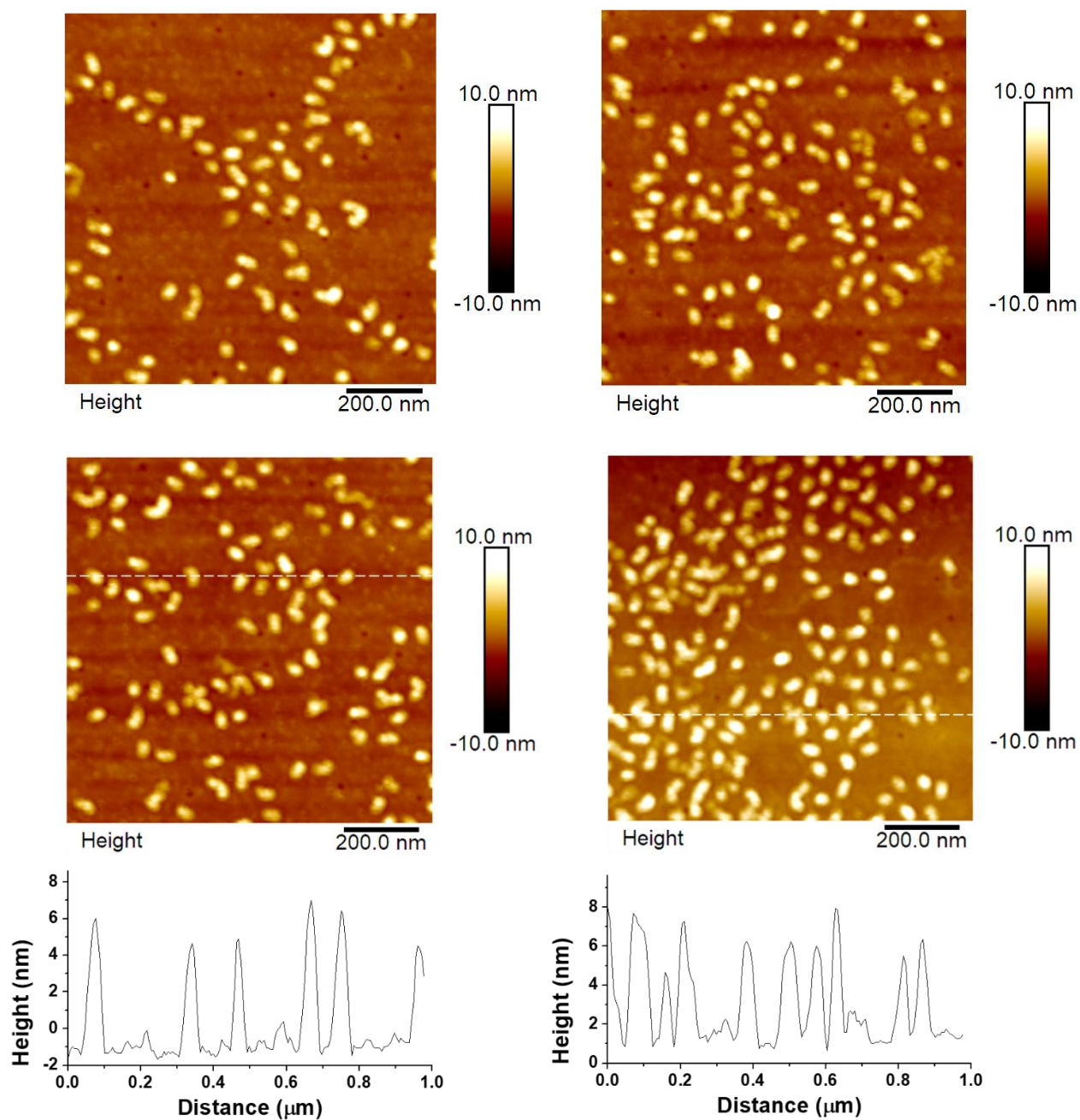


Figure B28. AFM height images of SMB-22 drop cast on glass disks from a 0.050 mg/g solution of SMB-22 in pH 4.50 water in the presence of 1.0 mM NaPF₆ at 0 °C, with cross-sectional profiles along the dashed lines in the bottom two images. The solution was diluted from a pH 4.50 solution of 0.2 mg/g SMB-22 with 4 mM NaPF₆ using pH 4.50 water.

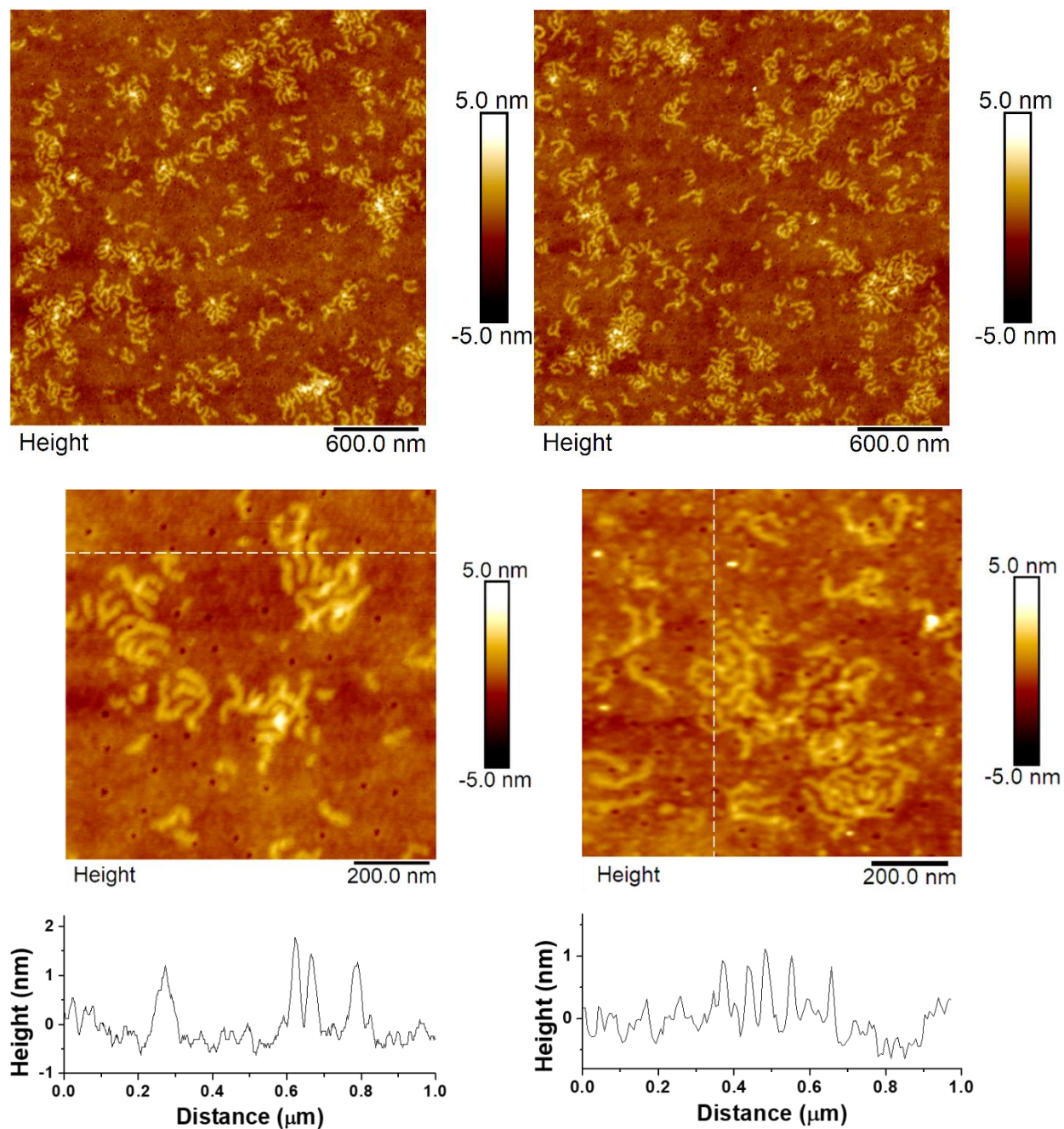


Figure B29. Atomic force microscopy (AFM) height images of SMB-22 drop cast on glass disks from a solution of 0.050 mg/g SMB-22 in pH 4.50 acidic water in the presence of 1.0 mM NaPF₆ at 70 °C, with cross-sectional profiles along the dashed lines in the bottom two images. The solution was diluted with pH 4.50 water from a 0.2 mg/g solution of SMB-22 with a pH of 4.50 in the presence of 4 mM NaPF₆.

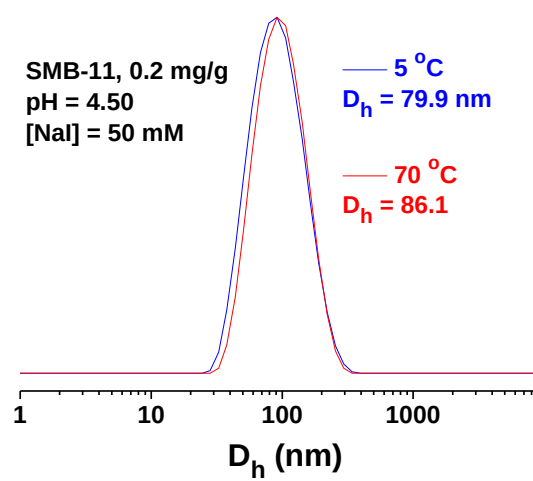


Figure B30. Hydrodynamic size distributions of 0.2 mg/g SMB-11 in pH 4.50 acidic water in the presence of 50 mM NaI at 5 and 70 °C for the corresponding data points in Figure 4.9A.

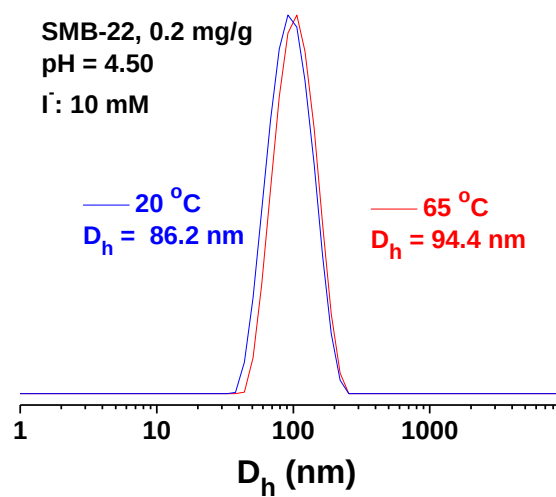


Figure B31. Hydrodynamic size distribution of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 10 mM NaI at 20 and 65 °C for the corresponding data points in Figure 4.9B.

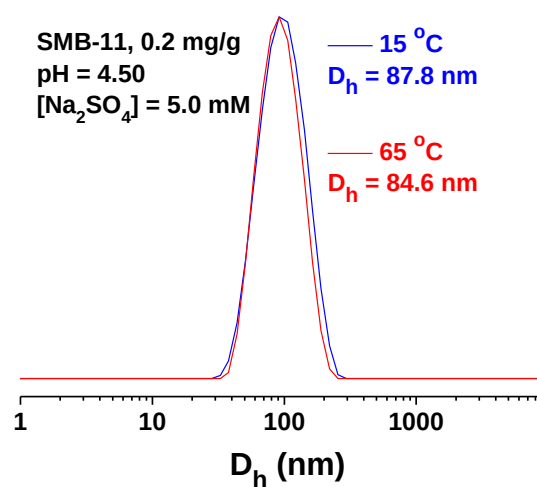


Figure B32. Hydrodynamic size distributions of 0.2 mg/g SMB-11 in pH 4.50 acidic water in the presence of 5 mM NaSO_4 at one low (15 °C) and one high temperature (65 °C) for the corresponding data points in Figure 4.10A.

