Salt- and pH-Induced Swelling of a Poly(Acrylic Acid) Brush via Quartz Crystal Microbalance w/ Dissipation (QCM-D)

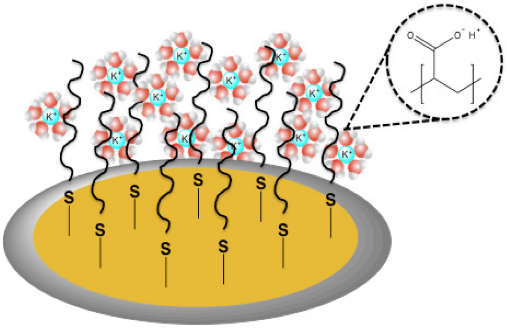
[1]Hollingsworth, N. R.; [2,3]Wilkanowicz, S. I.; [1,3,4,*]Larson, R.G.

[*]Corresponding author
[1]Department of Macromolecular Science & Engineering, University of Michigan, Ann Arbor, Michigan 48109, United States
[2]Warsaw University of Technology, Faculty of Civil Engineering, Mechanics and Petrochemistry, Institute of Chemistry, Plock, Poland
[3]Department of Chemical Engineering, University of Michigan, Ann Arbor, Michigan 48109, United States
[4]Biointerfaces Institute, University of Michigan, Ann Arbor, Michigan 48109, United States

Abstract

We infer the swelling/de-swelling behavior of weakly ionizable poly(acrylic acid) (PAA) brushes of 2-39 kDa molar mass in the presence of KCl concentrations from 0.1-1000 mM, pH = 3, 7, and 9, and grafting densities $\sigma = 0.12–2.15$ chains/nm^2 using a Quartz Crystal Microbalance with Dissipation (QCM-D), confirming and extending the work of Wu et al[1] to multiple chain lengths. At pH 7 and 9 (above the pKa~5), the brush initially swells at low KCl ionic strength (< 10 mM) in the "osmotic brush" regime, and de-swells at higher salt concentrations, in the "salted brush" regime, and is relatively unaffected at pH 3, below the pKa, as expected. At pH 7, at low and moderate grafting densities, our results in the high-salt "salted brush" regime ($C_s > 10$ mM salt) agree with the predicted scaling $H \sim N\sigma^{+1/3}C_s^{-1/3}$ of brush height H,[2,3] while in the low-salt "osmotic brush" regime ($C_s <10$ mM salt), we find $H \sim N\sigma^{+1/3}C_s^{+0.28-0.38}$, whose dependence on C_s agrees with scaling theory for this regime,[2,3] but the dependence on σ strongly disagrees with it. The predicted

linearity in the degree of polymerization N is confirmed.[2,3] The new results partially confirm scaling theory and clarify where improved theories and additional data are needed.



Introduction

Polymer brushes have been studied extensively for many decades and have gained considerable attention for their applications in a variety of areas, including oil recovery, protein antifouling, aqueous lubrication, nanocomposite fabrication, water harvesting, flow-control valves, drug delivery, and rectifiers.[4–13] Brushes are defined as densely-packed polymer chains that are tethered on one end onto an impermeable substrate and extend into solvent at the other end.[3,14] By definition, the grafting density of a polymer brush is high enough that the polymer chains are forced to extend away from the substrate, thus preventing the random-coil arrangement typical in solution, or on surfaces in the "mushroom" regime, where the grafting density is too low to form a brush.[11,15] Polyelectrolyte brushes in particular are desirable for their swelling characteristics in aqueous conditions, making them suitable as colloidal stabilizers, water-based lubricants, and biomaterials.[16,17]

Weak polyelectrolyte brushes, or annealed brushes, are modifiable as their charge density and resultant swelling behavior is altered by the environmental pH or ionic strength, which affect the extent of chain ionization.[18,19] Furthermore, the swelling or structural behavior of weak polyelectrolyte brushes has been shown to depend on a variety of factors, including the type of salt ion, the valence of salt ion, the hydrophobicity of the polyelectrolyte monomers, the temperature, and the brush grafting density.[18,20,21] This behavior has been demonstrated to have a significant influence on properties such as the coefficient of friction of the brush as well as the overall adhesion or wettability of the brush towards other surfaces.[22,23] Weak polyelectrolytes have also been demonstrated to exhibit pH-specific responses consistent with their pKa, which affects their overall extent of ionization.[24]

Despite this general understanding, the pH dependence of brush properties is not well understood and while theories for the dependence of brush height on chain length, grafting density, and salt concentration have been available for more than 20 years, tests of these theories have so far been somewhat limited. The most complete of these are the studies of Tirrell and coworkers using sodium polystyrene sulfonate (NaPSS) brushes bound to mica substrates, whose heights were measured by a surface forces apparatus,[25] of Chu et al. for a series of strong polyelectrolyte brushes of sodium polystyrene sulfonate (NaPSS), poly([2- (methacryloyloxy)ethyl] trimethylammonium chloride) (PMTAC), and potassium poly(3-sulfopropyl methacrylate) (PSPMA) of a variety of chain lengths studied on silicon and silica surfaces by ellipsometry and QCM-D,[21] and of Wu et al. for a weak polyacrylic acid (PAA) brush of a single chain length grown directly onto a gold substrate and studied by QCM-D.[1] The studies of Tirrell and coworkers covered a limited range of surface coverages and chain lengths and showed brush height to be independent of salt concentration below 0.01 M, followed by a region of decreasing height with increased salt concentration, in agreement with scaling theory, including that of Pincus[26] and of Israëls et al.[3] discussed below. The studies of Chu et al. and Wu et al. showed, instead of the low-salt region of constant height, a striking *non-monotonic* dependence of brush height on added *salt concentration*, with a pronounced maximum in height at a salt molarity of around 0.01 M (or 0.1−1.0 M in Wu et al.), for all of the polyelectrolytes listed above, for various values of their pKa (weak vs. strong polyelectrolyte), chain lengths (brush molecular weights from 12 to 235 kg/mol),[21] and grafting densities (0.06 to 0.25 chains/nm^2)[21] and (0.130-0.863 chains/nm^2).[1] Both of these latter two studies found a *monotonic* dependence of brush height on *grafting density* σ, as one might naively expect, based on chain-crowding effects, but in disagreement with the scaling theory that assumes that the effects of electrostatic and Gaussian chain elasticity dominate.

The theory for strong, permanently charged, polyelectrolytes of Israëls et al.[3] predicts a low-salt "Osmotic Brush" (OB) regime in which brush height H is independent of salt concentration C_s and grafting density σ, followed by a transition to a "Salted Brush" regime in which $H \sim N\sigma^{\frac{1}{3}}C_S{}^{-\frac{1}{3}}$; i.e., the brush height increases with grafting density and decreases with salt concentration. Zhulina and coworkers[2] extended this theory to weak polyelectrolytes in which the charge density on the polymer is regulated by, and changes with, the salt concentration. The prediction of this theory in the SB regime, where the polymer charge saturates, is the same as for strong polyelectrolytes, but in the OB regime predicts the opposite dependencies on σ and C_s, namely $H \sim \sigma^{-\frac{1}{3}}C_S{}^{\frac{1}{3}}$, implying that the brush height H has a maximum in height with increased salt concentration and a minimum with increased grafting density, for a weak polyelectrolyte. The existence of the maximum in H with respect to salinity seen by Wu et al. for PAA brushes would seem to confirm this theory; however, they did not observe the predicted minimum in H with respect to σ. Moreover, the observed pronounced maximum in H at 0.01 M salt for multiple strong polyelectrolytes by Chu et al.[21] seems to throw into doubt the connection of this maximum to charge regulation as is assumed by the weak polyelectrolyte theory. Hence, more studies, both theoretical and experimental, are clearly needed to determine the behavior and establish correct theoretical predictions of these brushes under various conditions.

In our study, a PAA brush was deposited directly onto a gold-plated QCM-D electrode via end-thiol-terminated PAA, which forms a self-assembled monolayer. Our "grafting-to" approach using pre-selected thiol-terminated chains of well-defined lengths, allows us to better control the brush molecular weight relative to traditional surface-initiated polymerization techniques. Our study covers a range of chain lengths, (2-39 KDa), grafting densities (0.12–2.15 chains/nm^2), salt concentrations (0.1-1000 mM), and pH values (3-9), expanding significantly on previous work. We also avoid salt concentrations that are within the same order of magnitude as the ionic strength due to buffers used to control pH, as such data likely to be subject to considerable error, as we discuss below.

A popular instrument for studying the swelling behavior of polyelectrolyte brushes is a Quartz Crystal Microbalance with Dissipation (QCM-D), which can be used to measure subtle changes in adsorbed mass and material viscoelasticity.[20,21,27,28] Here, we use QCM-D to evaluate the effects of pH, salt concentration, chain length, and grafting density on the swelling response to salt of a weakly ionizable polyelectrolyte brush of Poly(acrylic acid) (PAA), providing the first comprehensive study that spans all four of these parameters over a wide range. In QCM-D measurements, the flow medium has been shown to significantly affect the measurement of the material deposited onto the crystal.[20,29–31] We demonstrate that assessing the contribution of the flow medium to the QCM-D response is crucial for correct interpretation of results.[22] In our study, the dry thicknesses of the brushes were measured by variable angle spectroscopic ellipsometry (VASE).

QCM-D is an ideal technique for monitoring both the uptake of salt and hydration of the brush within aqueous, ionic environments without the need for intervening drying steps, and it is sensitive enough for measurements of ultra-short brushes; however, because of the weak signal, it is imperative to correct for the effect of salts on the QCM-D signal in the absence of adsorbed polymers, as we show below.

Materials and methods

Materials. α-thiol ω-bromo terminated poly(acrylic acid) samples (PAA-SH) (Short-chain: M_n=2,000 g/mol, M_w=2,600 g/mol, M_w/M_n: 1.3, -SH > 99%; Medium-chain: Mn=14,000 g/mol, Mw=18,200 g/mol, Mw/Mn: 1.3, -SH >99%; Long-chain: M_n= 39,000 g/mol, M_w=55,000 g/mol, M_w/M_n: 1.4, -SH > 99%) were obtained from Polymer Source Inc. (Quebec, Canada). Potassium chloride (KCl), sodium chloride (NaCl), lithium chloride (LiCl), and calcium chloride ($CaCl_2$) were purchased from Sigma Aldrich USA and used as received. Hydrogen peroxide (H_2O_2) solution (30 wt%), ammonium hydroxide (NH_4OH) solution (25 wt%), 2-mercaptoethanol, and absolute ethanol (EtOH) were obtained from Fisher Scientific and were used as received. HPLC plus grade water (pH 7) (Sigma Aldrich USA) was used to prepare all salt solutions used in this work. Solutions at concentrations between 0.1 and 1000 mM were prepared. Solutions of KCl were found to be at pH 7 upon mixing without buffer, while pH 3 and pH 9 solutions of KCl were obtained upon the addition of roughly <3 mM hydrochloric acid (HCl) (Sigma Aldrich USA) or potassium hydroxide (KOH) solutions (Sigma Aldrich USA), respectively. The pH and ionic strengths were confirmed using the combination of conductivity (CON 6+ conductivity meter, Oakton) and a benchtop pH meter (Accumet AE150, Fisher scientific) versus reference standards.

