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Structural behavior of competitive temperature and pH-responsive tethered polymer layers[†]

Simona Morochnik^a, Rikkert J. Nap^b, Guillermo A. Ameer^a, and Igal Szleifer^b

^aDepartment of Biological Sciences, Department of Biomedical Engineering, and Chemistry of Life Processes Institute, Northwestern University, Evanston, Illinois, USA

^bDepartment of Biomedical Engineering, Department of Chemistry, and Chemistry of Life Processes Institute, Northwestern University, Evanston, Illinois, USA

Abstract

Herein, we develop a molecular theory to examine a class of pH and temperature-responsive tethered polymer layers. The response of pH depends on intramolecular charge repulsion of weakly acidic monomers and the response of temperature depends on hydrogen bonding between polymer monomers and water molecules akin to the behavior of water-soluble polymers such as PEG (poly-ethylene glycol) or NIPAAm (n-isopropylacrylamide). We investigate the changes in structural behavior that result for various end-tethered copolymers: pH/T responsive monomers alone, in alternating sequence with hydrophobic monomers, and as 50/50 diblocks with hydrophobic monomers. We find that sequence and location of hydrophobic units play a critical role in thermodynamic stability and structural behavior of these responsive polymer layers. Additionally, polymers exhibit tunable collapse when varying surface coverage, location and sequence of hydrophobic units as a function of temperature and pH. As far as we know, our results present the first molecularly detailed theory for end-tethered polymers that are both pH and temperature-responsive via hydrogen bonding. We propose this work holds predictive power for the guided design of future biomaterials.

I. Introduction

Responsive and tunable, “smart” biomaterials are becoming increasingly prevalent as researchers aim to harness their many properties to advance applications ranging from tissue regeneration to molecular sensing to drug delivery.^{1–3} Once a field that contained sparingly few materials, it has grown to encompass not only myriads of novel biopolymers but also novel biocomposites. For example, it includes the composites of pAA (poly-acrylic acid) and PEG (poly-ethylene glycol) or PMA (polymethacrylate) and alginate.^{4, 5} We consider composites of increasing complexity such as those developed in the Ameer group involving hydrophobic linkers with temperature responsive polymers of NIPAAm (n-isopropyl acrylamide), PEG, and pH-responsive citric acid.⁶ These polymers have exciting biological

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Correspondence to: Igal Szleifer.

applications because they exhibit inverse solubility upon heating, and in the case of NIPAAm, gelate at physiological temperatures to allow for controlled release of growth factors or drugs.^{7, 8} While many studies have been carried out on pH or temperature-responsive polymers experimentally, fewer studies have been carried out theoretically and fewer still with sufficient molecular detail to predict polymeric behavior.⁹⁻¹⁷ Furthermore, little is known about how these materials behave when their sequence is changed or across different micro-environments with varying ion concentrations, pHs, and temperatures. In light of this, we use these biocomposites as inspiration to develop a novel theory to investigate polymer layers that are both thermoresponsive and pH-sensitive.

It is well known that pH-responsive weak polyelectrolytes such as pAA swell with increased pH due to their ability to acquire charge.¹⁸⁻²³ Likewise, temperature-responsive polymers like PEG uniquely swell at decreased temperatures due to increased hydrogen bonding between monomer units and water.²⁴ However, what happens when temperature and pH increase or decrease together, when hydrophobic units are added or sequence is altered? In this paper, we develop a molecular theory to study pH and temperature sensitive polymers. It is based in a molecular theory that has been successfully used in the past to describe various polymeric systems and has been shown to be in quantitative agreement with experiments as well as simulations. The theory explicitly incorporates the size, shape, polymer conformations, charge, charge distribution, and the chemical state of all the species in the system. The methodology is based in writing the total free energy of the system in terms of the density distribution of all molecular species and the probability distribution functions of polymer conformations.²⁵ After free energy minimization, the output of the theory includes the thermodynamic and conformational properties of the system for the given set of control conditions, such as pH, temperature, and ionic strength among others. Therefore, we can use the information from the theory to predict the optimal state for the conditions of interest for a given molecular composition.