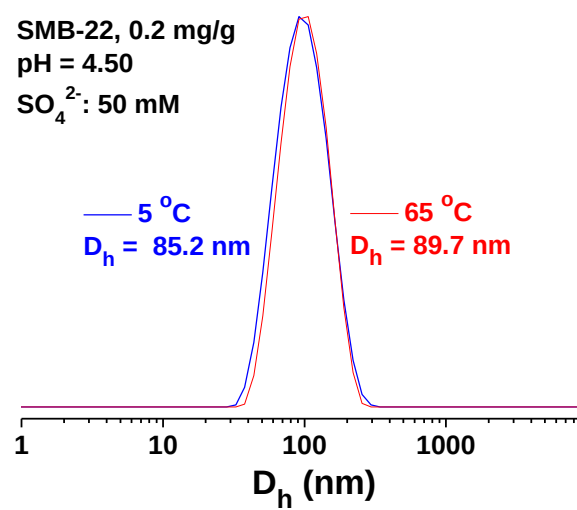


Figure B33. Hydrodynamic size distribution of 0.2 mg/g SMB-22 in pH 4.50 acidic water in the presence of 50 mM Na₂SO₄ at 5 and 65 °C for the corresponding data points in Figure 4.10B.

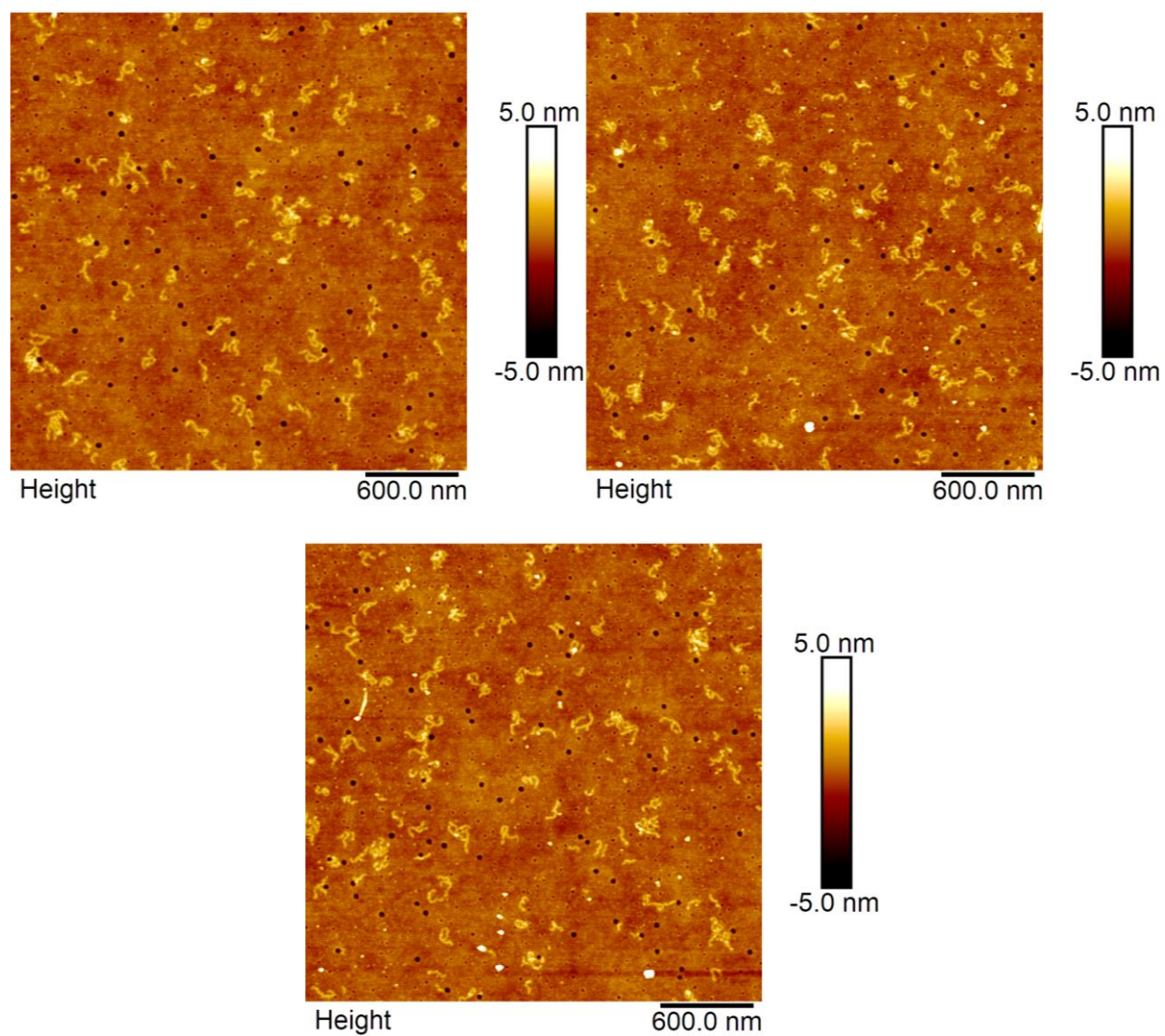


Figure B34. AFM height images ($3 \times 3 \mu\text{m}$) of SMB-11 drop cast on a glass disk from a solution of 0.050 mg/g SMB-11 in pH 4.50 water with 2.5 mM Na_2SO_4 at 20 °C. The solution was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 10 mM Na_2SO_4 using pH 4.50 water.

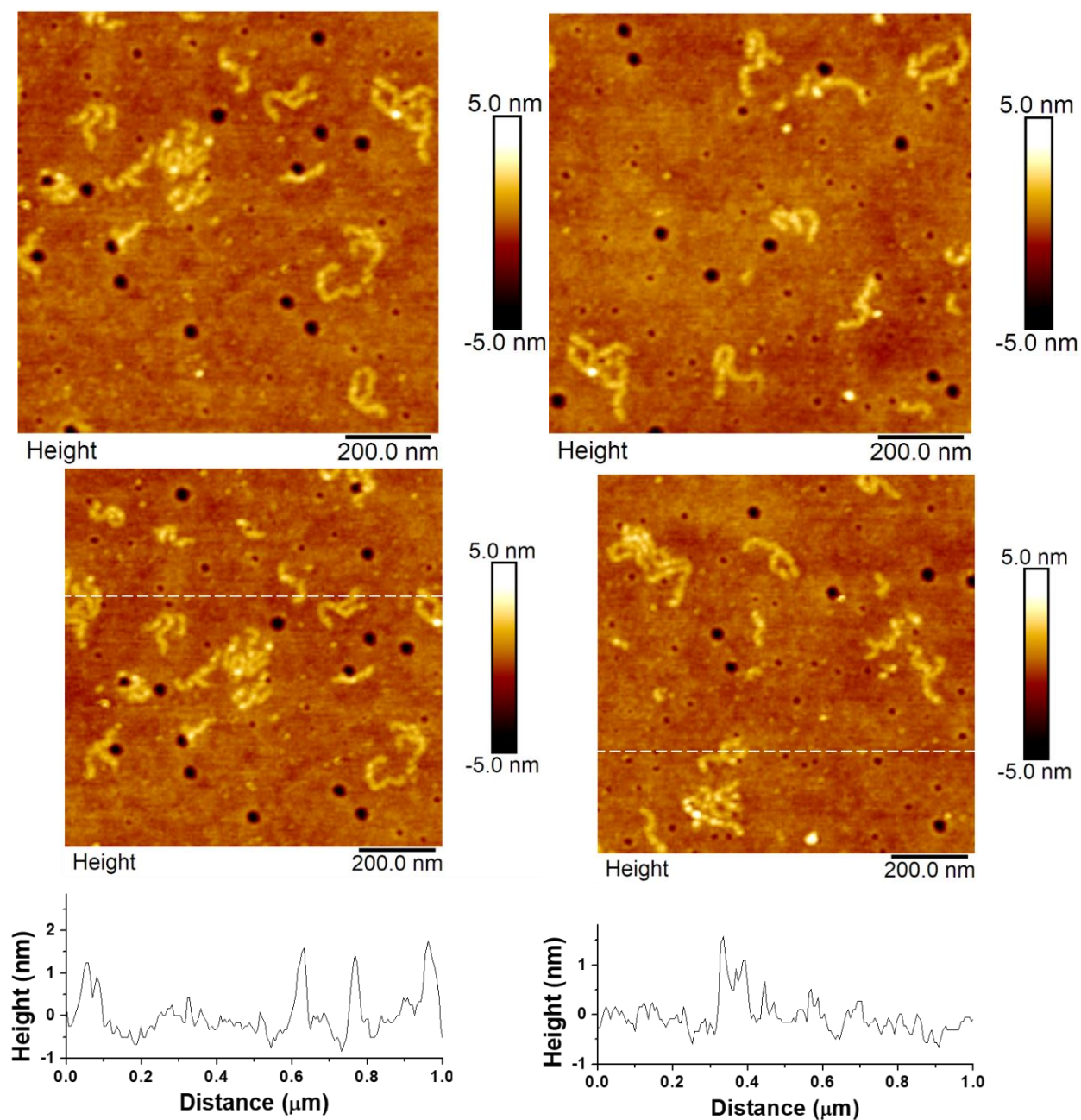


Figure B35. AFM height images ($1 \times 1 \mu\text{m}$) of SMB-11 drop cast on a glass disk from a solution of 0.050 mg/g SMB-11 in pH 4.50 water with 2.5 mM Na_2SO_4 at 20 °C, with cross-sectional profiles along the dashed lines in the bottom two $1 \times 1 \mu\text{m}$ images. The solution was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 10 mM Na_2SO_4 using pH 4.50 water.