Preparation of gold-coated QCM-D crystals. Gold-coated silicon wafers (QSX 301, AT-cut, F_0/n =5 MHz) were purchased from Q-Sense by Biolin Scientific (New Jersey, USA). In preparation for the deposition of PAA, the sensors were cleaned thoroughly to remove any residual dirt and debris via a TL-1 base cleaning solution. Gold-coated QCM-D wafers were fully immersed into base-cleaning solution (mixture of 30% hydrogen peroxide and ammonium hydroxide in ultra-pure water at the ratio 1:1:5) and left for two hours at 65 °C. The crystals were then rinsed thoroughly with deionized water, followed by absolute ethanol, and gently dried under a stream of pure air. The last step of crystal preparation was 15 minutes of O_2- plasma treatment.

Preparation of short-chain PAA-SH brush. All gold-coated silicon wafers were plasma treated before use. Short-chain PAA-SH (M_n: 2,000 g/mol, M_w/M_n=1.3) was dissolved in absolute ethanol to the final concentration of 4 mM.[32] To vary the grafting density the 4 mM PAA solution was used both as is and after dilution by factors of 1:500, 1:1000, 1:5000, and 1:10000 by volume in either absolute ethanol or a 10:1 (wt%) mixture of absolute ethanol to 2-mercaptoethanol. Here, 2-mercaptoethanol acts as a competitive adsorber to compete with the PAA-SH in binding. After this, 40 µL of the appropriate PAA solution was deposited onto the pure gold crystal and allowed to incubate for 18 h. Excess polymer solution was removed by rinsing the crystal repeatedly with absolute ethanol and gently drying under stream of pure air, making it ready for QCM-D analysis.

Preparation of medium-chain PAA-SH brush. Medium-chain PAA-SH (M_n: 14,000 g/mol, M_w/M_n=1.3) was dissolved in absolute ethanol to a final concentration of 4 mM. 40 μL was then deposited onto the gold crystal and allowed to incubate for 60 minutes, rather than the 18 h used for the short brush. Allowing the solution with the medium-length chain to incubate for longer than 60 min. led to the formation of a thick, heterogenous physical film due presumably to solvent evaporation. No sign of such a film was present when incubation was limited to 60 min., and reproducibility of results under this constraint was excellent. Excess polymer solution was removed by rinsing the crystal repeatedly with absolute ethanol and drying gently under pure air. To vary the grafting density, the 4 mM PAA solution was diluted by a factor of 1:10000 by volume in a 10:1 (wt%) mixture of absolute ethanol to 2-mercaptoethanol. Thereafter, the deposition process was repeated as described before.

Preparation of long-chain PAA-SH brush. Longer-chain PAA-SH (M_n: 39,000 g/mol, M_w/M_n=1.4) was, as with the other chains, dissolved in absolute ethanol to a final concentration of 4 mM. 40 μL was deposited onto the gold crystal and allowed to incubate for 20 minutes. Note that this is considerably less time than for the short-chain PAA due to the viscosity of the solution. Allowing the solution to incubate for longer than this led to the formation of a thick, heterogenous physical film. Excess polymer solution was removed by rinsing the crystal repeatedly with absolute ethanol and drying gently under pure air. This process (40 μL polymer deposition and subsequent repeated rinsing) was repeated 6 times. To vary the grafting density, the 4 mM polymer solution was diluted by 1:10000 in a 10:1 (wt%) mixture of absolute ethanol to 2-mercaptoethanol. Thereafter, the deposition process was repeated as described before.

Variable Angle Spectroscopic Ellipsometry (VASE). The thickness of the dry PAA-SH brush on the gold-coated QCM-D crystal was characterized by Variable Angle Spectroscopic Ellipsometry (VASE) (Woollam M-2000 Spectroscopic Ellipsometer, J. A. Woollam, USA) using parameters recommended in the literature for a PAA layer on bulk gold.[33] Measurements were carried out at angles of incidence (Φ_0) of 55°, 65°, and 75°. Woollam Complete EASE software was used to analyze the data obtained from ellipsometry that required more than one wavelength to perform fitting, so a central wavelength λ of 631.5 nm was chosen in addition to two wavelengths on either side of this (629.7 nm & 632.7nm). The optical relative phase shift, Δ, and relative amplitude ratio, tan(Ψ), measured by VASE, were modeled using two slabs: a bulk gold substrate overlaid by a uniform thin layer (brush). The underlying gold on the crystal was treated as a bulk (infinitely thick) substrate since its thickness is ~100 nm.

The fundamental equation of ellipsometry allows the monitoring of the complex reflectance ratio, given by the Fresnel reflection coefficients R_P (*p*-polarized electrical field) and R_S (*s*-polarized electrical field). The ratio of these two coefficients is a known, complicated, complex (i.e., not real) function[33,34] of the wavelength λ, the angle of incidence Φ_0, the optical constants (index of refraction, n, and absorption, k) of the substrate (n_{sub}, k_{sub}), and of the ambient medium (n_{amb}), and the film layer (n_{film}, k_{film}), in addition to the film layer thickness (*d*), as described in Equation 1 below.

$$\frac{R_P}{R_S} = tan(\Psi)e^{(i\Delta)} = f(\lambda, \Phi_0, \mathrm{n}_{sub}, \mathrm{k}_{sub}, \mathrm{n}_{amb}, \mathrm{n}_{film}, \mathrm{k}_{film}, d) \qquad [1]$$

The PAA layer was assumed to be transparent ($k_{\mathrm{film}}(\lambda)=0$) and homogenous, and with index of refraction (n_{PAA}) of 1.522 at $\lambda=631.5$ nm. The optical constant d in Eq. 1 was found as the value required to match the modeled to the measured values of Δ and Ψ, using a well-established method that is explained in great detail elsewhere.[34,35]

Quartz Crystal Microbalance with Dissipation (QCM-D). All QCM-D analyses were performed using the Q-Sense E4 system (Q-Sense AB, Gothenburg, Sweden). Fundamental frequency signals were established on the prepared sensors in air. A baseline for each measurement was established by flowing ultra-pure water (pH 7) until a stable response was seen, followed by the appropriate KCl solution (pH 3,7, or 9) again until a stable response was seen. Solutions were driven through the device by an IPC high precision multichannel peristaltic pump (ISMATEC, IDEX, USA) at a constant flow rate of 0.100 mL/min. The experiments were performed at room temperature (~22°C).

We model the change in frequency using the Sauerbrey relation rather than a viscoelastic model, following the work of DeNolf et al.,[28] who demonstrated that the Sauerbrey relation holds when the film is either very thin and/or rigid, expressed by the dimensionless quantity d/λ_n, where d is the thickness of the film, and λ_n is the shear wavelength of the material. The latter is a function of the complex shear modulus, the frequency at the appropriate harmonic, the density, and the viscoelastic phase angle. DeNolf et al. give the Sauerbrey limit as valid when d/λ_n is no greater than 0.05, beyond which a viscoelastic model should be used.[27,28] In our experiments, we find that d/λ_n ranges between 0.004 to 0.020, where d/λ_n approaches higher values in a thicker (longer chain) brush. Since $d/\lambda_n<0.05$ in all our

QCM-D experiments, we use the Sauerbrey model in all instances, since viscoelastic modeling would overestimate the amount of adsorbed mass.

In what follows, we use conventional interpretations of the QCM-D response to infer the swelling behavior of the brush, based on the change in QCM-D for the brush-adsorbed surface relative to the bare surface. While the validity of the Sauerbrey relation gives us a quantitative measure of the adsorbed mass and swelling through the measurement of ΔF_3 using Equation 2, this also means that the dissipation is too small to be interpreted quantitatively, since viscoelastic modeling fails in the Sauerbrey limit.[27] Hence, we can only interpret the ΔD_3 data qualitatively as a measure of dissipation.

Typically, in a QCM-D measurement, the fundamental frequency is first found for the sample in air. In our case, either a blank crystal or a chip coated with a thin polymer brush was loaded in air. The fundamental frequency of a blank quartz chip, F_0, is 5 MHz,[36] so that the resonance at the third overtone (n=3) is 15 MHz. A brush-coated chip, however, will possess a slightly different baseline frequency. Once this frequency is found, the QCM-D measurement begins by measuring the change in frequency (ΔF) and the change in dissipation (ΔD) at the desired overtones which are reported by the instrument hundreds of times per second, where again, the ΔF and ΔD reported by the instrument are changes from the initial frequency and dissipation measurements of the loaded sample, either of the bare crystal or of the dried polymer on the crystal; in our case at the third overtone n=3, which is considered to be more reliable than the noisier first overtone.[37] Only one overtone is needed to interpret the Sauerbrey mass for a rigid film, while more than one is necessary for viscoelastic modeling.[36] The equivalent adsorbed mass change (Δm) can be calculated using the simple Sauerbrey relation for relatively rigid films where the change in dissipation (ΔD) is small, as discussed above.[38]

$$\Delta F = -\Delta m\left(\frac{2nF_0^2}{A\sqrt{\mu_q\rho_q}}\right) = -\frac{1}{C_f}\Delta m \quad [2]$$

$$\Delta d = \frac{\Delta m}{\rho} \quad [3]$$

$$D = \frac{E_{lost,cycle}}{2\pi E_{stored,oscillator}} \quad [4]$$

Here, the crystal parameter (C_f) values are known and fixed; F_0 is the fundamental frequency (= 5 MHz), n is the overtone number (=3), Δm is the change in mass per unit area, ρ_q is the density of quartz (=2.648 g/cm^3), A is the crystal area (=1.54 cm^2), and μ_q is the shear modulus of quartz (=2.947 x 10^{11} g.cm^{-1}.s^{-2}), respectively.[39–41] Assuming a homogenous film, the Sauerbrey mass difference Δm can then be converted to a Sauerbrey thickness increment Δd using the added-mass density ρ. The dissipation D is a measure of the dimensionless ratio of energy lost in an oscillation cycle $E_{lost,cycle}$, to the energy stored in the oscillator, $E_{stored,oscillator}$.[36,37] An increase in ΔD indicates an increased dissipation of the film, meaning that it is less rigid, thicker, or both. A decrease in ΔF (i.e., more negative ΔF) indicates that more mass is adsorbed.

