

**Dually Responsive Shape-Changing Linear and Star
Molecular Bottlebrushes with Bicomponent Side Chains**

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Dedication

I would like to dedicate this dissertation to Timothy Cook, the man who introduced me to chemistry and continues to be a mentor and a friend.

“He is no fool who gives what he cannot keep to gain that which he cannot lose.”

-Jim Elliot

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Abstract

Molecular bottlebrushes (MBBs) can exhibit large conformational changes from wormlike to globular in solution in response to environmental stimuli. However, the instability of the collapsed state has prevented shape-changing MBBs from potential applications in, e.g., biomimetic catalysis and substance delivery. This dissertation work focused on dually responsive linear and star MBBs composed of bicomponent side chains in the form of either homografted diblock copolymer or binary heterografted polymeric side chains. When one polymer component collapsed, driving the shape changing of MBBs, another component served as a stabilizer. When both components in the side chains were stimuli-responsive, an additional level of control over the solution state of the brushes was achieved. The MBBs in this dissertation work were synthesized by a “grafting-to” method using the highly efficient Cu(I)-catalyzed azide-alkyne cycloaddition reaction.

Firstly, linear MBBs with diblock copolymer side chains composed of pH-responsive poly(2-(*N,N*-diethylamino)ethyl methacrylate) (PDEAEMA) as the inner block and thermoresponsive poly(methoxytri(ethylene glycol) acrylate) as the outer block were synthesized. These brushes underwent a unimolecular worm-to-globule shape transition in aqueous solution upon increasing the pH, where the PTEGMA block functioned as a stabilizer. The solution state of the brushes was then shown to be further controlled by the thermoresponsive property of PTEGMA. Subsequently, binary heterografted three-arm star MBBs consisting of two different homopolymers, pH-responsive PDEAEMA and thermoresponsive poly(ethoxydi(ethylene glycol) acrylate) (PDEGEA), as side chains were prepared and the formation of two distinct globular states with different polymers in the core from one brush system was demonstrated. A peculiar pearl-necklace brush morphology was observed from an aqueous phosphate buffer, which was further investigated

using a linear version of MBBs with the same side chains. It was found that the bead size of the pearl-necklace morphology was strongly affected by buffer anions. Lastly, binary heterografted star MBBs composed of poly(ethylene oxide) and either poly(2-(*N,N*-dimethylamino)ethyl methacrylate) or PDEAEMA side chains were prepared and the pH-induced conformational changes were revealed. Both star brush systems were found to undergo star-to-globule shape transitions upon the addition of chaotropic anions. The results obtained from this dissertation work will open up opportunities for potential applications of stimuli-responsive shape-changing MBBs.

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Chapter 1: Introduction

1.1. Introduction

This dissertation work is focused on the synthesis and behavior of stimuli-responsive shape-changing molecular bottlebrushes (MBBs). These brushes are composed of stimuli-responsive side chains densely grafted to a flexible polymer backbone and exhibit large conformational changes in aqueous solution in response to relatively small environmental changes. Section 1.1.1 provides a detailed description of molecular bottlebrushes along with general synthetic strategies. Section 1.1.2 is divided into two subsections; the first introduces stimuli-responsive polymers, with a focus on pH and temperature as stimuli, and the second focuses on the use of stimuli-responsive polymers in the preparation of responsive MBBs. Finally, Section 1.2 provides an overview of the dissertation work, presented in the following Chapters 2 through 5.

1.1.1. Introduction to Molecular Bottlebrushes

MBBs, also referred to as molecular brushes, are a class of graft copolymers which consist of a backbone polymer that is densely grafted with polymeric side chains.¹⁻⁶ When the aspect ratio of the molecular brushes (defined as the ratio of the brush contour length to the brush diameter) is sufficiently high, MBBs often adopt a highly stretched, worm-like or cylindrical conformation due to the strong steric interactions between individual side chains. It is important to note that molecular brushes with low aspect ratios (e.g., the length of the backbone is on the same order as that of the side chains) will adopt a compact conformation and are commonly referred to as spherical brushes.¹ In either case, the dense grafting of side chains allows for the direct imaging of individual brush molecules using atomic force microscopy (AFM). The distinct architecture of MBBs leads to many unique characteristics including high and tunable persistence lengths, low or no chain entanglement, and large conformational changes in response to external stimuli. These properties have allowed molecular bottlebrushes to attract the attention of many researchers in

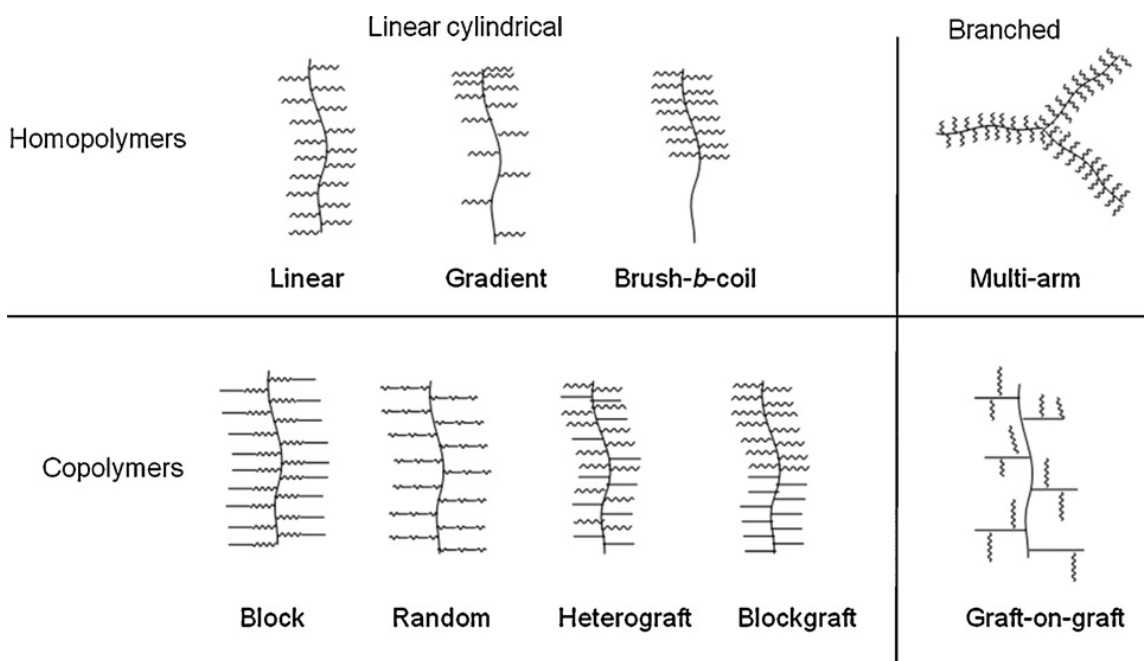
recent years, and they have shown promise in applications such as lubrication,⁷ supersoft elastomers,⁸ and templating synthesis of nanomaterials.⁹

Recent advances in polymerization techniques have accelerated molecular bottlebrush research, allowing for the routine preparation of well-defined molecular brushes with predetermined molecular weights, narrow dispersities, and various architectures. Scheme 1.1 illustrates a few of the many types of molecular brushes that have been prepared by varying side chain composition and distribution along the backbone as well as the topology of the backbone (e.g., star-like or cyclic).⁶ This dissertation work mainly focuses on (i) linear bottlebrushes, which consist of either a single type of side chains (homopolymer or diblock copolymer) or two distinct types of side chains, and (ii) binary heterografted three-arm star molecular brushes, composed of a branched backbone grafted with two different types of homopolymer side chains.

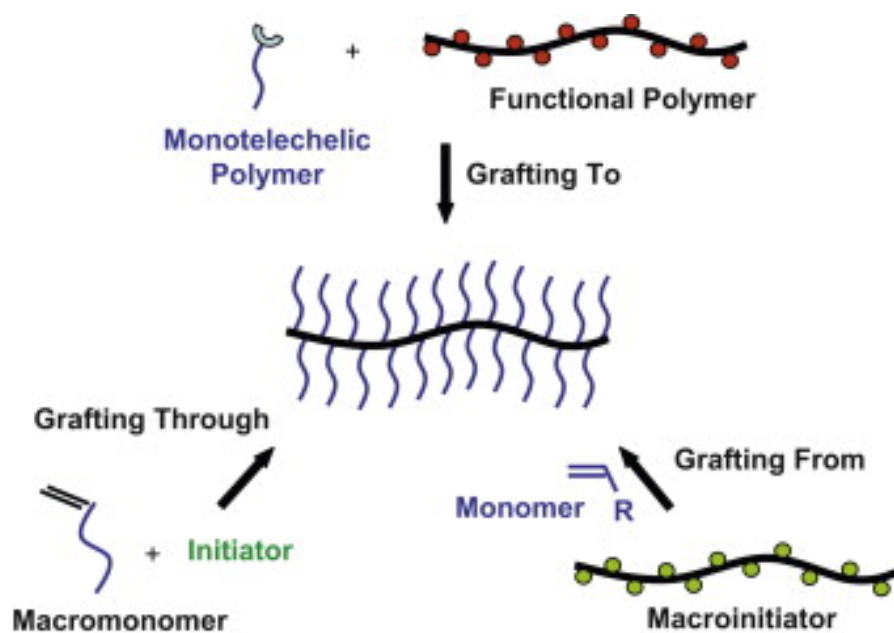
There are three main methods by which MBBs can be synthesized: grafting-from, grafting-through, and grafting-to (Scheme 1.2).⁵ Grafting-from is accomplished by growing polymer side chains from pendant initiating sites on the backbone polymer (macroinitiator). This method generally results in high grafting densities for side chains and is well-suited for the synthesis of homografted MBBs consisting of the same type of side chains with the same composition.¹⁰⁻¹² However, heterografted bottlebrushes, composed of more than one type of side chain polymer, are usually difficult to achieve using this method alone.

Grafting-through refers to the polymerization of macromonomers to form brushes. The advantage of this method is the ability to synthesize brushes with a side chain at every backbone repeat unit (i.e., 100% grafting density). However, the polymerization of macromonomers can be difficult; diffusion of the sterically bulky macromonomer molecules to bulky propagating chain ends is slow and the polymerization often only proceeds to low conversions. Using highly active

Scheme 1.1. Classification of Molecular Brushes by Branching Topologies and Chemical Composition of Side Chains.⁶ Reproduced from Ref. 6 with permission from Elsevier.



Scheme 1.2. Three Main Strategies for Preparing Molecular Brushes: Grafting-Through, Grafting-To, and Grafting-From.⁵ Reproduced from Ref. 5 with permission from Elsevier.



3rd generation Grubbs catalyst, ring-opening metathesis polymerization (ROMP) has been shown to be able to overcome some of these issues to produce high molecular weight well-defined bottlebrushes.¹³⁻¹⁷

In the grafting-to method, a polymer backbone bearing a large number of pendant functional groups is prepared, and then complementary end-functionalized polymer side chains are grafted to the backbone creating the brushes. One drawback of this method has been the difficulty in achieving high grafting density. However, with the development of highly efficient, robust “click” reactions, such as the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, grafting-to has gained increased attention in recent years.¹⁸⁻²³ In conjunction with “living”/controlled radical polymerization techniques such as ATRP and RAFT for synthesizing backbone and side chain polymers, this method has been used to make molecular brushes with high and tunable grafting densities. Our group recently explored this method with great success by clicking alkyne end-functionalized side chain polymers onto azide-bearing backbone polymers; high grafting efficiencies with respect to the azide functionality on the backbone were achieved.²⁴⁻²⁷ Moreover, grafting-to is a modular method, allowing for convenient simultaneous grafting of different side chain polymers onto the same backbone and tuning of the relative ratio of distinct side chain polymers in the preparation of heterografted brushes. The side chain and backbone polymers are prepared separately and therefore can be produced with greater synthetic control and characterized more thoroughly. By combining highly efficient click reactions, grafting-to is well-suited for the synthesis of multicomponent brushes. In this dissertation work, the “click” grafting-to method was employed for the preparation of molecular bottlebrushes.

1.1.1. Stimuli-Responsive Molecular Bottlebrushes

When stimuli-responsive polymers are used as the side chains in molecular bottlebrushes, the

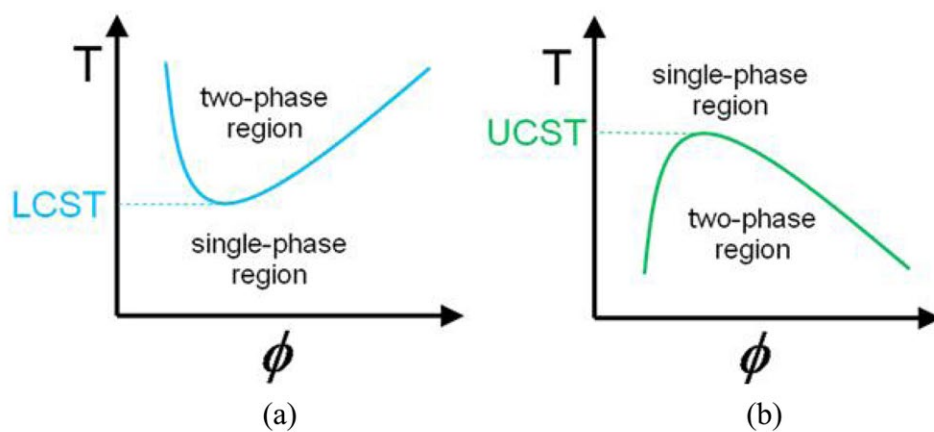
brushes can undergo dramatic changes in their size and shape triggered by external stimuli. Stimuli-responsive polymers undergo significant changes in conformation in solution in response to a relatively small variation in environmental conditions.²⁸ These so-called ‘smart’ materials have shown promise in areas such as drug delivery,²⁹ gene delivery,³⁰ and tissue engineering.³¹ The stimulus used can be either physical (temperature,³²⁻³⁶ electric fields,³⁷ light,³⁸ etc.) or chemical (pH,³⁹⁻⁴¹ redox agents,⁴² etc.). Temperature and pH are the most widely used stimuli due to the ease with which they can be applied, the reversibility of the polymer’s conformational change, and their relevance to biomedical applications. This dissertation work will mainly utilize polymers that exhibit temperature- and pH-responsiveness in aqueous solution in the preparation of molecular bottlebrushes and thus these will be the focus of the following discussion.

1.1.1.1. Stimuli-Responsive Polymers

Thermosensitive water-soluble polymers can be divided into two classes, those exhibiting a lower critical solution temperature (LCST) and those exhibiting an upper critical solution temperature (UCST) in water.³³ Both types of thermosensitive polymers undergo a soluble-to-insoluble transition in aqueous solution at a certain temperature called the cloud point (CP).³⁴ Scheme 1.3 shows representative phase diagrams for LCST- and UCST-type polymers. LCST-type polymers undergo phase separation in water upon heating past the CP, with the minimum temperature at which phase separation occurs being the LCST. In contrast, UCST-type polymers exhibit a clear-to-cloudy transition upon cooling below the CP, with the highest temperature at which phase separation occurs being the UCST.

LCST polymers are the most extensively studied type of thermosensitive polymers.³³ Poly(*N*-isopropyl acrylamide) (PNIPAm) is perhaps the most well-known example of these polymers, due to the commercial availability of the monomer and the biologically relevant transition temperature

Scheme 1.3. Illustration of Phase Diagrams for Polymer Solution Exhibiting (a) Lower Critical Solution Temperature (LCST) Behavior and (b) Upper Critical Solution Temperature (UCST) Behavior.³⁴ Reproduced from Ref. 34 with Permission from MDPI.



of ~ 32 °C. However, in recent years thermosensitive oligo(ethylene glycol)-containing poly(meth)acrylates and polystyrenics have attracted increasing attention.³⁶ These polymers exhibit transition temperatures that can easily be tuned to predictable values based on molecular structure and copolymer composition.^{36, 43, 44} All of these LCST-type polymers contain hydrophobic groups in addition to hydrophilic moieties that can participate in hydrogen bonding with water; their transition temperatures in water are determined by the hydrophilic-hydrophobic balance of the polymer chains. At temperatures below the CP, the polymer is soluble in water via hydrogen-bonding of hydrophilic groups (e.g., the pendant amide group, -CONH-, on PNIPAm) with water molecules, and water forms an ordered hydration layer around the hydrophobic moieties (i.e., alkyl groups) (Figure 1.1). This ice-like hydration layer decreases the overall entropy of the system, even though the polymer is molecularly dissolved in solution. However, as the temperature is increased above the CP, the hydration layer surrounding the hydrophobic groups is “melted” and released to bulk water (increasing the entropy of the system), and the polymer transitions from a solvated random coil to a collapsed globular state. This process is widely known as the hydrophobic effect and can even be observed by the presence of an endothermic peak (associated with the “melting” of the hydration layer) in differential scanning calorimetry (DSC) experiments.⁴⁵ Thus, the clear-to-cloudy LCST transition is generally regarded as an “entropy-driven” process.

UCST-type thermoresponsive polymers can be divided into two classes: hydrogen bonding-based and zwitterionic. Hydrogen bonding-based UCST behavior results from intra- and inter-chain hydrogen bonding between repeat units. Zwitterionic UCST behavior stems from intra-group, intra-chain, and inter-chain coulombic interactions.³³ In both cases the clear-to-cloudy transition of polymer aqueous solutions occurs upon cooling. In contrast to the LCST transition,

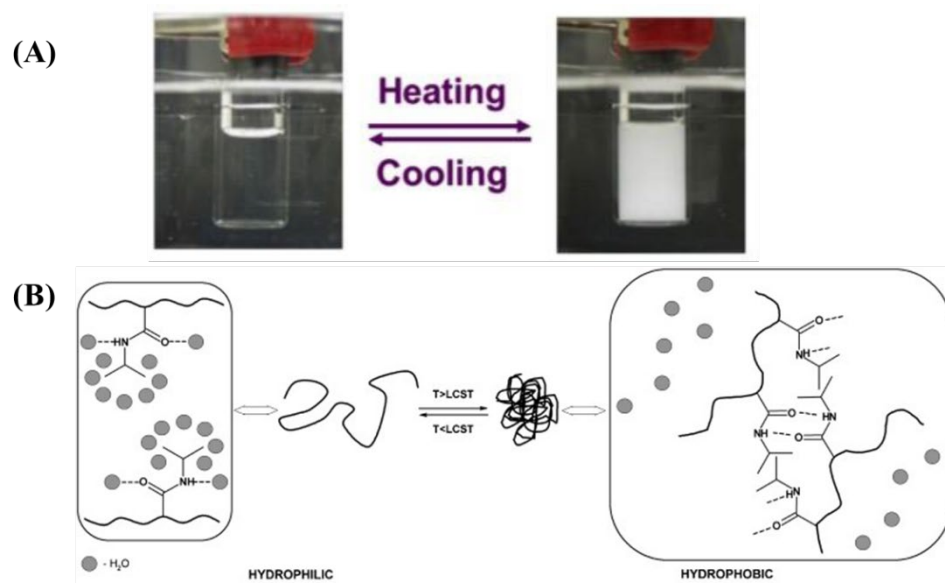


Figure 1.1. (A) Photographs of an LCST-type thermoresponsive polymer in water exhibiting a reversible clear-to-cloudy transition. (B) Illustration of temperature-induced phase transition of PNIPAm.³⁵ Reproduced from Ref. 35 with permission from Elsevier.

the UCST transition is generally regarded as “enthalpy-driven” process that is a result of strong polymer-polymer and strong solvent-solvent interactions; it is important to note that the entropic effect probably also plays a role in the UCST transition, though to a lesser extent.⁴⁶

pH-responsive polymers are divided into two main classes, polyacids (i.e., containing pendant carboxylic acid groups) and polybases (i.e., containing pendant amine groups).⁴⁷ For example, tertiary amine-containing polymers, such as poly(2-(*N,N*-diethylamino)ethyl methacrylate), are soluble in water at pH values below their pK_a , where the tertiary amine moieties are protonated and allow for ion-dipole interactions with water. However, upon increasing the pH to values above the pK_a , the deprotonation of a majority of the amine groups results in the insolubility of the polymer.

Chaotropic ions are generally large and polarizable with low charge densities, while kosmotropic ions are small with high charge densities. The effects of these two types of ions are well established in biological protein studies, with chaotropic salts usually having a “salting-in” effect and kosmotropic salts exhibiting a “salting-out” effect on neutral proteins in aqueous solution. Due to the diffusive nature of chaotropic ions, when dissolved in solution they tend to disrupt the hydrogen bonding in the solvation shell surrounding the ions.⁴⁸⁻⁵⁰ When two chaotropic ions of opposite charge are in solution, tight ion pairs can form driven by the release of water in the hydration layer to bulk water regaining some of the hydrogen bonds. A recent publication took advantage of this phenomenon to induce the micellization of block copolymers containing a polymethacrylate block with pendant tertiary amine groups. The ammonium cation is considered chaotropic, and when a kosmotropic anion (e.g., chloride from sodium chloride salt) is added, the ammonium-chloride ion pair is rather loose, being strongly solvated by water. However, if a salt containing a strong chaotropic anion (e.g., sodium perchlorate) is added, the ammonium cations

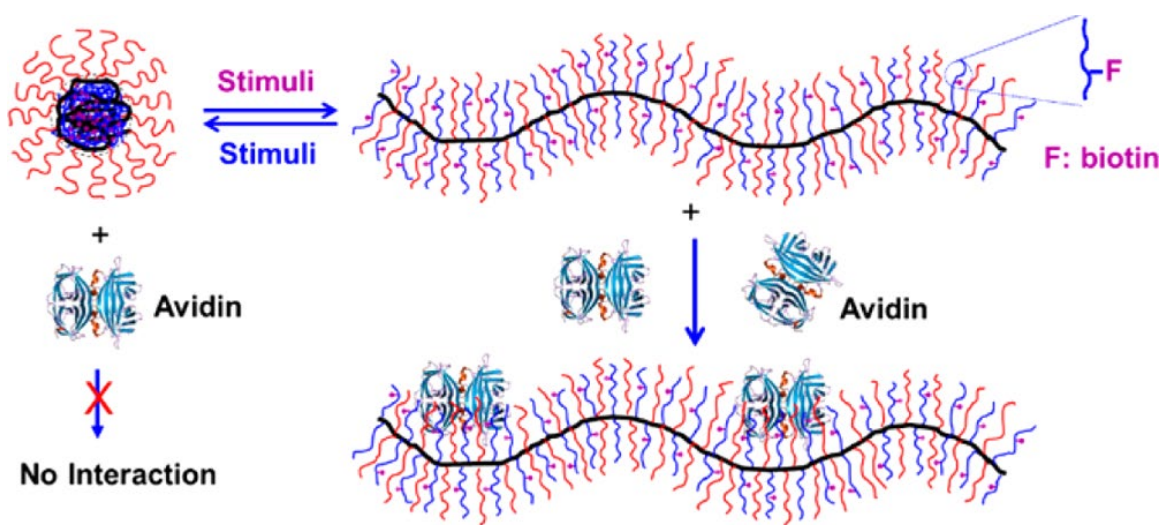
and the perchlorate anions form tight ion pairs. When the alkyl groups on the nitrogen atom of the protonated tertiary amine moieties are sufficiently long, the solubility of the tertiary amine-containing block in aqueous solution is greatly reduced, driving the micellization of the block copolymer molecules.

1.1.1.2. Synthesis and Behavior of Stimuli-Responsive Molecular Bottlebrushes

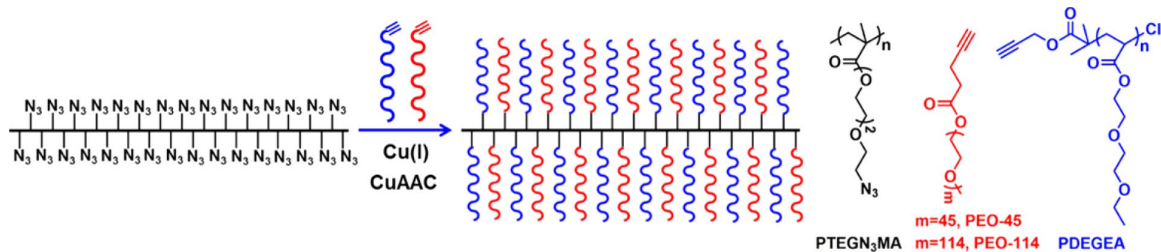
Molecular brushes that contain stimuli-responsive polymers as side chains exhibit intriguing responsive properties, most notably their ability to undergo unimolecular collapse as well as self-assembly to form complex morphologies.^{24-26, 51-64} Schmidt et al. reported in 2004 the synthesis of thermosensitive molecular bottlebrushes by a grafting-from method using ATRP to grow PNIPAm side chains.⁵¹ Dynamic light scattering (DLS) and AFM studies indicated unimolecular collapse of worm-like brush molecules to globular nano-objects at temperatures above the LCST of PNIPAm (32 °C). However, the collapsed globular brush molecules were only stable in solution up to 38 °C, above which aggregates formed and precipitated out from the solution. Matyjaszewski et al. reported thermosensitive homografted molecular brushes by grafting-from via ATRP of *N,N*-dimethylacrylamide (DMA) and *n*-butyl acrylate (BA) to grow LCST-type thermosensitive side chains from poly(2(2-bromopropionyloxy)ethyl methacrylate) (PBPEM) backbone (poly(BPEM-*graft*-P(DMA-*stat*-BA))).⁵³ The brush molecules in water showed an increase in hydrodynamic diameter (D_h) at a concentration of 1.0 wt% upon heating above the LCST, indicating intermolecular aggregation, but a decrease in D_h at 0.1 wt%, evidencing unimolecular brush collapse. Similar to these examples, other stimuli-responsive brush polymers reported in the literature were also not stable in the collapsed state and readily underwent aggregation, even at relatively low concentrations.

To make use of stimuli-responsive molecular bottlebrushes in applications, a method must be developed to stabilize the unimolecular shape transition in solution to prevent aggregation and precipitation. Over the past several years our lab has developed such a method and demonstrated its usefulness in the stabilization of collapsed stimuli-responsive molecular bottlebrushes. Henn et al. synthesized binary heterografted linear molecular bottlebrushes consisting of LCST-type thermoresponsive poly(ethoxydi(ethylene glycol) acrylate (PDEGEA, LCST = $\sim 9\text{ }^{\circ}\text{C}$) and water soluble poly(ethylene oxide) side chains that exhibited stabilized unimolecular shape-changing behavior in aqueous solution upon heating.²⁴ Moreover, bottlebrushes containing biotin in the thermoresponsive side chains were used to demonstrate that functional groups could be hidden in the collapsed state and exposed in the extended state to modulate the interactions with other species in solution (i.e., avidin) (Scheme 1.4). Homografted PDEGEA molecular brushes (HMB-1) and heterografted PDEGEA/PEO bottlebrushes (BMB-2) were prepared using a modular grafting-to method in which alkyne end-functionalized side chain polymers were attached to an azide-bearing backbone polymer via CuAAC (Scheme 1.5). Figure 1.2 shows optical photographs of aqueous solutions containing HMB-1 and BMB-2 at a concentration of 1.0 mg/g. At $0\text{ }^{\circ}\text{C}$ both solutions were clear, homogeneous solutions; upon heating to $22\text{ }^{\circ}\text{C}$ HMB-1 became cloudy while BMB-2 remained clear, indicating a stabilization of the collapsed brush molecules by the PEO side chains. DLS studies of both brushes at low concentration (0.2 mg/g) showed a decrease in size near the LCST of the PDEGEA side chains; BMB-2 displayed overlapping size distributions at a higher temperature for the 0.2 mg/g and 1.0 mg/g solutions, indicating a unimolecular size transition. Figure 1.3 shows AFM height images of BMB-2 spin cast onto bare mica at $0\text{ }^{\circ}\text{C}$ and $40\text{ }^{\circ}\text{C}$, allowing for the visualization of the thermally-induced worm-to-globule shape transition. Clearly water soluble PEO side chains were able to stabilize the shape transition of the brush

Scheme 1.4. Stimuli-Induced Unimolecular Shape Transition of Linear Binary Heterografted Molecular Bottlebrush and the Regulation of Interactions Between Brush Molecules and Avidin.²⁴ Reproduced from Ref. 24 with Permission from ACS.



Scheme 1.5. Illustration of the Synthesis of Binary Heterografted Molecular Bottlebrushes using CuAAC.²⁴ Reproduced from Ref. 24 with Permission from ACS.



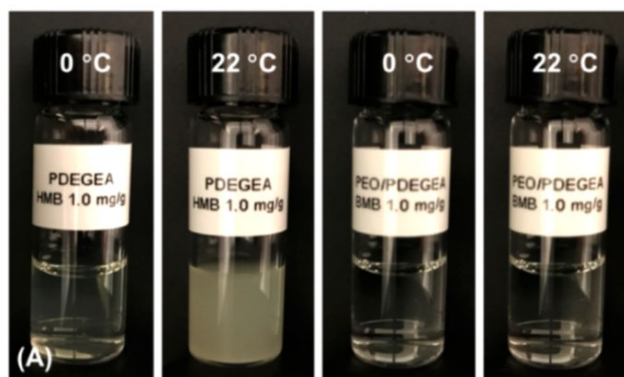


Figure 1.2. Optical photographs of HMB-1 and BMB-2 in 1.0 mg/g aqueous solution at 0 and 22 °C.²⁴ Reproduced from Ref. 24 with permission from ACS

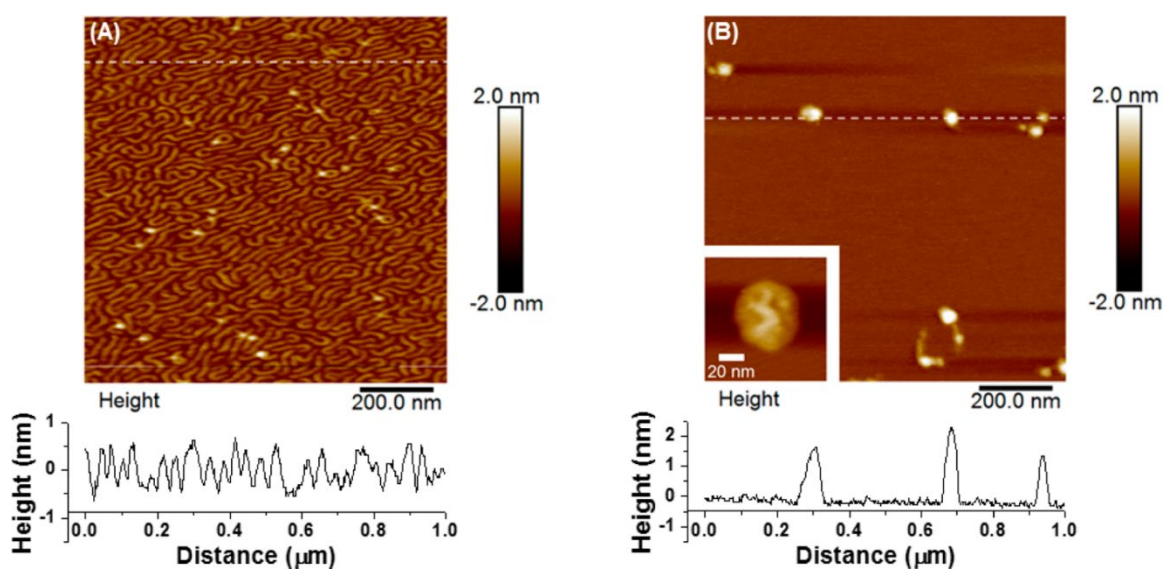


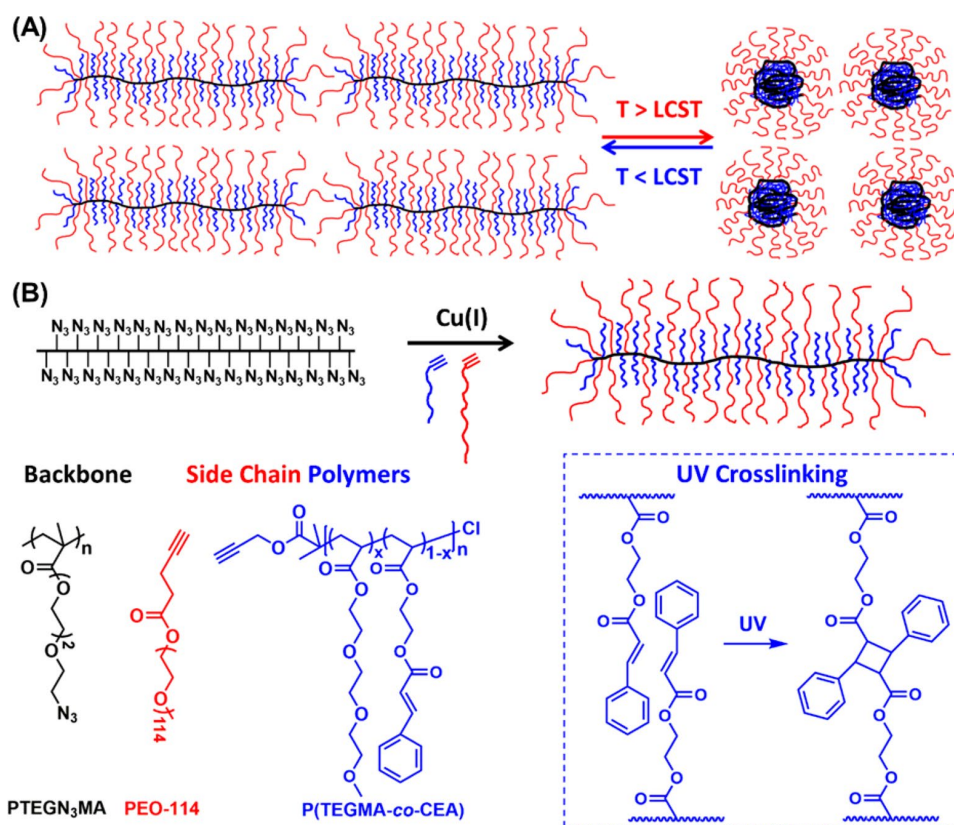
Figure 1.3. AFM height images of BMB-2 spin cast onto bare mica from aqueous solution at (A) 0 °C and (B) 40 °C.²⁴ Reproduced from Ref. 24 with permission from ACS.

molecules in solution, preventing intermolecular aggregation, even at temperatures well above the LCST of PDEGEA.

A separate molecular brush sample was prepared to demonstrate the regulation of interactions between the brush molecules and their environment using biotin-avidin complexation as a model. PDEGEA side chains were prepared with a small amount of biotin-containing acrylate and a fluorescent acrylate comonomer incorporated. The fluorescent tag was a fluorescence resonance energy transfer (FRET) donor for Rhodamine B. Heterografted bottlebrushes were then prepared using PEO and the functionalized PDEGEA side chain polymers (BMB-3). A solution of BMB-3 at 40 °C was then mixed with a solution of Rhodamine-B labeled avidin also at 40 °C. A fluorimeter was used to monitor the solution. While there was little change in the relative peak intensity for the FRET donor at 40 °C, when the temperature was lowered to 0 °C, a large decrease was observed for this peak relative to the FRET acceptor peak. This experiment demonstrated a high level of control over the interaction between biotin and avidin in aqueous solution, regulated by the shape changing of the brush molecules.

Zhao and coworkers then hypothesized that unimolecular worm-to-sphere shape transitions of linear bottlebrushes could be achieved in moderately concentrated aqueous solution if the stabilizing side chains were much longer than the stimuli-responsive side chains (Scheme 1.6). They synthesized binary heterografted linear bottlebrushes that were composed of a thermoresponsive, photocrosslinkable poly(methoxytri(ethylene glycol) acrylate-*co*-2-cinnamoyl ethyl acrylate) (P(TEGMA-*co*-CEA), LCST = ~ 27 °C) with a degree of polymerization (DP) of 43, and PEO side chains with a DP of either 17 (BMB-750), 45 (BMB-2k), or 114 (BMB-5k).²⁶ Aqueous solutions of these brush samples were prepared at a concentration of 10 mg/g and examined by visual inspection (Figure 1.4). All of the solutions were optically clear at 0 °C. How-

Scheme 1.6. (A) Worm-to-Sphere Shape Transition of Thermoresponsive Binary Heterografted Linear Molecular Bottlebrushes in Moderately Concentrated Aqueous Solutions and (B) Synthesis of Thermoresponsive, UV-Cross-Linkable Molecular Bottlebrushes.²⁶ Reproduced from Ref 26 with Permission from ACS.



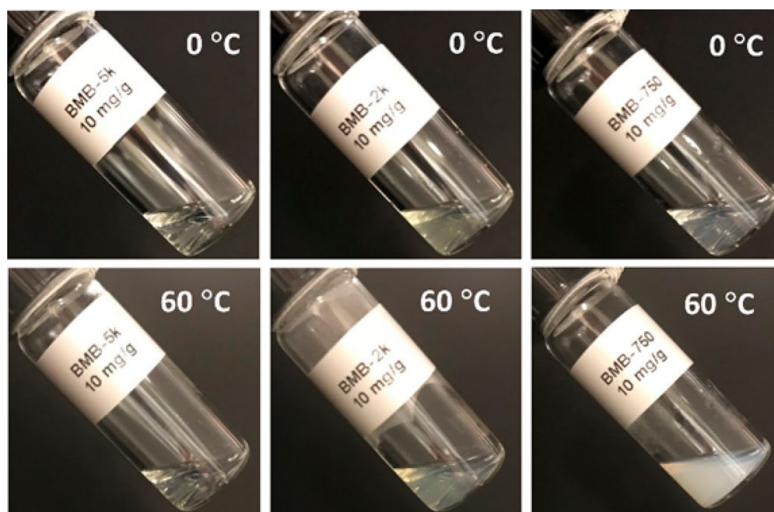


Figure 1.4. Optical photos of 10 mg/g aqueous solutions of BMB-5k, BMB-2k, and BMB-750 at 0 °C (top row) and 60 °C (bottom row).²⁶ Reproduced from Ref. 26 with permission from ACS.

ever, only the BMB-5K sample remained completely clear after heating to 60 °C; the BMB-2k sample developed a blue tinge and the BMB-750 sample became cloudy. Aqueous solutions of BMB-5K were prepared for investigation using DLS and AFM by crosslinking the brushes in the collapsed state at higher concentration (10, 25, and 100 mg/g) and then diluting the solutions to the appropriate concentrations for analysis (0.2 mg/g for DLS and 0.05 mg/g for AFM). DLS studies of the irradiated BMB-5k solutions from 10 and 25 mg/g aqueous solutions showed single distributions at 5 °C and at 60 °C. However, the sample diluted from 100 mg/g occasionally showed large species, indicating a small amount of possible intermolecular aggregates. AFM of the diluted solutions presented in Figure 1.5 shows that a vast majority of the brushes are in the unimolecular collapsed state, with a few molecules possibly indicating aggregation for the diluted 100 mg/g sample.

1.2. Dissertation Overview

The focus of this dissertation work is the further exploration of the idea of introducing a second polymer into the stimuli-responsive shape-changing molecular bottlebrushes for stabilization. Besides serving as a second type of side chains in binary heterografted molecular bottlebrushes, the second polymer component can also be added into the side chains as the outer block of diblock copolymers. In addition, if both polymer components are stimuli-responsive, another level of control over the solution state of the molecular bottlebrushes can be achieved or the formation of two distinct collapsed yet stable globular states can be realized. Chapter 2 expands on the method of stabilizing the stimuli-induced shape transition of linear molecular bottlebrushes by using pH- and thermo-responsive diblock copolymers as side chains in the preparation of homografted brushes. Chapter 3 introduces binary heterografted doubly responsive star bottlebrushes that can undergo either a pH-induced or a thermally-induced shape transition in aqueous solution providing

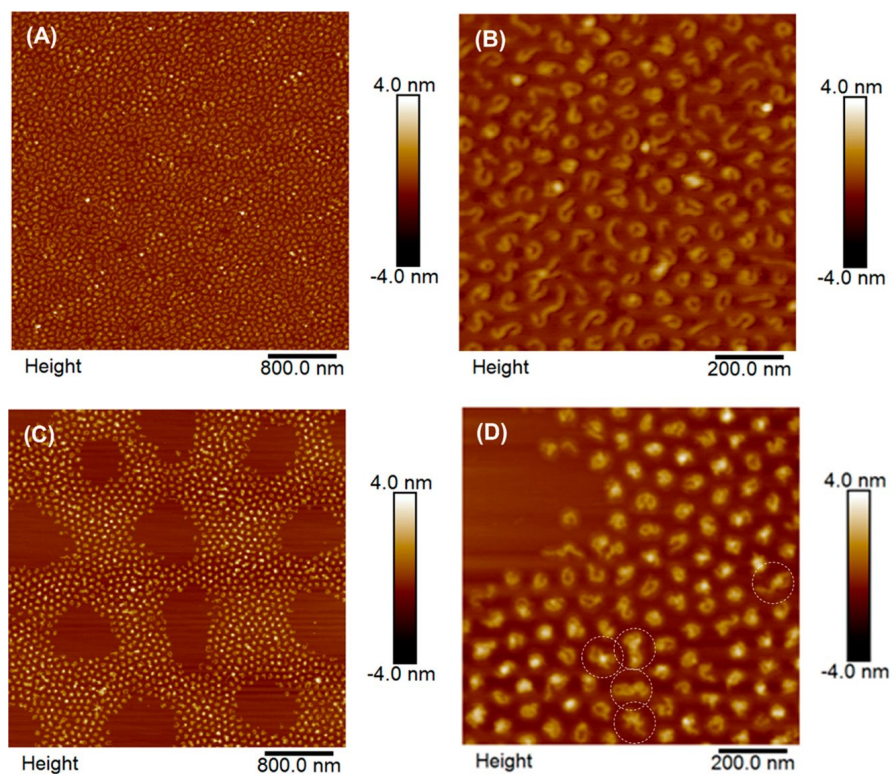


Figure 1.5. AFM height images of the cross-linked BMB-5k spin-cast on freshly cleaved mica from a 0.05 mg/g aqueous solution at 0 °C, diluted from the UV irradiated, 25 mg/g solution (A and B) and 100 mg/g aqueous solution (C and D).²⁶ Reproduced from Ref. 26 with permission from ACS.

a means to achieve two distinct collapsed states from one brush system. Chapter 4 uses binary heterografted pH- and thermo-responsive linear bottlebrushes to help elucidate a unique pearl-necklace morphology that was observed in the study of doubly responsive star bottlebrushes in Chapter 3. The final chapter is a study of pH- and chaotropic anion-induced star-to-globule shape transition for tertiary amine-containing binary heterografted star molecular brushes.

Chapter 2 expands on the methodology of stabilizing the collapsed states of stimuli-responsive bottlebrushes to achieve unimolecular collapse without intermolecular aggregation. Instead of using binary heterografted brushes as discussed previously, we chose to investigate homografted bottlebrushes composed of diblock copolymer side chains; a pH-responsive polymer, poly(2-(*N,N*-(diethylamino)ethyl methacrylate) (PDEAEMA, $pK_a = 7.4$), served as the inner block and a thermoresponsive polymer, poly(methoxytri(ethylene glycol) acrylate) (PTEGMA, LCST = ~ 58 °C), served as the outer block. An azide-functionalized backbone with a DP of 707 was prepared according to the same procedure described in literature.²⁴⁻²⁶ Alkyne end-functionalized side chain polymers were prepared by ATRP from an alkyne-functionalized initiator. Using visual inspection, DLS, and AFM, we confirmed that the bottlebrushes underwent a pH-induced shape transition that was stabilized by the outer PTEGMA block. Moreover, we demonstrated that the thermoresponsive outer block could be used to further control the solution state of the brushes; when the brushes were in the pH-induced collapsed state, increasing the temperature above the LCST of PTEGMA caused the brushes to form clusters at higher concentrations or remain unaggregated in dilute solution.

Intrigued by the idea of a dually responsive brush system, we explored binary heterografted star-shaped bottlebrushes composed of two different types of stimuli-responsive side chains in Chapter 3. The star brushes were prepared with both pH-responsive PDEAEMA and

thermoresponsive PDEGEA (LCST = 9 °C). Once again, we used CuAAC click chemistry in a grafting to approach to create the bottlebrushes. However, the azide-bearing three-arm star backbone polymer was prepared using a slightly different method than in the previous chapter; a silyl ether-protected methacrylate monomer was polymerized from a newly designed trifunctional initiator using ATRP, followed by a series of post-polymerization reactions to install azide moieties on the backbone. The alkyne end-functionalized side chain polymers, PDEAEMA and PDEGEA, were synthesized using ATRP. The pH- and thermally-induced size transitions were studied by DLS, and AFM was used to observe the shape transitions from extended star-like to collapsed globular. We demonstrated that when one set of side chains collapses into a hydrophobic core, the second set serves to stabilize the collapsed state and prevent aggregation.

In Chapter 4, we prepared binary heterografted linear bottlebrushes composed of PDEGEA and charged PDEAEMA side chains to elucidate the formation of a peculiar pearl-necklace morphology observed in Chapter 3. We hypothesized that the multivalent phosphate anions in the aqueous buffer solutions might enhance the microphase separation between the PDEGEA and PDEAEMA side chains through bridges between positively charged tertiary amine groups. In this study, we used DLS to measure and compare the hydrodynamic sizes of the brushes in aqueous acetate, phosphate, and citrate buffers at the same pH. We also employed AFM to directly observe any differences between the brushes in the various buffer solutions. DLS showed a trend of decreasing hydrodynamic diameter with increasing buffer anion valency, and AFM showed that the domain bead size increased with increasing buffer anion valency. These results indicated that the microphase separation of the two side chain polymers could be controlled and enhanced with the selection of buffer anions.

Chapter 5 investigates tertiary amine containing star-shape molecular bottlebrushes and their

pH- and chaotropic anion-induced conformation changes in aqueous solution. We prepared star-shaped MBBs composed of PEO side chains and either poly(2-(*N,N*-(dimethylamino)ethyl methacrylate) (PDMAEMA) or PDEAEMA side chains. Using DLS and AFM, we demonstrated the pH-induced star-to-globule collapse for PDEAEMA/PEO brushes, stabilized by the water-soluble PEO side chains; PDMAEMA/PEO brushes showed a slight decrease in size at a pH near its pK_a at room temperature. We then studied the effect of the addition of salts containing chaotropic anions to aqueous solutions of each bottlebrush polymer at a $pH < pK_a$. DLS and AFM studies were performed and showed that both brush systems exhibited chaotropic anion-induced star-to-globule shape transitions, with the PDEAEMA/PEO brushes exhibiting a greater sensitivity to moderately chaotropic anions.

Chapter 6 provides a summary of this dissertation work and future prospects in the area of stimuli-responsive shape-changing molecular bottlebrushes.

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Chapter 2: Shape-Changing Linear Molecular Bottlebrushes with Dually pH- and Thermo-Responsive Diblock Copolymer Side Chains

The work presented in this chapter has been published in *Polymer Chemistry* **2018**, 9 (41), 5133-5144. I synthesized all of the side chain polymers and molecular bottlebrushes in this work and performed the molecular and physical characterizations. Coauthor Daniel Henn synthesized and characterized the azide-bearing backbone polymer and provided useful insights into the work. Prof. Bin Zhao advised this work.

Abstract

This Chapter presents the synthesis and responsive behavior of shape-changing linear molecular bottlebrushes containing dually pH- and thermo-responsive diblock copolymer side chains. Using a “click” grafting to method, we synthesized linear homografted molecular brushes composed of diblock copolymer side chains with pH-responsive poly(2-(*N,N*-diethylamino)ethyl methacrylate) (PDEAEMA) as inner block and thermoresponsive poly(methoxytri(ethylene glycol) acrylate) (PTEGMA) as outer block. Dynamic light scattering studies showed that the brushes exhibited a sharp decrease in hydrodynamic size with increasing pH across the pK_a of PDEAEMA and the size leveled off at higher pH. This is in contrast to the molecular brushes with only PDEAEMA homopolymer side chains, which underwent aggregation and precipitation in basic solution even at a concentration of 0.2 mg/g. Atomic force microscopy confirmed the cylindrical morphology of the brushes at acidic pH and pH-induced cylinder-to-globule transitions. In addition, the use of PTEGMA as outer block provided another means to control the solution state of the collapsed brushes; above the lower critical transition temperature of PTEGMA, the brushes clustered at higher concentrations but remained unaggregated at lower concentrations. The switching of molecular shape, stabilization of collapsed nano-objects, and control of solution state reported here may enable new potential applications of stimuli-responsive shape-changing molecular brushes.

2.1. Introduction

Molecular bottlebrushes, a type of graft copolymers in which polymeric side chains are densely tethered to a backbone polymer,¹⁻³ have received growing interest in recent years because of their intriguing properties and great potential in a wide variety of applications such as photonic crystals,⁴ lubrication,⁵ and supersoft elastomers.⁶ In good solvents, linear macromolecular brushes adopt cylindrical or worm-like conformations, a result of strong excluded volume interactions between side chains.¹⁻³ The dimensions are determined by the aspect ratio of backbone to side chain length, grafting density, and solvent quality. These macromolecules are commonly synthesized by one or a combination of two of the following three methods: (i) grafting-through, in which macromonomers are polymerized directly into brushes (e.g., by using the Grubbs catalyst),^{1-4,7-9} (ii) grafting-from, in which side chains are grown from pendant initiation sites on a polymer backbone,^{1-3,10-19} and (iii) grafting-to, in which end-functionalized side chain polymers are covalently attached to a backbone polymer.¹⁷⁻²² Each method has advantages and drawbacks. For example, the grafting-to method provides the advantages of allowing for thorough characterization of backbone and side chain polymers and modular synthesis of brush polymers via simultaneous grafting of different side chain polymers onto the same backbone. However, the grafting density of the brushes tends to be low. In the past decade, the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction²³ has emerged as a powerful and versatile means for preparing molecular bottlebrushes with high grafting densities via grafting-to.¹⁷⁻²⁶

Stimuli-responsive molecular brushes,²⁴⁻⁴¹ composed of side chain polymers that can undergo large and abrupt conformational changes in response to external stimuli, are of great interest to fundamental study of the behavior of stimuli-responsive polymers and to potential technological uses. Many of these brushes have been shown to exhibit intriguing worm-to-globule shape

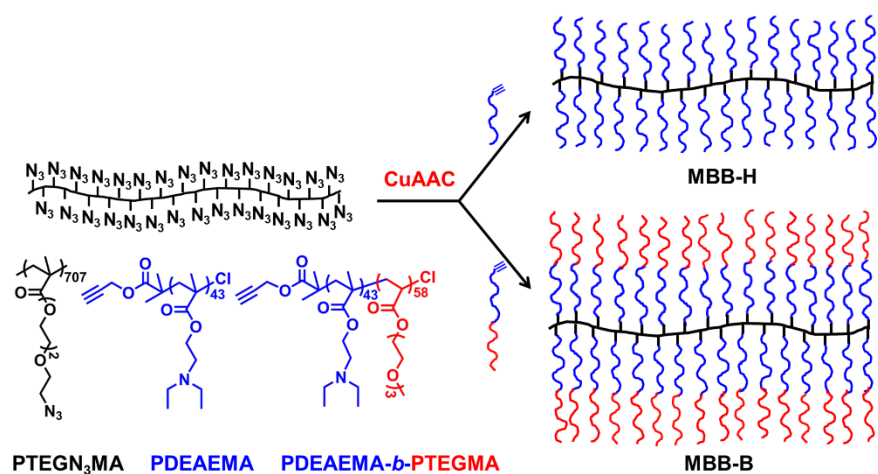
transitions or vice versa upon application of external stimuli. For example, Schmidt et al. synthesized thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAM) molecular brushes by a “grafting-from” method using atom transfer radical polymerization (ATRP) and observed by atomic force microscopy (AFM) that these brushes underwent a worm-to-sphere shape change when the temperature was raised to above the LCST of PNIPAM.³⁰ The unimolecular spherical nano-objects, however, were unstable; they aggregated and eventually precipitated out of the solution. Using a similar approach, Matyjaszewski et al. prepared loosely grafted poly(acrylic acid) molecular brushes and studied their conformations on mica at different pH values by AFM.³¹ The brushes underwent a globule-to-extended conformational transition when the pH was changed from acidic to basic. Müller et al. synthesized poly(*N,N*-dimethylaminoethyl methacrylate) (PDMAEMA) molecular brushes; cryo-transmission electron microscopy showed that the brushes were worm-like at pH = 7 but strongly contracted at pH = 10.³² They also reported the conformational switching of cationic quaternized PDMAEMA brushes via the formation of ionic and supramolecular inclusion complexes in dilute conditions.³⁴ Using a Langmuir-Blodgett trough, Sheiko et al. demonstrated reversible worm-to-sphere shape transitions of cylindrical poly(*n*-butyl acrylate) molecular brushes by lateral compression at the water-air interface.^{35,36}

While intriguing, the worm-to-globule shape changes of molecular brushes have been restricted to dilute solutions, typically <1.0 mg/g, or at liquid or solid-air interfaces,^{24-27,30,34,37-39} which has greatly limited the possible uses of shape-changing molecular bottlebrushes in potential applications, such as molecular actuators, delivery of substances, biomimetic catalysis, etc. Our group recently reported the synthesis and shape changing study of linear binary heterografted molecular brushes composed of a thermoresponsive polymer and a hydrophilic poly(ethylene oxide) (PEO) as side chains and showed that the collapsed thermoresponsive side chains were

stabilized by PEO in water.²⁴ While a 1.0 mg/g aqueous solution of the corresponding thermoresponsive homopolymer brushes turned cloudy upon heating above the LCST, the binary heterografted molecular brushes underwent unimolecular worm-to-globule transitions and the solution stayed clear at the same concentration. The shape changing of these brushes was utilized to regulate the interaction of brush molecules with avidin in the environment by hiding and exposing biotin functional groups that were incorporated into the thermoresponsive side chains. Our group further discovered that the worm-to-globule shape transitions of binary heterografted molecular brushes can be achieved in moderately concentrated aqueous solutions if the PEO is significantly longer so that the thermoresponsive side chains are well shielded by PEO to avoid intermolecular association during the LCST transition.²⁶

Besides the binary heterografted molecular brushes for achieving worm-to-stabilized globule shape transitions, it is also possible to use homografted molecular brushes with a stimuli-responsive diblock copolymer as side chains (i.e., core-shell brushes¹⁻³), in which the inner block is a stimuli-responsive polymer whose solubility change, triggered by environmental stimuli, drives the shape transition of the brushes from worm-like to globular and the outer block serves as a stabilizer. In addition, if the outer block is also stimuli-responsive, the state of the brushes in water can be controlled by a second stimulus. To test this hypothesis, we synthesized in the present work linear homografted molecular brushes composed of poly(2-(*N,N*-diethylamino)ethyl methacrylate)-*b*-poly(methoxytri(ethylene glycol) acrylate) (PDEAEMA-*b*-PTEGMA) side chains (MBB-B), where PDEAEMA is the inner block, by a “grafting to” method using CuAAC (Scheme 2.1). PDEAEMA is a pH-responsive polymer with a pK_a of 7.4 in water,^{42,43} while PTEGMA is a thermoresponsive polymer with a LCST of 58 °C.⁴⁴⁻⁴⁶ We chose PDEAEMA as the inner block because this polymer exhibits a solubility transition in water from very hydrophilic at

Scheme 2.1. Synthesis of Stimuli-Responsive Molecular Bottlebrushes MBB-B and MBB-H via Copper(I)-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) Click Reaction between Azide-Bearing Backbone Polymer and Alkyne End-Functionalized Side Chain Polymers



low pH to very hydrophobic at high pH, providing a strong driving force for the worm-to-globule shape transition. For comparison, we also made a molecular brush composed of only PDEAEMA side chains (MBB-H). We showed that MBB-B underwent a cylinder-to-globule shape transition with increasing pH from acidic to basic at room temperature and the collapsed globular state was stabilized by the outer block. In contrast, MBB-H precipitated out from the solution when the pH was increased to near the pK_a of PDEAEMA. We further demonstrated the ability to control the solution state of MBB-B in water by varying the temperature.