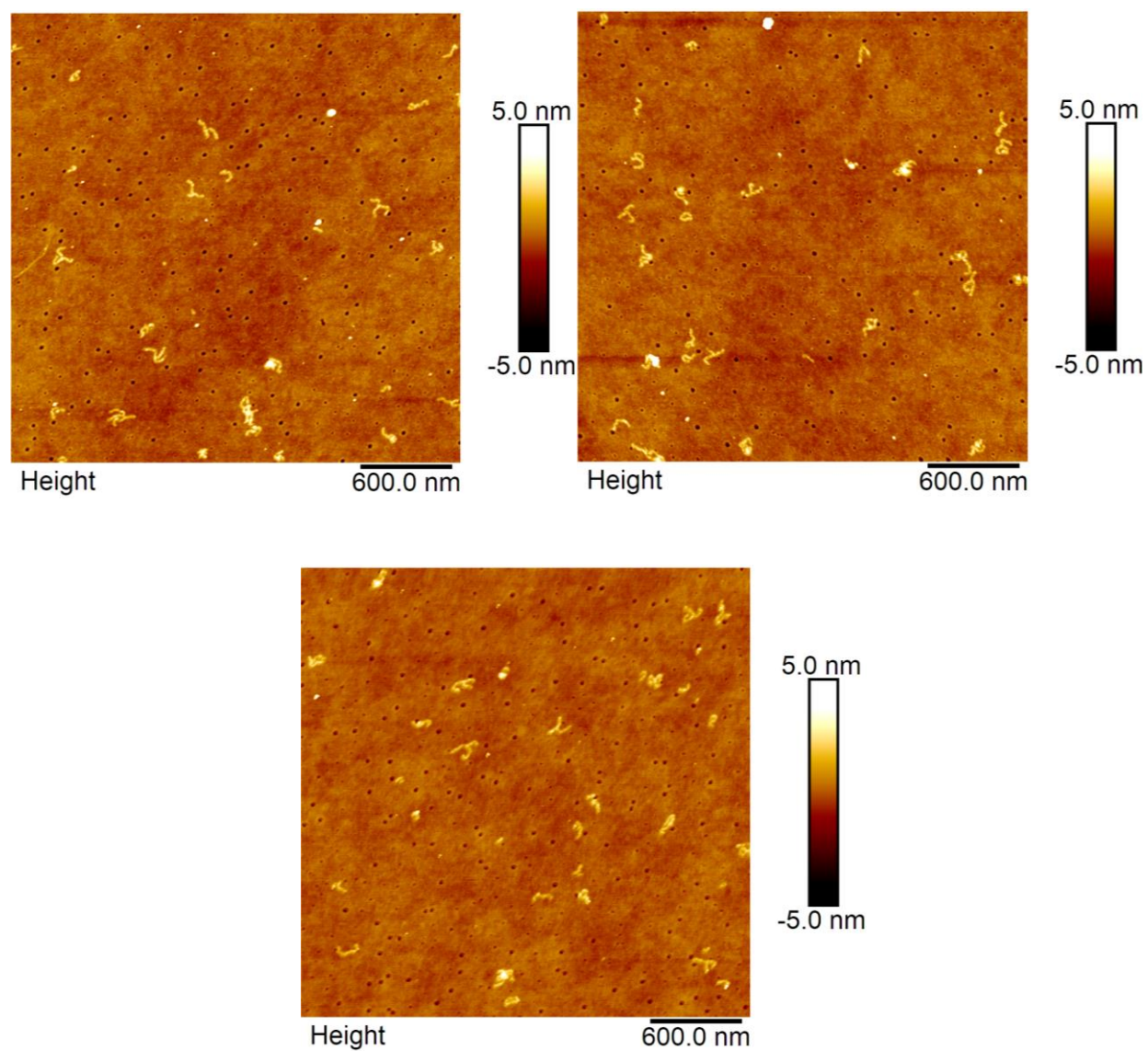


Figure B36. AFM height images ($3 \times 3 \mu\text{m}$) of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water with 2.5 mM Na_2SO_4 at 70 °C. The solution was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 10 mM Na_2SO_4 using pH 4.50 water.

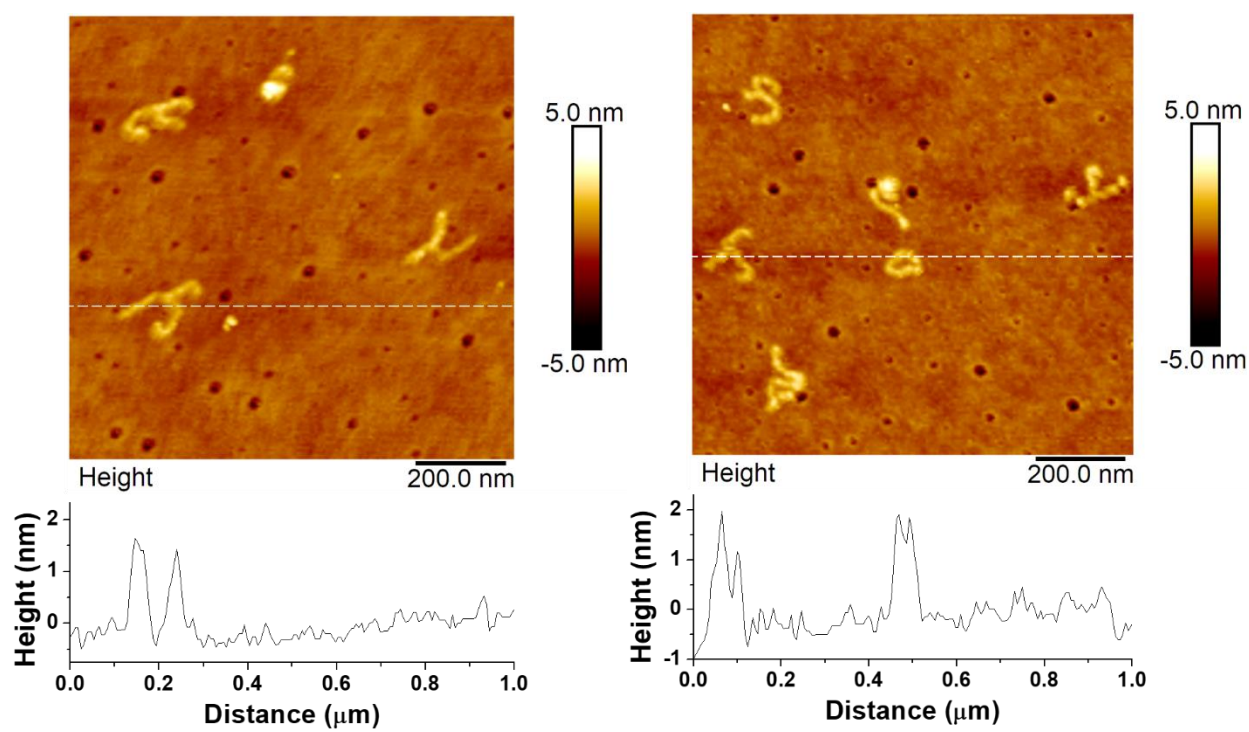


Figure B37. AFM height images of SMB-11 drop cast on glass disks from a solution of 0.050 mg/g SMB-11 in pH 4.50 water with 2.5 mM Na_2SO_4 at 70 °C, with a cross-sectional profile along the dashed line in each image. The solution was diluted from a 0.2 mg/g solution of SMB-11 in pH 4.50 water with 10 mM Na_2SO_4 using pH 4.50 water.

Chapter 5: Chaotropic Anion-Induced Conformational Changes of Three-Arm Star Molecular Bottlebrushes with Heterografted Poly(ethylene oxide) and Poly((2-(methacryloyloxy)ethyl)trimethylammonium iodide) Side Chains in Water

Abstract.

This chapter presents a study of chaotropic anion (CA)-induced conformational changes of three-arm star molecular bottlebrushes composed of heterografted poly(ethylene oxide) (PEO) and poly([2-(methacryloyloxy)ethyl]trimethylammonium iodide) (SMB-Q) in Milli-Q water at 25 °C. SMB-Q was synthesized by grafting alkyne-end-functionalized PEO and poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA) onto an azide-functionalized star backbone polymer via a click reaction followed by quaternization of the tertiary amine groups of PDMAEMA with iodomethane. The effects of six anions (super chaotropic $\text{S}_2\text{O}_8^{2-}$, moderately chaotropic ClO_4^- , relatively weak chaotropic SCN^- , kosmotropic SO_4^{2-} , salicylate ($\text{C}_7\text{H}_5\text{O}_3^-$), and thiosalicylate ($\text{C}_7\text{H}_5\text{O}_2\text{S}^-$)) on the conformation of SMB-Q in Milli-Q water at 25 °C were studied by DLS measurements. Upon increasing the salt concentration, pronounced size reduction transitions were observed for all the anions except SO_4^{2-} but at different anion concentrations. The observation suggests that salicylate and thiosalicylate anions might be chaotropic. The size transitions of SMB-Q occurred at much low concentrations of $\text{S}_2\text{O}_8^{2-}$ and ClO_4^- compared with a protonated three-arm star brush polymer composed of heterografted PEO and PDMAEMA side chains in acidic water. The latter only showed a small gradual size decrease in response to the increase of the concentration of SCN^- . These indicated that the quaternary ammonium was a stronger chaotropic cation than the protonated tertiary amine groups of PDMAEMA. The pronounced size transitions of SMB-Q are believed to result from the ion pairing of quaternary ammonium groups in SMB-Q with chaotropic anions, which decreased the solubility of the quaternized PDMAEMA side chains. This was confirmed by the observation that the solubility of the quaternized linear PDMAEMA in water in the presence of NaClO_4 decreased. On the other hand, kosmotropic SO_4^{2-} had a rather small influence on the conformation of SMB-Q in water.

5.1. Introduction

Molecular bottlebrushes (MBBs) are macromolecules composed of densely grafted polymeric side chains on a polymer backbone.¹⁻³ Due to the high grafting density, linear MBBs with a sufficiently large aspect ratio adopt extended wormlike conformations in good solvents.¹⁻⁴ MBBs are commonly synthesized by three methods: grafting from,⁵⁻⁷ grafting through,⁸⁻¹⁰ and grafting to.¹¹⁻¹³ Grafting to has been shown to be capable of producing MBBs with high and tunable grafting densities when the copper(I)-catalyzed azide-alkyne cycloaddition click reaction is used.¹⁴⁻¹⁸ Owing to their structural characteristics, molecular bottlebrushes possess broad potential in applications such as drug delivery,¹⁹ lubrication,²⁰ template synthesis,²¹ and sensors.²²

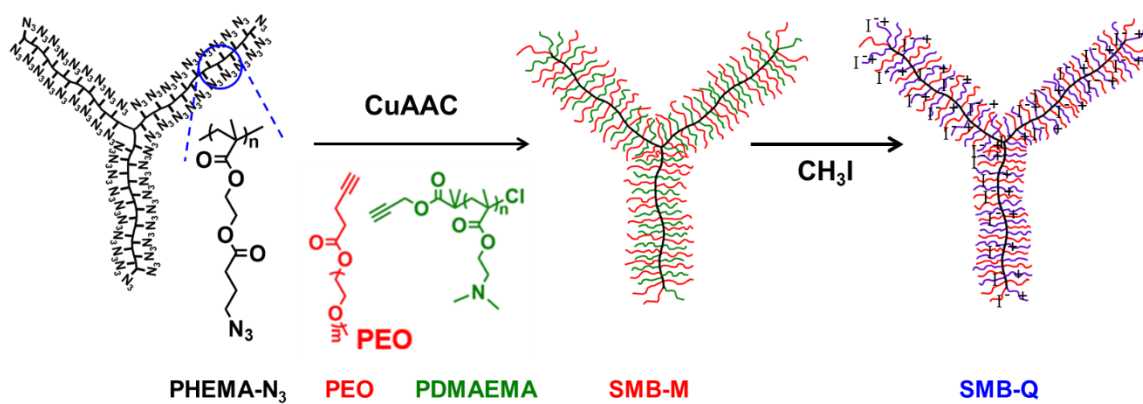
Stimuli-responsive MBBs can undergo large size changes in response to relatively small environmental variations.¹ The stimuli-responsiveness of MBBs is usually introduced through the use of stimuli-responsive polymers as side chains. When polyelectrolytes are incorporated into MBBs as side chains, the resultant MBBs can exhibit responsive behavior when salts or linear polyelectrolytes of opposite charges are added.²³⁻²⁵ However, the collapsed MBBs are not stable in aqueous solutions. This problem can be solved by introducing solvophilic side chains into the MBBs as a stabilizer.¹⁴⁻¹⁸ Chapter 4 describes the synthesis and responsive behavior in acidic water of two three-arm star brush polymers composed of heterografted PEO and either poly(2-(*N,N*-dimethylamino)ethyl methacrylate) (PDMAEMA) or poly(2-(*N,N*-diethylamino)ethyl methacrylate) (PDEAEMA) side chains. While the brushes collapsed by super chaotropic $[\text{Fe}(\text{CN})_6]^{4-}$ and $[\text{Fe}(\text{CN})_6]^{3-}$ stayed in the globular state upon heating from room temperature to 70 °C, the star MBBs collapsed by moderate chaotropic anions (CAs), such as PF_6^- and ClO_4^- , exhibited a globule-to-star shape transition. On the other hand, weak chaotropic I^- exerted only a small effect on the hydrodynamic sizes of the two brush polymers in acidic water.

