Controlling the Molecular Weight Distribution of Polymer Brushes to

Tune Stimulus-Response and Bacterial Adhesion

A Dissertation

Presented to

the Faculty of the Department of Chemical & Biomolecular Engineering

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

in

Chemical Engineering

by

Vivek Yadav

August 2017

Controlling the Molecular Weight Distribution of Polymer Brushes to

Tune Stimulus-Response and Bacterial Adhesion

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Abstract

Bacterial biofilms ubiquitously foul surfaces in technological settings – damaging oil pipelines,¹⁻³ medical implants,⁴⁻⁶ and marine engineering equipment.⁷⁻⁸ Stimuliresponsive polymer brushes provide one route to control adhesion of bacteria to surfaces by tuning bacterial-surface interactions, and prevent long-term bacterial attachment and fouling through brush conformational changes triggered by external stimuli (pH, temperature). Both thickness and molecular-weight distribution in brushes can change the conformation of a stimulus-responsive polymer brush,⁹⁻¹⁰ suggesting that these parameters may play distinct roles in detachment of bacteria. Surprisingly, how molecular weight distribution (quantified as dispersity) in stimuli-responsive brushes affects the response and fouling-release properties is largely unexplored.

First, we developed a method to systematically increase the dispersity of polymers synthesized through a widely-applicable polymerization method, atom transfer radical polymerization (ATRP), by identifying a reagent (phenylhydrazine) capable of reacting with propagating polymer chains. Polymerizations conducted with phenylhydrazine exhibited chain termination and increased dispersity, controlled through the phenylhydrazine concentration. Reaction kinetics in the presence of phenylhydrazine deviated from that observed in typical ATRP syntheses, and a theoretical model was developed that showed excellent agreement with experimental data.

Second, we investigated the effects of brush dispersity on the pH-response of poly(acrylic acid) (PAA) brushes. Increase in brush dispersity lead to greater pH-response at low pH, and additionally, variation in contact angle measurements at intermediate pH (hysteretic memory behavior) was observed when the pH was decreased

from 10 to 3 and increased to 10 thereafter. We posited that dispersity-driven conformational changes at low pH lead to observed hysteretic behavior.

Finally, we studied the effect of PAA brush thickness and dispersity on bacterial attachment at pH 4 and detachment at pH 9. Increasing either thickness or dispersity led to greater bacterial detachment. Bacterial attachment depended non-monotonically on brush thickness, with brushes of thickness between 13 - 18 nm showing lowest attachment, and attachment was independent of brush dispersity.

Together, these results identify brush dispersity as a design parameter to tune pHresponse with applications in sensors, controlled drug-release, and separation processes. Separate control over bacterial attachment and detachment via the molecular-weight distribution, demonstrated here, opens new options for smart antifouling surfaces.

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Chapter 1. Introduction

1.1 Bacterial Adhesion and Biofouling

Biofilms are colonies of microorganisms that are attached to a surface.¹¹ Surfaceattached bacterial biofilms ubiquitously foul surfaces in technological settings – damaging oil pipelines,¹⁻³ water purification systems,⁴ medical implants,⁴⁻⁶ food packaging,¹² and marine engineering equipment.⁷⁻⁸ Additionally, biofilms cause or exacerbate more than 80% of hospital-acquired infections.¹³ Conversely, biofilms can be advantageous in certain settings. Biofilms of some bacterial species are useful for water remediation and, additionally, can prevent pathogenic infections by forming benign biofilm coatings on medical devices.¹⁴⁻¹⁵ Early in biofilm formation, bacteria reversibly and then irreversibly attach to surfaces.¹⁶ Hence an important step in managing biofilm formation is to tailor surfaces to control the adhesion of bacteria.¹⁷

Bacterial adhesion to a surface is governed by Lifshitz-van der Waals interactions, electrostatic interactions, acid-base interactions, and hydrophobic interactions.¹⁸ Lifshitz-van der Waals and hydrophobic interactions are typically attractive in nature, whereas electrostatic and acid-base interactions can be either repulsive or attractive. Because adhesion is controlled by a combination of interactions between bacteria and surface, adhesion can be tuned by varying the properties of the coating. Physicochemical properties of polymer coatings such as polymer chemistry,¹⁹ wettability,²⁰⁻²¹ surface energy,²² charge,²³⁻²⁴ and roughness²⁵ have been used to tune fouling properties of the surface. Similarly, mechanical properties of surface, such as the elastic modulus, have also been modulated to control fouling.²⁶⁻²⁷

One strategy to control attachment of bacteria is to employ polymer brushes. In addition to increasing the separation between bacteria and surface and thus reducing the attractive interactions between surface and bacteria, polymer brushes also increase the repulsive interactions (due to steric hindrance of brushes) experienced by adhering bacteria.²⁸⁻³⁰ Polymer brushes can reduce attractive hydrophobic interactions by swelling, and thereby, reduce bacterial adhesion.³¹⁻³⁷ Polymer brush conformation can be tuned by changes in brush dispersity,³⁸ and changes in molecular weight distribution of polymer brushes might be a powerful way to affect bacterial adhesion.

1.2 Polymer Brushes

Figure 1.1 Polymer brush transitioning from mushroom to brush regimes.³⁸

Polymer brushes are polymers tethered to a surface. One widely explored property of polymer brushes is the grafting density, i.e. number of grafted polymer chains per unit area. Polymer brushes with low grafting density are said to be in "mushroom regime" as chains do not interact, whereas on increasing grafting density the brush transitions to the brush regime where chains adopt extended conformations to minimize steric hindrance (Figure 1.1). Many polymer brush properties depend on the grafting density. There are two methods to synthesize polymer brushes. In the "grafting to" method, synthesized polymers are tethered to the surface; in "grafting from" method, polymers are grown from the surface after attachment of a polymerization initiator to the surface. The "grafting to" method typically leads to polymer brushes with lower grafting density, as steric hindrance prevents long polymer chains from diffusing readily to the surface, whereas the "grafting from" method provides a route to synthesize polymers with higher grafting densities, through diffusion of small monomers to the surface.³⁹⁻⁴⁰

The brush length (controlled through the molecular weight) and dispersity (D, the ratio of weight-average and number-average molecular weights) also affect the polymer brush conformation.^{38, 41} With changes in polymer conformation, interactions of bacteria with surfaces can be tuned. Efficacy of polymer brushes in controlling bacterial adhesion is therefore controlled by brush properties. Although the effects of brush grafting density and thickness on bacterial adhesion have been studied by several research groups,²⁹⁻³¹ no experimental study has probed the role that dispersity plays in bacterial adhesion. Here, we posit that variation in molecular weight distribution can provide a new method to tune bacterial adhesion on polymer brushes as theoretical study has shown that polymer conformation of a neutral brush can be tuned with dispersity.³⁸

1.3 Stimuli-Responsive Polymer Brushes

Despite detailed insights provided by careful studies of adhesion on polymer brushes, static surfaces are irrevocably fouled by bacteria. One strategy to circumvent or reduce long-term fouling is inspired by the antifouling strategies used by mollusks, corals, and other marine organisms, which shed foulants through active deformation and motion. Similar active changes in brush conformation might be an effective method for fouling-release surfaces.⁴²⁻⁴³ Stimuli-responsive or smart polymers change conformation and properties in response to environmental changes, and may be a simple realization of the smart cleaning observed in aquatic organisms. Stimuli-responsive polymers can change the antifouling properties of a surface upon changes in brush environment by changing brush conformation, and are a prototype for self-cleaning observed in aquatic creatures. Temperature,⁴⁴⁻⁴⁶ pH,⁴⁷⁻⁴⁸ ionic strength,⁴⁹ pressure,⁵⁰ and light ⁵¹⁻⁵² are some of the stimuli used to tune bacterial attachment and detachment in responsive materials. Surprisingly, how the molecular-weight distribution in brushes affects the stimuli-response and fouling-release properties of stimuli-responsive polymer brushes is largely unexplored. Both thickness and dispersity can change the conformation of a stimulus-responsive polymer brush,⁹⁻¹⁰ suggesting that these parameters may play distinct roles in detachment of bacteria. Here, we employ a pH-responsive brush, poly(acrylic acid), develop a route to systematically vary the brush thickness and dispersity, and monitor the resulting changes in antifouling and fouling release properties.

Poly(acrylic acid) (PAA) is a weak polyelectrolyte, and can change its charge state upon changes in solution pH. The chain conformation, brush height, charge distribution, degree of ionization, and wetting properties of weak polyelectrolyte brushes depend strongly on the brush properties and pH of the solution. For example, polyacid brushes are fully protonated at low pH and adopt a conformation similar to that of neutral brushes.⁵³⁻⁵⁶ At high pH, the acid groups on the brush become dissociated and the brush behaves as an extended polyelectrolyte brush with a Gaussian density profile.⁵⁵⁻⁶² At all pH values, the brush density is greatest near the substrate.^{56, 63-65} The charge density increases with distance from the substrate, and also depends on pH, salt concentration, and grafting density.^{9, 40, 64-71} The brush thickness is closely related to the degree of ionization of the brush and resulting polymer conformation.^{9, 55, 64, 67, 72-75} As the ionization of weak polyelectrolytes depend on the brush properties, variation in brush dry

thickness and dispersity has an ability to tune brush conformation by changing the charge distribution and number of charges along the polymer chain grafted to the surface. To synthesize polyelectrolytes with controlled thickness and dispersity, controlled polymerization techniques are needed.

1.4 Control of Dispersity Using Atom Transfer Radical Polymerization

Free radical polymerization is a commonly used technique for producing commercial polymers, but it is not possible to synthesize polymers with narrow molecular weight distribution using this technique. Theoretically, dispersity, D of 1.5 - 2 can be achieved using free radical polymerization,⁷⁶ but in many syntheses D > 2 is observed, often with multimodal distributions. Controlled radical polymerization enables the synthesis of polymers with targeted molecular weights and narrow molecular weight distributions. Atom transfer radical polymerization (ATRP), a controlled radical polymerization technique developed by Matyjaszewski *et al.*, provides a route to synthesize low dispersity polymers (D < 1.1).⁷⁷ ATRP has been commonly used to synthesize polymers with well-defined molecular architectures such as stars, brushes, combs and block copolymers.⁷⁸ ATRP reactions where the initiator is grafted to the surface are commonly known as surface-initiated ATRP.⁷⁸⁻⁷⁹

The ATRP technique was first published in 1995.⁸⁰ ATRP is controlled by the equilibrium between the active radical species and dormant species (Figure 1.2). The dormant species (P_n -X) reacts with the transition metal complex (Mt^m/L), where Mt is transition metal in m oxidation state and L is the ligand with a rate constant of activation (k_{act}), and forms the propagating radicals (P_n). This reaction converts metal transition complexes to a higher oxidation state, also known as deactivators (X-Mt^{m+1}/L). Through

the reverse reaction, the deactivators then react with the active radicals to form metal transition complexes in lower oxidation states (the activator, Mt^m/L) and dormant species (P_n -X). This equilibrium between active (P_n) and dormant species forms the basic principle for ATRP. In this equilibrium the presence of dormant species is dominant, enabling all chains to grow at an equal rate and minimizing the possibility of radical termination through lowering the active radical concentration. As a result, ATRP results in a narrow molecular weight distribution.

$$P_{n} - X + CuBr/L \xrightarrow{K_{eq}} P_{n} + Cu_{2}Br/L$$

$$P_{n} + M \xrightarrow{k_{p}} P_{n+1} + Cu_{2}Br/L$$

$$P_{n} + P_{m} \xrightarrow{k_{t}} P_{n-1} + P_{m}$$

Figure 1.2 Mechanism of ATRP. The first step shows the equilibrium between active and dormant species, the second step is propagation of active radicals, and third step is chain termination which is minimized through use of ATRP.⁵²

Because ATRP is a variant of free radical polymerization, under certain (poorlyselected) conditions chain termination occurs, producing polymers with high dispersities. By varying reactions conditions such as temperature, pressure, catalyst/ligand, initiator, and/or solvent, dispersities in the range of 1.1 - 3.0 were observed for polyacrylates, polymethacrylates, and polystyrene.^{77, 81-87} This approach cannot be used to vary dispersity in a controlled and systematic manner, however, as the increase in dispersity is a consequence of uncontrolled polymerization. A variation of ATRP, Activator Regenerated by Electron Transfer (ARGET) ATRP, in which a reducing agent is added to generate active Cu(I) species at low catalyst concentrations,⁸⁸ used for the synthesis of poly (*n*-butyl acrylate) exhibited dispersities of 1.23 - 2.3 in the presence of reducing agents (phenylhydrazine and hydrazine) under various reaction conditions (choice and concentration of ligand).⁸⁹ Varying dispersity was an unintended consequence of optimizing the ARGET ATRP method, and little is understood regarding the mechanism of increased dispersity in the presence of reagents such as phenylhydrazine and hydrazine. Nonetheless, these results suggest that addition of these reagents may provide a method to systematically vary dispersity in ATRP, were the mechanism understood.

1.5 Objective and Organization of Dissertation

The objective of this thesis is to explore the role of molecular weight distribution and dispersity on the brush response and resultant smart antifouling properties of stimuliresponsive polymer brushes. The findings of this thesis will suggest new strategies to control bacterial adhesion, and also a platform towards the rational design of antifouling coatings.

In the first study (Chapter 2), atom transfer radical polymerization was employed to systematically vary the molecular weight distribution of polymers through the addition of phenylhydrazine (PH). Dispersities ranging from 1.07 – 1.90 were obtained in synthesis of poly(*tert*-butyl acrylate). The polymerization kinetics increasingly deviated from the ATRP kinetics as the concentration of PH was increased. Detailed ¹H-NMR end-group analysis revealed that PH participates in a nucleophilic substitution reaction with alkyl halides leading to chain termination and, therefore, an increase in dispersity. The reaction of chain ends with PH was incorporated into a kinetic model, which showed excellent agreement with the experimental data. This method was extended to the synthesis of polystyrene, through ATRP, showing broader applicability of this approach.

In the second study (Chapter 3), we explored the effects of brush thickness and dispersity on the pH-response of poly(acrylic acid) (PAA) brushes. High dispersity brushes exhibited hysteretic memory behavior, in which the contact angle measured upon increasing and decreasing pH differed. We observed three trends in the pH-response: (i) the thickness of dry brushes increased upon increasing pH for brushes above a critical length, ~ 12 nm; (ii) the low-pH contact angle increased with increasing dispersity; (iii) hysteretic memory behavior increased on increasing the brush dispersity. We concluded that conformational changes in high dispersity brushes led to the reported hysteretic memory behavior in pH-responsive polymer brushes.

In the final study (Chapter 4), we investigated the effect of PAA brush thickness and dispersity on the attachment and detachment of *Staphylococcus epidermidis* bacteria using confocal microscopy. First, we deposited bacteria onto collapsed and neutral brushes at pH 4. Bacterial adhesion at pH 4 varied non-monotonically with increasing brush thickness and was independent of dispersity. PAA brushes of thickness 13 – 18 nm showed the lowest bacterial attachment. When the solution pH was switched to 9, thereby changing the conformation of the polymer, increasing either dispersity or thickness enhanced the removal of bacteria from the PAA brush surface. We posit that increasing either parameter enhanced the stimulus-driven conformational changes, leading to greater actuation effect through swelling at low pH. This study implies that we can separately tune initial attachment and detachment via the molecular weight distribution of a stimulus-responsive polymer brush.

Chapter 2. Control of Dispersity in Atom Transfer Radical Polymerizations through Addition of Phenyl Hydrazine

2.1 Introduction

The dispersity (*D*), the ratio of weight-average and number-average molecular weights, is a critical parameter governing diverse polymer physical properties. For example, increasing the dispersity can improve the processability of polymer melts by delaying the onset of flow instabilities.⁹⁰⁻⁹² Dispersity greatly impacts the rheological properties of polymers, including the steady shear, zero shear, and elongational viscosities, storage and loss moduli, and normal stresses.⁹³⁻⁹⁷ Additionally, dispersity plays an important role in polymer phase behavior, tuning blend miscibility⁹⁸⁻¹⁰⁰ and modifying the morphology and domain spacing of block copolymers.¹⁰¹⁻¹⁰⁵ Furthermore, in polymer brushes increased dispersity induces conformational changes,³⁸ which alter the pH-responsive behavior of polyelectrolyte brushes¹⁰ and impart greater stability to polymer-grafted nanoparticles.¹⁰⁶

Various approaches have been developed to tune polymer dispersity. Blending polymers of differing molecular weights is an often employed yet inefficient method to control dispersity, and typically results in multimodal molecular weight distributions.^{93-95, 107-108} The molecular weight distributions of polymers synthesized through Ziegler-Natta and metallocene polymerizations have been modified through choice of catalyst and reaction temperature, and addition of hydrogen (a chain transfer agent), producing high dispersity polymers with D ranging from 2.0 – 14.^{92, 109-110} The dispersity of polymers synthesized through ring-opening polymerization may be increased from less than 1.1 to as high as 2.0 in the presence of transesterification. ^{101, 103, 105} Similarly, the dispersity of

polymers produced through anionic polymerization may be systematically increased over a limited range (up to around 1.34) by decreasing the rate of addition of sec-butyllithium or by increasing the reaction temperature.¹⁰² In all of these polymerization classes, the number of monomers that can be polymerized are limited. Hence, an open challenge is to create a more widely applicable method of synthesizing polymers with unimodal molecular weight distributions and tunable dispersities.

Radical polymerization methods are a convenient choice for synthesizing a diverse range of polymers. In free radical polymerization, a commonly used technique for producing commercial polymers, the theoretical "most probable" molecular weight distribution as originally described by Carothers⁷⁶ has a dispersity D of 1.5 - 2(depending on the chain termination mode, whether by radical combination and/or disproportionation), and in many syntheses D > 2 is observed, often with multimodal distributions. Atom transfer radical polymerization (ATRP), a controlled radical polymerization technique developed by Matyjaszewski et al., provides a route to synthesize low dispersity polymers (D < 1.1) with unimodal distributions by minimizing the time spent by radicals in the active state and thereby reducing chain terminations.⁸⁰ In ATRP, a transition metal complex (usually Cu(I)X/L, where X is a halogen and L is a ligand), converts to higher oxidation state (usually Cu(II)X₂/L) and reversibly activates and deactivates the propagating radical.⁷⁷ The polymerization initiator, copper catalyst, and ligand are chosen for a particular reaction system such that the radical spends most of the time in its deactivated or dormant state, thereby limiting the chain termination reactions. Though high dispersity (D = 1.5 - 2 or greater) and low dispersity (D < 1.1) polymers can be readily synthesized with existing radical polymerization techniques,

there remains the need for radical polymerization methods to create polymers with unimodal molecular weight distributions that have tunable dispersities.