2.2. Experimental Section

2.2.1. Materials

CuCl (99%, Acros) was purified by stirring in glacial acetic acid overnight; the solids were collected by vacuum filtration, thoroughly washed with absolute ethanol and diethyl ether, and then dried under high vacuum. 2-(*N,N*-Diethylamino)ethyl methacrylate (DEAEMA, 98.5%, TCI), ethyl 2-bromoisobutyrate (EBiB, 98%, Aldrich), and *N,N,N',N'',N''*-pentamethyl-diethylenetriamine (PMDETA, 99%, Acros) were purified by vacuum distillation over calcium hydride. The azide-bearing backbone polymer, PTEGN₃MA (Scheme 2.1), with a degree of polymerization (DP) of 707, was synthesized via a procedure reported previously (Scheme A1),²⁴ and the characterization data are included in Figure A1. Triethylene glycol monomethyl ether (97%, Aldrich), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 97%, Aldrich), and triethylamine (99%, Alfa Aesar) were used as received. Methoxytri(ethylene glycol) acrylate (TEGMA) was synthesized by the reaction of tri(ethylene glycol) monomethyl ether and acryloyl chloride,^{44,45} and the molecular structure was confirmed by ¹H NMR spectroscopy analysis. Propargyl 2-bromoisobutyrate (PBiB) was synthesized according to a procedure described before.²⁴ All other chemicals were purchased from either Aldrich or Fisher and used as received.

2.2.2. General Characterization

The molecular weights and dispersities of the backbone polymer PTEGN₃MA and molecular bottlebrushes with diblock copolymer side chains were determined by size exclusion chromatography (SEC), performed at 50 °C using a PL-GPC 50 Plus system (an integrated GPC/SEC system from Polymer Laboratories, Inc.) with a differential refractive index detector, one PLgel 10 µm guard column (50 × 7.5 mm, Agilent Technologies), and three PLgel 10 µm mixed-B columns (each 300 × 7.5 mm, linear range of molecular weight from 500 to 10,000,000 Da, Agilent Technologies). *N,N*-Dimethylformamide (DMF) containing 50 mM LiBr was used as the carrier solvent at a flow rate of 1.0 mL/min for analysis. SEC of alkyne end-functionalized PDEAEMA and PDEAEMA-*b*-PTEGMA side chain polymers was performed at ambient temperature using a PL-GPC 20 (an integrated GPC/SEC system from Polymer Laboratories, Inc.) with a refractive index detector, one PLgel 5 µm guard column (50 × 7.5 mm, Agilent Technologies), and two PLgel 5 µm mixed-C columns (each 300 × 7.5 mm, linear range of molecular weights from 200 to 2,000,000 Da, Agilent Technologies). THF was used as the eluent at a flow rate of 1.0 mL/min. Each SEC system was calibrated with a set of narrow disperse linear polystyrene standards (Scientific Polymer Products, Inc.). ¹H NMR spectra were recorded on a Varian VNMRs 500 NMR spectrometer, using residual solvent proton signal as the internal standard.

2.2.3. ATRP Synthesis of PDEAEMA and PDEAEMA-*b*-PTEGMA

PBiB (112.8 mg, 0.550 mmol), DEAEMA (8.157 g, 44.03 mmol), CuCl (55.1 mg, 0.557 mmol), HMTETA (161.1 mg, 0.699 mmol), and anisole (12.041 g) were added to a 50 mL two-necked round bottom flask and degassed by three freeze-pump-thaw cycles. After the polymerization proceeded at 50 °C for 2.25 h, the reaction mixture was opened to air and passed

through a neutral alumina (top)/silica gel (bottom) column with methylene chloride (200 mL). The volatiles were removed by rotary evaporation. The concentrated residue was diluted with THF (15 mL) and then precipitated in hexanes (150 mL) in a dry ice/acetone bath three times. The polymer was dissolved in THF and transferred to a round bottom flask, concentrated by rotary evaporation, and dried under high vacuum (3.353 g). SEC analysis results (Figure A2): $M_{n,SEC} = 6500$ Da and $PDI = 1.17$, relative to polystyrene standards. The DP of PDEAEMA was 43, calculated from the monomer-to-initiator molar ratio and the monomer conversion (53.7%), determined by ^1H NMR analysis of the ester ($-COOCH_2-$) peaks from the monomer (4.17-4.29 ppm) and the polymer (3.95-4.16 ppm).

PDEAEMA (DP = 43, 1.007 g, 0.124 mmol), TEGMA (3.400 g, 15.58 mmol), CuCl (12.8 mg, 0.129 mmol), PMDETA (27.9 mg, 0.161 mmol), and anisole (5.034 g) were added to a 25 mL two-necked round bottom flask. The reaction mixture was degassed via three freeze-pump-thaw cycles before the flask was placed in a 100 °C oil bath. After 24 h, the polymerization was stopped, and the mixture was diluted with THF and then passed through a basic alumina (top)/silica gel (bottom) column to remove the copper catalyst. The solvents were evaporated using a rotavapor; the concentrated residue was dissolved in THF (10 mL) and precipitated in hexanes (100 mL) in an ice/water bath four times. The polymer was collected and dried under high vacuum (0.977 g). SEC analysis (Figure A2): $M_{n,SEC} = 14.1$ kDa, and $PDI = 1.21$, relative to polystyrene standards.

2.2.4. Synthesis of Linear Homografted Molecular Bottlebrushes with PDEAEMA-*b*-PTEGMA Side Chains (MBB-B)

PTEGN₃MA (0.226 g of a 14.30 mg/g stock solution in THF, 3.23 mg, 0.0133 mmol) and PDEAEMA-*b*-PTEGMA (6.489 g of a 76.61 mg/g stock solution in THF, 497.1 mg, 0.0239 mmol) were added to a vial equipped with a magnetic stir bar; a portion of THF (3.324 g) was evaporated

under N₂ stream to concentrate the mixture. CuCl (1.5 mg, 0.015 mmol) was then added, and the vial was sealed with a rubber septum; the headspace was purged with nitrogen for 15 min using needles, followed by the injection of PMDETA (5.0 μ L, 0.024 mmol) and purging with nitrogen for additional 15 min. After the reaction proceeded at room temperature for 22 h, benzyl 2-propynyl ether (30 μ L) was injected via a microsyringe to cap unreacted azide moieties. The reaction mixture was stirred at ambient temperature for an additional 3 h and then passed through a silica gel (bottom)/basic alumina (top) column to remove copper catalyst using THF as eluent. The brushes were concentrated and purified by fractionation using THF (2 mL) and hexanes (5 mL) four times to remove the unreacted side chain polymer. The purified brush polymer MBB-B was dried under high vacuum (yield: 156.8 mg) and stored in THF with a concentration of 10.20 mg/g in a freezer. The grafting density of MBB-B was determined to be 75.0 % from the analysis of SEC peak areas of the brushes (42.03%) and the side chain polymer (57.97%) in the final reaction mixture. SEC results: $M_{n,SEC}$ = 1,783 kDa and D = 1.13, relative to polystyrene calibration.

2.2.5. Synthesis of Molecular Bottlebrushes with PDEAEMA Side Chains (MBB-H)

PTEGN₃MA (0.351 g of a 14.30 mg/g stock solution in THF, 5.02 mg, 0.0206 mmol) and PDEAEMA (0.940 g of a 0.294 g/g stock solution in THF, 276 mg, 0.0341 mmol) were added to a vial along with THF (3.896 g) and magnetic stir bar. While stirring, CuCl (2.1 mg, 0.021 mmol) was added, and the vial was sealed with a rubber septum, and then the headspace was purged with nitrogen through needles for 15 min. PMDETA (5.0 μ L, 0.024 mmol) was injected and the headspace was purged for an additional 15 min before the needles were removed. After 24 h, a sample was taken from the reaction mixture, and benzyl 2-propynyl ether (50 μ L) was injected to cap unreacted azide groups. After 3 h, the vial was opened to air, and the solution was diluted with CH₂Cl₂ (50 mL) and passed through a neutral alumina (top)/silica gel (bottom) column with

additional CH_2Cl_2 (100 mL). The brush solution was concentrated by rotary evaporation, and the brushes were purified by fractionation in a mixture of THF and hexanes (1 : 10, v/v) in an ice/water bath to remove the unreacted side chains. The purified brushes were dried under high vacuum to yield a slightly yellow, rubbery polymer (120.1 mg, 69.6% yield). The grafting density was 82.0%, determined from the conversion of alkyne end group ($\text{CH}\equiv\text{CCH}_2$ -, 4.62 ppm) relative to the ester CH_2 in the polymers ($-\text{COOCH}_2$ -, 4.10-3.90 ppm) (Figure A4A). The removal of free side chains was verified by ^1H NMR analysis that showed the disappearance of alkyne end group (Figure A4B), and the brushes were confirmed by atomic force microscopy, which revealed worm-like MBB-H molecules spin cast on bare mica from an acidic solution (Figure A5).

2.2.6. Dynamic Light Scattering (DLS) Study of Molecular Bottlebrushes MBB-B and -H in Water

DLS was performed using a Malvern Zetasizer Nano ZS instrument equipped with a He-Ne 633 nm laser and a temperature controller at a scattering angle of 173° . For each brush sample, a 5.0 mg/g solution was prepared by dissolving the brushes in a 10 mM KH_2PO_4 buffer solution, adjusting the pH to 5.00 with 0.1 M HCl, and stirring at room temperature overnight to ensure complete dissolution. More dilute solutions (1.0, 0.5 or 0.2 mg/g) were prepared by diluting a portion of the 5.0 mg/g solution with a 10 mM buffer solution with pH of 5.00. The pH of the solution was gradually adjusted using 0.1 M NaOH and 0.1 M HCl, monitored by an Accumet AB15 pH meter (calibrated with pH = 4.01, 7.00, and 10.01 standard buffer solutions), and allowed to equilibrate for 15 min. A portion of the solution was then transferred into a DLS tube without filtering and loaded into the DLS instrument; the temperature was set at 25°C , and the measurements were taken after equilibration for 10 min. For MBB-B with a concentration of 1.0 mg/g at pH = 8.50, a temperature ramp was performed from 20 to 65°C , with 5 min of equilibration

at each selected temperature. Each reported DLS value was an average of 3 measurements, each of which was comprised of 10 runs. In this work, z-average hydrodynamic diameters were used.

2.2.7. Atomic Force Microscopy (AFM) Study of Molecular Bottlebrushes

AFM study of MBB-B and -H molecular brushes was performed using a Digital Instruments Multimode IIIa Scanning Probe Microscope in tapping mode under ambient conditions. Reflective Al-coated Si probes (Budget Sensors) with a nominal resonant frequency of 300 kHz and a force constant of 40 N/m were employed. PMMA-coated mica substrates were prepared by cleaving a clean layer of mica disk (Ted Pella, Inc.) with a single-sided Scotch tape, placing three drops of a 1 wt% solution of PMMA ($M_n = 54.9$ kDa) in CHCl_3 onto the disk, and spinning the solution off at 3000 rpm to create a thin film. Aqueous solutions of molecular brushes with concentrations ranging from 0.01 to 0.1 mg/g in 2.5 mM phosphate buffer were prepared by dilution of brush solutions with higher concentrations and spin cast onto the PMMA-coated mica at 3000 rpm. The solution pH was measured with a pH meter and adjusted to a desired value using 0.1 M NaOH and 0.1 M HCl solutions prior to spin casting. When an elevated temperature was used, the spin casting stage was heated to that temperature along with the mica disk prior to the spin casting process.

2.3. Results and Discussion

2.3.1. Synthesis of Azide-Bearing Backbone Polymer and Side Chain Polymers

Molecular bottlebrushes MBB-B and -H were synthesized by a “grafting-to” method using CuAAC. The azide-functionalized backbone polymer, PTEGN₃MA (Scheme 2.1), with a degree of polymerization (DP) of 707, a dispersity of 1.10, and degree of azide functionalization > 99%, was prepared by following a previously reported procedure.²⁴ Briefly, an ATRP of tri(ethylene glycol) mono(*t*-butyldimethylsilyl) ether methacrylate was conducted first, followed by a series of post-polymerization reactions to install azide functionality, including the removal of *tert*-

butyldimethylsilyl ether protecting groups, tosylation of the resulting hydroxyl moieties, and then substitution of tosylate groups with azide via reaction with sodium azide (Scheme A1). A high DP of 707 for the backbone polymer was used to ensure a high aspect ratio of backbone to side chain length, which is necessary for shape changing.

Alkyne end-functionalized pH-responsive PDEAEMA and dually pH- and thermo-responsive diblock copolymer PDEAEMA-*b*-PTEGMA were made by ATRP. The polymerization of DEAEMA was carried out in anisole using propargyl 2-bromoisobutyrate (PBiB) as initiator and CuCl/HMTETA as the catalyst/ligand in molar ratios of $[M]_0 : [I]_0 : [CuCl] : [HMTETA] = 80 : 1 : 1 : 1.3$. The polymerization was carried at 50 °C and stopped after 2.25 h; SEC analysis showed that the ATRP of DEAEMA was well controlled and the polymer exhibited a single, narrow distribution with a $M_{n,SEC}$ of 6.5 kDa and a PDI of 1.17 (Figure A2). The DP of the obtained PDEAEMA was 43, calculated from the monomer conversion, determined from ^1H NMR analysis by comparing the integrals of the ester ($-COOCH_2-$) peaks of the monomer (4.17-4.29 ppm) and polymer (3.95-4.16 ppm) in the final reaction mixture, and the monomer-to-initiator molar ratio. The catalyst was removed by passing the mixture through a neutral alumina/silica gel column, and the polymer was purified by repetitive precipitation from THF into hexanes in a dry ice/acetone bath.

The diblock copolymer, PDEAEMA-*b*-PTEGMA, was synthesized by ATRP of TEGMA from the PDEAEMA macroinitiator in anisole at 100 °C for 24 h using CuCl/PMDETA as catalyst/ligand. The molar ratios of $[M]_0/[I]_0/[CuCl]/[PMDETA] = 126/1/1/1.3$. From SEC analysis, the peak shifted to the higher molecular weight side compared to the macroinitiator and broadened slightly, giving a $M_{n,SEC}$ of 14.1 kDa and PDI of 1.21 (Figure A2). The diblock copolymer was purified by passing of the mixture through a basic alumina/silica gel column and

multiple precipitation in hexanes at 0 °C. A DP of 58 for the PTEGMA block was calculated from ^1H NMR analysis of the purified, dried polymer by comparing the ester peaks ($-\text{COOCH}_2-$) of the PTEGMA block (4.09-4.24 ppm) and the PDEAEMA block (3.89-4.08 ppm) (Figure A3). The characterization data for the azide-functionalized backbone polymer and side chain polymers are summarized in Table 2.1.

2.3.2. Synthesis of MBB-B and MBB-H

Linear homografted molecular bottlebrushes, MBB-B with pH- and thermo-responsive PDEAEMA-*b*-PTEGMA side chains and MBB-H with PDEAEMA side chains, were synthesized by a “grafting to” method whereby the corresponding alkyne end-functionalized side chain polymer was “clicked” onto the azide-bearing backbone, PTEGN₃MA, via CuAAC reaction. CuCl/PMDETA were used as the catalyst/ligand system, and the reactions were carried out in THF at ambient temperature. For MBB-B, the feed molar ratio of PTEGN₃MA repeat units to PDEAEMA-*b*-PTEGMA side chains was 1 : 1.79; this high ratio was used to ensure that a high grafting density would be achieved. After 22 h, an excess of propargyl benzyl ether was injected in an attempt to cap the unreacted azide groups. SEC showed a high molecular weight peak (Figure 2.1A), indicating the formation of brushes; the relative peak areas of the brushes and the unreacted side chains from SEC analysis of the final mixture were 42.03 % and 57.97 %, respectively. Using these values and the feed molar ratio of backbone repeat units to the side chains, the grafting density of MBB-B was calculated to be 75.0%. The reaction mixture was passed through a basic alumina/silica column to remove the copper catalyst and purified by fractionation in a THF/hexanes mixture to remove the unreacted side chains, which was confirmed by SEC analysis (Figure 2.1A); the $M_{n,\text{SEC}}$ and $\bar{D}$ of the purified brushes were 1783 kDa and 1.13, respectively, relative to polystyrene standards. Note that the slight shift of the brush peak was likely caused by

Table 2.1. Characterization Data for Backbone, Side Chain Polymers, and Molecular Brushes

Polymer Sample	$M_{n,SEC}$ (kDa)	$\bar{D}$	DP	Grafting density
PTEGN ₃ MA	307.1 ^a	1.10 ^a	707 ^c	-
PDEAEMA	6.5 ^b	1.17 ^b	43 ^c	-
PDEAEMA- <i>b</i> -PTEGMA	14.1 ^b	1.21 ^b	43, ^c 58 ^d	-
MBB-B (PDEAEMA- <i>b</i> -PTEGMA side chains)	1783 ^a	1.13 ^a	-	75.0 % ^e
MBB-H (PDEAEMA side chains)	-	-	-	82.0 % ^f

^a The number-average molecular weight ($M_{n,SEC}$) and dispersity ($\bar{D}$) were determined by SEC relative to polystyrene standards using PL-GPC 50 Plus with DMF containing 50 mM LiBr as eluent. ^b Obtained by SEC relative to polystyrene standards using PL-GPC 20 with THF as solvent.

^c Calculated from the monomer-to-initiator ratio and monomer conversion by ¹H NMR spectroscopy analysis. ^d Determined by comparing characteristic peaks of the two blocks in the ¹H NMR spectrum. ^e The grafting density was determined by using the ratio of SEC peak areas of brushes and unreacted side chains in the final mixture and the feed ratio of backbone to side chains.

^f Determined by ¹H NMR spectroscopy analysis of the conversion of alkyne end group of PDEAMEA using the ester -COOCH₂- peak of PDEAEMA as reference.

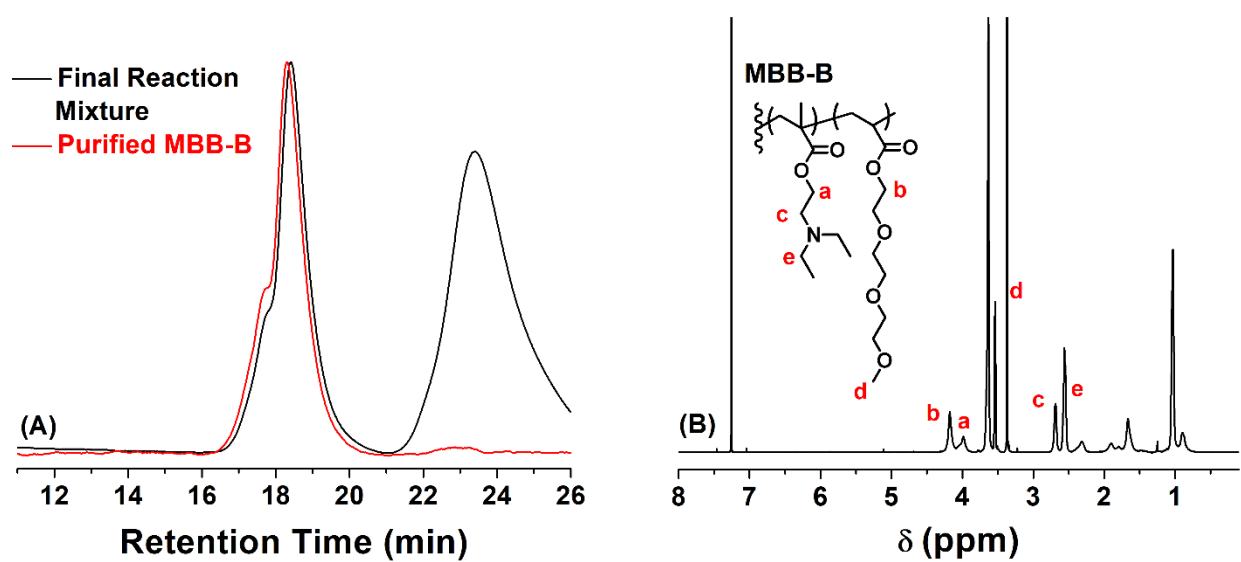


Figure 2.1. (A) SEC traces of the final reaction mixture and the purified molecular brushes with PDEAEMA-*b*-PTEGMA side chains (MBB-B). (B) ¹H NMR spectrum of the purified MBB-B brushes in CDCl₃.

the loss of some lower molecular weight brush molecules during the fractionation. Figure 2.1B shows the ^1H NMR spectrum of MBB-B in CDCl_3 , which is similar to that of PDEAEMA-*b*-PTEGMA side chain polymer (Figure A3B).

For comparison, we also synthesized homografted molecular brushes with pH-responsive homopolymer PDEAEMA side chains (Scheme 2.1). Similarly, the reaction was carried out in THF with a feed molar ratio of 1 : 1.66 for the backbone repeat units to the side chain polymer. However, the characterization of this click reaction by SEC analysis turned out to be much more difficult. We found that although the PDEAEMA side chain polymer was soluble in DMF, the brushes were not; a click reaction that was attempted in DMF resulted in the formation of an insoluble polymer immediately. While we were able to characterize the PDEAEMA side chain polymer by SEC using THF as solvent, the brushes could not be eluted out from the SEC system with THF as eluent – no high molecular weight brush peak was observed. Therefore, the reaction was characterized by ^1H NMR spectroscopy analysis; using the peak of $-\text{COOCH}_2$ (4.10-3.90 ppm) in the polymers as internal reference, the conversion of the reaction was calculated from the integral decrease of the peak at 4.62 ppm from alkyne end group ($\text{CH}\equiv\text{CCH}_2-$) of PDEAEMA (Figure A4). By using this conversion and the feed ratio of azide moieties to side chains, we calculated the grafting density of PDEAEMA brushes to be 82.0 %, which is slightly higher than that of MBB-B (75.0%). This is reasonable because PDEAEMA was shorter than PDEAEMA-*b*-PTEGMA and thus the steric hindrance in the “click” grafting to synthesis would be smaller, allowing for a higher grafting density. The MBB-H brushes were purified by passing of the reaction mixture through an alumina/silica gel column to remove the copper catalyst and fractionation in a mixture of THF and hexanes to remove the unreacted side chain polymer, which was confirmed by ^1H NMR analysis that showed the disappearance of the alkyne end group peak

(Figure A4). The molecular characteristics of MBB-B and MBB-H are summarized in Table 2.1.

2.3.3. Dynamic Light Scattering (DLS) Study and Visual Inspection of Stimuli-Responsive Behavior of MBB-B and -H in Aqueous Buffer Solution

To study the responsive property of MBB-B and the hypothesized stabilization effect of the PTEGMA outer block for the collapsed PDEAEMA side chains at high pH under ambient conditions, a 1.0 mg/g solution of MBB-B in a 10 mM phosphate buffer with a pH of 5.00 was made and the pH was gradually increased at room temperature by adding 0.1 M NaOH. For comparison, a 0.5 mg/g solution of MBB-H in 10 mM phosphate buffer was also prepared at pH = 5.00. The MBB-B solution remained transparent without any change in appearance when the pH was gradually increased from 5.00 to 10.00; Figure 2.2i and ii show the photos of the clear solutions at pH = 5.00 and 9.54 at ambient temperature ($\sim 21\text{ }^{\circ}\text{C}$). The 0.5 mg/g aqueous solution of MBB-H was clear when the pH was lower than 7.3 at room temperature (Figure 2.2v shows a photo at pH = 5.00). However, when the pH was raised to > 7.3 , a precipitate was formed immediately in the solution (Figure 2.2vi shows a photo at pH = 8.00). Evidently, the outer PTEGMA blocks can stabilize the collapsed PDEAEMA blocks at higher pH.

Figure 2.3A shows the apparent average hydrodynamic size (D_h) of 1.0 mg/g MBB-B in a 10 mM phosphate buffer solution as a function of pH in the process of increasing pH (black solid squares) under ambient conditions. In the entire pH range from 5.00 to 10.00, a single size distribution was observed; the hydrodynamic size distribution curves for pH = 6.00 and 8.50 are shown in Figure 2.3B as examples. Moreover, at pH = 6.00, the size distributions of MBB-B at concentrations of 0.5 and 1.0 mg/g essentially overlapped and the D_h values were virtually the same (Figure A6A), indicating that the brushes were molecularly dissolved in acidic buffers.

As shown in Figure 2.3A, when the pH was < 7.2 , the sizes of MBB-B at different pH values

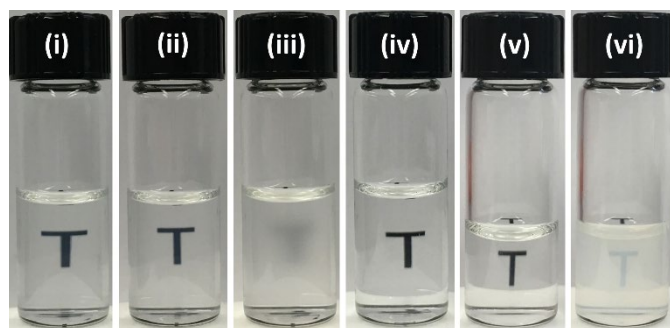


Figure 2.2. Optical photos of 1.0 mg/g solutions of MBB-B in 10 mM aqueous phosphate buffers at (i) pH = 5.00 and 21 °C, (ii) pH = 9.54 and 21 °C, and (iii) pH = 9.54 and 65 °C, of (iv) a 0.2 mg/g solution of MBB-B in phosphate buffer at pH = 9.54 and 65 °C, and of 0.5 mg/g solutions of MBB-H in phosphate buffer at (v) pH = 5.00 and (vi) pH = 8.00 under ambient conditions.

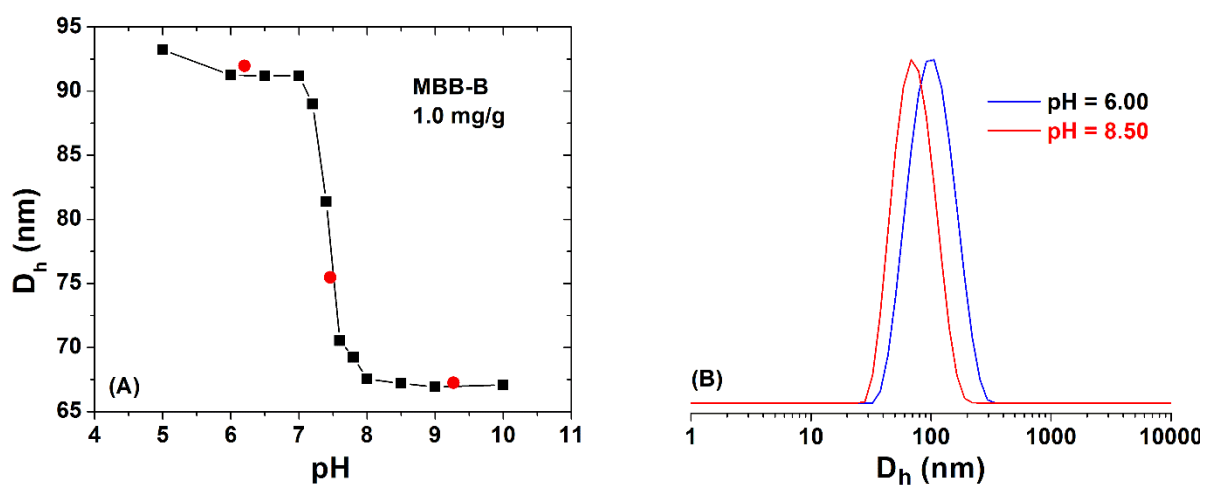


Figure 2.3. (A) Apparent average hydrodynamic size (D_h) of MBB-B in 10 mM phosphate buffer at a concentration of 1.0 mg/g at 25 °C as function of pH, adjusted using 0.1 M NaOH and 0.1 M HCl, in the process of increasing pH (black squares) and decreasing pH (red circles). (B) Hydrodynamic size distributions of 1.0 mg/g MBB-B in 10 mM buffer at pH = 6.00 and 8.50 at 25 °C.

were similar, decreasing only very slightly from 93.2 nm at pH = 5.00 to 91.2 nm at pH = 7.00. Upon further increasing pH, a sharp decrease in D_h was observed in a rather narrow pH range, from 89.0 nm at pH = 7.20 to 70.5 nm at pH = 7.60. When the pH was > 8.00, the apparent hydrodynamic size leveled off at 67 nm. Thus, from pH = 5.00 to 10.00, the D_h decreased by 26 nm (or by 28%). A similar size transition was observed for the concentration of 0.5 mg/g (Figure A7). These observations agreed well with the reported $pK_a = 7.4$ of PDEAEMA homopolymer. At lower pH values, the PDEAEMA blocks in the brush molecules were protonated, and the MBB-B brushes took on an extended conformation, exhibiting larger hydrodynamic sizes. Upon increasing the solution pH above pK_a , deprotonation occurred and the PDEAEMA blocks became insoluble, causing the brushes to collapse into a more compact structure. The unimolecular extended-to-compact conformation transition was supported by the large reduction in D_h and single size distributions at pH > pK_a as well as the observation that the size distributions of MBB-B at concentrations of 0.5 and 1.0 mg/g essentially superimposed each other for pH = 8.50 (Figure A6B). The pH-induced size changes were reversible. We lowered the pH value of the 1.0 mg/g MBB solution to 9.27, 7.46, and 6.20; a single size distribution was observed for each pH value, and the hydrodynamic sizes fell on the curve (red solid circles in Figure 2.3A).

We also studied the pH-responsive behavior of PDEAEMA homopolymer brushes MBB-H in aqueous buffers at a concentration of 0.2 mg/g (Figure 2.4) by DLS. At pH of 5.06, the average size was 76.3 nm with a single symmetric size distribution. Upon gradually increasing the pH to 7.0, the D_h decreased slightly to 74.0 nm, followed by a larger reduction to 68.7 nm at pH = 7.10 and then 67.1 nm at 7.25. Further raising the pH to 7.35 resulted in an increase in D_h and a much broader distribution with a large shoulder as shown in Figure 2.4B, indicating intermolecular aggregation even at a concentration as low as 0.2 mg/g. This stands in stark contrast to the behavior

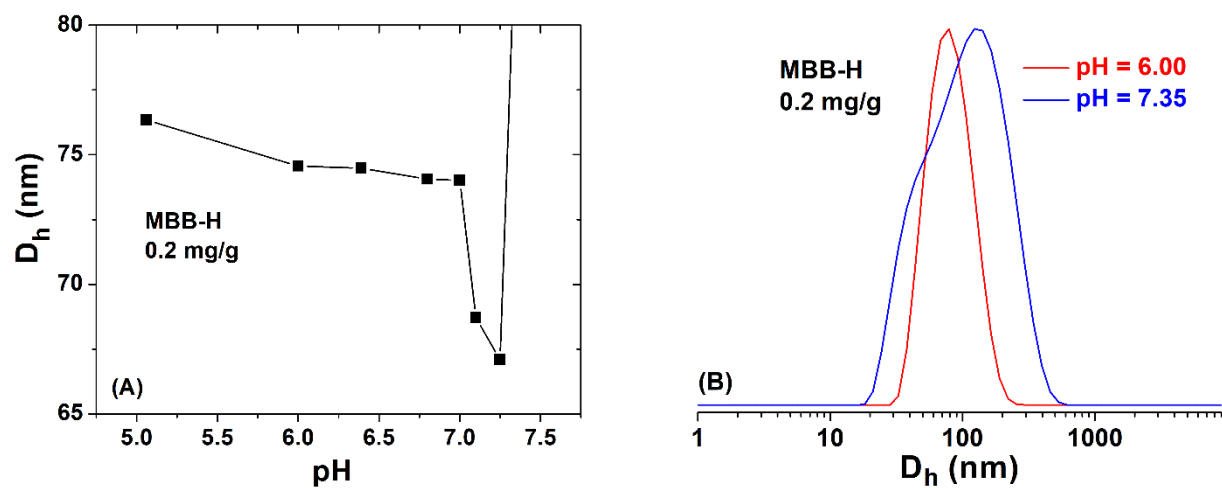


Figure 2.4. Apparent average hydrodynamic size (D_h) of MBB-H in 10 mM phosphate buffer at a concentration of 0.2 mg/g as function of pH. (B) Hydrodynamic size distribution curves of 0.2 mg/g MBB-B in 10 mM phosphate buffer at pH = 6.00 and 7.35 under ambient conditions.

of MBB-B, whose size leveled off at $\text{pH} > 8.0$ with a single narrow size distribution. Thus, we can conclude that the PTEGMA outer blocks in MBB-B provided a stabilization effect to the collapsed PDEAEMA inner blocks at ambient conditions.

As mentioned earlier, PTEGMA is a thermo-responsive water-soluble polymer with a LCST of $58\text{ }^{\circ}\text{C}$.^{44,45} As expected, when heated across the LCST of PTEGMA, the 1.0 mg/g solution of MBB-B with a pH of 9.54 turned cloudy as shown in Figure 2.2iii for the sample at $65\text{ }^{\circ}\text{C}$, indicating the aggregation of the MBB-B brush molecules due to the collapse of PTEGMA blocks. The thermoresponsive behavior of 1.0 mg/g MBB-B in 10 mM phosphate buffer at $\text{pH} = 8.50$ was studied by DLS. Figure 2.5 shows the plots of scattering intensity and D_h versus temperature from 20 to $65\text{ }^{\circ}\text{C}$ in a heating process. At each selected temperature, the solution was equilibrated for 5 min prior to the measurements. Interestingly, the scattering intensity increased slightly and the size showed a steady decrease upon heating until $\sim 60\text{ }^{\circ}\text{C}$. The D_h decreased by $\sim 10\text{ nm}$ in this temperature range, reaching 58 nm at $58\text{ }^{\circ}\text{C}$. This can be attributed to the solvent quality for PTEGMA decreasing gradually with increasing temperature but still remaining within the good solvent regime, which was also previously observed for other PEO-based thermoresponsive polymers.^{45,46} Note that aqueous solutions of PDEAEMA with a concentration of 2.0 mg and MBB-H with a concentration of 1.0 mg/g in 10 mM phosphate buffer at $\text{pH} = 7.10$ were found to become cloudy upon heating (Figure A8), a sign of LCST transitions, although both solutions stayed clear from 0 to $100\text{ }^{\circ}\text{C}$ at $\text{pH} = \sim 6.0$ ($\text{pH} = 6.13$ for PDEAEMA and $\text{pH} 6.00$ for MBB-H). It could be possible that the collapsed PDEAEMA inner blocks in MBB-B brush molecules at $\text{pH} = 8.50$ undergo further shrinking with increasing temperature, contributing to the size decrease observed in Figure 2.5B. Above $60\text{ }^{\circ}\text{C}$, sharp changes in scattering intensity and D_h were observed. In fact, the solution turned cloudy. The decrease in scattering intensity might be due to the settling

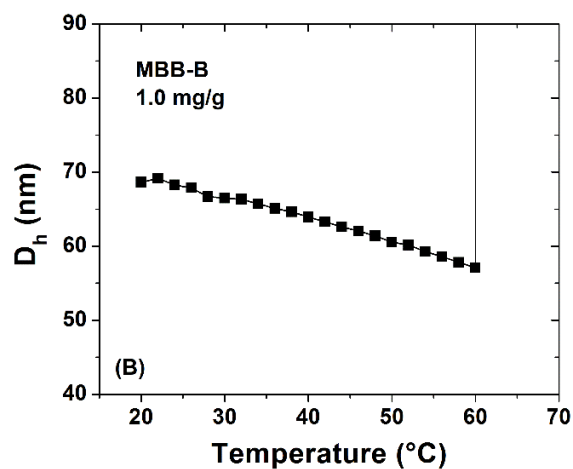
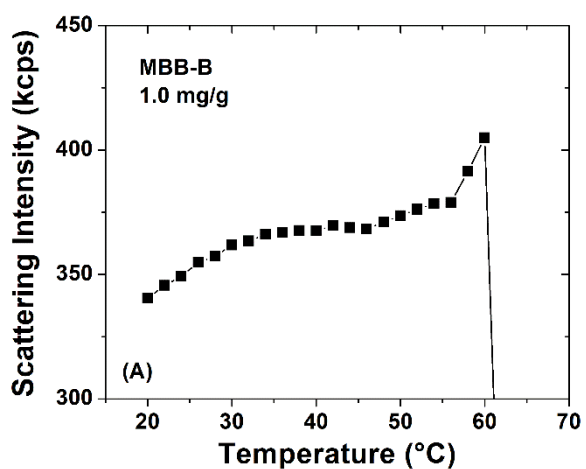


Figure 2.5. Plots of (A) scattering intensity and (B) D_h versus temperature for a 1.0 mg/g MBB-B solution in 10 mM phosphate buffer with pH of 8.50 from a DLS study in a heating process.

of aggregates during the equilibration. Note that when the brush concentration was 0.2 mg/g, the solution stayed clear at 65 °C (Figure 2.2iv) and DLS showed that the brushes remained unaggregated above the LCST (Figure A9) and the thermally induced size changes were reversible. This is consistent with our previous observation for thermoresponsive brushes in H₂O.^{24,26}

2.3.4. AFM Study of Responsive Behavior of MBB-B Molecular Brushes

The observed abrupt decrease in D_h of MBB-B in aqueous buffer with increasing pH across the pK_a of PDEAEMA suggests a transition of brush molecules from a more extended structure at lower pH to a more compact conformation at higher pH values, where the deprotonated PDEAEMA inner blocks collapsed and aggregated, forming a hydrophobic core that was supported in solution by the hydrophilic PTEGMA block at ambient conditions. To study the morphologies of MBB-B brush molecules under various conditions and to characterize their possible shape transitions, AFM was employed. Dilute aqueous solutions of MBB-B with concentrations from 0.05 mg/g to 0.1 mg/g in 2.5 mM phosphate buffer were prepared and spin cast onto PMMA-coated mica.

Figures 2.6 and A10 show AFM height images of MBB-B at pH = 5.00; as expected, the brush molecules were clearly in a cylindrical state, with an average contour length of 110.4 ± 13.8 nm and a typical height of ~ 6 nm. The brushes were taller than thermoresponsive binary heterografted molecular brushes on the same type of substrate that we observed before, which was about 1 nm,²⁶ likely due to the incorporation of counter-anions into the brushes at the low pH value as well as electrostatic repulsive forces present in the protonated tertiary amine groups, causing the side chains and backbone to adopt a more stretched conformation. When the pH of the solution was increased to 9.56 at room temperature, the MBB-B brushes collapse into much more condensed globular shape as shown in Figures 2.7 and A11 compared with cylindrical nano-objects in Figures

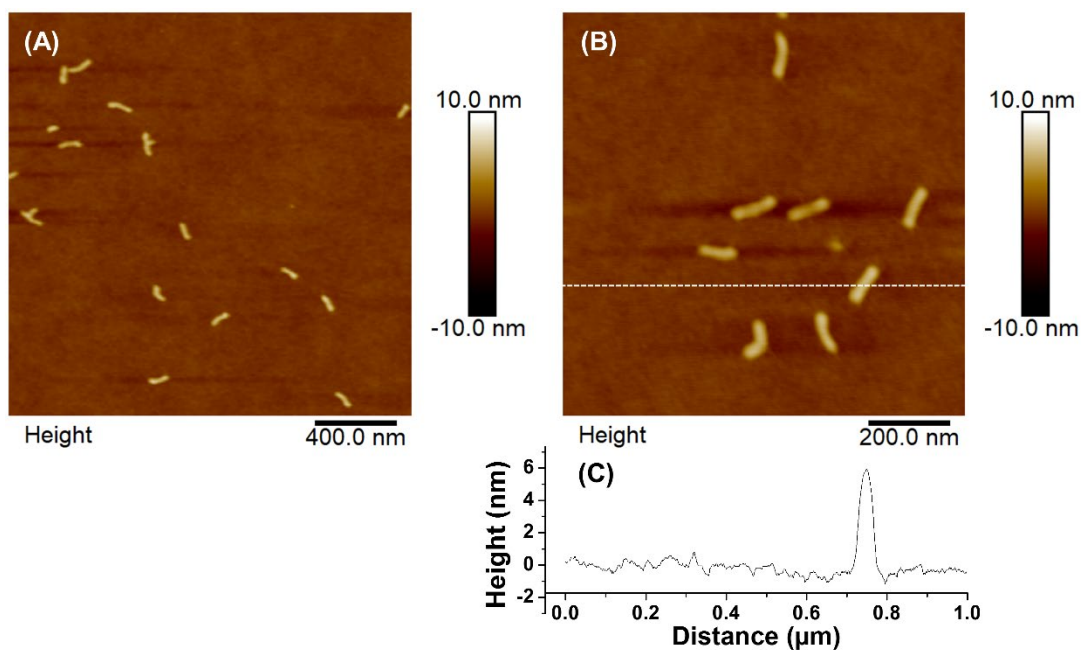


Figure 2.6. AFM height image (A) with sizes of $2 \times 2 \mu\text{m}$ and (B) with sizes of $1 \times 1 \mu\text{m}$ of MBB-B molecular brushes spin cast onto PMMA-coated mica at 3000 rpm from a 2.5 mM aqueous phosphate buffer solution with a polymer concentration of 0.05 mg/g at a pH of 5.00 and room temperature. (C) is the cross-sectional height profile along the dash line in image (B).

2.6 and A10. Some brush molecules appeared to be not fully collapsed into a spherical shape but exhibited a condensed C- or S-like shape. One possible factor that might contribute to this behavior is the relatively long PTEGMA outer block ($DP = 58$); the strong solvation of PTEGMA blocks in the collapsed state might make it difficult for brush molecules to reach a fully collapsed structure. Cross-sectional analysis showed that the typical height of globular nano-objects was ~ 5.5 nm; the average size was 61.9 ± 9.2 nm.

To study the thermally induced transition of MBB-B at $pH > pK_a$, we gradually increased the temperature of a 0.1 mg/g sample with a pH of 9.54 to 65 °C, which was above the LCST transition of PTEGMA, equilibrated it at the same temperature, and then spin cast on PMMA-coated mica for AFM imaging. As shown in Figures 2.8 and A12, the brush molecules remained as individually collapsed nano-objects but more condensed and spherical compared with the collapsed nano-objects in Figures 2.7 and A11 at the same pH but room temperature. This is consistent with the results by visual inspection and DLS analysis for a 0.2 mg/g solution of MBB-B in phosphate buffer (Figure 2.2iv and Figure A9). Analysis showed that the average size was 56.4 ± 7.5 nm and the typical height was ~ 7.5 nm, in contrast to 61.9 ± 9.2 nm and ~ 5.5 nm, respectively, for the brushes at room temperature. Evidently, the thermally induced LCST transition of PTEGMA outer blocks caused the globular MBB-B brushes to further collapse into a more spherical conformation in order to minimize the contact with surrounding water, as schematically illustrated in Scheme 2.2.

As shown in Figures 2.2iii and 2.5, at $pH > pK_a$ of PDEAEMA and a concentration of 1.0 mg/g, the MBB-B brush molecules underwent aggregation and the solution turned cloudy upon heating above the LCST of PTEGMA. To investigate the morphology of the aggregated brush molecules, a 1.0 mg/g sample with a pH of 9.57 was heated to 65 °C and it turned cloudy. The sample was

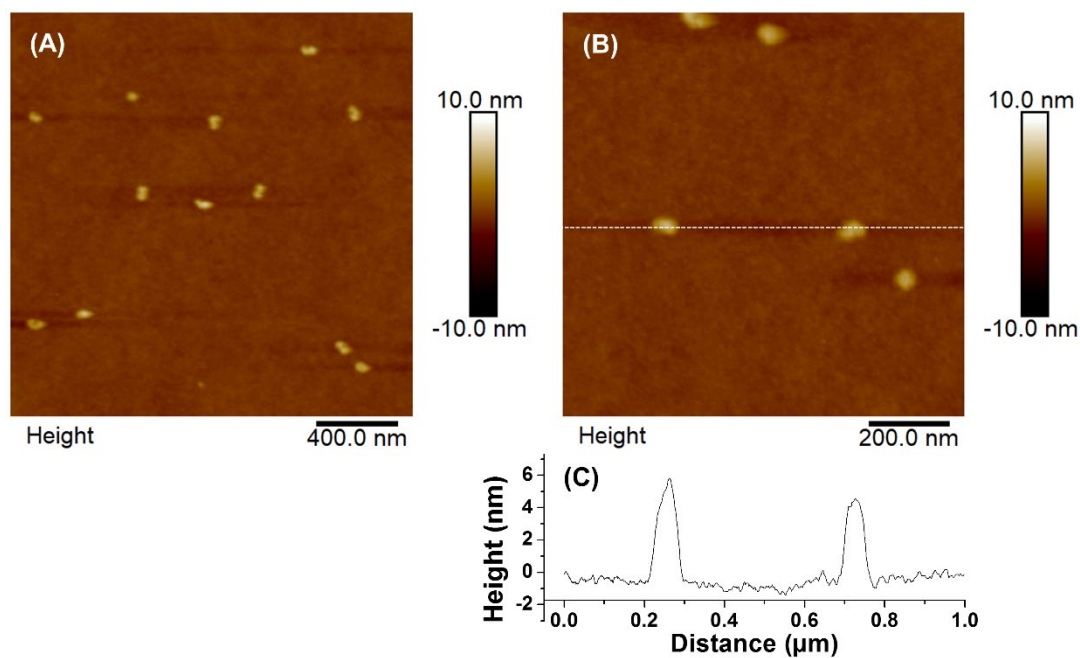


Figure 2.7. AFM height image (A) with sizes of $2 \times 2 \mu\text{m}$ and (B) with sizes of $1 \times 1 \mu\text{m}$ of MBB-B molecular brushes spin cast onto PMMA-coated mica at room temperature from a 2.5 mM aqueous phosphate buffer solution at a pH of 9.56 with polymer concentration of 0.05 mg/g. (C) is the cross-sectional height profile along the dash line in image (B).

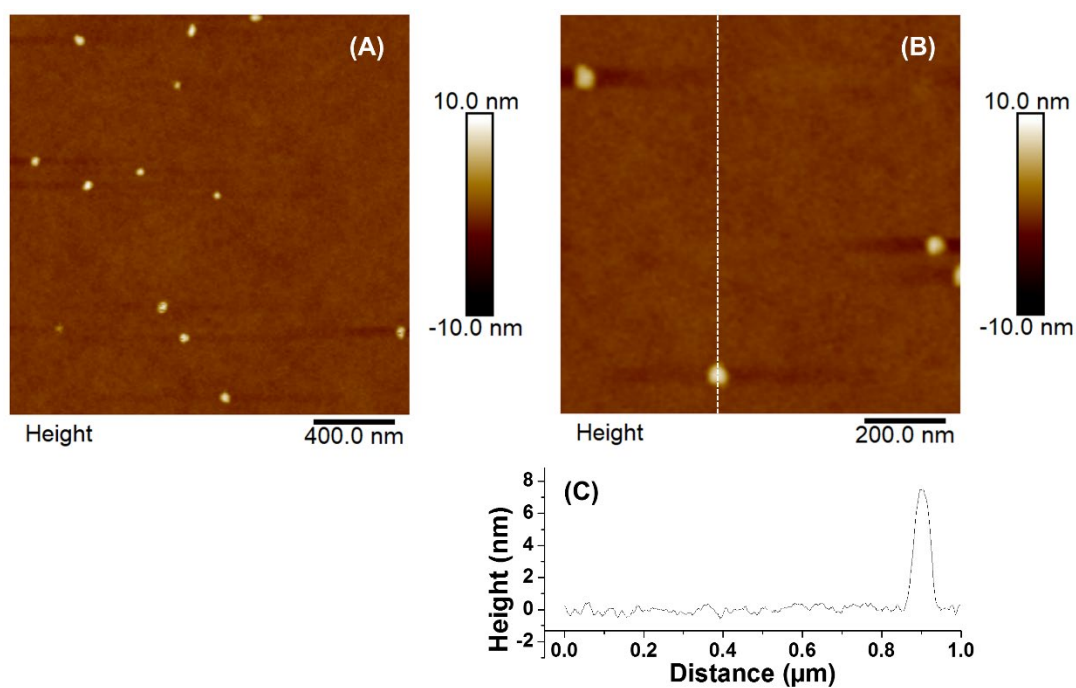
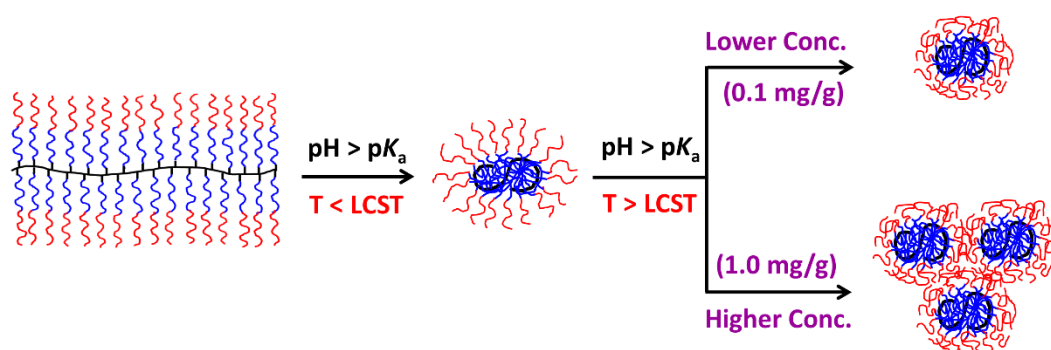


Figure 2.8. AFM height image (A) with sizes of $2 \times 2 \mu\text{m}$ and (B) with sizes of $1 \times 1 \mu\text{m}$ of MBB-B molecule brushes spin cast onto PMMA coated mica from a 0.1 mg/g solution of MBB-B in 2.5 mM phosphate buffer with a pH of 9.54 after being heated to 65 °C. (C) is the cross-sectional height profile along the dash line in image (B).

Scheme 2.2. Schematic Illustration of pH-Induced Cylinder-to-Globule Shape Transition and Temperature-Induced Further Condensation at Lower Concentration and Aggregation at Higher Concentration.



then diluted quickly to 0.1 mg/g at 65 °C and spin cast onto PMMA-coated mica. AFM study revealed the presence of large tight clusters (Figures 2.9 and A13) as well as spherical nano-objects, which confirmed the intermolecular aggregation of brushes (Scheme 2.2).

2.4. Conclusions

In summary, we synthesized linear homografted molecular brushes containing dually pH- and thermo-responsive diblock copolymer side chains by a click “grafting to” method and demonstrated that the introduction of a second block to the end of the inner pH-responsive block in the grafted side chains effectively stabilized the collapsed brushes when the inner blocks became insoluble in water. DLS study showed that the D_h of MBB-B exhibited a sharp drop in a narrow pH range when the pH was raised across the pK_a of PDEAEMA and leveled off at higher pH values. AFM revealed a cylindrical morphology of the brushes at acidic pH and a cylinder-to-globule transition with increasing pH. Moreover, the solution state of the brushes can be further controlled by the temperature and the brush concentration. At lower concentrations such as 0.1 mg/g, the brushes remained as individual nano-objects in solution and became more spherical at temperatures above the LCST of PTEGMA, while at higher concentrations (e.g., 1.0 mg/g) aggregation occurred and the solution turned cloudy. In contrast, the brushes with only pH-responsive side chains aggregated and precipitated out when the solution pH was around pK_a and above. It is our belief that the method reported in this work to achieve worm-to-stabilized globule transition and control the solution state of collapsed brushes could open up new opportunities for potential applications of stimuli-responsive shape-changing molecular brushes such as tailoring of the morphology of nanomaterials synthesized by using molecular brushes as templates.

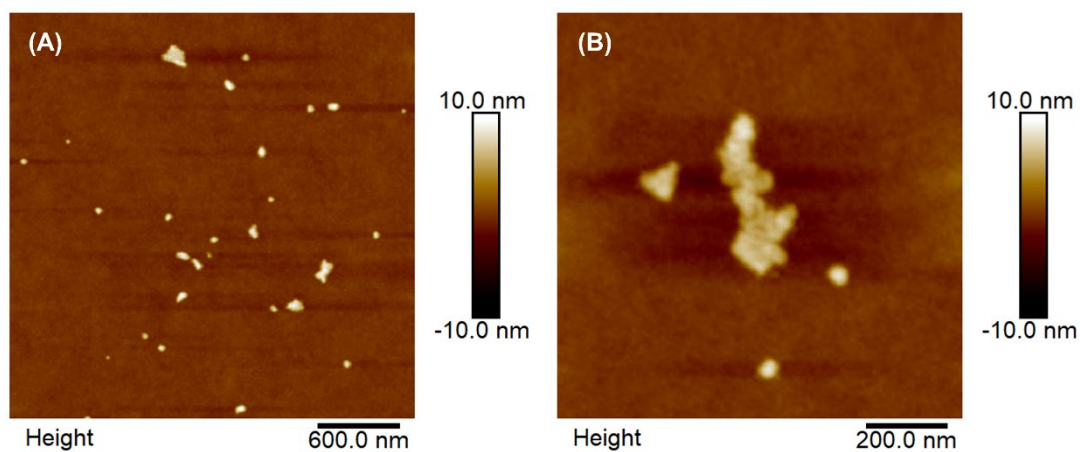


Figure 2.9. AFM height image (A) with sizes of $3 \times 3 \mu\text{m}$ and (B) with sizes of $1 \times 1 \mu\text{m}$ of MBB-B molecular brushes spin cast onto PMMA-coated mica from a 0.1 mg/g aqueous solution of MBB-B that was diluted at 65 °C from a 1.0 mg/g aqueous solution of MBB-B in 2.5 mM buffer with a pH of 9.57. The 1.0 mg/g solution was heated to 65 °C before the dilution.

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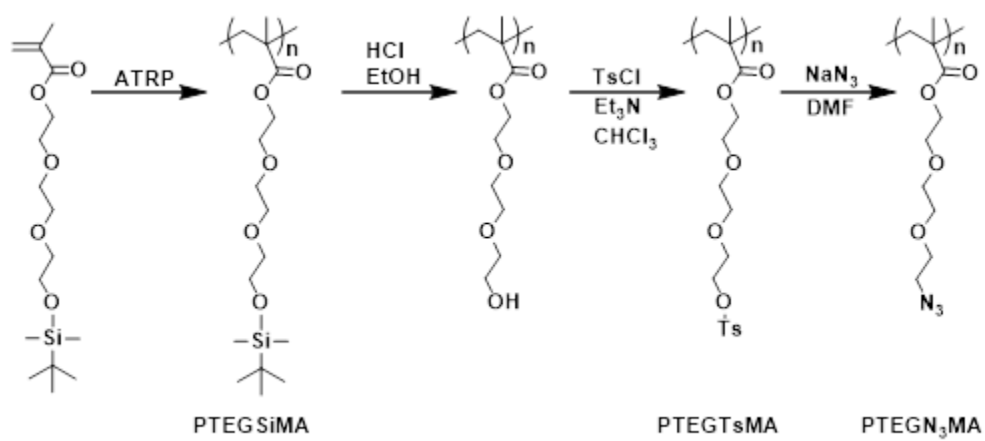
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Appendix A

for

**Chapter 2: Shape-Changing Linear Molecular Bottlebrushes with Dually pH-
and Thermo-Responsive Diblock Copolymer Side Chain**

Scheme A1. Synthesis of Azide-Bearing Backbone Polymer PTEGN₃MA



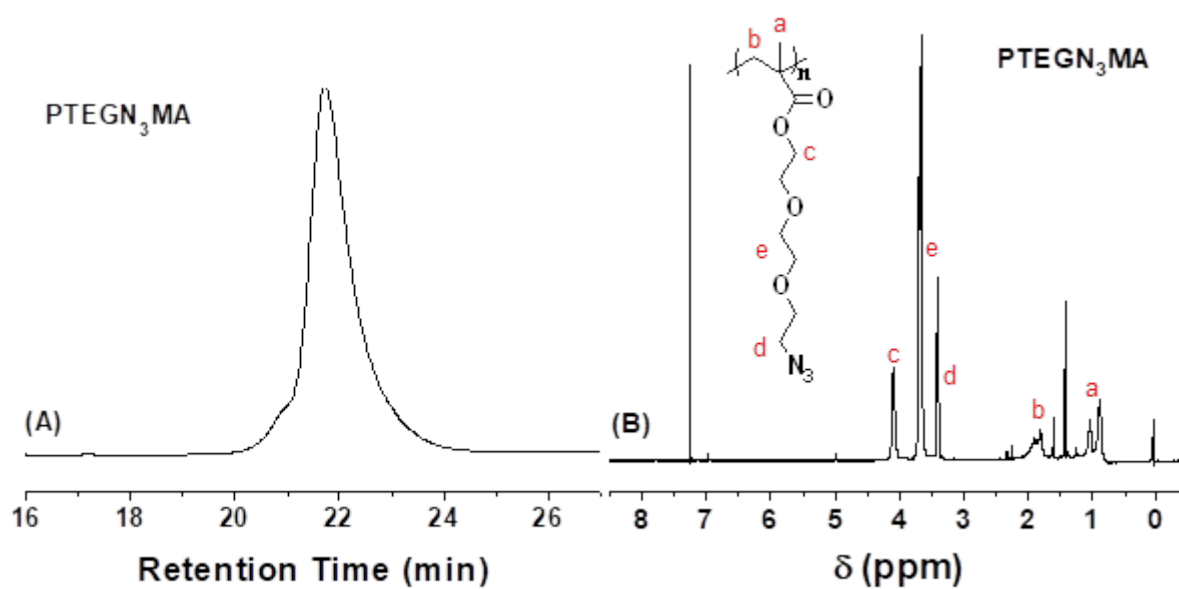


Figure A1. (A) Size exclusion chromatography (SEC) trace and ¹H NMR spectrum in CDCl₃ of the purified PTEGN₃MA with a degree of polymerization of 707.