In the present work, we synthesized a three-arm star brush polymer composed of heterografted PEO and poly((2-(methacryloyloxy)ethyl)trimethylammonium iodide) (PDMAEMA-Q) side chains (SMB-Q) and studied its responsive behavior in water upon the addition of salts containing chaotropic anions (Scheme 5.1). According to Marcus, $(\text{CH}_3)_4\text{N}^+$ is a stronger chaotrope than $(\text{CH}_3)_2\text{NH}_2^+$.²⁶ Compared with the protonated tertiary amine groups $(-\text{CH}_2\text{CH}_2\text{N}^+\text{H}(\text{CH}_3)_2)$ of PDMAEMA in acidic water, the quaternary ammonium cations $(-\text{CH}_2\text{CH}_2\text{N}^+(\text{CH}_3)_3)$ in PDMAEMA-Q are larger. Thus, we believe that the latter is a stronger chaotropic cation and moderate CAs could collapse the star brushes at lower salt concentrations than the protonated SMB-11 described in Chapter 4. To test this hypothesis, SMB-Q was prepared by first grafting alkyne-end-functionalized PEO and PDMAEMA onto an azide-functionalized star backbone polymer, PHEMA- N_3 , and then quaternizing the resultant brushes with iodomethane (Scheme 5.1). The responsive behavior of SMB-Q in water upon addition of three CAs ($\text{S}_2\text{O}_8^{2-}$, ClO_4^- , and SCN^-) and kosmotropic SO_4^{2-} (for comparison) was investigated by dynamic light scattering (DLS) measurements. While $\text{S}_2\text{O}_8^{2-}$ is a super CA, ClO_4^- is moderately chaotropic and SCN^- is a relatively weak CA.²⁷ In addition, salicylate ($\text{C}_7\text{H}_5\text{O}_3^-$) and thiosacrylate ($\text{C}_7\text{H}_5\text{O}_2\text{S}^-$) were also studied, although it is unclear whether they are chaotropes or kosmotropes. It would be interesting if these two drugs could induce large conformation changes of SMB-Q.

5.2. Experimental Section

5.2.1. Materials

2-(*N,N*-Dimethylamino)ethyl methacrylate (DMAEMA, 98.5%, TCI) was passed through a basic alumina (top)/silica gel (bottom) column to remove the inhibitor prior to use. Propargyl 2-bromoisobutyrate was made according to the literature¹⁴ and characterized by NMR spectroscopy analysis. 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA, 97%, Alfa-Aesar) and



Scheme 5.1. Synthesis of Polyelectrolyte Three-Arm Star Brushes SMB-Q.

1,1,4,7,7-pentamethyldiethylenetriamine (PMDETA, 99%, Acros Organics) were distilled under vacuum. Copper(I) chloride (CuCl, 97%, Alfa-Aesar) was stirred in glacial acetic acid overnight, filtered under a reduced pressure, rinsed with absolute ethanol and diethyl ether, and dried under high vacuum. Alkyne end-functionalized poly(ethylene glycol) methyl ether was prepared from 5 kDa poly(ethylene glycol) methyl ether as described in Chapter 4. The alkyne-end-functionalized PDMAEMA used in the present work was prepared using the same procedure as described in Chapter 4 (Section 4.2.4). The DP of this PDMAEMA was 56, calculated from the monomer conversion and the monomer-to-initiator ratio; SEC analysis using the DMF SEC system with GRAL columns showed the $M_{n,SEC}$ was 9.1 kDa and the D value was 1.18, relative to polystyrene standards. The azide-functionalized three-arm star backbone polymer with a DP of 434 per arm (PHEMA- N_3) was prepared by Dr. Ethan W. Kent as described in the literature.²⁸ From 1H NMR spectroscopy analysis, the degree of azide functionalization was 88.3%. The $M_{n,SEC}$ of PHEMA- N_3 was 224.5 kDa and the D was 1.16 relative to polystyrene standards by SEC analysis using PSS GRAL columns with DMF containing 50 mM LiBr as the eluent. Acetonitrile (99%, Aldrich) and anisole (99%, Acros Organics) were dried with 4 Å molecular sieves (Aldrich). Sodium persulfate ($Na_2S_2O_8$, 98 %, Alfa Aesar), sodium perchlorate ($NaClO_4$, 98 %, Alfa Aesar), sodium thiocyanate ($NaSCN$, 95%, Fisher Scientific), sodium sulfate (Na_2SO_4 , 99%, Fisher Scientific), and sodium salicylate ($NaC_7H_5O_3$, 99%, Alfa Aesar) were used as received. Sodium thiosalicylate was prepared from thiosalicylic acid ($C_7H_5O_2S$, 97%, Sigma Aldrich) by dissolving in basic water (1 M NaOH), recrystallizing, and drying under high vacuum. Aqueous solutions with different concentrations for each salt were prepared by dissolving appropriate amounts of the salt in Milli-Q water and then filtered through 0.45 µm hydrophilic PTFE filters. All other chemicals were purchased from commercial vendors and used as received.

5.2.2. General Characterization

The alkyne end-functionalized PDMAEMA and the reaction mixture for the synthesis of SMB-M (Scheme 5.1) were analyzed using a PL-PGC 50 Plus system, an integrated GPC/SEC system from Polymer Laboratories, Inc. This SEC instrument was equipped with a differential refractive index (RI) detector, one PSS GRAL guard column (10 micron particles, 50×8 mm, Polymer Standards Service-USA, Inc.), and two PSS GRAL linear columns (10 micron particles, 300×8 mm, molecular weight range of 500 to 1 000 000 g/mol, PSS-USA, Inc.). The analysis was performed at 50 °C, with *N,N*-dimethylformamide (DMF) containing 50 mM LiBr as the eluent at a flow rate of 1.0 mL/min. The system was calibrated with narrow dispersity linear polystyrene standards (Scientific Polymer Products, Inc.). ^1H NMR and ^{13}C NMR spectra were obtained using either a Varian VNMRs 500 NMR or a Varian VNMRs 600 NMR spectrometer.

5.2.3. Synthesis of Star Molecular Bottlebrushes Composed of Heterografted PEO and PDMAEMA Side Chains (SMB-M)

PHEMA- N_3 (10.7 mg, 4.42×10^{-5} mol azide moieties assuming quantitative functionalization, added by 1.633 g of a 6.111 mg/g stock solution in THF), alkyne end-functionalized PEO (126.9 mg, 2.49×10^{-5} mol), alkyne end-functionalized PDMAEMA (224.6 mg, 2.49×10^{-5} mol), copper(I) chloride (4.1 mg, 4.1×10^{-5} mol), and THF (6.5 mL) were added to a 2-dram scintillation vial equipped with a magnetic stir bar and sealed with a rubber septum. The headspace was flushed with nitrogen gas through needles for 10 min and PMDETA (9.52 μL , 4.56×10^{-5} mol) was injected by a microsyringe. The headspace was purged for an additional 10 min and sealed. After stirring overnight, propargyl benzyl ether (10 μL) was injected by microsyringe to cap unreacted azide groups and stirred. The copper catalyst was removed by passing the reaction mixture through a basic alumina (top)/silica gel (bottom) plug using THF as an eluent. The solution was

concentrated (~ 6 mL) by rotary evaporation, diluted with ethanol (200 proof, 30 mL), and then concentrated again. This process was repeated for two more times to remove THF. The concentrated solution was diluted with the same amount of Milli-Q water. The bottlebrushes were purified by eleven rounds of centrifugal filtration at 5000 rpm using a 50 kDa MWCO ultracentrifuge filter (MilliporeTM and AmiconTM). The removal of side chains was confirmed by SEC analysis using a SEC system with GRAL columns and DMF with 50 mM LiBr as the mobile phase. The purified brushes were slowly concentrated and stored in THF as a stock solution (yield: 64.3 mg, 21.0 %).

5.2.4. Synthesis of Star Molecular Bottlebrushes Composed of Heterografted PEO and PDMAEMA-Q Side Chains (SMB-Q)

SMB-M (18.7 mg, 4.20×10^{-5} mol tertiary amine groups) was dissolved in acetonitrile (1 mL, dried over anhydrous 4Å molecular sieves) in a 1-dram scintillation vial equipped with a magnetic stir bar and a rubber septum. Iodomethane (24.06 mg, 1.70×10^{-4} mol) was added, and the vial was immediately covered with aluminum foil. After the reaction mixture was stirred at room temperature for 16 h, an aliquot was taken for ^1H NMR analysis, which showed that reaction reached a quantitative conversion. The reaction mixture was carefully concentrated and purified by dialysis (regenerated cellulose dialysis tubing with MWCO of 3500 Da) against Milli-Q water. The dialysis was performed in dark and the water was changed frequently for 1 day. The resultant solution was concentrated and stored in Milli-Q water (yield: 11.22 mg, 41.0 %).

5.2.5. Synthesis of Poly((2-(methacryloyloxy)ethyl)trimethylammonium iodide) (PDMAEMA-Q)

PDMAEMA (0.643 g of a 71.8 mg/g stock solution in THF, 4.57×10^{-4} mol of tertiary amine groups) was added to a 2-dram scintillation vial equipped with a magnetic stir bar and a rubber

septum. The solution was concentrated, dried under high vacuum, and re-dissolved in acetonitrile (2 mL, dried over anhydrous 4Å molecular sieves). After the polymer solution was equilibrated in an ice/water bath, a solution of iodomethane (89.5 mg, 6.31×10^{-4} mol) in dry acetonitrile (1 mL) cooled in an ice/water bath was added dropwise. The mixture was warmed naturally to room temperature and stirred overnight. After 16 h, an aliquot was taken for ^1H NMR spectroscopy analysis showing a quantitative conversion. The reaction was stopped, and the mixture was concentrated and dried under high vacuum. The polymer was collected as a yellow solid (yield: 81.9 mg, 94.2 %).

5.2.6. Dynamic Light Scattering Study of SMB-Q in Aqueous Solutions With Salts

Dynamic light scattering (DLS) measurements of SMB-Q in aqueous solution in the presence of various salts were taken using a Malvern Zetasizer Nano ZS instrument with a He-Ne 644 nm laser and a temperature controller at a scattering angle of 173° . A stock solution of SMB-Q with a concentration of 1.09 mg/g was prepared first and then diluted to the concentration of 0.1 mg/g. The 0.1 mg/g solutions of SMB-Q were slowly filtered through a $0.45\ \mu\text{m}$ PTFE filter prior to DLS experiments. To study the effects of salts on the conformation of SMB-Q in water, aqueous stock solutions of six salts ($\text{Na}_2\text{S}_2\text{O}_8$, NaClO_4 , NaSCN , Na_2SO_4 , $\text{NaC}_7\text{H}_5\text{O}_3$, and $\text{NaC}_7\text{H}_5\text{O}_2\text{S}$) with various concentrations (0.010, 0.10, and 1.0 M) were made using Milli-Q water and added in small aliquots each time ($< 10\ \mu\text{L}$) into a 1.0 mL solution of 0.1 mg/g SMB-Q to obtain an appropriate salt concentration using a pipette (Gilson, Pipetman Classic P20). For each salt, the DLS measurements were performed at $25\ ^\circ\text{C}$ to study the effect of salt concentration on the apparent hydrodynamic size of SMB-Q. The data points in the DLS size plots are averages of three measurements and each measurement is the average of 10 runs. Unless otherwise stated, all the

data points in the DLS plots are single size distributions without any large species. Representative hydrodynamic size distributions are presented as examples.

5.2.7. Behavior of PDMAEMA-Q in Water in the Presence of NaClO₄ Upon Heating

The behavior of PDMAEMA-Q in water in the presence of NaClO₄ upon heating was investigated by visual inspection. A 5.0 mg/g solution of PDMAEMA-Q in Milli-Q water was prepared, and NaClO₄ salt was added to adjust the salt concentration incrementally until the solution turned cloudy at room temperature. The temperature was then gradually increased using a Fisher Scientific Isotemp 4100 R20F until the solution became clear. The temperature was subsequently decreased, and at each temperature, the solution was equilibrated for 2 min. When the solution just turned cloudy, the temperature was recorded as the cloud point. Photographs of the solution were taken above and below the cloud point using a Nikon Coolpix B500 camera.