To develop this molecular theory, we combine and generalize two prior developed molecular approaches. The first approach is the theory of pH-responsive end-tethered poly-acrylic acid (pAA) developed by Gong et al. that explicitly included the acid-base equilibrium of polyacid COOH groups.²⁶⁻²⁸ Most notably in this approach, the charge distribution is not imposed but follows from the free energy minimization. The theoretical predictions from the model were found to be in good agreement with experimental measurements for the height of tethered PAA layers as well as for the conduction of polyelectrolyte modifying nanopores and apparent pKa (degree of charge) of acidic ligated gold nanoparticles.²⁹⁻³¹ The ability of the theory to properly predict various aspects of end-tethered polyelectrolytes indicates that this approach contains the correct physical and chemical interactions to describe pH-responsive polyelectrolyte layers. We use it in this work to model pH responsiveness.

The second approach is the theory of temperature-responsive PEG. In 2002, Dormidontova developed a model to describe the solution phase-behavior of PEG that was in qualitative agreement with experiments.³² Dormidontova's work underscored the importance of including polymer-water as well as water-water hydrogen bonds in order to obtain *quantitative* agreement with experiments, enabling her to accurately predict closed-loop regions of phase coexistence of PEG solutions. The work of Ren et al. extends this model to

include conformational details, allowing for a molecular description of tethered PEG layers.³³ In this paper, we combine and build upon the theories of pAA and PEG tethered layers to provide novel theoretical predictions for dual-responsive polymer layers of pAA/PEG with and without hydrophobic units.

Our aim is to establish a theoretical foundation to study pH and temperature responsive systems and ultimately apply this theory to gels. Because gels are highly complex, both molecularly and computationally, we set out to first present a theory of tethered polymer layers. In doing so, we hope to begin to illuminate molecular-level behavior for systems that are representative of the increasingly complex polymers used in biomedical applications today.

In the following sections, we first present the theoretical approach. This outlines the model and the thermodynamic contributions to the free energy. We then present pertinent examples to highlight the importance of the coupling between thermal and pH responsiveness. We end with a discussion of these results and perspectives for how this work will inform future research.

II. Theoretical Approach

In this paper, we consider a system of N_p polymers covalently end-tethered to a planar surface of area A . The polymers are laterally homogenous, thus, we only consider polymer swelling and collapse in the inhomogeneous, z -direction perpendicular to the surface. The system is in equilibrium with a surrounding aqueous solution that contains water (w), protons (H^+), hydroxyl ions (OH^-) and dissociated salt. Because each polymer contains monomer units that are pH as well as temperature-responsive (denoted as “A”), our system is sensitive to both electrostatic and hydrogen bonding interactions. We later add monomers that are neutral hydrophobic, denoted as “B”. To model this system, we use a molecular approach that will be detailed here.

The total Helmholtz free energy of the system has the following contributions

$$F = -TS_{\text{conf}} + F_{\text{chm}} + F_{HB} - TS_{\text{mix}} + F_{\text{elect}} + U_{\text{vdw}}, \quad (1)$$

where the terms represent the conformational entropy of polymer chains, the chemical free energy of acidic monomers, the associative free energy of hydrogen bonding, the mixing entropy of free species, the electrostatic energy, and the Van der Waals interaction energy between monomers, respectively. The first three terms will be expanded below as they are particularly significant to this approach. The latter three terms are expanded in the supplementary information.