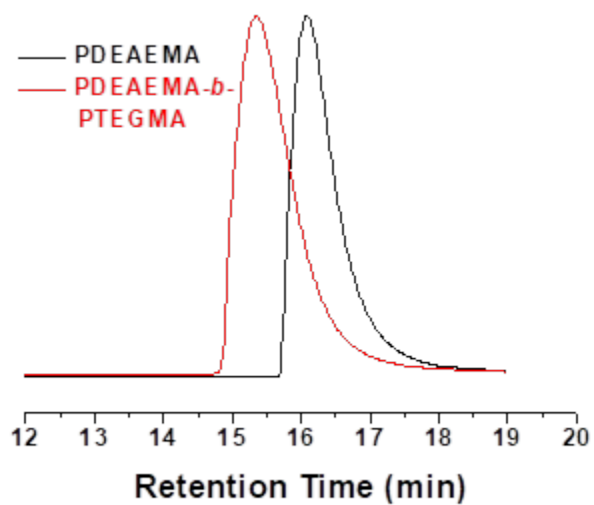


Figure A2. SEC traces of PDEAEMA macroinitiator ($M_{n,SEC} = 6.5$ kDa, PDI = 1.17) and PDEAEMA-*b*-PTEGMA side chain polymer ($M_{n,SEC} = 14.1$ kDa, PDI = 1.21).

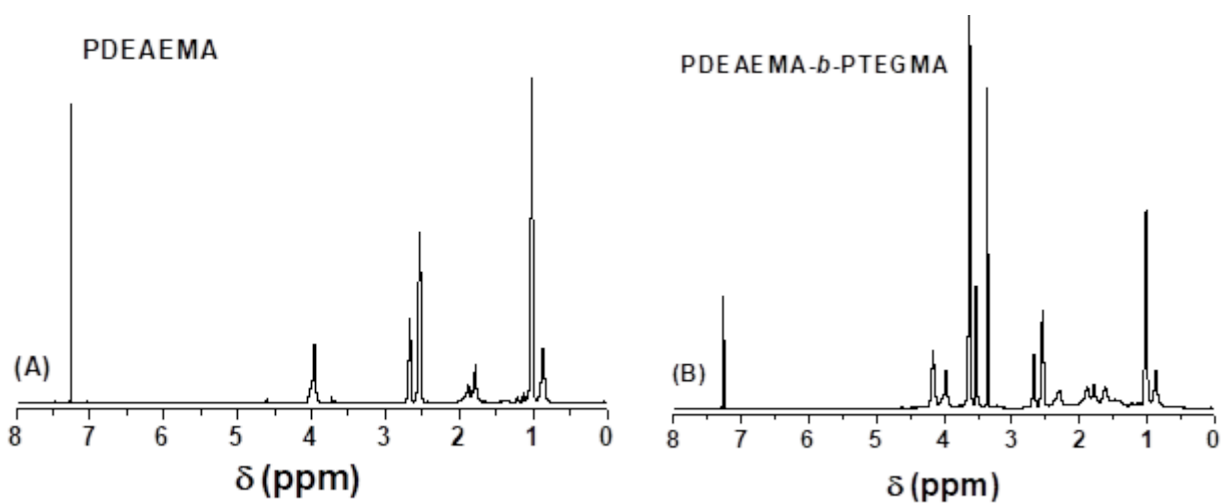


Figure A3. ^1H NMR spectra of (A) PDEAEMA macroinitiator and (B) PDEAEMA-*b*-PTEGMA side chain polymer in CDCl_3 .

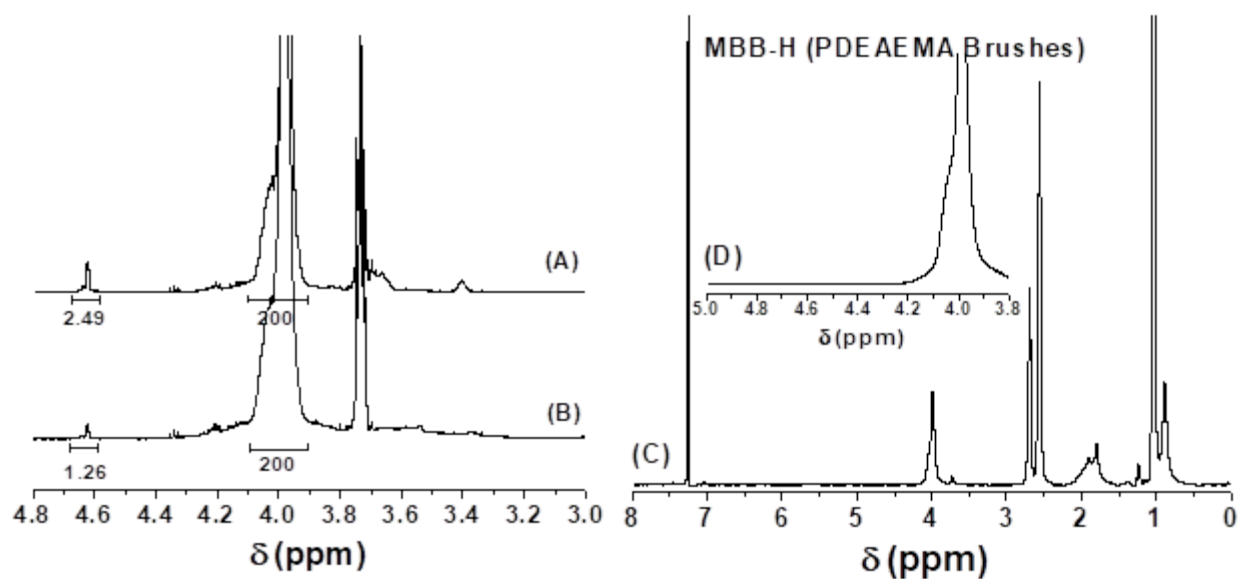


Figure A4. ^1H NMR spectrum of the reaction mixture at (A) $t = 0$ and (B) at the end of the click reaction in CDCl_3 , showing the decrease in the integral of the alkyne peak at 4.62 ppm. (C) ^1H NMR spectrum of the purified PDEAEMA molecular brushes MBB-H in CDCl_3 , and (D) shows the enlarged portion from 3.8 to 5.0 ppm; no alkyne peak left, indicating the removal of unreacted PDEAEMA side chains.

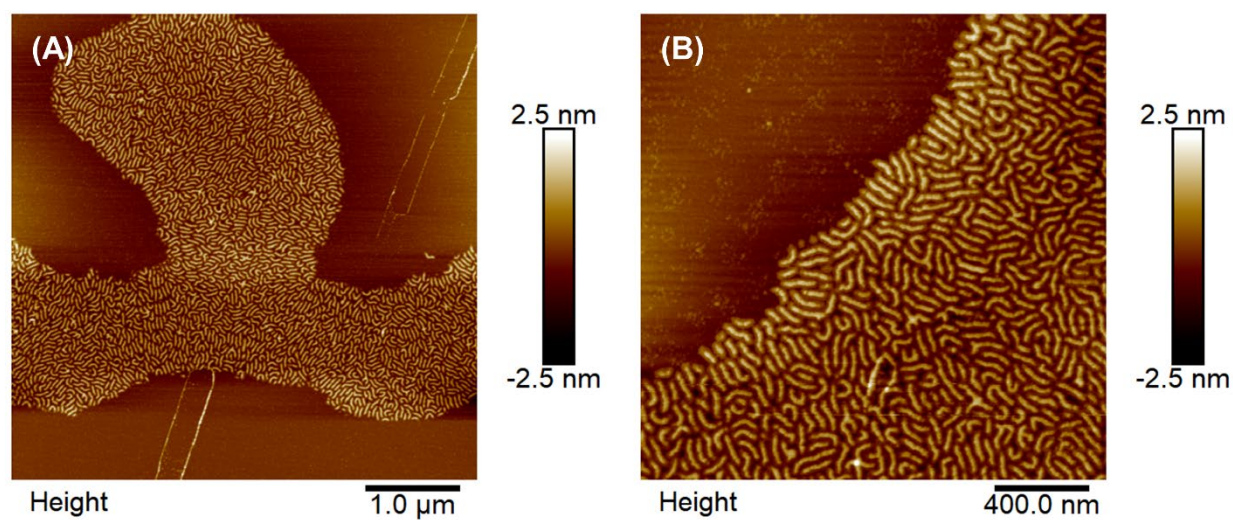


Figure A5. AFM height image (A) with sizes of $5 \times 5 \mu\text{m}$ and (B) with sizes of $2 \times 2 \mu\text{m}$ of MBB-H brush molecules spin cast onto bare mica from a 0.05 mg/g aqueous solution with pH of 5.00.

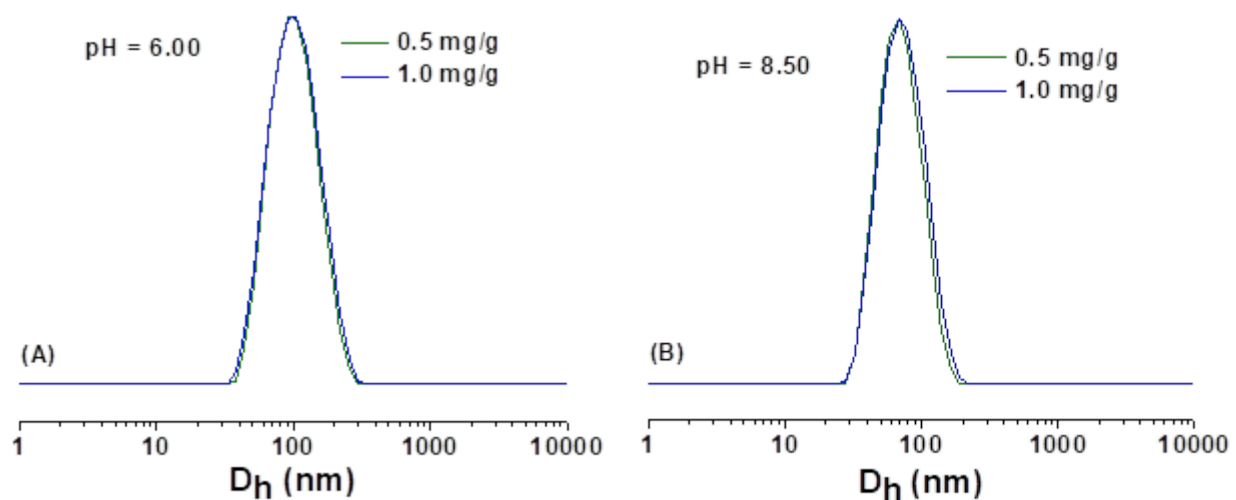


Figure A6. Apparent hydrodynamic size distributions of MBB-B molecular brushes at concentrations of 0.5 mg/g and 1.0 mg/g in aqueous 10 mM phosphate buffer with (A) pH of 6.00 and (B) pH of 8.50.

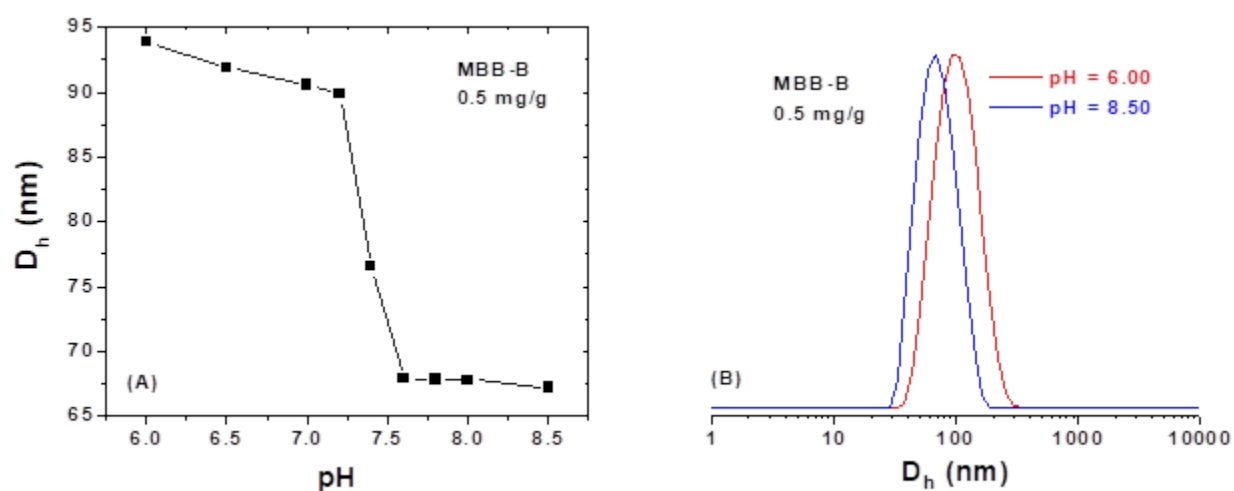


Figure A7. (A) Apparent average hydrodynamic size (D_h) of MBB-B in 10 mM phosphate buffer at a concentration of 0.5 mg/g as function of pH, adjusted using 0.1 M NaOH and 0.1 M HCl, in the process of increasing pH. (B) Hydrodynamic size distributions of 0.5 mg/g MBB-B in 10 mM buffer at pH = 6.00 and 8.50 under ambient conditions.

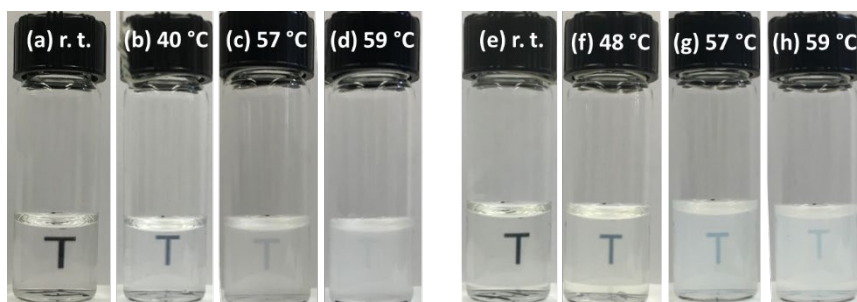


Figure A8. Optical photos of a 2.0 mg/g solution of PDEAEMA (a-d) and a 1.0 mg/g solution of MBB-H (e-h) in 10 mM phosphate buffer with a pH value of 7.10 at various temperatures. The onset temperature of clouding transition was 40 °C for PDEAEMA and 48 °C for MBB-H. Note that the PDEAEMA solution at pH = 6.13 and the MBB-H solution at pH = 6.00 remained clear in the entire temperature range of 0 to 100 °C. We could not determine if PDEAEMA exhibits a LCST transition at pH = 8.50 because the polymer becomes insoluble and precipitates in water at this pH.

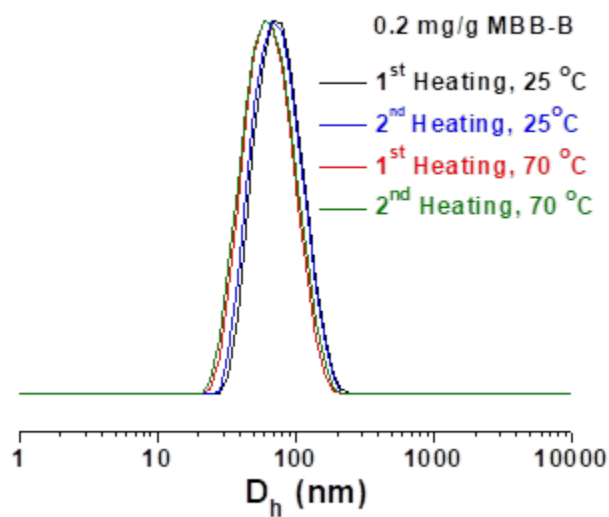


Figure A9. Apparent hydrodynamic size distributions of MBB-B molecular brushes at concentrations of 0.2 mg/g in aqueous 10 mM phosphate buffer with pH of 8.50 at 25 °C and 70 °C from the first and second heating processes. At each selected temperature, the sample was equilibrated for at least 5 min before the measurement. After the first heating was completed, the solution was cooled back to 25 °C and allowed to equilibrate at that temperature for 30 min before the second heating began.

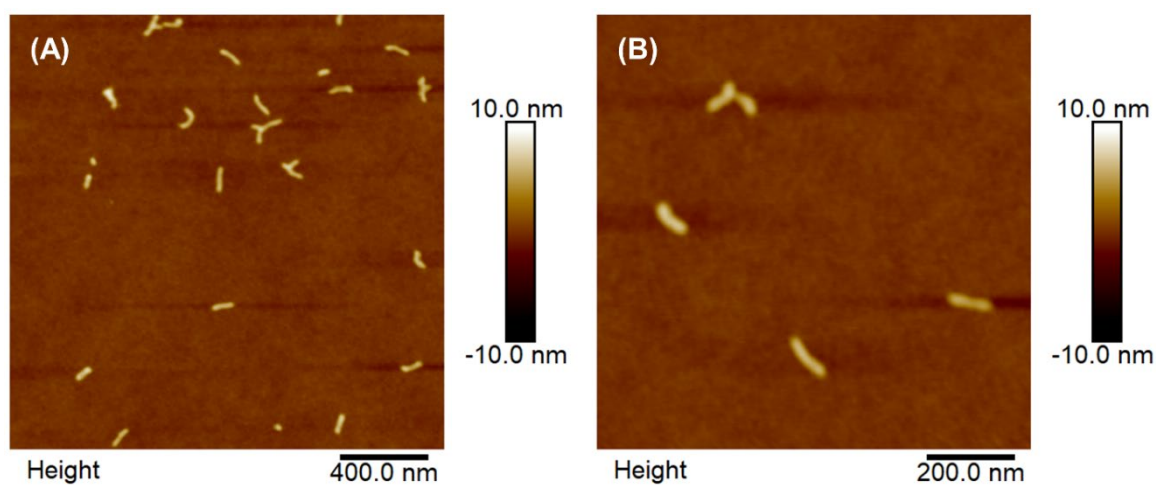


Figure A10. Additional AFM height image (A) with sizes of $2 \times 2 \mu\text{m}$ and (B) with sizes of $1 \times 1 \mu\text{m}$ of MBB-B molecular brushes with dually responsive PDEAEMA-*b*-PTEGMA side chains spin cast onto PMMA-coated mica at 3000 rpm from a 2.5 mM aqueous phosphate buffer solution with a polymer concentration of 0.05 mg/g at a pH of 5.00 and room temperature.

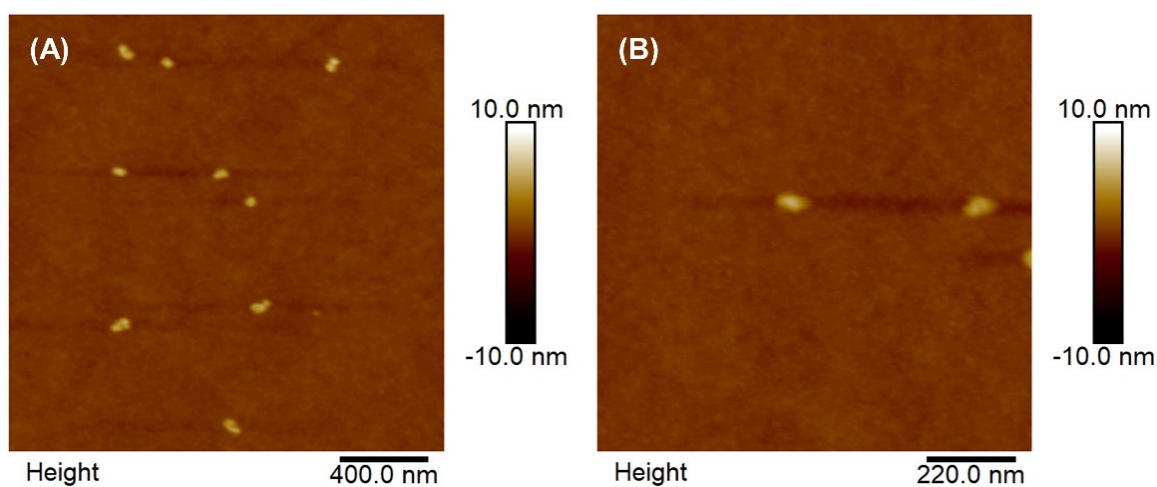


Figure A11. Additional AFM height image (A) with sizes of $2 \times 2 \mu\text{m}$ and (B) with sizes of $1.1 \times 1.1 \mu\text{m}$ of MBB-B molecular brushes spin cast onto PMMA-coated mica at room temperature from a 2.5 mM aqueous phosphate buffer solution at a pH of 9.56 with a polymer concentration of 0.05 mg/g diluted from 0.1 mg/g.

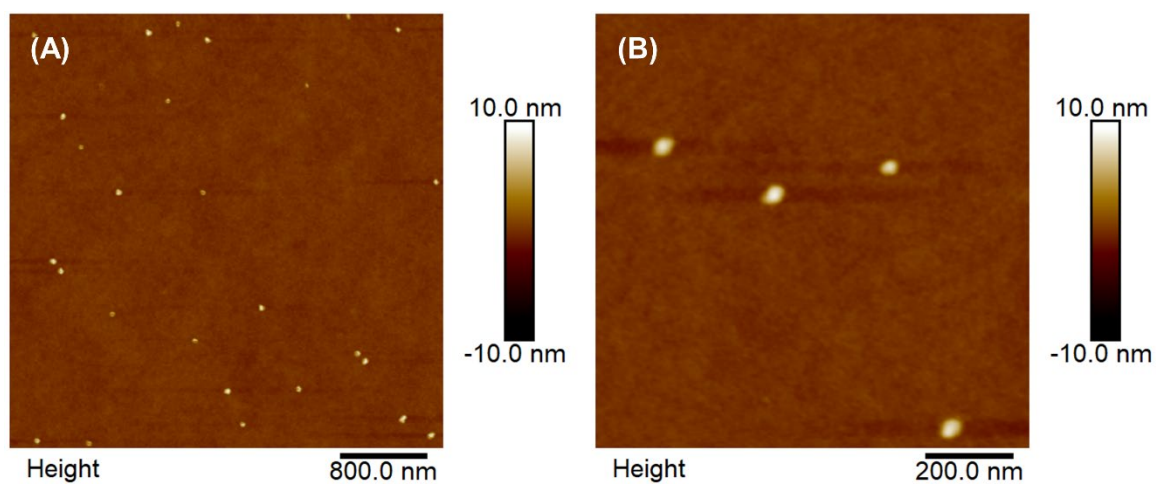


Figure A12. AFM height image (A) with sizes of $4 \times 4 \mu\text{m}$ and (B) with sizes of $1 \times 1 \mu\text{m}$ of MBB-B molecule brushes spin cast onto PMMA-coated mica from a 0.1 mg/g solution of MBB-B in 2.5 mM phosphate buffer with a pH of 9.54 after being heated to and equilibrated at 65 °C.

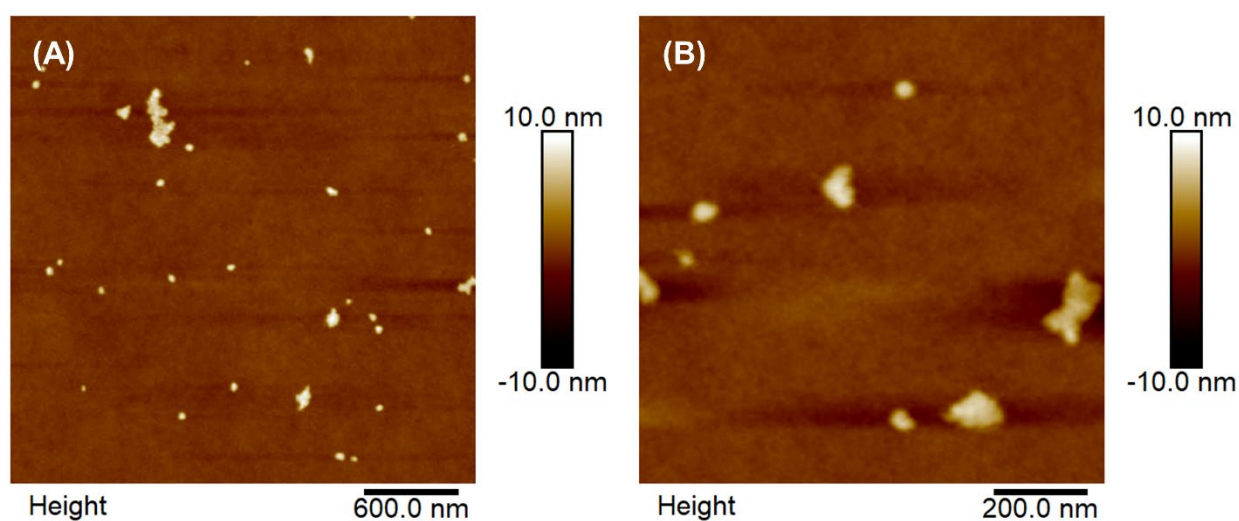


Figure A13. Additional AFM height image (A) with sizes of $3 \times 3 \mu\text{m}$ and (B) with sizes of $1 \times 1 \mu\text{m}$ of MBB-B molecular brushes spin cast onto PMMA-coated mica from a 0.1 mg/g aqueous solution of MBB-B that was diluted at 65 °C from a 1.0 mg/g aqueous solution of MBB-B in 2.5 mM buffer with a pH of 9.57. The 1.0 mg/g solution was heated to 65 °C before the dilution.

**Chapter 3: Stimuli-Induced Star-Globule Shape Transitions of Dually
Responsive Binary Heterografted Three-Arm Star Molecular Brushes in
Aqueous Solution**

The work presented in this chapter has been published in *Macromolecules* **2019**, 52 (17), 6714-6724. I synthesized all of the components of this work and performed the molecular and physical characterizations. Prof. Bin Zhao advised this work.

Abstract

This chapter presents the synthesis of doubly responsive binary heterografted, three-arm star molecular brushes and the study of their stimuli-induced star-to-globule shape transitions in aqueous solution. The star brushes were prepared using the highly efficient copper-catalyzed azide-alkyne cycloaddition reaction to simultaneously graft an alkyne end-functionalized thermoresponsive polymer of ethoxydi(ethylene glycol) acrylate (PDEGEA), with a lower critical solution temperature (LCST) of 9 °C, and a pH-responsive polymer of 2-(*N,N*-diethylamino)ethyl methacrylate (PDEAEMA), with a pK_a of 7.4 at room temperature, onto an azide-bearing three-arm star backbone polymer. The star architecture of the formed brushes was confirmed by atomic force microscopy. The use of two different stimuli-responsive polymers as side chains allowed for the stabilization of collapsed brushes against aggregation when one side chain polymer became insoluble in water and for the formation of two distinct globular states with different polymers in the core. At temperatures below the LCST of PDEGEA, upon increasing the pH from 5.00 to 9.40, the brushes underwent a shape transition from an extended starlike to a collapsed globular state stabilized by the hydrated PDEGEA side chains. On the other hand, at pH slightly lower than the pK_a of PDEAEMA, upon heating across the LCST of PDEGEA, the brushes also exhibited a star-to-globule shape transition, forming a globular state distinct from the one induced by pH changes.

3.1. Introduction

Molecular bottlebrushes (MBBs or simply molecular brushes) are a class of macromolecules in which relatively short polymeric side chains are densely, covalently grafted on a long polymer backbone.¹⁻⁶ These complex polymers exhibit many unique characteristics, such as high and tunable persistence length, no or low chain entanglement, and large conformational changes or shape transitions upon application of external stimuli.¹⁻¹⁰ The conformations of MBBs in a solvent are determined by a number of factors, including the aspect ratio of backbone to side chain length, grafting density, backbone architecture, side chain length, and solvent quality. When the aspect ratio and grafting density are sufficiently high, the strong excluded volume interactions between side chains cause MBBs to adopt extended conformations in good solvents, leading to a wormlike (for linear brushes) or a starlike (for star brushes) morphology. Due to their unique structural characteristics and intriguing properties, MBBs are widely envisioned to have great potential in a variety of applications, such as drug delivery, lubrication, and supersoft elastomers.¹⁻¹³

MBBs are commonly synthesized by one, or a combination of the following methods:¹⁴⁻³⁴ (i) grafting-from, in which side chains are grown from pendant initiating sites on a backbone polymer typically by a “living”/controlled polymerization method, (ii) grafting-through, in which macromonomers are polymerized to produce brushes directly, and (iii) grafting-to, in which end-functionalized side chain polymers are covalently attached to a backbone bearing complementary reactive pendant groups. While each method has its own advantages and disadvantages, grafting-to allows for thorough characterization of backbone and side chain polymers and provides a modular route for the synthesis of heterografted MBBs composed of two or more distinct side chain polymers with varying molar ratios and tunable overall grafting densities.²⁸⁻³⁴ The grafting-to synthesis of MBBs has benefited greatly from the advent of click-chemistry;³⁵ using the highly

efficient copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, molecular brushes with high and tunable grafting densities now can be routinely prepared.²⁸⁻³⁴

When stimuli-responsive polymers are employed as side chains, the resultant MBBs can undergo dramatic changes in size and shape in response to external stimuli.^{2-4,36-50} Schmidt et al. provided the first example of thermoresponsive MBBs, prepared by growing poly(*N*-isopropylacrylamide) (PNIPAm) side chains from a backbone polymer using atom transfer radical polymerization (ATRP).³⁶ They observed that the PNIPAm brushes underwent a worm-to-globule shape transition upon heating to above the lower critical solution temperature (LCST) of PNIPAm. The collapsed globular brushes, however, were unstable; intermolecular aggregation and precipitation eventually occurred. Matyjaszewski et al. synthesized thermoresponsive brush polymers with statistical copolymers of *N,N*-dimethylacrylamide and *n*-butyl acrylate as side chains.³⁷ At low concentrations, dynamic light scattering (DLS) studies showed that the apparent hydrodynamic size (D_h) of the brushes decreased upon heating, suggesting unimolecular collapse. At higher concentrations, however, they observed that the D_h increased when the solutions were heated across the LCST, a sign of intermolecular aggregation of the brushes. More recently, Verduzco et al. made PNIPAm MBBs by a grafting-through method;⁴⁶ the brushes were found to form aggregates in solution when heated to above the LCST of PNIPAm, which in this regard was similar to the observation by Schmidt et al. These examples of stimuli-responsive MBBs showed that while worm-to-globule shape transitions in solution could be induced, the collapsed globular brushes were unstable and readily underwent aggregation even at a concentration as low as 1.0 mg/g. This has severely limited potential applications of stimuli-responsive shape-changing MBBs.

Our laboratory recently developed two methods to stabilize collapsed globular states of MBBs.^{33,34,49,50} This was achieved by introducing poly(ethylene oxide) (PEO), a permanently water-soluble polymer, as the second type of side chains in thermoresponsive linear binary heterografted MBBs or another (stimuli-responsive) polymer as the outer block of diblock copolymer side chains in linear homografted brushes. The introduced second polymer served as a stabilizer when the brush molecules collapsed into the globular state; for the brush polymers with dually responsive diblock copolymer side chains, the stimuli-responsive outer block provided another level of control over the solution state of the brushes.³⁴ These multicomponent MBBs were prepared by grafting-to using the CuAAC click reaction. By increasing the PEO chain length relative to that of thermoresponsive side chains, our lab showed that worm-to-globule shape transitions could be achieved in moderately concentrated aqueous solutions due to the shielding effect provided by the long PEO side chains.⁵⁰

Compared with linear MBBs, star-shaped brushes are much less investigated, partly because of the synthetic challenges.⁵¹⁻⁵⁴ Star MBBs can be envisioned to possess their own unique structural features and behavior, such as more compact molecular structures, lower solution viscosity, and even higher entanglement molecular weight, compared with linear MBBs. Matyjaszewski, Sheiko and coworkers synthesized three- and four-arm poly(*n*-butyl acrylate) star MBBs and observed that the brushes underwent star-to-disk shape transitions at the water-air interface in a Langmuir trough upon lateral compression.^{51,52} It would be advantageous and sometimes necessary for many potential applications to effect stimuli-induced star-globule shape transitions in solution. In the present work, we synthesized binary heterografted 3-arm star MBBs composed of two different stimuli-responsive polymers as side chains and examined their shape changing behavior in aqueous solution. When one set of side chains collapses into the core of the

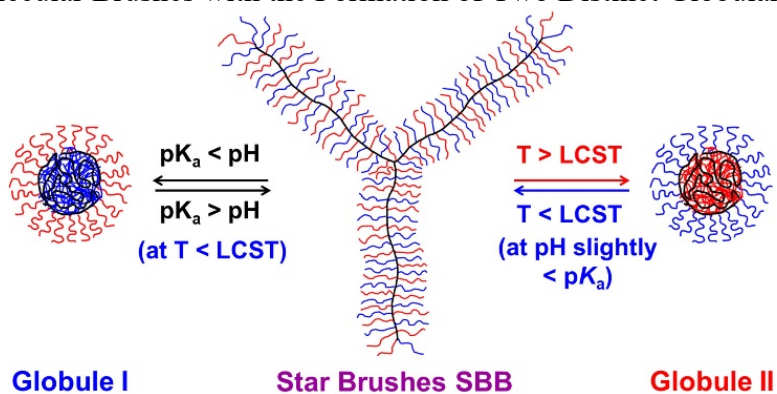
globule, the second set remains soluble and forms a stabilizing corona; this makes it possible to achieve two distinct globular states with different chemical and physical properties from one brush system (Scheme 3.1), which could be used, e.g., to load different substances for triggered delivery. The star brushes (SBB) were synthesized by simultaneously clicking alkyne end-functionalized thermoresponsive poly(ethoxydi(ethylene glycol) acrylate) (PDEGEA), with an LCST of 9 °C,⁵⁵⁻⁵⁸ and pH-responsive poly(2-(*N,N*-diethylamino)ethyl methacrylate) (PDEAEMA), with a pK_a of 7.4 at room temperature,^{59,60} onto an azide-bearing 3-arm star backbone (Scheme 3.2). PDEAEMA was chosen as the pH-responsive side chain polymer because it exhibits a drastic solubility transition in water from very hydrophilic at low pH to very hydrophobic at high pH. The responsive behavior of SBB in water was investigated by visual inspection, DLS, ¹H NMR analysis, and atomic force microscopy (AFM), which showed stimuli-induced star-globule shape transitions and the stabilization effect of the second side chain polymer.

3.2. Experimental Section

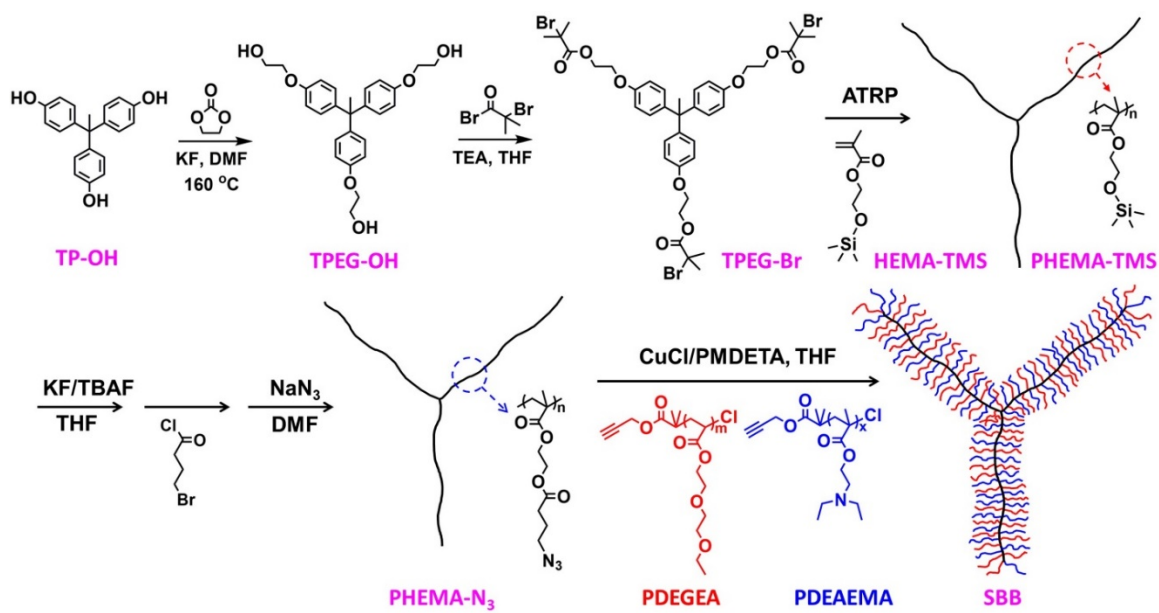
3.2.1. Materials

CuCl (99%, Sigma-Aldrich) was stirred in glacial acetic acid overnight, collected by vacuum filtration, washed thoroughly with absolute ethanol and diethyl ether, and then dried under high vacuum. 2-(*N,N*-Diethylamino)ethyl methacrylate (DEAEMA, 98.5%, TCI) and 2-(2-ethoxyethoxy)ethyl acrylate (DEGEA, 98%, TCI) were passed through a basic alumina (top)/silica gel (bottom) column to remove the inhibitor prior to polymerization. 2-Bromoisobutyryl bromide (97%, Alfa Aesar), 4-bromobutyryl chloride (95%, Acros), and *N,N,N',N'',N''*-pentamethyldiethylenetriamine (PMDETA, 99%, Acros) were purified by vacuum distillation. 2-Hydroxyethyl methacrylate (HEMA, 97%, Aldrich), chlorotrimethylsilane (TMS-Cl, 98%, Acros), triethylamine (TEA, 99%, Alfa Aesar), 1,1,1-tris(4-hydroxyphenyl)ethane (TP-OH, 99%, Sigma-

Scheme 3.1. Stimuli-Induced Star-Globule Shape Transitions of Doubly Responsive Binary Heterografted Molecular Brushes with the Formation of Two Distinct Globular States



Scheme 3.2. Synthesis of Binary Heterografted Three-Arm Star Molecular Brushes



Aldrich), ethylene carbonate (99%, Acros), tetrabutylammonium fluoride (1 M solution in THF, Acros), potassium fluoride (99%, Fisher), 4,4'-dinonyl-2,2'-dipyridyl (97%, Sigma-Aldrich), sodium azide (99%, Acros), *N,N*-dimethylformamide (Spectranalyzed, Fisher), and 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 97%, Sigma-Aldrich) were used as received. 2-(Trimethylsilyloxy)ethyl methacrylate (HEMA-TMS) was synthesized by the reaction of 2-hydroxyethyl methacrylate with chlorotrimethylsilane, and the molecular structure was confirmed by ^1H NMR spectroscopy analysis. Propargyl 2-bromoisobutyrate (PBiB) was synthesized according to a procedure described in the literature.⁶¹ All other chemicals were purchased from either Sigma-Aldrich or Fisher and used as received.

3.2.2. General Characterization

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

spectrometer (Peabody, MA) with an IonSense (Danvers, MA) DART source. The HRMS sample of the trifunctional ATRP initiator, TPEG-Br, was prepared in ethyl acetate with a concentration of 1.0 mg/g.

Size exclusion chromatography (SEC) of alkyne end-functionalized side chain polymers, PDEGEA and PDEAEMA, was performed at ambient temperature using a PL-GPC 20 (an integrated GPC/SEC system from Polymer Laboratories, Inc.) with a differential refractive index (RI) detector, one PLgel 5 μ m guard column (50 $\times$ 7.5 mm, Agilent Technologies), and two PLgel 5 μ m mixed-C columns (each 300 $\times$ 7.5 mm, linear range of molecular weights from 200 to 2 000 000 Da, Agilent Technologies). The mobile phase was tetrahydrofuran (THF) set at a flow rate of 1.0 mL/min, and the system was calibrated with a set of narrow disperse linear polystyrene standards (Scientific Polymer Products, Inc.). SEC of the backbone precursors, PHEMA-TMS and PHEMA-Br, the azide-functionalized backbone polymer, PHEMA-N₃, as well as the 3-arm star molecular brushes was performed at 50 °C using a PL-GPC 50 Plus system (an integrated GPC/SEC system from Polymer Laboratories, Inc.) with a differential refractive index detector, one PSS GRAL 10 μ m guard column (50 $\times$ 8 mm, Polymer Standards Service-USA, Inc.) and two PSS GRAL 10 μ m linear columns (each 300 $\times$ 8 mm, linear range of molecular weight from 500 to 1 000 000 Da, Polymer Standards Service-USA, Inc.). *N,N*-Dimethylformamide (DMF), containing 50 mM LiBr, was used as the eluent at a flow rate of 1.0 mL/min. The absolute molecular weight of the purified star brushes, SBB, was determined by a GPC-MALS system comprising one PSS GRAM 10 μ m guard column (8 $\times$ 50 mm, Polymer Standards Service-USA, Inc.), three PSS GRAM 10 μ m linear columns (8 $\times$ 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 μ L loop, and a Varian 390 LC detector system consisting of an RI

detector and a two angle light scattering detector (15° and 90°). The mobile phase was *N,N*-dimethylformamide (DMF) with 50 mM LiBr, and the analysis was carried out at 50 °C.

3.2.3. Dynamic Light Scattering (DLS) Study and Visual Inspection of 3-Arm Star Brushes in Aqueous Solution

DLS of SBB was performed using a Malvern Zetasizer Nano ZS instrument equipped with a He-Ne 633 nm laser and a temperature controller at a scattering angle of 173°. Each DLS sample was prepared by a wet-transfer method from a stock solution of SBB in THF. The stock solution of the star brushes in THF with a known concentration was weighed into a flask and diluted with a calculated amount of a 10 mM phosphate buffer. The solution was concentrated under high vacuum to remove THF, which was confirmed by ¹H NMR spectroscopy analysis. Finally, the solution was reconstituted to the desired concentration with Milli-Q water. To study the pH-responsive property of SBB, the pH of the solution was gradually adjusted using 0.1 M NaOH and 0.1 M HCl in an ice/water bath, monitored by an Accumet AB-15 pH meter (calibrated at 0 °C with pH = 4.01, 7.00, and 10.01 standard buffer solutions) and allowed to equilibrate for 15 min. A portion of the solution was transferred into a DLS tube without filtering and loaded into the DLS instrument with a pre-set temperature of 1 °C. The DLS sample was equilibrated for an additional 5 min before measurements were taken. Temperature ramps were performed at various pH values, with equilibration for 5 min at each selected temperature. Each reported DLS value was an average of 3 measurements, each of which was comprised of 10 runs; z-average hydrodynamic diameters were used. For visual inspection, the 1.0 mg/g aqueous solutions of SBB in a 10 mM phosphate buffer with various pH values were prepared similarly in an ice/water bath, and the photos were taken at the selected temperatures after equilibration for 10 min using a Nikon Coolpix B500 camera.

3.2.4. Atomic Force Microscopy (AFM) Study of SBB

The star brushes were studied by AFM using a Digital Instruments Multimode IIIa Scanning Probe Microscope in tapping mode under ambient conditions. Reflective Al-coated Si probes (Budget Sensors) with a resonant frequency of 300 kHz and a force constant of 40 Nm⁻¹ were used. PMMA-coated mica substrates were prepared by cleaving a fresh layer of mica disk (Ted Pella, Inc.) with Scotch tape, placing three drops of a 2.5 wt% solution of PMMA (M_n = 54.9 kDa) in CHCl₃ onto the disk, and immediately spinning the solution off at 3000 rpm (P-6000 Spin Coater, Specialty Coating Systems, Inc.) to create a thin PMMA film. Aqueous solutions of SBB with a concentration of 0.05 mg/g were prepared by diluting a brush solution with a concentration of 0.20 mg/g using a 5 mM phosphate buffer and were spin cast onto the PMMA-coated mica substrates (3000 rpm). The pH values of the aqueous solutions of SBB used for preparing AFM samples were measured with a pH meter (Accumet AB-15) in an ice/water bath and adjusted to the desired values using 0.1 M HCl and 0.1 M NaOH solutions. For the AFM samples prepared at 0 °C, the spin casting stage and the PMMA-coated mica disk were cooled in the refrigerator (4 °C), taken out and used immediately.

3.2.5. Synthesis of Trifunctional Initiator ((Ethane-1,1,1-triyltris(benzene-4,1-diyl))tris(oxy))tris(ethane-2,1-diyl)tris(2-bromo-2-methylpropanoate) (TPEG-Br)

The trifunctional initiator, ((ethane-1,1,1-triyltris(benzene-4,1-diyl))tris(oxy))tris(ethane-2,1-diyl)tris(2-bromo-2-methylpropanoate) (TPEG-Br), was prepared according to Scheme 3.2. TPEG-OH was synthesized from 1,1,1-tris(4-hydroxyphenyl)ethane (TP-OH) and ethylene carbonate according to a procedure modified from the literature for a similar reaction.⁶² 1,1,1-Tris(4-hydroxyphenyl)ethane (2.005 g, 6.54 mmol), ethylene carbonate (2.114 g, 24.0 mmol), KF (17.0 mg, 0.29 mmol), and DMF (5 mL) were added to a 50 mL two-necked flask equipped with

a reflux condenser. After the reaction mixture was refluxed and stirred in a 160 °C oil bath for 20 h, DMF was removed under high vacuum, and the crude product was purified by column chromatography using CH₂Cl₂/MeOH (10/1, v/v) as eluent. After being dried under high vacuum, the product was obtained as a white solid. ¹H NMR spectroscopy analysis showed four extra sets of small peaks in the range of 3.6 – 4.2 ppm (Figure B1), likely due to the fluoride-involved side reaction leading to the formation of -OCH₂CH₂F; washing with saturate NaHCO₃ and a second round of column chromatography did not remove the extra peaks.

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

(s, -C(CH₃), 3H), 0.15 (s, -Si(CH₃)₃, 27H). ¹³C NMR (CDCl₃, 126 MHz), δ (ppm) = 156.80 (-OC(CH₂)₂(CH₂)₂C-), 141.88 (-OC(CH₂)₂(CH₂)₂C-), 129.57 (-OC(CH₂)₂(CH₂)₂C-), 113.67 (-OC(CH₂)₂(CH₂)₂C-), 69.02 (-OCH₂CH₂O-), 61.41 (-OCH₂CH₂O-), 50.58 (-C(CH₃)), 30.75 (-C(CH₃)), 0.36 (-Si(CH₃)₃). The ¹H and ¹³C NMR spectra of TPEG-TMS are shown in Figure B2.

TPEG-TMS (0.629 g, 0.960 mmol) was dissolved in ethanol (10 mL) in a 50 mL two-necked flask, and 1 M aqueous HCl solution (2.98 mL, 2.98 mmol) was added via a syringe. After the reaction mixture was stirred under nitrogen at room temperature overnight, the volatile components were removed by rotary evaporation and subsequently under high vacuum. The product, 2,2',2''-((ethane-1,1,1-triyltris(benzene-4,1-diyl))tris(oxy))tris(ethan-1-ol) (TPEG-OH), was obtained as an off-white solid (0.405 g, 96.2 %). ¹H NMR (CDCl₃, 500 MHz), δ (ppm) = 6.99 (d, aromatic, -OC(CH₂)₂(CH₂)₂C-, 6H), 6.81 (d, aromatic, -OC(CH₂)₂(CH₂)₂C-, 6H), 4.07 (t, -OCH₂CH₂OH, 6H), 3.95 (t, -OCH₂CH₂OH, 6H), 2.11 (s, -C(CH₃), 3H), 2.05-1.70 (br s, -CH₂OH). ¹³C NMR (CDCl₃, 126 MHz), δ (ppm) = 156.52 (aromatic, -OC(CH₂)₂(CH₂)₂C-), 142.01 (aromatic, -OC(CH₂)₂(CH₂)₂C-), 129.54 (aromatic, -OC(CH₂)₂(CH₂)₂C-), 113.66 (aromatic, -OC(CH₂)₂(CH₂)₂C-), 69.09 (-OCH₂CH₂OH), 61.27 (-OCH₂CH₂O-), 50.52 (-C(CH₃)), 30.67 (-C(CH₃)). The ¹H and ¹³C NMR spectra of TPEG-OH are shown in Figure B3.

TPEG-OH (0.235 g, 0.536 mmol, 1.61 mmol -OH), TEA (0.200 g, 1.97 mmol), and dry THF (20 mL) were added to a 50 mL two-necked flask and stirred under nitrogen in an ice/water bath, followed by dropwise addition of α-bromoisobutyryl bromide (0.410 g, 1.78 mmol). After 16 h, the precipitate was filtered off, and the filtrate was concentrated by rotary evaporation to remove the volatiles. The crude product was dissolved in CH₂Cl₂ (100 mL) and washed two times with saturated sodium bicarbonate aqueous solution (25 mL) and once with 50% saturated aqueous NaCl solution (25 mL). The organic phase was dried over anhydrous sodium sulfate; the solid was

filtered off and the solvents were removed by rotary evaporation. The crude product was purified by column chromatography using ethyl acetate/hexanes (1 : 2, v : v) as the eluent. The isolated product was a white, crystalline solid upon drying by rotary evaporation. This white solid was further purified by recrystallization using a mixture of ethyl acetate and hexanes, yielding the pure product, TPEG-Br, as a white, needle-like crystal (0.297 g, 62.5 %). ^1H NMR (CDCl_3 , 500 MHz), δ (ppm) = 6.99 (d, aromatic, $-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}-$, 6H), 6.81 (d, aromatic, $-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}-$, 6H), 4.50 (t, $-\text{OCH}_2\text{CH}_2\text{OOC}-$, 6H), 4.20 (t, $-\text{OCH}_2\text{CH}_2\text{OOC}-$, 6H), 2.10 (s, $-\text{C}(\text{CH}_3)$, 3H), 1.93 (s, $-\text{C}(\text{CH}_3)_2\text{Br}$, 18H). ^{13}C NMR (CDCl_3 , 126 MHz), δ (ppm) = 171.66 ($-\text{OOC}(\text{CH}_3)_2\text{Br}$), 156.48 ($-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}$), 142.23 ($-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}-$), 129.64 ($-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}-$), 113.95 ($-\text{OC}(\text{CH})_2(\text{CH})_2\text{C}-$), 65.55 ($-\text{COOCH}_2\text{CH}_2\text{O}-$), 64.29 ($-\text{COOCH}_2\text{CH}_2\text{O}-$), 55.50 ($-\text{C}(\text{CH}_3)_2\text{Br}$), 50.66 ($-\text{C}(\text{CH}_3)$), 30.77 ($-\text{C}(\text{CH}_3)$) and 30.72 ($-\text{OOC}(\text{CH}_3)_2\text{Br}$). The ^1H and ^{13}C NMR spectra of TPEG-Br are shown in Figure B4. MS: m/z calc $\text{C}_{38}\text{H}_{45}\text{O}_9\text{Br}_3$ $[\text{M}+\text{H}]^+$: 885.06660; found: 885.06796; mass error: 1.54 ppm.

3.2.6. Synthesis of the Azide-Bearing 3-Arm Star Backbone Polymer (PHEMA- N_3)

Trifunctional initiator TPEG-Br (5.12 mg, 5.78×10^{-3} mmol, delivered from 0.324 g of a 15.8 mg/g stock solution in anisole), HEMA-TMS (3.042 g, 15.03 mmol), CuCl (2.5 mg, 2.5×10^{-2} mmol), 4,4'-dinonyl-2,2'-dipyridyl (15.0 mg, 3.67×10^{-2} mmol), and anisole (0.833 g) were added to a 25 mL two-necked flask and degassed by three freeze-pump-thaw cycles. The flask was then placed into an oil bath with a pre-set temperature of 75 °C, and the reaction mixture was stirred with a magnetic stir bar. After 26 h, the flask was taken out from the oil bath and opened to air. An aliquot was immediately withdrawn for ^1H NMR spectroscopy analysis, and the polymerization mixture was quickly diluted with chloroform. The copper catalyst was removed by passing through a column of neutral alumina (top)/silica gel (bottom) with chloroform as the eluent. The solution

was concentrated by rotary evaporation and then dried under high vacuum for 6 h at 50 °C to remove the unreacted monomer, yielding the 3-arm star polymer of HEMA-TMS (PHEMA-TMS) as a highly viscous polymer (1.484 g). The monomer conversion was 50.1%, calculated from the ^1H NMR spectrum of the final reaction mixture by using the integrals of the peaks at 4.26 – 4.18 ppm ($-\text{COOCH}_2-$ of the monomer) and 4.12 – 3.89 ppm ($-\text{COOCH}_2-$ of the polymer). Thus, the average degree of polymerization (DP) per arm was 434. SEC analysis results (PSS GRAL columns with DMF containing 50 mM LiBr as the eluent) relative to polystyrene standards: $M_{n,\text{SEC}} = 179.8$ kDa, dispersity (D) = 1.13 (Figure B5).

PHEMA-TMS (1.039 g, 5.14 mmol of $-\text{OTMS}$ groups), KF (0.386 g, 6.65 mmol), and THF (25 mL) were added to a 50 mL two-necked flask and stirred under nitrogen for 15 min in an ice/water bath. TBAF (105 μL of a 1 M solution in THF, 0.105 mmol) was added using a microsyringe, followed immediately by the addition of 4-bromobutyryl chloride (2.207 g, 11.90 mmol). The mixture was removed from the ice/water bath and stirred under nitrogen at ambient conditions for 3.5 h. The polymer solution was concentrated to about 10 mL and precipitated in methanol (100 mL) three times from THF (10 mL). The purified polymer was dried under high vacuum to yield an off-white rubbery polymer (PHEMA-Br, 1.023 g, 71.4%). SEC analysis results (PSS GRAL columns with DMF containing 50 mM LiBr as the eluent) relative to polystyrene standards: $M_{n,\text{SEC}} = 211.4$ kDa, dispersity (D) = 1.17 (Figure B5).

PHEMA-Br (0.922 g, 3.30 mmol of $-\text{CH}_2\text{Br}$ assuming a 100% conversion), NaN_3 (1.113 g, 17.12 mmol), and DMF (20 mL) were added to a 50 mL two-necked flask and stirred at room temperature for 20 h. DMF was removed under high vacuum and the crude mixture was precipitated twice in MeOH/ H_2O (80/20, v/v), once in methanol, and once in hexanes using THF as the good solvent. The purified polymer was dried under high vacuum for 3 h, yielding PHEMA-

N_3 as a slightly yellow, rubbery polymer (0.605 g, 80.8%) and stored as a stock solution in THF in the freezer. SEC analysis results relative to polystyrene standards (PSS GRAL columns with DMF containing 50mM LiBr as the eluent): $M_{n,SEC} = 224.5$ kDa, dispersity (D) = 1.16 (Figure B5). The degree of azide functionalization of the 3-arm star backbone polymer was 88.3%, determined by ^1H NMR spectroscopy analysis of PHEMA- N_3 with PHEMA-TMS and PHEMA-Br as references (Figure B6). The deprotection of TMS groups using KF/TBAF as the catalyst was highly efficient, as can be seen from the nearly complete disappearance of the silyl ether peak at 0.13 ppm ($-OSi(CH_3)_3$) with only a tiny amount of protective TMS groups remaining. After the subsequent esterification of the pendant $-OH$ with 4-bromobutyryl chloride, three new peaks appeared at 3.59 – 3.45 ppm ($-OOCCH_2CH_2CH_2Br$), 2.68 – 2.48 ppm ($-OOCCH_2CH_2CH_2Br$), and 2.32 – 2.11 ppm ($-OC(O)CH_2CH_2CH_2Br$), and the peaks at 4.14 - 3.86 ppm ($-COOCH_2CH_2O-$) and 3.85 – 3.66 ppm ($-COOCH_2CH_2O-$) shifted to 4.46 – 4.25 ppm and 4.25 – 4.02 ppm, respectively. Further modification to the azide groups resulted in shifting of the three new peaks to 3.45 – 3.34 ppm ($-OOCCH_2CH_2CH_2N_3$), 2.52 – 2.42 ppm ($-OOCCH_2CH_2CH_2N_3$), and 1.99 – 1.88 ppm ($-OOCCH_2CH_2CH_2N_3$). A set of three peaks next to each of the new peaks, located at 3.65, 2.57, and 2.12 ppm, was believed to be from $-OOCCH_2CH_2CH_2Cl$, which was formed by the reaction of deprotected PHEMA with the impurity of $ClCH_2CH_2CH_2COCl$ in the reactant $BrCH_2CH_2CH_2COCl$ and did not participate in the azide substitution reaction. This accounted for 5.8% of repeat units from the ^1H NMR analysis. The peak at 3.52 ppm was believed to be from the unreacted bromide moieties, which accounted for 4.0 % of repeat units. Lastly, the peak at 3.82 was believed to be from the unreacted PHEMA units (1.5 %). The small amount of the remaining TMS groups at 0.06 ppm corresponded to 0.4 % of monomer units. Taking into consideration the repeat units that do not bear an azide functionality, the degree of azide functionalization of the

backbone polymer, PHEMA-N₃, was calculated to be 88.3%.