The most accurate QCM-D measurements are obtained as the differences in mass produced upon adding solvent to a dried crystal that is either bare or has an adsorbed brush. We therefore obtain the mass of the adsorbed brush by ellipsometry and use QCM-D only to obtain changes in brush mass due to solvation of the brush. In Appendix A, we show the importance of correcting QCM-D data obtained with a polymer brush by subtracting from these data the results obtained for a bare, polymer-free crystal. This subtraction removes the effect of the inertia and viscosity of the overlying solvent, which varies with salt concentration. Without this subtraction, salt-induced changes in the overlying solvent can be incorrectly attributed to the effect of salt on the behavior of the polymer brush, and this leads to huge errors for thin brush layers, as we show in Appendix A.

To obtain our results, we assume an additive relationship between the effects of the polymer brush $\Delta F_{Polymer}$, the solvent swelling the brush $\Delta F_{Swelling}$, and the overlying solvent $\Delta F_{Solvent}$, described in detail by Chu et al.[21] The effect of adding a polymer layer and solvent to a bare crystal surface would give $\Delta F_{Total} = \Delta F_{Polymer} + \Delta F_{Solvent} + \Delta F_{Swelling}$. However, the most reliable measurement is the change in frequency upon adding solvent to a dry specimen, either a bare dry crystal, or a polymer-coated dry crystal. For the latter, the frequency change $\Delta \mathrm{F}(polymer\ coated) = \Delta F_{Solvent} + \Delta F_{Swelling}$ is obtained by measuring the change in frequency that occurs when a dry polymer-coated crystal is exposed to solvent, while $\Delta F(bare\ crystal) = \Delta F_{Solvent}$ is the change in frequency when solvent is added to the bare crystal. Thus, the difference in these two measured quantities $\Delta \mathrm{F}(polymer\ coated) - \Delta \mathrm{F}(bare\ crystal) = \Delta F_{Swelling}$ is the frequency change due to solvent swelling the brush, where "solvent" here includes any dissolved salt. This $\Delta F_{Swelling}$was then converted to a Sauerbrey mass increment using Equation 2, and then to a finite thickening distance $\Delta d_{Swelling}$, and the wet brush height $d_{wet\ brush}$ is found by adding this thickening $\Delta d_{Swelling}$ to the dry brush height $d_{brush,dry}$ (found from VASE) for a given brush grafting density, and this $d_{brush,wet} = \Delta d_{Swelling} + d_{brush,dry}$ is equivalent to the wet brush height (H) data plotted in all subsequent figures. To find the % Swelling as described by Sadman et al.[27], the wet brush thickness was compared to that of the dry brush (found from VASE) as shown in Equation 5. To find the change in dissipation response due to swelling $\Delta D_{Swelling}$, the dissipation was calculated according to the same additive relationship used to calculate the change in frequency due to swelling, where for dissipation, $\Delta D_{Swelling} = \Delta D(polymer\ coated) - \Delta D(bare\ crystal)$, as given in Eq. 6. Again, the first term on the right-hand side of Equation 6 is calculated from the change in dissipation (3rd harmonic) upon solvating the dried polymer on the chip, while the second term is from solvating the bare chip.

$$\%\ \text{Swelling} = \frac{\Delta d_{Swelling}}{d_{\text{dry brush}}} x\ 100 \qquad [5]$$

$$\Delta D_{Swelling} = \Delta D(polymer\ coated) - \Delta D(bare\ crystal) \qquad [6]$$

Following every experiment, each crystal was cleaned with TL-1 solution and O_2-plasma treatment as described earlier. All measurements were repeated three times and the averages of each value are presented in what follows; error bars are comparable to the size of the symbols used.

Results and Discussion

Variable Angle Spectroscopic Ellipsometry (VASE)

The VASE results for the dry PAA brush at various grafting densities σ are given in Table 1. To calculate the grafting density σ in chains/nm^2, the following formula was used,[42] where $d_{brush,dry}$ is the dry thickness in nm determined by VASE, ρ is the bulk density of PAA (=1.08 x 10^{-21} g/nm^3), N_A is Avogadro's number, and M_n is the number average molecular weight of PAA:

$$\sigma = \frac{d_{brush,dry}\rho N_A}{M_n} \qquad [7]$$

It should be noted that σ~1 is considered to be highly grafted, and σ ~0.3 is considered to be a "moderate" grafting density in the literature.[43] While our shortest chains achieve a grafting density σ>2 (chains/nm^2), which is considered to be extremely highly grafted, a similar grafting density has been achieved in the past in a combination grafted-to/grafted-on fabrication method[42] for polymer chains three times as long as ours. We note that the brushes in our study are fabricated as self-assembled monolayers (SAMs), which are typically end-functionalized oligomers that self-assemble onto their substrate at high grafting densities to form a uniform, highly elongated, dense monolayer. Values of grafting densities σ exceeding 3 chains/nm^2 for oligomers are typical for SAMs,[44] and we note that at these extremely high grafting densities the polymer chains are most likely already highly elongated even while dry due to steric repulsion, leaving them little room to swell. Depending on dilution during preparation, the short-chain PAA-SH solutions yielded very dense, dense, moderately dense, and low-density brushes with grafting densities up to 20 times lower than that achieved using the original 4 mM solution. The medium molecular weight brush yielded a moderately dense brush. The long-chain PAA-SH solution yielded a moderately dense brush. The brush densities are summarized in Table 1. Although we define density here based on the magnitude of the surface coverage σ, the response of the brush to solvent also depends on its molecular weight. For example, a long-chain brush with a "moderate" grafting density as defined above may be more resistant to stretching out fully in solvent than is a shorter brush at the same grafting density.

Polymer MW (Mn)	PAA Brush Density	Dry Thickness, *d* (nm)	Grafting Density, σ (chains/nm^2)
2 kDa	Very High	6.60 ± 0.10 nm	2.15
	Very High	4.49± 0.21 nm	1.46
	High	3.21 ± 0.61 nm	1.04
	Moderate	2.66 ± 0.13 nm	0.87

	Moderate	0.923 ± 0.12 nm	0.30
	Low	0.369 ± 0.59 nm	0.12
14 kDa	Moderate	8.17 ± 0.92 nm	0.38
39 kDa	Moderate	27.0 ± 2.04 nm	0.45
None (Ethanol/ 2-Mercaptoethanol 1:10)	-	ND	-
None (Bare Au)	-	ND	-

Table 1- P-AA brush thicknesses *d* and grafting densities, σ for the chain lengths studied here. ND indicates that the dry thickness is not detectable by VASE.

Quartz Crystal Microbalance with Dissipation (QCM-D) – Solvent-Induced Changes in Swelling and Dissipation

At pH 9 (Figure 1(A-C), triangles), the brush should be virtually fully ionized, and we see that at each surface coverage, the brush swells/becomes more dissipative with increased salt concentration at low ionic strength, indicating the uptake of salt and water, and then de-swells/becomes more rigid as the ionic strength increases further as evidenced by the %Swelling and $\Delta D_{\mathrm{Swelling}}$. (We note that the changes in %Swelling and $\Delta D_{\mathrm{swelling}}$ are insignificant when the salt concentration is increased from zero to the lowest non-zero salt concentration of 0.1 mM for all work shown here.) A similar maximum was seen for PAA brushes by others in the vicinity of 10 mM,[1,21,45] corresponding to the end of the low-salt "osmotic brush" regime.[1,2,46,47] We observe subsequent de-swelling as the salt concentration increases into the "salted brush" regime, apparently due to charge screening,[45,48] as expected. This trend is seen at all grafting densities investigated. It can be seen from %Swelling that at moderate grafting densities (σ=0.87 and σ=1.04 chains/nm^2) (Figure 1(A-B), triangles), that less dense brushes are able to take in more water at low salt concentrations than the highly grafted brush (σ=2.15 chains/nm^2) ((Figure 1(C), triangles), but expel this water once electrostatics are screened by counterions at high salt concentration.

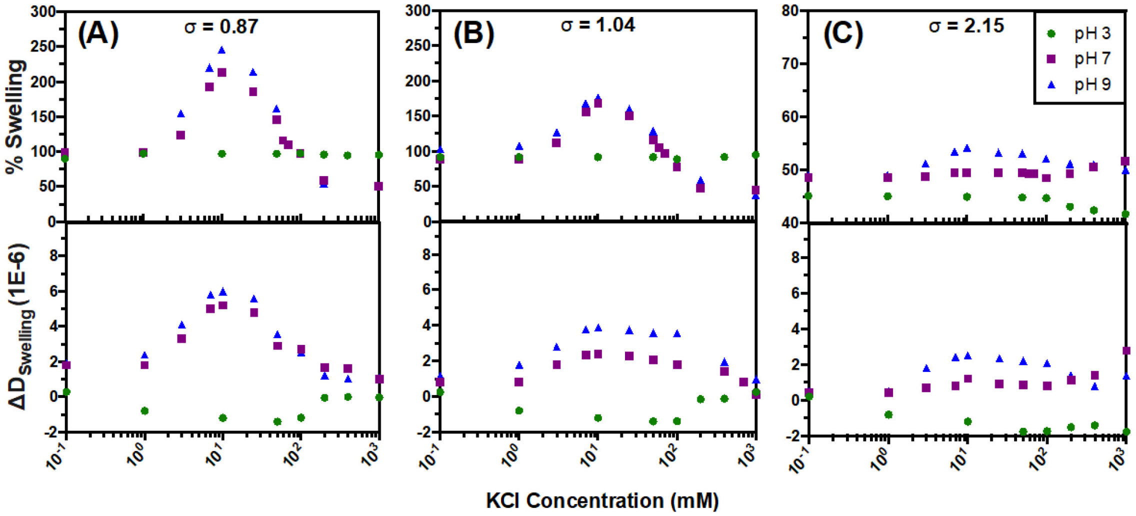


Figure 1 (A-C)- Dependence of %Swelling and ΔDissipation$_{Swelling}$ on concentration of KCl at various values of pH for short-chain (M_n= 2,000 g/mol) PAA brush grafted to a gold crystal at various grafting densities, σ (chains/nm^2): (A) σ = 0.87, (B) σ = 1.04, & (C) σ = 2.15. Note: due to the logarithmic axes, in both this and subsequent figures the values of %Swelling and ΔDissipation$_{Swelling}$ at 10^{-1} mM represent the value at 0 mM salt for pH 3 (green circles). For pH 7 (purple squares) & pH 9 (blue triangles), the values at 10^{-1} mM are indistinguishable from the values at 0 mM salt.