5.3. Results and Discussion

5.3.1. Synthesis and Characterization of SMB-Q with Heterografted PEO and Poly((2-(methacryloyloxy)ethyl)trimethylammonium iodide) Side Chains

SMB-Q composed of PEO and permanently charged PDMAEMA-Q side chains was prepared via a two-step procedure as shown in Scheme 5.1. In the first step, a copper(I)-catalyzed azide-alkyne cycloaddition reaction was carried out to simultaneously graft alkyne-end-functionalized PEO and PDMAEMA onto an azide-bearing three-arm star backbone polymer, PHEMA-N₃, producing SMB-M with heterografted PEO and PDMAEMA side chains, as for the synthesis of SMB-11 in Chapter 4. The synthesis of 5 kDa PEO was described in the previous chapter, and the alkyne end-functionalized PDMAEMA used in this work was prepared by atom transfer radical polymerization via a procedure similar to that for the PDMAEMA in Chapter 4. The DP was also

56, and the $M_{n,SEC}$ and $\bar{D}$ from SEC analysis were 9.1 kDa and 1.18, respectively, with respect to polystyrene standards.

After the click reaction, the reaction mixture was passed through a basic alumina/silica gel column to remove the catalyst and the polymers were transferred to a solvent mixture of ethanol and water (1 : 1, v/v). The unreacted side chains were removed by 11 rounds of centrifugal filtration at 5000 rpm using a 50 kDa MWCO centrifugal filter. The complete removal of unreacted PEO and PDMAEMA side chains was confirmed by SEC analysis (Figure 5.1), yielding a pure bottlebrush polymer (SMB-M). The side chain composition of SMB-M was determined by 1H NMR analysis to be 59.3 mol% PEO and 40.7 mol% PDMAEMA (Figure 5.2A). The grafting density of SMB-M was 80.8 %, calculated by using the peak area ratio of the bottlebrushes and the unreacted side chains (69.0% : 31.0%) in the SEC trace at the end of the click reaction, the feed ratios, and the side chain composition of SMB-M as described for SMB-11 in Appendix B1. Using the method presented in Appendix B2 for SMB-11 and -22 in Chapter 4, the calculated weight-average molecular weight was 8.49×10^6 g/mol.

In the second step, the tertiary amine groups in SMB-M were quaternized by iodomethane in acetonitrile at room temperature, producing SMB-Q. 1H NMR spectroscopy analysis showed that the quaternization reaction was quantitative (Figure 5.2B), as indicated by the complete disappearance of the peaks located at 2.28 ppm ($-N(CH_3)_2$ of PDMAEMA) and 2.57 ppm ($-OCH_2CH_2N(CH_3)_2$ of PDMAEMA) and the emergence of the peak at 3.35 ppm ($-N^+(CH_3)_3$) (Figure 5.2). SMB-Q was purified by dialysis against Milli-Q water. The calculated weight-average molecular weight was 1.22×10^7 g/mol. The characterization data for PHEMA- N_3 , both alkyne end-functionalized side chain polymers, SMB-M, and SMB-Q are summarized in Table 5.1. as well as calculated grafting densities and weight-average molecular weights.

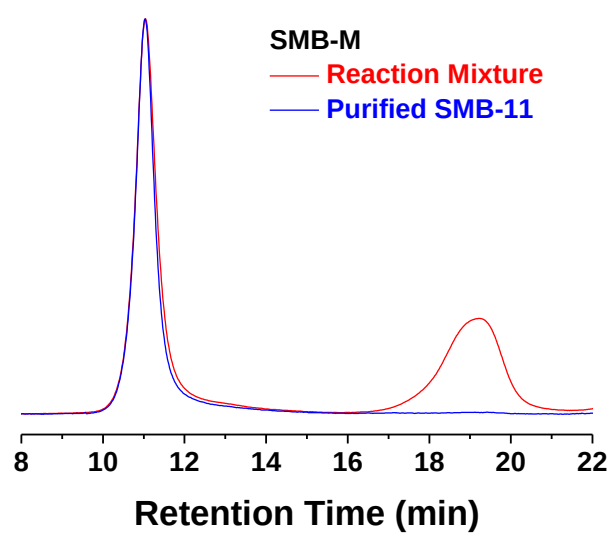


Figure 5.1. Size exclusion chromatography (SEC) curves of the reaction mixture at the end of the click reaction (red) for the synthesis of SMB-M and the purified brushes (blue). The SEC chromatograms were obtained on a SEC system equipped with GRAL columns and DMF containing 50 mM LiBr as the eluent at 50 °C.

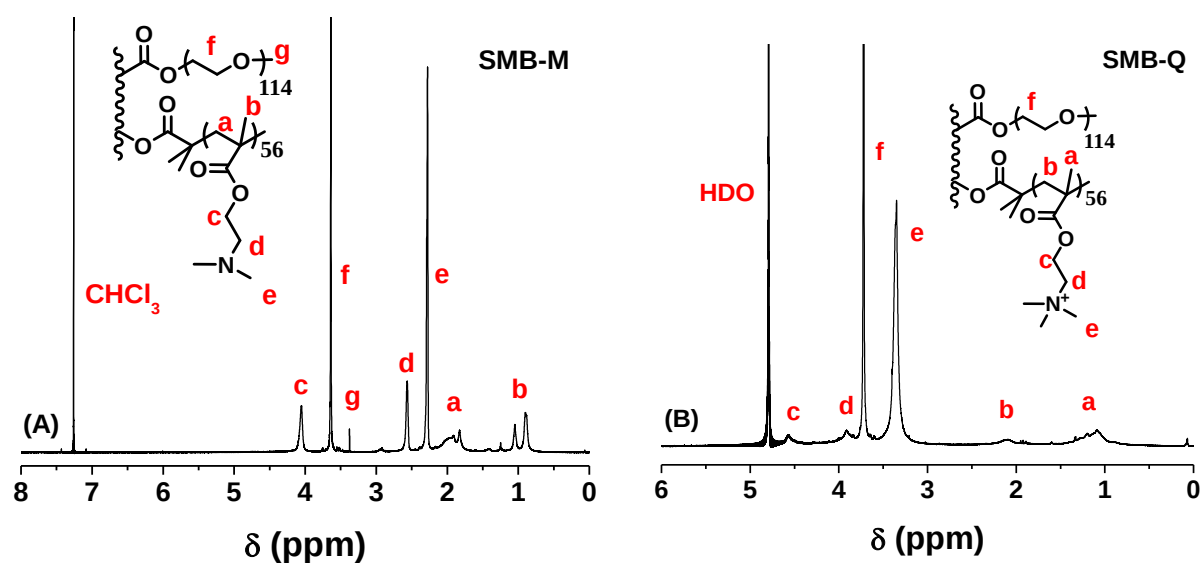


Figure 5.2. ^1H NMR spectra of (A) purified SMB-M in CDCl_3 and (B) SMB-Q in D_2O .

Table 5.1. Characterization Data for PHEMA-N₃, Alkyne End-Functionalized PEO, PDMAEMA, SMB-M, SMB-Q, and PDMAEMA-Q.

Polymer Sample	DP or Grafting Density (σ)	Molecular Weight and $\bar{D}$
PHEMA-N ₃	DP = 434 per arm ^a	$M_{n,SEC} = 2.25 \times 10^5$ Da, $\bar{D} = 1.16^e$
PEO	DP = 114 ^b	$M_{n,SEC} = 8.4 \times 10^3$ Da, $\bar{D} = 1.04^f$
PDMAEMA	DP = 56 ^a	$M_{n,SEC} = 9.1 \times 10^3$ Da, $\bar{D} = 1.18^e$
SMB-M (PEO/PDMAEMA)	$\sigma = 80.8\%$ ^c (molar ratio: 59.3% (PEO): 40.7%) ^d	$M_{w,cal} = 8.22 \times 10^6$ Da ^g
SMB-Q (PEO/PDMAEMA-Q)	Quaternization conversion: 100%	$M_{w,cal} = 1.22 \times 10^7$ Da ^g

^a Degree of polymerization (DP) was calculated from the monomer-to-initiator molar ratio and the monomer conversion (obtained from ¹H NMR analysis). ^b The DP of PEO was calculated from the molecular weight of 5000 g/mol provided by the vendor. ^c The value of grafting density (σ) was calculated using the ratio of SEC peak areas of the bottlebrushes and the unreacted side chain polymers in the final reaction mixture, the feed ratios of the star backbone polymer to the side chain polymers, and the side chain composition of the purified bottlebrush polymer determined by ¹H NMR analysis. ^d The molar ratio of PEO to PDMAEMA was determined from the ¹H NMR spectrum of the brush polymer. ^e $M_{n,SEC}$ and $\bar{D}$ were determined by SEC relative to polystyrene standards using a SEC system with GRAL columns and DMF containing 50 mM LiBr as solvent. ^f $M_{n,SEC}$ and $\bar{D}$ were determined by SEC relative to polystyrene standards using a SEC system with Mixed C columns and THF as solvent. ^g The calculated absolute molecular weight ($M_{w,cal}$) was obtained using the weight-average molecular weight of PHEMA-N₃ and the side chain polymers calculated from the DP and the dispersity ($M_n \times \bar{D}$), the grafting density, and the side chain composition.

5.3.2. Dynamic Light Scattering Study of the Effects of Various Salts on the Conformation of SMB-Q in Milli-Q Water at 25 °C

Six salts were used in this study to investigate their effects on the conformation of SMB-Q in Milli-Q water at 25 °C: $\text{Na}_2\text{S}_2\text{O}_8$, NaClO_4 , NaSCN , sodium salicylate ($\text{NaC}_7\text{H}_5\text{O}_3$), and sodium thiosalicylate ($\text{NaC}_7\text{H}_5\text{O}_2\text{S}$). $\text{S}_2\text{O}_8^{2-}$ is a super CA, causing a decrease of 2.04 hydrogen bonds in the solvation shell, and ClO_4^- is a moderate CA with a loss of 1.27 hydrogen bonds in the surrounding solvation shell.²⁷ SCN^- is a relatively weak CA as shown in a previous study from our group that a star brush polymer composed of PEO and PDMAEMA, similar to SMB-M, did not exhibit a full collapse in acidic water even at a SCN^- concentration of 300 mM.¹⁸ SO_4^{2-} is a kosmotropic anion, with an effective increase of 0.78 hydrogen bonds in the solvation shell around it.²⁷ It is unclear whether salicylate and thiosalicylate are chaotropic or kosmotropic; they were included in this study because we wanted to see if these two anions from drugs can induce large conformational changes of SMB-Q. Note that the counteranion of quaternary ammonium cations in SMB-Q is I^- , which is a chaotrope.²⁷ Since I^- existed in all the solutions at the same concentration, we believe that the effects observed here are from the anions in the salts.