The first term, representing the conformational entropy, is expressed per unit area as

$$-\frac{S_{\text{conf}}}{k_B A} = \sigma \sum_{\alpha} P(\alpha) \ln P(\alpha), \quad (2)$$

where $\sigma = \frac{N_p}{A}$ is the surface coverage, or number of chains per unit area. $P(\alpha)$ is the probability of finding the chain in conformation α . This probability distribution function (pdf) is essential in describing various thermodynamic and structural quantities like the monomer number density $\langle \rho_i(z) \rangle$, which in turn is related to the local volume fraction of monomers at position z , $\langle \phi_i(z) \rangle$

$$\langle \rho_i(z) \rangle = \sigma_p \sum_{\alpha} P(\alpha) n_i(\alpha, z), \quad (3)$$

$$\langle \Phi_p(z) \rangle = \langle \rho_A(z) \rangle \nu_A + \langle \rho_B(z) \rangle \nu_B, \quad (4)$$

where $\langle \dots \rangle$ denotes ensemble average over α . $n_i(\alpha; z) dz$ is the number of polymer segments of type i that a chain in conformation α has between position z and $z + dz$. The index i refers to the monomer type, i A or B monomers. The total local volume fraction of monomers $\langle \phi_p(z) \rangle$ is the sum of the volume fractions of the individual monomer types, A and B. ν_A and ν_B denote volume of the A or the B monomers, respectively.

The second term describes the chemical free energy that accounts for the acid-base equilibrium of the weakly acidic (A) monomers ($AH \rightleftharpoons A^- + H^+$). It is given by

$$\begin{aligned} \frac{\beta F_{\text{chm}}}{A} = & \int dz \langle \rho_A(z) \rangle f_d(z) (\ln f_d(z) + \beta \mu_{A^-}^0) + \int dz \langle \rho_A(z) \rangle (1 \\ & - f_d(z)) [\ln(1 - f_d(z)) + \beta \mu_{AH}^0] \\ & + \int dz (\rho_{H^+}(z) \beta \mu_{H^+}^0 \\ & + \rho_{OH^-}(z) \beta \mu_{OH^-}^0), \end{aligned} \quad (5)$$

Here $f_d(z)$ denotes the local degree of dissociation. The first term is the entropy of mixing of the dissociated state along with the standard chemical potential of the dissociated, charged acidic groups. The second term is the entropy of mixing of the non-dissociated state along with the standard chemical potential of the non-dissociated protonated groups. The last term is included to account for self-ionization of water. Here, $\mu_{H^+}^0$ and $\mu_{OH^-}^0$ are the standard chemical potentials of protons and hydroxyl ions, respectively.

The third term, describing the hydrogen bond formation, builds on the work of Dormidontova for PEG solutions which was applied by Ren et al. to PEG tethered layers. Importantly, we consider not only the possibility of hydrogen bonds between A monomers and water molecules but also between water-water molecules. This contribution is given by

$$\begin{aligned}
 \frac{\beta F_{\text{HB}}}{A} = & \int dz 2 \langle \rho_A(z) \rangle [x_p(z) \ln x_p(z) \\
 & + (1 - x_p(z)) \ln(1 - x_p(z)) \\
 & + x_p(z) \beta \Delta F_p] \\
 & + \int dz 2 \rho_w(z) [x_w(z) \ln x_w(z) \\
 & + (1 - x_w(z)) \ln(1 - x_w(z)) \\
 & + x_w(z) \beta \Delta F_w] \\
 & + \int dz 2 \rho_w(z) \left[1 - x_w(z) - x_p(z) \frac{\langle \rho_A(z) \rangle}{\rho_w(z)} \right] \ln \left[1 - x_w(z) - x_p(z) \frac{\langle \rho_A(z) \rangle}{\rho_w(z)} \right] \\
 & - \int dz 2 \rho_w(z) \left[x_w(z) + x_p(z) \frac{\langle \rho_A(z) \rangle}{\rho_w(z)} \right] \ln \frac{2 \rho_w(z) \nu_w}{e},
 \end{aligned} \tag{6}$$