Because ATRP is a variant of free radical polymerization, under certain (poorlyselected) conditions chain termination occurs, producing polymers with high dispersities. In one study, dispersities of 1.1 - 3.0 were observed for polyacrylates, polymethacrylates, and polystyrene synthesized under different reaction conditions by varying temperature, pressure, catalyst/ligand, initiator, and/or solvent.^{77, 81-87} This approach to varying dispersity is a consequence of uncontrolled polymerization, however, and does not provide a method to controllably and systematically vary the dispersity. Similarly, in the synthesis of poly (*n*-butyl acrylate) by Activator Regenerated by Electron Transfer (ARGET) ATRP, in which a reducing agent is added to generate active Cu(I) species at low catalyst concentrations,⁸⁸ dispersities ranging from 1.23 - 2.3 were observed under various reaction conditions (choice and concentration of ligand) in the presence of reducing agents (phenylhydrazine and hydrazine).⁸⁹ Varying dispersity was again an unintended consequence of optimizing the ARGET ATRP method, and little is understood regarding the mechanism of increased dispersity in the presence of reagents such as phenylhydrazine and hydrazine.

Herein, we develop a method to systematically vary the dispersity of polymers produced by ATRP through the addition of phenylhydrazine (PH), and explore the mechanism of increased dispersity. In our study, conventional ATRP methods were employed (i.e. without reduced catalyst concentrations) and PH was explored as a reagent to modify dispersity, in contrast to its well-established role as a reducing agent in ARGET ATRP syntheses. Using ATRP in the presence of PH, poly(*tert*-butyl acrylate) (PtBA) polymers were synthesized with dispersities ranging from 1.08 to 1.80. By investigating the effect of PH addition on ATRP reaction kinetics and the resulting polymer molecular weight distribution, we demonstrated that the presence of PH leads to chain termination, thereby increasing dispersity. By contrast, a second conventional reducing agent used in ARGET ATRP, tin(II) 2-ethylhexanoate, had no effect on dispersity. We applied this method to synthesize polystyrene with dispersities ranging from 1.07 to 1.88, showing the generality of this approach to controllably tune dispersity in ATRP syntheses.

2.2 Materials and Methods

Materials. All chemicals were purchased from Sigma Aldrich and used asreceived unless otherwise noted. Monomethyl ether hydroquinone (inhibitor) was removed from *tert*-butyl acrylate (tBA, 98%) by passing through a silica gel column (60 Å pore size). tBA was then dried with calcium hydride (reagent grade, 95%) and distilled under vacuum. Ethyl α -bromoisobutyrate (EBiB, 98%) and *N*,*N*,*N'*,*N''*pentamethyldiethylenetriamine (PMDETA, 99%) were each degassed with three freezepump-thaw cycles. 4-tert-butylcatechol was removed from styrene (99%) by passing through a basic alumina column (58 Å pore size). Toluene (JT Baker, HPLC grade, 99.7%) was dried using a Pure Process Technology solvent purification system.

Characterization. Gel permeation chromatography (GPC) was employed to characterize the molecular weight and molecular weight distribution (via the number-average molecular weight, M_n , and dispersity, D) of the polymers. The polymers were analyzed with a Viscotek GPC system containing Agilent ResiPore columns, using stabilized THF (OmniSolv, HPLC grade, >99.9%) as the mobile phase at 30°C. The flow

rate was 1 ml min⁻¹ and injection volume was 100 μ l. Linear polystyrene (PS) standards were used for calibration. Triple detection was used where applicable; the low refractive index increment in THF, however, precluded light scattering analysis of low molecular weight poly(*tert*-butyl acrylate).

Proton nuclear magnetic resonance (¹H-NMR) spectra were collected on a JEOL ECA-500 spectrometer using deuterated chloroform as the solvent. Chemical shifts were referenced to the solvent proton resonance (7.26 ppm).

Synthesis of Poly(*tert*-**butyl acrylate**) (**PtBA**). PtBA was synthesized using atom transfer radical polymerization (ATRP).^{10, 111} tBA, PMDETA, copper(I) bromide (CuBr, 99%), anhydrous N,N-dimethylformamide (DMF, 99.8%), EBiB (initiator) and PH (PH, 97%) were added in a predetermined molar ratio to a round bottom flask for each polymerization inside a nitrogen glovebox. An equimolar ratio of PMDETA, EBiB, and CuBr was used, and this mixture was diluted with DMF such that the volume ratio of DMF:tBA was 1:1. The flask was capped with a septum inside the glove box and transferred to a preheated oil bath at 50°C. The reaction mixture was stirred until the reaction reached maximum achievable conversion. The ratio [PH]:[EBiB] was varied (0:1, 1:1 and 3:1) to obtain polymers with varying dispersity. In select reactions, tin(II) 2-ethylhexanoate (95%) was added in place of PH.

The polymerization was quenched by addition of tetrahydrofuran (THF, OmniSolv, HPLC grade, 99.9%) and exposing the reaction mixture to air. The solution was diluted by adding excess THF and passed through a neutral aluminum oxide column to remove the catalyst. The polymer solution devoid of catalyst was concentrated using a rotary evaporator and precipitated in a DI water/methanol mixture (1:1 by volume).

Finally, the precipitated PtBA was collected and dried under vacuum overnight at room temperature.

Synthesis of Polystyrene (PS). PS was also synthesized using ATRP. Styrene, PMDETA, anhydrous toluene, EBiB, and PH were added in a predetermined molar ratio in one flask and CuBr was added in a second flask. Both flasks were purged with argon for 30 minutes; thereafter, contents from the first flask were transferred to the second flask using a cannula tube. The reaction mixture was heated in a preheated oil bath at 80°C. An equimolar ratio of PMDETA, EBiB, and CuBr was used, and this mixture was diluted with toluene such that the volume ratio of toluene:tBA was 1:1. The reaction mixture was stirred until the reaction reached the maximum achievable conversion. The ratio [PH]:[EBiB] was varied (0:1 and 3:1) to obtain polymers with varying dispersity.

The polymerization was quenched by adding THF and exposing the reaction mixture to air. The solution diluted with THF was passed through a neutral aluminum oxide column to remove the catalyst. Thereafter, the polymerization solution was concentrated using a rotary evaporator and precipitated in methanol. Finally, the precipitated PS was dried under vacuum overnight at room temperature.

2.3 Results and Discussion

PH is commonly used as a reducing agent to minimize the required copper catalyst concentration in ARGET ATRP syntheses.⁸⁹ Here, we characterized the effect of PH addition on the molecular weight dispersity of PtBA, synthesized using conventional ATRP methods without reduced catalyst concentrations. ATRP of *tert*-butyl acrylate was conducted in DMF employing EBiB as the initiator, CuBr as the catalyst, and PMDETA as the ligand. ¹H-NMR data obtained from PtBA, synthesized without the addition of PH,

are shown in Figure 2.1. The peaks located at 1.20 - 1.67 ppm, 1.28 - 1.69 ppm, 1.72 - 1.90 ppm, and 2.08 - 2.32 ppm correspond to the $(CH_3)_3C$ of the *tert*-butyl group (peak f), racemo CH_2 of the polymer backbone (peak d), meso methylene CH_2 of the polymer backbone (peak d), and methine CH of the polymer backbone (peak e), respectively.¹¹²⁻¹¹⁶ The ratio of the area of peaks d + f to the area of peak e was 11.06:1, very close to the theoretical prediction of 11:1. These measurements confirm that PtBA was successfully synthesized using ATRP. The reaction conversion was characterized through analysis of ¹H-NMR data obtained prior to purification, in which the areas of the peaks corresponding to the *tert*-butyl groups of the monomer and polymer (peak f, located at 1.41 ppm) were quantified.



Figure 2.1 ¹H-NMR data obtained from PtBA, synthesized in absence of PH.

A series of PtBA polymers was synthesized with PH added to the reaction mixture, at varying [PH]:[EBiB] (Figures 2.2 – 2.3). On increasing the concentration of PH, the maximum achievable reaction conversion decreased (Table 2.1). M_n and D were also characterized using GPC with polystyrene standards. Under the same reaction conditions ([tBA]:[EBiB]:[CuBr]:[PMDETA] = 120:1:1:1), the molecular weight of the polymer decreased as the concentration of PH was increased, due to lower conversion (Table 2.1). Importantly, D markedly increased upon addition of PH.



Figure 2.2 ¹H-NMR data obtained from PtBA, synthesized in presence of PH ([PH]:[EBiB] = 1:1). Additional peaks in the region 7.10 – 7.25 ppm corresponds to side product of the reaction between alkyl halide and PH.



Figure 2.3 ¹H-NMR data obtained from PtBA, synthesized in presence of PH ([PH]:[EBiB] = 3:1). Additional peaks in the region 7.10 - 7.25 ppm corresponds to side product of the reaction between alkyl halide and PH.

Table2.1	Characteristics	of	PtBA	polymers	synthesized	through	ATRP	with	PH
	addition. ^a								

[PH]:[EBiB]	Time (mins)	Conversion	M _n (kg/mol)	Ð
0:1	180	85%	12.0	1.08
1:1	180	68%	9.6	1.80
3:1 ^d	120	36%	4.8	1.71

^a [tBA]:[EBiB]:[CuBr]:[PMDETA] = 120:1:1:1

To understand the effect of PH addition on the ATRP kinetics and resulting polymer molecular weight distribution, the reaction conversion was monitored over time for reactions with three different ratios of PH to initiator ([PH]:[EBiB] = 0:1, 1:1, and3:1). All other reagent concentrations were held constant. Aliquots were taken at regular intervals for all reactions to quantify reaction conversion from ¹H-NMR data and to characterize the molecular weight distribution through GPC and NMR analyses as a function of reaction time. Increasing the concentration of PH altered both the reaction kinetics and the molecular weight distribution of the polymer, as shown in Figure 2.4. In the absence of PH, the polymer Mn increased linearly with reaction conversion, as the conversion varied from 16% to the maximum achievable conversion of 85%, and low values of D were obtained at all conversions (Figure 2.4(a)), as anticipated for controlled radical polymerizations. GPC data obtained for PtBA at different reaction times confirmed the increase in molecular weight with reaction time, via a shift to lower retention volume, and the low dispersity, via narrow peak widths (Figure 2.4(b)). Adding PH at a ratio of [PH]:[EBiB] = 1:1 produced a plateau in the monomer conversion at long reaction times, decreasing the maximum achievable conversion to 68%, and increased the molecular weight dispersity D (Figure 2.4(c)). In the presence of PH, D also increased with conversion (from 1.47 at 25% conversion to 1.80 at 68% conversion). The width of the peak in the GPC data was greater than that observed in the absence of PH addition and increased with reaction time (Figure 2.4(d)). When the concentration of PH was increased further to [PH]: [EBiB] = 3:1, the maximum achievable conversion was reduced further to 36% (Figure 2.4(e)). A small increase in conversion, from 23% to 36%, was

observed over a time of 120 minutes, and D was close to 1.80 for all conversions, with GPC data exhibiting little change in peak width with reaction time (Figure 2.4(f)).

The data presented in Figure 2.4 reveal several distinctive features of polymers synthesized in the presence of PH. Adding PH reduced the maximum attainable conversion and broadened the molecular weight distribution. The values of M_n obtained via NMR monomer conversion (i.e., theoretical value), via NMR end-group analysis, and via GPC analysis (using polystyrene standards) were consistent for the PH-free polymerizations (Figure 2.4(a)). Notably, addition of PH led to discrepancies in the M_n determined through NMR end-group analysis and through other methods (Figures 2.4(c) and 2.4(e)), indicating lack of end-group fidelity. Further, reactions with PH addition initially progressed more quickly than those in the absence of PH, as conversion around 25% was reached for both 1:1 and 3:1 samples within 5 minutes. The combination of these factors, i.e., lack of end-group fidelity, higher conversion at shorter reaction times, and lower maximum achievable conversion, suggests the possibility of chain termination in the presence of PH, and is also consistent with the increase in D. Moreover, the disagreement in M_n determined through NMR end-group and GPC analyses for reactions conducted in presence of PH suggests that the addition of PH changes the nature of the end-groups, again consistent with chain termination.¹

¹We note that GPC analysis using polystyrene standards does not generally characterize the absolute molecular weight of the polymer (for polymers other than polystyrene). The low refractive index increment of PtBA in THF precluded the use of light scattering with our available GPC instrument. Nonetheless, the polymerizations conducted in the absence of PH showed good agreement between M_n characterized with NMR end-group analysis and GPC analysis using polystyrene standards (i.e. Figure 2(a)), indicating that the hydrodynamic volume of PtBA is perhaps similar to polystyrene of comparable molecular weight. We observed a discrepancy in the two analysis methods with PH addition to the polymerization mixture, as discussed in the main text.



Figure 2.4 (a, c, e) M_n (left y-axis, open symbols) and dispersity (right y-axis, closed symbols) as functions of monomer conversion for different ratios of [PH]:[EBiB] (0:1, 1:1 and 3:1). (b, d, f) Representative GPC traces at different reaction times for different ratios.

Next, we compared the kinetics of reactions with and without PH to two widelyaccepted equations for well-controlled ATRP reactions: Matyjaszewski's equation (equation 1)¹¹⁷ and Fischer's equation (equation 2):¹¹⁸

$$\ln\left(\frac{[M]_0}{[M]}\right) = k_p K_{eq} \frac{[RX][Cu(I)]}{[Cu(II)]} t = K_{App} t \text{ and}$$
(1)

$$\ln\left(\frac{[M]_0}{[M]}\right) = \frac{3}{2}k_p([RX]_0 [Cu(I)]_0)^{1/3} \left(\frac{K_{eq}}{3k_t}\right)^{1/3} t^{2/3} = K_{Fischer} t^{2/3}, \qquad (2)$$

where $k_{\rm p}$, $k_{\rm t,}$ and $K_{\rm eq}$ are rate constants for propagation, termination, and ATRP equilibrium. RX is alkyl halide, and Cu(I) and Cu(II) is the catalyst in different oxidation states. Fischer's equation is applicable to reactions exhibiting the "persistent radical effect", in which [Cu(II)]₀ is low enough for radical termination to dominate initially.^{117, 119} Matyjaszewski's equation is applicable when enough [Cu(II)]₀ is added to the reaction and self-regulation due to the persistent radical effect is unimportant.¹²⁰ Experimental work by Zhang *et al.* on methyl methacrylate showed that Fischer's equation was applicable for [Cu (II)]₀/[Cu (I)]₀ < 0.1, Matyjaszewski's equation was applicable for [Cu (II)]₀/[Cu (I)]₀ > 0.1, and both equations were applicable at the crossover ratio of [Cu (II)]₀/[Cu (I)]₀ = 0.1.¹²⁰ The crossover ratio, however, depends on various rate constants and varies from one reaction system to another.¹²¹

The kinetics of the reaction without PH were in excellent agreement with the prediction of both Matyjaszewski's and Fisher's equations (Figure 2.5), suggesting that the ratio of $[Cu (II)]_0/[Cu (I)]_0$ in our system is near the crossover value. When PH was added to the reaction mixture, however, the reaction kinetics were faster than predicted from either Matyjaszewski's or Fisher's equations at short times and plateaued at lower $ln([M]_0/[M])$ values. Together, these observations indicate that adding PH modifies the

reaction kinetics. Moreover, the observed plateau in conversion is consistent with prior studies that attributed this behavior to chain termination,¹²²⁻¹²³ which we will explore in more detail.



Figure 2.5 ln([M]₀/[M]) as a function of reaction time (a) t and (b) t^{2/3}, corresponding to Matyjaszewski's equation (a) and Fisher's equation (b), respectively, for different ratios of [PH]:[EBiB]: 0:1 (▲), 1:1 (■), and 3:1 (♦).

Two possible mechanisms may underlie the observed increase in polymer dispersity with the addition of PH: 1) addition of a reducing agent leads to deviation from ATRP kinetics, and/or 2) the presence of side reactions with PH may lead to chain termination. To test the first mechanism, we conducted experiments using a second common ATRP reducing agent, tin(II) 2-ethylhexanoate,¹²⁴⁻¹²⁵ under the same reaction conditions. Conversion for reactions in the presence of tin(II) 2-ethylhexanoate was characterized using ¹H-NMR. Neither increasing the reaction time nor the concentration of tin(II) 2-ethylhexanoate with respect to the initiator significantly modified the dispersity, which was close to 1.1 for all reactions conducted in presence of tin(II) 2-ethylhexanoate (Table 2.2 and Figure 2.6(a)). Likewise, GPC analysis showed little change in the breadth of the molecular weight distribution upon addition of tin(II) 2-

ethylhexanoate when compared to that obtained without addition of a reducing agent (Figure 2.6(b)). Only when PH was added did the breadth of the molecular weight distribution increase (Figure 2.6(b)). These results indicate that the ability of PH to increase dispersity is not due to its generic action as a reducing agent, but is instead specific to this compound.



Figure 2.6 (a) GPC traces of polymers obtained in the presence of varying ratios of tin(II) to EBiB and over varying reaction times. (b) GPC traces of polymers obtained in the presence of tin(II) or PH, and in absence of reducing agent (RA) after 120 minutes.

[Tin(II)]:[EBiB]	Time (mins)	Conversion ^b	M _n (kg/mol) ^c	D^{c}
3:1	30	18%	2.9	1.11
3:1	120	39%	5.5	1.07
10:1	300	47%	8.6	1.12

Table 2.2 Characteristics of PtBA polymers synthesized through ATRP with addition of tin(II) 2-ethylhexanoate^a

^a [tBA]:[EBiB]:[CuBr]:[PMDETA] = 120:1:1:1
To gain insight into the PH-specific effect that gives rise to the striking change in the molecular weight dispersity, we note that that 1-phenylethyl bromide (a low molecular weight analogue of bromine-terminated polystyrene chains) reacts with nucleophilic reducing agents such as PH and hydrazine via nucleophilic substitution.¹²⁶ If present during the ATRP synthesis with PH addition, this reaction would terminate the growing chains. To calculate the extent of chain termination, we compared the ratio of the ¹H-NMR peak area corresponding to the methyl groups of the initiator fragment on the polymer chain end (peak c in Figures 2.1 - 2.3) to the overlapping peak areas of the methylene group of the initiator fragment on the polymer chain end (peak b) and the CH on the Br end-group (peak g). The peak area ratio c:(b+g) was close to the theoretical value of 2 in the absence of PH (Table 2.3, Figure 2.1), but markedly increased with addition of PH (Table 2.3, Figures 2.2 and 2.3). Additionally, chain termination via the nucleophilic substitution reaction was verified by the presence of aromatic end-groups (from PH) in the region 7.08 - 7.24 ppm for the polymerizations conducted with [PH]:[EBiB] = 1:1 and 3:1 (peaks x and y in Figures 2.2 and 2.3); the reaction conducted in the absence of PH did not show the respective aromatic peaks (Figure 2.1). Using the peak areas of the end-groups, we calculated the extent of the side reaction as shown in Table 2.3. Increasing the concentration of PH led to a greater extent of the nucleophilic substitution reaction as the yield of substituted alkyl chains increased from 38% to 53% on increasing [PH]:[EBiB] from 1:1 to 3:1. This result confirms that the addition of PH leads to nucleophilic substitution reactions. The proposed reaction mechanism by which addition of PH increases the polymer dispersity via chain termination is shown in Figure 2.7. In the absence of PH, propagating chains remain in the dormant state, retaining the

Br chain ends. In the presence of PH, the nucleophilic substitution reaction of a propagating chain with PH terminates the chain and produces an aromatic end-group. Together, these results show that PH increases dispersity via chain termination due to a nucleophilic substitution reaction, and suggest that PH concentration can be used as a design parameter to systematically tune dispersity of polymers synthesized via conventional ATRP.