3.2.7. Synthesis of Alkyne End-Functionalized Side Chain Polymer PDEGEA

PBiB (54.8 mg, 0.267 mmol, delivered via 0.454 g of a 120.8 mg/g stock solution in anisole), DEGEA (5.028 g, 26.71 mmol), PMDETA (47.8 mg, 0.276 mmol), CuCl (26.4 mg, 0.267 mmol), and anisole (5.202 g) were added to a 50 mL two-necked flask equipped with a magnetic stir bar, a rubber septum, and a gas flow control adapter. The mixture was degassed by three freeze-pump-thaw cycles and immersed in an 80 °C oil bath. The polymerization was stopped after 17 h by opening the flask to air. After a sample was taken for ¹H NMR spectroscopy analysis, the reaction mixture was diluted immediately with dichloromethane. The copper catalyst was removed by passing the mixture through a column of basic alumina (top)/silica gel (bottom). The polymer solution was concentrated to about 10 mL by rotary evaporation and precipitated into hexanes (100 mL) three times. The purified polymer was dried under high vacuum for 2 h, yielding a slightly yellow, viscous liquid (2.685 g). The monomer conversion was 57.4%, calculated from the ¹H NMR spectrum of the final reaction mixture by comparing the monomer ester peak at 4.36-4.31 ppm (-COOCH₂-) to the polymer ester peak at 4.28-4.16 ppm (-COOCH₂-). From the monomer conversion and the monomer-to-initiator molar ratio, the DP of the polymer was determined to be 57. SEC analysis results relative to polystyrene standards using mixed-C columns and THF as eluent: M_{n,SEC} = 10.3 kDa and Đ = 1.17 (Figure B7A).

3.2.8. Synthesis of Alkyne End-Functionalized Side Chain Polymer PDEAEMA

PBiB (55.7 mg, 0.272 mmol, delivered via 0.255 g of 218.4 mg/g stock solution in anisole), DEAEMA (6.007 g, 32.42 mmol), 1,1,4,7,10,10-hexamethyltriethylenetetramine (72.8 mg, 0.316 mmol), CuCl (27.0 mg, 0.273 mmol) and anisole (9.006 g) were added to a 50 mL two-necked round bottom flask and degassed by three freeze-pump-thaw cycles. The polymerization was

started by immersing the flask in an oil bath at 50 °C. After 5.5 h, the polymerization was stopped by removing the flask from the oil bath and opening it to air, and an aliquot was withdrawn for ^1H NMR spectroscopy analysis. The reaction mixture was diluted with THF and passed through a column of basic alumina (top)/silica gel (bottom) to remove the copper catalyst. The polymer was purified by precipitation of its THF solution in hexanes three times in a dry ice/acetone bath (-78 °C) and then dried under high vacuum. A white, glassy polymer was obtained (2.850 g, 47.4%). The monomer conversion was 50.3%, determined by ^1H NMR analysis of the final polymerization mixture by comparing the integrals of the peaks at 4.32-4.17 ppm (-COOCH₂- of monomer) and at 4.17-3.98 ppm (-COOCH₂- of the polymer). The degree of polymerization of PDEAEMA was calculated to be 60. SEC analysis relative to polystyrene standards using mixed-C columns and THF as eluent: $M_{n,SEC} = 12.4$ kDa and $\bar{D} = 1.14$ (Figure B7B).

3.2.9. Synthesis of 3-Arm Star Binary Heterografted PDEGEA/PDEAEMA Molecular Brushes and Calculation of Overall Grafting Density of SBB

PHEMA-N₃ (10.54 mg, 4.37×10^{-2} mmol -N₃ assuming 100% functionalization, delivered via 0.2850 g of a 36.98 mg/g stock solution in THF), alkyne end-functionalized PDEGEA (286.8 mg, 2.63×10^{-2} mmol, delivered via 1.986 g of a 144.4 mg/g stock solution in THF), alkyne end-functionalized PDEAEMA (295.0 mg, 2.62×10^{-2} mmol, delivered via 3.105 g of a 95.0 mg/g stock solution in THF), CuCl (4.3 mg, 4.3×10^{-2} mmol), and THF (5 mL) were added to a vial equipped with a magnetic stir bar. The vial was sealed with a rubber septum and purged with nitrogen via needles for 15 min. PMDETA (10 μL , 4.83×10^{-2} mmol) was then injected. Purging of the vial with nitrogen continued for an additional 15 min, and the reaction was conducted under a slightly positive pressure of nitrogen. After 21 h, propargyl benzyl ether (25 μL) was injected to attempt to cap possible unreacted azide groups. After an additional 2 h, the vial was opened to the

air, and a final sample was taken for SEC analysis to determine the grafting density. The reaction mixture was diluted with THF and then passed through a basic alumina (top)/silica gel (bottom) column to remove the copper catalyst. The solution was concentrated to ~ 3 mL via rotary evaporation and then fractionated at -12 °C (in a freezer) with 10 mL of hexanes. The fractionation ceased removing excess side chain polymers after three attempts. The remaining excess side chain polymers were removed by 4 rounds of centrifugal filtration using 50 kDa MWCO filters and a mixture of ethanol and water (50/50, v/v) at 5000 rpm (Eppendorf 5804 Centrifuge). The complete removal of excess side chain polymers was confirmed by SEC analysis using PSS GRAL columns with DMF (containing 50 mM LiBr) as the mobile phase (Figure B8). The absolute weight-average molecular weight (M_w) was determined by a GPC-MALS system equipped with one PSS GRAM 10 μ m guard column, three PSS GRAM 10 μ m linear columns, and a Varian 390 LC-detector system consisting of an RI detector and a multi-angle light scattering detector (15° and 90°), and it was 1.49×10^7 Da.

The overall grafting density of the 3-arm star brushes was 84.7 %, calculated from the peak areas of the brushes and the side chains along with the feed ratio of the backbone azide groups and the alkyne end-functionalized side chain polymers as well as the consideration of the molar ratio of two side chain polymers in the purified star brushes. The details are presented in the following. ^1H NMR spectroscopy analysis showed that the brushes were composed of 48.9 mol % PDEGEA monomer units and 51.1 mol % PDEAEMA monomer units using the integrals of the peaks at 4.33-4.11 ppm ($-\text{COOCH}_2-$ of PDEGEA) and 4.11-3.91 ppm ($-\text{COOCH}_2-$ of PDEAEMA). From the DP of each side chain polymer ($\text{DP}_{\text{PDEGEA}} = 57$, $\text{DP}_{\text{PDEAEMA}} = 60$), the relative brush composition is calculated to be 50.2 mol % PDEGEA and 49.8 mol % PDEAEMA side chains. Using the molar mass of PDEGEA (10889 g/mol) and PDEAEMA (11277 g/mol), the relative

mass percent of each side chain was found to be 49.3 % PDEGEA and 50.7 % PDEAEMA. The peak areas for the star brushes and the side chain polymers by SEC analysis were 71.0 % and 29.0 %, respectively (Figure B8). Multiplying the area percentage for the brushes by the total feed mass of the backbone polymer PHEMA-N₃ and two side chain polymers yielded 420.32 mg of the brushes. By subtracting the amount of backbone polymer, we found that 409.78 mg of side chain polymers had reacted. Using the mass percentages of two side chain polymers calculated earlier, we found the mass of the reacted PDEGEA (202.0 mg) and PDEAEMA (207.8 mg). Using the molar mass of each side chain polymer, the number of moles of the reacted side chain polymers was 3.70×10^{-5} mol. Therefore, the overall grafting density was calculated to be 84.7 % by dividing the total moles of side chains that reacted by the moles of repeat units in the backbone polymer (assuming 100% azide functionalization).

3.3. Results and Discussion

3.3.1. Synthesis of Dually Thermo- and pH-Responsive, Binary Heterografted 3-Arm Star Molecular Brushes (SBB)

SBB was prepared using a highly efficient CuCl-catalyzed click reaction to simultaneously graft alkyne end-functionalized thermosensitive PDEGEA and pH-responsive PDEAEMA onto an azide-bearing 3-arm star backbone polymer, PHEMA-N₃ (Scheme 3.2). PHEMA-N₃ was prepared by ATRP of HEMA-TMS from a trifunctional initiator, TPEG-Br, and a series of post-polymerization reactions to install the azide groups.⁶³ We initially attempted to use a simpler trifunctional initiator made from 1,1,1-tris(4-hydroxyphenyl)ethane (TP-OH in Scheme 3.2) and 2-bromoisobutyryl bromide to prepare the backbone polymer.⁵¹ Unfortunately, we found that the star polymer was not stable during the post-polymerization modifications, likely because of the weak phenol ester groups in the core resulting in the cleavage of arms. Therefore, an ethylene

glycol spacer was inserted between the core and each ATRP initiating group.⁶³

The ATRP of HEMA-TMS from trifunctional initiator TPEG-Br was conducted in anisole at 75 °C using CuCl/4,4'-dinonyl-2,2'-dipyridyl as the catalyst system and monitored by ¹H NMR analysis.⁶³ When the monomer conversion reached a desired value, the polymerization was stopped and the unreacted monomer was removed under vacuum. Size exclusion chromatography (SEC) analysis revealed a unimodal peak with a $M_{n,SEC}$ of 179.8 kDa and a dispersity of 1.13 (Figure B5), indicating that the polymerization was controlled. Based on the final monomer conversion (50.1%) from ¹H NMR analysis and the monomer-to-initiator ratio (2600 : 1), the average DP per arm was calculated to be 434. The PHEMA-TMS star polymer was then converted to the azide-functionalized backbone polymer through three steps of functional group transformations, including the removal of silyl ether protective groups, esterification with 4-bromobutyryl chloride, and installation of azide moieties via substitution of pendant bromide with NaN₃ (Scheme 3.2).⁶³ The reactions were monitored by ¹H NMR spectroscopy and SEC analysis. While in general the pendant group transformations were efficient, they were not quantitative. From ¹H NMR spectroscopy analysis of the purified PHEMA-N₃, the degree of azide-functionalization was 88.3% (Figure B6 and the discussion in Appendix B). SEC analysis showed that the 3-arm star polymer remained unimodal and narrow, with no shoulder appearing in the front or in the tail of the main peak after the esterification with 4-bromobutyryl chloride and the azidization (Figure B5), indicating the absence of coupling between star molecules in the post-polymerization modifications.

Alkyne end-functionalized side chain polymers, thermosensitive PDEGEA and pH-responsive PDEAEMA, were synthesized by ATRP using propargyl 2-bromoisobutyrate as the initiator under similar conditions except the polymerization temperature and the catalyst/ligand system (80 °C

with $\text{CuCl}/N,N,N',N'',N''\text{-pentamethyldiethylenetriamine}$ for PDEGEA and $50\text{ }^{\circ}\text{C}$ with $\text{CuCl}/1,1,4,7,10,10\text{-hexamethyltriethylenetetramine}$ for PDEAEMA).⁶³ Similar to the synthesis of PHEMA-TMS from TPEG-Br, the polymerizations were followed by ^1H NMR analysis and stopped when the monomer conversions reached a desired value (50 – 60%). SEC analysis showed a single, narrow peak for both side chain polymers; for PDEGEA, $M_{n,\text{SEC}} = 10.3\text{ kDa}$ and the dispersity ($\mathcal{D}$) = 1.17 (Figure B7A), and for PDEAEMA, $M_{n,\text{SEC}} = 12.4\text{ kDa}$ and $\mathcal{D} = 1.14$ (Figure B7B), relative to polystyrene standards. These indicated that both polymerizations were well controlled. From the monomer-to-initiator ratios and the monomer conversions determined from the ^1H NMR spectra of the final reaction mixtures, the DPs of PDEGEA and PDEAEMA were calculated to be 57 and 60, respectively. The alkyne end-functionalized polymers were purified by repeated precipitations from THF into hexanes (for PDEAEMA, hexanes was chilled in a dry ice/acetone bath), dried under high vacuum, and then stored in THF stock solutions.

SBB was then constructed by “clicking” alkyne end-functionalized PDEGEA and PDEAEMA simultaneously onto the azide-bearing star backbone polymer, PHEMA- N_3 , using the CuAAC reaction. This “grafting to” reaction was carried out at room temperature in THF under a nitrogen atmosphere using $\text{CuCl}/N,N,N',N'',N''\text{-pentamethyldiethylenetriamine}$ as the catalyst. The feed molar ratios of PHEMA- N_3 repeat units to PDEAEMA and PDEGEA were 1 : 0.6 : 0.6, respectively; slightly excess side chains were used to achieve a high overall grafting density. After 21 h, propargyl benzyl ether was injected to cap the unreacted azide groups.^{33,34} SEC analysis of the final reaction mixture showed the appearance of a high molecular weight peak, suggesting the formation of molecular brushes. The 3-arm star brushes were purified by repetitive fractionation and centrifugal filtration; the complete removal of unreacted side chain polymers was confirmed by SEC (Figure B8). ^1H NMR spectroscopy analysis showed that the purified brushes comprised

50.2 mol % PDEGEA side chains and 49.8 % PDEAEMA side chains, which were determined by using the integrals of the peaks at 4.33-4.11 ppm (-COOCH₂- of PDEGEA) and 4.11-3.91 ppm (-COOCH₂- of PDEAEMA) (Figure 3.1A) and the DPs of the two polymers.

The overall grafting density of SBB was found to be 84.7%, calculated by using the relative SEC peak areas of the brushes (71.0 %) and the unreacted side chains (29.0 %) in the final reaction mixture, the feed molar ratios, and the brush composition from ¹H NMR analysis (i.e., the molar ratio of two side chain polymers in the purified star brushes).⁶³ The absolute weight-average molecular weight (M_w) of SBB was found to be 1.49×10^7 Da, determined by SEC analysis using a GPC-MALS system equipped with a two-angle light scattering detector in series with a RI detector (Figure 3.1B). Considering the weight-average molecular weight of PHEMA-N₃, the average DP of each arm in the star brushes, the M_w of each side chain polymer from the DP and the dispersity (M_n × Đ), and the grafting density, the M_w value of the star brushes was calculated to be 1.45×10^7 Da, which was very close to the experimental value.

The 3-arm star structure of SBB was verified by AFM imaging of the brushes spin cast onto freshly cleaved mica from THF (Figure 3.2A and 3.2B). A vast majority of brush molecules had a 3-arm star architecture, although there were some molecules that either lacked one arm or were unusually short. Using the ImageJ software, the average length per arm after subtraction of a half of the width of the arms was 92.2 ± 34.9 nm, and the typical height of the brushes on bare mica was ~1 nm (Figure 3.2C). Considering the contour length of one arm with a DP of 434 in an all-trans zig-zag conformation, the average length per arm measured from AFM images represents a degree of stretching of 83.6%.

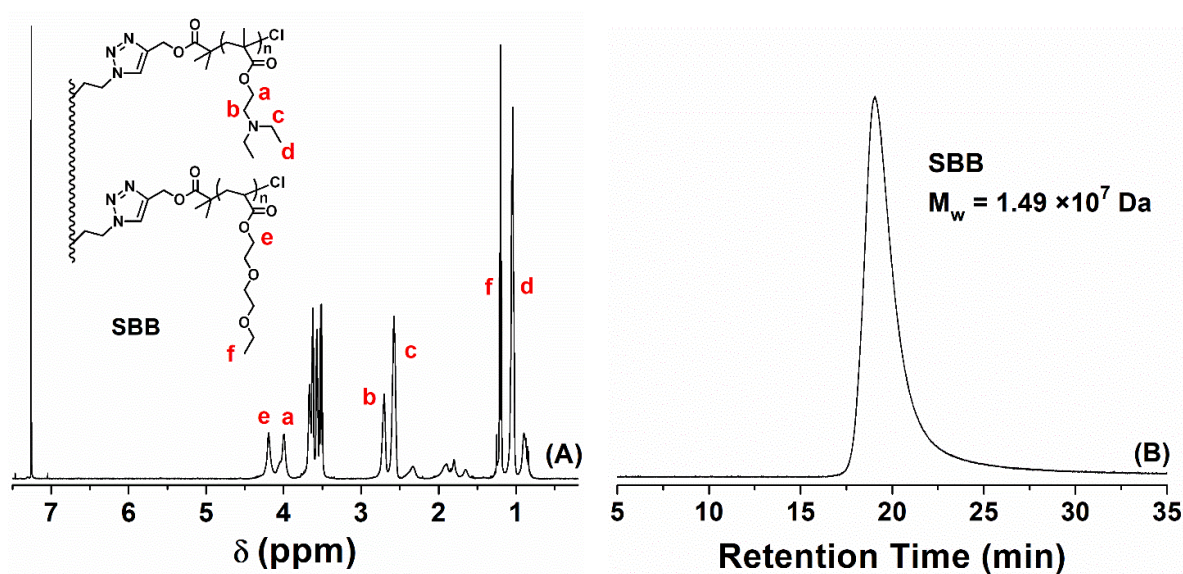


Figure 3.1. (A) ^1H NMR spectrum of the purified 3-arm star molecular brushes, SBB, in CDCl_3 and (B) SEC trace of SBB from a GPC-MALS system equipped with a dual-angle light scattering detector and a RI detector.

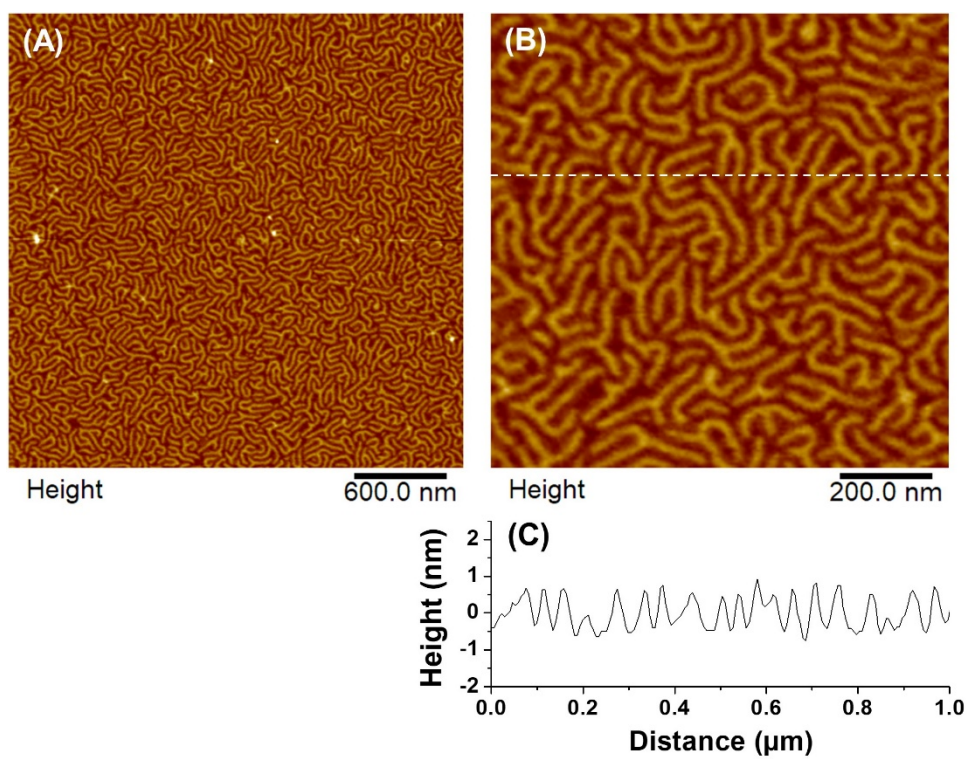


Figure 3.2. (A) and (B) AFM height images of SBB brush molecules spin cast from 0.05 mg/g solution in THF onto freshly cleaved bare mica. (C) The cross-sectional height profile along the dashed line in (B).

3.3.2. Responsive Behavior and Stabilization Capabilities of SBB in Aqueous Solution by Visual Inspection

The responsive properties and the stabilization capabilities of SBB in aqueous solution upon application of external stimuli were first visually examined. Aqueous solutions of 1.0 mg/g SBB in a 10 mM phosphate buffer with a pH of 5.00 were prepared in an ice/water bath. Under these conditions ($\text{pH} \ll \text{p}K_a = 7.4$ of PDEAEMA and $T < \text{LCST} = 9\text{ }^\circ\text{C}$ of PDEGEA), the PDEAEMA side chains were nearly fully protonated and the PDEGEA side chains were hydrated in the buffer; as expected the sample was an optically clear solution (Figure 3.3A). Upon warming to room temperature ($22\text{ }^\circ\text{C}$, above the LCST of PDEGEA), the brush solution remained clear (Figure 3.3B), demonstrating the stabilization of the collapsed PDEGEA side chains in SBB by the charged PDEAEMA because we previously observed that a 1.0 mg/g aqueous solution of homografted PDEGEA linear molecular brushes turned cloudy upon heating from $0\text{ }^\circ\text{C}$ to room temperature.³³ Similarly, when the PDEAEMA side chains became insoluble at $\text{pH} = 9.50$, the brush molecules were stabilized by the hydrated PDEGEA at $0\text{ }^\circ\text{C}$, and the solution stayed clear (Figure 3.3C). This stands in stark contrast to the behavior of homografted PDEAEMA linear MBBs in aqueous solutions with pH values $> \text{p}K_a$, where the brush molecules aggregated and precipitated out of the solution immediately even at a concentration of 0.2 mg/g.³⁴ Warming the 1.0 mg/g SBB solution with a pH of 9.50 from $0\text{ }^\circ\text{C}$ to room temperature resulted in the solution turning cloudy (Figure 3.3D), and a precipitate was formed. This is undoubtedly caused by the LCST transition of PDEGEA side chains in the stabilizing corona layer.

3.3.3. pH-Induced Star-Globule Shape Transitions of SBB

The thermo- and pH-responsive behavior of SBB in aqueous phosphate buffers was investigated by DLS, ^1H NMR spectroscopy, and AFM. Figure 3.4A shows the D_h of 1.0 mg/g

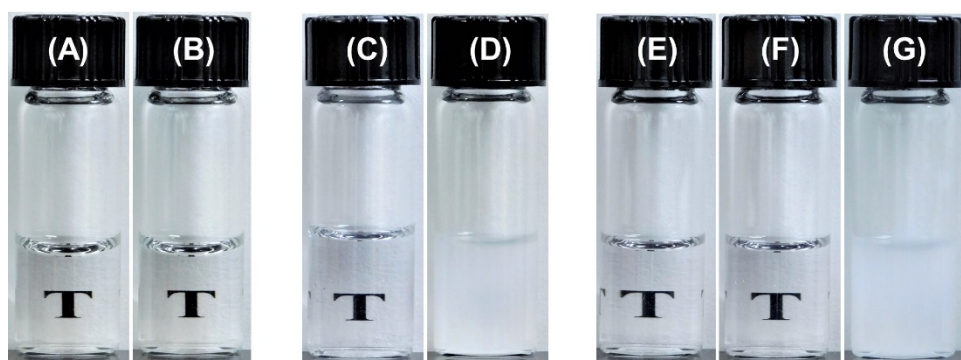


Figure 3.3. Optical photos of 1.0 mg/g solutions of 3-arm star binary heterografted PDEGEA/PDEAEMA molecular brushes, SBB, in a 10 mM phosphate buffer at (A) pH = 5.00 and T = 0 °C, (B) pH = 5.00 and T = 22 °C, (C) pH= 9.50 and T = 0 °C, (D) pH= 9.50 and T = 22 °C, (E) pH = 7.43 and T = 0 °C, (F) pH= 7.43 and T = 22 °C, (G) pH= 7.43 and T = 35 °C.

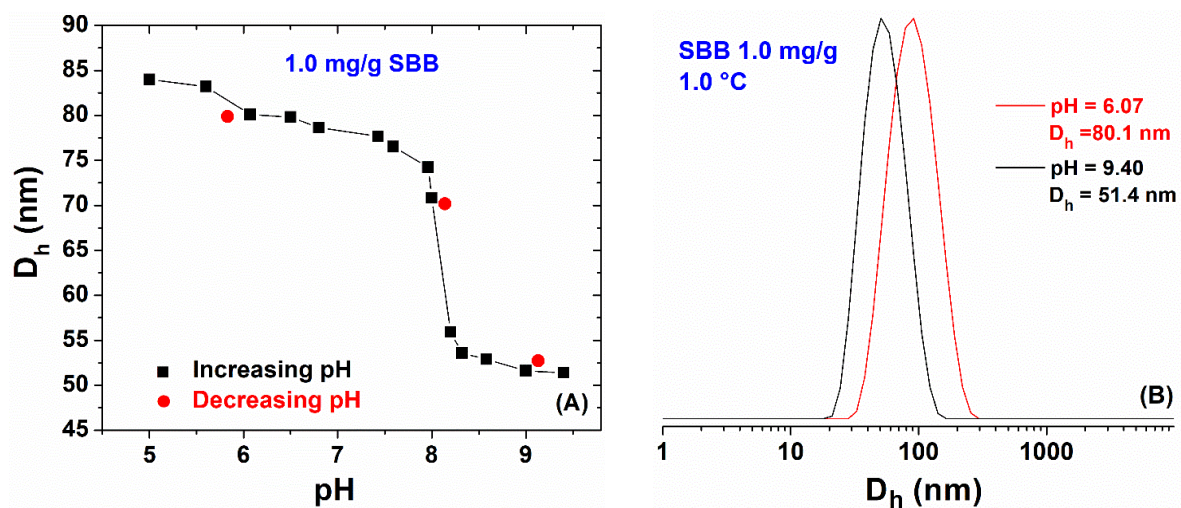


Figure 3.4. (A) Apparent hydrodynamic diameter (D_h) of the star molecular brushes, SBB, in a 10 mM phosphate buffer with a brush polymer concentration of 1.0 mg/g at 1 °C as a function of pH in the process of increasing pH (black squares) and decreasing pH (red circles) obtained from a DLS study. (B) Hydrodynamic size distributions of SBB in 10 mM phosphate buffers with a polymer concentration of 1.0 mg/g at 1 °C and pH values of 6.07 and 9.40.

SBB in 10 mM aqueous phosphate buffers at 1 °C as a function of pH, obtained from a DLS study. The pH was gradually changed in an ice/water bath using 0.1 M NaOH and 0.1 M HCl and measured by a pH meter that had been calibrated at the same temperature. When the pH was increased from 5.00 to 7.96, the D_h decreased slightly and gradually from 84.0 to 74.2 nm. A sharp reduction in size from 74.2 to 53.6 nm was observed upon further increasing the pH by only 0.36 pH units (i.e., from pH 7.96 to 8.32). Beyond pH 8.32, the size changes were small and the D_h leveled off, reaching 51.4 nm at pH = 9.40. The absence of aggregates at higher pH values indicated that the collapsed brush molecules were stabilized in solution by the PDEGEA side chains at 1 °C, corroborating the visual inspection (Figure 3.3A and 3.3C). Thus, the apparent hydrodynamic size decreased by 32.6 nm when the pH was raised from 5.00 to 9.40, indicating the pH-induced collapse of SBB from an extended to a more compact conformation. We stress here that a single size distribution with a relatively small polydispersity index was observed at each pH in Figure 3.4A; Figure 3.4B shows the hydrodynamic size distributions of SBB at pH = 6.07 and 9.40 as examples. In addition, the hydrodynamic sizes and size distributions of SBB at concentrations of 1.0 mg/g and 0.2 mg/g were virtually identical at pH = 6.07 (Figure B9A), suggesting that the brush molecules were fully dissolved in the buffer. Similarly, the curves overlapped for the two solutions at pH = 9.40 (Figure B9B), a sign of a unimolecular collapse of SBB at this pH value. The pH-induced size changes of SBB were reversible, as can be seen from the data points (red solid circles) collected from the process of decreasing pH shown in Figure 3.4A.

We previously observed that the pH-induced collapse of homografted linear MBBs composed of PDEAEMA-*b*-poly(methoxytri(ethylene glycol) acrylate) (PDEAEMA-*b*-PTEGMA) side chains, where PDEAEMA was the inner block, occurred at pH = ~ 7.4 ,³⁴ which coincided with the

pK_a of PDEAEMA.^{59,60} Unexpectedly, the sharp size decrease for SBB occurred at $pH \sim 8.1$ (Figure 3.4A). We believe that the following two factors contributed to the delayed size transition.

(i) The pH values reported here were measured in an ice/water bath ($0\text{ }^{\circ}\text{C}$), while the pK_a values are usually determined at $25\text{ }^{\circ}\text{C}$. Our previous work on the pH -induced shape changing of PDEAEMA-*b*-PTEGMA core-shell linear brushes was carried out at ambient conditions.³⁴ In our experiment, we observed that when a 1.0 mg/g solution of SBB in a 10 mM phosphate buffer with a pH of 8.00 was warmed to room temperature ($22\text{ }^{\circ}\text{C}$), the solution displayed a clear-to-cloudy transition and the pH decreased to 7.75 . Note that the pH meter was calibrated at $0\text{ }^{\circ}\text{C}$ using $pH = 4.01, 7.00,$ and 10.01 standard buffer solutions, whose pH values at $0\text{ }^{\circ}\text{C}$ were $4.00, 7.11$ and 10.32 , slightly different from the values at $25\text{ }^{\circ}\text{C}$. (ii) The transition shown in Figure 3.4A is the apparent hydrodynamic size transition of SBB with increasing pH , which is different from the solubility change of PDEAEMA side chains. We believe that the fully hydrated PDEGEA side chains at $1\text{ }^{\circ}\text{C}$ stabilized the brushes in an extended state for a small pH range above the pK_a of PDEAEMA until more monomer units were deprotonated and the PDEAEMA side chains became sufficiently hydrophobic to force the collapse of brush molecules. Consequently, the size transition was delayed to a slightly higher pH value.

^1H NMR spectroscopy was employed to confirm that the drastic size decrease of SBB with increasing pH from 5.00 to 9.40 at $1\text{ }^{\circ}\text{C}$ was driven by the pH -triggered transition of PDEAEMA side chains from a water-soluble to a water-insoluble state. A 4.0 mg/g solution of SBB in a 10 mM phosphate buffer of D_2O was prepared in an ice/water bath and the pH was adjusted to 5.00 and 9.50 using 0.1 M NaOH and 0.1 M HCl solutions also prepared in D_2O . For comparison, a solution of the alkyne end-functionalized PDEAEMA homopolymer in the same buffer of D_2O with a pH of 5.00 was also prepared at $0\text{ }^{\circ}\text{C}$. As can be seen in Figure 3.5, the peaks associated

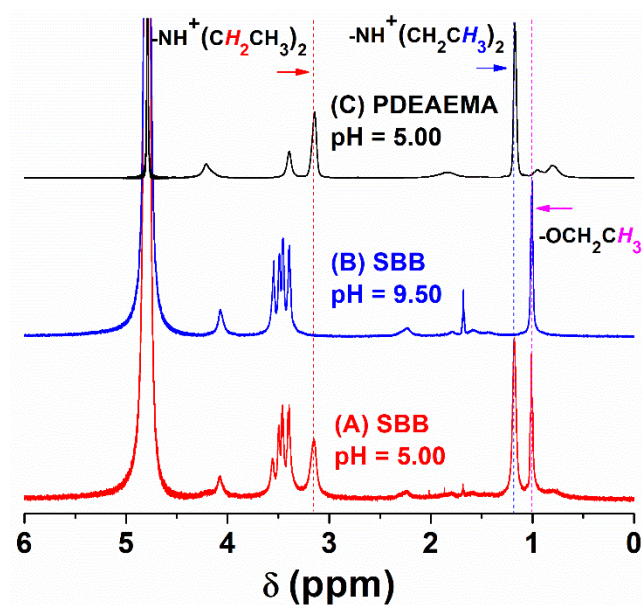


Figure 3.5. ^1H NMR spectra collected at 4 °C for SBB in a 10 mM phosphate buffer of D_2O with a concentration of 4.0 mg/g at (A) pH = 5.00 and (B) pH = 9.50 and for an alkyne end-functionalized PDEAEMA homopolymer solution with a concentration of 4.0 mg/g in a 10 mM phosphate buffer of D_2O at pH = 5.00 (C).

with the PDEAEMA side chains at 1.17 ppm ($-\text{NH}^+(\text{CH}_2\text{CH}_3)_2$) and 3.15 ppm ($-\text{NH}^+(\text{CH}_2\text{CH}_3)_2$) as well as those from PDEGEA at 1.01 ppm ($-\text{OCH}_2\text{CH}_3$) and 3.60-3.30 ppm ($-\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_3$) were clearly visible in the brush solution at the pH of 5.00 and 4 °C. Upon increasing the pH to 9.50, the PDEAEMA peaks completely disappeared, and those associated with PDEGEA were the only peaks observed. At the low pH and low temperature, PDEAEMA side chains were protonated and the PDEGEA were highly hydrated, making them fully soluble in the buffer and visible in the NMR spectrum. When the pH was increased to 9.50, well above the pK_a , the PDEAEMA was deprotonated and became insoluble in water, resulting in the disappearance of the peaks. Meanwhile, the signals from PDEGEA remained sharp, indicating that these side chains were still hydrophilic and mobile, providing a stability to the collapsed brushes in solution.

AFM was then employed to directly visualize the SBB brush molecules. Figure 3.6A shows the AFM height images of SBB spin cast at 0 °C from a phosphate buffer with a brush concentration of 0.050 mg/g at pH = 6.60.⁶³ The brush molecules were of a 3-arm star shape, consistent with the expected more extended structure from the DLS study (Figure 3.4A). However, unlike the brush molecules spin cast on mica from a THF solution (Figure 3.2), where the arms were wormlike and smooth with nearly equal widths along the arms, the brushes from the pH 6.60 buffer exhibited a very peculiar morphology with each arm taking on a pearl-necklace structure composed of a certain number of beads with a representative size of ~ 30 nm and a height of ~ 5 nm (Figure 3.6). The height was undulated along the backbone, with variations of 2-3 nm, as can be seen from the cross-sectional profile (Figure 3.6C). A similar star morphology with a pearl-necklace nanostructure for the arms was observed for the brushes spin cast from a pH = 7.43 solution at 0 °C on PMMA-coated mica (Figure B11). Note that when the pH 7.43 solution was

warmed to room temperature (22 °C), the pH value became 7.25, slightly below the pK_a of PDEAEMA, and the solution remained optically clear. Similar “pearl-necklace” morphologies were observed by Müller et. al. in linear molecular bottlebrushes composed of diblock copolymer side chains with poly(acrylic acid) as the inner block and poly(*n*-butyl acrylate) as the outer block when multivalent metal ions, such as Fe^{3+} and Cd^{2+} , were complexed with the monovalent carboxylate ions in the core.^{64,65} However, our previous study showed that the linear MBBs homografted with PDEAEMA-*b*-PTEGMA side chains were rodlike when spin cast from a pH 5.00 phosphate buffer solution, with a uniform width and no height undulation along the brush backbone.³⁴ Thus, the pearl-necklace structure observed here was likely caused by the electrostatic interactions of charged PDEAEMA side chains with multivalent phosphate anions, resulting in local microphase separation in the binary heterografted brush arms. Further study is needed to provide insight into this morphology and elucidate its formation mechanism.

Figure 3.7A and B shows the AFM images of SBB spin cast from a pH = 9.50 buffer solution on PMMA-coated mica.⁶³ Almost all of the brush molecules were in a compact globular state as expected; however, one molecule was seen in the star-shaped morphology (Figure 3.7A), which was likely due to the shear-induced unraveling of the globular molecule during the preparation of the AFM sample by spin casting. It is also interesting to note here that the arms of this brush molecule in the star shape were not undulated as those from the pH 6.60 and pH 7.43 aqueous buffers (Figure 3.6 and Figure B11). Presumably, this is because the PDEAEMA side chains were deprotonated, resulting in the exclusion of multivalent phosphate anions from the brush molecules. Figure 3.7B shows an enlarged $1 \times 1 \mu m$ image. For some globular brushes, the collapsed three arms formed three tightly associated lobes, as indicated by arrows. The size and height of a typical globule were ~ 80 and ~ 5 nm, respectively (Figure 3.7C).

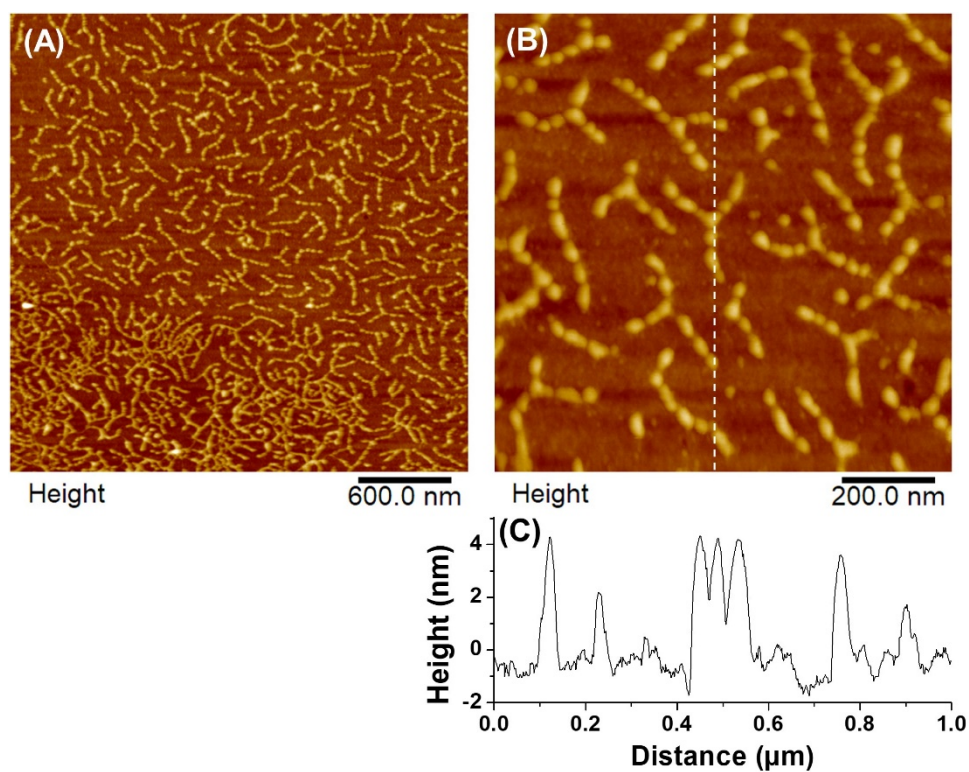


Figure 3.6. (A) and (B) AFM height images of SBB spin cast onto PMMA-coated mica from a 0.05 mg/g solution in a 5 mM phosphate buffer with a pH of 6.60 at 0 °C. (C) The cross-sectional height profile along the dashed line in (B) from top to bottom.

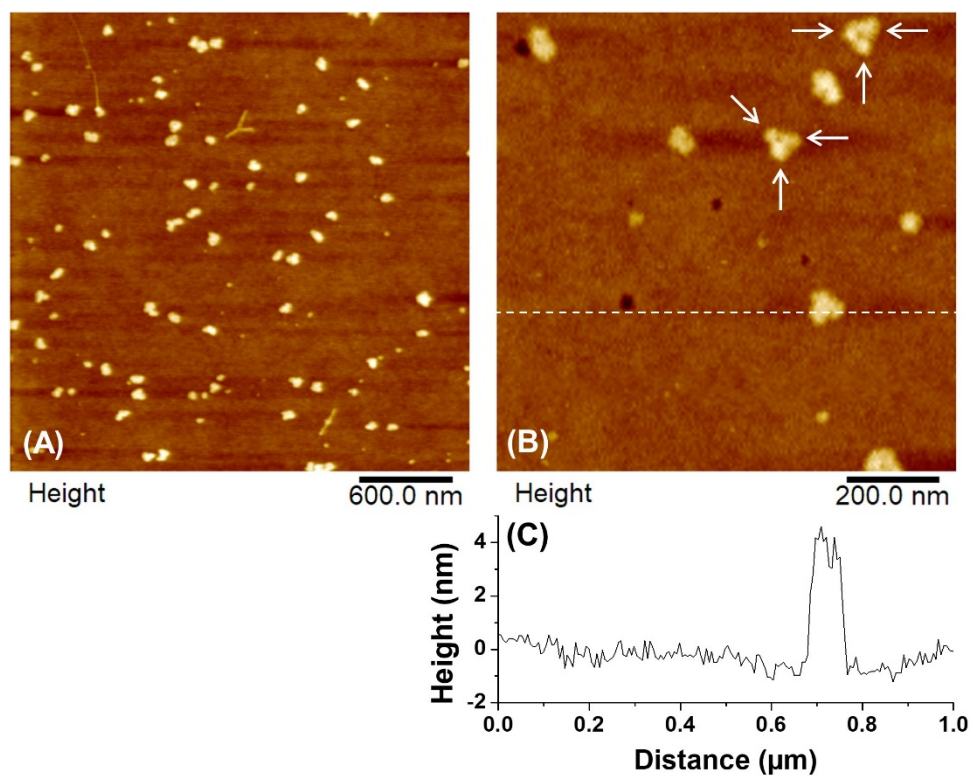


Figure 3.7. (A) and (B) AFM height images of SBB spin cast onto PMMA-coated mica at 0 °C from a 0.05 mg/g solution in a 5 mM phosphate buffer with a pH of 9.50. (C) The cross-sectional height profile along the dashed line in (B).

3.3.4. Thermally Induced Star-Globule Shape Transitions of SBB in Aqueous Solution

Figure 3.3B shows that a 1.0 mg solution of SBB in a 10 mM phosphate buffer with a pH of 5.00 remained clear when it was warmed from 0 to 22 °C. Unexpectedly, at this pH value, the brush molecules exhibited no drastic size reduction upon heating from 1 to 70 °C (Figure 3.8A). Instead, the D_h appeared to increase slightly over the temperature range. We note here that a single size distribution was observed at each measured temperature. In contrast, at pH = 9.13, where the star brushes were in the globular state (Figure 3.4A), a dramatic size jump was observed when the temperature was raised from 7 to 9 °C, indicating that the brushes underwent aggregation as a result of the LCST transition of PDEGEA, consistent with the observation in Figure 3.3D. Thus, even if DLS did not show a drastic size reduction transition at pH = 5.00 upon heating, we believed that the PDEGEA side chains underwent a hydrated-to-dehydrated transition upon increasing temperature across the LCST of PDEGEA. This was supported by a variable temperature ^1H NMR spectroscopy study (Figure 3.9A). With the temperature increasing from 10 to 13 °C, the peaks in the range of 3.3 – 3.7 ppm ($-\text{COOCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_3$ of PDEGEA) became broadened and less resolved, and the intensity of the peak at 1.07 ppm ($-\text{OCH}_2\text{CH}_3$ of PDEGEA), relative to the peak at 1.24 ppm ($-\text{CH}_2\text{CH}_3$ of PDEAEMA), decreased noticeably. This indicated that the PDEGEA side chains of SBB underwent an LCST transition in the temperature range of 10 – 13 °C. Note that the cloud point of the alkyne end-functionalized PDEGEA homopolymer in D_2O at a concentration of 0.5 wt% was found to be 11.5 °C.⁶³ The slightly lower onset temperature for the aggregation observed by DLS (Figure 3.8B) was likely caused by the local higher concentration of PDEGEA side chains.⁶⁴ Therefore, the absence of a drastic size reduction transition of SBB at pH = 5.00 upon increasing temperature was attributed to the strong solvation of nearly fully protonated PDEAEMA side chains in the solution, which prevented the transition of SBB from an

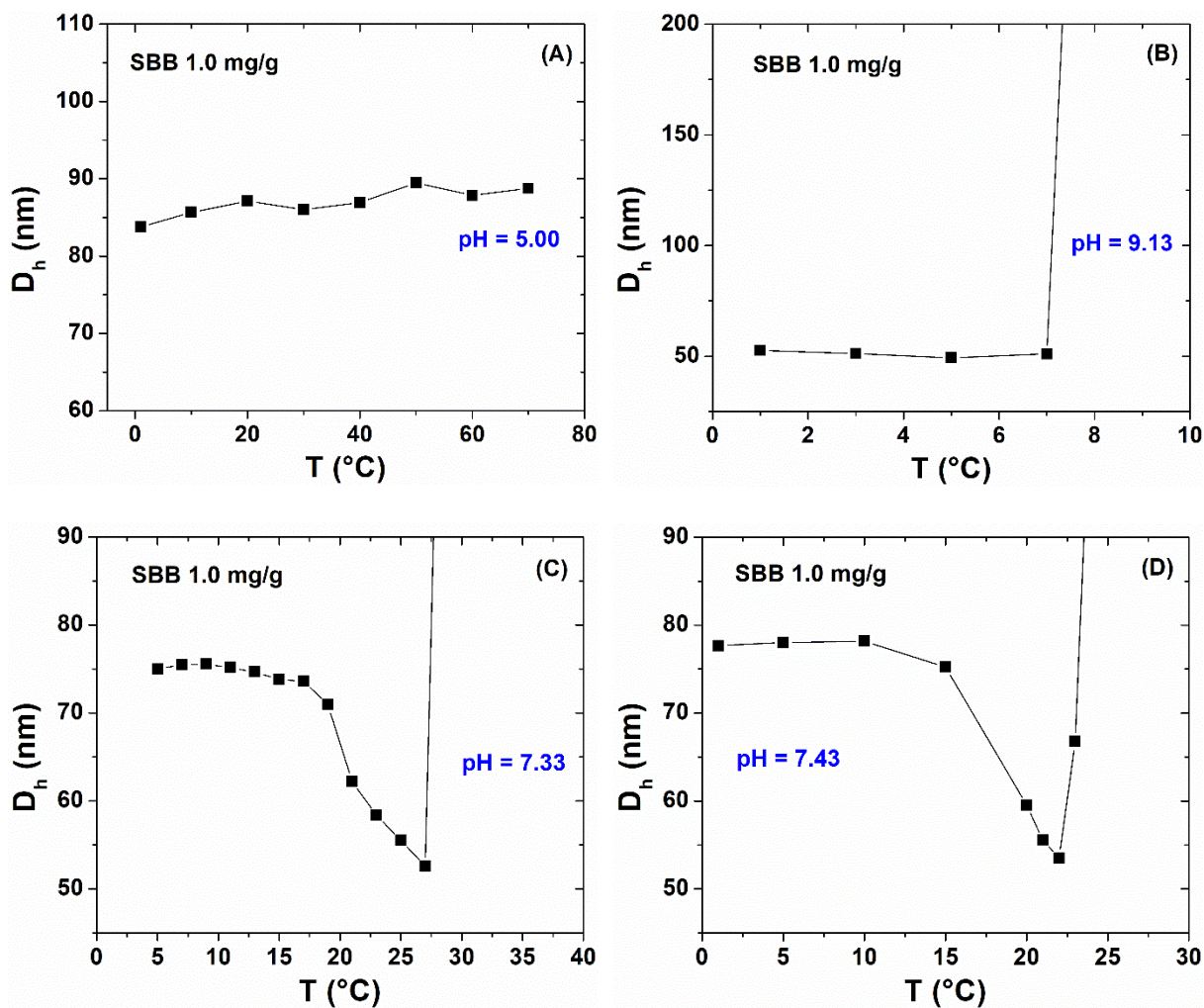


Figure 3.8. Apparent hydrodynamic size (D_h) of SBB in a 10 mM phosphate buffer with a concentration of 1.0 mg/g as a function of temperature in the heating process at (A) pH = 5.00, (B) pH = 9.13, (C) pH = 7.33, and (D) pH = 7.43, obtained from dynamic light scattering studies. The pH values were measured in an ice/water bath using a pH meter.

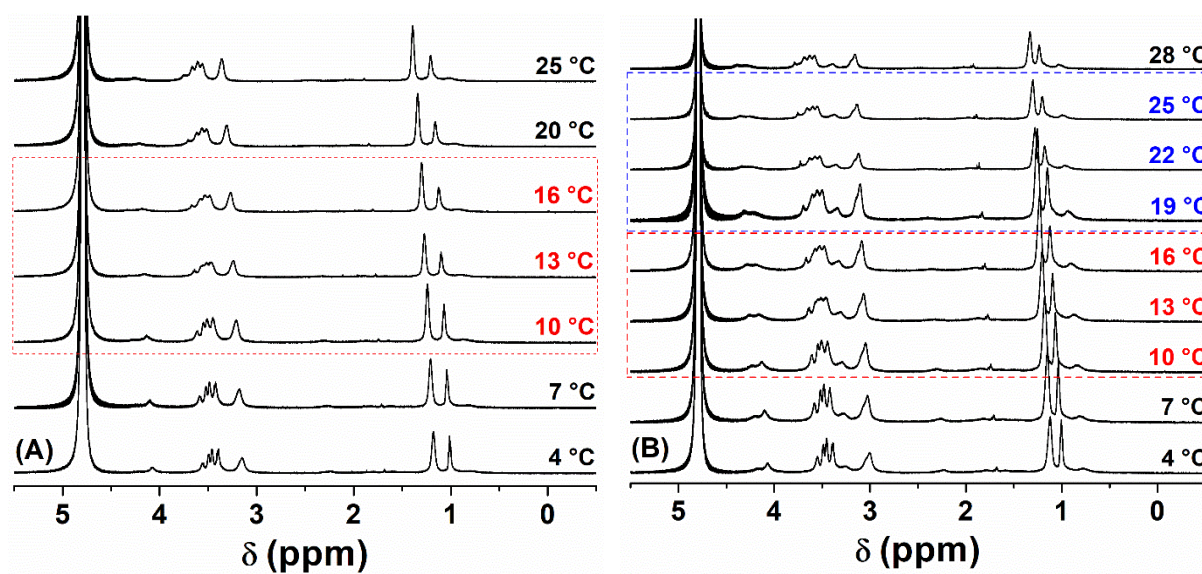


Figure 3.9. ^1H NMR spectra of 4.0 mg/g SBB in a 10 mM phosphate buffer prepared in D_2O with a pH of (A) 5.00 and (B) 7.43 at various temperatures recorded from variable temperature ^1H NMR spectroscopy analysis. All spectra are normalized to the H_2O peak at 4.79 ppm.

extended starlike to a compact globular state. In other words, the LCST transition of PDEGEA side chains was not sufficient to drive the star-globule transition of SBB when the PDEAEMA was strongly charged.

From the above analysis, to achieve star-to-globule shape transitions of SBB by the LCST transition of PDEGEA, the solvation power of charged PDEAEMA side chains must be weakened, which can be accomplished by increasing the buffer pH and thus decreasing the degree of protonation. We increased the pH of the 1.0 mg/g SBB solution to 7.33, and indeed a size reduction transition was observed from DLS when the temperature was above 19 °C (Figure 3.8C); the smallest hydrodynamic size, 52.6 nm, was observed at 27 °C. Increasing the pH at 0 °C to 7.43 decreased the onset temperature for the size reduction transition to 15 °C (Figure 3.8D), with the smallest D_h of 53.5 nm observed at 22 °C. For both pH values, further heating, however, resulted in the aggregation of SBB brush molecules. The clear-to-cloudy transition for the pH 7.43 solution can be seen from Figure 3.3; at 0 and 22 °C, the solution was completely clear (Figure 3.3E and 3.3F) but turned cloudy at 35 °C (Figure 3.3G). The heating-induced aggregation of brush molecules and the clear-to-cloudy transitions were caused by the LCST transition of the partially charged PDEAEMA side chains.³⁴ Our measurements showed that the cloud point of the alkyne end-functionalized PDEAEMA in a 10 mM phosphate buffer at a concentration of 0.5 wt% was 33.1 °C at pH = 7.33 and 30.2 °C at pH 7.43.⁶³ The difference between the onset temperature for aggregation of SBB brush molecules and the cloud point of the alkyne end-functionalized PDEAEMA for both pH values (6.1 °C for pH 7.33 and 8.2 °C for pH 7.43) was likely due to the higher local concentration of PDEAEMA side chains in the brushes.⁶⁶ The LCST transitions of PDEGEA and the partially charged PDEAEMA at pH = 7.43 were confirmed by a variable temperature ¹H NMR spectroscopy study (Figure 3.9B). Again, for PDEGEA, the transition

occurred in the temperature range of 10 – 13 °C. For PDEAEMA, the intensities of the peaks at 1.25 and 3.11 ppm decreased noticeably when the temperature was raised from 19 to 22 °C. These observations were in agreement with the DLS data, although there was a slight difference (1 or 2 °C) in the transition temperature, which was possibly caused by the different local concentrations in the two experiments and the effect of deuterated water on the LCST transition. In summary, the higher the solution pH, the less the charged PDEAEMA units, and the weaker the solvation power of the charged PDEAEMA, making it possible to observe the thermally induced collapse of star brushes driven by the LCST transition of PDEGEA side chains. Meanwhile, the less charged monomer units resulted in a lower cloud point for the partially protonated PDEAEMA.

The thermally induced star-to-globule shape changing at pH = 7.43 was verified by AFM (Figure 3.10A and 3.10B).⁶³ At 0 °C, the SBB brush molecules were in a starlike shape with a pearl-necklace morphology as mentioned earlier (Figure B11). Increasing the temperature to 22 °C transformed the brush molecules to spheres with a typical diameter of 85 nm and a typical height of 7 nm (Figure 3.10C), and the molecules remained stable in the solution because the temperature was still below the LCST of the partially charged PDEAEMA. The spheres observed under these conditions ($T = 22\text{ °C} > \text{LCST of PDEGEA}$, pH = 7.43 at 0 °C or 7.25 at 22 °C, slightly lower than the $pK_a = 7.4$ of the PDEAEMA at room temperature) were larger in the both typical diameter and height than in the pH-induced globule state (~ 80 and ~ 5 nm, Figure 3.7), likely due to the electrostatic interactions between the partially charged PDEAEMA side chains and the counter-anions included in the brushes in the dry state.