Aqueous solutions of 0.1 mg/g SMB-Q in Milli-Q water were prepared and the concentration of each salt was incrementally increased. Figure 5.3 shows the plots of apparent hydrodynamic diameter (D_h) for 0.1 mg/g SMB-Q in Milli-Q water at 25 °C as a function of salt concentration for the six salts. The initial D_h values varied from 85.6 to 95.8 nm and is believed to result from the filtration in the sample preparation process. For all the six salts, as the salt concentration increased, the hydrodynamic diameter of SMB-Q decreased but at different speeds. For $\text{S}_2\text{O}_8^{2-}$, the D_h decreased sharply by ~ 49 nm from 0.05-0.25 mM and then increased slightly upon further increasing the salt concentration. The smallest value of D_h , 43.1 nm, was found at 0.25 mM. In the

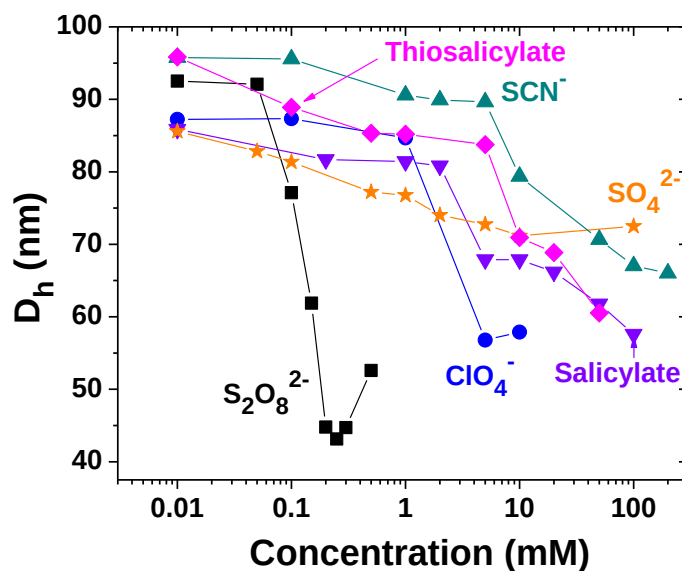


Figure 5.3. Apparent hydrodynamic diameter (D_h) of SMB-Q in Milli-Q water at 25 °C as a function of increasing salt concentration for sodium persulfate (black square), sodium perchlorate (blue circle), sodium thiocyanate (dark cyan up-pointing triangle), sodium salicylate (violet down-pointing triangle), sodium thiosalicylate (magenta diamond), and sodium sulfate (orange star). All data points shown here are single size distributions except the concentration of 10 mM for $NaClO_4$.

concentration range for $\text{S}_2\text{O}_8^{2-}$ shown in Figure 5.3, single size distributions were observed; examples are included in Figure 5.4A. This strong and sharp decrease in D_h was expected since $\text{S}_2\text{O}_8^{2-}$ is a super CA. The cause of the slight increase in D_h is unclear but has been observed before after the collapse of molecular bottlebrushes.¹⁸ Compared with a protonated star brush polymer similar to SMB-M in water at pH 4.00,¹⁸ the minimum salt concentration for achieving a nearly full collapse of SMB-Q was noticeably lower (0.2 mM vs > 0.6 mM) and the transition was sharper.

For ClO_4^- , the D_h decreased sharply by ~ 28 nm from 1-5 mM and then large aggregates appeared at 10 mM as shown in Figure 5.4B. The smallest D_h was 56.8 nm observed at 5 mM NaClO_4 , which is significantly lower than the minimum concentration of the same salt (~ 150 mM) for the protonated star brushes composed of PEO and PDMAEMA side chains (similar to SMB-M) reported by Kent et. al.¹⁸ The size transition induced by ClO_4^- occurred at a salt concentration roughly one order of magnitude higher than $\text{S}_2\text{O}_8^{2-}$. The size decrease for SCN^- occurred slightly less sharply than for ClO_4^- and was ~ 30 nm when the salt concentration was increased from 0.01 mM to 200 mM. At 200 mM, the D_h was 66.0 nm, with a single size distribution (Figure 5.4C). In contrast, for protonated brushes of similar compositions to SMB-M, only a gradual decrease of ~ 15 nm was observed when the concentration of SCN^- was increased from 0.01 to 300 mM.¹⁸ Kosmotropic SO_4^{2-} exerted only a small effect on the conformation of SMB-Q, with a gradual decrease of ~ 13 nm from 0.01 to 100 mM (Figure 5.4D), similar to its effect on SMB-11 and -22 in acidic water described in Chapter 4. All of these indicated that the quaternary ammonium cations in SMB-Q are a stronger chaotrope than the protonated tertiary amine groups of PDMAEMA under acidic conditions, as expected.

Very interestingly, for both salicylate and thiosalicylate anions, SMB-Q exhibited a pronounced size reduction transition, with a decrease of ~ 28 nm for salicylate from 0.01 mM to

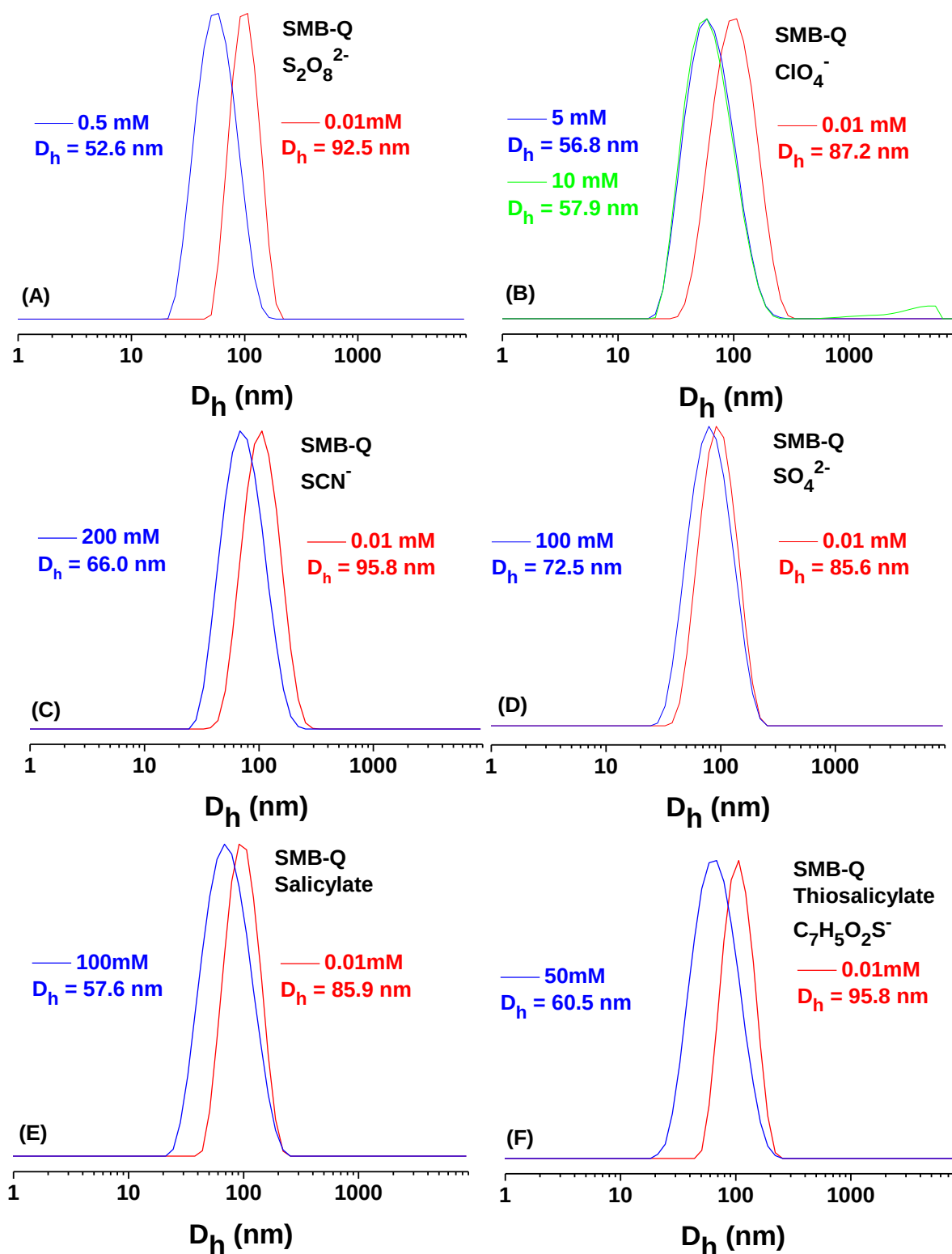


Figure 5.4. Hydrodynamic size distributions obtained from DLS measurements at 25 °C of 0.1 mg/g SMB-Q in Milli-Q water in the presence of $Na_2S_2O_8$ (A), $NaClO_4$ (B), $NaSCN$ (C), Na_2SO_4 (D), sodium salicylate (E), and sodium thiosalicylate (F) at various concentrations. Large species were observed at 10 mM $NaClO_4$ in (B).

100 mM (Figure 5.4E) and ~ 35 nm for thiosalicylate from 0.01 mM to 50 mM (Figure 5.4F). The size transitions induced by the two drugs occurred in the salt concentration ranges that were between those for ClO_4^- and SCN^- , and the breadths of the transition zones was similar to that of SCN^- but broader than ClO_4^- . These observations seemed to suggest that both salicylate and thiosalicylate were CAs, with a strength likely between ClO_4^- and SCN^- . On the other hand, salicylate and thiosalicylate have aromatic rings that can interact with the cation through π -cation interactions,^{29,30} which could also contribute to the interactions between the brushes and the anion.

The size transitions induced by chaotropic $\text{S}_2\text{O}_8^{2-}$, ClO_4^- , and SCN^- are believed to be caused by the enthalpically favorable formation of tight ion pairs with quaternary ammonium cations in SMB-Q. The ion pairing decreased the solubility of the quaternized PDMAEMA side chains in water and drove SMB-Q to collapse into more compact structures with soluble PEO side chains as a stabilizer. To confirm the solubility change of the quaternized PDMAEMA side chains in the presence of a CA, we quaternized alkyne-end-functionalized PDMAEMA with iodomethane. ^1H NMR spectroscopy analysis showed that the reaction was complete (Figure 5.5); the obtained polymer is denoted as PDMAEMA-Q.

To study the influence of NaClO_4 on the solubility of PDMAEMA-Q in water, aqueous solutions of PDMAEMA-Q with a concentration of 5 mg/g were prepared. Without adding NaClO_4 , the solution stayed clear in the temperature range of 0 – 100 °C. NaClO_4 was then added and the salt concentration was gradually increased. At 5 mM of ClO_4^- , the solution was also completely clear from 0 to 100 °C. When the concentration of ClO_4^- was increased to 50 mM, the solution turned cloudy at room temperature (~ 20 °C) and became clear upon heating, indicating a upper critical solution temperature (UCST) transition. The cloud point was found to be 29 °C upon

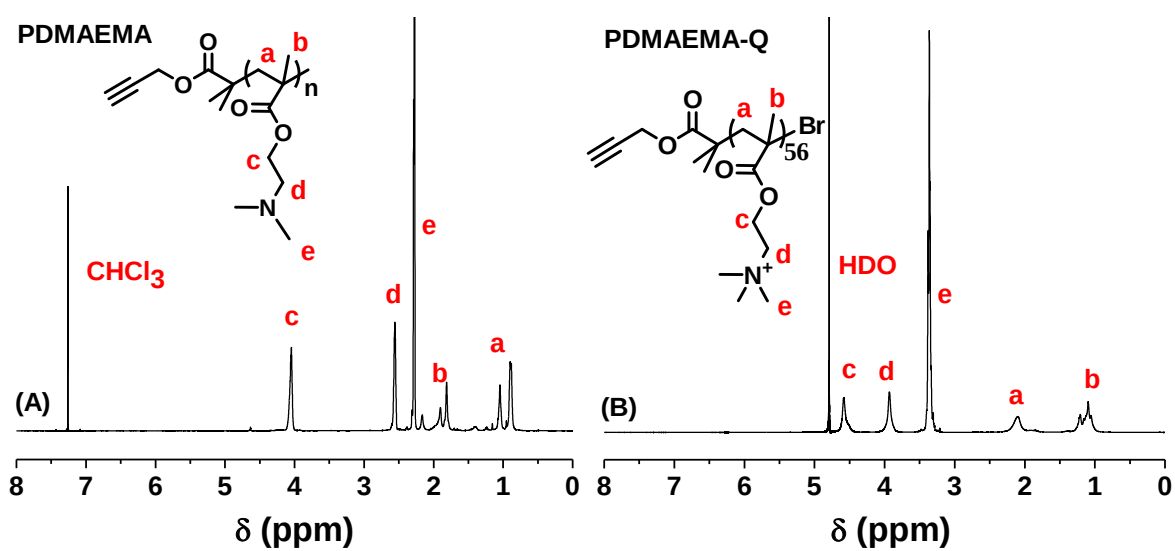


Figure 5.5. ^1H NMR spectra of alkyne-end-functionalized PDMAEMA in CDCl_3 (A) and PDMAEMA-Q in D_2O .

cooling. Similar UCST transitions were observed at the NaClO₄ concentrations of 100 mM and 500 mM and the cloud points were found to be 48 and 88 °C, respectively. Optical photos for solutions of PDMAEMA-Q with 50 and 100 mM NaClO₄ are shown in Figure 5.6. This study confirmed the solubility decrease of PDMAEMA-Q in the presence of NaClO₄ with a sufficiently high concentration, which induced the size transition of SMB-Q observed by DLS (Figure 5.3). In addition, we also studied Na₂SO₄. No change in the solubility of PDMAEMA-Q in water was observed even when the concentration of Na₂SO₄ was increased to 500 mM.