[PH]:[EBiB]	Peak area ratio for protons on initiator fragment and end group: c:(b+g)	Peak area ratio for protons on polymer repeat units: (f+d):e	Peak area ratio for protons in meta and para position on PH end group: x:(b+g)	Nucleophilic substitution product yield ^b
0:1	2.03	11.05	0	0
1:1	2.52	11.08	0.48	38%
3:1	2.56	11.07	0.67	53%

Table 3.3 ¹H-NMR peak area ratios for polymerizations with various amounts of PH^a

^a Theoretical ratios in the absence of chain termination: c:(b+g) = 2 and (f+d):e = 11. ^b Side product yield: $(2 \times \text{Area of peak } x / \text{Area of peak } c) \times 100$



Figure 2.7 Reaction mechanism for PtBA polymerization conducted in the (a) presence and (b) absence of PH. NMR peak positions corresponding to labeled protons are indicated in Figures 2.1 - 2.3.



Scheme 2.1 Reactions depicting ATRP equilibrium, propagation of radical, and chain termination due to nucleophilic substitution of PH with their respective kinetic rate constants.

We developed a kinetic model to describe ATRP reactions in the presence of a chain terminating agent (here, PH). In addition to the conventional ATRP reaction steps, we also incorporated chain termination due to nucleophilic substitution (Scheme 2.1). First, we assumed that the ATRP reactions undergo fast initiation and fast pre-equilibrium, conditions necessary to achieve low Đ in the absence of PH.¹¹⁷ Second, we neglected chain termination outside of the reaction with PH. Finally, we assumed that [PH] was much higher at any given time point than the dormant chain concentration [PX] due to fast ATRP equilibrium. After solving the rate equations, we obtained a rate equation of the form

$$\ln\left(\frac{[M]_{0}}{[M]}\right) = A(1 - e^{-Bt}); \ A = \frac{k_{\rm p}K_{\rm eq} \left[{\rm Cu}({\rm I})\right]\left[{\rm PX}\right]_{0}}{k_{\rm PH} \left[{\rm PH}\right]\left[{\rm Cu}({\rm II})\right]}, B = k_{\rm PH} \left[{\rm PH}\right].$$
(3)

Although the values of the parameters in A and B are not known, we may nonetheless fit kinetic data for reactions conducted in the presence of PH to the functional form in equation 3. We obtained excellent agreement between the model (eqn 3) and the experimental kinetic data obtained in reactions with [PH]:[EBiB]= 1:1 and 3:1 (Figure 2.8), further confirming our proposed reaction mechanism in Figure 2.7 and validating the assumptions used in deriving the model. We anticipate that this approach and the

derived equation will be valid for any ATRP synthesis with the addition of a chain terminating agent reacting with the alkyl halide following the same stoichiometry as described in Scheme 2.1.



Figure 2.8 Kinetic data (ln([M]₀/[M]) as a function of reaction time) for reactions conducted in presence of PH. Dashed curves show the model fit to experimental data obtained from syntheses conducted with [PH]:[EBiB] = 1:1 (■) and 3:1 (♦).

To test the applicability of PH method on other polymer systems, we employed polystyrene as another model system. Reaction was conducted in absence and presence of PH ([PH]:[EBiB] = 0:1 and [PH]:[EBiB] = 3:1), keeping the concentrations of the other reagents same as those used for PtBA polymerization. The PS reaction was allowed to run longer than the PtBA polymerization because the reaction kinetics for PS is slower. As observed for PtBA, reaction conducted in absence of PH showed narrow molecular weight distribution (Figure 2.9) and dispersity calculated from GPC was 1.07. By contrast, addition of PH led to broadening of the molecular weight distribution (Figure 2.9) and dispersity calculated from PS polymerization

demonstrated that the method of increasing dispersity through PH addition is not limited to just PtBA, but is applicable to a wide variety of polymers.



Figure 2.9 GPC traces of polystyrene obtained in the absence and presence of PH ([PH]:[EBiB] = 0:1 and [PH]:[EBiB] = 3:1) for a reaction conducted for 36 hrs. Addition of PH leads to broader molecular weight distribution.

2.4 Conclusions

We investigated the effect of phenyl hydrazine (PH) addition on the synthesis of poly(*tert*-butyl acrylate) using ATRP. The presence of PH increased the dispersity of the synthesized polymer. In addition, reactions conducted with PH showed deviations from the expected kinetic behavior for ATRP syntheses: 1) the monomer conversion achieved a plateau at low to moderate conversion and subsequently did not change with increased reaction time, 2) the reaction kinetics were not consistent with well-described models (Matyjaszewski and Fischer's equations), and 3) the initial reaction rate was higher than a comparable reaction conducted without PH addition.

To test the hypothesis that the increase in dispersity is due to the action of PH as a reducing agent in the ATRP synthesis, we examined the effect of another common ATRP reducing agent, tin(II) 2-ethylhexanoate. Surprisingly, addition of tin(II) 2-

ethylhexanoate did not increase the polymer dispersity. Next, we examined the possibility of chain termination in reactions conducted in the presence of PH. Polymerizations conducted with PH showed a lack of end-group fidelity through characterization of the relative concentration of initiator fragments and Br end-groups, consistent with the presence of chain termination. By contrast, polymerizations without PH addition exhibited the expected relative concentrations of initiator fragments and Br end-groups. The presence of aromatic end-groups in reactions conducted in the presence of PH confirmed the chain termination mechanism due to nucleophilic substitution. Finally, we developed a kinetic model that accounted for chain termination, which showed excellent agreement with experimental data. Additionally, by increasing dispersity of polystyrene via PH addition, we demonstrated that the method of increasing dispersity via PH addition is not limited to PtBA synthesis but is applicable to other ATRP syntheses as well. In summary, PH is an effective modifier for ATRP syntheses, providing systematic control over the dispersity of polymers in a unimodal molecular-weight distribution.

Chapter 3. Effect of Brush Thickness and Dispersity on pH-Response of Poly(acrylic acid) Brushes

3.1 Introduction

Stimuli-responsive polyelectrolyte brushes are smart surfaces appropriate for applications in antifouling coatings, sensors, diagnostics, and drug delivery vehicles.¹²⁷⁻ ¹²⁹ Annealed polyacid brushes exhibit hydrophilic behavior at high pH (for which the brush is swollen and dissociated) and hydrophobic behavior at low pH (for which the brush is collapsed and protonated). Annealed polybasic brushes exhibit comparable behavior, with the collapsed state present at high pH and the swollen state present at low pH. The chain conformation, brush height, charge distribution, degree of ionization, and wetting properties of responsive polyelectrolyte brushes depend strongly on the grafting density, salt concentration, and pH of the solution. For example, polyacid brushes at low pH are fully protonated and adopt a conformation similar to that of neutral brushes.⁵³⁻⁵⁶ At high pH, the acid groups on the brush become dissociated and the brush behaves as an extended polyelectrolyte brush in the osmotic or Pincus regime, with a Gaussian density profile.⁵⁵⁻⁶² At all pH values, the brush density is greatest near the substrate.^{56, 63-65} The charge density increases with distance from the substrate, and also depends on pH, salt concentration, and grafting density.^{9, 40, 64-71} The brush thickness is closely related to the degree of ionization of the brush and resulting polymer conformation, as well as the grafting density.^{9, 55, 64, 67, 72-75} Despite the existing depth of knowledge on polyelectrolyte brush physics, an improved fundamental understanding of the effects of solution conditions on the brush response is required to design responsive brushes for practical applications. Specifically, open questions remain regarding the mechanism of the brush response to variations in the solution pH, including changes in polymer conformation, degree of dissociation, and charge distribution through the brush.

The simplest method commonly used to probe the degree of dissociation of weak polyelectrolyte brushes is the measurement of the water contact angle on the brush surface.¹³⁰⁻¹³¹ Indeed, the pH-response of the water contact angle for one model polyacid brush, poly(acrylic acid) (PAA), has been measured for brushes of varying grafting densities and lengths.¹³²⁻¹³⁶ Dong et al. identified differences between the bulk pK_a (examined through FTIR) and the surface pK_a (examined through contact angle measurements).¹³⁴ They concluded that the acid groups near the substrate are more difficult to ionize and hence have a higher pKa.¹³⁴ This finding is in agreement with theoretical predictions for weak polyelectrolyte brushes, which indicate that the fraction of charged groups increases with distance from the substrate.40, 65, 67, 70, 137 Other polyelectrolyte brush systems (e.g. poly(2-dimethylamino) ethyl acrylate) (PDMAEA)) also exhibit differences in the contact angle at low and high pH and between the bulk and surface pK_a values.¹³⁸⁻¹⁴⁰ The difference between bulk and surface pK_a arises because polyelectrolytes are able to locally regulate the degree of dissociation based on the local dielectric function.^{137, 141}

By contrast, relatively few literature studies have investigated the pH-response of weak polyelectrolyte brushes when the direction of pH change is varied (i.e. upon decreasing or increasing pH). Aulich *et al.* reported hysteresis (which they termed a "memory effect") in the pH-response of the contact angle of PAA: the pK_a measured upon increasing pH was significantly larger than that measured upon decreasing pH.¹³⁵ This behavior was corroborated with direct measurements of the carboxylic acid and

carboxylate anion concentrations at various pH conditions.¹³⁵ In polybasic brushes, Wanless and collaborators reported the hysteretic pH-response of the hydrated brush thickness and swelling ratio.¹⁴²⁻¹⁴³ Finally, Zhulina, Borisov, and Priamitsyn theoretically predicted a hysteresis loop in the grafting density-dependence of various polyelectrolyte brush properties, including the degree of dissociation, brush height, electrostatic potential profile, and polymer and end-segment density profiles.¹⁴⁴ These studies all posit that differences in the location of dissociated charges upon increasing or decreasing pH generate the observed hysteretic memory behavior; the formation of a neutral hydrophobic skin¹⁴³ or the charge distribution in the brush¹³⁵ may interfere with counterions and/or solvent moving into the brush. These proposed mechanisms involve hindered transport, suggesting that the brush characteristics such as length or dispersity that influence ion movement into or out of the brush may be critical parameters governing the extent of hysteretic memory behavior.

The effects of brush length and dispersity on the pH-response of weak polyelectrolyte brushes, surprisingly, have not been systematically explored. Previous studies on poly(methacrylic acid) (PMAA) brushes reported length-independent pK_a values over a wide range of brush lengths.¹⁴⁵⁻¹⁴⁶ In both studies, the effect of the direction of pH change was not characterized. In polybasic brushes, the dry brush thickness was reported to significantly affect the hysteretic pH-response of the hydrated brush thickness and swelling ratio;¹⁴²⁻¹⁴³ the pH-response of the degree of dissociation, however, was not explored. To our knowledge, the effect of dispersity (in the molecular weight distribution) on the brush pH-response has not been directly explored in the literature. Systematic studies of the effect of brush length and dispersity on pH-response are

expected to generate insight into the physical mechanisms underlying charge dissociation and hysteretic memory.

Here, we characterize the effect of the direction of pH change on the response of the water contact angle and dry thickness of model weak polyacid brushes of varying length and dispersity. PAA brushes of as-synthesized dry thicknesses in the range of 7 -44 nm were synthesized through surface-initiated atom transfer radical polymerization (SI-ATRP) of tert-butyl acrylate, followed by hydrolysis to PAA. Dry brush thickness (via ellipsometry) and water contact angle were measured after brushes were exposed to solutions of varying pH. The brushes were first exposed to solutions of decreasing pH (from 10 to 3), and subsequently exposed to solutions of increasing pH (from 3 to 10). We report three trends in the response of the PAA brushes to changes in pH. First, the thickness of dry brushes increased as the pH was increased for brushes above a critical length, and this effect was magnified as the dispersity increased. Second, the water contact angle measured at low pH increased with brush dispersity. Finally, brushes of sufficiently high dispersity exhibited hysteretic memory behavior (i.e. dependent on the direction of pH change) in the contact angle. We propose a mechanism to relate the observed pH response of PAA brushes in this study to behavior reported in the literature for the polymer conformation, charge distribution, and degree of penetration of solution throughout the brushes, and show that an increase in brush dispersity generates the hysteretic memory behavior.

3.2 Materials and Methods

Materials. All chemicals were purchased from Sigma Aldrich and used asreceived unless otherwise noted. Monomethyl ether hydroquinone (inhibitor) was

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removed from *tert*-butyl acrylate (tBA, 98%) by passing through a silica gel column (60 Å pore size). tBA was then dried with calcium hydride (reagent grade, 95%) and distilled under vacuum. Ethyl α -bromoisobutyrate (EBiB, 98%) and *N*,*N*,*N'*,*N''*-pentamethyldiethylenetriamine (PMDETA, 99%) were each degassed with three freeze-pump-thaw cycles. Dichloromethane (JT Baker, HPLC grade, 99.8%) was dried using a Pure Process Technology solvent purification system.

Immobilization of Initiator on Silicon Wafer. Initiator attachment to silicon substrates followed procedures in ref.¹⁴⁷. Single-sided polished silicon wafers (Mechanical Grade, University Wafers) were cut into approximately $1 \text{ cm} \times 2 \text{ cm}$ pieces before use. The silicon substrates were cleaned by 10 min of sonication in acetone (ACS reagent, 99.5%) followed by 10 min of sonication in deionized (DI) water. After drying under nitrogen, the wafers were exposed to air plasma (Harrick Plasma Cleaner) for three minutes to generate surface hydroxyl groups. (3-Aminopropyl)triethoxysilane (APTES, 99%) was deposited on the wafer in vacuum for 30 min at a vacuum level of 50 mtorr. The silanized surface on the silicon substrate was annealed at 110°C for 30 min. Subsequently, the silicon substrate was immersed in dry dichloromethane containing 2 vol% anhydrous pyridine (99.8% pure). The polymerization initiator, α -bromoisobutyryl bromide (98%), was added dropwise into the solution containing the substrate at 0°C until an initiator concentration of 0.081 M was attained. The resulting solution was held at 0°C for 1 hour and then at room temperature for 12 hours. The initiator-grafted substrate was rinsed with acetone and dried under flow of nitrogen gas. The silicon substrates grafted with initiator were used immediately for polymerization.



Scheme 3.1 Synthesis of PAA brushes.

Synthesis of Poly(Acrylic acid) (PAA) Brushes. Poly(*tert*-butyl acrylate) (PtBA) brushes were synthesized using surface-initiated atom transfer radical polymerization (SI-ATRP) and subsequently hydrolyzed to PAA (Scheme 1), following procedures in refs. ¹⁴⁸⁻¹⁴⁹. The initiator-grafted silicon substrates were transferred to a glove box under nitrogen gas in a round bottom flask where tBA, PMDETA, copper(I) bromide (CuBr, 99%), anhydrous N,N-dimethylformamide (DMF, 99.8%), and EBiB (a solution initiator) were added in a predetermined molar ratio for each polymerization. The flask was capped with a septum inside the glove box and transferred to a preheated oil bath at 50°C. The reaction mixture was stirred for 24 hours and [tBA]:[EBiB] was varied to obtain different PtBA brush lengths. An equimolar ratio of PMDETA, EBiB, and CuBr was used, and this mixture was diluted with DMF such that the volume ratio of DMF:tBA was 1:1.

The polymerization was quenched by addition of tetrahydrofuran (THF, OmniSolv, HPLC grade, 99.9%). The silicon substrate grafted with PtBA was rinsed with acetone, methanol (ACS reagent, 99.8%), and DI water, and dried under nitrogen gas. The PtBA brushes were stored in a petri dish. The PtBA brush was converted to a PAA brush by hydrolysis in the presence of dichloromethane (11ml) and trifluoroacetic acid

(reagent plus, 99%, 3 ml) for 24 hours. The PAA brush on a silicon substrate was rinsed with acetone and dried under nitrogen flow.

The remaining solution, which contained free PtBA (initiated from EBiB), was diluted by adding excess THF and passed through a neutral aluminum oxide column to remove the catalyst. The polymer solution devoid of catalyst was concentrated using a rotary evaporator and precipitated in a DI water/methanol mixture (1:1 by volume). Finally, the precipitated PtBA was collected and dried under vacuum overnight at room temperature.

Two separate methods were employed to synthesize brushes of constant length and varying dispersity. In one synthesis, the procedure described above was modified to use a higher ratio of initiator (EBiB) to catalyst (CuBr) and ligand (PMDETA) (20:1:1) and a longer reaction time of 32 hours, resulting in a PtBA brush of length 12.7 nm and dispersity 1.26 (methods used to characterize the length and dispersity are discussed in the following sections). In a second reaction, a reducing agent (phenylhydrazine), which is known to decrease the concentration of Cu^{II} deactivator,⁸⁹ was employed. Phenylhydrazine (97%) was added to the reagents described above (the molar ratio of phenylhydrazine : initiator : catalyst : ligand was 0.5:1:1:1 and the reaction time was 24 h), resulting in a PtBA brush of length 14.4 nm and dispersity 1.54. Both brushes were subsequently hydrolyzed to PAA.

Gel Permeation Chromatography (GPC). Molecular weight and molecular weight distribution (including the number-average molecular weight, M_n , and dispersity, D) for free polymer (synthesized in solution) were measured with a Viscotek GPC system with Agilent ResiPore columns, using stabilized THF (OmniSolv, HPLC grade, >99.9%)

as the mobile phase at 30°C. The flow rate was 1 ml/min and injection volume was 100 μ l. A triple detection system, including light scattering, viscometer, and differential refractometer, was used to characterize the molecular weight distribution. The dn/dc value of PtBA was determined to be 0.0479 ± 0.0012 under these conditions.

Proton Nuclear Magnetic Resonance (¹**H-NMR).** ¹H-NMR spectra were collected on a JEOL ECA-500 spectrometer using deuterated chloroform as the solvent and tetramethyl silane (TMS) as an internal reference.

X-ray Photoelectron Spectroscopy (XPS). XPS spectra were obtained with a Physical Electronics Model 5700 instrument using a monochromatic Al K α X-ray source (1486.6 eV) operated at 350 W. The diameter of the analyzed area, the collection solid cone angle, and the take-off angle were fixed at 0.8 mm, 5°, and 45°, respectively. An applied pass energy of 23.5 eV resulted in an energy resolution of better than 0.51 eV. Measurements were conducted at a vacuum level of 5×10^{-9} torr or lower.