where $x_p(z)$ is the local fraction of polymer-water hydrogen bonds for A monomers and $x_w(z)$ is the local fraction of water-water hydrogen bonds. The first line corresponds to the mixing entropy contribution associated with forming monomer-water hydrogen bond pairs plus the free energy gain associated with the formation of this bond (F_p). The next line is identical and describes the mixing entropy associated with having a water-water hydrogen bond pair plus the free energy gain associated with the formation of this bond (F_w). The last lines contain a necessary correction for the change that results upon formation of a hydrogen bond, as it reduces the number of available hydrogen bond sites. In each associative free energy term written, $\beta F_i = \beta E_i - S_i/k_B$. Here, E_i is the enthalpic change of forming a hydrogen bond and S_i is the entropic change of forming the hydrogen bond for either $i = p$ (polymer-water) or $i = w$ (water-water) hydrogen bonds. We set these

terms to $\beta \Delta F_p = -\frac{2000 \text{ K}}{T} + 3.35$ and $\beta \Delta F_w = -\frac{1800 \text{ K}}{T} + 2.25$. These are prescribed values that reflect experimental and simulation results for hydrogen bonding specific to PEG derived by Dormidontova in ref. 16. For the temperature range tested, both βF_p and βF_w

are negative and hydrogen bond formation is preferred as it lowers the free energy. As the B monomers are neutral and do not participate in hydrogen bonding, they are not included in the second and third terms.

The total free energy (eq. 1) is minimized under the constraints of incompressibility and the condition that the system is in contact with a bath of anions, cations, protons, and hydroxyls. This yields the semi-grand thermodynamic potential, W , which is minimized with respect to $P(\alpha)$, $\rho_p(z)$, $f_d(z)$, $x_w(z)$, and $x_p(z)$ and varied with respect to the electrostatic potential $\psi(z)$. Minimization with respect to $P(\alpha)$ gives the probability of a given polymer chain conformation, or the pdf (probability distribution function)

$$P(\alpha) = \frac{1}{q} \exp \left[-\beta \int dz \pi(z) n_A(\alpha; z) \nu_p - \frac{\beta \chi_A}{\nu_w} \int dz \langle \phi_p(z) \rangle n_A(\alpha; z) \nu_p - \int dz n_A(\alpha; z) (\beta \psi(z) q_p + \ln f_d(z)) - 2 \int dz n_A(\alpha; z) \ln(1 - x_p(z)) \right] \times \exp \left[-\beta \int dz \pi(z) n_B(\alpha; z) \nu_p \right] \quad (7)$$

where the two exponential terms represent the pdf of A and B monomers. The A exponential is composed of repulsive, Van der Waals, electrostatic, acid-base equilibrium, and hydrogen bonding interactions. As the B monomers are neutral hydrophobic, the B exponential is composed of only repulsive and Van der Waals interactions. q ensures normalization of $P(\alpha)$ and $\pi(z)$ are the Lagrange multipliers that enforces the incompressibility constraint.

After these minimizations, we are left with a set of equations containing 4 unknowns: the Lagrange multipliers $\pi(z)$, the electrostatic potential $\psi(z)$ and the fraction of hydrogen bonds between polymer-water $x_p(z)$ and water-water $x_w(z)$ molecules. Expressions for each of these unknowns are found by explicitly introducing the pdf (eq. 7), the polymer volume fraction (eqs. 3-4), the density profiles of the solvent (eq. S.10) and ions (eq. S.8), into the packing constraint (eq. 12), the Poisson equation (eq. S.13) and the ‘chemical’ reaction equations for the fraction of polymer-water and water-water hydrogen bonds (eqs. S.11-S.12) yielding a non-linear integro-differential equation that can be discretized and solved numerically.³⁴ Further details about the methodology can be found in the supplementary information and cited references.^{†,††} The necessary input to solve these equations includes the bulk pH and salt concentration, polymer pK_a , surface coverage (σ), the free energies for formation of hydrogen bonds between polymer-water (F_p) and water-water (F_w), the interaction parameter χ for each monomer type, and the set of conformations of polymer chains. Specifically, a set of 5×10^5 polymer conformations are generated by the RIS

[†]For further detail on the theoretical approach, see supplementary information and subsequent references.