3.4. Conclusions

We synthesized binary heterografted three-arm star molecular brushes by a click “grafting-to” method and demonstrated the stimuli-induced star-globule shape transitions with the formation of

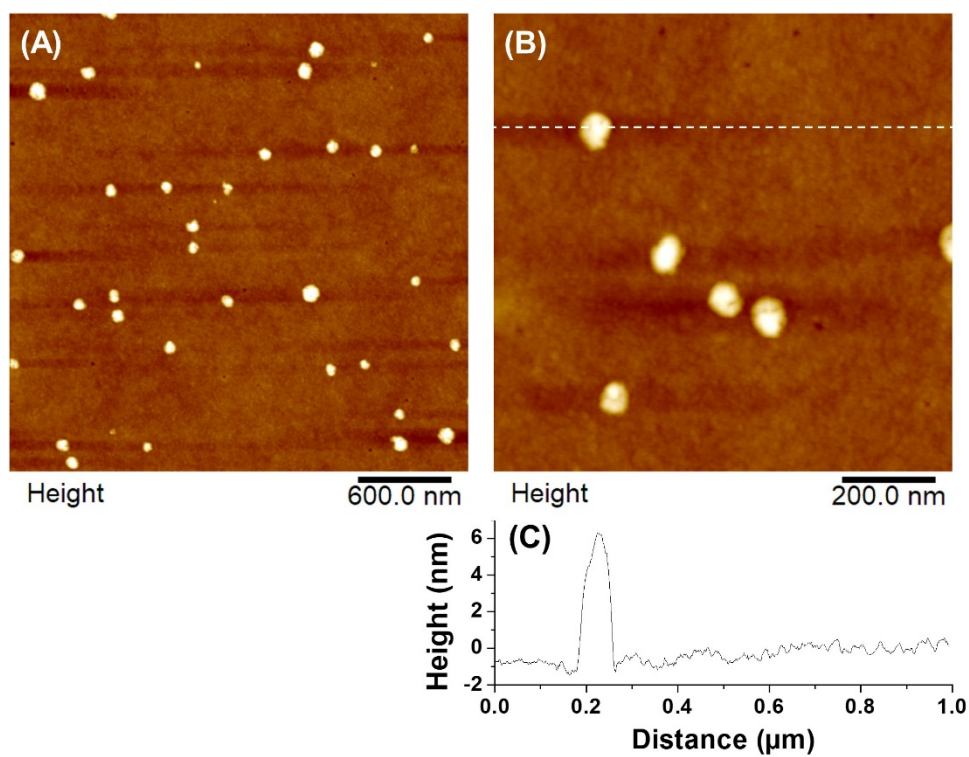


Figure 3.10. (A) and (B) AFM height images of SBB spin cast onto PMMA coated mica at 22 °C from a 0.05 mg/g solution in a 5 mM KH_2PO_4 buffer with pH = 7.43. (C) The cross-sectional height profile along the dashed line in (B).

two distinct collapsed yet stable globular states from one star brush system by employing two different stimuli-responsive polymers as side chains. The responsive behavior of SBB in aqueous buffers was examined by combining visual inspection, DLS, ^1H NMR spectroscopy, and AFM. When the temperature was below the LCST of PDEGEA, SBB exhibited a star-to-stable globule shape transition upon increasing the pH from 5.00 to 9.40. However, the sharp size decrease occurred at $\text{pH} = \sim 8.1$, slightly higher than the pK_a , due to the stabilization of the hydrated PDEGEA side chains. On the other hand, at pH well below the pK_a of PDEAEMA (e.g., $\text{pH} = 5.00$), the highly charged PDEAEMA side chains provided strong enough solvation to keep the brushes in an extended state even at temperatures well above the LCST of PDEGEA. At pH values slightly lower than the pK_a , where the solvation of the charged PDEAEMA was weakened, the thermally-induced shape transition between an extended starlike and a stable spherical state was achieved. The observed shift in the onset pH or temperature for the brush collapse was attributed to the unique structure of binary heterografted molecular bottlebrushes; the respective individual side chains already underwent a soluble-to-insoluble or a LCST transition at $\sim \text{pK}_a$ or LCST, but the resultant driving force was not sufficient to cause the entire brush molecules to collapse to a globular state. This work demonstrated the stabilization of the collapsed star brushes against aggregation when one side chain polymer became insoluble in water and, for the first time, the formation of two distinct globular states with dissimilar polymers in the core from one brush system. The cores in the two globular states have distinct physicochemical characteristics and could be used to encapsulate or load different substances. We believe that the unique behavior of binary heterografted star brushes reported in this article could lead to applications, e.g., in encapsulation and delivery of substances and regulation of molecular interactions

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Appendix B

for

Chapter 3: Stimuli-Induced Star-Globule Shape Transitions of Dually Responsive Binary Heterografted Three-Arm Star Molecular Brushes in Aqueous Solution

B.1. Cloud Points of Alkyne End-Functionalized PDEGEA and PDEAEMA in H₂O and D₂O at a Concentration of 0.5 wt%

The cloud points of alkyne end-functionalized PDEGEA and PDEAEMA in both H₂O and D₂O solutions were determined by visual examination. For PDEGEA, a calculated amount of a stock solution of the polymer in THF with a known concentration was weighed into each of two flasks and dried under high vacuum to remove THF. Milli-Q water or D₂O (Cambridge Isotope Laboratories, Inc.) was added to dissolve the polymer in an ice/water bath to give a concentration of 0.5 wt%. For PDEAEMA, similarly, a calculated amount of the polymer was transferred into each of two flasks from a THF stock solution; the aqueous solutions were prepared at a concentration of 0.5 wt% using a 10 mM phosphate buffer in either Milli-Q water or D₂O. The pH values of the PDEAEMA samples were adjusted in an ice/water bath using 0.1 M NaOH and 0.1 M HCl (prepared in Milli-Q water or D₂O for the respective samples) and monitored with an AB-15 pH meter calibrated at 0 °C using three standard buffer solutions with pH values of 4.01, 7.00, and 10.01. Cloud points for all solutions were determined by visual inspection upon gradually heating the samples from 4 °C in an Isotemp water bath (Fisher Scientific). The cloud points of PDEGEA in H₂O and D₂O at a concentration of 0.5 wt% were 12.0 and 11.5 °C, respectively. The cloud points for PDEAEMA in H₂O and D₂O buffers are summarized in Figure B14. Interestingly, the cloud point of PDEAEMA in the D₂O buffer (red solid circle) was slightly higher than that in H₂O (black squares).

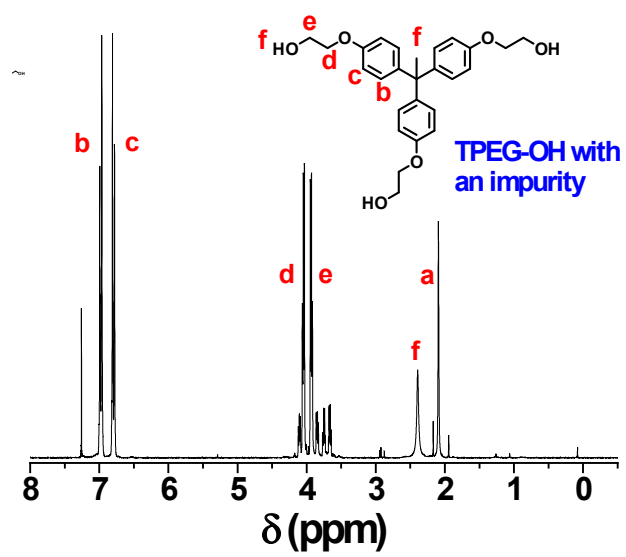


Figure B1. ^1H NMR spectrum of impure TPEG-OH in CDCl_3 .

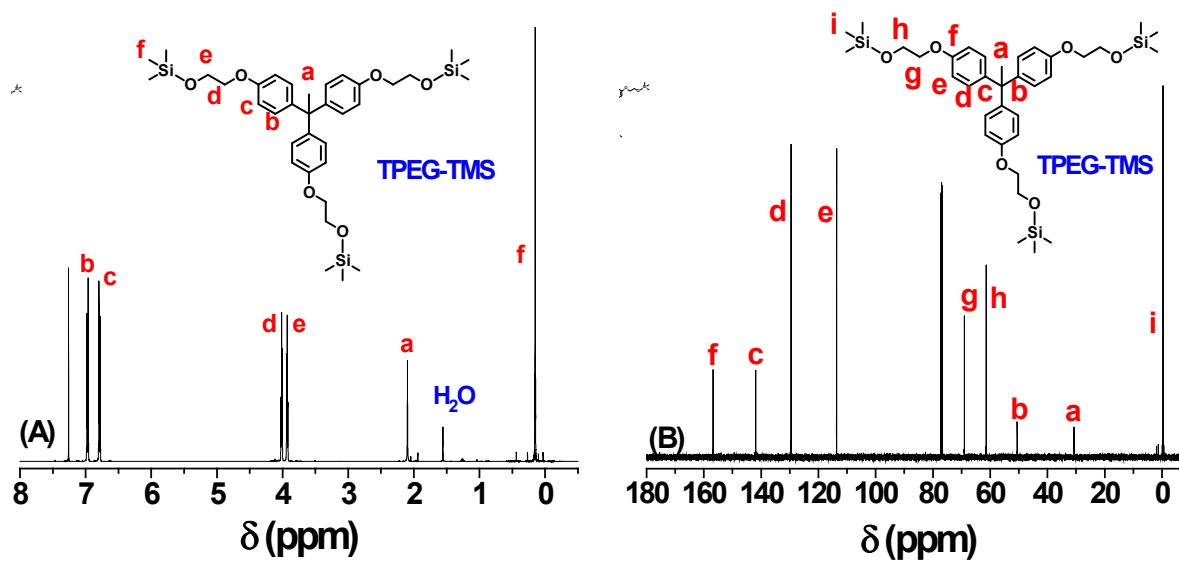


Figure B2. (A) ^1H and (B) ^{13}C NMR spectra of the TMS-protected TPEG-OH, TPEG-TMS, in CDCl_3 .

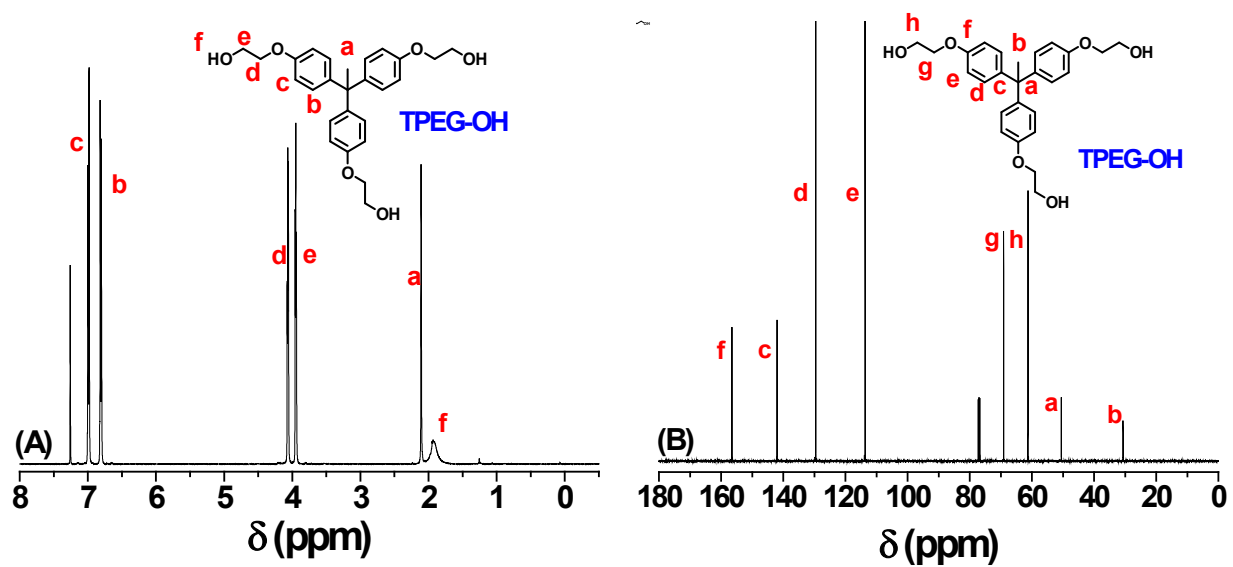


Figure B3. (A) ^1H and (B) ^{13}C NMR spectra of TPEG-OH in CDCl_3 .

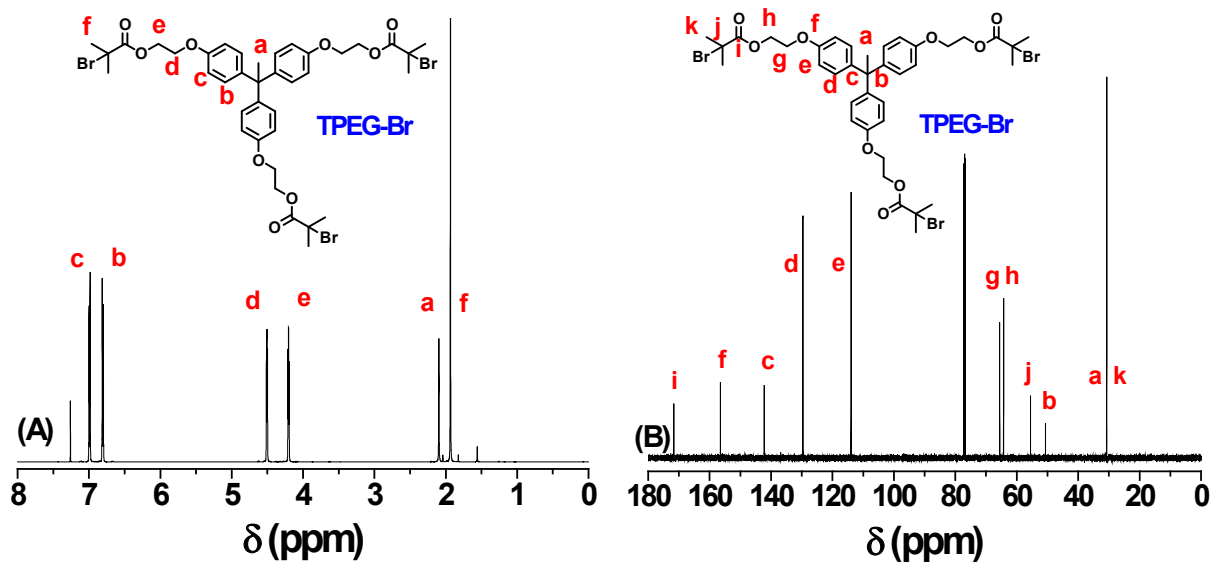


Figure B4. (A) ^1H and (B) ^{13}C NMR spectrum of trifunctional initiator (TPEG-Br) in CDCl_3 .

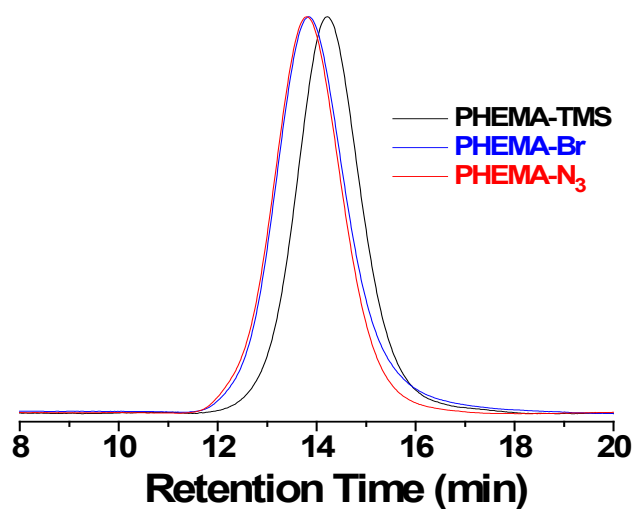


Figure B5. SEC traces of star polymer PHEMA-TMS ($M_{n,SEC} = 179.8$ kDa, $\bar{D} = 1.13$), PHEMA-Br ($M_n = 211.4$ kDa, $\bar{D} = 1.17$), and PHEMA-N₃ ($M_n = 224.5$ kDa, $\bar{D} = 1.16$) obtained from a GPC system equipped with PSS GRAL columns using DMF + 50 mM LiBr as the mobile phase at 50 °C.

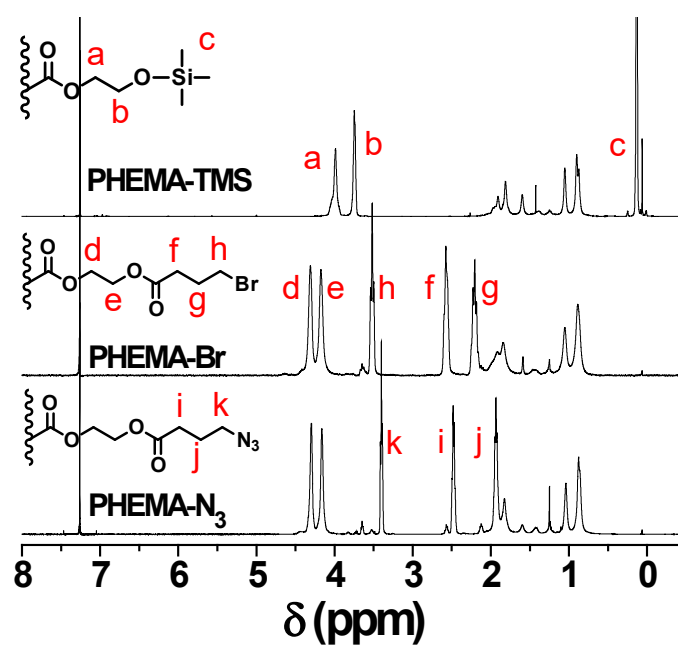


Figure B6. ^1H NMR spectra of PHEMA-TMS, PHEMA-Br, and PHEMA- N_3 in CDCl_3 (500 MHz).

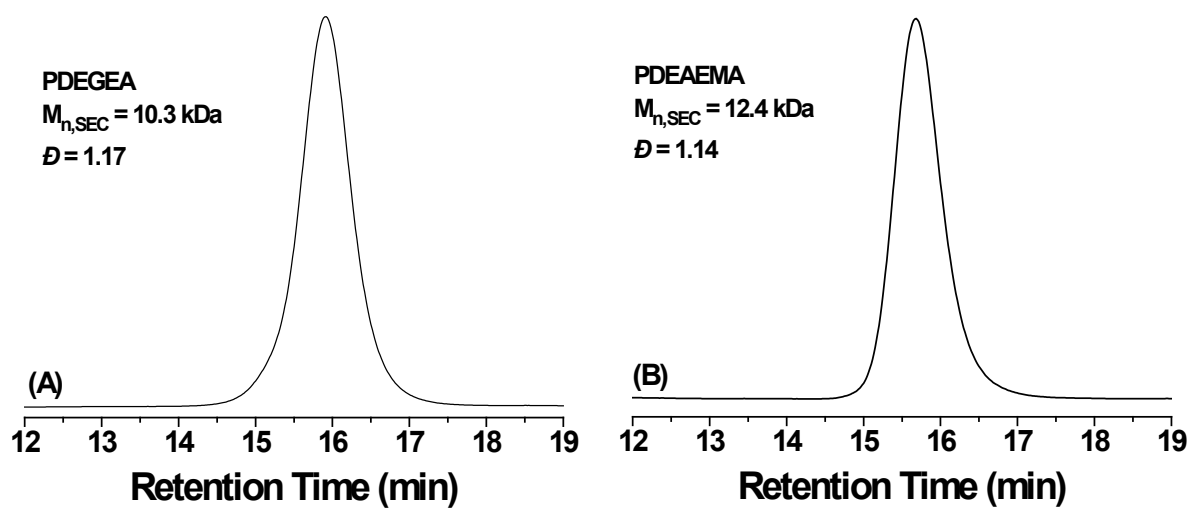


Figure B7. SEC traces of alkyne end-functionalized PDEGEA (A) and PDEAEMA (B).

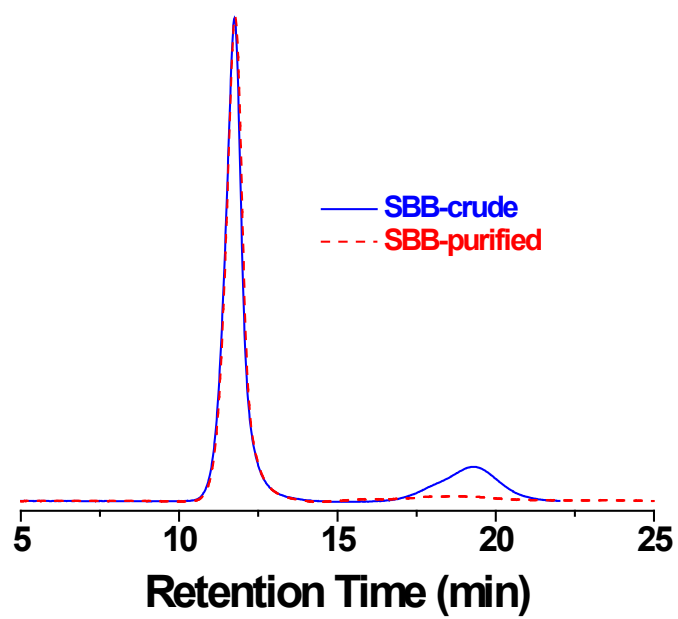


Figure B8. SEC traces of star brush polymer SBB before and after purification.

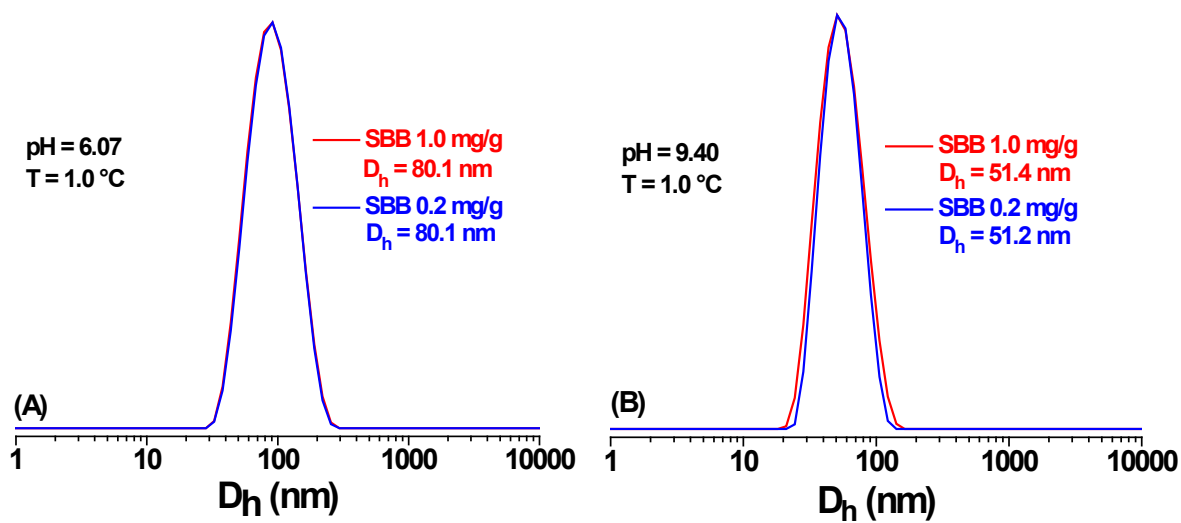


Figure B9. Apparent hydrodynamic size distributions for 0.2 mg/g and 1.0 mg/g solutions of SBB at T = 1.0 °C and pH = 6.07 (A) and pH = 9.40 (B).

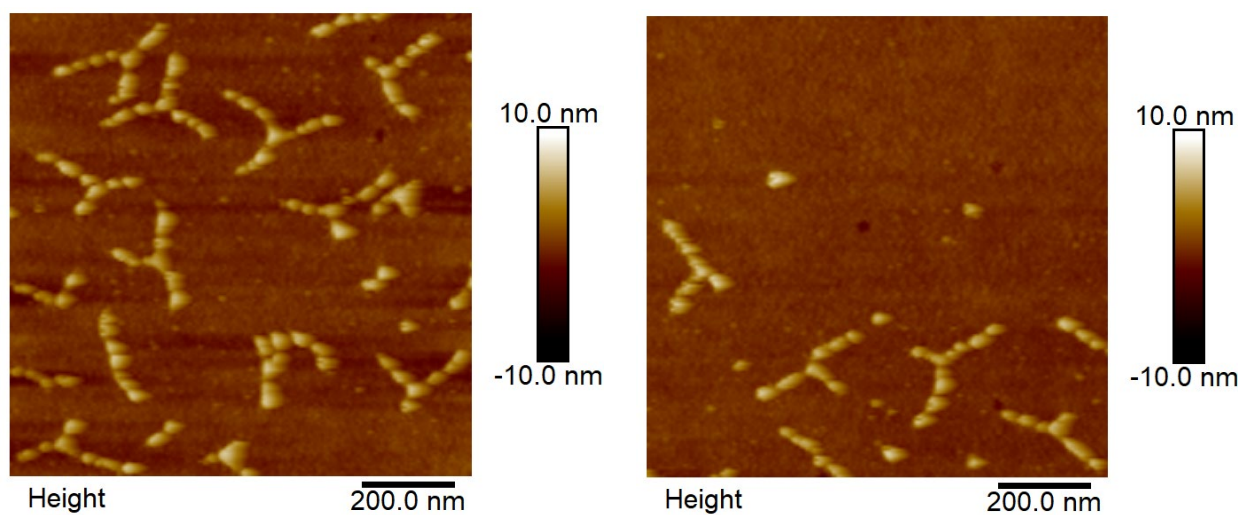


Figure B10. AFM height images of SBB spin cast onto PMMA-coated mica from a 0.05 mg/g solution in a 5 mM phosphate buffer with a pH of 6.60 at 0 °C.

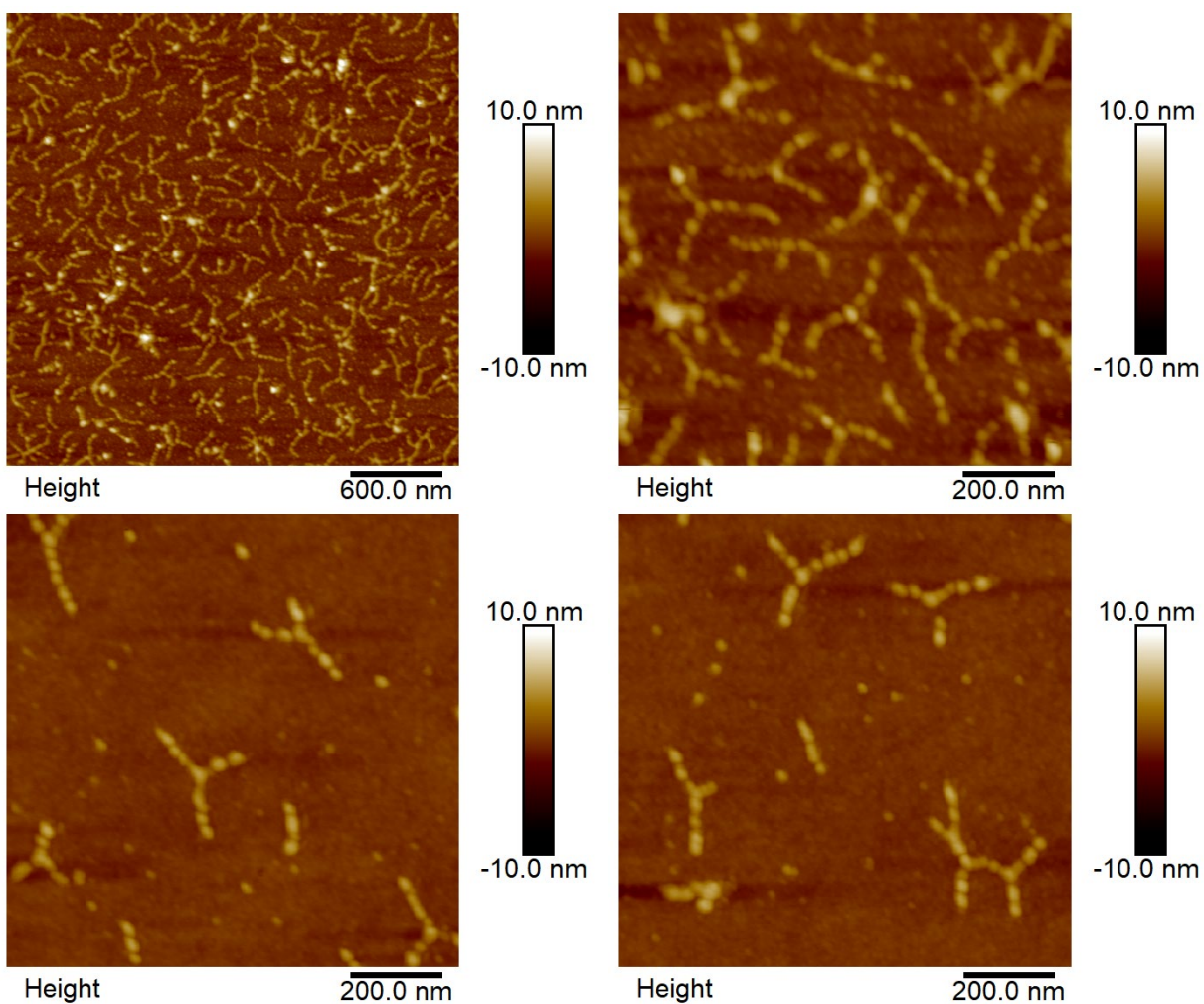


Figure B11. AFM height images of SBB spin cast onto PMMA-coated mica from a 0.05 mg/g solution in a 5 mM phosphate buffer with a pH of 7.43 at 0 °C.

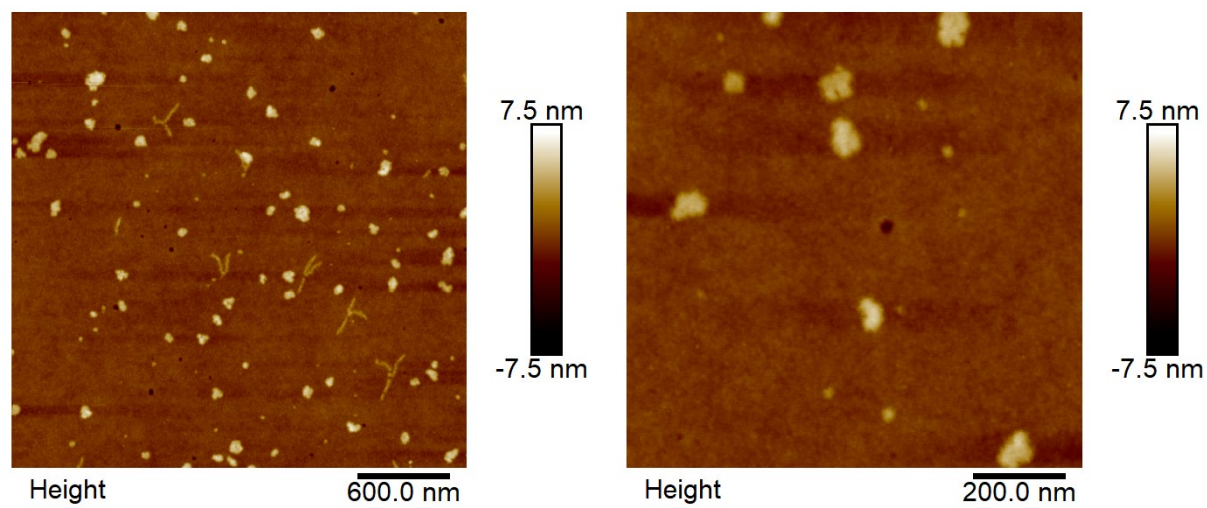


Figure B12. AFM height images of SBB spin cast onto PMMA-coated mica from a 0.05 mg/g solution in a 5 mM phosphate buffer solution with a pH of 9.50 at 0 °C.

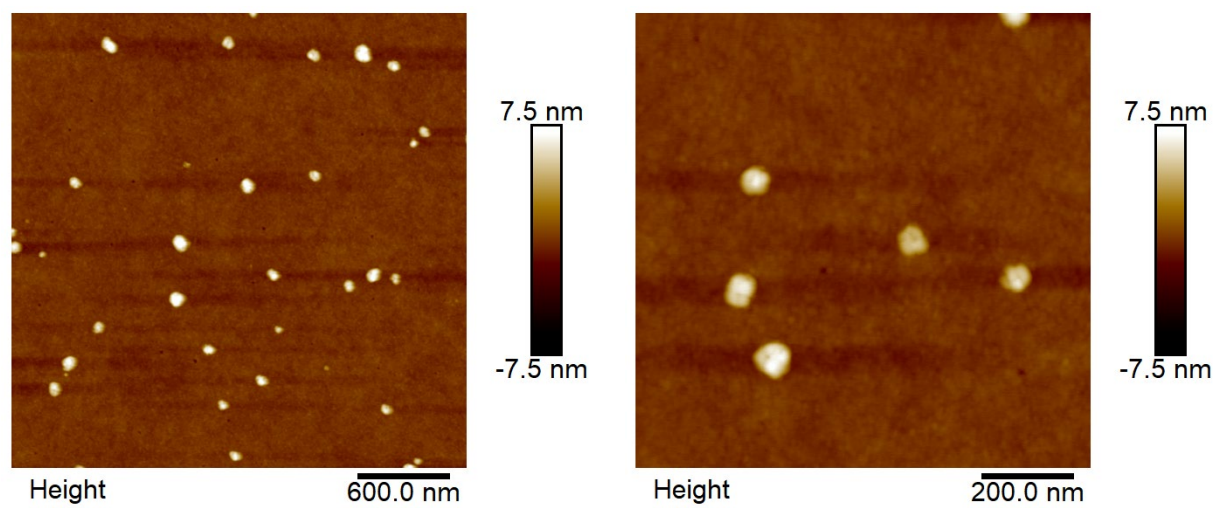


Figure B13. AFM height images of SBB spin cast onto PMMA-coated mica at 22 °C from a 0.05 mg/g solution in 5 mM phosphate buffer solution with a pH of 7.43.

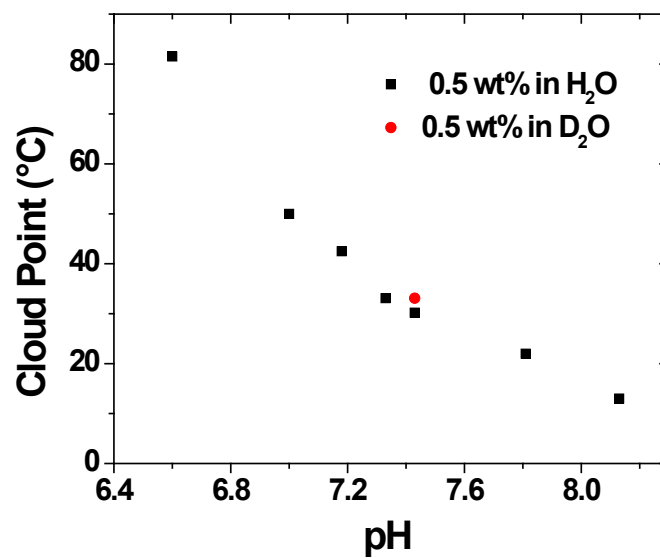


Figure B14. Cloud points for 0.5 wt% PDEAEMA at various pH values in 10 mM phosphate buffers prepared with H₂O (black squares) and D₂O (red circle).

**Chapter 4: Effect of Buffer Anions on Size and Morphology of Tertiary
Amine-Containing Binary Heterografted Linear Molecular Bottlebrushes in
Acidic Aqueous Buffers**

Abstract

This chapter presents the synthesis of tertiary amine-containing linear heterografted molecular bottlebrushes and the study of the effect of buffer anions on the size and morphology of the brushes in various acidic aqueous buffers at lower temperatures. Using a grafting-to approach via click chemistry, we synthesized linear binary heterografted molecular bottlebrushes consisting of pH-responsive poly(2-*N,N*-diethylamino)ethyl methacrylate) (PDEAEMA) and thermoresponsive poly(ethoxydi(ethylene glycol) acrylate) (PDEGEA) side chains. Dynamic light scattering studies of the bottlebrushes in aqueous buffers at a pH below the pK_a of the PDEAEMA side chains (i.e., tertiary amine groups were protonated and positively charged) showed different apparent hydrodynamic diameters for various buffer anions; smaller sizes of the brush polymer were observed for buffer anions with a higher valency. Atomic force microscopy revealed that the bottlebrushes adopted a pearl-necklace morphology at lower pH values, presumably due to the microphase separation of charged PDEAEMA and neutral PDEGEA side chains. The size of the beads was dependent upon the buffer anions used, with higher anion valency resulting in generally larger beads.

4.1. Introduction

Molecular bottlebrushes, or molecular brushes, are a class of macromolecules that consist of polymeric side chains densely tethered to a backbone polymer.¹⁻⁵ Strong excluded volume interactions between adjacent side chains cause both the side chain and backbone polymers to stretch when dissolved in a good solvent leading to a cylindrical, or worm-like, conformation; the aspect ratio of the bottlebrushes is determined by the relative lengths of the backbone and side chain polymers.⁶ Molecular brushes have many unique characteristics due to their distinctive structure, including low chain entanglement, high and tunable persistence lengths, and the ability to undergo large conformational changes in response to external stimuli.^{3, 7} Due to their unique properties, molecular brushes have garnered much attention in recent years and have shown potential in many applications such as, template synthesis,⁸ lubrication,⁹ photonic crystals,⁵ and sensors.¹⁰

Molecular brushes are generally synthesized by utilizing one, or a combination, of the following methods: grafting-from, grafting-through, or grafting-to. Grafting-from is a process whereby side chains are grown from pendant initiating sites along the backbone polymer, typically via “living”/controlled radical polymerization methods such as atom transfer radical polymerization (ATRP).¹¹⁻¹⁵ Grafting-through entails the polymerization of macromonomers to form bottlebrushes directly, typically via ROMP using new generations of Grubb’s catalyst.¹⁶⁻¹⁸ Lastly, the grafting-to method consists of covalently attaching pre-formed end-functionalized side chain polymers to a backbone polymer with complementary pendant functional groups.^{15, 18-21} The grafting-to method is versatile and allows for easily synthesizing heterografted molecular bottlebrushes containing two or more distinct side chain polymers, with an additional benefit of the ability to separately characterize the backbone and side chain polymers. Moreover, employing

click chemistry techniques in the preparation of molecular brushes by grafting-to, such as the copper(I)-catalyzed azide alkyne cycloaddition (CuAAC) reaction,²² has resulted in bottlebrushes with both high and tunable grafting densities.²³

When stimuli-responsive polymers are employed as the side chains of molecular brushes, the brushes can undergo large conformational changes in response to relatively small variations in the environment.³ While these worm-(or star)-to-globule shape transitions have traditionally been limited to either dilute solutions (< 1.0 mg/g)^{13, 24} or at interfaces,^{25, 26} our lab has reported bicomponent stimuli-responsive molecular brushes that display shape-changing behavior in more concentrated aqueous solutions.²⁷⁻³¹

While studying the stimuli-induced star-globule shape transitions of dually responsive binary heterografted three-arm star molecular brushes (SBB) in Chapter 3, we noticed a peculiar pearl-necklace structure of the star brushes by atomic force spectroscopy (AFM); this morphology was observed when SBB was spin cast onto a PMMA-coated mica substrate from an aqueous phosphate buffer solution at a pH of 6.60.³¹ SBB was comprised of pH-responsive poly(2-(*N,N*-diethylamino)ethyl methacrylate) (PDEAEMA) and thermoresponsive poly(ethoxydi(ethylene glycol) acrylate) (PDEGEA) side chain polymers. We hypothesized that the multivalent phosphate anions were forming bridging linkages between the protonated pendant tertiary amine groups in the PDEAEMA side chains, leading to and likely enhancing the microphase separation of PDEAEMA and PDEGEA side chains. It would be advantageous to elucidate the effect of buffer anions on the morphology of heterografted molecular brushes in solution to further both fundamental understanding of the solution behavior of molecular brushes as well as future pursuit of potential applications such as nanoparticle templating.

In the present work, we synthesized binary heterografted linear molecular bottlebrushes

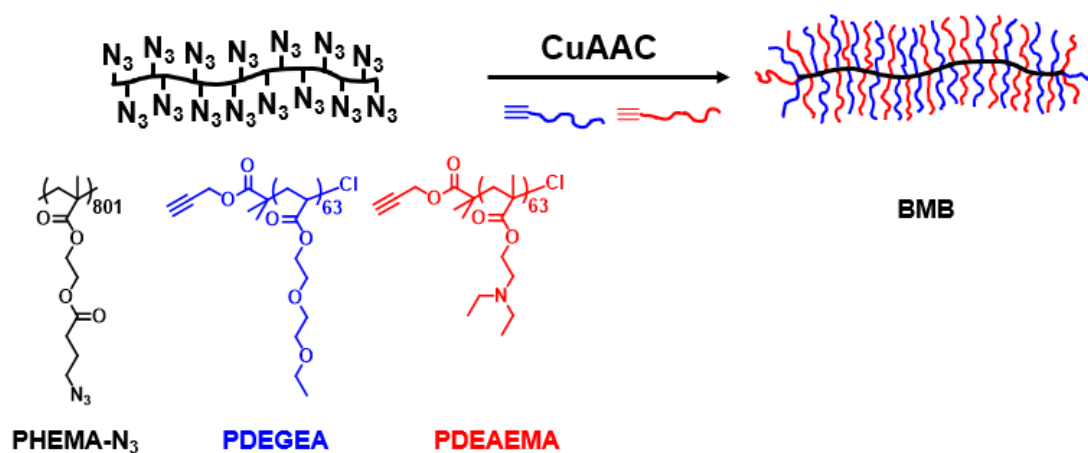
consisting of two different stimuli-responsive side chain polymers and studied their behavior in aqueous buffers. The binary molecular brushes (BMB) were synthesized using a grafting-to method in which alkyne end-functionalized pH-responsive PDEAEMA and thermoresponsive PDEGEA polymers were attached to an azide-bearing backbone polymer via the CuAAC “click” reaction (Scheme 4.1). These side chains were chosen to closely mimic the conditions of the previously observed pearl-necklace morphology. The behavior of BMB in aqueous buffer solutions was investigated by dynamic light scattering (DLS) and atomic force microscopy (AFM) while varying the identity and quantity of various anions in the buffers.

4.2. Experimental Section

4.2.1. Materials

2-Bromoisobutyl bromide (97%, Alfa Aesar), *N,N,N,N,N*-pentamethyldiethylenetriamine (PMDETA, 99%, Acros), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA, 97%, Sigma-Aldrich), and 4-bromobutyl chloride (95%, Acros) were purified by vacuum distillation. 2-(*N,N*-Diethylamino)ethyl methacrylate (DEAEMA, 98.5%, TCI) and ethoxydi(ethylene glycol) acrylate (DEGEA, 98%, TCI) were passed through a basic alumina (top)/silica gel (bottom) prior to polymerization to remove the inhibitor. Copper(I) chloride (CuCl, 99%, Sigma-Aldrich), was stirred in glacial acetic acid overnight, collected by vacuum filtration, washed thoroughly on the filter with absolute ethanol followed by diethyl ether, and then dried under high vacuum. 2-(Trimethylsilyloxy)ethyl methacrylate (HEMA-TMS) was synthesized by the reaction of 2-hydroxyethyl methacrylate with chlorotrimethylsilane and purified by vacuum distillation with the final structure confirmed by ¹H NMR spectroscopy. Propargyl 2-bromoisobutyrate (PBiB) were synthesized according to the procedures described in the literature.²⁷ All other chemicals were purchased from either Sigma-Aldrich or Fisher and used as received.

Scheme 4.1. Synthesis of Linear Binary Heterografted Molecular Bottlebrushes (BMB) via Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) Click Reaction between Azide-Bearing Backbone Polymer and Alkyne End-Functionalized Side Chain Polymers



4.2.2. General Characterization

^1H NMR spectra were recorded on either a Varian VNMRs 500 or 600 spectrometer and the residual solvent peak was used as the internal standard. Size exclusion chromatography (SEC) of the alkyne end-functionalized side chain polymers, PDEGEA and PDEAEMA, was conducted at ambient temperature using a PL-GPC 20 (an integrated GPC/SEC system from Polymer Laboratories, Inc.) with a differential refractive index (RI) detector, one PLgel 5 μm guard column (50×7.5 mm, Agilent Technologies), and two PLgel 5 μm mixed-C columns (each 300×7.5 mm, linear range of molecular weights from 200 to 2 000 000 Da, Agilent Technologies). The eluent was tetrahydrofuran (THF), with a flow rate of 1.0 mL/min, and the system was calibrated using a set of narrow disperse linear polystyrene standards (Scientific Polymer Products, Inc.).

SEC analysis of the backbone polymer, PHEMA- N_3 , and its precursors, PHEMA-TMS and PHEMA-Br, was carried out at 50 $^\circ\text{C}$ using a PL-GPC 50 Plus system (an integrated GPC/SEC system from Polymer Laboratories, Inc.) equipped with a differential refractive index detector, one PSS GRAL 10 μm guard column (50×8 mm, Polymer Standards Service-USA, Inc.) and two PSS GRAL 10 μm linear columns (each 300×8 mm, Polymer Standards Service-USA, Inc., linear range of molecular weight from 500 to 1 000 000 Da). The mobile phase was *N,N*-dimethylformamide (DMF) containing 50 mM LiBr at a flow rate of 1.0 mL/min, and the system was calibrated using a set of narrow disperse polystyrene standards (Scientific Polymer Products, Inc.).

SEC analysis and absolute molecular weight determination of the molecular bottlebrushes (BMB) were performed using a GPC-MALS system comprised of an Agilent model 1260 Infinity pump, a Rheodyne model 7725 manual injector with a 200 μL loop, and a Varian 390 LC detector system consisting of an RI detector and a dual angle light scattering detector (15° and 90°). The

column set used consisted of one PSS GRAM 10 μm guard column (8×50 mm, Polymer Standards Service-USA, Inc.) and three PSS GRAM 10 μm linear columns (each 8×300 mm; 100, 1000, and 3000 Å, Polymer Standards Service-USA, Inc.). The analysis was conducted at 50 °C using DMF containing 50 mM LiBr as eluent.

4.2.3. Dynamic Light Scattering (DLS) Study of Molecular Bottlebrushes (BMB) in Aqueous Solution

DLS experiments were carried out using a Malvern Zetasizer Nano ZS instrument equipped with a He-Ne 633 nm laser and a temperature controller at a scattering angle of 173°. Each sample was prepared for DLS measurements by transferring a known amount of BMB to a 20 mL scintillation vial from a stock solution in THF. The solvent was removed under high vacuum and the desired buffer solution was added immediately to dissolve the bottlebrushes. The pH of the solution was adjusted to 6.00, using 0.1 M HCl, with stirring in an ice/water bath and the brushes were allowed to equilibrate in a refrigerator at 4 °C overnight to ensure complete dissolution. The pH-responsive property of BMB was studied using a 10 mM phosphate buffer solution with a bottlebrush concentration of 0.2 mg/g; the pH was gradually adjusted via addition of 0.1 M NaOH and 0.1 M HCl in an ice/water bath, monitored by an Accumet AB-15 pH meter (calibrated at 0 °C using standard buffer solutions with pH values of 4.01, 7.00, and 10.01). The solution was allowed to equilibrate at each pH for 10 min, and then an aliquot of the solution was transferred to a DLS cuvette without filtering and loaded into the DLS instrument with a pre-set temperature of 1 °C. The sample was then equilibrated in the instrument for an additional 5 min before measurements were taken. To study the effect of various buffer anions on the solution state of BMB, aqueous solutions were prepared with a bottlebrush concentration of 0.1 mg/g in several different buffers (acetate, phosphate, and citrate) with concentrations of 5 mM and 50 mM. The

pH of each solution was adjusted to 6.50 in an ice/water bath using 0.1 M NaOH and 0.1 M HCl and equilibrated for 15 min with stirring. A portion of the solution was transferred to a DLS cuvette and placed into the DLS instrument with a pre-set temperature of 1 °C. The sample was equilibrated for an additional 5 min before measurements were recorded. Each reported DLS value was an average of 3 measurement, with each measurement comprised of at least 10 runs. All reported hydrodynamic sizes (D_h) are the z-average values.

4.2.4. AFM Study of Molecular Bottlebrushes

AFM was performed on a Digital Instruments Multimode IIIa Scanning Probe Microscope in tapping mode under ambient conditions. Reflective Al-coated Si probes (Budget Sensors) with a nominal resonant frequency of 300 kHz and a force constant of 40 N/m were employed. Scotch tape was used to cleave a clean layer of mica disk (Ted Pella, Inc.), which was either used directly or coated with poly(methyl methacrylate) (PMMA). The PMMA-coated mica disks were prepared by placing three drops of 2.5 wt% solution of PMMA ($M_n = 54.9$ kDa) in CHCl_3 onto the freshly cleaved disk and spinning the solution off at 3000 rpm to create a thin film. The aqueous solutions from DLS measurements were used immediately to prepare the samples for AFM by spin casting onto the PMMA-coated mica disks at 3000 rpm; the substrates and spin coating stage were cooled in a refrigerator at 4 °C prior to the spin casting of the BMB solutions. AFM images collected on bare mica were prepared by spin casting a dilute solution of BMB in THF at 3000 RPM onto freshly cleaved mica at ambient temperature.

4.2.5. Synthesis of Linear Azide-Bearing Backbone Polymer (PHEMA- N_3)

Ethyl 2-bromoisobutyrate (1.89 mg, 9.69×10^{-6} mol, delivered from 70.4 mg of a 26.9 mg/g stock solution in anisole), 2-(trimethylsilyloxy)ethyl methacrylate (4.005 g, 1.980×10^{-2} mol), 4,4'-dinonyl-2,2'-dipyridyl (12.8 mg, 3.13×10^{-5} mol), CuCl (3.0 mg, 3.0×10^{-5} mol), and anisole

(1.328 g) were added to a 25 mL two-necked flask equipped with a magnetic stirbar, rubber septum, and gas adapter. The mixture was degassed by three freeze-pump-thaw cycles and immersed in a thermostated oil bath at 75 °C. After 24 h, the flask was removed from the oil bath and opened to air. A sample was taken for ^1H NMR spectroscopy analysis, and the polymerization mixture was immediately diluted with THF. The catalyst was removed by passing the diluted crude mixture through a short column of neutral alumina (top)/silica gel (bottom), using THF as the eluent. The solution was concentrated by rotary evaporation followed by drying under high vacuum at 60 °C for 6 h to remove unreacted monomer and other volatile components, yielding a glassy white polymer, poly(2-(trimethylsilyloxy)ethyl methacrylate) (PHEMA-TMS, 1.371 g, 34.2 %). The monomer conversion was calculated to be 39.2 % by ^1H NMR spectroscopy analysis of the final reaction mixture using the integrals of the peaks at 4.26 – 4.18 ppm ($-\text{COOCH}_2-$ of the monomer) and 4.12 – 3.89 ppm ($-\text{COOCH}_2-$ of the polymer). The degree of polymerization (DP) was determined to be 801 based on the monomer conversion and the feed ratio of monomer to initiator (2043:1). SEC analysis results (PSS GRAL columns with DMF containing 50 mM LiBr as the eluent, 50 °C) relative to polystyrene standards: $M_{n, \text{SEC}} = 121.1$ kDa and $D = 1.13$.

PHEMA-TMS (0.847 g, 4.19×10^{-3} mol -OTMS) and KF (0.340 g, 5.85×10^{-3} mol) were added to a 50 mL two-necked flask. The flask was sealed and degassed by pulling high vacuum and then refilling with nitrogen. THF (25 mL) was added *via* syringe under nitrogen and the mixture was stirred to dissolve the polymer. The flask was then placed in an ice/water bath and stirred for 15 min. Tetrabutylammonium fluoride (84 μL of a 1 M solution in THF, 8.40×10^{-5} mol) was delivered into the flask using a microsyringe, immediately followed by the addition of 4-bromobutyryl chloride (1.856 g, 1.00×10^{-2} mol) via a syringe. After 30 min the mixture was removed from the ice/water bath and stirred for 11 h at room temperature. The reaction was

stopped by precipitating the polymer into a mixture of methanol and water (80/20, v/v, 150 mL) two times from THF, followed by precipitation from THF (10 mL) into methanol (150 mL) once and finally into hexanes (150 mL) once. The resulting polymer was dried under high vacuum for 2 h, yielding a glassy, white polymer (PHEMA-Br, 0.979 g, 83.7 %). SEC analysis results (PSS GRAL columns with DMF containing 50 mM LiBr as the eluent, 50 °C) relative to polystyrene standards: $M_{n, SEC} = 151.9$ kDa and $D = 1.13$.

PHEMA-Br (0.707 g, 2.53×10^{-3} mol -CH₂Br assuming quantitative esterification with 4-bromobutyryl chloride), NaN₃ (0.890 g, 1.37×10^{-2} mol), and DMF (10 mL) were added to a 50 mL two-necked round bottomed flask and stirred at room temperature overnight. The azide-functionalized polymer was purified by precipitation of the crude product into a mixture of methanol and water (80/20, v/v) twice, followed by methanol once and hexanes once, using THF as the good solvent. The resulting polymer was dried under high vacuum to yield a glassy off-white polymer (PHEMA-N₃, 0.536 g, 87.9 %). SEC analysis results (PSS GRAL columns with DMF containing 50 mM LiBr as the eluent, 50 °C) relative to polystyrene standards: $M_{n, SEC} = 154.1$ kDa and $D = 1.13$.

The degree of azide functionalization of the backbone polymer was 96.7%, determined by ¹H NMR spectroscopy analysis of PHEMA-N₃ in CDCl₃ using PHEMA-TMS and PHEMA-Br precursor polymers as references. As discussed in Chapter 3, there are three characteristic peaks of unreacted pendant groups on the backbone in the ¹H NMR spectrum of PHEMA-N₃ located at: (i) 3.52 ppm, corresponding to the unreacted bromide-containing pendant groups (-OOCCH₂CH₂CH₂Br, 0.6 %), (ii) 3.65 ppm, corresponding to the unreacted chloride-containing pendant groups (-OOCCH₂CH₂CH₂Cl, 1.2 %), and (iii) 3.82 ppm, corresponding to the deprotected, yet unreacted, PHEMA pendant groups (-COOCH₂CH₂OH, 1.5 %). Taking into

consideration the repeat units that do not bear an azide functionality, the degree of azide functionalization of the backbone polymer, PHEMA-N₃, was calculated to be 96.7%.

4.2.6. Synthesis of Alkyne End-Functionalized Thermoresponsive Side Chain Polymer Poly(ethoxydi(ethylene glycol) acrylate) (PDEGEA)

Propargyl 2-bromoisobutyrate (73.0 mg, 3.56×10^{-4} mol, delivered from 0.636 g of a stock solution in anisole with a concentration of 114.8 mg/g), ethoxydi(ethylene glycol) acrylate (8.035 g, 4.27×10^{-2} mol), *N,N,N',N'',N''*-pentamethyldiethylenetriamine (67.7 mg, 3.91×10^{-4} mol), CuCl (36.1 mg, 3.65×10^{-4} mol), and anisole (8.093 g) were added to a 50 mL two-necked flask equipped with magnetic stir-bar, septum, and gas flow adapter. The solution was degassed by three freeze-pump-thaw cycles and placed in a thermostated oil bath set to 80 °C. After 21 h, the flask was removed from the oil bath and opened to air. A sample was quickly withdrawn for ¹H NMR spectroscopy analysis, immediately followed by dilution of the polymerization mixture with chloroform. The copper catalyst was removed by passing the mixture through a column of basic alumina (top)/silica gel (bottom) using chloroform as eluent. The solution was concentrated to about 15 mL by rotary evaporation; the polymer was purified by precipitation from THF into hexanes (150 mL) three times. The purified polymer was dried under high vacuum to afford a slightly yellow, highly viscous liquid (3.754g, 46.7 %). The monomer conversion was 52.5 %, calculated using ¹H NMR spectroscopy analysis of the ester peaks in the monomer (-COOCH₂-, 4.38-4.28) and the polymer (-COOCH₂-, 4.28-4.13). The DP was determined to be 63 using the monomer conversion and the monomer-to-initiator feed ratio. SEC analysis results relative to polystyrene standards using mixed-C columns and THF as eluent: $M_{n, SEC} = 12.1$ kDa and $\bar{D} = 1.17$.

4.2.7. Synthesis of Alkyne End-Functionalized pH-Responsive Side Chain Polymer Poly(2-(*N,N*-Diethylamino)ethyl methacrylate) (PDEAEMA)

PBiB (55.4 mg, 2.70×10^{-4} mol, delivered via 0.500 g of a stock solution in anisole with a concentration of 110.8 mg/g), 2-(*N,N*-diethylamino)ethyl methacrylate (6.005 g, 3.24×10^{-2} mol), HMTETA (73.2 mg, 3.18×10^{-4} mol), CuCl (26.8 mg, 2.70×10^{-4} mol), and anisole (7.4997 g) were added to a 50 mL two-necked flask with a magnetic stirbar, rubber septum and gas adapter. The mixture was degassed by three consecutive freeze-pump-thaw cycles and placed into an oil bath with a preset temperature of 50 °C. The polymerization was stopped after 3.5 h by removing the flask from the oil bath and opening it to air. An aliquot of the mixture was withdrawn for ^1H NMR spectroscopy analysis, and the solution was diluted with THF. The crude polymerization mixture was passed through a column of basic alumina (top)/silica gel (bottom) to remove the copper catalyst, using THF as the eluent. This solution was then concentrated by rotary evaporation. The polymer was purified by precipitation into cold hexanes (-78 °C) from THF three times and then dried under high vacuum, yielding a slightly yellow, rubbery product (2.812 g, 46.8 %). ^1H NMR spectroscopy analysis of the final mixture revealed the monomer conversion to be 52.7 % by comparing the ester peaks corresponding to monomer (4.32-4.17 ppm, $-\text{COOCH}_2-$) and polymer (4.17-3.98 ppm, $-\text{COOCH}_2-$). The DP of the obtained alkyne end-functionalized PDEAEMA was calculated to be 63 from the monomer conversion and the monomer-to-initiator feed ratio. SEC analysis relative to polystyrene standards using mixed-C columns and THF as eluent: $M_{n, \text{SEC}} = 13.6$ kDa and $D = 1.13$.