Atomic Force Microscopy (AFM). AFM images were acquired with a multimode atomic force microscope (Nanoscope IV) from Digital Instruments operated in tapping mode using monolithic silicon Tap300Al-G probes. The resonance frequency was 300 kHz and force constant was 40 N/m. Images were collected in height and amplitude (or deflection) modes with scan rates between $2-3 \text{ s}^{-1}$ and 256 scan lines per image.

Fourier Transform Infrared Spectroscopy (**FTIR**). IR absorbance was measured using a Nicolet 6700 FTIR spectrometer equipped with an attenuated total reflectance (ATR) stage. IR absorbance was recorded with OMNIC data acquisition

software using 128 scans at a resolution of 8 cm⁻¹. IR peak locations were used to characterize PtBA and PAA brushes grafted on silicon wafers.

Water Contact Angle and Thickness of Dried PAA Brushes. To investigate the dependence of the water contact angle and dry brush thickness on pH, separate solutions of pH ranging from 3 to 10 were prepared by adding an appropriate amount of sodium hydroxide or hydrochloric acid to water purified with a Millipore water purification system (resistivity 18.2 M Ω cm). The polymer-grafted silicon wafers were immersed for 30 min in a solution with a desired pH and subsequently dried under nitrogen.

Static water contact angles on the dried PAA brush samples were measured on an OCA 15EC video-based optical contact angle-measuring instrument (DataPhysics, Germany) at ambient temperature using SCA 20 software. DI water droplets (of approximate volume 1 μ L) were deposited carefully onto the polymer-grafted surface and the contact angles were measured at five different positions on the surface.

The dry thickness of the PAA brushes was measured with a JA Woollam M-2000 spectroscopic ellipsometer. Ellipsometry data were modeled using a two-layer model that consisted of a polymer layer on top of SiO₂. The refractive index of each layer was modeled with the Cauchy dispersion relation, $n(\lambda) = A + B/\lambda^2$ (A and B are strictly positive). The measured amplitude ratio and phase difference were modeled with the Fresnel equation, with the brush thickness as a fitting parameter. This model provided a good fit for data in the wavelength range of 350 - 1690 nm. Thickness measurements were conducted at five different locations on each substrate.

3.3 Results

3.3.1 Brush Synthesis. PtBA brushes of varying length on silicon wafers were synthesized using SI-ATRP, as shown in Scheme 1. The attachment of APTES and SI-

ATRP initiator to the silicon substrate was verified through XPS.¹⁰ The presence of the PtBA brush was confirmed through FTIR.^{55-56, 74, 134, 148, 150-153} In the first series of experiments, we synthesized brushes of varying lengths by varying the amount of monomer in solution. To estimate the grafting density of brushes on the substrate, we examined the relationship between the as-synthesized dry thickness of the PtBA brush, measured using ellipsometry, and the M_n of PtBA. As the M_n of PtBA grafted to the substrate could not be directly characterized, a solution initiator (EBiB) was added to every brush synthesis. The solution PtBA was characterized through ¹H-NMR and GPC.¹⁰ The PtBA dry brush thickness appeared to be uniform across the substrate and increased linearly with M_n of the solution PtBA, as shown in Figure 3.1, consistent with a constant brush grafting density. We estimated the grafting density of the PtBA brushes as $\sigma = h\rho N_A/M_n$ ⁷⁴ where h is the brush thickness, $\rho = 1.05$ g/cm³ is the density of PtBA,⁷⁴ N_A is Avogadro's number, and M_n is the molecular weight of the solution polymer, which is assumed to be equal to that of the brush (following prior studies^{55, 74, 148}). Although the M_n of the solution polymer was likely not equal to that of the brush and water from humid air adsorbed into the brush may have increased the height by up to 30%,¹⁴¹ the linear scaling of the solution polymer M_n with brush thickness nonetheless suggested that the grafting density was constant for all brushes. From the linear scaling of M_n with brush thickness, we estimated a grafting density of 0.38 chains/nm² for all brushes described in this manuscript (unless otherwise indicated). For PtBA brushes of a lower molecular weight ($M_n = 8.56$ kg/mol), the crossover from the mushroom to brush regime occurred at 0.08 chains/nm² [ref ⁷⁴]. We therefore assumed that all brushes in this study were in the

(extended) brush regime (even after accounting for errors in the brush thickness due to atmospheric water taken up by the brush).

We also measured the dispersity (D) of the solution polymer that was copolymerized during brush synthesis. Here we explicitly assumed that the dispersity of the solution polymer is proportional to that of the polymer brush on the surface; this assumption is analogous to that frequently used to estimate the molecular weight of surface-grafted polyelectrolyte brushes,⁷⁴ in which the molecular weight of the solution polymer is assumed to be proportional to that of the brush. The dispersity (D) of the solution polymer was relatively low, 1.1 – 1.2, over a wide range of M_n (18.6 – 73.3 kg/mol), as shown in Figure 3.1. At higher M_n the molecular weight distribution was broader, with $D \sim 1.3 - 1.4$ for M_n of 79.9 – 167 kg/mol.



Figure 3.1 PtBA brush dry thickness (closed symbols) and dispersity (D, open symbols) as a function of molecular weight (M_n) of PtBA solution polymer. The dashed line indicates a linear fit ($\mathbb{R}^2 = 0.99$) to the thickness versus M_n data.

PtBA brushes on silicon wafers were subsequently hydrolyzed to PAA for characterization of the pH-dependent response. FTIR spectroscopy (Figure 3.2) showed the disappearance of the absorbance maxima at 2977 and 1392/1368 cm⁻¹, associated with asymmetric stretching and bending of the *tert*-butyl methyl group of PtBA, and

appearance of the absorbance maximum at 3420 cm⁻¹, associated with OH stretching of PAA, confirming that PtBA was successfully converted to PAA.^{55, 74, 148, 154-156} After hydrolysis, the decrease in the thickness of the brushes, measured with ellipsometry as 48 – 56% (Table 3.1), was also consistent with the expected decrease in the volume of the polymer.⁷⁴



- Figure 3.2 FTIR spectra of (a) PAA brush (thickness = 58 nm) and (b) PtBA brush (thickness = 65 nm). The conversion of PtBA to PAA is confirmed by disappearance of peaks located at 2977 and 1392/1368 cm⁻¹ and ii) appearance of broad peak at 3420 cm⁻¹.
- **Table 3.1** Average as-synthesized dry thickness (with standard deviation) of PtBA and PAA brush used for experiments, corresponding molecular weight and dispersity of the PtBA that was polymerized in solution simultaneously during brush synthesis.

PtBA Brush	M _n (kg/mol) of	PAA Brush	Reduction in Thickness
Thickness (nm)	Solution PtBA (D)	Thickness (nm)	After Hydrolysis
13.4 ± 0.3	18.6 (1.11)	6.8 ± 0.2	49.2 %
12.7 ± 0.3	18.8 (1.26)	6.3 ± 0.2	50.0 %
14.4 ± 0.3	18.4 (1.54)	7.3 ± 0.1	49.3 %
22.4 ± 0.1	35.4 (1.06)	11.7 ± 0.3	47.7 %
47.8 ± 0.7	79.9 (1.25)	22.2 ± 0.8	53.6 %
65.7 ± 0.6	111 (1.30)	30.4 ± 0.4	53.7 %
100.1 ± 0.7	167 (1.41)	44.2 ± 0.3	55.8 %

3.3.2 pH Response of PAA Brushes. PAA brushes were immersed in unbuffered solutions of constant pH for 30 minutes to allow sufficient time for swelling or deswelling. Subsequently, we measured the brush thickness (using ellipsometry) and the static contact angle¹³⁰⁻¹³¹ of a neutral (pH 7) water droplet^{147, 157} on the brush surface; we use the latter method as a simple and rapid way to assess the degree of dissociation within the brush. We confirmed that the thickness and static contact angle did not change on time scales longer than 30 minutes. Additionally, we confirmed that the choice of a phosphate-buffered or unbuffered soaking solution, and the choice of DI water, phosphate-buffered solution at a given pH, or unbuffered solution at a given pH used as the droplet for the contact angle measurement, did not affect the measured contact angle; this finding is in agreement with earlier measurements that showed that the presence of buffer negligibly affected the contact angle.¹³⁶ Finally, we limited our measurements to the pH range of 3 - 10 as brushes exposed to solutions of pH 2 did not exhibit reproducible changes upon cycling pH, which we attributed to cleavage of the polymer chains by hydrolysis of the ester bonds.¹³⁴

The dry thickness of a short 12 nm brush decreased slightly upon decreasing the pH from 10 to 3, as shown in Figure 3.3(a). Longer brushes (e.g. 22, 30, and 44 nm) exhibited a more pronounced decrease in thickness as pH was decreased. The decrease in thickness with decreasing pH reported here is consistent with prior measurements on flat brushes.^{69, 155} The thickness did not depend on the direction of pH change (increasing or decreasing) for brushes of thickness 12, 22, and 30 nm. Only the thickest brush (44 nm) exhibited a slight hysteresis in the thickness response, with the dry thickness upon decreasing pH slightly larger than that upon increasing pH. To quantify the change in dry

thickness we calculated the percentage increase in the dry thicknesses (Percentage increase in dry thickness = 100 [(dry thickness at pH 10) – (dry thickness at pH 3) / (dry thickness at pH 3)) from pH 3 to 10 (Table 3.2). The percentage increase in thickness was approximately constant save for the thinnest brush.



Figure 3.3 (a) Dry thickness and (b) static water contact angle of PAA brushes of varying length and dispersity, as a function of pH. The pH was first decreased from 10 to 3 (closed symbols) and then increased from 3 to 10 (open symbols).

Table 3.2 Percentage increase in dry thickness of PAA brushes (of varying length and dispersity), measured as pH is increased from 3 to 10.

As-Synthesized	Dispersity, D	Percentage Increase in
Dry Thickness (nm)		Dry Thickness
12	1.06	30 ± 5
22	1.25	51 ± 5
30	1.30	58 ± 4
44	1.41	55 ± 2

The contact angle of neutral water on a PAA brush after immersion in a solution of pH 10 was low (<10°) and characteristic of a hydrophilic surface (Figure 3.3(b)), independent of brush length. The low contact angle is consistent with the ionization of the great majority of the acrylic acid groups.^{135, 158} For a brush of as-synthesized dry thickness 12 nm, the water contact angle increased to 48 degrees as the pH was decreased from 10 to 3, consistent with protonation of the acrylic acid groups. The contact angle at low pH increased with increasing brush length and dispersity, reaching 70 degrees for a brush of as-synthesized dry thickness 44 nm and dispersity 1.41.¹³⁴

Upon increasing the pH from 3 to 10, the contact angle on all brushes decreased and the brushes returned to the deprotonated and hydrophilic state at high pH. Notably, the water contact angles measured upon increasing and decreasing pH differed over an intermediate pH range of 5 - 8. This hysteretic memory behavior was observed over a wide range of brush lengths and dispersities (Figure 3.3(b)). Our findings confirm and significantly extend an earlier report of hysteresis by Aulich *et al.*, who observed similar differences in the contact angle (which they termed a "memory effect") measured for a brush of dry thickness ~5 nm cycled between pH 2 and 10; the brush dispersity was not reported.¹³⁵

Motivated by this hysteretic change in contact angle with pH, we examined the behavior of the logarithm of the effective acid dissociation constant, pK_a , defined as the midpoint of the contact angle titration curve. We fit the cosine of the contact angle profile, which is related to the degree of dissociation of the carboxylic groups near the free surface of the brush,¹³⁰⁻¹³¹ to a sigmoidal function and thereby extracted pK_a upon increasing and upon decreasing pH. PAA brushes exhibited distinct differences in pK_a

upon increasing and decreasing pH, as shown in Figure 3.4. The pK_a measured upon increasing pH for a given brush length and dispersity was always greater than that measured upon decreasing pH, in agreement with prior work conducted at a single brush length.¹³⁵ Upon increasing pH, the measured pK_a (6.1 – 6.5) was comparable to that previously reported for PAA in solution $(6.2)^{159}$ and a "bulk" pK_a of thick 55 nm PAA brushes measured by FTIR titration (6.5 - 6.6).¹³⁴ By contrast, the pK_a values measured upon decreasing pH (4.6 – 5.0) were similar to the previously reported "surface" pK_a of the 55 nm PAA brushes (4.4)¹³⁴ and 12 nm brushes (4.8)¹³⁶ measured via contact angle titration.¹³⁸



Figure 3.4 pK_a , as a function of PAA brush dry thickness (as-synthesized). The closed (open) symbols indicate pK_a values calculated from contact angle curves measured as the pH is decreased (increased). The upper *x*-axis labels indicate the dispersity of each brush.

To our knowledge there are no published studies of the pK_a for PAA brushes of varying length and/or dispersity, although two studies examined the length-dependence

of pK_a in poly(methacrylic acid) (PMAA) brushes. Schüwer and Klok measured the frequency-dependent response using quartz crystal microbalance (QCM) and found that the pK_a of PMAA (measured upon increasing pH) was constant as the brush length increased from 5 nm to 80 nm (with a slight increase in pK_a for a brush of length 91 nm),¹⁴⁶ and Santonicola *et al.* reported that PMAA brushes of length ~30 nm and ~90 nm exhibited similar pK_a values¹⁴⁵. In both studies, the relative insensitivity of the pK_a to the PMAA brush length agrees with our results (Figure 3.4) for PAA brushes of lengths 12 – 44 nm.

3.3.4 Decoupling Effects of Brush Thickness and Dispersity. To directly test the effects of dispersity on brush response, in a second series of experiments we synthesized three PAA brushes of similar length but varying dispersity. Using an equimolar (1:1:1) ratio of initiator (EBiB) to catalyst (CuBr) and ligand (PMDETA), we obtained a PtBA brush of length 13.4 nm and dispersity *D* of 1.11; using a higher ratio of initiator to catalyst and ligand of 20:1:1, we obtained a PtBA brush of length 12.7 nm and dispersity D of 1.26. Lastly, we produced a PtBA brush of similar length (14.4 nm) and even greater dispersity D (1.54) through addition of a reducing agent, phenylhydrazine. All three brushes were hydrolyzed to PAA. The thicknesses of the PAA brushes produced through these three different synthetic methods were comparable and did not vary with pH, as shown in Figure 3.5(a) and in Table 3.3. The contact angle of the lowest-dispersity PAA brush (D = 1.11) exhibited no measurable hysteretic memory upon reversing the direction of pH change, whereas the contact angle of both higher-dispersity brushes (D =1.26 and 1.54) exhibited significant hysteretic memory. Furthermore, the low-pH static contact angles of the two higher-dispersity brushes were indistinguishable from one

another, yet significantly larger than that of the lowest dispersity brush. The similarity of the low-pH contact angles for the two higher-dispersity brushes suggests that there may be an upper limit to the enhancement in the low-pH contact angle in the limit of high dispersity values; this result is in contrast to that found in our the first series of experiments on PAA brushes of varying length and dispersity, in which the low-pH contact angle systematically increased with dispersity (Figure 3.3(b)).

The pK_a was characterized as the midpoint of the contact angle titration curves, as described previously. The pK_a values measured for the lowest-dispersity brush in Figure 3.5(b) upon increasing or upon decreasing pH were identical, as that brush did not exhibit hysteretic memory behavior. Brushes of higher dispersity exhibited differences in the pK_a values measured upon decreasing and increasing pH. The pKa values obtained upon decreasing pH were approximately independent of brush dispersity, as shown by the closed symbols in Figure 3.5(c), and were consistent with those reported in Figure 3.3 for brushes in which both dispersity and thickness were varying. Upon increasing pH, however, the pK_a surprisingly yet systematically increased with increasing dispersity (Figure 3.5(c)). In the first series of PAA brushes of varying dispersity and length (Figure 3.4), by contrast, the pK_a observed upon increasing pH was fairly independent of both brush length and dispersity. We defined the difference in pK_a , $\Delta pK_a = (pK_a, increasing)$ pH) – (pKa, decreasing pH), and examined its dependence on dispersity D for brushes of varying length (extracted from Figure 3.4) and brushes of fixed length (extracted from Figure 3.5(c)). Across all samples, $\Delta p K_a$ increased with increasing *D* (Figure 3.6). This result suggests that dispersity is the dominant factor underlying the hysteretic memory





Figure 3.5 (a) Dry thickness and (b) static water contact angle as a function of pH for PAA brushes of approximately equal length but varying dispersity. (c) pK_a is shown as a function of dispersity D. Closed symbol: decreasing pH, open symbol: increasing pH.

Table 3.3 Percentage increase in dry thickness of PAA brushes of varying dispersity (and
approximately constant length), measured as pH is increased from 3 to 10.

As-Synthesized	Dispersity, D	Percentage Increase
Dry Thickness (nm)		in Dry Thickness
6.8	1.11	20 ± 5
6.3	1.26	< 1
7.3	1.54	4 ± 1



Figure 3.6 Difference in pK_a , $\Delta pK_a = (pK_a$, increasing pH) – (pK_a, decreasing pH) as a function of dispersity D for brushes of varying length (extracted from Figure 3.4) and brushes of fixed length (extracted from Figure 3.5(c)).

3.4 Discussion

In our first series of experiments, the response of PAA brushes of varying length and dispersity to changes in pH exhibited three trends (Figures 3.3 and 3.4). (i) The dry thickness decreased as the pH was decreased, and the percentage increase in dry thickness was nearly constant for the brushes of greater length and dispersity (lengths between 22 - 44 nm and dispersities between 1.25 - 1.41). (ii) The low-pH contact angle of water on dry brushes following immersion in solution of pH 3 increased with increasing brush length and dispersity. (iii) All brushes exhibited hysteretic memory behavior at intermediate pH, in which the contact angles measured upon increasing and decreasing pH were different. The pK_a observed upon increasing pH was significantly higher than that observed upon decreasing pH.

Similar but slightly different trends were observed for our second series of experiments on PAA brushes of varying dispersity yet constant PAA brush length of around 7 nm (Figure 3.5). (i) The dry thickness of these relatively short brushes was independent of dispersity and increased only slightly with increasing pH. (ii) The low-pH contact angles of the higher dispersity brushes were comparable to one another, but were significantly higher than that of the low-dispersity brush. (iii) The two higher dispersity brushes exhibited hysteretic memory behavior, whereas the pH-response in the low dispersity brush did not depend on the direction of pH change.

Finally, the difference in pK_a (characterized upon decreasing and increasing pH) increased slightly with increasing dispersity when the two series of samples (varying length and constant length) were considered together (Figure 3.6).