The swelling is greater for the less dense brushes, evidently because there is more room for water, than at higher grafting densities. In a highly grafted brush (σ=2.15 chains/nm^2) (Figure 1(C), triangles) the %Swelling maximizes at 51% relative to a dry brush at low ionic strength. We note that at extremely high grafting densities and high salt concentrations, a polymer brush is expected to be dense enough that individual chains are almost fully elongated, to such an extent that the polyelectrolyte brush is only slightly solvated, and counterions should condense onto the polymer, making it less charged.[1,2,47] The attainment of high density and nearly full elongation is supported by VASE measurements, which yield a dry brush thickness of 6.6 nm at the highest grafting density (σ = 2.15 chains/nm^2), and the fully extended chain length is estimated to be roughly 7-9 nm,[49] where the upper and lower bounds of this range are, respectively, based on number and weight average molecular weights of the PAA used (M_w/M_n=1.3). Starting with a dry-brush thickness of 6 nm, the 51% peak in swelling exhibited by the highest grafting density (σ=2.15 chains/nm^2) corresponds to a thickness of ~10 nm. This shows that the brush is virtually fully extended at a salt concentration of 10 mM, and that the longer chains — within the polydispersity of the starting material— contribute to an effective thickness that is somewhat greater than that predicted by the average molecular weight.

At pH 7 (Figure 1(A-C), squares) the trend of increased swelling/increased dissipation at low salt concentration followed by decreased swelling/increased rigidity with increasing ionic strength is similar to that at pH 9, although to a somewhat lesser extent, as expected for the decreased ionization at pH 7, with one exception at the highest grafting density (σ=2.15 chains/nm^2). We note that this swelling at low ionic strengths again corresponds to the "osmotic brush" regime,[1,2,46,47] and that subsequent de-swelling as the salt concentration increases into the "salted brush" regime is presumably due to charge screening.[48] At lower grafting densities (σ=0.87 and σ=1.04 chains/nm^2) (Figure 1(A-B), squares) we again see a greater magnitude of swelling than at the highest grafting density, presumably due to more space available.

At the highest grafting density (Figure 1(C), squares), we see that while the %Swelling at pH 7 does initially increase somewhat at low salt concentration and then decrease as expected, it eventually increases again at the highest ionic strengths. Correspondingly, the $\Delta D_{Swelling}$ at pH 7 (Figure 1(C), squares) continuously increases, indicating that the brush becomes more dissipative. It has been shown by Yu and colleagues that high grafting densities lead to the extension of the osmotic brush regime to higher ionic strengths as a result of the osmotic pressure of associated counterions.[50] We can hypothesize that this additional swelling at high ionic strengths, relative to pH 9, is due to incomplete ionization of the brush, but why this should lead to more swelling than at pH 9 at high salt concentration is mysterious. Looking closely at Fig. 8 of Wu et al., a similar increase in brush height at the highest salt concentration near 1 M can be seen for their short PAA brushes at pH 10 for the two lower grafting densities.[1] Thus, both our brush height and that of Wu et al. show a maximum, then deswelling, and finally again an increase in height with increasing salt. However, this final increase in height at ionic strength near 1 M occurs only for our densest brush (σ = 2.15 chains/nm^2) at pH 7, whereas it occurs only in their two lower-density brushes (σ = 0.130 and 0.404 chains/nm^2) at a pH of 10 (also well above the pKa), but is not evident in their highest density brush (σ = 0.863), and so the significance of upturn in height at high salt concentration in a few cases is not clear. We remark here that Wu et al. report wet brush thicknesses for their short (M_n= 4.10 kDa, M_w/M_n=1.29) brushes that are as high as 30 nm, which is higher than the fully extended length of PAA chains of this molecular weight, and so the actual molecular weight of their brush must be somewhat higher than they estimated. Adamcyzk et al.[49] estimated the contour length of PAA of molecular weight 12 kDa to be 40 nm, from which we can estimate that the brushes of Wu et al. must be around 9 kDa, at least. (This difference from the reported value of Wu et al. is perhaps not too surprising, since the molecular weight of chemically grown PAA cannot be determined directly.)

Figure 2 shows the %Swelling and $\Delta D_{\text{Swelling}}$ for two additional grafting densities, σ=0.12 chains/nm^2 (diamonds) and σ=0.30 chains/nm^2 (crosses) at pH 7 only. These brushes swell considerably more than the denser brushes, as expected for their lower density, leaving them more room to swell, and become less rigid, as expected, since these brushes are more hydrated.

At pH 3, below the pKa (Figure 1(A-C), circles), our brush can be considered charge neutral and fully protonated. Accordingly, when KCl is added, the %Swelling decreases, indicating the loss of water which is presumably osmotically pulled out of the brush by the external solution, which has a higher KCl concentration than the brush because of excluded volume effects. (The decrease in swelling can be clearly seen in Fig. 1(C) but is too small to be evident in Fig. 1(A-B).) $\Delta D_{\text{Swelling}}$ supports this interpretation; as KCl is increased from zero concentration, the brush becomes less dissipative with increasing ionic strength, becoming negative, which implies that it is below the value for the hydrated blank crystal, indicating that the brush becomes more rigid due to dehydration as salt concentration increases.

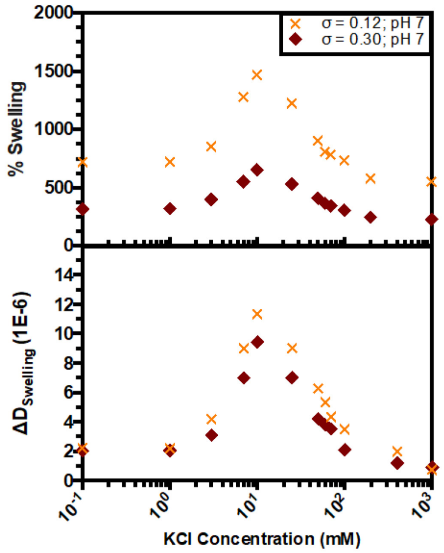


Figure 2- Dependence of %Swelling and $\Delta D_{\text{Swelling}}$ on concentration of KCl at pH 7 for short-chain (M_n= 2,000 g/mol) PAA brush grafted to a gold crystal at low grafting densities, σ (chains/nm^2) σ = 0.12 & σ = 0.30.

The grafting density σ of the long-chain (M_n=39,000 g/mol) PAA brush deposited using the method described earlier was determined to be σ = 0.45 chains/nm^2 by VASE. While this grafting density might be considered "moderate" since it corresponds to fewer chains per unit area than the "moderately" dense short-chain brushes investigated (σ = 0.30 chains/nm^2; see Table 1), it can be considered very dense for a chain of this length (M_n=39 kDa). Thus, we compare in Figure 3 (A) %Swelling and (B) $\Delta D_{Swelling}$ for the long-chain brush of grafting density σ = 0.45 chains/nm^2 with the results for a short-chain brush of grafting density σ = 2.15 chains/nm^2 at pH 9, pH 7, and pH 3 (A-C, respectively). It can be seen from %Swelling (Figure 3(A-C)) that at every pH in the absence of salt (pure water), the long brush (squares) and the short brush (circles) swell to a similar extent (i.e., to within 10%), which is initially surprising, and is probably related to the dense grafting of both the long and short brushes. As we will discuss below, the swelling observed in our short and long-chain brushes appears to be consistent with the scaling theory for the dependence of brush thickness on chain length at fixed grafting density.

At pH 9 (Figure 3(A)), the brushes should be considered virtually fully ionized. As expected, in the low-salt regime, up to 10 mM KCl, the brush swells tremendously as exhibited by %Swelling (Figure 3(A), top). At 50 mM, in the salted-brush regime, the %Swelling curves of the long and short chains intersect. The curves of $\Delta D_{Swelling}$ (Figure 3(A), bottom) intersect at a similar salt concentration, but are greater at lower ionic strengths in the long-chain brush than in the short-chain one, indicating more dissipation than for the nearly fully extended short chains. The decrease in $\Delta D_{Swelling}$ (Figure 3(A), bottom, squares) indicates that the brush becomes more rigid as ionic strength increases. At pH 7 (Figure 3(B)), this trend is seen again but to a slightly lesser extent as expected due to the lower ionization. Again, from the %Swelling, the long brush is seen to swell greatly at lower ionic strengths, then de-swell as the concentration of KCl increases, while the rise and fall of $\Delta D_{Swelling}$ also suggests that the brush takes up water at low ionic strengths and loses it due to counterion condensation as salt concentration increases. At pH 3, as evidenced by %Swelling (Figure 3(C), top), both the long-chain and short-chain brushes de-swell slightly with increased KCl concentration, as water is driven out due to annealing from increased salt. Figure 3(C), bottom, shows very little response overall in $\Delta D_{Swelling}$ for both brush lengths, suggesting that the loss of water in the brush has little effect on dissipation.

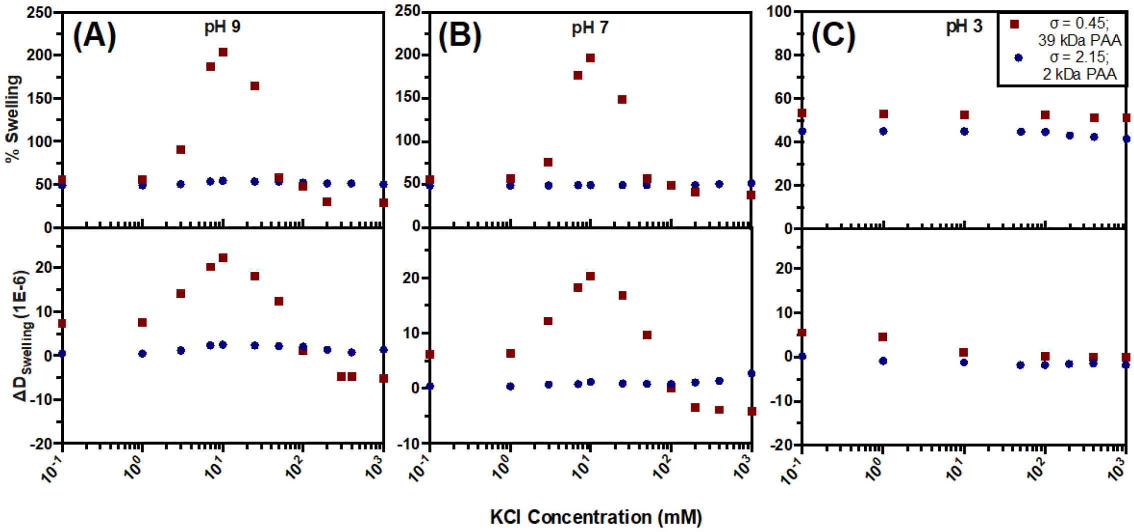


Figure 3(A-C) Dependence of %Swelling and $\Delta \text{Dissipation}_{Swelling}$ of short-chain (M_n= 2,000 g/mol) at its highest grafting density and long-chain PAA (M_n= 39,000 g/mol) to KCl concentration at (A) pH 9, (B) pH 7, and (C) pH 3.