4.2.8. Synthesis of Linear Binary Heterografted Molecular Bottlebrushes (BMB)

PHEMA- N_3 (5.24 mg, 2.17×10^{-5} mol $-\text{CH}_2\text{N}_3$ assuming quantitative functionalization, delivered via addition of 160.8 mg of a stock solution in THF with a concentration of 32.6 mg/g),

PDEAEMA (159.6 mg, 1.35×10^{-5} mol, delivered via 1.719 g of a stock solution in THF with a concentration of 92.81 mg/g), PDEGEA (168.2 mg, 1.40×10^{-5} mol, delivered via 1.028 g of a stock solution in THF with a concentration of 163.6 mg/g), THF (5 mL), and CuCl (2.0 mg , 2.0×10^{-5} mol) were added to a 20 mL scintillation vial with a magnetic stirbar and sealed with a rubber septum. After purging the headspace of the vial with nitrogen gas through needles for 15 min, PMDETA (5.0 μL , 4.2 mg, 2.4×10^{-5} mol) was injected. The purging with nitrogen gas was continued for an additional 15 min; the needles were removed, and the reaction proceeded at room temperature. After 24 h, propargyl benzyl ether (50 μL) was injected in an attempt to cap unreacted azide moieties. After the reaction mixture was stirred for an additional 2 h, the vial was opened to air and an aliquot was taken for SEC analysis to determine the grafting density. The mixture was diluted with methylene chloride (50 mL) and passed through a column of basic alumina (top)/silica gel (bottom), and the column was flushed with additional methylene chloride (100 mL). The crude mixture was concentrated by rotary evaporation to $\sim 2 \text{ mL}$, and six fractionations were performed by cooling the crude solution in methylene chloride in a dry ice/acetone bath followed by the slow addition of hexanes (6 mL) to remove the unreacted side chain polymers, yielding 142.0 mg of bottlebrushes (BMB). The complete removal of unreacted side chain polymers was confirmed by SEC analysis using PSS GRAM columns with DMF containing 100 mM LiBr as eluent at 50 $^{\circ}\text{C}$. The absolute weight-average molecular weight (M_w) was determined by a GPC-MALS system: $M_w = 8.70 \times 10^6 \text{ Da}$, $\bar{D}$ of 1.07.

4.3. Results and Discussion

4.3.1. Synthesis of Azide-Bearing Backbone Polymer and Side Chain Polymers

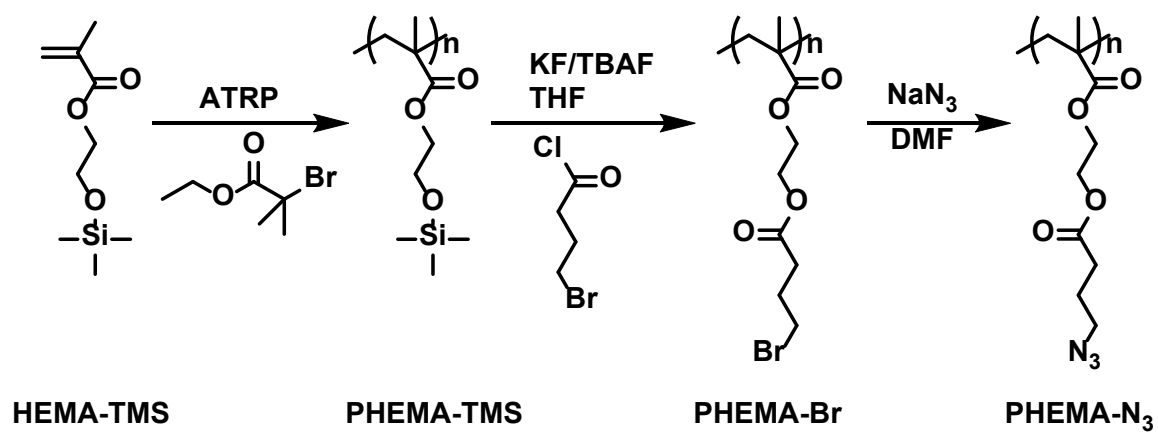
Binary heterografted molecular bottlebrushes (BMB) were synthesized in a “grafting-to” approach by utilizing copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) click chemistry.

The azide bearing backbone polymer, PHEMA-N₃, with a DP of 801 was prepared by adopting a procedure similar to that used in Chapter 3. First, ATRP of 2-(trimethylsilyloxy)ethyl methacrylate (HEMA-TMS) was conducted from ethyl 2-bromoisobutyrate to form the silyl-ether protected linear backbone polymer precursor, PHEMA-TMS. Second, a one-pot reaction was performed in order to remove the silyl-ether protecting group and install an alkyl bromide moiety at each repeat unit. Lastly, a substitution reaction, using sodium azide, was conducted to replace the bromide groups with azide, resulting in a 96.7 % azide functionality (Scheme 4.2). The characterization data for the backbone polymer and precursors are presented in Figures C1 and C2 in Appendix C.

Alkyne end-functionalized thermoresponsive PDEGEA and pH-responsive PDEAEMA side chain polymers were made by ATRP. The polymerization of DEGEA was carried out in anisole at 80 °C for 21 h using propargyl 2-bromoisobutyrate (PBiB) as initiator and CuCl/PMDETA as the catalyst/ligand. The molar ratios of $[M]_0 : [I]_0 : [CuCl] : [PMDETA] = 120 : 1 : 1 : 1.1$. From SEC analysis, the polymerization of DEGEA was well controlled, resulting in a single, narrow molecular weight distribution with an $M_{n, SEC}$ of 12.1 kDa and a dispersity ($\mathcal{D}$) of 1.17 (Figure C3A). The DP of PDEGEA was 63, calculated from the monomer conversion, as determined via ¹H NMR spectroscopy analysis by comparing the ester peaks in the monomer (-COOCH₂-, 4.38-4.28 ppm) and the polymer (-COOCH₂-, 4.28-4.13 ppm), and the initial monomer-to-initiator ratio. The polymer was purified by passing through a basic alumina (top)/silica gel (bottom) column to remove the catalyst, followed by precipitation from THF into hexanes three times.

The polymerization of DEAEMA was performed in anisole at 50 °C for 3.5 h using PBiB as initiator and CuCl/HMTETA as the catalyst/ligand in the molar ratios of $[M]_0 : [I]_0 : [CuCl] : [PMDETA] = 120 : 1 : 1 : 1.2$. SEC analysis of the obtained PDEAEMA revealed a single, narrow peak, with an $M_{n, SEC}$ of 13.6 kDa and $\mathcal{D}$ of 1.13, indicating a well-controlled polymerization

Scheme 4.2. Synthesis of Azide-Bearing Backbone Polymer PHEMA-N₃



(Figure C3B). A DP of 63 was calculated using the monomer-to-initiator ratio and the monomer conversion, determined from ^1H NMR analysis by comparing the ester peaks corresponding to the monomer (4.32-4.17 ppm, $-\text{COOCH}_2-$) and the polymer (4.17-3.98 ppm, $-\text{COOCH}_2-$). The catalyst was removed by passing the polymerization mixture through a column of basic alumina (top)/silica gel (bottom), and the polymer was purified by precipitation from THF into hexanes at $-78\text{ }^\circ\text{C}$ in a dry ice/acetone bath.

4.3.2. Synthesis of Linear Binary Heterografted Molecular Bottlebrushes (BMB)

BMB, consisting of pH-responsive PDEAEMA and thermoresponsive PDEGEA side chains, was synthesized using a “grafting-to” approach in which the preformed alkyne end-functionalized side chain polymers were attached to the azide-bearing backbone polymer, PHEMA- N_3 , via the CuAAC “click” reaction. The reaction was carried out in THF at ambient temperature using $\text{CuCl}/\text{PMDETA}$ as catalyst/ligand with a feed molar ratio $[\text{PHEMA-}\text{N}_3 \text{ repeat units}] : [\text{PDEGEA}] : [\text{PDEAEMA}]$ of 1 : 0.65 : 0.62; an excess of side chains was used in order to achieve a high grafting density. After 24 h, propargyl benzyl ether was injected in an attempt to cap unreacted azide groups. The copper catalyst was removed by passing the mixture through a column of basic alumina (top)/silica gel (bottom) and the bottlebrushes were purified by repeated fractionation in a mixture of methylene chloride and hexanes, at $-78\text{ }^\circ\text{C}$ in a dry ice/acetone bath, to remove unreacted side chain polymers, which was confirmed by SEC analysis. ^1H NMR spectroscopy analysis of the purified bottlebrushes revealed that the composition of BMB was 51.6 mol % PDEGEA and 48.4 mol % PDEAEMA side chains, as determined by comparing the integrals of the peaks at 4.33-4.11 ppm ($-\text{COOCH}_2-$ of PDEGEA) and 4.11-3.91 ppm ($-\text{COOCH}_2-$ of PDEAEMA) as well as the DPs of the two polymers (Figure 4.1A).

The overall grafting density of the obtained bottlebrushes was 72.5 %, calculated by

considering the SEC peak areas of the brushes and the side chains, the feed ratio of the backbone azide groups and the alkyne end-functionalized side chain polymers, and the molar ratio of two side chain polymers in the purified bottlebrushes. Using ^1H NMR spectroscopy analysis of the purified brushes and considering that both side chain polymers were of the same DP ($\text{DP}_{\text{PDEGEA}} = 63$, $\text{DP}_{\text{PDEAEMA}} = 63$), the composition of the bottlebrushes was calculated to be 51.6 mol % PDEGEA and 48.4 mol % PDEAEMA side chains. Using the number of moles of the repeat units of the backbone polymer (2.17×10^{-5} mol), the final molar composition of the brushes, and the molar masses of PDEGEA and PDEAEMA calculated from their DPs and end groups (12018 and 11832 g/mol, respectively), the theoretical mass of each side chain polymer that could react was found to be 134.7 mg PDEGEA and 124.4 mg PDEAEMA (259.1 mg total). The peak areas for the bottlebrushes and the side chain polymers by SEC analysis were 58.0 % and 42.0 %, respectively (Figure C4). Multiplying the area percentage for the brushes by the total feed mass of the backbone polymer, PHEMA- N_3 , and two side chain polymers yielded 193.2 mg of the brushes. By subtracting the amount of the backbone polymer, we found that 187.9 mg of side chain polymers had reacted. The overall grafting density was calculated to be 72.5 % by dividing the total mass of side chains that reacted (187.9 mg) by the total theoretical mass of side chains that could react (259.1 mg, assuming 100% azide functionalization).

The absolute weight-average molecular weight (M_w) of BMB was found to be 8.70×10^6 Da using a GPC-MALS system equipped with an RI detector and a dual-angle light scattering detector (Figure 4.1B). The calculated value of M_w for BMB was 8.19×10^6 Da, as determined by considering the weight-average molecular weight of PHEMA- N_3 , the average DP of each arm, the M_w of the side chain polymers ($M_n \times D$), and the grafting density from SEC peak areas. The calculated value of M_w was very close to the experimentally determined value by GPC-MALS.

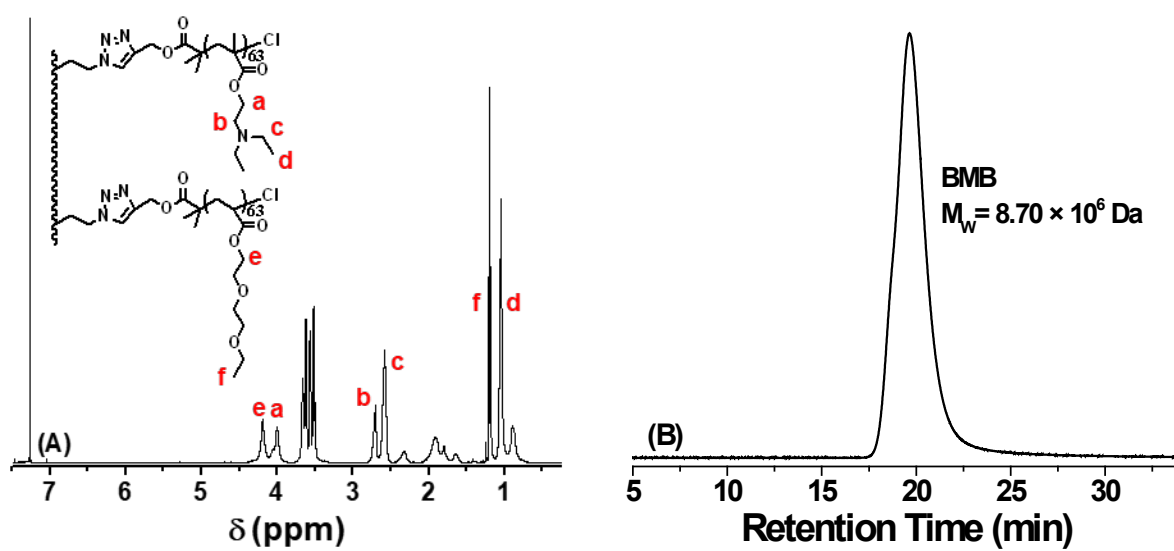


Figure 4.1. (A) ^1H NMR spectrum of the purified bottlebrushes, BMB, in CDCl_3 and (B) SEC trace of BMB from a GPC-MALS system.

The formation of bottlebrushes was further confirmed by AFM imaging of the BMB spin cast onto freshly cleaved mica from a solution in THF (Figure 4.2A and B). The average length of the bottlebrushes, measured using the Image J software, was found to be $156.7 \text{ nm} \pm 38.4 \text{ nm}$, and the typical height was $\sim 1 \text{ nm}$ (Figure 4.2C). Considering the contour length of the backbone ($DP = 801$) in an all-trans conformation, the average length measured from the AFM images corresponds to a degree of stretching of 77.0 %.

4.3.3. Dynamic Light Scattering (DLS) Study of BMB in Aqueous Buffer Solution.

DLS was used to study the pH-responsive property of BMB and the effect of buffer anions on the size of the brushes; we hypothesized that higher valency buffer anions could form bridging links between adjacent PDEAEMA side chains, leading to shrinking or partial collapse of the bottlebrushes. A 0.2 mg/g solution of BMB in a 5 mM aqueous phosphate buffer with a pH of 6.00 was prepared, and the pH was gradually increased at 0°C by adding 0.1 M NaOH. At a pH of 6.00, the hydrodynamic size (D_h) was found to be 68.6 nm; upon increasing the pH, the size decreased sharply around a pH of 8.20 to 47.8 nm at a pH of 8.50 and leveled off at a pH of 9.50 with a size of 46.9 nm (Figure 4.3A). As can be seen in Figure 4.3B, the size distributions for both low and high pH values are single, narrow peaks, indicating no aggregation of brush molecules in solution. The decrease in size occurred at a pH higher than the pK_a of the PDEAEMA side chains, as observed for star brush molecules in Chapter 3, due to two main factors. (i) The pK_a of PDEAEMA of 7.4 was measured at room temperature, and the pH measurements conducted in this experiment were carried out in an ice/water bath at 0°C using a pH probe calibrated at 0°C . Note that the standard basic buffer solutions displayed a decrease in their pH values upon warming from 0°C to room temperature; 7.11 and 10.32 at 0°C became 7.00 and 10.01, respectively, at room temperature. (ii) The PDEGEA side chains are strongly hydrated at 0°C and are likely to

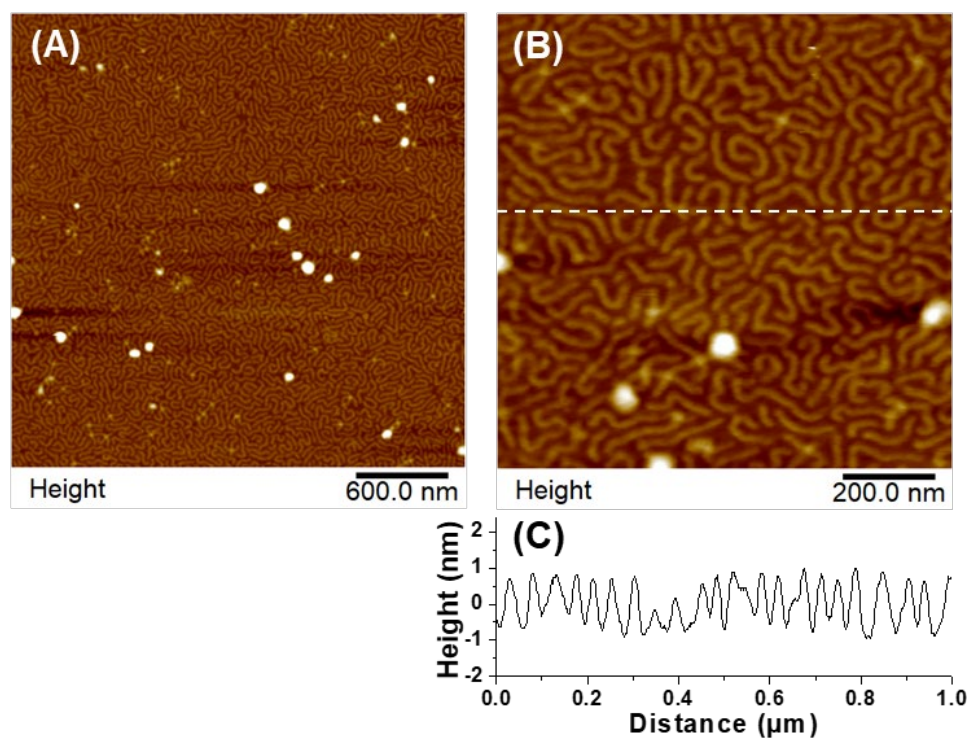


Figure 4.2. (A) and (B) AFM height images of BMB spin cast onto freshly cleaved bare mica from a 0.01 mg/g solution in THF. (C) Cross-sectional height profile along the dashed line in (B).

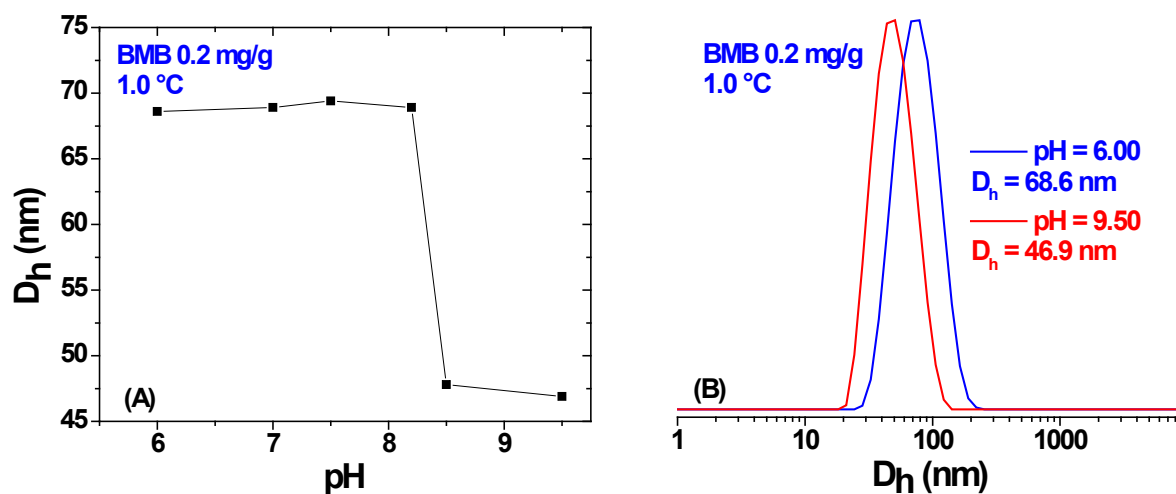


Figure 4.3. (A) Apparent hydrodynamic diameter (D_h) of the bottlebrushes, BMB, at a concentration of 0.2 mg/g in a 5 mM phosphate buffer solution at 1 °C as a function of pH obtained from a DLS study. (B) Hydrodynamic size distributions of BMB at pH = 6.00 and pH = 9.50.

provide enough solvating power to keep the bottlebrushes in an extended state for a short pH range beyond the pK_a of the PDEAEMA side chains, until more of the tertiary amines are deprotonated to drive the pH-induced collapse.

The bottlebrushes were then studied by DLS in various buffer solutions to determine what effect, if any, the buffer anion valency had on the hydrodynamic size of BMB in solution. The solutions of BMB were prepared at a concentration of 0.1 mg/g in various buffers with concentrations of 5 mM and 50 mM and adjusted to a pH of 6.50 at 0 °C in an ice/water bath. It is worth noting that a solution of BMB prepared in THF at a concentration of 0.1 mg/g at room temperature displayed a hydrodynamic size of 70.1 nm (Figure 4.4A). For BMB in the 5 mM acetate buffer solution, the D_h was the largest observed, 78.0 nm, with a single distribution, indicating no intermolecular aggregation of bottlebrushes (Figure 4.4B). At a pH of 6.50, the acetate buffer exists in the mostly deprotonated state and the acetate anions bear a charge of -1. It is unlikely that the acetate anions can form bridging interactions between adjacent tertiary amines, which leads to high electrostatic repulsion between the positively charged PDEAEMA chains, causing the brushes to adopt a more stretched conformation. When the acetate buffer concentration was increased to 50 mM, the observed size decreases slightly to 74.9 nm (Figure C5A); this was likely due to the higher salt concentration screening some of the electrostatic interactions between positively charged side chains, allowing the brush to relax to a less stretched state.

For BMB in the 5 mM phosphate buffer solution, the D_h was found to be 71.0 nm with a single size distribution (Figure 4.4B). This decrease in size compared to the acetate buffer was likely due to the higher valency of the phosphate anions at a pH of 6.50; about 30 % of the phosphate anions carried a -2 charge and about 70 % carry a -1 charge (calculated using CurTiPot software).³² Since a portion of the counterions carried a -2 charge, bridging of protonated tertiary amines within a

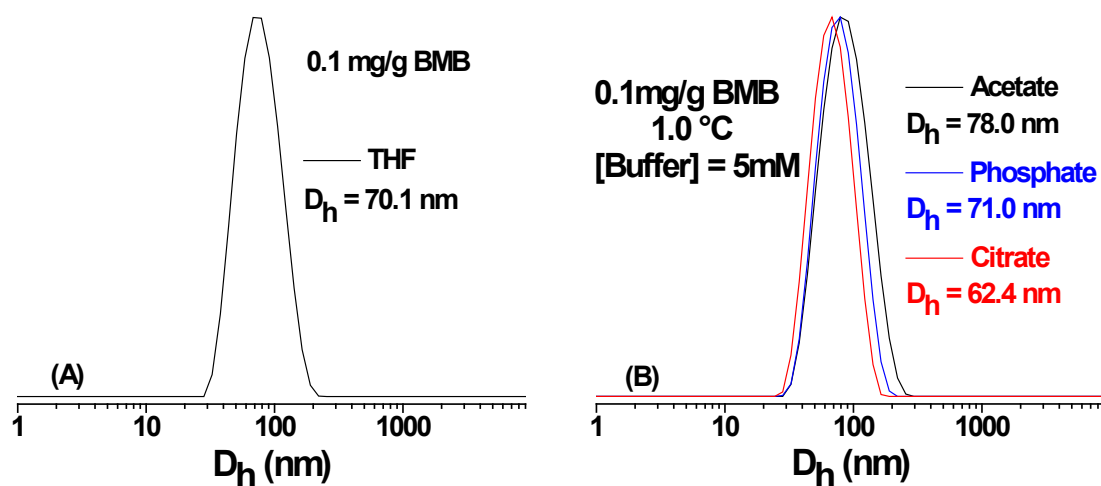


Figure 4.4. Hydrodynamic size distributions obtained from a DLS study of BMB at a concentration of 0.1 mg/g in (A) THF at 25 °C, and (B) 5 mM buffer solutions adjusted to a pH of 6.50 at 1 °C.

side chain or between adjacent side chains was possible, leading to a contraction of the bottlebrush molecules. When the concentration of phosphate buffer was increased to 50 mM, the size decreased slightly to 69.0 nm (Figure C5B). This decrease can be explained by either an increased salt concentration that screened electrostatic interactions between the protonated tertiary amine groups, or increased bridging events due to a larger number of multivalent counterions, or a combination of these effects.

For BMB in 5 mM citrate buffer, the D_h decreased even further to 62.4 nm, displayed as a single size distribution (Figure 4.4B). Citrate at a pH of 6.50 exists as several species in solution: about 55 % carrying a -3 charge, 44 % carrying a -2 charge, and 1 % carrying a -1 charge.³² The high valency of the citrate counterions can lead to multiple bridging events per citrate molecule, which would lead to further collapse of the bottlebrushes. Interestingly, when the citrate concentration was increased to 50 mM, a small increase in the D_h to 65.1 nm was observed (Figure C5C), and the reason was unclear.

4.3.4. AFM Study of BMB

AFM was employed to study the morphologies of BMB from various aqueous buffer solutions. The dilute solutions of BMB used for the DLS studies were used directly by spin casting onto PMMA-coated mica after measurements by DLS. Figures 4.5 and C6 show the AFM height images of BMB spin cast onto PMMA-coated mica at a concentration of 0.1 mg/g in 5 mM acetate buffer at a pH of 6.50 and about 0 °C (cooled in an ice/water bath). The brush molecules were in an extended worm-like state with an average contour length of 174.1 ± 35.0 and a typical height of 2.5 nm. The bottlebrushes appeared to be much more rigid compared to those cast from THF onto bare mica and display a rougher texture with small domains (typical size ~16 nm) along the whole length. The elongation of the brush molecules as well as the fish-bone-like morphology are most

likely the result of microphase separation of the positively charged PDEAEMA side chains and neutral PDEGEA side chains as well as electrostatic repulsive forces within the PEAEMA domains.

Figures 4.6 and C7 show the AFM height images of BMB spin cast onto PMMA-coated mica from the 5 mM phosphate buffer also at a pH of 6.50 and 0 °C. The average contour length was 173.2 ± 37.1 nm. The morphology of these brushes was similar to a pearl-necklace, composed of bead-like structures with typical diameters of ~ 30 nm and a ~ 4 nm typical height. As can be seen from the cross-sectional height profile in Figure 4.6C, the height undulated along the contour of the bottlebrushes with 2 – 3 nm variations. This morphology was consistent with the observations in Chapter 3 when a phosphate buffer was employed in the analysis of star brushes comprised of PDEGEA and PDEAEMA side chains. The observed pearl-necklace morphology was likely caused by microphase separation of the PDEGEA and PDEAEMA side chains, which was further enhanced through bridging of adjacent PDEAEMA chains by multivalent phosphate anions.

Figures 4.7 and C8 show the AFM height images of BMB spin cast onto PMMA-coated mica from a 5 mM citrate buffer. Here, an average contour length of 170.9 ± 33.5 nm was observed. A pearl-necklace morphology was displayed, with somewhat larger typical bead sizes of ~ 36 nm when compared to those observed for the bottlebrushes from the phosphate buffer. The average height of the beads (~ 5 nm) was also larger. The larger and taller beads, along with a shorter average contour length, suggest that as the valency of the counterions is increased, more bridging and enhanced microphase separation could occur, leading to a contraction of the overall brush molecule.

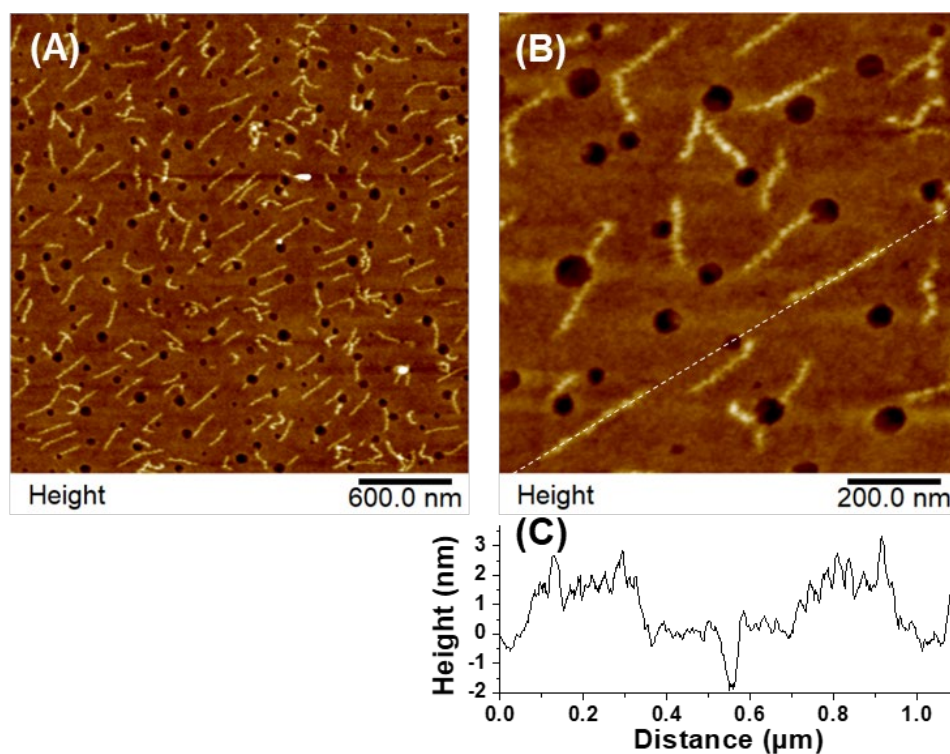


Figure 4.5. (A) and (B) AFM height images of BMB spin cast onto PMMA-coated mica from a 0.1 mg/g solution of BMB in a 5mM acetate buffer with a pH of 6.50 at 0 °C. (C) Cross-sectional height profile along the dashed line in (B).

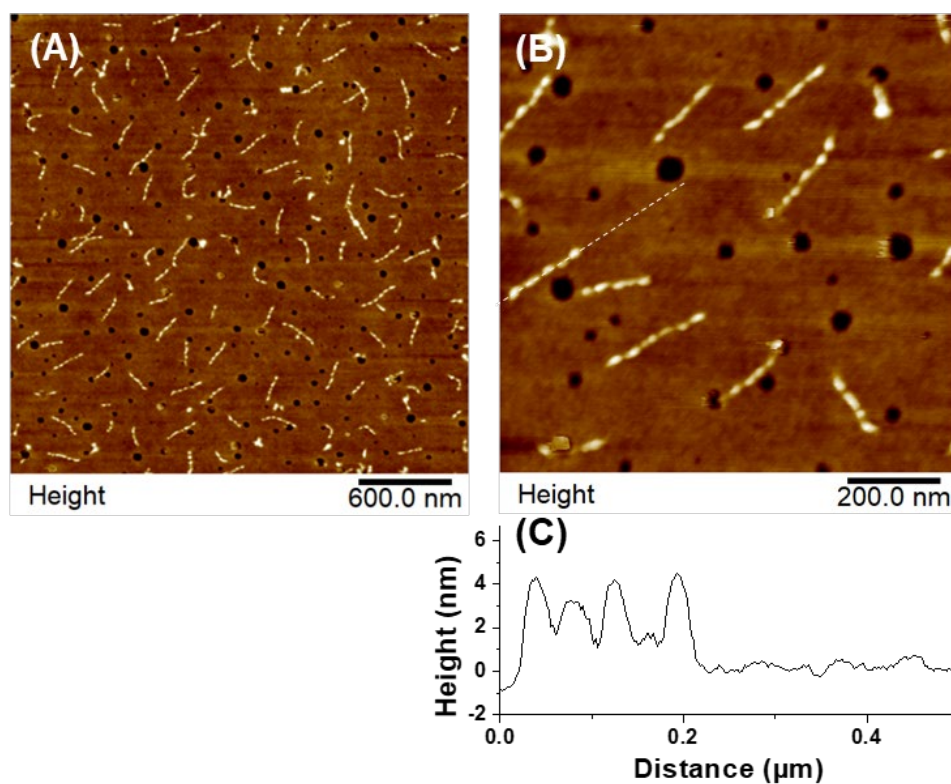


Figure 4.6. (A) and (B) AFM height images of BMB spin cast onto PMMA-coated mica from a 0.1 mg/g solution of BMB in a 5mM phosphate buffer with a pH of 6.50 at 0 °C. (C) Cross-sectional height profile along the dashed line in (B).

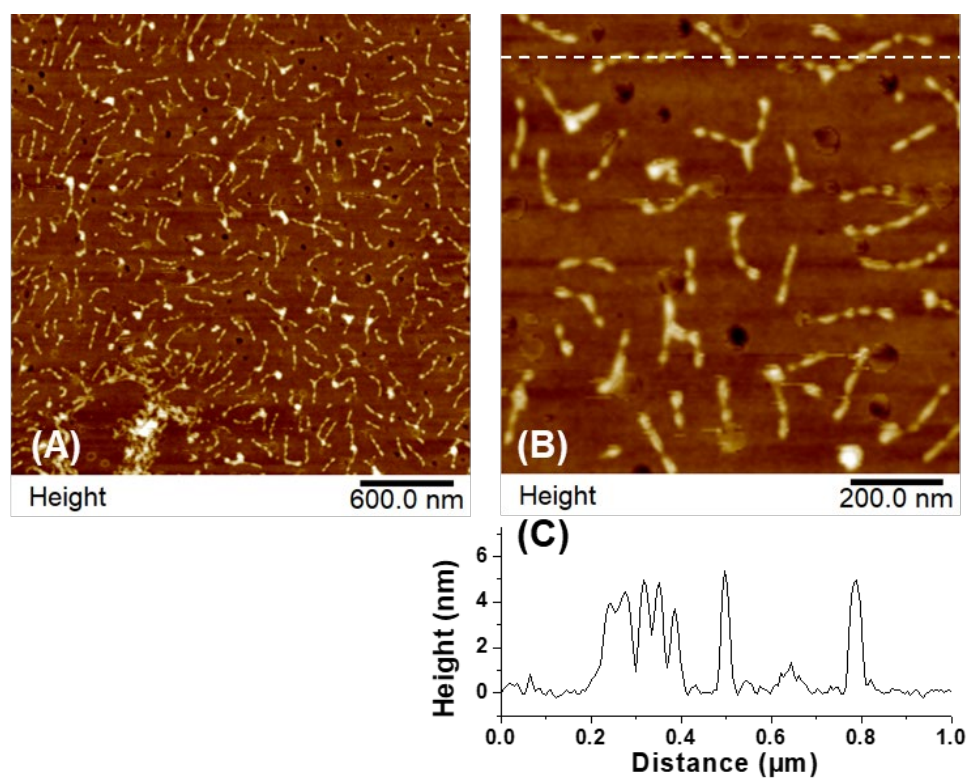


Figure 4.7. (A) and (B) AFM height images of BMB spin cast onto PMMA-coated mica from a 0.1 mg/g solution of BMB in a 5mM citrate buffer with a pH of 6.50 at 0 °C. (C) Cross-sectional height profile along the dashed line in (B).

4.4. Conclusions

In summary, we synthesized linear binary heterografted molecular bottlebrushes consisting of tertiary amine-based pH-responsive and LCST-type thermoresponsive side chains by a click grafting-to method and studied the effect of buffer anions on the size and morphology of the bottlebrushes in acidic aqueous solution. The bottlebrushes displayed a large change in size, indicating a worm-to-globule shape transition, when the pH of the solution was increased from pH = 6.00 to pH = 9.50. DLS studies showed that the D_h of BMB in aqueous acidic buffer solution decreased with increasing valency of the buffer anions; the largest size was observed for acetate buffer and the smallest size was observed for citrate buffer. AFM showed that aqueous buffer solutions of BMB spin cast onto PMMA mica had adopted a peculiar pearl-necklace morphology, with roughly equal sized beads along the contour of the brush. The bead sizes were measured using ImageJ software and were found to correlate with the valency of the buffer anions; smaller beads were observed with monovalent acetate buffer and the largest beads were observed in the presence of the mostly tri-valent citrate buffer. The smaller size observed by DLS and larger domain sizes in buffer with higher valency anions are likely due to those anions forming bridging links between protonated ammonium moieties, resulting in enhanced microphase separation of the charged PDEAEMA and neutral PDEGEA side chains.

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Appendix C

For

Chapter 4: Effect of Buffer Anions on Size and Morphology of Tertiary

Amine-Containing Binary Heterografted Linear Molecular

Bottlebrushes in Acidic Aqueous Buffers

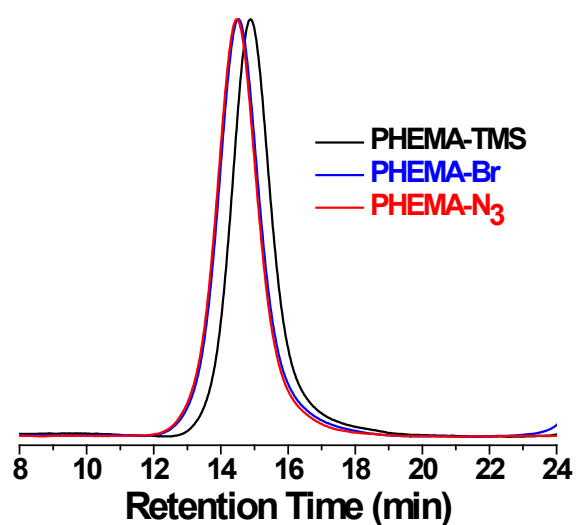


Figure C1. SEC traces of backbone polymer PHEMA-TMS ($M_{n, SEC} = 121.1$ kDa, $\bar{D} = 1.13$), PHEMA-Br ($M_{n, SEC} = 151.9$ kDa, $\bar{D} = 1.13$), and PHEMA-N₃ ($M_{n, SEC} = 154.1$ kDa, $\bar{D} = 1.13$) obtained from a GPC system equipped with PSS GRAL columns using DMF containing 50 mM LiBr as the mobile phase at 50 °C.

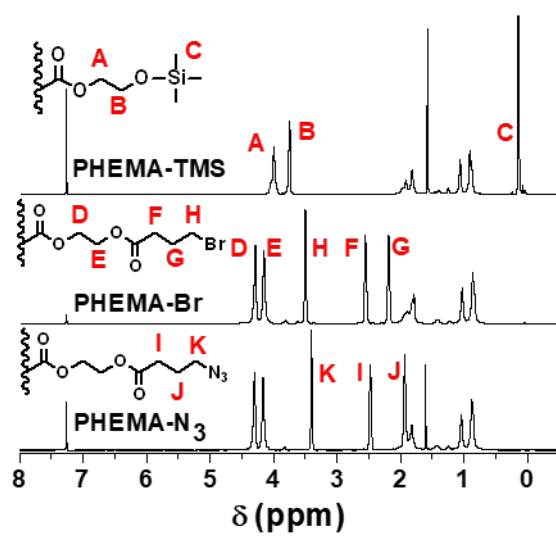


Figure C2. ^1H NMR spectra of PHEMA-TMS, PHEMA-Br, and PHEMA-N₃ in CDCl_3 .

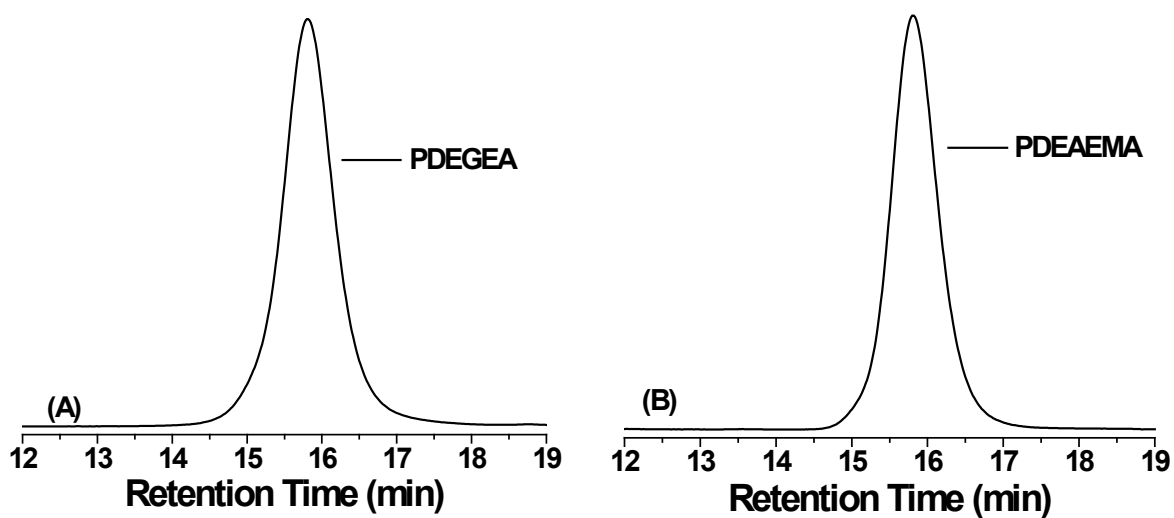


Figure C3. SEC traces of alkyne end-functionalized (A) PDEGEA ($M_{n, SEC} = 12.1$ kDa, $\bar{D} = 1.17$), and (B) PDEAEMA ($M_{n, SEC} = 13.6$ kDa, $\bar{D} = 1.13$) obtained from a GPC system equipped with Agilent Mixed-C columns using THF at ambient temperature.

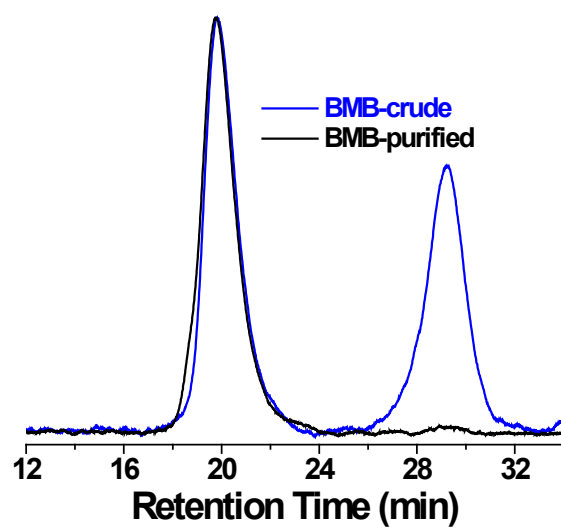


Figure C4. SEC traces of BMB in the final crude reaction mixture and after purification obtained from a GPC system equipped with PSS GRAM columns using DMF containing 100 mM LiBr as the mobile phase at 50 °C.

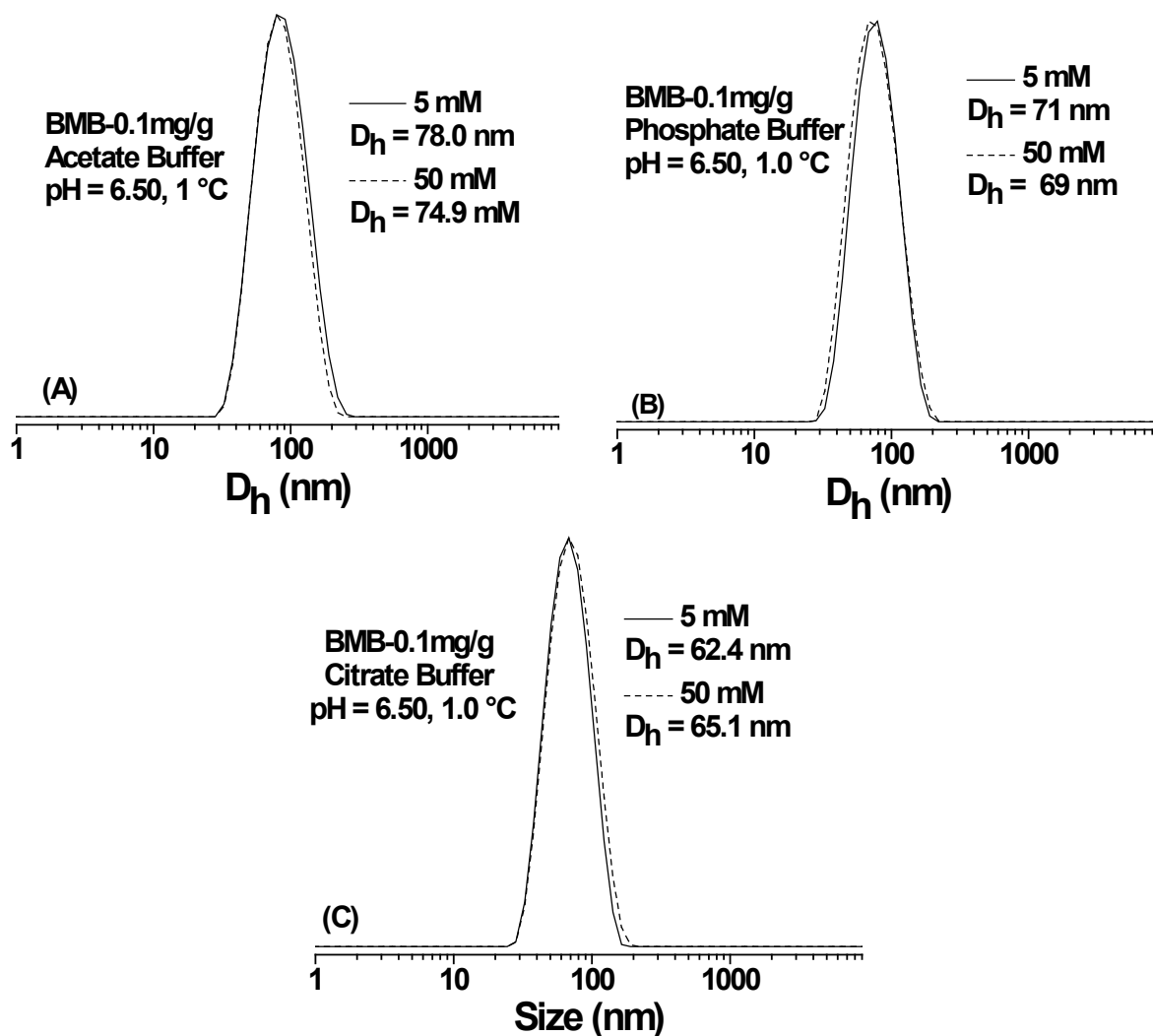


Figure C5. Hydrodynamic size distributions obtained from a DLS study of BMB at a concentration of 0.1 mg/g in aqueous buffer solutions with concentrations of 5 mM and 50 mM for (A) acetate, (B) phosphate, and (C) citrate buffers adjusted to a pH of 6.50 at 1.0 °C.

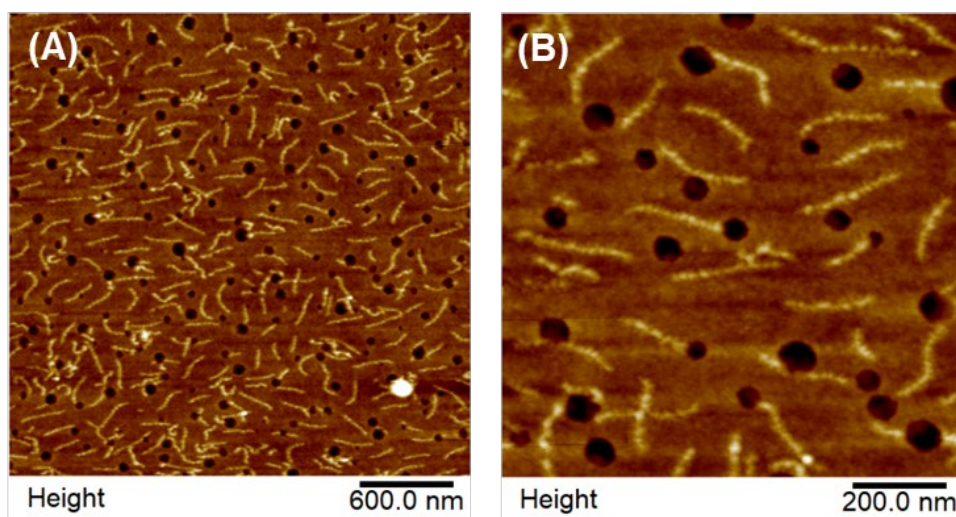


Figure C6. (A) and (B) AFM height images of BMB spin cast onto PMMA-coated mica from a 0.1 mg/g solution prepared in 5 mM acetate buffer adjusted to pH = 6.50 at 0 °C.

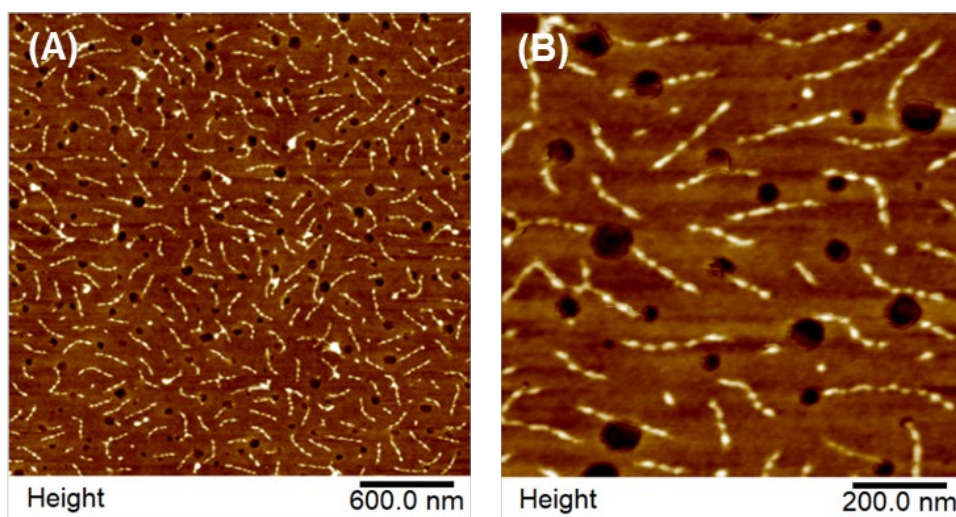


Figure C7. (A) and (B) AFM height images of BMB spin cast onto PMMA-coated mica from a 0.1 mg/g solution prepared in 5 mM phosphate buffer adjusted to pH = 6.50 at 0 °C.

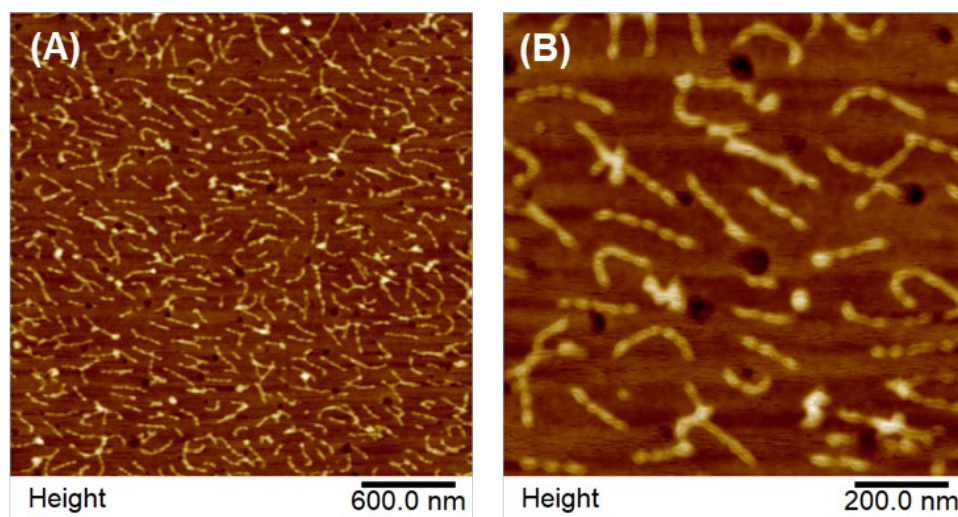


Figure C8. (A) and (B) AFM height images of BMB spin cast onto PMMA-coated mica from a 0.1 mg/g solution prepared in 5 mM citrate buffer adjusted to pH = 6.50 at 0 °C.

**Chapter 5: pH- and Chaotropic Anion-Induced Conformational Changes of
Binary Heterografted Three-Arm Star Molecular Bottlebrushes**

Abstract

This chapter presents the synthesis of pH- and chaotropic anion-responsive binary heterografted three-arm star molecular bottlebrushes and the study of their conformational transitions in aqueous solution. The star molecular brushes, synthesized using a “click” grafting-to method, were composed of poly(ethylene oxide) (PEO) side chains and tertiary amine-containing poly(2-(*N,N*-dimethylamino)ethyl methacrylate) (PDMAEMA) or poly(2-(*N,N*-diethylamino)ethyl methacrylate) (PDEAEMA) side chains. Dynamic light scattering (DLS) studies revealed that the brushes consisting of PEO and PDMAEMA (SMB-1) only had a small change in hydrodynamic size with increasing pH across the pK_a of PDMAEMA. However, the brushes composed of PEO and PDEAEMA (SMB-2) displayed a rather large size change over a narrow pH range near the pK_a of PDEAEMA. Atomic force microscopy (AFM) showed that while SMB-1 remained in the star-like state at both low and high pH values, SMB-2 displayed a shape transition from the extended starlike state to the collapsed globular state. In addition, DLS studies showed that both SMB-1 and SMB-2 at pH = 4.00 exhibited a large decrease in size upon the addition of various chaotropic anions, with SMB-2 showing greater sensitivity to moderately chaotropic anions than SMB-1. ^1H NMR analysis revealed that the peaks of charged tertiary amine groups in the brushes in D_2O at pH = 4.00 either disappeared or became significantly broadened with increasing the concentration of ferricyanide anions, a super chaotrope. AFM confirmed the ferricyanide anion-induced shape transition from extended starlike to collapsed yet stable globular states for both brushes in the protonated state. The shape transitions were caused by the formation of tight ion pairs between the protonated tertiary amine groups, which are chaotropic cations, and the added chaotropic anions.

5.1. Introduction

Molecular bottlebrushes, or molecular brushes, are a type of macromolecules characterized by polymeric side chains densely grafted to a polymer backbone.¹⁻³ The dense grafting of polymeric side chains to the backbone causes the molecular bottlebrushes to adopt a stretched (wormlike or starlike) conformation in good solvents, with the aspect ratio of brushes determined by the relative lengths of the backbone and side chain polymers.⁴ Advances in “living”/controlled polymerization techniques such as atom transfer radical polymerizations (ATRP) and reversible addition-fragmentation chain-transfer (RAFT) polymerizations have allowed for the preparation of molecular brushes with precise control over architecture and composition. Generally, there are three methods for the preparation of molecular bottlebrushes: (i) grafting-from, in which side chains are grown from an initiator-functionalized backbone,⁵⁻⁷ (ii) grafting-through, which entails the polymerization of end-functionalized macromonomers,⁸⁻¹² and (iii) grafting-to, in which end-functionalized side chain polymers are covalently attached to a backbone containing complementary functional groups.¹³⁻¹⁸ While each method has advantages and drawbacks, grafting-to has been shown to be an extremely modular method, with high and tunable grafting densities attainable through the use of the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) “click” reaction.¹⁹⁻²²

Molecular brushes that employ stimuli-responsive polymers as side chains are particularly intriguing due to their ability to undergo dramatic changes in size, shape, and solubility triggered by relatively small changes in the environment.²³ The unimolecular shape transition of bottlebrushes from an extended worm- or star-like state to a collapsed globular state make them intriguing candidates for a host of applications ranging from unimolecular sensors to drug delivery vehicles. However, the instability of the collapsed state in many of the literature reports has

hampered their usefulness. To address this issue, this work aimed to synthesize binary heterografted molecular bottlebrushes that could undergo a stable unimolecular shape transition.

In Chapter 3, we demonstrated that binary heterografted three-arm star molecular brushes composed of pH-responsive poly(2-(*N,N*-diethylamino)ethyl methacrylate) (PDEAEMA, $pK_a \approx 7.4$) and thermoresponsive poly(ethoxydi(ethylene glycol) acrylate) (PDEGEA, LCST = 9 °C) side chains could undergo pH-induced or thermally-induced collapse, resulting in two distinct unimolecular micelle-like globular states, with PDEAEMA as the hydrophobic core and PDEGEA as the stabilizing corona at pH = 9.50 and T = 0 °C or with PDEGEA as the core and the protonated PDEAEMA as the stabilizing corona at pH = 7.43 and T = 22 °C. Using AFM imaging, we noticed a peculiar pearl-necklace morphology of the PDEAEMA/PDEGEA brushes when they were in the extended star-like state (pH = 6.60 and T = 0 °C).

In Chapter 4, we prepared linear heterografted bottlebrushes composed of PDEAEMA and PDEGEA side chains in order to rationalize the formation of the pearl-necklace morphology observed in Chapter 3. We found that the formation of these discrete domains was strongly influenced by the identity of the buffer used in the preparation of aqueous bottlebrush solutions; monovalent acetate anions showed the smallest pearl domain sizes (~16 nm) and the buffer anions with higher valency, phosphate and citrate, resulted in bead domain sizes of ~30 nm and ~36 nm, respectively. These results suggested that the microphase separation between the highly charged PDEAEMA and neutral PDEGEA side chains could be enhanced with the use of different salts.