To explain the observed trends in dry thickness and contact angle we consider the effect of changing pH on the dissociation of the carboxylic acid groups in the brush and on polymer conformation, as reported in earlier literature studies. At pH 3, the acid groups are protonated throughout the brush, and the brush is uncharged and somewhat hydrophobic. Earlier ionic concentration measurements indicated that at pH 3 the solution does not penetrate the brush.¹³⁵ The relatively high contact angle of neutral water on the dry brush observed at pH 3 is thus consistent with a hydrophobic brush periphery. As the pH of the solution is increased, the acid groups at the surface start to become deprotonated.¹³⁴ Theory and simulations,^{40, 65, 67, 70} as well as experiments,¹³⁴ indicated that the degree of dissociation in weak polyacid brushes increases as the distance from

the substrate is increased. At pH 10, the carboxylic acid groups in the solvent-soaked brushes are mostly deprotonated and the brushes are swollen and extended. The low contact angle indicates that solution fully penetrates the charged, hydrophilic brush; this result is consistent with earlier ionic concentration measurements at high pH.¹³⁵ For all brushes, it is possible to cycle the brush between very low and very high pH values (outside of the pH range over which hysteretic memory is observed in Figure 3.3), and reversibly recover surfaces with high and low contact angles, respectively, as illustrated in Figure 3.7; this finding indicates that the conformational changes at extreme pH values are fully reversible.



Figure 3.7 Reversible switching of PAA brush with as-synthesized dry thickness of 28 nm (grafting density = 0.32 chains/nm²). For each measurement, the brush was exposed to solution of the desired pH for 30 min.

The hysteretic memory in contact angle observed in our experiments suggests that the polymer conformation affects the ability of solvent and counterions to penetrate the brush at intermediate pH values. Earlier studies suggest that polyelectrolytes can locally regulate the degree of dissociation of the charge groups based on the local dielectric environment.^{137, 141} We thus propose a hypothesized physical picture similar to those used to describe changes in conformation in polybasic¹⁴³ and polyacid¹³⁵ brushes upon increasing or decreasing pH, as shown in Figure 3.8. This picture is based on an earlier literature report of hysteresis in dissociation of the carboxylic groups upon changing pH.¹³⁵ Upon decreasing the pH from 10, the brush remains water-wet over several pH units. Contact angle is thought to probe the ionization state of the charge groups far from the substrate¹³⁴ and so our findings suggest that upon decreasing pH these acid groups remain charged until the pH is lowered significantly below 10. When the pH is sufficiently lowered, the brush begins to collapse as the groups on the periphery become uncharged, leading to an increase in the contact angle.



Figure 3.8 Proposed mechanism for changes in brush conformation and charge distribution as a function of pH. The schematic indicates the proposed hydrated state of the brush at different pH conditions.

Conversely, when the pH is increased from 3 to 10 the initial change in contact angle is gradual. This result suggests a delayed onset of deprotonation, with the periphery of the brush remaining hydrophobic and collapsed. A possible explanation for the delayed collapse is suggested by two sets of earlier measurements. First, using the standing x-ray technique Aulich *et al.* showed that ions do not penetrate hydrophobic PAA brushes at low pH.¹³⁵ Second, via turbidity measurements Sarkar and Somarsundan showed that high molecular weight PAA brushes phase separate from solution at low pH under which intramolecular hydrogen bonding occurs, whereas low molecular weight PAA brushes remain suspended in solution; both enthalpic and entropic contributions to the free energy, and hence the driving force for the phase separation, increase with molecular weight.¹⁶⁰ These earlier results therefore suggest that the delay in the onset of swelling observed in our study, as pH is increased from 3 to 10, arises from the inability of the solvent to interact with collapsed and phase-separated PAA brushes.

We considered several possible origins of the observed trends in the pH-response of the contact angle and dry thickness shown in Figures 3.3 - 3.5, which are hypothesized to result from underlying changes in PAA brush conformation and charge distribution shown schematically in Figure 3.8. First, we determined that PtBA was fully converted to PAA (within measurement error), based on the complete disappearance of characteristic PtBA peaks in the FTIR measurements (Figure 3.2) and the near-zero CA at observed at high pH. Second, we measured the characteristic roughness of dry brushes using atomic force microscopy. The root-mean-square (RMS) roughness did not vary significantly with brush height (0.29 and 0.48 nm for brushes of lengths 12 and 30 nm, respectively),¹⁰ and thus differences in surface roughness were unlikely to cause the observed hysteretic memory behavior in contact angle. Similarly, a comparison of Figures 3.3(a) and 3.3(b) shows that hysteretic memory behavior in contact angle is not explained by variations in brush length: brushes with as-synthesized dry thicknesses of 12, 22, and 30 nm exhibited hysteretic pH-dependent contact angles, whereas their pH-responsive thicknesses were largely independent of the direction of pH change. Figure 3.5 further supports this conclusion, as brushes with comparable lengths nonetheless exhibited vast differences in the degree of hysteretic memory behavior.

Next, we considered explanations related to brush kinetics. Earlier studies quantified the kinetics of the swelling and collapse of PAA brushes using QCM,¹⁵⁴ QCM with dissipation monitoring (QCM-D),¹⁶¹ and surface plasmon resonance (SPR) spectroscopy.¹⁶⁰ Kurosawa *et al.* observed changes in frequency on the time scale of tens of minutes after switching brushes of molecular weights around 4 and 14 kg/mol between pH 4.0, 4.8, and 5.4.¹⁵⁴ Liu and Zhang reported changes in the oscillation frequency as the pH was repeatedly cycled between 3.2 and 6.6, and the time over which the frequency changed was short compared to the 20-min time scale of the oscillation.¹⁶¹ Finally, Sarkar and Somasundaran showed that the angular position of the reflectance dip of PAA, with molecular weight ranging from 50 to 3,000 kg/mol, changed over time scales of tens of seconds as the pH was repeatedly cycled between 3.5 and 9.5; swelling in these experiments took longer than collapse.¹⁶⁰ Although the response times reported in these studies vary widely, all time scales are shorter than the 30-minute equilibrium time used for our brush measurements. Moreover, we checked that the contact angle did not significantly change over time scales of longer than 30 minutes. We therefore concluded that the kinetics of swelling and deswelling likely did not play a role in the observed pH response of our PAA brushes.

We also considered the effect of substrate interactions on the pH-response of brushes of varying lengths.¹⁶² We modeled the polymer brush as a PAA sphere (with

surface area equal to σ^{-1} , 2.6 nm²/chain, and Hamaker constant reported for PAA in ref. ¹⁶³, 9.15 × 10⁻²¹ J) separated from the silicon oxide substrate by a distance *d*. The van der Waals free energy, calculated as a function of *d*,¹⁶⁴ decreased below kT a short distance from the substrate (around 0.2 nm). We therefore concluded that substrate interactions were unlikely to explain differences in the response of brushes of varying length, such as the low-pH contact angle and degree of swelling.

Instead, we suggest that the observed trends in the pH response of the brushes are governed by differences in brush dispersity. Milner and collaborators showed theoretically that increased dispersity can increase the thickness of polyelectrolyte brushes.¹⁶⁵ de Vos and collaborators showed theoretically that increased dispersity can significantly change the volume fraction profile of the brush.³⁸ The three trends in the pH-responsive thickness and contact angle reported in this study could be explained by differences in the dispersity of the brushes examined here. (i) We suggest that the collapse of the brushes at low pH (Figure 3.3(a)) is aided by conformational changes proposed by de Vos *et al.* for higher dispersity brushes, in which the longest chains near the brush surface are less extended than the chains near the substrate in densely grafted systems.³⁸ In support of this idea, the 12 nm brush had the lowest D of the brushes included in Figure 3.3, and exhibited the lowest percentage increase in dry thickness upon decreasing pH (Table 3.2). The 7 nm brush series with varying dispersity also exhibited a very low percentage increase in dry thickness upon decreasing pH (Table 3.3); we suggest that these low molecular weight chains have a reduced driving force for phase separation. (ii) We posit that the thickness of the collapsed layer near the brush surface at low pH increases with brush dispersity, resulting in the increase of the low pH contact angle (and degree of hydrophobicity) shown in Figures 3.3(b) and 3.5. Comparison of the two higher dispersity brushes in Figure 3.5, which exhibited similar low-pH contact angles, may indicate that there is a critical dispersity above which no further increase in the low-pH contact angle is observed. (iii) Finally, we suggest that the presence of this collapsed region at the brush surface prevents solvent and counterions from penetrating into the brush (as shown in the schematic mechanism in Figure 3.8) and hence generates the hysteretic memory behavior in the contact angle observed in this study. In particular, the 7 nm brush with the lowest dispersity in Figure 4 showed no hysteretic memory behavior; moreover, across both series of experiments the degree of hysteretic memory behavior (quantified by ΔpK_a , as shown in Figure 3.6) increased as dispersity increased. Together, these results suggest that brush dispersity generates hysteretic memory and can serve as a design parameter to tune the responsive properties of polyelectrolyte brushes.

3.5 Conclusions

We synthesized a series of poly(acrylic acid) brushes of varying length and dispersity and characterized changes in the dry thickness and static water contact angle as the pH was first decreased from 10 to 3, and subsequently increased from 3 to 10. From these measurements, we report three trends in the pH-response: (i) the thickness of dry brushes increased upon increasing pH for brushes above a critical length; (ii) the low-pH contact angle increased with increasing dispersity; (iii) brushes of sufficient dispersity exhibited hysteretic memory behavior at intermediate pH; as a result, the pK_a measured upon increasing pH was greater than that upon decreasing pH. The degree of hysteretic memory behavior (as quantified by the difference in the pK_a values measured upon increasing and decreasing pH) increased with brush dispersity. To explain this response, we proposed a hypothetical physical picture in which solvent was excluded from the collapsed PAA brush at pH 3; as a result, the onset of swelling was delayed to higher pH by the inability of the solvent to penetrate the brush. We posited that the trends observed in this study originate from differences in the dispersity of the brushes. This study is the first to systematically assess the pH-response for PAA brushes of varying length and dispersity through careful synthesis and thorough characterization. Here we identify one unanticipated consequence of high brush dispersity, the hysteretic memory behavior, and show that its extent can be controlled using synthetic methods that modulate brush dispersity. Because the water contact angle is thought to probe the degree of dissociation of charges near the polyelectrolyte brush surface, we expect that brush dispersity may have other practical consequences for the response of pH brushes in a fully hydrated state.

Chapter 4. Effect of Brush Thickness and Dispersity on Bacterial Attachment and Detachment

4.1 Introduction

Biofilms are surface-attached colonies of microorganisms,¹¹ and adversely affect the industrial processes leading to reduction in performance of water purification systems,⁴ medical implants,⁴⁻⁶ food packaging,¹² and marine engineering equipment.⁷⁻⁸ Apart from industrial processes, biofilms also affect human health as biofilms cause or exacerbate more than 80% of hospital-acquired infections.¹³ *Staphylococcus epidermidis*, one of the most common agents of infection, is highly resistant to antibiotics.¹⁶⁶⁻¹⁶⁷ Its virulence is often attributed to the formation of resistant biofilms after bacteria attach to medical devices.^{26, 168} Although the deleterious consequences of biofilms are widely known, in other settings they can have benign or even beneficial properties. As one example, biofilms from certain bacterial species (*Pseudomonas* sp. and *Pseudomonas putida*) are used to remediate water; as a second benign biofilm coatings on medical devices can prevent infection by pathogenic invaders.¹⁴⁻¹⁵ Early in biofilm formation, bacteria reversibly and then irreversibly attach to surfaces.¹⁶ Hence an important step in managing biofilm formation is to tailor surfaces to control the adhesion of bacteria.¹⁷

In the absence of adhesins, adhesion of bacteria to a surface is governed by Lifshitz-van der Waals interactions, electrostatic interactions, acid-base interactions, and hydrophobic interactions.¹⁸ Lifshitz-van der Waals and hydrophobic interactions are typically attractive whereas electrostatic and acid-base interactions can be either repulsive or attractive. Because adhesion is controlled by these various interactions between bacteria and surface, in principle adhesion can be tuned by varying the properties of the

surface by applying a coating. Polymers are a convenient choice for coatings due to their easy applicability and processability. Variety of polymer coatings have been studied to control bacterial adhesion such as poly(acrylamides), poly(acrylates), poly(ethylene oxide) (PEO) etc.^{3, 19} One strategy to control attachment of bacteria is to employ polymer brushes. Polymer brushes increase the separation between bacteria and the surface, thereby reducing the attractive interactions between them, and increase the repulsive interactions (due to steric hindrance of brushes) experienced by adhering bacteria.²⁸⁻³⁰ In addition, polymer brushes can swell when solvated (depending on chemistry). Hence the hydration of a polymer layer also can reduce adhesion (by reducing attractive hydrophobic interactions); indeed, easily-hydrated polymer brushes such as poly(ethylene glycol) and zwitterionic polymers considerably reduce adhesion.^{31-37, 169} Adhesion of organisms depends on many properties of polymer brushes, including their grafting density,^{30, 170} average thickness,^{29, 31} wettability,^{41, 171} and surface charge.^{47, 172} Expanding the range of brush properties that can be tuned opens new avenues for the rational design of antifouling surfaces.

One characteristic property of polymer brushes, underexplored in the context of fouling, is the dispersity (breadth) in the molecular weight distribution. To the best of our knowledge, there are no experimental studies probing the effect of brush dispersity on the bacterial adhesion. Dispersity is an appealing parameter to tune in applications requiring control over adhesion because it affects the conformation of polymer brushes. Theoretical studies showed that polymer brushes exhibit extended conformation upon increasing dispersity.^{38, 165, 173} Moreover, simulations demonstrated that dispersity may affect the
adhesion of microscale particles as the number of contacts between the particle and polymer will change upon changing the brush dispersity.¹⁷⁴

Despite the detailed insights provided by careful studies of adhesion on polymer brushes, static surfaces are nonetheless irrevocably fouled by bacteria. Certain aquatic organisms have developed a clever strategy to overcome this limitation: active cleaning. Mollusks and corals, among others, shed foulants through active deformation and motion, ⁴²⁻⁴³ and similar active changes in brush conformation may promote fouling-release. Stimuli-responsive polymers, as one example, change conformation and properties in response to environmental changes, and may represent a simple realization of the smart cleaning observed in marine organisms. Researchers have thought about active strategies, with temperature,⁴⁴⁻⁴⁶ pH,⁴⁷⁻⁴⁸ ionic strength,⁴⁹ pressure,⁵⁰ and light ⁵¹⁻⁵² being some of the stimuli used to tune bacterial attachment and detachment in responsive materials. How the molecular-weight distribution in brushes affects the fouling-release properties of stimuli-responsive polymer brushes, however, is unexplored. Both thickness and dispersity can change the conformation of a stimulus-responsive polymer brush,⁹⁻¹⁰ suggesting that these parameters may affect the detachment of microscale particles or bacteria after stimulation. This question has not been explored experimentally, in part because of the difficulty in synthesizing polymer brushes of a wide range of dispersities and lengths.

Here, we investigated the effect of brush thickness and dispersity on initial attachment and subsequent detachment of *S. epidermidis* using pH-responsive, poly(acrylic acid) (PAA) brushes as a model system. PAA brushes are neutral, collapsed, and hydrophobic at pH 4, and become negatively charged, swollen, and hydrophilic at pH

9. We synthesized PAA brushes with controlled brush thickness and dispersity, and studied attachment and detachment of bacteria using confocal microscopy. Adhesion of bacteria on collapsed brushes at pH 4, as quantified via surface coverage, varied nonmonotonically with increase in average brush thickness but was independent of brush dispersity. The lowest attachment occurred over a thickness range of 13 - 18 nm due to competing Van der Waals, electrostatics and hydrophobic interactions. When the solution pH was switched to 9, increasing either dispersity or thickness promoted the detachment of bacteria from the PAA brush surface. We suggest that an actuation effect, arising from the swelling of the polymer brushes, removes the bacteria. Increases in removal likely arises from stimulus-driven changes in brush conformation that are enhanced by greater length or dispersity, in turn increasing the actuation effect and swelling. Our results indicate that initial attachment and detachment of microscale bacteria can be separately tuned via the molecular weight distribution of a stimulus-responsive polymer brush. Beyond the application for smart antifouling surface coatings demonstrated here, this capability may prove useful in controlled release, sensors, and separation technologies.

4.2 Materials and Methods

4.2.1 Materials. All chemicals were purchased from Sigma Aldrich and used asreceived unless otherwise stated. Ethyl α -bromoisobutyrate (EBiB, 98%) and N,N,N',N',N''-pentamethyldiethylenetriamine (PMDETA, 99%) were each degassed with three freeze-pump-thaw cycles. Monomethyl ether hydroquinone (inhibitor) was removed from *tert*-butyl acrylate (tBA, 98%) by passing through a silica gel column (60 Å pore size). tBA was then dried with calcium hydride (reagent grade, 95%) and distilled under vacuum. Dichloromethane (JT Baker, HPLC grade, 99.8%) was dried using a Pure Process Technology solvent purification system.

4.2.2 Immobilization of Initiator on Substrate. Attachment of the silane, (3aminopropyl)triethoxysilane (APTES, 99%), to silicon wafers (Mechanical Grade, University Wafers) and glass coverslips (VWR, 22 mm \times 40 mm \times 0.15 mm) was completed as described in chapter 3.¹⁰ Silicon substrates were cut into 1 cm \times 2 cm pieces for the experiments. The silicon and glass substrates were cleaned by 10 min of sonication in acetone (ACS reagent, 99.5%) followed by 10 min of sonication in deionized (DI) water. The cleaned substrates were dried under nitrogen and exposed to air plasma (Harrick Plasma Cleaner) for three minutes to generate surface hydroxyl groups. Next, APTES was deposited on both silicon and glass substrates in vacuum for 30 min at a vacuum level of 50 mtorr. The silanized substrates were annealed at 110°C for 30 min. Subsequently, the substrates were immersed in dry dichloromethane containing 2 vol% anhydrous pyridine (99.8% pure). The polymerization initiator, α bromoisobutyryl bromide (98%), was added dropwise into the solution containing the substrates at 0°C until an initiator concentration of 0.081 M was attained. The resulting solution was held at 0°C for 1 hour and then at room temperature for 12 hours. The initiator-grafted substrates were rinsed with acetone and dried under flow of nitrogen gas. The silicon and glass substrates grafted with initiator were used immediately for polymerization.

4.2.3 Synthesis of Poly(Acrylic acid) (PAA) Brushes. Poly(*tert*-butyl acrylate) (PtBA) brushes were synthesized using surface-initiated atom transfer radical polymerization (SI-ATRP) and subsequently hydrolyzed to PAA. The initiator-grafted

silicon and glass substrates were transferred to a glove box under nitrogen gas in a round bottom flask where tBA, PMDETA, copper(I) bromide (CuBr, 99%), anhydrous N,Ndimethylformamide (DMF, 99.8%), EBiB (a solution initiator), and phenylhydrazine (97%) were added in a predetermined molar ratio for each polymerization. The flask was capped with a septum inside the glove box and transferred to a preheated oil bath at 50°C. The reaction mixture was stirred for 24 hours and [tBA]:[EBiB] was varied to obtain PtBA brushes of varying length. An equimolar ratio of PMDETA, EBiB, and CuBr was used, and this mixture was diluted with DMF such that the volume ratio of DMF:tBA was 1:1. The dispersity of the polymer brushes was varied by changing the concentration of phenyl hydrazine added to the reaction mixture.