Comparison to Theory and Previous Work

While PAA is a “weakly” dissociating polymer, it is likely that it is highly ionized at pH 7 & 9, well above its pKa (~5). The similarity in the swelling and deswelling behavior seen at pH 7 and pH 9 in Figs. 2 and 4 also suggest that the polymer is sufficiently charged at pH 7 that the brush is essentially a "strong polyelectrolyte" at both these pH values. Oddly enough, however, Wu et al.[1] found similar salt dependence of PAA brush height, with a maximum at salt concentration ~ 0.1 M, for pH = 4, 5.8, and 10, which spans from below to above the pKa ~ 5, implying either that there is little change in the charge of the brush over this large range of pH, which seems unlikely, or that there is little effect of this change on the salt dependence of brush height. We have found that once pH is reduced to 3, the brush clearly loses its charge, as shown by the "neutral brush" behavior we observe in Figs. 2 and 4. The mean-field scaling theory of Israëls et al.[3] in the low-salt osmotic brush (OB) regime, predicts that the brush height (H) follows the dependence given in Equation 8 where the chain length is measured in number of monomers (N), and α is the average fraction of charged monomers on the chain.

$$H \sim N\alpha^{1/2} \tag{8}$$

For a fully ionized PAA chain, for which α= 1, H is thus predicted to be independent of grafting density σ and salt concentration. In the salted brush (SB) regime, the theory of Israëls et al. predicts that the brush height scales according to the following Equation 9.

$$H \sim N\sigma^{\frac{1}{3}}{C_s}^{-\frac{1}{3}}\alpha^{2/3} \tag{9}$$

Israëls et al.[3] predict that when the brush is fully ionized ($\alpha = 1$), in both the OB regime (see Equation 8) and the SB regime (see Equation 9), the brush height should depend linearly on chain length, N, if grafting density, pH, and salt concentration are held fixed. Figure 4 (A-B) shows that this linear dependence is indeed obtained for our PAA brushes of chain length 2 kDa (σ=0.30 chains/nm^2), 14 KDa (σ=0.38 chains/nm^2), and 39 kDa

(σ=0.45 chains/nm^2), in both the OB (Figure 4(A)) and SB regimes (Figure 4(B)) when grafting density is kept as constant as possible with our data sets.

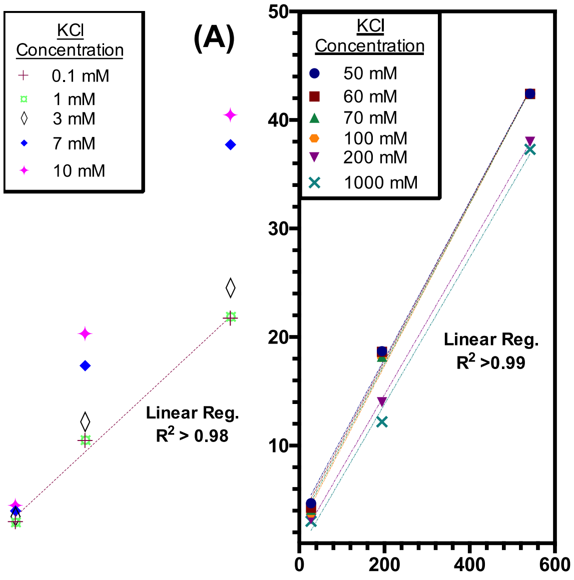


Figure 4(A-B). Brush height vs. PAA chain length, N at various KCl concentrations in the (A) OB regime and (B) SB regime, both at pH 7. The grafting densities are 2 kDa (σ=0.30 chains/nm^2), 14 KDa (σ=0.38 chains/nm^2), and 39 kDa (σ=0.45 chains/nm^2).

As shown in Equations 8 and 9, Israëls et al.[3] predict that for a strong, fully charged, polyelectrolyte (i.e., α= 1), the brush height should be independent of salt concentration in the OB regime and should scale to the -1/3 power in the SB regime. In the weak polyelectrolyte regime, however, where $\alpha \ll 1$, Zhulina et al. predicted that in the OB regime the charge density α on the chains should vary with grafting density and salt concentration as

$$\alpha \sim [\sigma^{-1}([H^+] + C_s)]^{2/3} \qquad [10]$$

where the sum of the external proton concentration [H+] and the external salt concentration (C_s) is the total ionic strength of the solution. Combining this with Equation 8 yields the scaling for the OB regime for weak polyelectrolytes:

$$H{\sim}N\sigma^{-\frac{1}{3}}([H^{+}]+C_{s})^{1/3} \qquad [11]$$

Figure 5 shows our measured PAA brush height vs. salt concentration for the short-chain (2 kDa) PAA at pH 7. It shows an approximate power law with respect to salt concentration, with +0.28-0.38 exponent in the low-salt (presumably OB) regime spanning 1-10 mM salt concentration at every grafting density except at σ >1 chain/ nm^2, where the brush is nearly fully extended. While this 0.28-0.38 power-law scaling disagrees with the theoretical prediction for a strong polyelectrolyte in the osmotic brush (OB) regime, it agrees well with the theoretical prediction of Zhulina et al.,[2] given by Equation 11, for weakly dissociating polyelectrolytes, and with what has been observed by others for other weakly dissociating systems at low salt concentration.[1,25,51,52] As noted earlier, at the lowest salt concentration investigated (0.1 mM), the swelling at all grafting densities is similar to that seen in pure water. In the high salt SB region, the power law exponent is around -0.30, very close to the value of -1/3 predicted in both strong and weak polyelectrolyte theories in the SB regime. At the highest salt concentration (1000 mM), there is an apparent leveling off of the height of the brushes, which agrees with what has been observed experimentally by others,[1,21,45] and can be attributed to a transition to the "neutral brush" (NB) regime where polymer charge is nearly completely screened out. The long-chain brush (39 kDa), at the single grafting density (σ = 0.45 chains/nm^2) studied, shows a similar dependence on salt concentration as the short-chain brush, with a scaling exponent of 0.31 in the OB regime and -0.30 in the SB regime (data not shown).

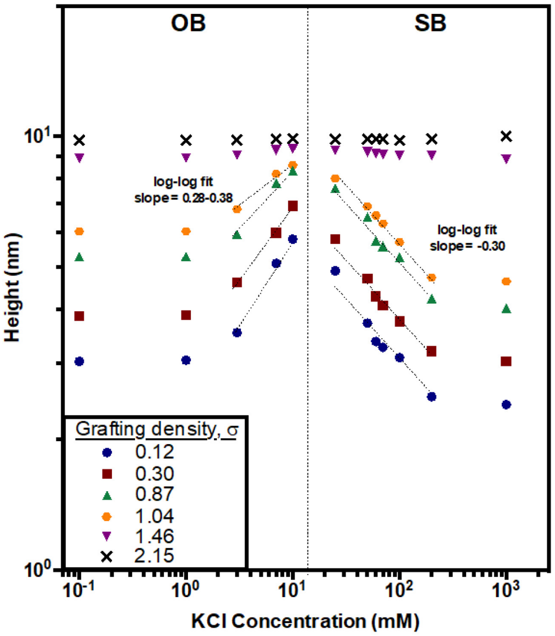


Figure 5. Short-chain (Mn=2 kDa) PAA brush height vs. KCl Concentration (mM) at various grafting densities σ (chains/nm^2) in the OB regime and SB regime at pH 7.

Figure 6(A-B) shows our measured PAA brush height vs. grafting density for the short-chain PAA (2 kDa) at pH 7. In the SB regime, Figure 6(B) shows that the brush follows the predicted dependence on σ to the power +1/3 of Israëls et al., until σ >1 chains/nm^2, where the exponent shifts to around 0.21. The +0.33 scaling at the more modest grafting densities also has been predicted by Pincus and colleagues[26] and demonstrated experimentally numerous times.[25,51,52] In the low-salt, OB regime, Figure 6(A) shows that at 10 mM, where maximal swelling is seen, the brush height scales weakly with σ to the 0.18 power, with this exponent shifting to 0.22 at 7 mM KCl, and to 0.31 at 0.1-3 mM KCl. While this disagrees with the theoretical prediction that the height should be either insensitive to σ in the OB regime for strongly ionized brushes (Equation 8), or should actually decrease with σ for weakly ionized brushes (Equation 11), our observation does agree with the +0.33 power-law scaling observed experimentally in the OB regime by Wu et al.[1] for a short PAA brush at pH 5.8 (slightly above the pKa). At this pH, close to the pKa (~ 5), the scaling of height with grafting density σ might be expected to follow the prediction of Zhulina et al.[2] for a weak, only slightly charged polyelectrolyte, which yields a -1/3 power law dependence on σ in the OB regime, but this is clearly not observed. Possibly, our chains and/or those of Wu et al. are either too highly charged, or too short, for the applicability of the scaling theories, which require that the chains have a charge fraction much less than unity (for weak polyelectrolytes) and the chain elasticity be Gaussian. In particular, our PAA chains (Mn=2 kDa) in Fig. 6(A) have heights ranging from 30% to 100% of maximum chain extension, which lies outside of the range of Gaussian elasticity.