5.4. Conclusions

In summary, we synthesized a heterografted three-arm star brush polymer composed of 5 kDa PEO and PDMAEMA-Q side chains, SMB-Q, and studied the effects of six anions (super chaotropic S₂O₈²⁻, moderately chaotropic ClO₄⁻, relatively weak chaotropic SCN⁻, kosmotropic SO₄²⁻, salicylate, and thiosalicylate) on the conformation of SMB-Q in water DLS. Pronounced size reduction transitions of SMB-Q were observed for S₂O₈²⁻, ClO₄⁻, SCN⁻, salicylate, and thiosalicylate. The results for S₂O₈²⁻, ClO₄⁻, SCN⁻, and SO₄²⁻ are consistent with those reported previously and in Chapter 4. The observations suggest that salicylate and thiosalicylate anions are likely chaotropic; the data also indicated that the ammonium cations in SMB-Q are a stronger chaotrope than the protonated PDMAEMA in acidic water. The chaotropic anion-induced collapse of SMB-Q is presumably caused by the pairing of chaotropic ammonium cations and chaotropic anions, resulting in a decrease in the solubility of PDMAEMA-Q in water. This was confirmed by testing the solubility of a quaternized PDMAEMA in water with the addition of NaClO₄. The finding that salicylate and thiosalicylate can induce pronounced conformational changes of SMB-Q is particularly interesting, which may have potential applications in drug delivery.

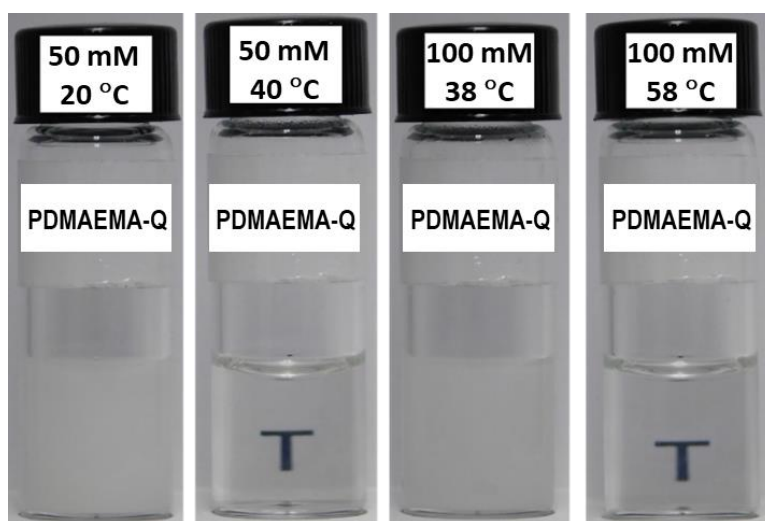


Figure 5.6. Optical photographs of quaternized alkyne end-functionalized PDMAEMA homopolymer, PDMAEMA-Q, at various concentrations of NaClO_4 and temperatures in Milli-Q water.

References:

1. Lee, H.-i.; Pietrasik, J.; Sheiko, S. S.; Matyjaszewski, K., Stimuli-responsive molecular brushes. *Progress in Polymer Science* **2010**, *35* (1), 24-44.
2. Sheiko, S. S.; Sumerlin, B. S.; Matyjaszewski, K., Cylindrical molecular brushes: Synthesis, characterization, and properties. *Progress in Polymer Science* **2008**, *33* (7), 759-785.
3. Zhang, M.; Müller, A. H. E., Cylindrical polymer brushes. *Journal of Polymer Science Part A: Polymer Chemistry* **2005**, *43* (16), 3461-3481.
4. Xie, G.; Martinez, M. R.; Olszewski, M.; Sheiko, S. S.; Matyjaszewski, K., Molecular Bottlebrushes as Novel Materials. *Biomacromolecules* **2019**, *20* (1), 27-54.
5. Beers, K. L.; Gaynor, S. G.; Matyjaszewski, K.; Sheiko, S. S.; Möller, M., The Synthesis of Densely Grafted Copolymers by Atom Transfer Radical Polymerization. *Macromolecules* **1998**, *31* (26), 9413-9415.
6. Cheng, G.; Böker, A.; Zhang, M.; Krausch, G.; Müller, A. H. E., Amphiphilic Cylindrical Core-Shell Brushes via a "Grafting From" Process Using ATRP. *Macromolecules* **2001**, *34* (20), 6883-6888.
7. Matyjaszewski, K.; Qin, S.; Boyce, J. R.; Shirvanyants, D.; Sheiko, S. S., Effect of Initiation Conditions on the Uniformity of Three-Arm Star Molecular Brushes. *Macromolecules* **2003**, *36* (6), 1843-1849.
8. Cheng, C.; Khoshdel, E.; Wooley, K. L., One-Pot Tandem Synthesis of a Core-Shell Brush Copolymer from Small Molecule Reactants by Ring-Opening Metathesis and Reversible Addition-Fragmentation Chain Transfer (Co)polymerizations. *Macromolecules* **2007**, *40* (7), 2289-2292.

9. Xia, Y.; Kornfield, J. A.; Grubbs, R. H., Efficient Synthesis of Narrowly Dispersed Brush Polymers via Living Ring-Opening Metathesis Polymerization of Macromonomers. *Macromolecules* **2009**, *42* (11), 3761-3766.
10. Li, A.; Ma, J.; Sun, G.; Li, Z.; Cho, S.; Clark, C.; Wooley, K. L., One-pot, facile synthesis of well-defined molecular brush copolymers by a tandem RAFT and ROMP, “Grafting-through” strategy. *Journal of Polymer Science Part A: Polymer Chemistry* **2012**, *50* (9), 1681-1688.
11. Gao, H.; Matyjaszewski, K., Synthesis of Molecular Brushes by “Grafting onto” Method: Combination of ATRP and Click Reactions. *Journal of the American Chemical Society* **2007**, *129* (20), 6633-6639.
12. Tsarevsky, N. V.; Bencherif, S. A.; Matyjaszewski, K., Graft Copolymers by a Combination of ATRP and Two Different Consecutive Click Reactions. *Macromolecules* **2007**, *40* (13), 4439-4445.
13. Tang, H.; Li, Y.; Lahasky, S. H.; Sheiko, S. S.; Zhang, D., Core–Shell Molecular Bottlebrushes with Helical Polypeptide Backbone: Synthesis, Characterization, and Solution Conformations. *Macromolecules* **2011**, *44* (6), 1491-1499.
14. Henn, D. M.; Fu, W.; Mei, S.; Li, C. Y.; Zhao, B., Temperature-Induced Shape Changing of Thermosensitive Binary Heterografted Linear Molecular Brushes between Extended Wormlike and Stable Globular Conformations. *Macromolecules* **2017**, *50* (4), 1645-1656.
15. Henn, D. M.; Lau, C. M.; Li, C. Y.; Zhao, B., Light-triggered unfolding of single linear molecular bottlebrushes from compact globular to wormlike nano-objects in water. *Polymer Chemistry* **2017**, *8* (17), 2702-2712.

16. Henn, D. M.; Holmes, J. A.; Kent, E. W.; Zhao, B., Worm-to-Sphere Shape Transition of Thermoresponsive Linear Molecular Bottlebrushes in Moderately Concentrated Aqueous Solution. *The Journal of Physical Chemistry B* **2018**, *122* (27), 7015-7025.
17. Kent, E. W.; Lewoczko, E. M.; Zhao, B., Effect of Buffer Anions on Pearl-Necklace Morphology of Tertiary Amine-Containing Binary Heterografted Linear Molecular Bottlebrushes in Acidic Aqueous Buffers. *Langmuir* **2020**, *36* (44), 13320-13330.
18. Kent, E. W.; Lewoczko, E. M.; Zhao, B., pH- and chaotropic anion-induced conformational changes of tertiary amine-containing binary heterografted star molecular bottlebrushes in aqueous solution. *Polymer Chemistry* **2021**, *12* (2), 265-276.
19. Johnson, J. A.; Lu, Y. Y.; Burts, A. O.; Xia, Y.; Durrell, A. C.; Tirrell, D. A.; Grubbs, R. H., Drug-Loaded, Bivalent-Bottle-Brush Polymers by Graft-through ROMP. *Macromolecules* **2010**, *43* (24), 10326-10335.
20. Banquy, X.; Burdyńska, J.; Lee, D. W.; Matyjaszewski, K.; Israelachvili, J., Bioinspired Bottle-Brush Polymer Exhibits Low Friction and Amontons-like Behavior. *Journal of the American Chemical Society* **2014**, *136* (17), 6199-6202.
21. Zhang, M.; Drechsler, M.; Müller, A. H. E., Template-Controlled Synthesis of Wire-Like Cadmium Sulfide Nanoparticle Assemblies within Core–Shell Cylindrical Polymer Brushes. *Chemistry of Materials* **2004**, *16* (3), 537-543.
22. Xu, H.; Sun, F. C.; Shirvanyants, D. G.; Rubinstein, M.; Shabratov, D.; Beers, K. L.; Matyjaszewski, K.; Sheiko, S. S., Molecular Pressure Sensors. *Advanced Materials* **2007**, *19* (19), 2930-2934.

23. Xu, Y.; Bolisetty, S.; Drechsler, M.; Fang, B.; Yuan, J.; Ballauff, M.; Müller, A. H. E., pH and salt responsive poly(N,N-dimethylaminoethyl methacrylate) cylindrical brushes and their quaternized derivatives. *Polymer* **2008**, 49 (18), 3957-3964.
24. Xu, Y.; Bolisetty, S.; Drechsler, M.; Fang, B.; Yuan, J.; Harnau, L.; Ballauff, M.; Müller, A. H. E., Manipulating cylindrical polyelectrolyte brushes on the nanoscale by counterions: collapse transition to helical structures. *Soft Matter* **2009**, 5 (2), 379-384.
25. Xu, Y.; Borisov, O. V.; Ballauff, M.; Müller, A. H. E., Manipulating the Morphologies of Cylindrical Polyelectrolyte Brushes by Forming Interpolyelectrolyte Complexes with Oppositely Charged Linear Polyelectrolytes: An AFM Study. *Langmuir* **2010**, 26 (10), 6919-6926.
26. Marcus, Y., Effect of Ions on the Structure of Water: Structure Making and Breaking. *Chemical Reviews* **2009**, 109 (3), 1346-1370.
27. Assaf, K. I.; Nau, W. M., The Chaotropic Effect as an Assembly Motif in Chemistry. *Angewandte Chemie International Edition* **2018**, 57 (43), 13968-13981.
28. 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 (17), 6714-6724.
29. Gallivan, J. P.; Dougherty, D. A., A Computational Study of Cation- π Interactions vs Salt Bridges in Aqueous Media: Implications for Protein Engineering. *Journal of the American Chemical Society* **2000**, 122 (5), 870-874.
30. Tsuzuki, S.; Yoshida, M.; Uchimaru, T.; Mikami, M., The Origin of the Cation/ π Interaction: The Significant Importance of the Induction in Li^+ and Na^+ Complexes. *The Journal of Physical Chemistry A* **2001**, 105 (4), 769-773.