The polymerization was quenched by addition of tetrahydrofuran (THF, OmniSolv, HPLC grade, 99.9%). The PtBA grafted substrates were rinsed with acetone, methanol (ACS reagent, 99.8%), and DI water, and dried under nitrogen gas. PtBA brushes were converted to PAA brushes through hydrolysis in the presence of dichloromethane (11 mL) and trifluoroacetic acid (reagent plus, 99%, 3 mL) for 24 hours. PAA brushes on silicon and glass substrates were rinsed with acetone and dried under nitrogen flow, and used for bacteria experiments the following day.

The remaining solution, containing free PtBA (initiated from EBiB), was diluted by adding excess THF and passed through a neutral aluminum oxide column to remove the catalyst. After removing the catalyst, the polymer solution was concentrated using a rotary evaporator and precipitated in a DI water/methanol mixture (1:1 by volume). Finally, the precipitated PtBA was collected and dried under vacuum overnight at room temperature. Gel Permeation Chromatography (GPC). The molecular weight distribution (including the number-average molecular weight, M_n , and dispersity, D) for free polymer (synthesized in solution) was characterized with a Viscotek GPC system with Agilent ResiPore columns, using stabilized THF (OmniSolv, HPLC grade, >99.9%) as the mobile phase at 30°C. The flow rate was 1 mL min⁻¹ and the injection volume was 100 µL. A triple detection system, including light scattering, viscometer, and differential refractometer, was used to characterize the molecular weight distribution. The dn/dc value of PtBA was measured to be 0.0479 ± 0.0012 under these conditions.

Ellipsometry. The dry thickness of PAA brushes grafted on silicon substrates was measured with a JA Woollam M-2000 spectroscopic ellipsometer. Thickness measurements were conducted at five different locations on each substrate. Ellipsometry data were modeled using a two-layer model, consisting of a polymer layer on top of SiO₂. The refractive index of each layer was modeled with the Cauchy dispersion relation, $n(\lambda) = A + B/\lambda^2$ (A and B are strictly positive). The measured amplitude ratio and phase difference were modeled with the Fresnel equation, with the brush thickness as a fitting parameter. The data was fit over the wavelength range of 350 - 900 nm.

Bacterial culture. *Staphylococcus epidermidis* (ATCC® 12228) was used as a model bacterium. Bacteria from a frozen stock were streaked onto an LB-agar plate (5 g yeast extract, 5 g NaCl, 10 g of Bacto-tryptone, 15 g agar, all from BD Chemicals) for 18 h at 37°C (Nuvaire Inc.). A single colony from the plate was inoculated into sterile LB medium (5 g yeast extract, 5 g NaCl and 10 g tryptone per 1 L medium, BD Chemicals) and incubated in an orbital incubator shaker (SH1000, Southwest Science) at 200 rpm and 37°C for about 10 h. Then, a second step culture was prepared by diluting the

original culture 100-fold in sterile LB medium and incubated until the bacteria reached stationary phase (approximately 12 h). To remove the growth media, bacteria were centrifuged at 5000g in a Sorvall ST 16 Centrifuge (Thermo Fisher Scientific); the supernatant was replaced with 0.9% NaCl, and the pellet was resuspended and centrifuged again. The pellet was again re-suspended in 0.9% NaCl solution (ionic strength 154 mM) and the optical density of the solution was adjusted to 0.25 (Laxco DSM-Micro Cell Density Meter). Finally, a 3 mL aliquot was taken from the solution with adjusted optical density and centrifuged. The pelleted bacteria were re-suspended in 0.5 mL of deionized water at pH 4. For imaging, bacteria were stained with 2 μ M Syto® 9 nucleic acid stain (ThermoFisher). The percentage hydrophobicity of the bacteria, assessed via a microbial adhesion to hydrocarbons (MATH)¹⁷⁵ assay with n-dodecane and hexadecane, was 74 ± 4 % and 57 ± 3 % at pH 4 and pH 9, respectively, for n-dodecane and 87 ± 2 % and 67 ± 1 % at pH 4 and pH 9, respectively, for hexadecane.

Microscopy, flow-cell experiments, and image analysis. A flow cell device was created by attaching a PAA-grafted glass coverslip to a custom-machined polycarbonate flow cell with a silicone sealant (3M[®]), which was allowed to cure overnight. The channels in the flow cell, of dimensions 1 mm \times 30 mm \times 4 mm, were filled with deionized water at pH 4 and the brushes grafted to the glass surface were equilibrated in this solution for 30 minutes. After equilibration, the channel was flushed with fresh deionized water at pH 4. Next, 300 µL of the bacteria suspension were inoculated into the channel and incubated at room temperature (21°C) for 3 h. Bacteria attached to the polymer-brush surface were imaged using a confocal fluorescence scanner (VT Infinity, Visitech) attached to a Leica DM4000 inverted microscope, which was equipped with a

40x oil immersion lens (HCX PL APO, numerical aperture 1.25-0.75). A laser source of wavelength $\lambda = 488$ nm was used to excite the fluorescence in the bacteria. Images of area 210 µm × 160 µm (corresponding to 672 pixels × 512 pixels) were acquired at a frame rate of 1 fps and an exposure time of 300 ms using an ORCA 200 camera (Hamamatsu) that was controlled by Voxcell Scan software (Visitech).

In a typical flow experiment, bacteria that were loosely attached to the surface after incubation were removed by flowing deionized water at pH 4 at a flow rate of 45 mL h⁻¹ using a Fusion 200 pump (Chemix). The flow was maintained until no detachment of bacteria was observed, typically about 20 minutes. Subsequently, the flowing media was switched to deionized water at pH 9 while the flow rate was held constant at 45 mL h⁻¹. Flow of the pH 9 solution was maintained for 20 min. Each experiment was done on a PAA brush that was hydrolyzed the previous day and a fresh bacteria culture was used for each experiment.

Images acquired from the confocal microscope were analyzed using ImageJ software (NIH) and MATLAB. In all images, bacteria appeared as bright objects on a dark background. To determine the percentage of the surface covered by bacteria, a threshold was individually applied to each image using ImageJ, and the resulting binary image was analyzed using MATLAB. The surface coverage of attached bacteria was calculated by counting the number of white pixels in the binarized image using a MATLAB code.

4.3 Results

4.3.1 Brush Synthesis. PtBA brushes of varying thickness and dispersity on glass coverslips and silicon substrates were synthesized using SI-ATRP, as described in

Chapter 3.¹⁰ To estimate the brush thickness on the glass coverslips, a silicon substrate was added to each reaction mixture so that the thickness could be measured with ellipsometry; explicitly, we assumed that the brush thickness on the glass coverslip equaled that on the silicon substrate. To determine the molecular weight, M_n, of the solution polymer PtBA, a solution initiator (EBiB) was added to every synthesis, and the M_n of the polymer brush was assumed equal that of the solution polymer (following prior studies^{55, 74, 148}). The brush thickness h scaled approximately linearly with M_n , as shown in Figure 4.1. From the linear scaling, we estimated the grafting density of the PtBA brushes as $\sigma = h\rho N_A/M_n$,⁷⁴ where $\rho = 1.05$ g/cm³ is the density of PtBA,⁷⁴ N_A is Avogadro's number, and M_n is the molecular weight of the solution polymer. This estimate yielded a grafting density of 0.36 chains nm^{-2} for the brushes described in this study; hence all brushes in our study were assumed to be in the (extended) brush regime, as the crossover for a lower molecular weight ($M_n = 8.56$ kg/mol) PtBA brush occurred near 0.08 chains nm⁻² [ref⁷⁴]. To vary brush dispersity (Đ), phenylhydrazine was added to the synthesis solution. Assuming that the dispersity of the brushes equalled that of the solution PtBA polymers, brushes with dispersities ranging from 1.28 - 1.95 were synthesized.

To produce pH-responsive surfaces, PtBA brushes were hydrolyzed to PAA brushes. The PAA brushes were characterized through FTIR.¹⁰ The thickness of PAA brushes after hydrolysis varied from 4 nm to 28 nm. For all bacterial experiments, the stated thickness is that of the as-synthesized PAA (post-hydrolysis) brush and the stated D is that of the PtBA brush.



Figure 4.1 PtBA brush dry thickness as a function of M_n of PtBA solution polymer. Symbol color indicates the range of brush dispersity. The grafting density calculated from the slope of the linear fit ($\mathbb{R}^2 = 0.95$), is 0.36 chains nm⁻².

4.3.2 pH-Triggered Detachment. pH-responsive PAA brushes undergo changes in charge and conformation as the solution pH is varied. In an acidic medium, PAA brushes are uncharged and collapsed; in a basic medium, PAA brushes are negatively charged and extended. We hypothesized that triggering brush swelling would alter the number of adhered bacteria. Hence we inoculated *S. epidermidis* bacteria suspended in pH 4 solution onto PAA brushes that were equilibrated at pH 4 and thus collapsed and uncharged. The S. epidermidis bacteria were hydrophobic, and so readily attached to the neutral collapsed PAA brushes; loosely attached bacteria were detached by flowing the pH 4 solution at a rate of 45 ml h⁻¹. Next, pH 9 solution was flowed through the flow cell, causing brushes to swell and become negatively charged. We acquired confocal micrographs before, during, and after flow of the pH 9 solution to measure changes in the coverage of the surface by bacteria as a function of the brush thickness and dispersity.

Confocal micrographs acquired before pH 9 flow (i.e., after pH 4 flow) and after pH 9 flow reveal differences in the extent of bacterial attachment and detachment on

surfaces of varying thickness and dispersity (Fig. 4.2a - f). On a short brush of thickness 4 nm and dispersity D = 1.33, a similar fraction of the surface was covered by bacteria before and after pH 9 flow (Fig. 4.2a,b). Fewer bacteria initially attached on a surface of higher dispersity (D = 1.95) but similar thickness (4 nm); strikingly, after pH 9 flow there was a pronounced reduction in the surface coverage (Fig. 4.2c,d). Increasing instead the thickness (to 21 nm) while holding the dispersity approximately constant (at $D \approx 1.3$) reduced the number of initially-attached bacteria (before pH 9 flow, Fig. 4.2e) and sharply reduced the surface coverage after pH 9 flow (Fig. 4.2f). These results suggest that brush thickness and dispersity affect the attachment and detachment of bacteria.



Figure 4.2 Representative confocal micrographs of S. epidermidis bacteria deposited onto PAA brushes at pH 4 (a,c,e) and pH 9 (b,d,f). Scale bars in the images signify 30 µm. Labels indicate PAA brush thickness (dispersity). (g,h) Normalized surface coverage (SC) as a function of time after start of pH 9 flow for PAA brushes with varying (g) dispersity or (h) thickness.

To gain insight into the factors controlling adhesion, we monitored the percentage of the surface covered by bacteria as a function of time, normalized to the average surface coverage two minutes after the onset of the pH 9 flow (to account for the abrupt variation at the onset of flow at time t = 0). Because brushes of varying thickness and dispersity can exhibit pronounced differences in brush extension, charge, and conformation,^{142-143,} ¹⁷⁶ we separately examined the changes in surface coverage at constant Đ and varying thickness, and at constant thickness and varying D. The surface coverage of bacteria on a short 4 nm PAA brush with D = 1.33 was nearly constant over the duration of the experiment; on a brush with similar length but larger dispersity of D = 1.95, however, the surface coverage began to decrease four minutes after the start of pH 9 flow (Fig. 4.2g), indicating that bacteria were detached from the surface. The time scale at the onset of detachment, ~ 4 min, reflected the time required for the flow cell to equilibrate at pH 9, 177 and was much longer than the characteristic response time of the brush (< 10 sec, measured by monitoring the change in contact angle over time for a brush equilibrated at pH 4 and exposed to a water drop of pH 10). Bacteria also detached from a longer brush of thickness 21 nm but low dispersity D = 1.36 (Fig. 4.2h), and the onset of detachment occurred at a similar time after pH 9 flow; the latter result confirmed that the detachment time was set by the time needed to equilibrate the solution inside the flow chamber.

4.3.3 Brush Thickness Controls Attachment. To probe the effects of brush thickness and \overline{D} on bacterial attachment, the average percent of the surface covered by bacteria was calculated across confocal images acquired at 10 different locations after the flow of pH 4 solution, for which PAA brushes are collapsed and uncharged. As the brush thickness was increased from 4 – 12 nm, the normalized surface coverage decreased until

reaching a local minimum (Fig. 4.3a). Few bacteria attached on brushes of thickness 13 – 18 nm. The normalized surface coverage increased sharply as the thickness was increased from 18 to 21 nm and then increased very slowly for the greatest thicknesses accessed here. Bacteria covered a similar percentage of surfaces functionalized by brushes of comparable length but varying dispersity (Fig. 4.3b), suggesting that the thickness but not dispersity predominantly controls bacterial attachment.



Figure 4.3 Percent surface coverage at pH 4 as a function of (a) PAA dry brush thickness or (b) PAA brush dispersity. In (a) all brushes are included; whereas in (b), only brushes with similar thickness are compared.

To test this idea, we examined the percentage of the surface covered by bacteria as a function of the brush dispersity for brushes of comparable thickness. Bacteria covered a similar (high) fraction of the surfaces of brushes of thickness 4 - 5 nm, even as the dispersity was varied from 1.28 to 1.95 (Fig. 4.3b). Likewise, the surface coverages on brushes of thickness 8 - 9 nm and on thickness 14 - 15 nm were nearly constant, independent of dispersity. Moreover, the surface coverage decreased as the brush thickness was increased from 4 - 5 nm to 8 - 9 nm and then to 14 - 15 nm, consistent with the data presented in Fig. 4.3a. Together, these results indicate that brush D does not affect adhesion of bacteria; instead, adhesion is controlled by the brush thickness.

4.3.4 Brush Thickness and Dispersity Affect Detachment. To probe the effects of brush thickness and D on detachment of bacteria, we examined the change in the percentage of the surface covered before and after switching to pH 9 (i.e. before and after swelling the PAA brushes). Because it was difficult to assess meaningful changes in the fraction of surfaces with initial coverages of < 2% (comparable to the magnitude of the error in the surface coverage measurement), we compared only those samples with relatively high initial surface coverages at pH 4. For a series of brushes of approximately constant dispersity $(1.28 \le D \le 1.42)$, the reduction in surface coverage upon switching to pH 9 increased monotonically with brush thickness (Fig. 4.4a). (Very few bacteria attached to brushes of length 13 - 18 nm, and thus these samples were not included in Fig. 4.4a.) We therefore conclude that longer brushes more effectively removed bacteria when dispersity was held approximately constant. Similarly, for two series of brushes of approximately constant length (4 - 5 nm and 8 - 10 nm), the reduction in surface coverage upon switching to pH 9 increased monotonically with brush dispersity (Fig. 4.4b). Brushes of length 8 - 10 nm were slightly more effective than those of length 4 - 5nm at reducing the surface coverage after pH switching, due to their greater thickness. Slightly higher reductions in surface coverage were achieved over the studied range of brush lengths relative to that observed for the studied range of dispersities: 12% reduction in surface coverage was observed for the longest brush in the constant-dispersity series, whereas 7 - 8 % reduction was observed for the brushes of highest dispersity in the constant-length series. A dispersity of D = 1.95 is close to the maximum anticipated for

unimodal size distributions formed through radical polymerization techniques (i.e. the Carothers most probable distribution).⁷⁶ Nonetheless, these results demonstrate that increasing either the thickness or dispersity of responsive brushes promote removal of bacteria from the surface after pH switching.



Figure 4.4 Reduction in SC after switching from pH 4 to pH 9 as a function of PAA brush (a) thickness or (b) dispersity. Only samples with similar dispersity [1.28 - 1.42, in (a)] or similar brush length [4 - 5 nm (●) and 8 - 10 (♦), in (b)] are shown, to separate the effects of brush length and dispersity.

4.4 Discussion

Polyelectrolyte brushes such as PAA change their surface charge and conformation with the pH of solution. At low pH, PAA brushes are uncharged and collapsed; at high pH, PAA brushes are negatively charged and extended. Additionally, PAA brushes are hydrophobic at lower pH due to greater extent of intramolecular hydrogen bonding, and hydrophilic at higher pH due to greater extent of intermolecular hydrogen bonding.^{134, 160} Various analytical measurements have confirmed the changes in wettability and thickness of PAA brushes with solution pH.^{74, 134, 136, 160, 178-179} In our earlier study, for example, PAA brushes exhibited lower thickness and higher contact angle at low pH, and higher thickness and lower contact angle at higher pH; these results

coincide with expectations based on a pH-dependent conformational change.¹⁰ Here, we tested the adhesion of bacteria on PAA brushes equilibrated at pH 4 and at pH 9 as models for tunable substrates with the ability to change brush charge and conformation. By synthesizing brushes of varying thickness and dispersity, we sought to identify the effects of these brush properties on bacterial attachment and detachment. Our results, summarized in Figures 4.2 - 4.4, indicate that brush thickness and dispersity differently control the attachment and detachment of bacteria from the brush surface.

4.4.1 Mechanism of Bacterial Release from PAA Surfaces. The results presented in Figure 4.2 indicate that bacteria detach from brushes of high dispersity and/or thickness when the solution pH is changed from 4 to 9. To understand the mechanisms driving detachment, we first considered how changing the pH affected electrostatic interactions between the bacteria and the brushes. The isoelectric point of S. epidermidis bacteria is between 2.0 - 2.3,¹⁸⁰ and therefore the surface charge of the bacteria did not change sign as the pH was increased in our experiments. As the surface changes from neutral (at pH 4) to charged (at pH 9), one possibility is that negativelycharged S. epidermidis bacteria were repelled from the newly negatively-charged PAA surface.¹⁸¹⁻¹⁸² In support of this idea, switching copolymer brushes with a pH-responsive poly (2-carboxy ethyl acrylate) block from low pH to high pH removed S. epidermidis bacteria from a 24 nm copolymer brush.⁴⁷ In our system, however, very few bacteria were detached from a brush with thickness 4 nm and dispersity of 1.33 compared to brushes of higher thickness and/or dispersity, although all brushes switched from neutral to charged upon increasing the pH.^{10, 134} It is therefore unlikely that the change in surface charge alone drives bacterial removal.