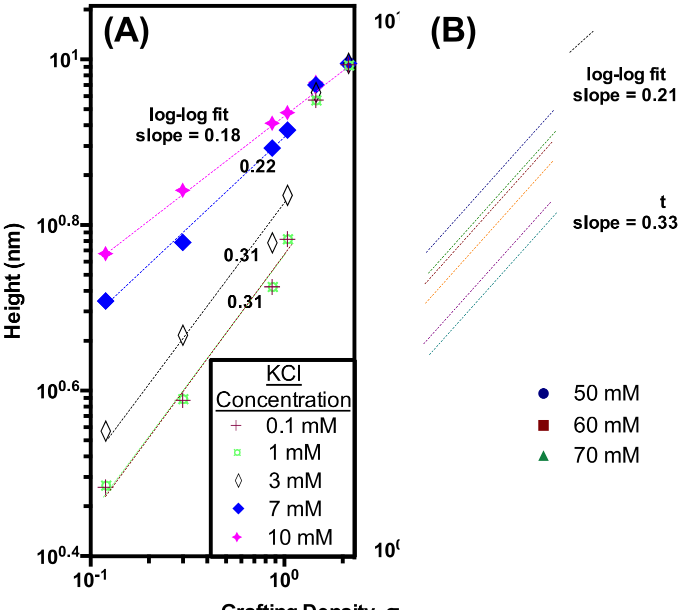


Figure 6(A-B). Short-chain (Mn=2 kDa) PAA brush height vs. grafting density at various KCl concentrations in the (A) OB regime and (B) SB regime, both at pH 7

We also note also that the scaling theories predict that the transition from the OB to the SB regimes should occur at a salt concentration of around $C_s^* \sim \sigma\alpha^{1/2}/a$, where is the fraction of charged monomers and a is the monomer radius.[5] This implies that C_s^* should depend on grafting density, but in our work the maximum in brush height remains at nearly the same salt concentration of 10 mM, regardless of grafting density, which varied from 0.12 to 2.15 chains/nm^2. Wu et al.[1] similarly found for PAA brushes little dependence of C_s^* on grafting density for pH = 4 or 5.8, but did see a roughly linear dependence on σ at pH = 10, where the polyelectrolyte should be virtually completely charged, and hence act as a strong polyelectrolyte. Consistent with this, Yu et al. in the Tirrell group report that at high grafting density of the strong polyelectrolyte PSS, the osmotic brush regime is extended to as high as 1 M, while at much lower grafting densities (around 0.01 chains/nm^2) the transition from OB to SB regimes occurs at around 0.01 M.[50] Strangely, in the work of Wu et al., the maximum in brush height occurred at $C_s^* \sim 0.10$ M for pH = 4 and 5.8, while we find $C_s^* \sim 0.01$ M for the same polymer (PAA), similar range of PH, and molecular weights that encompass that used by Wu et al.

Thus, while the available scaling theories for weak polyelectrolytes do predict a non-monotonic dependence of brush height on salt concentration, they fail to account for numerous other features observed in our work and that of our predecessors. A more detailed self-consistent field theory of Willott et al.[53] which allows for charge regulation, also shows the non-monotonic dependence of height on salt concentration and finds that the maximum in height is sensitive to the hydrophobic or hydrophilic character of the polymers, as well as its charge. However, this method has not yet considered the effect of grafting density of chain length on brush height, and so cannot at present be compared with experimental results. Szleifer and coworkers derived a theory for the brush thermodynamics based on a free energy functional that includes excluded volume, Poisson-

Boltzmann electrostatics, and acid-base equilibrium, all resolved spatially within the brush.[54] This theory provided semi-quantitative agreement with the measurements of brush height vs. salt concentration by Wu et al., but did not address the dependence of brush height on grafting density. The theory predicts that at pH = 10, the brush becomes fully charged, and the maximum in brush height at C_s^*, at the transition from OB to SB, therefore disappears. The brush height is also predicted to be independent of salt concentration in the OB regime for strong polyelectrolytes by the density functional theory of Jiang and Wu.[55] This prediction disagrees with the experiments of Wu et al., who showed the retention of this maximum even at pH = 10, and of course our experiments also show this maximum at pH = 9. A subsequent Monte Carlo by Szleifer and coworkers[56] predicts such a decrease in brush height at low salt by allowing counterion condensation controlled by a Manning parameter; this helps neutralize the brush when the pH is too high for charge regulation to do so. However, this leaves unexplained why in the surface forces experiments of Tirrell and coworkers[5] the strong polyelectrolyte PSS, the brush height remains constant at low salt concentration. Perhaps the Manning parameter in this case is outside of the range for which counterion condensation becomes strong at low ionic strength.

We can summarize the comparison of our work with those of others and with available theory, and suggest where more work is needed, by posing the following questions:

1. Why do we not see a decrease in brush height with increasing grafting density σ in the OB regime, as predicted by weak-polyelectrolyte theory?

2. Why at high pH does the weak polyelectrolyte PAA still show a maximum in brush height with increasing salt concentration? Is it due to counterion condensation at low salt?

3. Why is the maximum seen in ellipsometry and QCM-D experiments for NaPSS[21] but not in the surface forces results of Tirrell and coworkers[51]?

4. Why does C_s^*, the transition salt concentration from OB to SB regimes, seem usually to be independent of grafting density in weak polyelectrolytes, in disagreement with theory?

5. Over what range of pH values does PAA function as a “weak” polyelectrolyte, for which charge regulation as a function of salt concentration and grafting density is important?

Finally, we note that the study by Wu et al.[1] of a nominally short-chain PAA brush (Mn = 4 kDa), presented brush height data at pH 5.8 (above the pKa) as a %Change in Swelling produced by the presence of salt water over that in pure water, as opposed to the %Swelling reported in our present work. Plotted as %Change in Swelling, Wu et al. observed a power law against grafting density σ with exponent -1/3. Since our data on brush height versus grafting density are qualitatively similar to theirs, we would expect to get a similar scaling law when our data are plotted in this same form, and, indeed, in Appendix B, we show that such a power-law can be fit to our data. However, we show for both our set of data and that of Wu et al., the data are more accurately described as having two regimes, with a weak dependence on σ at small grafting densities σ, and a steep dependence at high σ.

Final Discussion and Conclusions

Swelling/de-swelling of a weakly dissociating PAA polyelectrolyte brush as a function of ionic strength of KCl between 0.1-1000 mM, at different pH values (3, 7, and 9), chain grafting densities (from 0.45 to 2.15 chains/nm^2), and chain molar masses (from M_n = 2 KDa to 39 KDa), was investigated using QCM-D, the results of which are summarized in Figures 2 & 3. The PAA brush was deposited directly onto a gold QCM-D sensor via self-assembly using thiol-gold interaction. The brushes are thin enough that the Sauerbrey thickness can be extracted from ΔFrequency and expressed as %Swelling relative to the dry brush.

In all highly ionized brushes above the pKa (pH 7 & 9), swelling is seen upon addition of low KCl concentrations with a maximum swelling at 10 mM, in the so-called osmotic brush (OB) regime,[1,2,46,47] followed by de-swelling with increased salt concentration, where the latter is a well-known result of charge screening predicted in the literature for the salted brush (SB) regime.[21,48,57,58] As expected, swelling at low ionic strength is greater in looser, less-dense brushes (σ = 0.12, 0.30, 0.87, 1.04) than in a very dense brush (σ = 1.46 & 2.15 chains/nm^2). We find maximum swelling for all chain lengths and grafting densities at around 10 mM salt, consistent with the work of Chu et al.,[21] Zhang and Rühe,[58] and Wu et al.,[1] all of whom found maximum swelling at around 10 mM for a strong polyelectrolyte brush of poly(sodium 4-styrenesulfonate) (NaPSS),[21] a "weak" polyelectrolyte brush of poly(methacrylic acid) (PMAA),[58] and a "weak" brush of PAA,[3] respectively. Oddly, in similar work by Wu et al. on PAA brushes, the maximum appeared at 100 mM for pH = 4 and 5.8, and at even higher salt concentrations at pH = 10. An insensitivity to grafting density of the salt concentration at which this apparent transition from OB to SB behavior occurs is not in accord with theory, which predicts a transition at a salt concentration that increases with grafting density.

We find that at pH 7, in the low-salt osmotic brush regime, the brush height scales with a +0.33 power at lower grafting densities with respect to grafting density. This disagrees strongly with the -1/3 power in the OB regime scaling theory of Zhulina et al. for weakly dissociating polyelectrolytes.[2] Noting that since the pKa of PAA is around 5, our brush at pH 7 and 9 is likely strongly ionized and hence effectively a "strong" polyelectrolyte, for which the theory of Zhulina et al. may not be applicable, since it assumes polyelectrolyte charge fractions << 1. For strongly dissociating polyelectrolytes, Israëls et al. predicted that the brush height in the OB regime should be independent of grafting density, which is closer to, but still in disagreement with, our exponent of 0.28-0.38. However, the theory for strongly dissociating polyelectrolytes predicts that brush height should be independent of salt concentration in the OB regime, contrary to our observation of a decrease in brush height with decreasing salt concentration in this regime. It is noteworthy that a decrease in brush height with decreasing salt concentration in the OB regime was observed even for strong polyelectrolytes, including NaPSS, by Chu et al. using ellipsometry and QCM-D. (However, NaPSS studies in the surface forces apparatus by Balastre et al. a showed salt-independent brush height in the OB regime.) A Monte Carlo of Szleifer and coworkers[56] suggests that this decrease in brush height at low salt concentration for highly charged polyelectrolytes results from counterion condensation controlled by the Manning parameter.

In the SB regime, our results agree with the prediction by Israëls et al.[3] and Pincus[26] that the brush height H should depend on grafting density σ and chain length N as $H \sim N\sigma^{+1/3}$, which is also shown by numerous experiments.[25,51,52] While our findings of the dependencies of brush height and swelling on grafting densities partially disagree with scaling theory in the OB regime, we do find the predicted linear dependence of brush height on chain length N in both the OB and SB regimes.

The discrepancies between experimental results and theory explored here should motivate additional experimental work closer to conditions for which weak polyelectrolyte theory is most applicable, namely long chains, at lower grafting densities, and additional pH values near the pH at which the chain becomes neutral. In addition, computer simulations and self-consistent field theory should also explore a similarly wider range of conditions than has been carried out heretofore.

Acknowledgments

The research presented here was supported by the National Science Foundation under Grant No. 1707640. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of NSF. The authors also acknowledge the Dekaban Fund Postdoctoral Fellowship to S.W. through the College of Engineering at the University of Michigan- Ann Arbor. This work was performed in part at the University of Michigan Lurie Nanofabrication Facility (LNF) — special thanks are given to Brian Armstrong for assistance with VASE measurements.

References

1 T. Wu, P. Gong, I. Szleifer, P. Vlček, V. Šubr and J. Genzer, *Macromolecules*, 2007, **40**, 8756–8764.
2 E. B. Zhulina, T. M. Birshtein and O. V. Borisov, *Macromolecules*, 1995, **28**, 1491–1499.
3 R. Israels, J. F. A. M. Leermakers, J. Fleer and E. B. Zhulina, *Macromolecules*, 1994, **27**, 3249–3261.
4 D. V. Andreeva and D. G. Shchukin, *Mater. Today*, 2008, **11**, 24–30.
5 N. Saleh, T. Sarbu, K. Sirk, G. V Lowry, K. Matyjaszewski and R. D. Tilton, *Langmuir*, 2005, **21**, 9873–9878.
6 N. Su and Na, *Polymers (Basel).*, 2015, **7**, 1599–1616.