Chapter 6: Conclusions and Future Work

This dissertation work focused on the synthesis and responsive behavior of two classes of stimuli-responsive polyelectrolytes: thermoresponsive zwitterionic poly(sulfobetaine methacrylate)s (PSBMAs) and charged shape-changing heterografted star molecular bottlebrushes (MBBs). In the first part, a series of PSBMAs with differing *N*-substituents were synthesized to establish a clear structure-solution behavior relationship and for investigating PSBMAs as potential kinetic hydrate inhibitors for gas and oil transportation. In the second part, we studied the effects of temperature on chaotropic anion-collapsed star MBBs with heterografted poly(ethylene oxide) (PEO) and poly(*N,N*-dialkylaminoethyl methacrylate) side chains in acidic water and the salt-responsive behavior of three-arm star polyelectrolyte MBBs with permanent quaternary ammonium groups in water.

In Chapter 2, we synthesized a series of PSBMAs with systematically varied *N*-substituents and studied the effects of *N*-substituents on solution behavior of PSBMAs in water.¹ Through multi-step organic synthesis procedures, we prepared a set of zwitterionic SBMA monomers with two symmetric *N-n*-alkyl substituents containing 1, 2, 4, 5, or 6 carbon atoms in each substituent and with two asymmetric *N*-substituents comprising one methyl group and either a cyclohexyl or a phenyl or a 2-hydroxyethyl group. These monomers were polymerized and the obtained zwitterionic polymers were purified by dialysis. The solution behavior of these PSBMAs in water was first visually inspected and then for thermoresponsive PSBMAs the cloud points were measured by UV-Vis spectrometry. The behavior of the PSBMAs with symmetric *N-n*-alkyl substituents in water changed from UCST to soluble, LCST, and insoluble with the increase of the alkyl length. This behavior pattern presumably originates from two competing effects. Increasing the length of *N-n*-alkyl substituents weakens the electrostatic attractive interactions between zwitterionic groups while increasing the hydrophobic effect. For the PSBMAs with two

asymmetric *N*-substituents, when the *N*-*n*-hexyl substituent is changed to *N*-cyclohexyl while maintaining the other *N*-methyl group, the lower critical solution temperature (LCST)-type thermoresponsive property disappeared and the polymer became totally soluble in water due to the decreased hydrophobic effect. Switching the *N*-*n*-hexyl with a *N*-phenyl substituent changed the behavior of the polymer in water from LCST to upper critical solution temperature (UCST). Introducing a 2-hydroxyethyl group as an *N*-substituent while keeping the *N*-methyl group enhanced the attractive zwitterionic interactions, producing a UCST thermoresponsive polymer. We further showed that the cloud points of UCST-type thermoresponsive zwitterionic polymers can be readily tuned by copolymerization of two zwitterionic monomers with different monomer ratios. This study improves our understanding of the structure-property relationship of zwitterionic PSBMAs and provides a guideline for design of both LCST- and UCST-type thermoresponsive zwitterionic polymers as well as completely water-soluble zwitterionic polymers.^{1,2}

In a collaborative effort presented in Chapter 3, we and Professor Malcolm Kelland's group in Norway investigated the potential of our zwitterionic PSBMAs with *N*-*n*-alkyl substituents as kinetic hydrate inhibitors (KHIs) for gas and oil transportation.³ We prepared a series of zwitterionic polymers, including PSBMA homopolymers with a methyl and either an *n*-butyl, an *n*-pentyl, or an *n*-hexyl group at the nitrogen atom and copolymers of a zwitterionic SBMA monomer containing an *N*-methyl and an *N*-*n*-hexyl substituent (M16) with isopropyl methacrylamide. KHI testing showed that the homopolymers and copolymers of M16 were very promising; their performances were similar to commercially used poly(*N*-vinylcaprolactam).

Chaotropic effect is now employed as a generic driving force for supramolecular assembly. Combining chaotropic ion pairing and chaotropic effect, our research group previously showed chaotropic anion (CA)-induced star-to-globule shape transitions of three-arm star MBBs with

heterografted poly(ethylene oxide) (PEO) and either poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA) or poly(*N,N*-diethylaminoethyl methacrylate) (PDEAEMA) side chains in acidic water (SMB-11 and SMB-22, respectively).⁴ Given that the ion pairing of chaotropic cations and anions is an enthalpic effect, we hypothesized that heating might be able to induce globule-to-star shape transitions of CA-collapsed star brushes in acidic water. We showed in Chapter 4 that the globular bottlebrushes collapsed by super CAs such as $[\text{Fe}(\text{CN})_6]^{3-}$ and $[\text{Fe}(\text{CN})_6]^{4-}$ remained in the collapsed globular state upon heating to 70 °C, while moderate CA (ClO_4^- and PF_6^-)-collapsed SMB-11 and -22 in pH 4.50 acidic water exhibited a UCST-type globule-to-star shape change when heated to 70 °C. In contrast, weak chaotropic I^- and kosmotropic SO_4^{2-} had only small effects on the conformations of both brush polymers in acidic water in the studied concentration range; the sizes of SMB-11 and -22 in the presence of I^- and SO_4^{2-} also showed small, gradual variations in the studied temperature range. Given the large number of possible CAs, the finding that CA-collapsed brushes exhibited different thermoresponsive behavior could allow us to design tailored stimuli-responsive systems for specific needs in application.

Finally, on the basis of the hypothesis that iodomethane-quaternized PDMAEMA might be stronger chaotropic cations than the protonated PDMAEMA, we studied the salt-responsive behavior of a permanently charged star brush polymer with heterografted PEO and CH_3I -quaternized PDMAEMA (PDMAEMA-Q) side chains in water at 25 °C (the brush polymer is denoted as SMB-Q). Dynamic light scattering was employed to examine the effects of six anions (super CA $\text{S}_2\text{O}_8^{2-}$, moderate chaotropic ClO_4^- and SCN^- , kosmotropic SO_4^{2-} , salicylate, and thiosalicylate) on the conformation of SMB-Q in water. SMB-Q exhibited a pronounced size reduction transition for all the anions except SO_4^{2-} but at different anion concentrations. As expected, the size transitions induced by super and moderate CAs ($\text{S}_2\text{O}_8^{2-}$, ClO_4^- , and SCN^-)

occurred at significantly lower concentrations compared with unquaternized star brushes, suggesting that the quaternized ammonium groups in SMB-Q are stronger chaotropic cations than the protonated PDMAEMA. The DLS results also suggested that salicylate and thiosalicylate, which are drugs, might be CAs. Moreover, we found that the addition of moderate chaotropic ClO_4^- induced UCST-type phase separation of PDMAEMA-Q in water, which confirmed that the size transitions of SMB-Q observed by DLS resulted from the ion pairing of chaotropic cations and CAs. Given the widespread use of polyelectrolytes, the finding described in Chapter 5 could further enhance our understanding of the behavior of polyelectrolytes under various conditions.

As can be seen from above, zwitterionic polymers and polyelectrolyte MBBs possess many interesting properties that could increase opportunities for design of novel materials for various applications such as anti(bio)fouling,⁵ lubrication,⁶ and drug delivery.⁷ The first part of this dissertation work contributes a much-needed understanding to the structure-solution behavior of PSBMAs in water, opening up more possibilities for PSBMAs in the fields of, e.g., coatings and biomedicines. The second part of this dissertation research enhances our understanding of the responsive properties of polyelectrolyte MBBs in aqueous solution. Future work for zwitterionic polymers could include the development of non-PSBMA-based zwitterionic polymers to further our understanding of the structure-solution property relationship and the design of thermoresponsive zwitterionic homopolymers that can exhibit both UCST- and LCST-type solution behavior with tunable transition temperatures. Future work of polyelectrolyte MBBs could be salt-induced assemblies of MBBs in solution that are sensitive to magnetic and electric fields. Additionally, it would be exciting to confirm and further investigate drug anion-induced shape transitions of polyelectrolyte MBBs, which might be useful in drug delivery.

References:

1. Lewoczko, E. M.;[†] Wang, N.;[†] Lundberg, C. E.; Kelly, M. T.; Kent, E. W.; Wu, T.; Chen, M.-L.; Wang, J.-H.; Zhao, B. Effects of N-Substituents on Solution Behavior of Poly(sulfobetaine methacrylate)s in Water: UCST and LCST. *ACS Applied Polymer Materials* **2021**, 3, 867-878. [†] These authors contributed equally to this work.
2. Wang, N.; Seymour, B. T.; Lewoczko, E. M.; Kent, E. W.; Chen, M.-L.; Wang, J.-H.; Zhao, B. Zwitterionic Poly(sulfobetaine methacrylate)s in Water: From Upper Critical Solution Temperature (UCST) to Lower Critical Solution Temperature (LCST) with Increasing Length of One Alkyl Substituent on the Nitrogen Atom. *Polym. Chem.* **2018**, 9, 5257-5261.
3. Zhang, Q.; Kelland, M. A.; Lewoczko, E. M.; Bohannon, C. A.; Zhao, B. Non-amide Based Zwitterionic Poly(sulfobetaine methacrylate)s as Kinetic Hydrate Inhibitors. *Chemical Engineering Science* **2021**, 229, 116031.
4. Kent, E. W.; Lewoczko, E. M.; Zhao, B. pH- and Chaotropic Anion-Induced Conformational Changes of Tertiary Amine-Containing Binary Heterografted Star Molecular Bottlebrushes in Aqueous Solution. *Polymer Chemistry* **2021**, 12, 265-276.
5. Shao, Q.; Jiang, S., Molecular Understanding and Design of Zwitterionic Materials. *Advanced Materials* **2015**, 27 (1), 15-26.
6. Chen, M.; Briscoe, W. H.; Armes, S. P.; Klein, J., Lubrication at Physiological Pressures by Polyzwitterionic Brushes. *Science* **2009**, 323 (5922), 1698-1701.
7. Johnson, J. A.; Lu, Y. Y.; Burts, A. O.; Xia, Y.; Durrell, A. C.; Tirrell, D. A.; Grubbs, R. H., Drug-Loaded, Bivalent-Bottle-Brush Polymers by Graft-through ROMP. *Macromolecules* **2010**, 43 (24), 10326-10335.

Vita

Evan Michael Lewoczko was born in Medina, Tennessee, U.S.A. in 1994. In May 2016, he earned his B.S. degree in Chemistry from Union University in Jackson, Tennessee. In August 2016, he enrolled as a graduate student at the University of Tennessee, Knoxville. Soon after, Evan joined Professor Bin Zhao's research group where his research focused on the synthesis and behavior of two classes of stimuli-responsive polyelectrolytes, thermosensitive zwitterionic polymers and charged shape-changing star molecular bottlebrushes. He received his Doctor of Philosophy Degree in Chemistry from the University of Tennessee in August 2021.