Instead, we suggest that detachment of bacteria from brushes with higher thickness and/or dispersity is due to an actuation effect that, in turn, arises from pHinduced conformation changes. In our earlier study,¹⁰ the dry thickness of a PAA brush with an as-synthesized thickness of 30 nm increased $\sim 40\%$ more than that of a 6 nm (assynthesized) brush with similar dispersity when pH was changed from 4 to 9. At constant grafting density, brushes of higher as-synthesized thickness have a higher numberaverage molecular weight, M_n (Figure 4.1). Per theories for a weak polyelectrolyte brush,^{9, 183} we anticipate that the wet thickness of PAA brush increases with both M_n and dry thickness. Hence, we suggest that increasing the dry brush thickness increases the extent of swelling upon switching pH from 4 to 9 and thereby increases removal of adherent bacteria (consistent with the images in Figure 4.2e,f). Likewise, two theoretical studies suggested that increasing the dispersity of polymer brushes may promote conformations that, in turn, magnify brush thickness: (a) neutral polymer brushes of high dispersity adopt a crown-and-stem morphology, in which the longer chains are greatly extended,³⁸ and (b) polymer brushes of higher dispersity exhibit extended density profiles.^{165, 173} We posit that electrostatic and steric repulsions arising at pH 9, when the PAA brushes are fully charged, will enhance the extension of the longer chains. Hence we suggest that brushes of higher dispersity will extend more upon switching from pH 4 to pH 9 and thereby increase removal of bacteria from the surface (consistent with the images in Figure 4.2 (c,d)).

Our suggested physical picture for the mechanism of bacterial release is shown in Figure 4.5. When the brush thickness is held constant, increasing the brush D increases the number of longer chains, which extend more than short chains when the pH is

increased from 4 to 9; this extension enhances the removal of bacteria from the PAA surface. When the brush dispersity is held constant, increasing the brush thickness magnifies the extent of swelling when the pH is increased from 4 to 9, enhancing removal of bacteria from the PAA surface. Because both thickness and dispersity are expected to monotonically increase the extent of brush swelling upon pH change, this picture also explains the results reported for the detachment in Figure 4.4.



Figure 4 Illustration of the removal of *S. epidermidis* bacteria as the solution pH is rapidly switched from 4 to 9. Brushes of greater dispersity or thickness swell more upon increasing pH and promote the release of adherent bacteria.

4.4.2 Mechanism of Bacterial Attachment to Neutral PAA Brushes. The results reported in Figure 4.3 indicate that attachment of bacteria on the collapsed, neutral brushes at pH 4 varied non-monotonically as the brush thickness was increased, first decreasing to a local minimum between 13 - 18 nm and subsequently increasing. Our results for short brushes are comparable to those obtained in earlier studies on antifouling polymer brushes. Attachment of *S. epidermidis* bacteria on poly(ethylene oxide) brushes, for example, decreased as the brush thickness was increased from 2 to 7 nm.²⁹ Similarly, attachment of *S. epidermidis* decreased as the thickness of poly(2-hydroxyethyl

methacrylate) (PHEMA) brushes grafted at low density was increased from 2 to 5 nm; adhesion of bacteria on brushes grafted at higher densities, however, did not vary with brush thickness, most likely due to negligible bacterial adhesion on PHEMA brushes.¹⁸⁴ Over the full range of thicknesses, the non-monotonic dependence of attachment reported in Figure 4.3 is akin to that observed for a marine bacterium, *Cobetia marina*, on random copolymer brushes of thickness 10 – 60 nm. Random copolymer used was poly (2-hydroxyethyl methacrylate (HEMA)-co-hydroxyl-terminated PEG methacrylate monomer, with an average chain length of 10 ethylene glycol units (PEG10MA)). Bacteria adhered least to brushes of thickness 20 - 40 nm, and attachment increased as the brush thickness was increased or decreased outside this range.³¹

These results evoke the non-monotonic dependence on brush thickness reported for protein adsorption.¹⁹ A variety of polyacrylamide and polyacrylate brushes bearing different functional groups exhibited ultralow fouling by proteins from blood plasma and serum at intermediate thicknesses; examples include poly(N-hydroxymethyl acrylamide) brushes of thickness 20 - 60 nm, poly(2-hydroxyethyl methacrylate) brushes of thickness 20 - 30 nm, poly(N-hydroxyethyl acrylamide) brushes of thickness 10 - 40 nm, poly(carboxybetaine acrylamide) (poly(CBAA)) brushes of thickness 15 - 25 nm, and poly(N-acryloylaminoethoxyethanol) brushes of thickness 10 - 40 nm.^{167, 185-188} Poly(sulfobetaine methacrylate) and poly(serine methacrylate) brushes also exhibited local minima in protein adsorption as a function of brush thickness, with lowest adsorption at thicknesses of 62 nm and 37 nm, respectively.¹⁸⁹⁻¹⁹⁰ Insights into the properties controlling protein adhesion are suggested by the anomalous behavior of one polymethacrylate brush, poly(hydroxypropyl methacrylate), which exhibited a local minimum in adsorption for a brush thickness of ~ 30 nm but had somewhat higher adsorption than the other brush systems; the higher adsorption was attributed to its relatively high hydrophobicity.¹⁸⁶ Likewise, the decrease in protein adsorption between 25°C to 37°C on polyCBAA brushes, which become increasingly hydrophilic with increasing temperature, underlines the role of hydrophobicity on protein adsorption.¹⁸⁸

In our experiments, the lowest bacterial adhesion occurred on PAA brushes of thickness 13 - 18 nm, comparable to the ranges that minimized protein adsorption across the varied acrylate and acrylamide chemistries. This observed non-monotonic dependence of bacterial adsorption on brush thickness must arise from the physicochemical interactions between substrate, brush, and bacteria. One widely-used model for the interaction potential between particles and a brush-coated substrate includes three contributions: (i) short-range particle-substrate interactions (e.g., hydrogen bonding, ion pairing, and hydrophobic interactions); (ii) long-range Lifshitz-van der Waals attractions between particles and the substrate; and (iii) osmotic repulsions between particles and brushes.¹⁹¹ This model assumes that particles have no affinity for the polymer brushes. The resultant interaction potential exhibits two minima, a primary minimum very close to the substrate and a secondary minimum near the outer surface of the brush.¹⁷⁰ Particles can only adsorb in the primary minimum when they are able to reach the surface. Because our brushes are grafted at high density (0.36 chains nm^{-2} , Fig. (-1.9 nm), 4.1) and the bacteria are much larger than the average spacing between chains (-1.9 nm), we expect that bacteria mostly adsorb in the secondary minimum. The depth of the secondary minimum is governed by the competition between long-range van der Waals attraction and steric repulsion.¹⁹¹ Increasing the brush thickness reduces the depth of the

secondary minimum, because the magnitude of the van der Waals attraction decreases as the particle and the substrate are increasingly separated. Further, increasing the brush thickness also increases the steric repulsion energy.³⁰ Both the decrease in van der Waals attractions and the increase in elastic repulsions reduce adsorption of particles as the brush thickness is increased. Hence the initial decrease in bacterial attachment with increasing thickness, reported in Figure 4.3a, is consistent with the model of ref ¹⁹¹.

This model, however, cannot explain the increase in bacterial attachment at high brush thicknesses – likely because it neglects the hydrophobic interactions between brushes and bacteria. The enhanced adsorption of proteins on longer brushes was attributed to stronger intra- and intermolecular interactions between long polymer chains, leading to chain entanglements.¹⁸⁶ Stronger interactions between polymers weaken the interactions between polymers and water, reducing surface hydration; consequently the surface becomes more hydrophobic and more proteins adsorb.¹⁹ In our experiments, we also observed non-monotonic dependence of bacterial attachment on thick collapsed brushes at pH 4; following ref ^{19, 31}, we attribute this increase to greater interactions between longer chains of thick PAA brushes. A similar argument is given in ref ³¹ for attachment of bacteria to swollen and hydrated $poly(HEMA-co-PEG_{10}MA)$ copolymer brushes, where attachment inversely tracked the hydration of the brush. There, the minimum in attachment coincided with a maximum in hydration at the intermediate brush thicknesses (20 - 40 nm), as determined using a quartz crystal microbalance (QCM) experiment. Hydration decreases at lower thicknesses because the osmotic penalty to remove water from the brush is lowered,^{19, 31, 192-193} and also decreases at higher thicknesses due to entanglements.³¹

In contrast to the striking dependence of bacterial attachment on brush thickness, the data in Figure 4.3 indicate that the brush dispersity does not affect attachment of bacteria onto collapsed brushes at pH 4. This result suggests that the depth of the secondary minimum controlling bacteria attachment is independent of brush dispersity. Thus, bacteria attached to collapsed brushes of similar average thickness but varying dispersity experience similar van der Waals attractions and steric repulsions; both are controlled by the average brush thickness, independent of dispersity. This result contrasts with a theoretical study of a neutral polymer brush, which for large particles found that increasing the dispersity in the brush molecular weight reduced the depth of the secondary minimum and shifted its position slightly further from the substrate; both effects were expected to reduce fouling by large particles.¹⁹⁴ The grafting density in our study (0.36 chains nm^{-2}), however, was higher than that used in ref ¹⁹⁴ (0.05 chains nm^{-2}); hence it is possible that the increased steric repulsion, arising from the higher grafting density, overwhelmed a smaller difference in attraction of particles to low- and highdispersity brushes.

The lack of dependence of attachment on dispersity remains surprising, however, because highly disperse brushes nonetheless contain long chains, which are expected to undergo enhanced swelling upon pH change. To estimate the distribution of long chains (and thus, indirectly, the likelihood of their interactions), we calculated the molar percentage of chains of molecular weight greater than 75.1 kg mol⁻¹ (corresponding to the threshold thickness of 18 nm identified in Figure 3a) for the brushes of greatest dispersity at a given thickness in Figure 3b. The 4 nm brush with D = 1.95 had zero mol % of chains longer than 18 nm, the 9 nm brush with D = 1.82 had 2.3 mol % of long chains

(corresponding to an areal density of 0.008 chains nm^{-2}), and the 15 nm brush with D = 1.55 had 39 mol % of long chains (areal density of 0.14 chains nm^{-2}). Attachment of bacteria on these brush surfaces was indistinguishable from that on brushes of similar length but lower dispersity. This result suggests that the distribution, and not simply the presence, of longer chains is essential for reducing the polymer-water interactions. Likely, longer chains separated by many shorter chains cannot form the chain entanglements required to enhance polymer-polymer interactions. We therefore posit that the large separation between the long chains in highly disperse brushes prevents the polymer-polymer interactions that increase the surface hydrophobicity. We note, though, that these long chains can still extend upon switching to pH 9 to promote detachment of bacteria.

4.4.3 Comparison to Antifouling Studies Varying Brush Grafting Density. The calculation of an effective concentration of long brushes prompts comparisons to the many studies probing the effect of brush grafting density on antifouling efficacy. As the grafting density is increased, polymer brushes undergo a structural transition from the mushroom regime (in which brushes are nearly hemispherical) to the brush regime (in which the brushes are fully extended).¹⁹⁵ Further, brush thickness increases concomitant with grafting density, due to greater steric repulsion.^{183, 196} Together, these effects suggest that brushes grafted at higher densities should exhibit better fouling resistance. Indeed, many studies report decreases in cell and protein adsorption upon increasing the grafting density of polymer brushes.^{30, 170, 184, 196-200} However, for very low grafting densities, an initial increase in protein adsorption with increase in grafting density has been observed for both monomodal and bimodal PEO brushes due to tertiary adsorption between

polymer and protein.²⁰¹ Tertiary adsorption is only relevant for small molecules which can diffuse inside the brush, and will not be a factor for micron scale particles such as bacteria. Moreover, the model of ref ¹⁹¹ predicts lower particle adhesion as the grafting density is increased, due to the increased steric repulsion and reduced van der Waals attractions experienced by an adhering particle.¹⁹¹ Although our study employed brushes in the "extended brush" regime at a fixed (total) grafting density, the lack of dependence of bacterial attachment on brush dispersity indirectly supports the idea that low areal densities (here, of the long chains that effectively repel bacterial attachment) are less effective at preventing adhesion of bacteria.

4.5 Conclusions

We show that tuning the molecular weight distribution of pH-responsive polyelectrolyte brushes yields orthogonal control over attachment and detachment of adherent *S. epidermidis* bacteria. By synthesizing poly(acrylic acid) (PAA) brushes with varying dry thickness (4 - 28 nm) and dispersity (1.28 - 1.95), we quantified the effects of brush thickness and dispersity on bacterial attachment and stimulated detachment of *S. epidermidis*. Bacteria were detached from the brush surface when the solution pH was switched from pH 4 (at which PAA is uncharged and hydrophobic) to pH 9 (at which PAA is charged and hydrophilic). Brushes with very low dispersity and thickness showed negligible detachment; increasing the brush thickness and/or brush dispersity promoted bacterial detachment. By contrast, bacterial adhesion at pH 4 was independent of dispersity but varied non-monotonically with brush thickness.

To explain the greater detachment efficiency of PAA brushes with higher thickness and/or dispersity, we propose that conformational changes in the brush upon

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switching from pH 4 to 9 generated an actuation effect. Brushes of higher dry thickness and/or dispersity swelled more (due to changes in the conformation of the polymer chains) in response to a pH change from 4 to 9, releasing adhered bacteria. To explain the non-monotonic dependence of bacterial adhesion on the dry brush thickness, we suggest that the combination of Lifshitz-van der Waals attractions, steric repulsions, and hydrophobic interactions (with the brush surface) generate a local minimum in the interaction potential of bacteria with the substrate. Bacteria attached to collapsed brushes of similar average thickness but varying dispersity experience similar van der Waals attractions and steric repulsions. Although longer polymer chains lead to greater polymerpolymer interactions and increased hydrophobic interactions between bacteria and the polymer brush, the areal density of long chains remains low even at high dispersities. Hence bacterial attachment is independent of brush dispersity. We note that PAA is not especially antifouling,²⁰² suggesting that the removal arises from the stimulus-induced change in conformation, and the synthetic method can be extended to the vast array of antifouling polymers that can be synthesized via ATRP.^{19, 187}

Our findings suggest several routes to improve the efficacy of smart coatings. First, selecting the optimum brush thickness may considerably reduce bacterial fouling. In our system, the optimal thickness is set by the competition between different physicochemical interactions. Whether this optimal thickness is constant across a range of fouling organisms, including different species of bacteria as well as macrofoulers, is not *a priori* clear. Ref ³¹ suggests that this is indeed the case for swollen (charged, hydrophilic) brushes, for which organism/brush interactions are controlled by hydration of the polymer; additional studies across a broad range of polymer chemistries with varying degrees of hydrophobicity are needed to confirm this idea. Second, employing higher brush thickness and dispersity may significantly improve the ability of smart polymers to remove foulants by magnifying conformational changes in the polymer in response to a stimulus. Tailoring the distribution of polymer molecular weight is largely unexplored in the context of smart antifouling surfaces, but may provide a new route by which to enhance their antifouling properties. More broadly, polymer brushes of broad dispersity may have use in other applications that demand controllably tunable adsorption and desorption of particles, including sensing, separations, and controlled release.

5. Summary and Future Work

5.1 Summary

We developed a method to vary dispersity of polymers synthesized via Atom Transfer Radical Polymerization (ATRP), and investigated the effects of brush dispersity and thickness on the pH-response of and attachment/detachment of bacteria to pHresponsive poly(acrylic acid) brushed with applications in antifouling coatings, sensors, controlled drug release and adsorption/desorption processes.

- 1. We developed a method to systematically vary the dispersity of polymers produced by ATRP through the addition of phenylhydrazine (PH), and explored the mechanism of increased dispersity. Addition of Phenylhydrazine (PH) to the reaction mixture increased the polymer dispersity and dispersities ranging from 1.08 1.80 were achieved. Reaction kinetics of poly(*tert*-butyl acrylate) synthesis were studied using three different ratios of PH to initiator (EBiB), i.e. 0:1, 1:1, and 3:1. Increasing the ratio of PH:EBiB lead to greater disagreement between Mn obtained from GPC and ¹H-NMR, indicating lack of end-group fidelity in the presence of PH. Through ¹H-NMR end-group analysis, we determined that nucleophilic substitution reaction of PH with alkyl halides leads to chain termination, and therefore increase in dispersity. We created a model to describe the reaction kinetics which showed excellent agreement with the data. Additionally, we demonstrated that method has broader applicability to other polymers, as dispersity for polystyrene increased from 1.07 to 1.90 upon addition of PH.
- 2. Secondly, we investigated the role of brush thickness and dispersity on the pHresponse of poly(acrylic acid) brushes. pH was varied from 10 to 3 and vice versa, and brush wettability was quantified by the contact angle measurements. From these

measurements, we report three trends in the pH-response: (i) the thickness of dry brushes increased upon increasing pH for brushes above a critical length; (ii) the lowpH contact angle increased with increasing dispersity; (iii) brushes of sufficient dispersity exhibited hysteretic memory behavior at intermediate pH; as a result, the pK_a measured upon increasing pH was greater than that upon decreasing pH. The degree of hysteretic memory behavior (as quantified by the difference in the pK_a values measured upon increasing and decreasing pH) increased with brush dispersity. To explain this response, we proposed a hypothetical physical picture in which solvent was excluded from the collapsed PAA brush at pH 3; as a result, the onset of swelling was delayed to higher pH by the inability of the solvent to penetrate the brush. We posited that the trends observed in this study originate from differences in the dispersity of the brushes.

3. Finally, we investigated bacterial attachment and detachment on PAA brushes using confocal microscopy. Bacteria were inoculated at pH 4 and were allowed to attach to the collapsed PAA brushes for 3 hours, after which pH 4 solution was flowed to remove loosely attached bacteria from the surface, and finally PAA brush was swelled on changing the pH from 4 to 9. Adhesion of bacteria on collapsed brushes at pH 4, as quantified via surface coverage, varied non-monotonically with increase in brush thickness but was independent of brush dispersity. The lowest attachment occurred over a thickness range of 13 – 18 nm. When the solution pH was switched to 9, increasing either dispersity or thickness lead to removal of bacteria from the PAA brush surface. Most likely increasing either parameter enhanced conformational changes leading to greater actuation effect and swelling. This study implies that we

can separately tune initial attachment and detachment via the molecular weight distribution of a stimulus responsive polymer brush.

We strongly believe that our findings in this dissertation will guide future studies to understand the role of molecular weight distribution of pH-responsive polymer brushes in various applications and rationally design self-cleaning antifouling coatings.

5.2 Future Work

Although this study demonstrated the role brush thickness and dispersity plays in pH-response and antifouling properties, there are still few questions which need to be answered.

- 1. We demonstrated that the dispersity of polymer brushes can affect the pH-response of poly(acrylic acid) brushes, and attributed the observed hysteretic memory behavior to the dispersity induced conformational changes. However, we were not able to demonstrate that conformation of polymer changes as the dispersity is changed. Neutron reflectivity will be one possible route to examine the changes in polymer density profile at different heights, and density profile at different heights can provide the information regarding different polymer conformations as we tune the dispersity of polymer. Information regarding polymer conformation at different pH on increasing and decreasing pH will give insights regarding experimentally observed hysteretic memory behavior.
- 2. In the study regarding bacterial adhesion on PAA brushes, we only considered brushes with lower average thickness while varying dispersity. As lower thickness and higher thickness interact differently with bacteria due to varying degree of chain entanglements and hydrophobic interactions, behavior of brushes with higher

dispersity and thickness might be different due to the bigger distribution of chains with lower and higher thicknesses, and needs to be explored. Additionally, the magnitude of actuation effect upon pH change can also change, likely leading to even further cell removal at pH 9.