7 G. Liu, M. Cai, X. Wang, F. Zhou and W. Liu, *ACS Appl. Mater. Interfaces*, 2014, **6**, 11625–11632.

8 S. P. Adiga and D. W. Brenner, *J. Funct. Biomater.*, 2012, **3**, 239–56.

9 M. Motornov, K. Tam, M. Pita, I. Tokarev, E. Katz and S. Minko, *IOP Publ. Nanotechnol. Nanotechnol.*, 2009, **20**, 434006–434016.

10 J.-Y. Lin, C.-Y. Lin, J.-P. Hsu and S. Tseng, *Anal. Chem.*, 2016, **88**, 1176–1187.

11 S. Das, M. Banik, G. Chen, S. Sinha and R. Mukherjee, *Soft Matter*, 2015, **11**, 8550–8583.

12 J. Klein, E. Kumacheva, D. Mahalu, D. Perahia and L. J. Fetters, *Nature*, 1994, **370**, 634–636.

13 A. Halperin and D. E. Leckband, *Comptes Rendus l'Académie des Sci. - Ser. IV - Phys.*, 2000, **1**, 1171–1178.

14 M. Krishnamoorthy, S. Hakobyan, M. Ramstedt and J. E. Gautrot, *Chem. Rev.*, 2014, **114**, 10976–11026.

15 O. Azzaroni, S. Moya, T. Farhan, A. A. Brown and W. T. S. Huck, *Macromolecules*, 2005, **38**, 10192–10199.

16 M. Chen, W. H. Briscoe, S. P. Armes, H. Cohen and J. Klein, *Eur. Polym. J.*, 2011, **47**, 511–523.

17 A. Kusumo, L. Bombalski, Q. Lin, K. Matyjaszewski, J. W. Schneider and R. D. Tilton, *Langmuir*, 2007, **23**, 4448–4454.

18 G. Sudre, D. Hourdet, C. Creton, F. Cousin and Y. Tran, *Macromol. Chem. Phys.*, 2013, **214**, 2882–2890.

19 M. P. Weir and A. J. Parnell, *Polymers (Basel).*, 2011, **3**, 2107–2132.

20 E. S. Dehghani, V. V. Naik, J. Mandal, N. D. Spencer and E. M. Benetti, *Macromolecules*, 2017, **50**, 2495–2503.

21 X. Chu, J. Yang, G. Liu and J. Zhao, *Soft Matter*, 2014, **10**, 5568–5578.

22 Z. Zhang, M. Moxey, A. Alswieleh, A. J. Morse, A. L. Lewis, M. Geoghegan and G. J. Leggett, *Langmuir*, 2016, **32**, 5048–5057.

23 H. Sakata, M. Kobayashi, H. Otsuka and A. Takahara, *Polym. J.*, 2005, **37**, 767–775.

24 V. Mazzini, G. Liu and V. S. J. Craig, *J. Chem. Phys.*, 2018, **148**, 222805.

25 F. Li, M. Balastre, P. Schorr, J.-F. Argillier, J. Yang, J. W. Mays and M. Tirrell, *Langmuir*, 2006, **22**, 4084–4091.

26 P. Pincus, *Macromolecules*, 1991, **24**, 2912–2919.

27 K. Sadman, C. G. Wiener, R. A. Weiss, C. C. White, K. R. Shull and B. D. Vogt, *Anal. Chem.*, 2018, **90**, 4079−4088.

28 G. C. Denolf, L. F. Sturdy and K. R. Shull, *Langmuir*, 2014, **30**, 9731−9740.

29 J. T. O'Neal, E. Y. Dai, Y. Zhang, K. B. Clark, K. G. Wilcox, I. M. George, N. E. Ramasamy, D. Enriquez, P. Batys, M. Sammalkorpi and J. L. Lutkenhaus, *Langmuir*, 2018, **34**, 999–1009.

30 R. Kou, J. Zhang, T. Wang and G. Liu, *Langmuir*, 2015, **31**, 10461–10468.

31 P. Zhuang, A. Dirani, K. Glinel and A. M. Jonas, *Langmuir*, 2016, **32**, 3433–3444.

32 B. Almeida and A. Shukla, *J Biomed Mater Res Part A J Biomed Mater Res Part A*, 2017, **105A**, 464–474.

33 E. Bittrich, K. B. Rodenhausen, K.-J. Eichhorn, T. Hofmann, M. Schubert, M. Stamm and P. Uhlmann, *Biointerphases*, 2010, **5**, 159–167.

34 H. G. Tompkins, *A User's Guide to Ellipsometry.*, Elsevier Science, 1992.

35 C. M. Herzinger, H. Yao, P. G. Snyder, F. G. Celii, Y. -C. Kao, B. Johs and J. A. Woollam, *J. Appl. Phys.*, 1995, **77**, 4677–4687.

36 M. C. Dixon, *J. Biomol. Tech.*, 2008, **19**, 151–8.

37 G. Liu and G. Zhang, in *QCM-D Studies on Polymer Behavior at Interfaces*, Springer Science & Business Media, 2013, pp. 1–8.

38 A. Alassi, M. Benammar and D. Brett, *Sensors*, 2017, **17**, 2799.

39 V. M. Mecea, *Anal. Lett.*, 2005, **38**, 753–767.

40 X. Zhang, S. S. Chu, J. R. Ho and C. P. Grigoropoulos, *Appl. Phys. A Mater. Sci. Process.*, 1997, **64**, 545–552.

41 G. Sauerbrey, *Zeitschrift für Phys.*, 1959, **155**, 206–222.

42 T. Zhou, H. Qi, L. Han, D. Barbash and C. Y. Li, *Nat. Commun.*, 2016, **7**, 11119.

43 L. C. H. Moh, M. D. Losego and P. V. Braun, *Langmuir*, 2011, **27**, 3698–3702.

44 J. Genzer, K. Efimenko and D. A.Fischer, *Langmuir*, 2002, **18**, 9307–9311.

45 E. P. K. Currie, A. B. Sieval, G. J. Fleer and M. A. Cohen Stuart, *Langmuir*, 2000, **16**, 8324–8333.

46 M. Geoghegan, *Polymer (Guildf).*, 2017, **112**, 414–417.

47 J. D. Willott, T. J. Murdoch, F. A. M. Leermakers and W. M. de Vos, *Macromolecules*, 2018, **51**, 1198–1206.

48 J. A. V. Butler, B. E. Conway and D. W. F. James, *Trans. Faraday Soc.*, 1954, **50**, 612.

49 Z. Adamczyk, A. Bratek, B. Jachimska, T. Jasiński and P. Warszyński, *J. Phys. Chem. B*, 2006, **110**, 22426–22435.

50 J. Yu, J. Mao, G. Yuan, S. Satija, W. Chen and M. Tirrell, *Polymer (Guildf).*, 2016, **98**, 448–453.

51 M. Balastre, F. Li, P. Schorr, J. Yang, J. W. Mays and M. V. Tirrell, *Macromolecules*, 2002, **35**, 9480–9486.

52 T. Jiang, Z. Li and J. Wu, *Macromolecules*, 2007, **40**, 334–343.

53 J. D. Willott, B. A. Humphreys, G. B. Webber, E. J. Wanless and W. M. de Vos, *Langmuir*, 2019, **35**, 2709–2718.

54 R. J. Nap, M. Tagliazucchi and I. Szleifer, *J. Chem. Phys.*, 2014, **140**, 024910.

55 T. Jiang and J. Wu, *J. Phys. Chem. B*, 2008, **112**, 7713–7720.

56 O. J. Hehmeyer, G. Arya, A. Z. Panagiotopoulos and I. Szleifer, *J. Chem. Phys.*, 2007, **126**, 244902.

57 A. L. P. Fernandes, R. R. Martins, C. G. Da, T. Neto, M. R. Pereira and J. L. C. Fonseca, *J Appl Polym Sci*, 2003, **89**, 191–196.

58 H. Zhang and J. Rühe, *Macromolecules*, 2005, **38**, 4855–4860.

59 K. K. Kanazawa and J. G. Gordon, *Anal. Chem.*, 1985, **57**, 1770–1771.

Appendix A

Quartz Crystal Microbalance with Dissipation (QCM-D) – Effect of Overlying Solvent

Appendix Figure A1(A) from our work shows that the change in dissipation at the third overtone (n=3, F_0 =5 MHz), $\Delta D(bare\ crystal)$, with salt concentration $for\ a\ blank\ crystal$, with no added brush, is almost identical to the results of Zhang et al., for a poly(zwitterionic) brush composed of poly(2-(methacryloyloxy)ethyl phophorylcholine), or PMPC, in the presence of $CaCl_2$ and LiCl.[22] Furthermore, $\Delta D(bare\ crystal)$ data are plotted on a linear scale (Figure A1(B)) instead of the semi-log axis published in their work,[22] a linear dependence on salt concentration is evident, and shows that this extends up to much higher salt concentration than covered by Zhang et al. The effect of salt on $\Delta D(polymer\ coated)$ in the presence of a PAA brush (with M_n=2,000 g/mol, at pH = 7 from our studies) at the highest grafting density (σ=2.15 chains/nm^2) is also shown in Figure A1(B) as filled symbols. It is evident that the QCM-D response in the presence of the PAA brush closely resembles that obtained for a poly(zwitterionic) brush by Zhang et al. in the case of $CaCl_2$, both of which are similar to the response of a bare substrate. It is thus apparent that much of the QCM-D response is merely due to the change in flow medium, and, moreover, is similar to the trend in solution mass density, as shown in Figure A1(C), as well as solution viscosity in accordance with concentration and the Hofmeister series as previously predicted theoretically by Kanazawa and Gordon.[59] The densities of all of the salt solutions tested were confirmed gravimetrically and are given in Figure A1(C), which shows the same trend with salt type and concentration as shown by the QCM-D response in Figure A1(B). Thus, $\Delta D(polymer\ coated)$ is not much different from $\Delta D(bare\ crystal)$, and reflects the influence of the density and viscosity of the salt solution more than it does the presence of the brush, at least for $CaCl_2$ and LiCl.