In the present work, we prepared dually pH- and chaotropic anion-responsive binary heterografted three-arm star molecular brushes using the highly efficient CuAAC “click” reaction in a grafting-to approach and demonstrated that these brushes were able to switch from an extended starlike to collapsed unimolecular micelle-like globules in response to changes in pH or with the

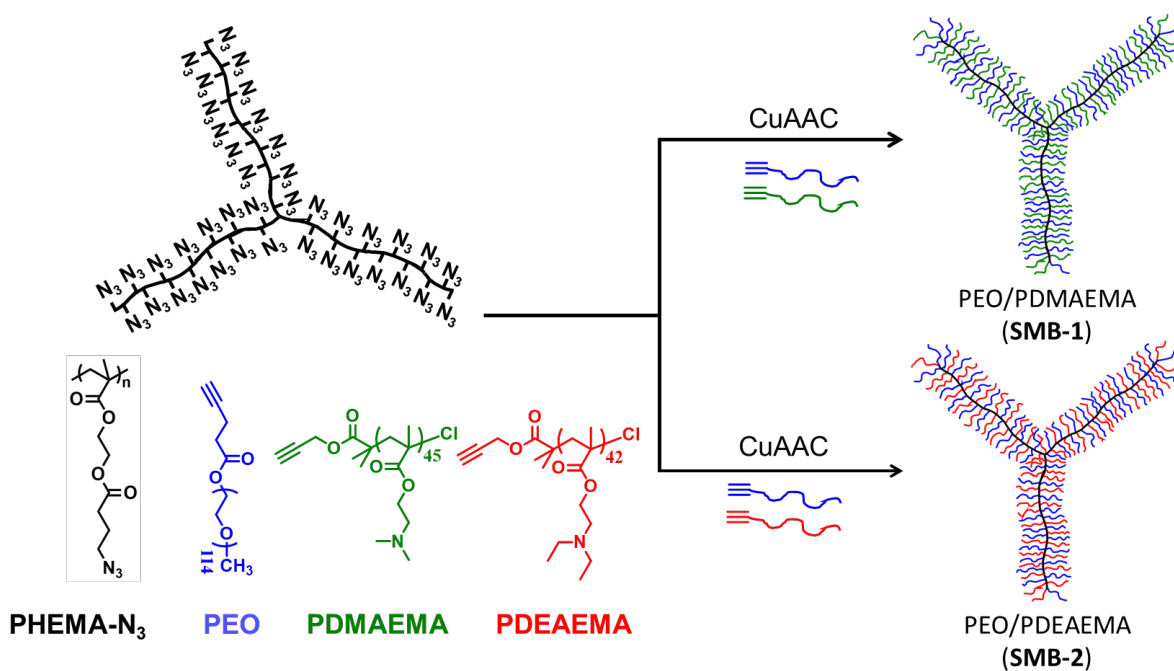
addition of chaotropic salts. The star molecular brushes were composed of PEO (5 kDa) and either poly(2-(*N,N*-dimethylamino)ethyl methacrylate) (PDMAEMA, $pK_a = \sim 7.4$), or PDEAEMA ($pK_a = \sim 7.4$) side chains, which were randomly grafted to an azide-bearing star backbone polymer, PHEMA- N_3 (Scheme 5.1). The star backbone polymer used in this work had a degree of polymerization (DP) of 434 per arm and was synthesized by ATRP followed by post-polymerization modifications as discussed in Chapter 3. The alkyne end-functionalized PEO was prepared by the reaction of PEO monomethyl ether with 4-pentynoic acid in a Steglich esterification. The alkyne end-functionalized tertiary amine-containing polymers, PDMAEMA and PDEAEMA, were synthesized by ATRP using an alkyne-functionalized initiator. The stimuli-responsive properties of the formed brushes were investigated by dynamic light scattering (DLS), ^1H NMR spectroscopy, and atomic force microscopy (AFM). The brushes consisting of PDEAEMA and PEO side chains underwent unimolecular pH-induced collapse near the pK_a of PDEAEMA as evidenced by DLS studies, and the shape transition from the extended starlike state to the collapsed globular state was observed by AFM. While the brushes consisting of PDMAEMA and PEO side chains did not display a pronounced pH-induced collapse, both sets of brushes were able to undergo collapse induced by certain chaotropic anions, as observed by DLS and AFM. PDEAEMA-containing brushes were found to be more sensitive to moderately chaotropic anions, while neither brush system showed a significant size or shape transition for the tested kosmotropic anions.

5.2. Experimental Section

5.2.1. Materials

2-(*N,N*-Dimethylamino)ethyl methacrylate (DMAEMA, 98%, TCI) and 2-(*N,N*-diethylamino)ethyl methacrylate (DEAEMA, 98.5%, TCI) were passed through a basic alumina/.

Scheme 5.1. Synthesis of Tertiary Amine-Containing Binary Heterografted Star Molecular Brushes, SMB-1 and SMB-2, via Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) Click Reaction between Azide-Bearing Star Backbone Polymer and Alkyne End-Functionalized Side Chain Polymers



silica gel column to remove the inhibitor prior to use. Poly(ethylene glycol) methyl ether (PEO, 5 kDa, Sigma-Aldrich), was dried under high vacuum at 75 °C for 3 h prior to use. Copper(I) chloride (CuCl, 99%, Sigma-Aldrich) was stirred in glacial acetic acid overnight, collected by vacuum filtration, washed thoroughly on the filter with absolute ethanol followed by diethyl ether, and then dried under high vacuum. 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA, 97%, Sigma-Aldrich) was purified by vacuum distillation. The preparation of the azide-functionalized star backbone polymer with a DP of 434 per arm (PHEMA-N₃) is described in Chapter 3. Propargyl 2-bromoisobutyrate (PBiB) was synthesized according to the procedure described in the literature.¹⁹ All other chemicals were purchased from either Sigma-Aldrich or Fisher and used as received.

5.2.2. General Characterization

The molecular weights and dispersities of the azide-bearing backbone polymer, PHEMA-N₃, and the alkyne end-functionalized tertiary amine-containing side chain polymers, PDMAEMA and PDEAEMA, were determined by size exclusion chromatography (SEC), performed at 50 °C using a PL-GPC 50 Plus system (an integrated GPC/SEC system from Polymer Laboratories, Inc.) with a differential refractive index detector, one PSS GRAL 10 µm guard column (50 × 8 mm, Polymer Standards Service-USA, Inc.), and two PSS GRAL 10 µm linear columns (each 300 × 8 mm, linear range of molecular weight from 500 to 1 000 000 Da, Polymer Standards Service-USA, Inc.). *N,N*-Dimethylformamide (DMF), containing 50 mM LiBr, was used as the eluent at a flow rate of 1.0 mL/min.

SEC of the alkyne end-functionalized PEO side chain polymer was performed at ambient temperature using a PL-GPC 20 (an integrated GPC/SEC system from Polymer Laboratories, Inc.) with a differential refractive index (RI) detector, one PLgel 5 µm guard column (50 × 7.5 mm,

Agilent Technologies, Inc.), and two PLgel 5 μ m mixed-C columns (each 300 $\times$ 7.5 mm, linear range of molecular weights from 200 to 2 000 000 Da, Agilent Technologies, Inc.). The mobile phase was tetrahydrofuran (THF) set at a flow rate of 1.0 mL/min, and the system was calibrated with a set of narrow disperse linear polystyrene standards (Scientific Polymer Products, Inc.).

The absolute molecular weights of the purified star brushes, SMB-1 and SMB-2, were determined by a GPC-MALS system comprising one PSS GRAM 10 μ m guard column (8 $\times$ 50 mm, Polymer Standards Service-USA, Inc.), three PSS GRAM 10 μ m linear columns (8 $\times$ 300 mm; 100, 1 000, and 3 000 Å, Polymer Standards Service-USA, Inc.), an Agilent model 1260 Infinity pump, a Rheodyne model 7725 manual injector with a 200 μ L loop, and a Varian 390 LC detector system consisting of an RI detector and a two-angle light scattering detector (15° and 90°). The mobile phase was *N,N*-dimethylformamide (DMF) with 50 mM LiBr, and the analysis was carried out at 50 °C.

¹H NMR spectra were recorded on either a Varian VNMRs 500 NMR, or a Varian VNMRs 600 NMR spectrometer using the residual solvent proton signal as the internal standard. ¹H NMR spectroscopy was also employed to study the responsive properties of the binary heterografted three-arm star molecular brushes, SMBs, in aqueous solution containing various amounts of either potassium ferricyanide or sodium chloride. Aqueous solutions of the brushes with a concentration of 4.0 mg/g were prepared using D₂O as the solvent. The pH was measured with a pH meter (AB-15, calibrated using pH = 4.01, 7.00, and 10.01 standard buffer solutions at room temperature), and adjusted to a pH of 4.00 using 0.1 M HCl and 0.1 M NaOH solutions prepared in D₂O. For the experiments involving the addition of sodium chloride, after each spectrum was recorded (64 scans), the contents of the NMR tube were poured into a vial and the appropriate amount of solid NaCl was added to achieve the desired concentration and the solution was stirred for 5 min before

it was transferred to the NMR tube and a new spectrum was recorded. For the experiments involving the addition of potassium ferricyanide a similar procedure was followed, except that the salt was added via an adjustable pipet from a 0.1 M solution prepared in D₂O.

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

CH₃O-PEO-OH (6.016 g, 1.20×10^{-3} mol, 5000 g/mol) was added to a 50 mL two-necked round bottom flask equipped with a magnetic stir bar and dried at 75 °C under high vacuum for 2 h to remove as much absorbed water as possible. The PEO was then cooled to room temperature and dissolved in methylene chloride (10 mL). 4-Pentynoic acid (0.150 g, 1.53×10^{-3} mol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, 0.285 g, 1.49×10^{-3} mol), and 4-(dimethylamino)pyridine (DMAP, 0.018 g, 1.5×10^{-4} mol) were dissolved in methylene chloride (15 mL) and transferred to an addition funnel. The solution of 4-pentynoic acid, EDC, and DMAP was added dropwise to the flask containing the PEO solution while stirring under nitrogen in an ice/water bath; the mixture naturally warmed to room temperature and was stirred overnight. The reaction mixture was then diluted with methylene chloride (150 mL) and washed with 1 M NaOH three times, followed by a mixture of pure water and a saturated NaCl solution (1/1, v/v) twice. The organic phase was collected and dried over sodium sulfate, gravity filtered, and concentrated by rotary evaporation. The polymer was further purified by precipitation from methylene chloride into diethyl ether three times and dried under high vacuum to yield a white, powdery polymer (5.601 g, 91.3 %). ¹H NMR δ (ppm, CDCl₃): 4.21 (t, HC≡CCH₂CH₂COOCH₂- of the alkyne end group, 2H), 3.79-3.47 (m, -OCH₂CH₂- of PEO repeat units), 3.37 (s, -OCH₃ of the methyl end group, 3H), 2.60-2.47 (m, HC≡CCH₂CH₂COOCH₂- of the alkyne end group, 4H), 1.98 (t, HC≡CCH₂CH₂COOCH₂- of alkyne end group, 1H). SEC analysis relative to polystyrene standards using Agilent Mixed-C columns and THF as eluent: M_{n, SEC} = 8300 Da and *D* = 1.04.

5.2.4. Synthesis of Alkyne End-Functionalized Tertiary Amine-Containing Side Chain Polymers (PDMAEMA and PDEAEMA) by ATRP

5.2.4.1. Synthesis of Alkyne End-Functionalized PDMAEMA

Propargyl 2-bromoisobutyrate (48.8 mg, delivered via 1.204 g of a 40.5 mg/g stock solution in anisole, 2.38×10^{-4} mol), DMAEMA (3.009 g, 1.914×10^{-2} mol), HMTETA (59.6 mg, 2.59×10^{-4} mol), CuCl (22.3 mg, 2.25×10^{-4} mol), and anisole (3.893 g) were added to a 50 mL two-necked round bottom flask equipped with a magnetic stir bar, a rubber septum, and a gas flow adapter. The mixture was degassed by three freeze-pump-thaw cycles and then immersed in an oil bath with the temperature preset at 50 °C. The polymerization was stopped after 2.5 h by opening the flask to air, taking a sample for ^1H NMR analysis, and then diluting the mixture with methylene chloride. The copper catalyst was removed by passing the mixture through a column of basic alumina (top)/silica gel (bottom) with excess methylene chloride as eluent. The crude solution was concentrated by rotary evaporation and the polymer was purified by precipitation from methylene chloride into hexanes in an ice/water bath three times. The purified polymer was collected and dried under high vacuum, yielding a glassy, white polymer (1.502 g, 49.9%). The monomer conversion was 56.0%, determined by ^1H NMR spectroscopy analysis of the final polymerization mixture by comparing the integrals of the peaks at 4.32-4.23 ppm ($-\text{COOCH}_2-$ of the monomer) and at 4.18-4.01 ppm ($-\text{COOCH}_2-$ of the polymer). The degree of polymerization of PDMAEMA was calculated to be 45 by using the monomer conversion and the monomer-to-initiator feed ratio. SEC analysis relative to polystyrene standards using PSS GRAL columns and DMF with 50 mM LiBr as eluent at 50 °C: $M_{n, \text{SEC}} = 11.2$ kDa and $D = 1.17$.

5.2.4.2. Synthesis of Alkyne End-Functionalized PDEAEMA

Propargyl 2-bromoisobutyrate (134.3 mg, 6.55×10^{-4} mol), DEAEMA (6.113 g, 3.29×10^{-2}

mol), HMTETA (153.2 mg, 6.65×10^{-4} mol), CuCl (64.3 mg, 6.5×10^{-4} mol), and anisole (9.043 g) were added to a 50 mL two-necked round bottom flask and degassed by three freeze-pump-thaw cycles. The flask was immersed in an oil bath with the temperature preset at 50 °C and the polymerization proceeded under nitrogen while stirring. After 3.75 h, the flask was removed from the oil bath and opened to air, an aliquot was withdrawn for ^1H NMR spectroscopy analysis and the polymerization mixture was diluted with methylene chloride. The copper catalyst was removed by passing the mixture through a column of basic alumina (top)/silica gel (bottom). The polymer was purified by precipitating the concentrated polymer solution into hexanes from methylene chloride in a dry ice/acetone bath (-78 °C) four times followed by drying under high vacuum to yield a slightly yellow, rubbery polymer (4.390 g, 71.8%). The monomer conversion was determined to be 82.7% using ^1H NMR spectroscopy analysis of the final polymerization mixture by comparing the integrals of the peaks at 4.32-4.17 ppm (-COOCH₂- of the monomer) and at 4.17-3.98 ppm (-COOCH₂- of the polymer). Using the monomer conversion as well as the initial monomer-to-initiator ratio, the DP of the polymer was calculated to be 42. SEC analysis results relative to polystyrene standards using PSS GRAL columns with DMF (containing 50mM LiBr) as eluent: $M_{n, \text{SEC}} = 10.7$ kDa and $D = 1.18$.

5.2.5. Synthesis of Binary Heterografted Star Molecular Brushes with PEO and PDMAEMA Side Chains (SMB-1)

PHEMA-N₃ (8.01 mg, 3.32×10^{-5} mol of -N₃ groups assuming quantitative functionalization, delivered via 0.706 g of 11.34 mg/g stock solution in THF), alkyne end-functionalized PEO (90.9 mg, 1.78×10^{-5} mol), alkyne end-functionalized PDMAEMA (131.8 mg, 1.82×10^{-5} mol), CuCl (3.5 mg, 3.5×10^{-5} mol), and THF (7 mL) were added to a 20 mL scintillation vial equipped with a magnetic stir bar. The vial was sealed with a rubber septum and purged with nitrogen through

needles for 15 min. PMDETA (10 μ L, 4.79×10^{-5} mol) was injected via a microsyringe. After the purging with nitrogen was continued for 15 min, the needles were removed. The reaction was stirred under nitrogen for 23 h at ambient temperature. Propargyl benzyl ether (25 μ L) was injected to cap unreacted azide groups on the backbone and the reaction continued for 3.5 h. A sample was withdrawn for SEC analysis, and then the reaction mixture was diluted with methylene chloride and passed through a basic alumina (top)/silica gel (bottom) column to remove the catalyst. Rotary evaporation was used to concentrate the brush solution to ~ 10 mL; ethanol was added (50 mL) and the solution was concentrated by rotary evaporation again. This process was repeated three times to remove methylene chloride and THF. The brush solution in ethanol was diluted with Milli-Q water. Unreacted side chain polymer was removed by 7 rounds of centrifugal filtration at 5000 rpm using a 50 kDa MWCO filter and a mixture of water and ethanol as solvent (50/50, v/v). SEC analysis (PSS GRAL columns with DMF containing 50 mM LiBr as eluent) confirmed the complete removal of unreacted side chain polymer.

5.2.6. Synthesis of Binary Heterografted Star Molecular Brushes with PEO and PDEAEMA Side Chains (SMB-2)

PHEMA- N_3 (8.25 mg, 3.42×10^{-5} mol of $-N_3$ groups assuming quantitative functionalization, delivered via 0.7275 g of 11.34 mg/g stock solution in THF), alkyne end-functionalized PEO (100.7 mg, 1.97×10^{-5} mol), alkyne end-functionalized PDEAEMA (174.8 mg, 2.20×10^{-5} mol), CuCl (3.1 mg, 3.1×10^{-5} mol), and THF (6 mL) were added to a 20 mL scintillation vial equipped with a magnetic stir bar. The vial was sealed with a rubber septum and the headspace was purged with nitrogen through needles for 15 min., followed by the injection of PMDETA (9.0 μ L, 4.31×10^{-5} mol) via a microsyringe. The purging with nitrogen was continued for 15 min. The needles were then removed, and the reaction proceeded under nitrogen at ambient temperature. After 22 h,

propargyl benzyl ether (25 μ L) was injected in an attempt to cap unreacted azide moieties on the backbone and the reaction continued for another 3 h. The reaction was opened to air, and a sample was withdrawn for SEC analysis; the mixture was diluted with methylene chloride and passed through a column of basic alumina (top)/silica gel (bottom) to remove the catalyst. The crude brush solution was concentrated by rotary evaporation to approximately 10 mL; ethanol (50 mL) was added and the solution was concentrated by rotary evaporation again to remove volatile organic solvents without allowing the brushes to become dry. This process was repeated three times to ensure the removal of methylene chloride and THF. The ethanol solution of the star brushes was diluted with milli-Q water for purification of the molecular brushes by centrifugal filtration. Excess side chain polymers were removed by 9 rounds of centrifugal filtration using a 50 kDa MWCO filter and a mixture of ethanol and water (50/50, v/v) at 5000 rpm. The complete removal of excess side chain polymer was confirmed by SEC analysis using PSS GRAL columns with DMF (containing 50 mM LiBr) as eluent.

5.2.7. Dynamic Light Scattering (DLS) Study of SMB-1 and SMB-2 in Aqueous Solutions

DLS of SMB-1 and SMB-2 was performed using a Malvern Zetasizer Nano ZS instrument equipped with a He-Ne 633 nm laser and a temperature controller at a scattering angle of 173°. Each DLS sample was prepared by transferring a known amount of a stock solution containing the brushes in water to a vial and diluting to the desired concentration (0.1 or 0.2 mg/g) with Milli-Q water or 10 mM phosphate buffer. The pH was adjusted to 4.00 and stirred at room temperature overnight to ensure complete dissolution of the brush molecules. To study the pH-responsive properties of the molecular brushes, 0.2 mg/g solutions were prepared in a 10 mM phosphate buffer and the pH was gradually adjusted using 0.1 M NaOH and 0.1 M HCl, monitored by an Accumet AB-15 pH meter (calibrated at room temperature using standard buffer solutions with pH = 4.01,

7.00, and 10.01) and allowed to equilibrate for 15 min. A portion of the solution was then transferred to a DLS cuvette without filtering and loaded into the instrument with a preset temperature of 25 °C. The DLS sample equilibrated for an additional 5 min before measurements were taken.

To study the effect of various salts on the hydrodynamic size of the star brushes, dilute solutions (0.1 mg/g) were prepared with Milli-Q water and the pH was adjusted to 4.00. In total, six salts were studied (potassium ferricyanide, potassium persulfate, sodium perchlorate, sodium thiocyanate, sodium bromide, and sodium chloride). Potassium ferricyanide and potassium persulfate were added via adjustable pipets from 0.1 M solutions prepared in Milli-Q water; the dilution of the brush sample was accounted for when determining the concentration of the salt in the DLS sample. The other salts were added via 0.1 M solutions prepared in Milli-Q water up to a concentration of 10 mM salt in the brush solution, then the solid salt was added to achieve higher concentrations to avoid excessive dilution of the molecular brushes. Each DLS data point presented is the average result of three measurements (each measurement is comprised of 10 runs) and the error bars show the standard deviation from the average.

5.2.8. Atomic Force Microscopy (AFM) Study of SMB-1 and SMB-2

The star molecular brushes were studied by AFM using a Digital Instruments Multimode IIIa Scanning Probe Microscope in tapping mode under ambient conditions. Reflective Al-coated Si probes (Budget Sensors) with a resonant frequency of 300 kHz and a force constant of 40 Nm⁻¹ were used. Mica substrates were prepared by cleaving a fresh layer of mica disk (Ted Pella, Inc.) with Scotch tape. Molecular brushes prepared at a concentration of 0.01 mg/g in THF were spin cast onto the freshly cleaved mica surface at a spin rate of 3000 rpm. Glass substrates were prepared by thoroughly cleaning glass disks (Ted Pella, Inc.) with a piranha solution followed by

rinsing with Milli-Q water and finally storing the disks in isopropanol. To study the pH-induced transitions of SMB-1 and SMB-2 aqueous solutions of the star molecular brushes prepared in Milli-Q water at a concentration 0.05 mg/g were used to spin cast the brushes onto the glass substrates at 3000 rpm. To study the chaotropic anion-induced shape transitions, aqueous solutions of SMB-1 and SBM-2 were prepared in milli-Q water at a concentration of 0.05 mg/g and a pH of 4.00, then an appropriate amount of ferricyanide stock solution in water (0.1 M) was added to make the final ferricyanide concentration of 0.14 mM and the solutions were stirred for 10 min. Two drops of this solution were then added to a glass disk and the solution was immediately removed with a steady stream of nitrogen; the nitrogen was flowed over the disk continuously for 30 s until the disk was dry.

5.3. Results and Discussion

5.3.1. Synthesis of Azide-Bearing Star Backbone Polymer and Alkyne End-Functionalized Side Chain Polymers

Star molecular brushes, SMB-1 and SMB-2, were synthesized using a grafting-to method, employing the copper-catalyzed azide-alkyne cycloaddition (CuAAC) click reaction. The three-arm star backbone polymer containing pendant azide moieties, PHEMA-N₃, with a degree of polymerization (DP) of 434 per arm, a dispersity of 1.16, and a degree of azide functionalization of 88.3 %, was prepared as described in Chapter 3. In short, an ATRP of 2-(trimethylsilyloxy)ethyl methacrylate was conducted using a tri-functional initiator, followed by post-polymerization reactions to install azide functional groups on the backbone. The post-polymerization modifications included the removal of trimethylsilyl protecting groups using potassium fluoride and tetrabutylammonium fluoride, subsequent esterification of the deprotected polymer with 4-bromobutyryl chloride, and finally substitution of the pendant bromide groups with azide via

reaction with sodium azide. The molecular characterization data PHEMA-N₃ is presented in Chapter 3.

Alkyne end-functionalized poly(ethylene oxide) (PEO) with a molecular weight of 5000 Da was prepared according to a previously reported procedure.¹⁹ Briefly, the hydroxyl end group of poly(ethylene oxide) monomethyl ether with a DP of 114 (5000 Da) was reacted with 4-pentynoic acid in a Steglich esterification, using *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride and dimethylamino pyridine as catalyst. SEC analysis showed the polymer as single, narrow peak with an $M_{n, SEC}$ of 8.3 kDa and $\bar{D}$ of 1.04, relative to polystyrene standards (Figure D1A). ¹H NMR spectroscopy analysis of the purified polymer showed quantitative end group functionalization, as evidenced by the appearance of peaks at 4.21 ppm (t, HC≡CCH₂CH₂COOCH₂- of the alkyne end group, 2H), 2.60-2.47 ppm (m, HC≡CCH₂CH₂COOCH₂- of the alkyne end group, 4H), and 1.98 ppm (t, HC≡CCH₂CH₂COOCH₂- of alkyne end group, 1H) (Figure D1B).

Alkyne end-functionalized tertiary amine-containing side chain polymers, PDMAEMA and PDEAEMA, were synthesized by ATRP of respective monomers, employing propargyl 2-bromoisobutyrate (PBiB) as initiator and CuCl/HMTETA as the catalyst/ligand. Both polymerizations were carried out in anisole at 50 °C; they were monitored by ¹H NMR spectroscopy and stopped when the monomer conversion had reached a desired value (56.0 % for PDMAEMA and 82.7 % for PDEAEMA). SEC analysis of the polymers indicated that the polymerizations were well controlled, with single narrow peaks; for PDMAEMA, $M_{n, SEC}$ = 11.2 kDa and the dispersity ($\bar{D}$) = 1.17 (Figure D2A), and for PDEAEMA, $M_{n, SEC}$ = 10.7 kDa and $\bar{D}$ = 1.18 (Figure D3A), relative to polystyrene standards. The DPs for PDMAEMA and PDEAEMA were calculated to be 45 and 42, respectively, by using the monomer conversions determined via

^1H NMR spectroscopy analysis and the monomer-to-initiator feed ratios. Both polymers were purified by repeated precipitation from methylene chloride into hexanes (at 0 °C for PDMAEMA and at -78 °C for PDEAEMA), dried under high vacuum, and then stored as stock solutions in THF. ^1H NMR spectra for PDMAEMA and PDEAEMA are included in Appendix D (Figures D2B and D3B, respectively). The characterization data for the three alkyne end-functionalized side chain polymers are summarized in Table 5.1.

5.3.2. Synthesis of SMBs

Binary heterografted star molecular brushes consisting of water soluble PEO side chains and either PDMAEMA (SMB-1) or PDEAEMA (SMB-2) side chains were synthesized using a grafting-to method in which alkyne end-functionalized side chain polymers were attached to an azide-bearing star polymer backbone, PHEMA- N_3 , via CuAAC reaction. The reactions were carried out in THF at room temperature under nitrogen using CuCl/PMDETA as the catalyst/ligand. For SMB-1, the feed molar ratio of PHEMA- N_3 repeat units to PEO and PDMAEMA side chains was 1 : 0.55 : 0.54. After 23 h, propargyl benzyl ether was injected in an attempt to cap remaining unreacted azide groups on the backbone. SEC analysis using a GPC system equipped with two PSS GRAL columns with DMF (containing 50 mM LiBr) as eluent showed a high molecular weight peak (Figure D4A), indicating the formation of molecular brushes. The reaction mixture was passed through a basic alumina/silica gel column to remove the catalyst and the purified brushes were obtained after 7 rounds of centrifugal filtration using a 50 kDa MWCO centrifugal filter at 5000 rpm with a mixture of water and ethanol (50/50, v/v) as solvent. The removal of unreacted side chain polymers was confirmed by SEC analysis (Figure D4A). ^1H NMR spectroscopy analysis revealed that SMB-1 was composed of 45.7 mol %

Table 5.1 Characterization Data for Alkyne End-functionalized Side Chain Polymers

Polymer Sample	DP ^a	M _{n,SEC} (kDa) ^b	<i>D</i> ^b
PEO	114	8.3	1.04
PDMAEMA	45	11.2	1.17
PDEAEMA	42	10.7	1.18

^a Degree of polymerization (DP) was calculated from the monomer conversion, determined by ¹H NMR spectroscopy analysis, and the monomer-to-initiator molar ratio. ^b M_{n,SEC} and *D* were measured by SEC relative to polystyrene standards.

PDMAEMA and 54.3 mol % PEO side chains using the integrals of the peaks at 4.25 – 3.94 ppm ($-\text{COOCH}_2-$ of PDMAEMA) and at 3.37 ppm ($-\text{OCH}_3$ of PEO) (Figure 5.1A).

The grafting density of SMB-1 was calculated to be 85.3 % by comparing the SEC peak areas of the molecular brushes (78.0 %) and the remaining side chain polymers (22.0 %) in the final reaction mixture as well as considering the composition of the two side chain polymers in the purified molecular brushes from ^1H NMR spectroscopy analysis; a detailed explanation of this calculation is available in Appendix D. The absolute molecular weight (M_w) of SMB-1 was found to be 9.71×10^6 Da, determined by SEC analysis using a GPC-MALS system equipped with a two-angle light scattering detector in series with an RI detector (Figure 5.1B). Considering the weight-average molecular weight of PHEMA- N_3 and the side chain polymers, calculated from the DP and dispersity ($M_n \times D$), and the grafting density, the calculated M_w for SMB-1 was 7.86×10^6 Da.

Star molecular brushes consisting of PEO and PDEAEMA side chains (SMB-2) were synthesized and purified in a similar manner. SEC analysis of the final reaction mixture showed that the relative peak areas of the molecular brushes and side chains were 68.3 % and 31.7 %, respectively (Figure D4B). ^1H NMR spectroscopy of the purified brushes showed that SMB-2 was comprised of 53.9 mol % PEO and 46.1 mol % PDEAEMA side chains (Figure 5.2A). From this data, the overall grafting density for SMB-2 was calculated to be 84.8%. SEC analysis using a GPC-MALS system gave an experimental M_w of 9.08×10^6 Da, which was close to the calculated M_w of 8.25×10^6 Da (Figure 5.2B). The characterization data for SMB-1 and SMB-2 are summarized in Table 5.2.

AFM imaging was used to confirm the formation of three-arm star molecular brushes by spin casting brushes onto freshly cleaved mica from a solution in THF. Figures 5.3A and 5.3B show

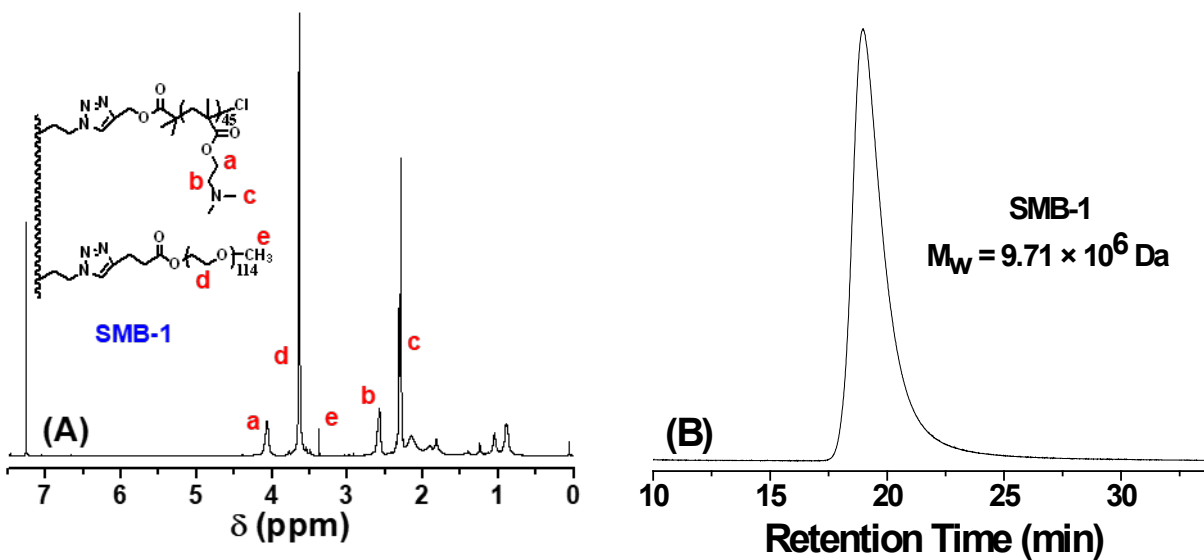


Figure 5.1. (A) ^1H NMR spectrum of the purified star molecular brushes, SMB-1, in CDCl_3 and (B) SEC trace of SMB-1 from a GPC-MALS system.

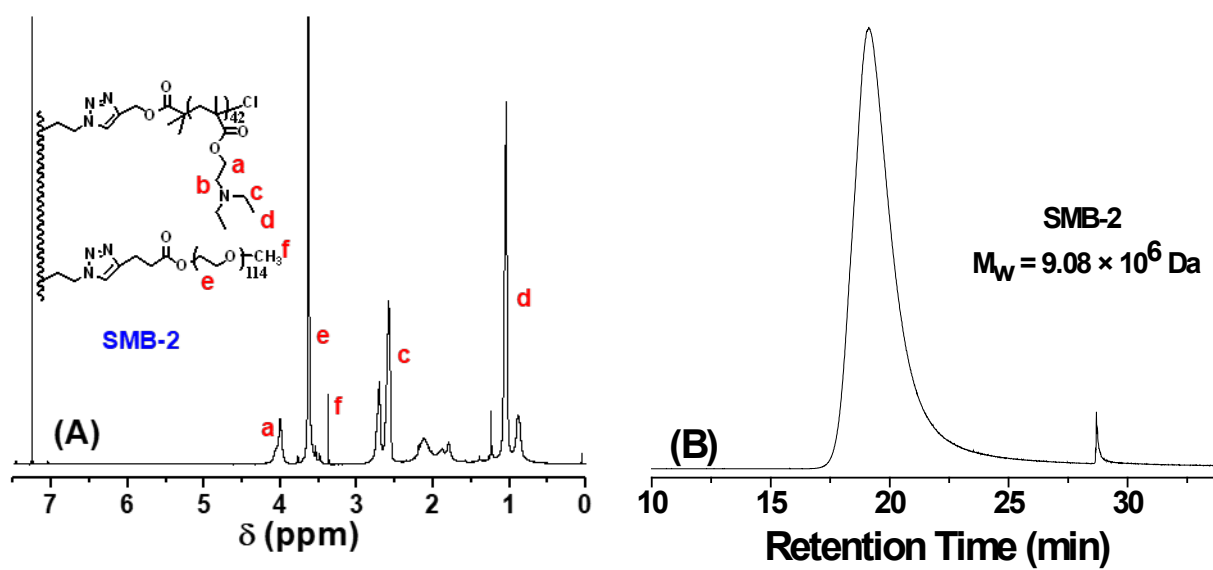


Figure 5.2. (A) ^1H NMR spectrum of the purified star molecular brushes, SMB-2, in CDCl_3 and (B) SEC trace of SMB-2 from a GPC-MALS system.

Table 5.2 Characterization Data for Binary Heterografted Star Molecular Brushes

Molecular Brush Sample	Feed Molar Ratio of Backbone Repeat Units to PEO and Methacrylate Polymer Molecules	Molar Ratio of PEO to Methacrylate Polymer Side Chains in Purified Brushes	Grafting Density (%) ^a	M _w , theoretical (Da) ^b × 10 ⁶	M _w , experimental (Da) ^c × 10 ⁶
PEO/PDMAEMA SMB-1	1 : 0.55 : 0.54	0.543 : 0.457	85.3	7.86 ^b	9.71
PEO/PDEAEMA SMB-2	1 : 0.58 : 0.64	0.539 : 0.461	84.8	8.25 ^b	9.08

^a The grafting density was determined by using the ratio of SEC peak areas of brushes and unreacted side chains in the final mixture and the feed ratio of backbone to side chains, considering the final composition of the brushes determined using ¹H NMR. ^b The theoretical absolute molecular weight (M_{w, theoretical}) was calculated considering the weight-average molecular weight of PHEMA-N₃ and the side chain polymers, calculated from the DP and dispersity (M_n × *D*), and the grafting density. ^c The experimental absolute molecular weight (M_{w, experimental}) was determined by SEC analysis using a GPC-MALS system.

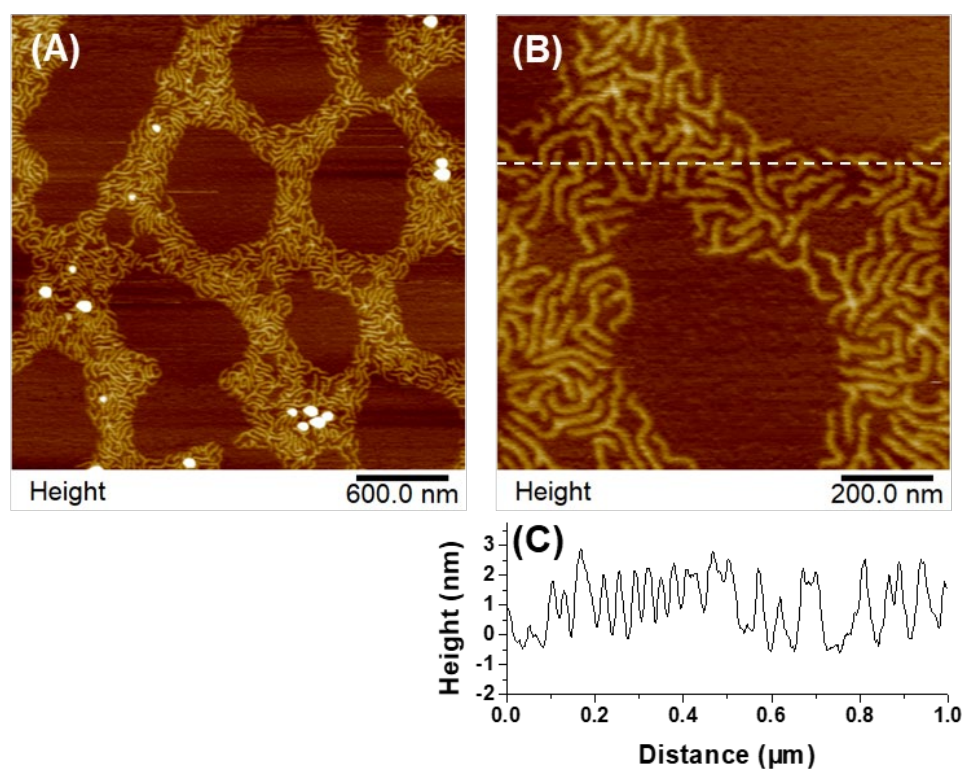


Figure 5.3. (A) and (B) AFM height images of SMB-1 spin cast onto freshly cleaved bare mica from a 0.01 mg/g solution in THF. (C) Cross-sectional height profile along the dashed line in (B).

that most of the SMB-1 brush molecules have three-arms; some of the molecules were either lacking an arm or were short. Using ImageJ software, the average length per arm was 92.9 ± 27.3 nm (300 arms measured). The typical height of the brushes was ~ 1.5 nm (Figure 5.3C). Similarly, the height images of SMB-2 show that a vast majority of the molecules have three arms (Figures 5.4A and 5.4B). The average length per arm for SMB-2 was 94.1 ± 30.3 nm (300 arms measured) and the typical height of the brushes spin cast onto bare mica was ~ 1 nm (Figure 5.4C). Since each arm of the backbone was calculated to have a DP of 434, the measured lengths from the AFM images represent a degree of stretching of 84.2 % for SMB-1 and 85.3 % for SMB-2.

5.3.3. pH-Responsive Behavior of SMBs

The pH-responsive behavior of SMB-1 and SMB-2 in aqueous solution was investigated by DLS and AFM. Figure 5.5A shows the apparent hydrodynamic diameter (D_h) as a function of pH for a 0.2 mg/g solution of SMB-1 in 10 mM KH_2PO_4 buffer at 25 °C. The pH was gradually changed with 0.1 M NaOH and 0.1 M HCl while the solution was stirred at room temperature. Between a pH of 4.00 and 7.50 there was relatively little change in the D_h from 85.2 nm to 82.4 nm. This was followed by a sharp, albeit small, reduction in size to 75.2 nm at a pH of 8.00. Increasing the pH further did not result in a significant change to the D_h , which reached 74.6 nm at a pH of 9.50. Figure 5.5B shows the size distributions for pH = 4.00 and pH = 9.50; both distributions were single, narrow peaks, indicating no aggregation of the bottlebrushes over the pH range tested. The observed size change occurred in the same range as the reported pK_a for PDMAEMA homopolymer (~ 7.4). At pH values below the pK_a , a majority of the tertiary amines are protonated, providing strong ion-dipole interactions with water and keeping the bottlebrushes completely solvated. The electrostatic repulsive interactions between protonated tertiary amine groups of the PDMAEMA side chains caused the brush molecules to further extend in aqueous

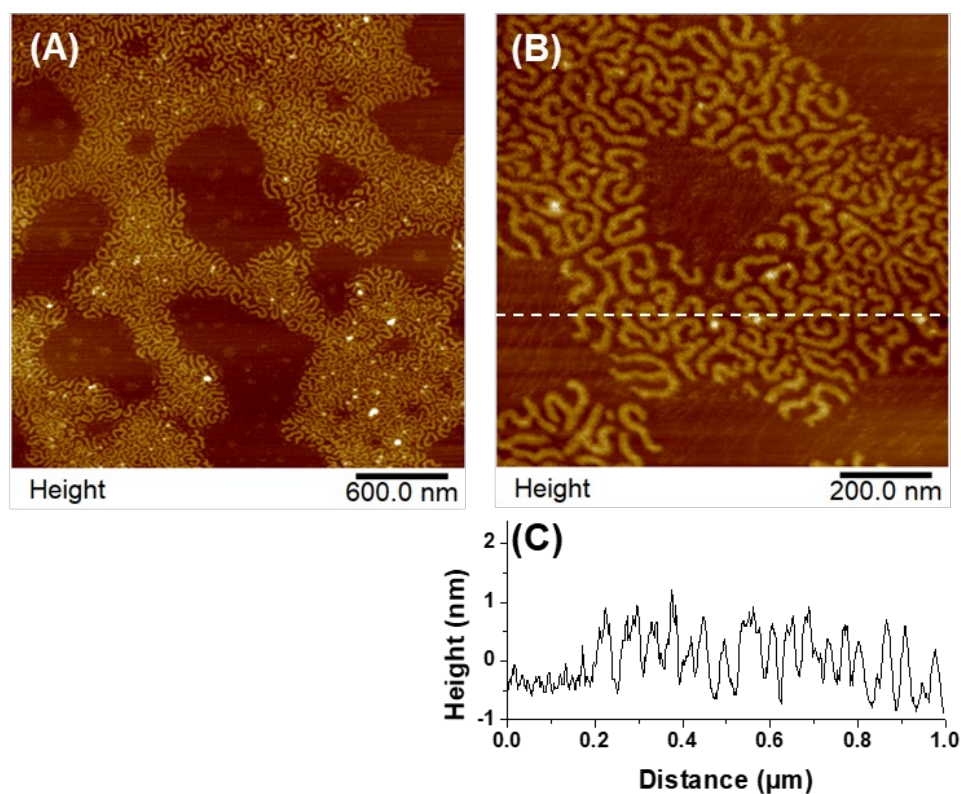


Figure 5.4. (A) and (B) AFM height images of SMB-2 spin cast onto freshly cleaved bare mica from a 0.01 mg/g solution in THF. (C) Cross-sectional height profile along the dashed line in (B).

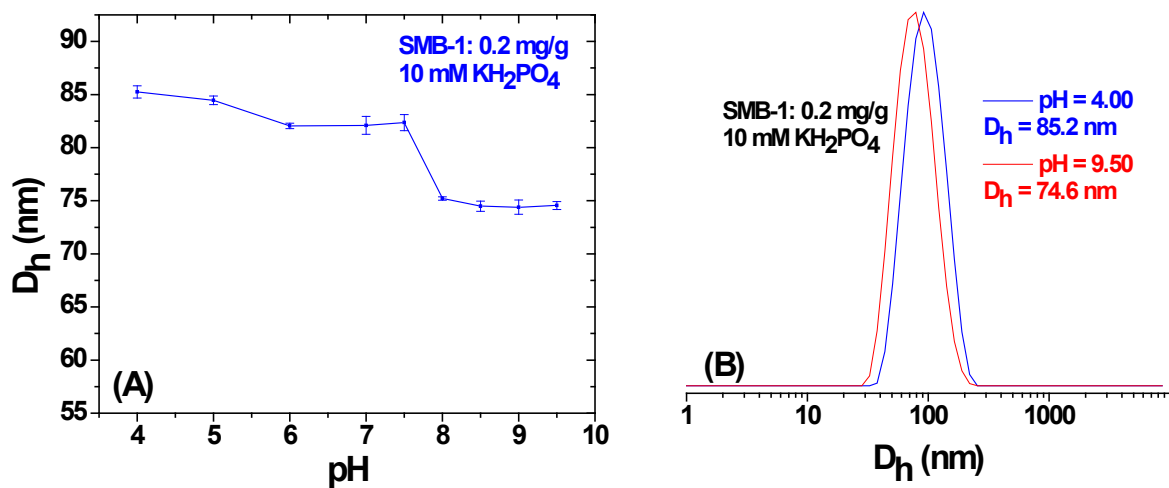


Figure 5.5. (A) Apparent hydrodynamic diameter (D_h) of SMB-1 at a concentration of 0.2 mg/g in a 10 mM phosphate buffer solution at 25 °C as a function of pH. (B) Hydrodynamic size distributions of SMB-1 at pH = 4.00 and pH = 9.50.

solution. When the pH is increased across the pK_a , most of the PDMAEMA repeat units are neutral tertiary amines, leading to diminishing of electrostatic repulsion between tertiary amine moieties in water and contraction of the brush molecules. This pH-induced change in D_h is only 10.6 nm, which does not represent a full collapse of the brush molecules into a compact globular state. This is because PDMAEMA is a water-soluble polymer in the entire pH range.

Figure 5.6A shows the change in the D_h with increasing pH for SMB-2. When the pH was increased from 3.95 to 6.91, the D_h gradually decreased from 88.2 nm to 82.5 nm followed by sharp decrease to 63.6 nm at a pH of 7.51. Beyond pH 7.51, there was relatively little size change and the D_h leveled off, reaching 59.6 nm at a pH of 9.50. Overall, the pH-induced change in the apparent size from a pH of 3.95 to 9.50 was 28.6 nm. It is worth noting that at all studied pH values the size distributions were single, narrow peaks, indicating unimolecular species with no aggregation throughout the pH range (Figure 5.6B). This pH-induced size change correlates well with the reported pK_a of PDEAEMA around 7.4. At values below the pK_a the PDEAEMA side chains are mostly protonated and solubilized, resulting in the brush molecules being in an extended state. When the pH is raised through the pK_a , the PDEAEMA side chains are deprotonated and the hydrophobic ethyl groups on the amine cause the side chains to become insoluble, driving the brush to collapse into a more compact globular state. Previously we observed that homografted PDEAEMA bottlebrushes underwent aggregation and precipitated from solution even at very low concentrations (< 0.02 mg/g) when the pH was increased to a value above the pK_a . Presently, however, we did not observe aggregation, indicating that the PEO side chains stabilized the collapsed state of the bottlebrushes, preventing aggregation and keeping the brush molecules in solution.

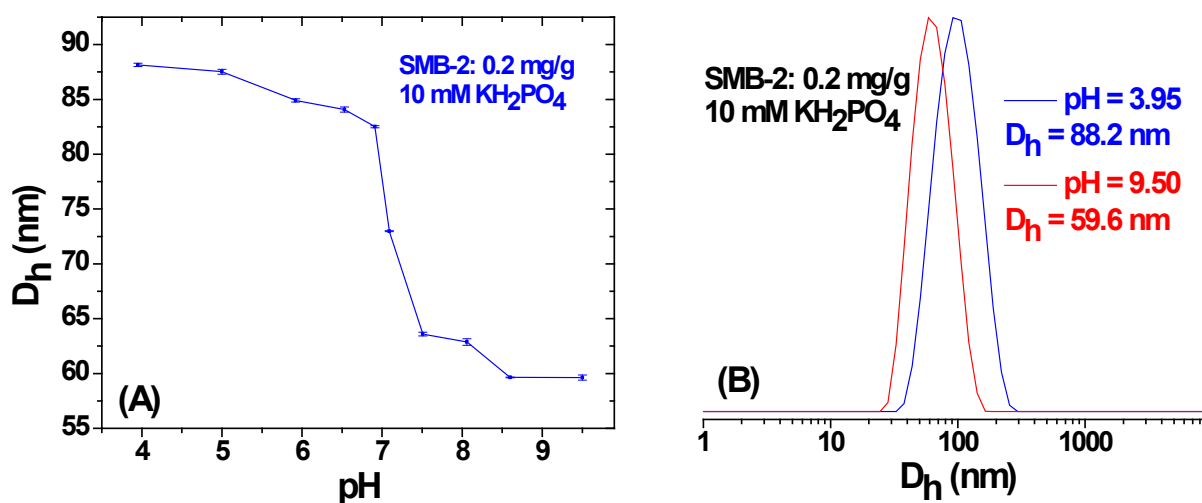


Figure 5.6. (A) Apparent hydrodynamic diameter (D_h) of SMB-2 at a concentration of 0.2 mg/g in a 10 mM phosphate buffer solution at 25 °C as a function of pH. (B) Hydrodynamic size distributions of SMB-2 at pH = 3.95 and pH = 9.50.

AFM imaging was employed to confirm the pH-induced shape transition of SMB-1 and SMB-2 by spin casting the molecular brushes onto glass disks from aqueous solutions. Figures 5.7 and D5 show SMB-1 spin cast onto a glass substrate from a 0.05 mg/g aqueous solution adjusted to a pH of 4.00. As expected, the brushes are in the extended starlike state with an average length per arm of 99.7 ± 24.7 nm (75 arms measured). Figures 5.8 and D6 show SMB-1 spin cast from a 0.05 mg/g aqueous solution at a pH of 9.50 onto a glass substrate. Here the brushes are also in an extended star-like conformation with an average length per arm of 98.9 ± 25.7 nm (75 measured), which was expected since the DLS results showed only a small change in size with increasing pH.

Figures 5.9 and D7 show SMB-2 spin cast from a 0.05 mg/g aqueous solution at a pH of 4.00. As expected, the bottlebrushes are in the extended starlike state and have an average length per arm of 99.8 ± 32.3 nm (75 measured) and a typical height of ~ 2 nm (Figure 5.9C). Some of the brush molecules appear to be missing arms or have unusually short arms, which could be due to the mechanical shear forces present during the spin casting process. Figures 5.10 and D8 show the brushes spin cast from a 0.05 mg/g aqueous solution at a pH of 9.50. The brushes are in a collapsed globular state with an average diameter of 53.4 ± 6.6 nm (100 measured) and a typical height of ~ 6 nm (Figure 5.10C). In some of the spherical objects in Figure 5.8B, it is possible to see the contour of the retracted brush molecule, indicating that the observed species are indeed collapsed bottlebrushes.

5.3.4. Study of SMBs in Aqueous Solution with the Addition of Salts Containing Chaotropic and Kosmotropic Anions

The behavior of SMB-1 and SMB-2 in aqueous solutions containing various salts, ranging from “super-chaotropic” to kosmotropic, was studied by DLS, ^1H NMR spectroscopy, and AFM.

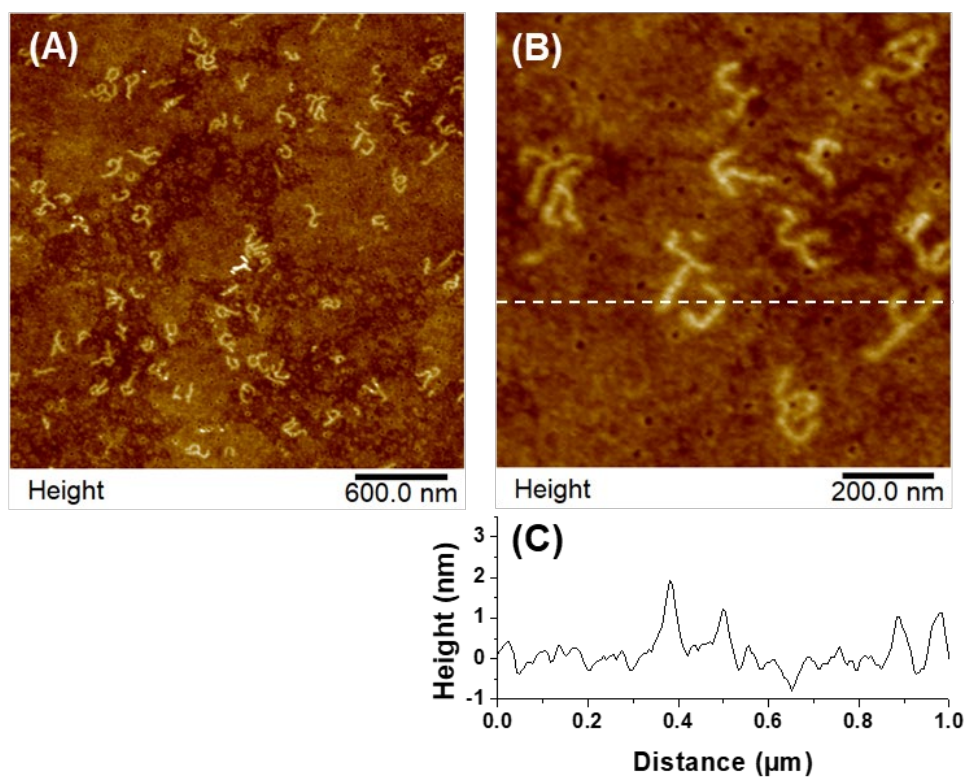


Figure 5.7. (A) and (B) AFM height images of SMB-1 spin cast from a 0.05 mg/g solution adjusted to pH = 4.00 (no buffer) onto a glass disk. (C) Cross-sectional height profile along the dashed line in (B).

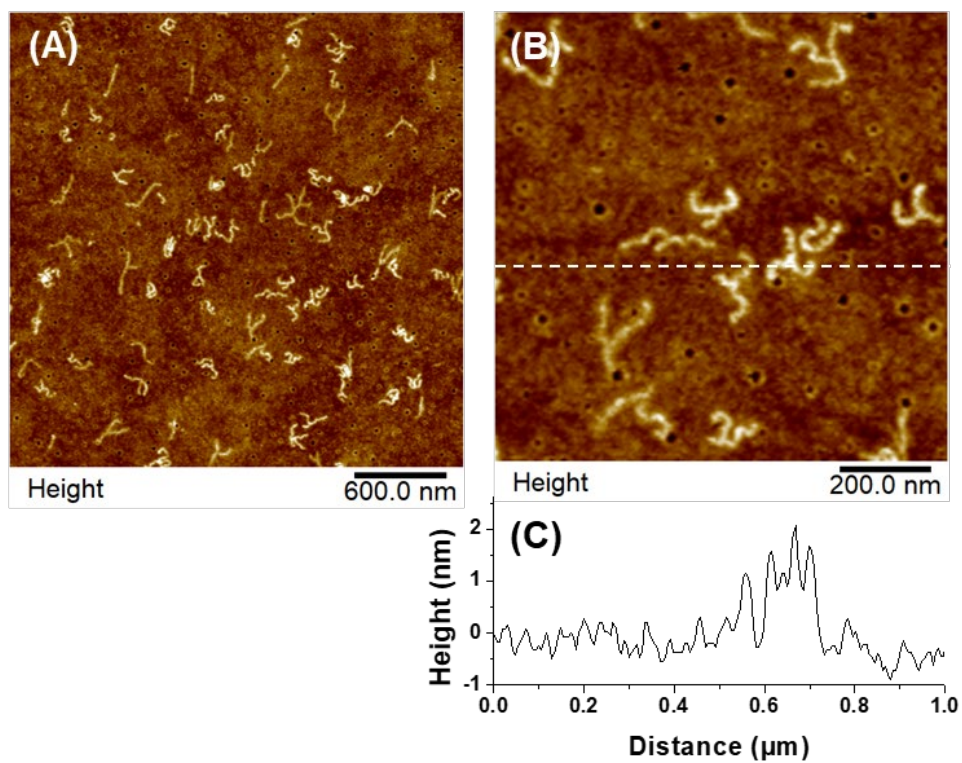


Figure 5.8. (A) and (B) AFM height images of SMB-1 spin cast from a 0.05 mg/g solution adjusted to pH = 9.50 (no buffer) onto a glass disk. (C) Cross-sectional height profile along the dashed line in (B).