- 3. One of the drawbacks of "grafting from" approach is that it is not very feasible to graft polymers commercially by putting large substrates inside reactors under inert environment. Instead, "grafting to" is a more feasible approach, as functionalized bulk polymers can be synthesized and subsequently adsorbed to the surface, but this approach will lead to lower grafting densities. There are no experimental studies exploring the role of molecular weight distribution on bacterial attachment and fouling-release for a brush with lower grafting density. A theoretical study demonstrated that molecular weight distribution can alter particle adhesion for a lower grafting density brush,¹⁷⁴ and an experimental study using lower grafting regarding effects of polymer brush properties on bacterial attachment and detachment.
- 4. Other than polymer brushes, substrates with varying degree of "softness" has been used to design antifouling materials. Mostly polyelectrolyte multilayers, hydrogels and plastic substrates have been characterized by their elastic modulus and softness, and an effort has been made to correlate these properties with the bacterial adhesion.^{20, 26-27} Experiments to measure moduli of polymer brushes with varying thickness, dispersity and grafting density will bridge the gap between literature on bacterial adhesion using polymer brushes and using substrates with varying elasticity. Nanoindentation experiments using AFM have capability to measure mechanical

properties of the polymer brush. Such a study would further the scientific knowledge on effects of polymer properties in bacterial adhesion, and would aid in the development of antifouling coatings.

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Appendices

Supporting Information for Chapter 2



Figure A1.1 ¹H-NMR spectra for PtBA synthesized without phenylhydrazine addition ([PH]:[EBiB] = 0:1) at different reaction times. Spectra were taken directly after removing the aliquot from the reaction flask without purification to determine the monomer conversion. Respective peaks are marked on the spectrum.



Figure A1.2 ¹H-NMR spectra for PtBA with [PH]:[EBiB] = 1:1 at different reaction times. Spectra were taken directly after removing the aliquot from the reaction flask without purification to determine the monomer conversion.



Figure A1.3 ¹H-NMR spectra for PtBA with [PH]:[EBiB] = 3:1 at different reaction times. Spectra were taken directly after removing the aliquot from the reaction flask without purification to determine the monomer conversion.



Figure A1.4 GPC refractometer data for PtBA synthesized without PH ([PH]:[EBiB] = 0:1) at different reaction times. Molecular weight and dispersity for each polymer are given in Table S1.



Figure A1.5 GPC refractometer data for PtBA synthesized using [PH]:[EBiB] = 1:1 at different reaction times. Molecular weight and dispersity for each polymer are given in Table S1.



Figure A1.6 GPC refractometer data for PtBA synthesized using [PH]:[EBiB] = 3:1 at different reaction times. Molecular weight and dispersity for each polymer are given in Table S1.

[PH]:[EBiB]	Time	Conversion	M _n (NMR)	M _n (GPC)	M _n (Theor)	$\overline{\mathbf{D}^{c}}$
	(mins)	$(\%)^{\rm b}$	(kg/mol) ^b	(kg/mol) ^c	(kg/mol) ^b	
0:1	10	16	2.6	3.5	2.5	1.11
	25	33	4.8	5.6	5.1	1.08
	40	43	6.2	7.2	6.6	1.07
	60	56	8.4	8.5	8.6	1.07
	90	69	10	10	11	1.08
	120	77	11	11	12	1.08
	150	81	12	12	12	1.08
	180	85	13	12	13	1.08
	210	85	13	12	13	1.08
1:1	4	25	4.4	4.3	3.8	1.47
	8	33	5.8	5.2	5.0	1.47
	15	43	7.6	6.0	6.5	1.53
	25	51	8.3	6.9	7.8	1.57
	40	56	9.4	7.4	8.6	1.65
	60	62	10	7.8	9.5	1.77
	90	65	11	8.6	10	1.83
	120	67	11	9.1	10	1.78
	150	67	11	9.4	10	1.76
	180	68	11	9.6	10	1.80
3:1	5	23	4.6	3.1	3.6	1.79
	10	28	5.1	3.8	4.4	1.80
	15	31	5.8	3.9	4.8	1.82
	20	31	6.1	4.0	4.9	1.86
	25	33	6.1	4.1	5.2	1.83
	30	34	5.9	4.0	5.3	1.89
	60	35	6.1	4.0	5.5	1.84
	90	35	6.3	4.1	5.5	1.82
	120	36	6.5	4.8	5.7	1.71

Table A1.1 Characterization of PtBA synthesized using various ratios of [PH]:[EBiB] at different reaction times^a

^a [tBA]:[EBiB]:[CuBr]:[PMDETA] = 120:1:1:1 was used in all reactions. ^b Characterized with ¹H-NMR: M_n (NMR) was determined through end-group analysis while M_n (Theor) was calculated from the NMR monomer conversion. ^c Characterized with GPC (polystyrene standards).

Calculation for PH End group analysis

1. <u>3:1 Sample</u>

1 subscript for reaction product in absence of chain termination and 2 is for product in presence of chain termination

Total peak area for methyl from initiator, $c = 2.56 = c_1 + c_2$ Area of peak from aromatic end groups of PH, x = 0.67

Area of peak from aromatic end groups of PH, x = 0.0

- \Rightarrow Area of $c_2 = 2 * x$ (from stoichiometry) = 1.34
- \Rightarrow Area of $c_1 = 2.56 1.34 = 1.22$
- \Rightarrow Area of $b_2 = 2/3 * x$ (from stoichiometry) = 0.44
- \Rightarrow Area of $b_1+g_1 = c1/2$ (from stoichiometry) = 0.61

Therefore, $b_1 + g_1 + b_2 = 0.61 + 0.44 = 1.05$

Measured value of peak (b+g) from NMR spectra is 1 which is very close to value obtained using stoichiometry.

2. <u>1:1 Sample</u>

1 subscript for reaction product in absence of chain termination and 2 is for product in presence of chain termination

Total peak area for methyl from initiator, $c = 2.52 = c_1 + c_2$ Area of peak from aromatic end groups of PH, x = 0.48

- \Rightarrow Area of $c_2 = 2 * x$ (from stoichiometry) = 0.96
- \Rightarrow Area of $c_1 = 2.52 0.92 = 1.56$
- \Rightarrow Area of $b_2 = 2/3 * x$ (from stoichiometry) = 0.32
- \Rightarrow Area of $b_1+g_1 = c1/2$ (from stoichiometry) = 0.78

Therefore, $b_1+g_1+b_2 = 0.78+0.32 = 1.10$ Measured value of peak (b+g) from NMR spectra is 1 which is very close to value obtained using stoichiometry.

The slight variation in the calculated and measured peak areas for (b+g) could be attributed to the error in peak area calculation for the convoluted peaks of the aromatic protons in PH.

Model to fit experimental data

$$P_{n} - X + CuBr/L \xrightarrow{K_{eq}} P_{n} + Cu_{2}Br/L$$

$$P_{n} + M \xrightarrow{k_{p}} P_{n+1} + Cu_{2}Br/L$$

$$P_{n} - X + PH \xrightarrow{k_{PH}} P_{n} - PH$$

Here are the steps for the derivation:

$$K_{eq} = \frac{[P \cdot][Cu(II)]}{[Cu(I)][PX]} \longrightarrow [P \cdot] = \frac{K_{eq} [Cu(I)][PX]}{[Cu(II)]} = D[PX]$$

$$\frac{dM}{dt} = -k_p [P \cdot][M] = -k_p D[PX][M]$$

$$\frac{d[PX]}{dt} = -k_{PH} [PX][PH]$$
(1)

Assuming [PH] >>> [PX], therefore, reaction is pseudo first order with [PH] considered a constant.

$$\Rightarrow [PX] = [PX]_0 e^{-k_{PH} [PH]t}$$
(2)

Substituting equation 2 to 1, we get:

$$d\mathbf{M}/dt = -\mathbf{k}_{p}\mathbf{D}[\mathbf{M}][\mathbf{PX}]_{0} e^{-k_{PH}[PH]t}$$

Solving the differential equation, we obtain:

$$\ln\left(\frac{[M]_0}{[M]}\right) = \frac{k_p D[PX]o}{k_{PH}[PH]} (1 - e^{-k_{PH}[PH]t})$$
$$\ln\left(\frac{[M]_0}{[M]}\right) = A(1 - e^{-Bt})$$

Supporting Information for Chapter 3



Figure A2.1 XPS spectra obtained after APTES deposition and initiator attachment on silicon substrate. The presence of nitrogen and bromine peak confirms successful grafting of APTES and surface initiator to the silicon substrate.



Figure A2.2 GPC refractometer data for PtBA polymerized in solution, concurrent with the synthesis of PtBA brushes on silicon substrates. Molecular weight and dispersity for each polymer are given in Table S1. The PtBA polymer with $M_n = 18.8 \text{ kg/mol}$ and D = 1.26 was synthesized with a higher ratio of initiator to catalyst and ligand (to increase the dispersity). The PtBA polymer with $M_n = 18.4 \text{ kg/mol}$ and D = 1.54 was synthesized with the addition of phenylhydrazine (also to increase the dispersity).



Figure A2.3 Camera images showing PtBA brushes of thickness (a) 85 nm and (b) 99 nm grafted on Si wafer. Different colors correspond to different brush thicknesses. Uniform brush color shows uniform brush thickness.



Figure A2.4 Optical microscopy images of a 69-nm thick PtBA brush (left column) and a 30-nm thick PAA brush (right column) at different magnifications. All brushes are uniform on the microscale. The scratches seen in the images arise from collisions of the stir bar with the surface during the reaction.



Figure A2.5 Contact angle as a function of pH for a PAA brush (as-synthesized dry thickness = 27 nm, grafting density = 0.39 chains/nm²). Measurements were conducted under the following conditions: Case A) brush was soaked in a phosphate-buffered solution (ionic strength 0.05 M), and a drop of the same buffered solution was used for the contact angle measurement; Case B) brush was soaked in a non-buffered solution, and a DI water drop was used for the contact angle measurement; and Case C) brush was soaked in a non-buffered solution, and drop of the same non-buffered solution was used for the contact angle measurement. All three measurements agree within experimental errors at each pH, confirming that the contact angle measurement is robust.



Figure A2.6 –cos(CA) as a function of pH for each brush thickness reported in Figure 3.3 (CA is the static water contact angle). Dry thickness (as-synthesized) is specified on each plot. In each pair of plots for a single thickness, the left graph corresponds to decreasing pH and the right graph corresponds to increasing pH. The dashed curves indicate sigmoidal fits to the data.


Figure A2.7 –cos(CA) as a function of pH for each brush thickness reported in Figure 3.3 (CA is the static water contact angle). Dry thickness (as-synthesized) is specified on each plot. In each pair of plots for a single thickness, the left graph corresponds to decreasing pH and the right graph corresponds to increasing pH. The dashed curves indicate sigmoidal fits to the data.



Figure A2.8 (a) Variation in water contact angle with pH measured for PAA brushes of varying thicknesses on glass substrates (grafting density = 0.32 chains/nm²). (b) Variation in pK_a with as-synthesized dry brush thickness (○ increasing pH, ▲ decreasing pH). Data obtained for brushes grafted on glass surfaces exhibit a similar trend as that for brushes grafted on silicon wafers presented in the chapter 3.



Figure A2.9 –cos(CA) as a function of pH for each brush reported in Figure 3.5(b) (CA is the static water contact angle). Solution PtBA dispersity is specified on each plot. In each pair of plots for a particular dispersity, the left graph corresponds to decreasing pH and the right graph corresponds to increasing pH. The dashed curves indicate sigmoidal fits to the data. In order to fit the data obtained upon decreasing pH, in which the low-pH contact angle plateau was not well-resolved, the low-pH contact angle value was fixed to that determined from the curve fit to the data obtained upon increasing pH. The error on the pK_a was taken to be the maximum value of standard error determined from the best fit curve (highest adjusted R² value).



Figure A.10 AFM images for (a) 27 nm PtBA brush on Si substrate, (b) 30 nm PAA brush on Si substrate, and (c) 12 nm PAA brush on Si substrate. The grafting density was 0.38 chains/ nm² for the 30 nm brush and was not measured for the other brushes. All images are 5 μ m by 5 μ m. The z-scale is 6 nm for all three images. The root-mean-square roughness is 0.29 nm, 0.48 nm, and 0.60 nm for the 12 nm, 30 nm, and 27 nm thick brushes, respectively.

Effect of Ionic Strength on pH-response of Disperse PAA brushes

Introduction

The surface charge of weak polyelectrolytes can be tuned not only with pH but with ionic strength. Ionization of weak polyelectrolytes initially increases as the ionic strength is increased, then decreases after reaching a maximum due to charge screening.^{9, 39} Increased ionization leads to greater extension of polymer brushes, analogous to what we observed upon increasing the pH.¹⁸³ As we attribute the hysteretic memory behavior in contact angle data to greater brush collapse at low pH due to dispersity, we hypothesize that increased ionization due to addition of salt might lead to disappearance of observed hysteretic memory behavior.

Here we investigate the effect of systematic increase in ionic strength on the pHresponse of PAA brushes with thickness of 7 nm and dispersity around 1.65. We carried out experiment similar to those reported in Chapter 3 by varying the solution pH from 10 to 3 and subsequently increasing from 3 to 10; here, we varied the ionic strength by varying the concentration of sodium chloride.

Materials and Methods

The synthesis of PAA brushes grafted on silicon wafers follows that described in Chapter 3. Solution initiator was added in each reaction to characterize molecular weight and dispersity of polymer brushes. Dispersity was increased via addition of phenylhydrazine in ATRP syntheses.

Firstly, sodium chloride (ACS reagent, >99%) was added to the water purified with a Millipore water purification system (resistivity 18.2 M Ω .cm) to adjust the ionic strength of solution. Subsequently, to investigate the dependence of the water contact

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angle and dry brush thickness on pH at varying ionic strengths, separate solutions of pH ranging from 3 to 10 were prepared by adding an appropriate amount of sodium hydroxide (ACS reagent, >97%) or hydrochloric acid (Ricca Chemical Company, 1% v/v aqueous solution) to the solution at specific ionic strength. The polymer-grafted silicon wafers were immersed for 30 min in a solution with a desired pH and ionic strength, and subsequently dried under nitrogen.

Static water contact angles on the dried PAA brush samples were measured on an OCA 15EC video-based optical contact angle-measuring instrument (DataPhysics, Germany) at ambient temperature using SCA 20 software. DI water droplets (of approximate volume 1 μ L) were deposited carefully onto the polymer-grafted surface and the contact angles were measured at five different positions on the surface.

The dry thickness of the PAA brushes was measured with a JA Woollam M-2000 spectroscopic ellipsometer. Ellipsometry data were modeled using a two-layer model that consisted of a polymer layer on top of SiO₂. The refractive index of each layer was modeled with the Cauchy dispersion relation, $n(\lambda) = A + B/\lambda^2$ (A and B are strictly positive). The measured amplitude ratio and phase difference were modeled with the Fresnel equation, with the brush thickness as a fitting parameter. This model provided a good fit for data in the wavelength range of 350 - 1690 nm. Thickness measurements were conducted at five different locations on each substrate.

Results and Discussion

All PAA brushes used in this study had a thickness of 7 nm and dispersity of around 1.65 (Table S1). Brushes with lower thickness and higher dispersity were chosen

to investigate the effect of ionic strength on the hysteretic memory behavior. The ionic

strength of NaCl solution was varied from 0 mM to 100 mM.

Table A3.1 Average PAA brush thickness, number-average molecular weight and dispersity of PtBA brush. Ionic strength indicates the particular ionic strength experiment performed for each PAA brush

As-Synthesized PAA	M_n (kg/mol) (D)	Ionic Strength Used
Dry Thickness (nm)		(mM)
7.5	15.9 (1.67)	100
7.3	15.9 (1.67)	20
7.5	15.9 (1.67)	5
6.9	14.9 (1.65)	1
7.2	14.9 (1.65)	0.1
7.6	18.1 (1.64)	0

The pH-dependence of the dry thickness was measured for PAA brushes at various ionic strengths. The dry thickness was independent of both pH and ionic strength (Figure S1). Hysteretic memory behavior was not observed in dry thickness, similar to the results presented in Chapter 3.



Figure A3.1 Dry thickness of PAA brushes as a function of solution pH at varying ionic strengths. Solid symbols signify decreasing pH and open symbols signify increasing pH.

Water contact angles (CA) were measured at different pH values for solutions of varying ionic strength, as shown in Figure S2a. Three trends were observed in the data. First, low CAs were obtained for brushes at basic pH at all ionic strengths, and the CA increased with decreasing PH. The onset pH at which the CA increased was dependent on the ionic strength of solution. As the ionic strength increased, the onset pH decreased. Second, low pH CA decreased with increasing ionic strength. The reduction in the lowpH CA as the ionic strength was increased could be attributed to increased ionization of PAA brushes which increases the hydrophilicity of brushes.⁷⁴ Due to increased ionization of brushes with increase in ionic strength, lower CA plateau extended from pH 9 to pH 6 as the ionic strength was increased from 0 mM to 100 mM. Therefore, ionic strength does affect the pH-response of PAA brushes. Last, upon increasing the pH, hysteretic memory behavior was not observed for any of the brushes exposed to solutions of varying ionic strength. Additionally no significant differences in pK_a for increasing and decreasing pH direction were obtained (Figure S2b), again underlining absence of hysteretic memory behavior. Because the PAA brush exposed to the salt-free (0 mM) solution did not show hysteretic behavior (whereas a PAA brush with similar thickness and dispersity showed hysteretic behavior in Chapter 3), we cannot conclude if disappearance of hysteretic memory behavior with increased ionic strength is due to ionic strength or due to some synthetic issue. Further experiments are needed to conclude the effects of ionic strength on pH-response, as we have observed discrepancy in results for PAA brushes with lower thickness and higher dispersity in absence of salt.



Figure A3.2 (a) Static water contact angles of PAA brushes as a function of solution pH at varying ionic strengths. Solid symbols signify decreasing pH and open symbols signify increasing pH. (b) pK_a obtained by fitting sigmoidal function to CA in increasing and decreasing pH direction for each ionic strength. Open symbols signify increasing pH direction.

Disclosure.

Chapter 3 has been published.

Vivek Yadav, Adrienne V. Harkin, Megan L. Robertson and Jacinta C. Conrad, "Hysteretic memory in pH-response of water contact angle on poly(acrylic acid) brushes", SoftMatter, 2016, 12, 3589-3599