Figure A1(B) shows a greater difference between the response of the brush and a blank crystal for KCl and NaCl than for $CaCl_2$. Figure A1(D) enlarges these data for the influence of KCl for the short-chain PAA brush (M_n= 2,000 Da) and adds similar data at other grafting densities at neutral pH 7. From this plot, it is evident that the influence of KCl salt solution on the QCM-D response on the PAA brush is sufficiently distinct from its influence on the bare crystal to allow the influence of the PAA brush to be determined clearly by subtraction, i.e., by taking $\Delta D(polymer\ coated) - \Delta D(bare\ crystal)$. Thus, hereafter, we focus only on KCl. Thus, in the main text, $\Delta D_{Swelling} \equiv \Delta D(polymer\ coated) - \Delta D(bare\ crystal)$ is used to signify the relative change in ΔD from the dry brush in air to the wet brush using Equation 1). %Swelling from a dry brush in air was found under the same conditions, determined by from $\Delta \mathrm{F}_{Swelling} \equiv \mathrm{F}(polymer\ coated) - \Delta \mathrm{F}(bare\ crystal)$, using QCM-D measurements on both blank and brush-coated surfaces, and converted to %Swelling, using Equations 2 and 3 to convert $\Delta \mathrm{F}_{swelling}$ to $\Delta \mathrm{d}_{swelling}$ and finally obtaining %Swelling from the ratio of $\Delta \mathrm{d}_{swelling}$ to $d_{brush,dry}$ (Equation 5), using VASE measurements to determine the dry brush thickness.

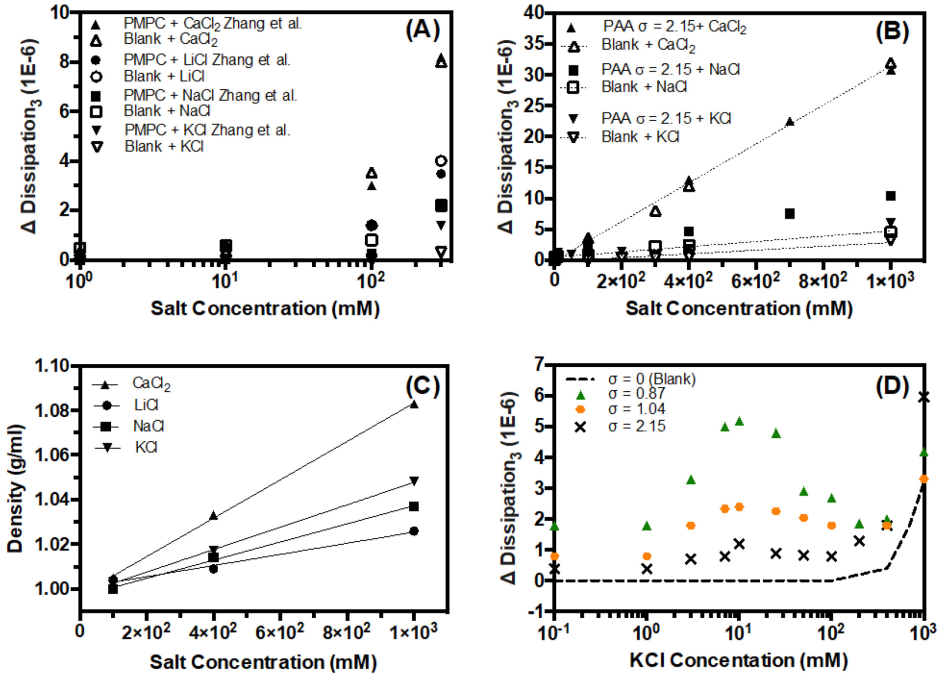


Figure A1 **(A)** Change in dissipation $\Delta D(bare\ crystal) = \Delta D_{Solvent}$ as a function of salt concentration for various salts on a blank gold crystal from our measurements and $\Delta D(polymer\ coated)$ on a PMPC-grafted brush on a gold crystal (the latter from Zhang et al.). Note that some data points are obscured by overlap, such as the open square covered by a filled square at the highest salt concentration. **(B)** Corresponding $\Delta D_{Solvent}$ data for a blank gold crystal, compared to $\Delta D(polymer\ coated)$ data from our study for the densely PAA-grafted surfaced (σ=2.15). Note the difference in scale on the x- and y-axes, relative to (A). **(C)** Densities of KCl solutions at pH 7 obtained gravimetrically. **(D)** Change in dissipation $\Delta D(bare\ crystal) = (\Delta D_{Solvent})$ as a function of salt concentration for a blank crystal and $\Delta D(polymer\ coated)$ for various grafting densities σ of PAA (Mn= 2,000 g/mol) at pH 7. Error bars are comparable in size to the symbols and are therefore not shown on these or following graphs.

Appendix B

Comparison to previous experimental work of Wu et al. – Replotting data as %Change in Swelling

As discussed in the main text, Wu et al.[1] defined a %Change in Swelling, which is here given by Equation B1, and plotted this against grafting density σ. In our main text, we instead plotted as %Swelling, and here we wish to compare our data, converted to %Change in Swelling, against that of Wu et al. Our %Swelling is the inferred thickness of the wet brush in salt water normalized by that of the dry brush. The resulting dependence of this *relative* height increase determined by Wu et al. on grafting density in the OB regime is similar to that predicted theoretically for the *absolute height H* for weakly dissociating brushes by Zhulina et al.[2] We therefore recalculated our data at pH 7 in the OB regime in the way Wu et al. did as %Change in Swelling, and our results are shown below in Figure B1. It can be seen that for this short-chain PAA brush, there is very little dependence of the grafting density on the %Change in Swelling at the lowest grafting densities, but this is followed by a steep decline for σ>1 chain/nm^2, at roughly at the same salt concentration at which the 0.31 power law dependence of height on grafting density switches to a power law closer to 0.20 (see Figure 6(A) and 6(B)). Figure B1 demonstrates that a power law exponent of -0.35, close to the value of -0.32 reported by Wu et al. at pH 5.8, can be obtained by extending the range over which a power law is fit to the data; however, this does not represent well the actual behavior.

$$\%\text{ Change in Swelling} = \frac{d_{\text{brush,KCl}} - d_{\text{brush,pure water}}}{d_{\text{brush,pure water}}} x\ 100 \qquad [\text{B1}]$$

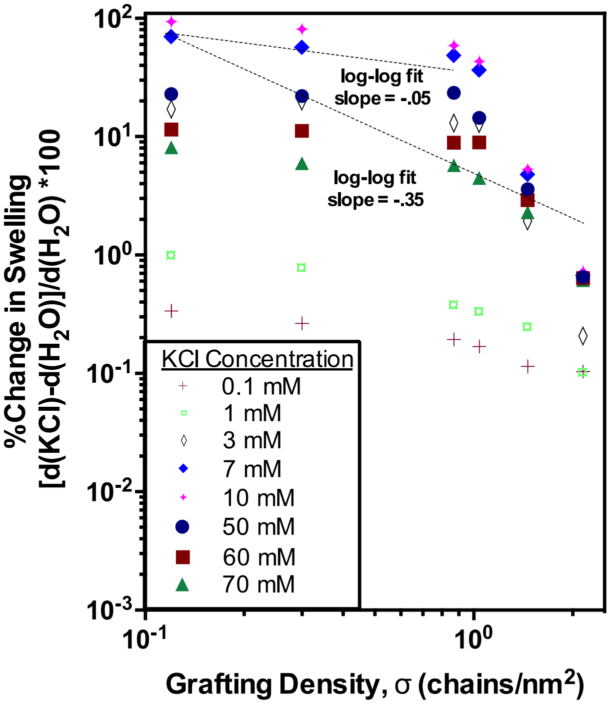
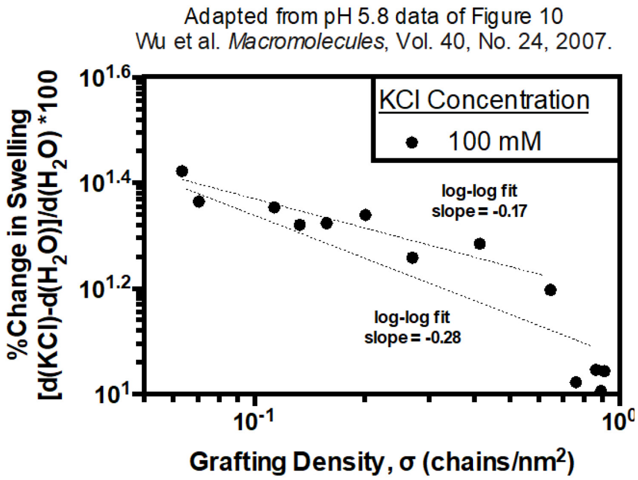


Figure B1. %Change in Swelling (as defined by Wu et al.[1]) vs. grafting density for our short-chain (Mn= 2 kDa) PAA at various KCl concentrations in the OB regime at pH 7

To examine their data more closely we digitized the data of Wu et al. at pH 5.8, from the brush height data from Figure 10 of Wu et al. When replotted on log-log axes in Figure B2 as %Change in Swelling, their results show good agreement with our data. To plot the data in this way, the height at the lowest ionic strength (roughly 10^{-6} M) was taken to be the "zero-salt" value. We note that for a pH of 5.8, the minimum ionic strength, with no added salt, is $10^{-5.8}$ M in [H^+] alone, which makes KCl concentration of 10^{-6} M, if indeed it can be accurately controlled to such a small value, roughly equivalent to zero salt. (In our study, the minimum KCl concentration is 0.1 mM, or 10^{-4} M.) In both our data and the data of Wu et al., we see two regimes; at low grafting densities, there is very little dependence of swelling on grafting density, but as σ approaches 1 chain/nm^2, the %Change in Swelling induced by salt decreases drastically. It can also be seen from Figure B2 that if the data of Wu et al. are forced to fit to a power law over all grafted densities, then an exponent of -0.28 is obtained, which is similar to the exponent reported in their work of -0.32. However, the data are better represented by two regimes – with weak dependence of swelling on grafting density at low grafting densities, and a steep drop-off at higher densities. This transition to a steeper dependence seems to be magnified when the brush becomes more ionized: At pH 10 in the results of Wu et al., there is an almost negligible effect of grafting density on %Change in Swelling at lower grafting densities, followed by a dramatic decrease as σ approaches 1 chain/nm^2. This result from their work at pH 10 is similar to our results at pH 7, where we note that our brush is highly ionized at pH 7, as theirs is at pH 10. We cannot meaningfully compare our results at pH 7 with theirs at pH 5.8, since the pKa of a PAA brush is ~5, meaning that our brush is almost 16*x* more ionized than theirs.



Adapted from pH 5.8 data of Figure 10
Wu et al. *Macromolecules*, Vol. 40, No. 24, 2007.

Figure B2. Brush height data reported by Wu et al.[1] replotted as %Change in Swelling for a nominally 4 kDa PAA brush at pH 5.8.