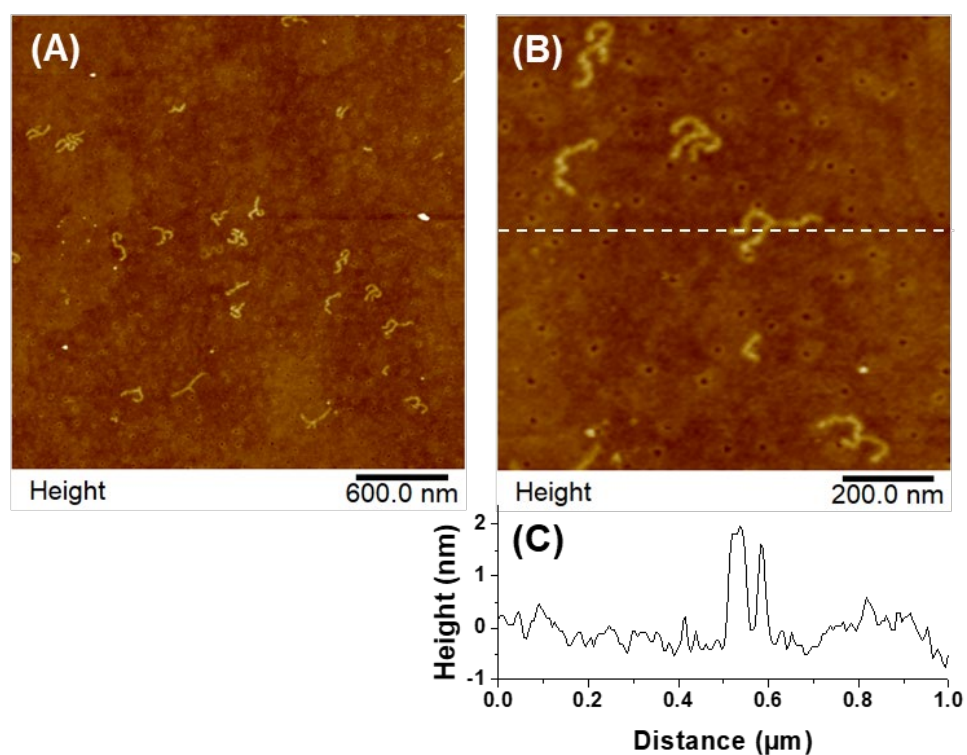


Figure 5.9. (A) and (B) AFM height images of SMB-2 spin cast from a 0.05 mg/g solution adjusted to pH = 4.00 (no buffer) onto a glass disk. (C) Cross-sectional height profile along the dashed line in (B).

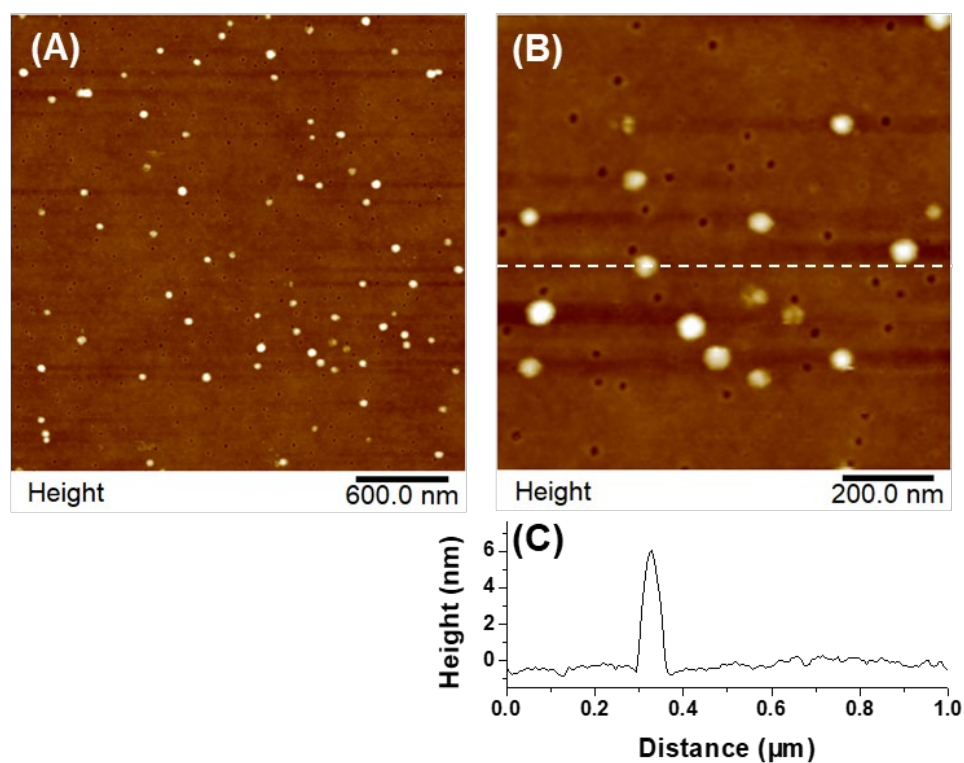


Figure 5.10. (A) and (B) AFM height images of SMB-2 spin cast onto a glass disk from a 0.05 mg/g aqueous solution adjusted to pH = 9.50 (no buffer). (C) Cross-sectional height profile along the dashed line in (B).

Figure 5.11A shows the change in D_h as a function of salt concentration for SMB-1 in aqueous solution with a polymer concentration of 0.1 mg/g at a pH of 4.00. For each of the solutions containing 0.01 mM of the salt, the sizes were very similar (between 90 and 92 nm). However, the salts quickly differentiated themselves with increasing concentration. For the brush solution containing the “super-chaotropic” ferricyanide anions, the size rapidly decreased from 88.9 nm to 55.7 nm upon increasing the salt concentration from 0.05 mM to 0.1 mM, respectively. Interestingly, at a ferricyanide concentration of 0.5 mM, we began to observe large species by DLS in the 4 000 – 5 000 nm range, likely due to the formation of some loose aggregates at the higher salt concentrations (Figure 5.11B). For the persulfate anions, the transition was somewhat broader than with ferricyanide; the size decreased from 88.4 nm to 61.7 nm between a persulfate concentration of 0.1 mM and 0.4 mM. This was followed by a gradual leveling-off of the size to 55.6 nm at 10.0 mM; at concentrations greater than 10 mM, large aggregates began to occur. Perchlorate had a very broad size transition, although the overall size change was similar to those with the addition of ferricyanide and persulfate, from 90.7 nm at 1.0 mM to 56.1 nm at 300 mM; at perchlorate concentrations greater than 300 mM, large aggregates were observed. Thiocyanate, bromide, and chloride all showed much more gradual and overall smaller changes in size before aggregation began to occur; 90.4 nm (0.01 mM) to 74.5 nm (300 mM) for thiocyanate, 91.0 nm (0.01 mM) to 77.8 nm (500 mM), and 91.8 nm (0.01 mM) to 81.2 nm (500 mM) for chloride. These observations follow the trend that more chaotropic anions (ferricyanide, persulfate, and perchlorate) cause large and often dramatic changes in the size of the molecular brushes in aqueous solution, while the moderately chaotropic-to-kosmotropic anions (thiocyanate, bromide, and chloride) cause relatively small changes in the size of the bottlebrushes. Additionally, the large size change before aggregation begins to occur suggests that the PEO side chains are acting to

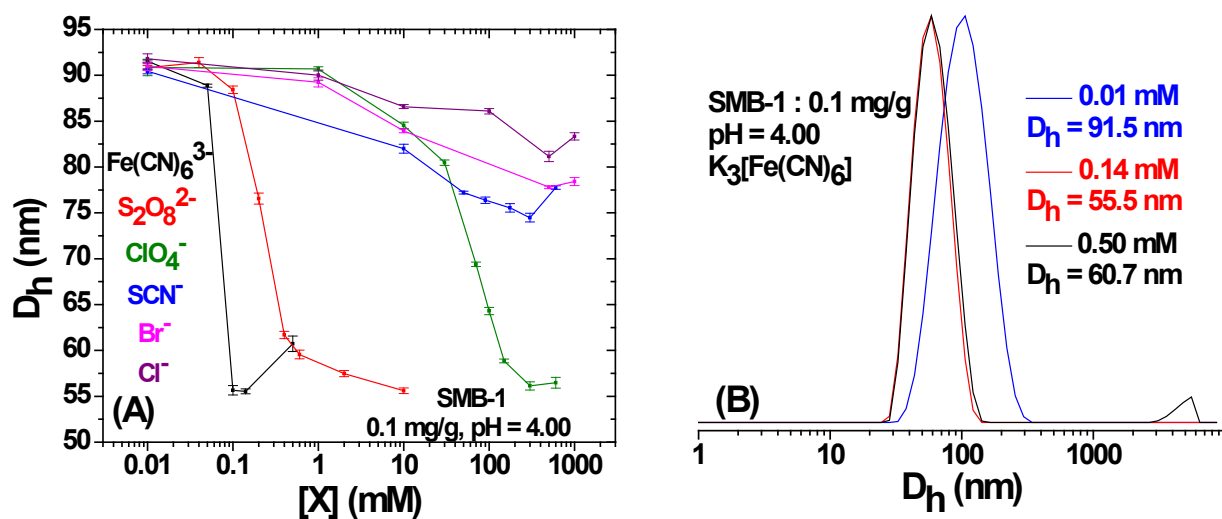


Figure 5.11. (A) Apparent hydrodynamic diameter (D_h) of SMB-1 at a concentration of 0.1 mg/g in various aqueous salt solutions at 25 °C and pH = 4.00 as a function of salt concentration. (B) Hydrodynamic size distributions of SMB-1 in aqueous solution at various potassium ferricyanide concentrations.

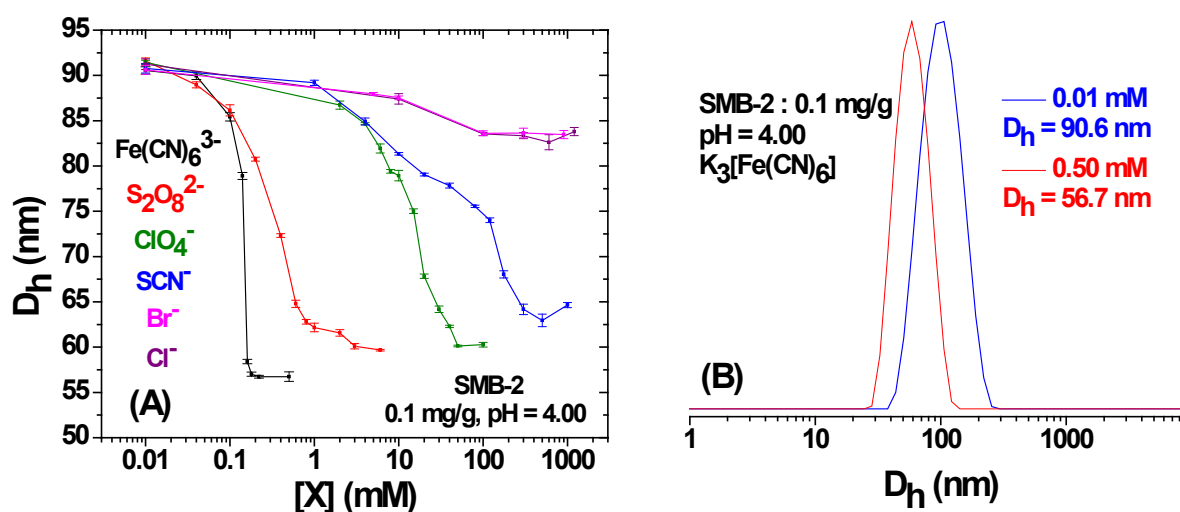


Figure 5.12. (A) Apparent hydrodynamic diameter (D_h) of SMB-2 at a concentration of 0.1 mg/g in various aqueous salt solutions at 25 °C and pH = 4.00 as a function of salt concentration. (B) Hydrodynamic size distributions of SMB-2 in aqueous solution at various potassium ferricyanide concentrations.

stabilize the collapsed brushes, at least until the salt concentration becomes too high and the PEO begins to salt-out. Solutions of SMB-2 were used to study what effect, if any, increasing the alkyl chain length on the nitrogen atom has on the salt-responsive behavior. Figure 5.12A shows the change in D_h with increasing concentrations of the same six salts studied with SMB-1. Interestingly, for SMB-2 solutions, the “super-chaotropic” anions (ferricyanide and persulfate) have size transitions that occur over very similar ranges to those observed for SMB-1; 90.0 nm (0.04 mM) to 57.01 nm (0.18 mM) for ferricyanide, and 89.0 nm (0.04 mM) to 62.2 nm (1.0 mM) for persulfate. However, the size transition for SMB-2 in the presence of perchlorate occurred in a lower concentration range than for SMB-1: between 2.0 mM (86.7 nm) and 50.0 mM (60.1 nm). For SMB-2 in thiocyanate, the size transition was much clearer than with SMB-1, resulting in a gradual size change from 90.8 nm (0.01 mM) to 74.0 nm (120 mM) followed by a steeper decrease to 63.0 nm (500 mM). Bromide and chloride had relatively little effect on the size of SMB-2 in solution; both anions only reduced the size from ~ 91 nm at 0.01 mM to ~ 83 nm at 100 mM before leveling off. The effect of increasing the alkyl chain length from methyl to ethyl is not apparent with the “super-chaotropic” anions (ferricyanide and persulfate) but becomes more evident with the moderately chaotropic anions (perchlorate and thiocyanate). The more hydrophobic ethyl groups increase the sensitivity of the molecular brushes to the chaotropic anions.

^1H NMR spectroscopy was used to confirm our hypothesis in regards to the observations made using DLS; the more chaotropic anions were forming tighter ion pairs with the protonated tertiary amines in the bottlebrush polymers, leading to a decreased solubility or insolubility of the PDMAEMA (for SMB-1) or PDEAEMA (for SMB-2) side chains and the collapse of the brush molecules, stabilized by the soluble PEO side chains. Solutions of SMB-1 and SMB-2 were prepared at a concentration of 4.0 mg/g in D_2O and adjusted to a pH of 4.00. Figure 5.13A shows

the ^1H NMR spectra for the solution of SMB-1 with varying concentrations of ferricyanide. The characteristic peaks for the PDMAEMA side chains are clearly visible before the addition of any potassium ferricyanide at 4.58 – 4.16 ppm [$-\text{OCH}_2\text{CH}_2\text{N}(\text{CH}_3)_2$], 3.67 – 3.41 ppm [$-\text{OCH}_2\text{CH}_2\text{N}(\text{CH}_3)_2$], and 3.20 – 2.83 ppm [$-\text{OCH}_2\text{CH}_2\text{N}(\text{CH}_3)_2$]. Increasing the ferricyanide concentration to 0.1 mM results in broadening of these peaks and by 1.0 mM they are severely diminished. When the concentration is increased above 2.0 mM, SMB-1 precipitated from solution. At all concentrations studied, the characteristic peak for the PEO side chains at 3.72 ppm (s, $-\text{OCH}_2\text{CH}_2-$ of PEO repeat units) remains sharp, only decreasing slightly in intensity relative to the residual solvent proton signal. A control experiment was conducted with the addition of sodium chloride instead of ferricyanide and the PDMAEMA characteristic peaks appeared sharper in the 100 mM NaCl solution compared to the spectra with no added salt (Figure 5.13B).

Similarly, for SMB-2, the characteristic peaks for the PEO side chains at 3.72 ppm (s, $-\text{OCH}_2\text{CH}_2-$ of PEO repeat units) and the PDEAEMA side chains at 3.48 – 3.21 ppm [$-\text{OCH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_3)_2$], and 1.53 – 1.30 ppm [$-\text{OCH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_3)_2$] are in sharp focus before the salt is added (Figure 5.14A). With increasing ferricyanide concentration, the PEO peak remains sharp and at a similar intensity relative to the residual solvent peak, while the PDEAEMA peaks broaden significantly beyond 0.2 mM ferricyanide. As with SMB-1, above 2.0 mM ferricyanide, the molecular brushes begin to precipitate from solution. In the control experiment for SMB-2 involving the addition of sodium chloride, the peaks corresponding to PDEAEMA appear to sharpen at the higher salt concentration (Figure 5.14B).

AFM was used to visualize the size change associated with the addition of ferricyanide to aqueous solutions of SMB-1 and SMB-2 at a pH of 4.00. Figures 5.15 and D9 show AFM height

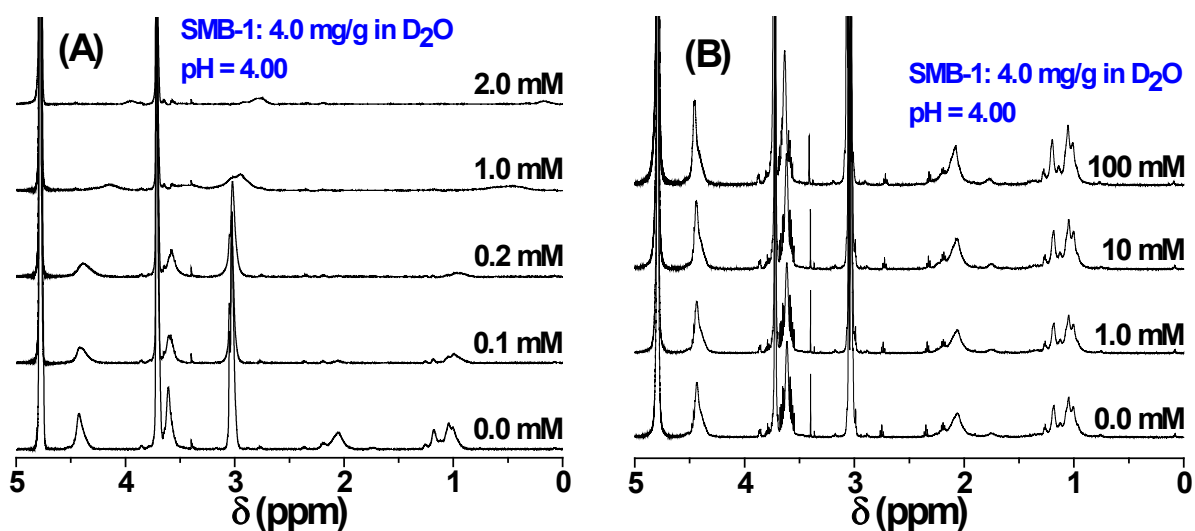


Figure 5.13. ^1H NMR spectra of 4.0 mg/g SMB-1 in aqueous solution prepared in D_2O at a pH of 4.00 and various concentrations of (A) potassium ferricyanide and (B) sodium chloride. All spectra are normalized to the residual H_2O at 4.79 ppm.

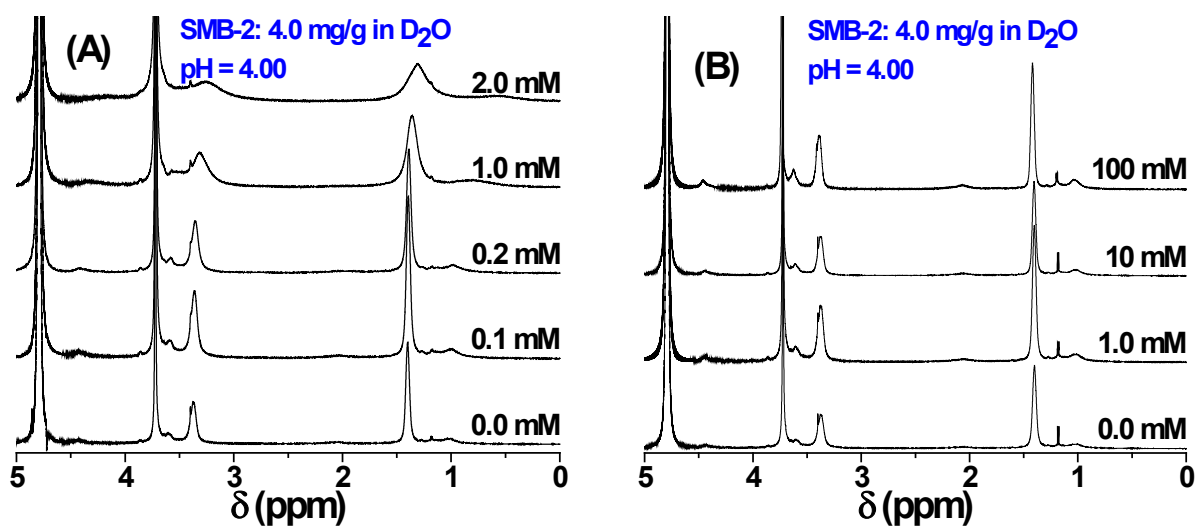


Figure 5.14. ^1H NMR spectra of 4.0 mg/g SMB-2 in aqueous solution prepared in D_2O at a pH of 4.00 and various concentrations of (A) potassium ferricyanide and (B) sodium chloride. All spectra are normalized to the residual H_2O at 4.79 ppm.

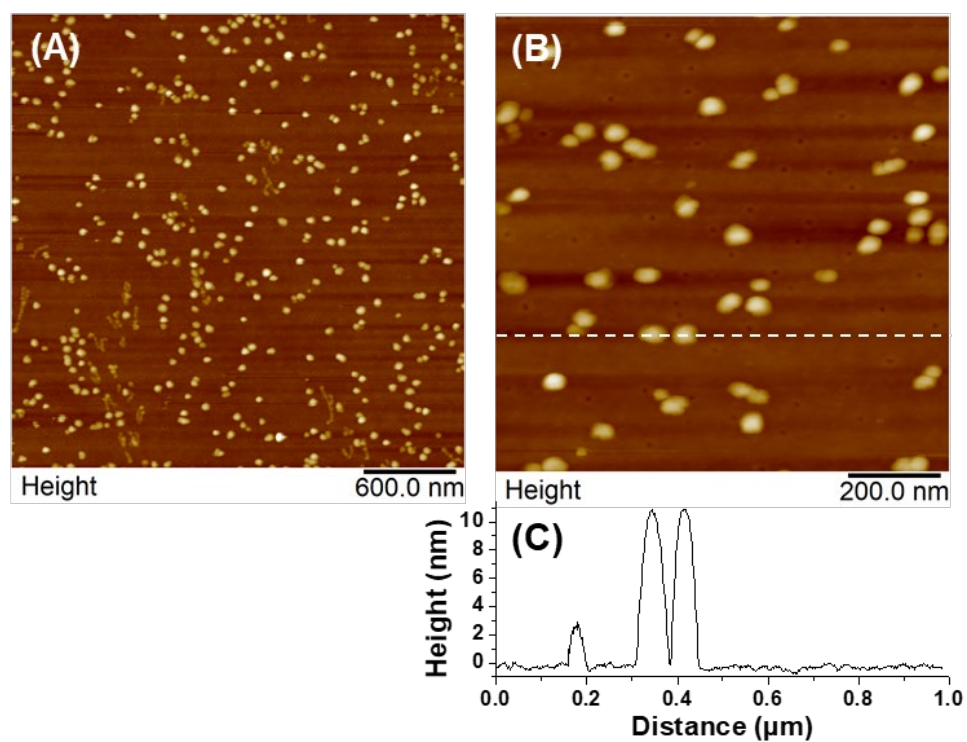


Figure 5.15. (A) and (B) AFM height images of SMB-1 drop cast onto a glass disk from a 0.05 mg/g aqueous solution at pH = 4.00 with 0.14 mM potassium ferricyanide. (C) Cross-sectional height profile along the dashed line in (B).

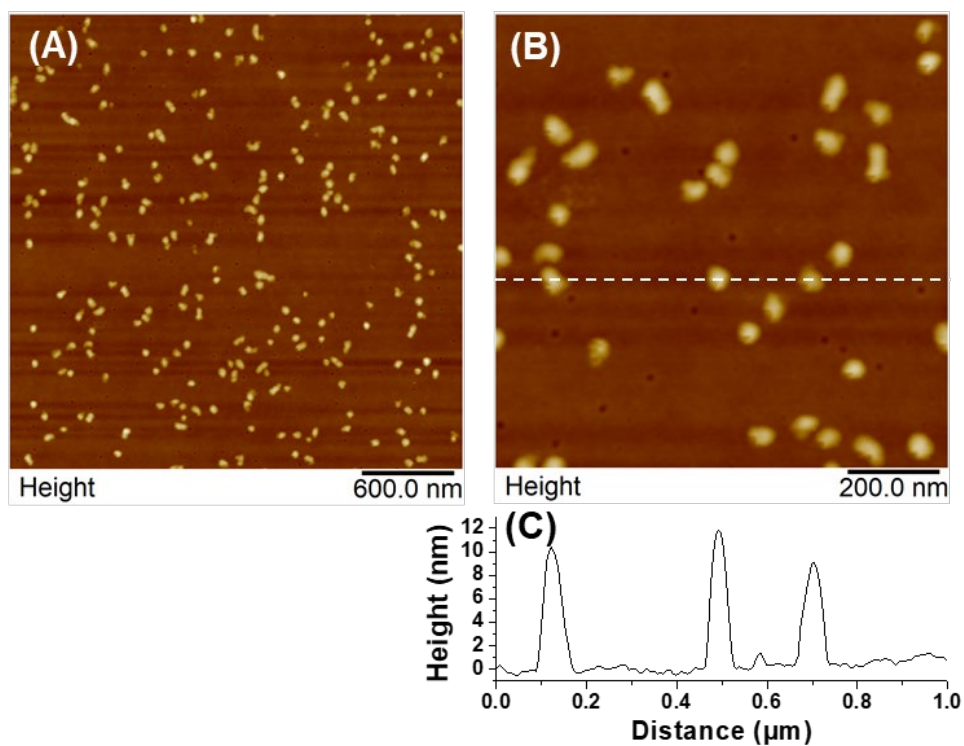


Figure 5.16. (A) and (B) AFM height images of SMB-2 drop cast onto a glass disk from a 0.10 mg/g aqueous solution at pH = 4.00 with 0.14 mM potassium ferricyanide. (C) Cross-sectional height profile along the dashed line in (B).

images of SMB-1 drop cast from dilute solution (0.05 mg/g) in the presence of potassium ferricyanide (0.14 mM) onto a glass disk. The globular nano-objects have an average diameter of 49.7 ± 8.7 nm (100 measured) and a typical height of ~ 11 nm. Figures 5.16 and D10 show the AFM height images of SMB-2 drop cast from a 0.05 mg/g solution at a pH of 4.00 with a potassium ferricyanide concentration of 0.14 mM. The average diameter of the collapsed globules is 57.3 ± 7.2 nm (100 measured) and the typical height is ~ 11 nm. It is worth noting that the AFM samples in this section were prepared on glass disks using a drop casting method in which several drops of the dilute solution were placed onto the disk and then a stream of nitrogen was used to push the solution off and dry the remaining film of water. We chose this method because spin casting the solutions resulted in some of the brush molecules becoming unraveled on the surface, presumably due to the higher shear forces present during the spin coating process.

5.4. Conclusions

In summary, we synthesized binary heterografted three-arm star molecular bottlebrushes (SMB-1 consisting of PEO and PDMAEMA side chains and SMB-2 consisting of PEO and PDEAEMA side chains) via a grafting-to method and demonstrated chaotropic anion-induced shape changes from an extended starlike state to a stable collapsed globular state in aqueous solution. A pH-induced star-globule shape transition was observed for SMB-2 but not SMB-1. The responsive behavior of SMB-1 and SMB-2 in aqueous solutions was studied using DLS, ^1H NMR spectroscopy, and AFM. For the pH-induced transition, SMB-1 showed a small change in size upon raising the pH through the pK_a of the PDMAEMA side chains (~ 7.4). Below the pK_a of PDMAEMA side chains, most of the tertiary amines are protonated and these side chains, as well as the PEO side chains, are well solubilized in aqueous solution. When the pH is increased to values well above the pK_a of PDMAEMA, the size decreases slightly as the electrostatic repulsive

interactions are lessened due to deprotonation of the tertiary amines. As expected, AFM images of SMB-1 samples prepared at both low (4.00) and high (9.50) pH values showed the molecular brushes in the extended star-like state. On the other hand, SMB-2 displayed a large change in size by DLS when the pH was raised across the pK_a of the PDEAEMA side chains (~ 7.4), indicating collapse of the brush molecules into a more compact globular state. AFM imaging confirmed the pH-induced shape transition for SMB-2, showing brush molecules in an extended star-like state at a pH of 4.00 and collapsed, spherical nano-objects at a pH of 9.50. Additionally, DLS studies of SMB-1 and SMB-2 in aqueous salt solutions showed that both brushes exhibited a large decrease in size in the presence of various chaotropic anions. The most dramatic size changes were observed in the presence of “super-chaotropic” anions such as ferricyanide and persulfate; only nominal changes resulted from the addition of the kosmotropic bromide and chloride anions. Moreover, SMB-2 showed greater sensitivity to moderately chaotropic anions (perchlorate and thiocyanate) than SMB-1, indicating a positive relationship between alkyl chain length and anion responsiveness. ^1H NMR was employed to confirm the selective dehydration of the tertiary amine-containing side chains with increasing ferricyanide concentration and AFM imaging showed the shape transition from extended star-like to collapsed globular states for both brushes in the presence of the “super-chaotropic” ferricyanide anion.

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Appendix D

For

Chapter 5: pH- and Chaotropic Anion-Induced Conformational Changes of

Binary Heterografted Three-Arm Star Molecular Bottlebrushes

D.1. Example Grafting Density Calculation:

The grafting density of SMB-1 was calculated to be 85.3 % by comparing the SEC peak areas of the molecular brushes (78.0 %) and the remaining side chain polymers (22.0 %) in the final reaction mixture as well as considering the composition of the two side chain polymers in the purified molecular brushes. ^1H NMR spectroscopy analysis revealed that SMB-1 was composed of 45.7 mol % PDMAEMA and 54.3 mol % PEO side chains using the integrals of the peaks at 4.25 – 3.94 ppm ($-\text{COOCH}_2-$ of PDMAEMA) and at 3.37 ppm ($-\text{OCH}_3$ of PEO). Using the molar mass of PDMAEMA from the DP (7230 g/mol) and PEO (5100 g/mol), the mass percentage was calculated to be 54.4 % for PDMAEMA and 45.6 % for PEO. SEC analysis showed that the relative peak areas of the molecular brushes and side chains were 78.0 % and 22.0 %, respectively. The total mass of reacted side chains was 171.9 mg, determined by multiplying the brush peak area by the feed masses of the side chain polymers and the backbone polymer and then subtracting the mass of the backbone. The masses of reacted PDMAEMA and PEO were found to be 93.5 mg and 78.4 mg, respectively. Using the molar mass of each side chain polymer, the total number of moles of side chain polymers reacted was 2.83×10^{-5} mol. The overall grafting density was determined to be 85.3 % by dividing the number of moles of reacted side chains by the number of moles of available backbone repeat units (assuming complete azide functionalization).

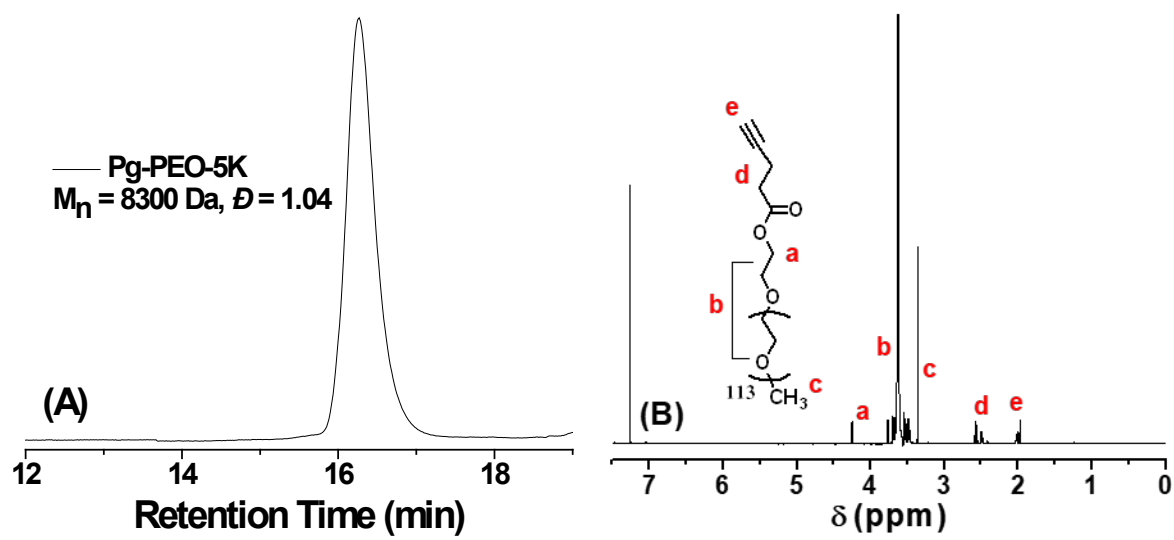


Figure D1. SEC trace (A) and ¹H NMR spectra (B) of alkyne end-functionalized PEO.

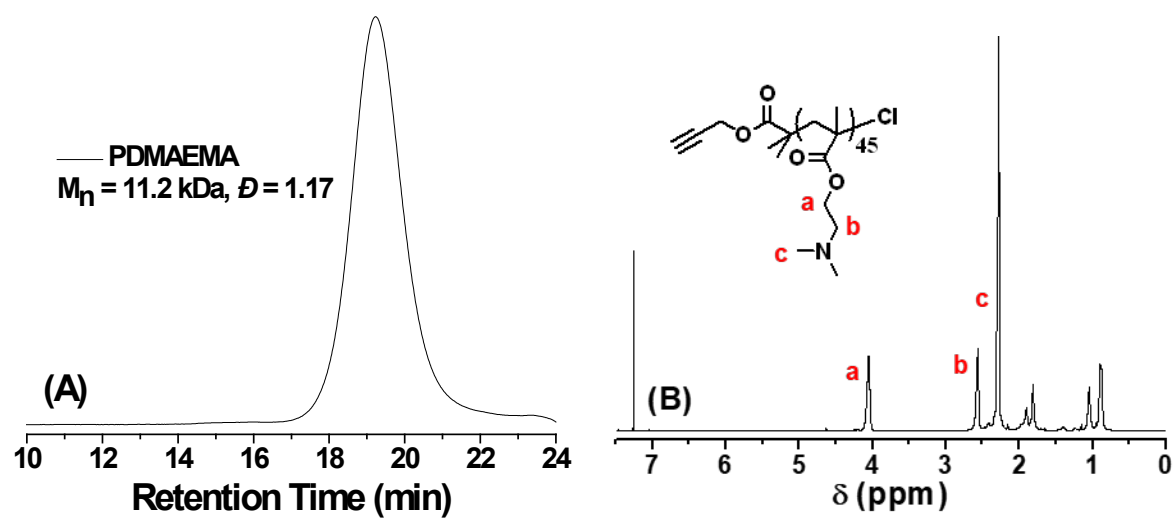


Figure D2. SEC trace (A) and ^1H NMR spectra (B) of alkyne end-functionalized PDMAEMA

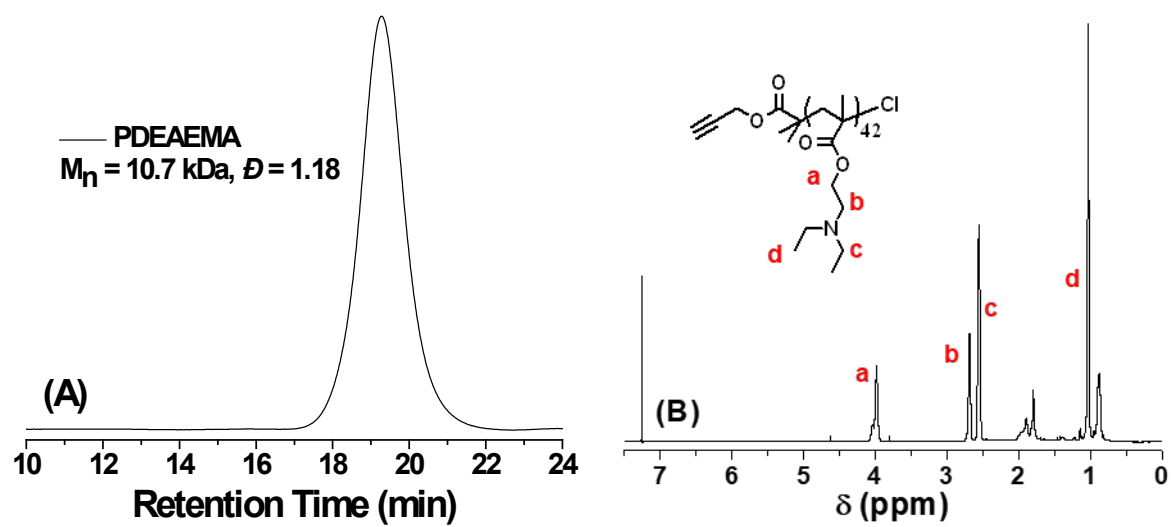


Figure D3. SEC trace (A) and ^1H NMR spectra (B) of alkyne end-functionalized PDEAEMA.

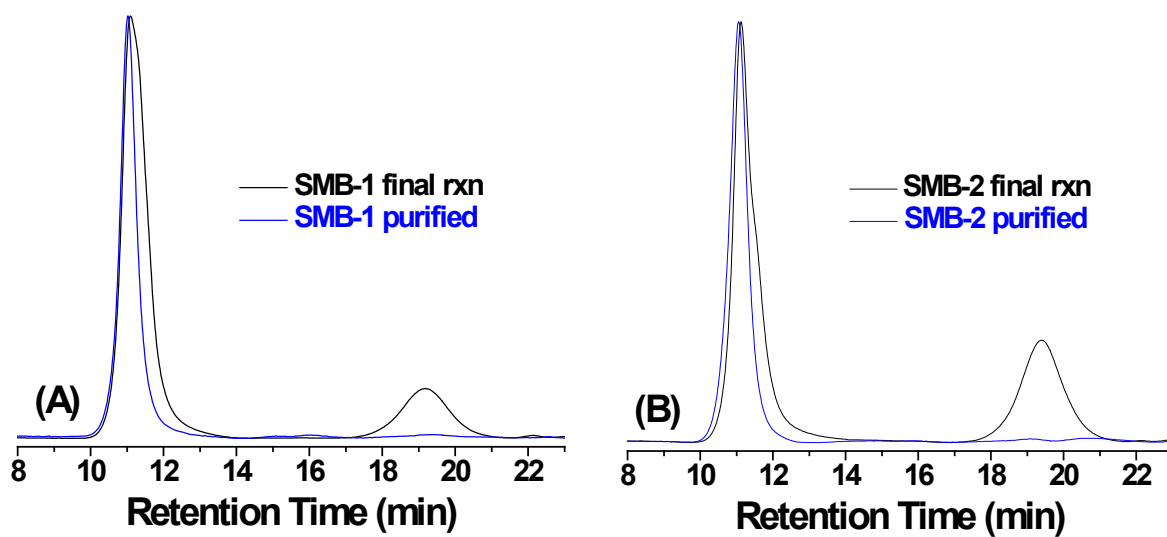


Figure D4. SEC traces for crude and purified star molecular brushes, SMB-1 (A), and SMB-2 (B).

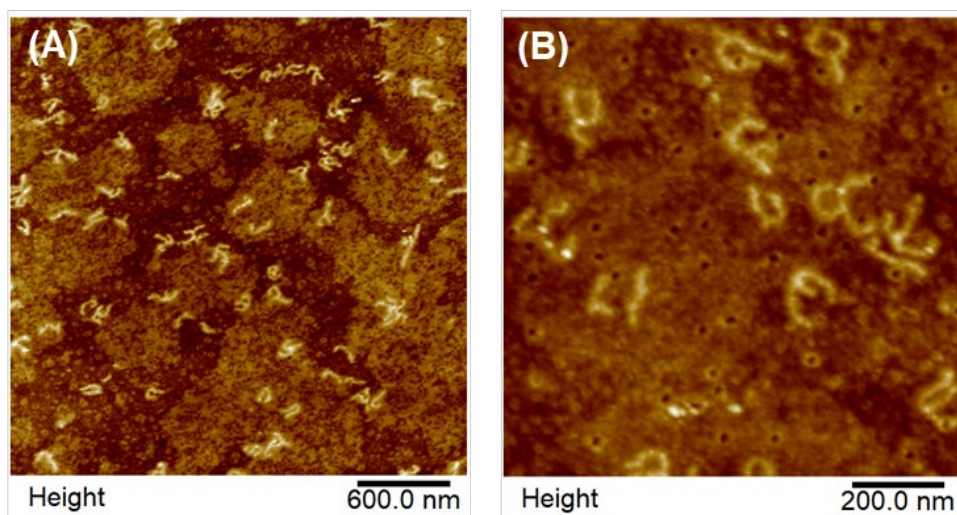


Figure D5. (A) and (B) AFM height images of SMB-1 spin cast from a 0.05 mg/g solution adjusted to pH = 4.00 (no buffer) onto a glass disk.

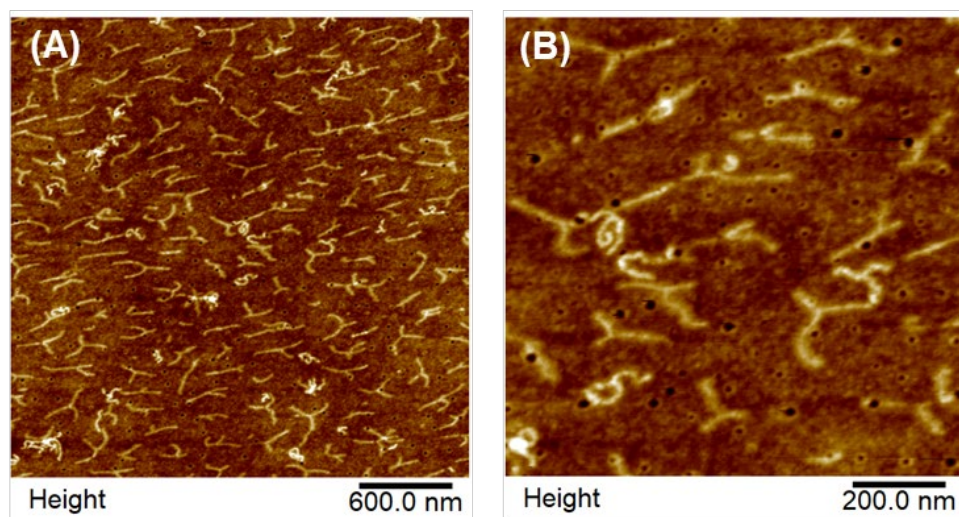


Figure D6. (A) and (B) AFM height images of SMB-1 spin cast from a 0.05 mg/g solution adjusted to pH = 9.50 (no buffer) onto a glass disk.

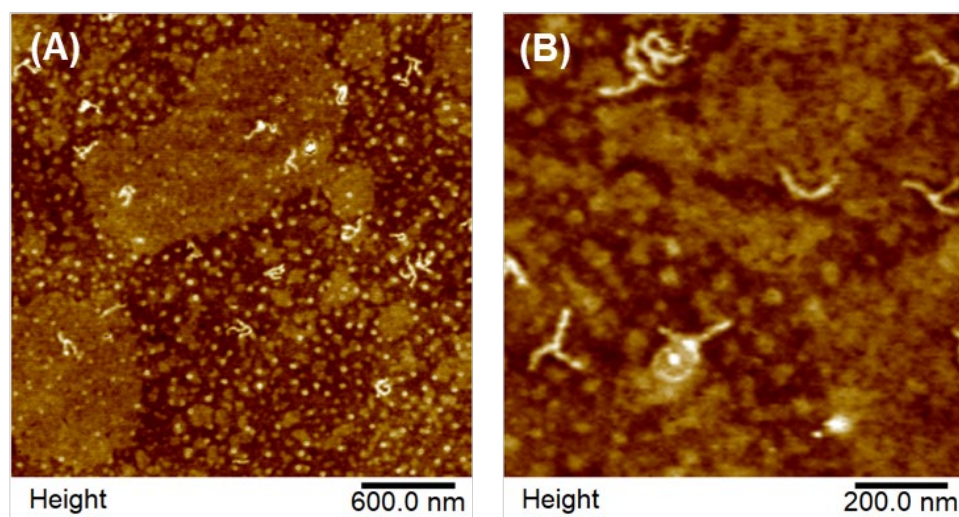


Figure D7. (A) and (B) AFM height images of SMB-2 spin cast from a 0.05 mg/g solution adjusted to pH = 4.00 (no buffer) onto a glass disk.

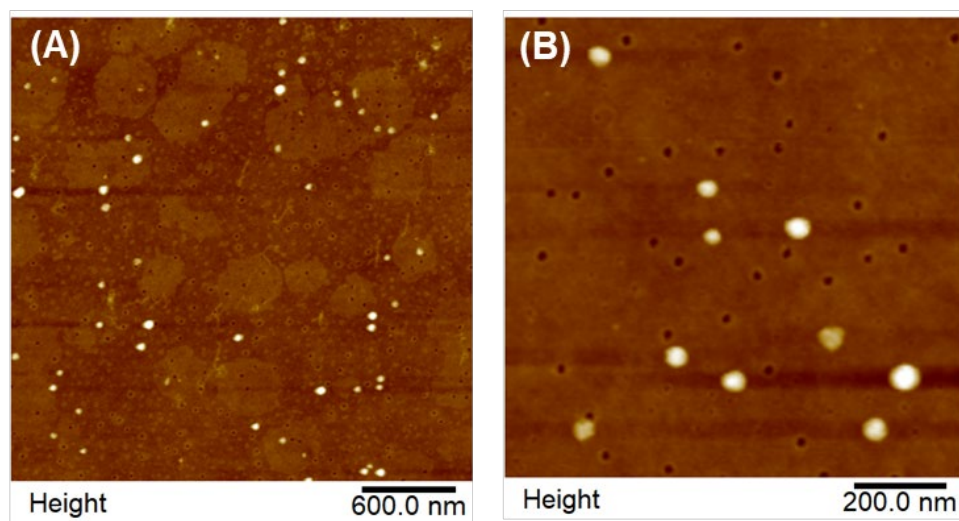


Figure D8. (A) and (B) AFM height images of SMB-2 spin cast from a 0.05 mg/g solution adjusted to pH = 9.50 (no buffer) onto a glass disk.

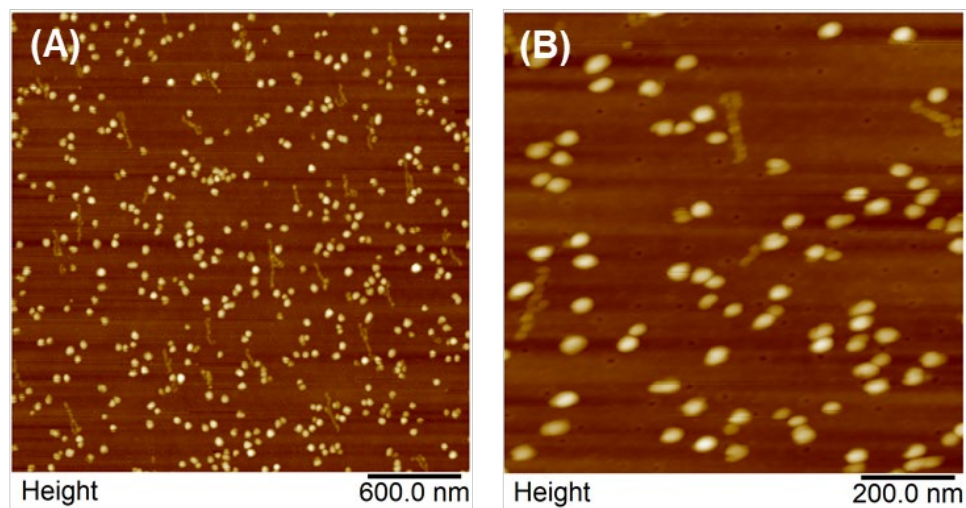


Figure D9. (A) and (B) AFM height images of SMB-1 drop cast onto a glass disk from a 0.05 mg/g aqueous solution at pH = 4.00 with 0.14 mM potassium ferricyanide.

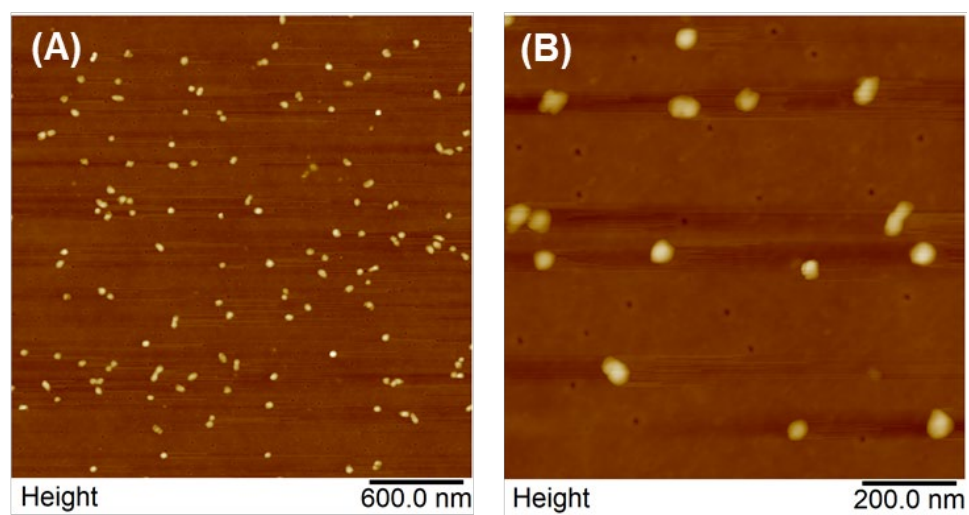


Figure D10. (A) and (B) AFM height images of SMB-2 drop cast onto a glass disk from a 0.05 mg/g aqueous solution at pH = 4.00 with 0.14 mM potassium ferricyanide.

Chapter 6: Conclusions and Future Work

This dissertation work focused on the synthesis and behavior of dually responsive linear and star molecular bottlebrushes composed of biocomponent side chains, either homografted diblock copolymer or binary heterografted polymeric side chains. When one polymer component became insoluble, driving the collapse of the brush molecules, the other component remained soluble and served as a stabilizer allowing for unimolecular shape transitions. In Chapter 2, a pH- and thermo-responsive diblock copolymer was used as side chains of homografted molecular brushes. Chapter 3 expands on the idea of biocomponent molecular bottlebrushes by introducing two types of stimuli-responsive side chains to achieve pH- and thermally-induced star-to-stable globular shape transitions in aqueous solution. A peculiar pearl-necklace brush morphology was observed from an aqueous phosphate buffer, which was further investigated using a linear version of star molecular bottlebrushes with the same side chains in Chapter 4. The final chapter uses binary heterografted star bottlebrushes composed of a water-soluble polymer and a tertiary amine-containing polymethacrylate as side chains to demonstrate the pH- and chaotropic anion-induced star-to-globule shape transitions in aqueous solution.

Chapter 2 explores homografted linear molecular bottlebrushes composed of diblock copolymer side chains; a pH-responsive polymer, poly(2-(*N,N*-diethylamino)ethyl methacrylate) (PDEAEMA) with a pK_a value of 7.4, was used as the inner block and a thermoresponsive polymer, poly(methoxytri(ethylene glycol) acrylate) (PTEGMA) with an LCST of ~ 58 °C, was the outer block.¹ The molecular brushes were prepared using copper(I)-catalyzed azide-alkyne cycloaddition reaction (CuAAC). The azide-functionalized backbone with a DP of 707 was prepared according to the same procedure described in literature,²⁻⁴ and the alkyne end-functionalized diblock copolymer was synthesized by sequential atom transfer radical polymerization (ATRP) from an alkyne-functionalized initiator. Dynamic light scattering (DLS)

and atomic force microscopy (AFM) studies showed that the bottlebrushes underwent a pH-induced shape transition at room temperature, which was below the LCST of the PTEGMA block. The pH induced collapse of brush molecules was stabilized by the outer PTEGMA block. In contrast, the molecular brushes with only PDEAEMA homopolymer side chains showed aggregation and precipitation upon increasing the pH, while the sample prepared from molecular brushes with diblock copolymer side chains did not show aggregation. We additionally demonstrated that the PTEGMA outer block could be used to further control the solution state of the brushes; when the molecular brushes were in the pH-induced collapsed state, increasing the temperature above the LCST of PTEGMA caused the brushes to form clusters at a higher concentration or remain unaggregated in dilute solution.

In Chapter 3, we expanded upon the idea of bicomponent molecular bottlebrushes by preparing binary heterografted star-shaped bottlebrushes composed of two different types of stimuli-responsive side chains, pH-responsive PDEAEMA and thermoresponsive PDEGEA (LCST = 9 °C).⁵ CuAAC click chemistry was used to make the brush polymer in a grafting-to approach, similar to that in Chapter 2. However, the azide-bearing three-arm star backbone polymer was prepared using a slightly different method; a silyl ether-protected methacrylate monomer was polymerized from a newly designed trifunctional initiator using ATRP, followed by a series of post-polymerization reactions to install azide moieties on the backbone. The DP of the backbone was 434 per arm in order to give a sufficiently high aspect ratio to visualize stimuli-induced conformational changes. The alkyne end-functionalized side chain polymers, PDEAEMA and PDEGEA, were synthesized separately using ATRP. DLS studies showed a dramatic decrease in size upon increasing the pH across the pK_a of the PDEAEMA side chains at a temperature below the LCST of PDEGEA. ¹H NMR spectroscopy analysis at 4 °C revealed the dehydration of

PDEAEMA side chains while the PDEGEA side chains remained soluble, indicating the stabilization of the pH-induced collapsed state. The thermally-induced transition was realized by heating the solution when the pH was slightly lower than the pK_a ; the strong solvation of the protonated PDEAEMA repeat units prevented the collapse when the pH was far below the pK_a . Variable temperature ^1H NMR spectroscopy analysis showed that the PDEGEA side chains were dehydrated near the LCST of PDEGEA homopolymer, regardless of the pH values. The pH- and thermally-induced starlike to collapsed globular shape transitions were visualized by AFM. We demonstrated that when one set of side chains collapsed into a hydrophobic core, the second set served to stabilize the collapsed state and prevented aggregation, achieving two distinct collapsed states from the same molecular bottlebrush system.

Chapter 4 investigates a peculiar pearl-necklace morphology observed in Chapter 3 using linear binary heterografted molecular bottlebrushes with a similar side chain composition. We hypothesized that the multivalent phosphate anions in the aqueous buffer solutions might form bridges between the positively charged tertiary amine groups and enhance the microphase separation between charged PDEAEMA and neutral PDEGEA side chains. We first demonstrated the pH-induced collapse of the brush molecules using DLS. Further DLS studies were used to measure and compare the hydrodynamic sizes of the brushes in aqueous acidic acetate, phosphate, and citrate buffers at the same pH. A trend of decreasing hydrodynamic diameter with increasing buffer anion valency was observed. AFM was used to directly observe the brushes spin cast from various buffer solutions onto PMMA-coated mica and revealed that the domain bead size increased with increasing buffer anion valency. We found that the selection of buffer anions could be used to modulate the microphase separation of the two side chain polymers.

Finally, in Chapter 5, we prepared binary heterografted star molecular brushes composed of a tertiary amine-containing polymer and a water-soluble polymer, poly(ethylene oxide) (PEO), as side chains and investigated their behavior in response to pH changes and the addition of various anions. The star-shaped molecular bottlebrushes were composed of PEO and either poly(2-(*N,N*-dimethylamino)ethyl methacrylate) (PDMAEMA) or PDEAEMA side chains. DLS and AFM studies demonstrated the pH-induced star-to-globule collapse for PDEAEMA/PEO brushes, stabilized by the water-soluble PEO side chains. However, PDMAEMA/PEO brushes showed only a slight decrease in size at a pH near its pK_a at room temperature and no full collapse was observed. The effect of the addition of various salts containing kosmotropic or chaotropic anions to aqueous solutions of each bottlebrush polymer at an acidic pH was then investigated by DLS and AFM. The studies revealed that both star bottlebrush systems exhibited chaotropic anion-induced star-to-globule shape transitions; the addition of kosmotropic anions did not cause a significant decrease in size. Moreover, the PDEAEMA/PEO brushes showed a greater sensitivity to moderately chaotropic anions, likely due to the more hydrophobic ethyl groups on the nitrogen atom of PDEAEMA.

Stimuli-responsive molecular bottlebrushes offer exciting opportunities for the design of novel smart materials for myriad applications.⁶⁻⁸ This dissertation work provides new means of achieving triggered conformational changes of molecular bottlebrushes between extended worm- or star-like and collapsed yet stable globular states using multiple environmental triggers. Future work along this direction could include the design of a brush system that could exhibit multi-level unraveling in response to either different stimuli or different intensities of stimuli. For example, the work presented in Chapter 3 showed that two distinct collapsed states could be achieved orthogonally from the same brush system, either by pH or temperature changes. However, it could be imagined

that a star brush consisting of arms with different stimuli-responsiveness could allow for the collapse of individual arms and subsequent step-wise unfolding. This could result in stabilized collapsed states with distinct compartments for the loading and on-demand release of hydrophobic materials.

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Vita

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