^{††}In the electrostatic potential, we use as the dielectric permittivity of the medium. Because we scan over temperature in this paper, theoretically the dielectric constant of water should change as temperature changes. However, prior work has shown that results only mildly depend on the dielectric constant.⁴² Thus, we include it here only for the sake of completion.

(rotational isomeric state) chain model.³⁵ Volume of a polymer segment is 0.065 nm^3 if PEG and 0.07448 nm^3 if acrylic acid.

III. Results

In this section, we present results to predict the behavior of competitive pH and temperature responsive tethered polymers. We consider three different cases: purely responsive polymers (A_n), responsive As alternating with hydrophobic Bs ($(A-B)_n$), and diblocks (A_nB_n or B_nA_n). We begin by considering the purely responsive case composed of 50 monomers, A_{50} .

A. Purely Responsive Polymers (A_{50})

Figure 1 shows the behavior of purely responsive polymers in response to temperature (Fig. 1a) and pH (Fig. 1b). When the temperature increases from 7°C to 67°C , the average polymer volume fraction shows a slight but distinguishable collapse. The source of this behavior arises from the competitive hydrogen bonding between polymer-water and water-water molecules and follows directly from the difference in their associative free energies as described in eq 6. For all temperature ranges considered βF_w is more negative than βF_p meaning water-water bonds are preferred over polymer-water bonds. With increasing temperature this difference increases. The result is an increased tendency of water to repel monomers, leading to reduced solubility at high temperatures. Thus, the quality of the solvent becomes a product of both Van der Waals interactions and hydrogen bonding. The polymer chains collapse at elevated temperatures at the expense of both conformational entropy of chains and increased free energy of polymer-water hydrogen bonds, leading to familiar LCST (lower critical solution temperature) phase behavior observed for PEG. However, because the LCST of PEG is over 100°C , we only see a small response in the physiological temperature range we test. Figure 1b shows that the A monomers are also pH-responsive. The acid-base chemical equilibrium we establish for weakly acidic monomers follows a local Le Chatelier's principle. In response to elevated pH the polyelectrolyte monomers will tend to acquire charge. This leads to unfavourable electrostatic repulsions within the polymer layer. To mitigate this charge, the layer will respond in a number of ways: it will try to stretch (which is entropically unfavourable), confine counter-ions (causing loss of translation entropy), and shift the acid-base equilibrium back toward the uncharged state (work against the chemical free energy). Overall this results in a lower degree of charge in the tethered layer. Thus, while the pH effects play a large role in chain stretching, the total amount of stretching is informed by many different variables including chain conformation, counter-ion confinement, and charge regulation, all competing with one another.^{36, 37} In prior work, it has been shown the transition between charged and uncharged segments in tethered layers and in polymer gels depends not only on the solution pH but also on the local electrostatic potential which follows from the Poisson equation.^{19, 27, 36} The electrostatic potential is in turn influenced by the local polymer and counter-ion density. Consequently, the change in degree of deprotonation as a function of pH occurs over a much broader pH interval and is shifted as compared to the same acid monomer in dilute solution. These trends were recognized by Katchalsky and coworkers in their seminal theoretical and experimental studies on biopolymer solutions.^{38, 39} Similar trends were observed in the theoretical studies by Israels et al, employing numerical self-consistent theory or Scheutjens-

Fleer theory to investigate the behavior of weak polyelectrolytes brushes.⁴⁰ However, these studies focused on purely pH-responsive systems.

Figure 1 demonstrates that we can combine this pH theory with the temperature theory of PEG initially presented by Dormidontova to successfully model an end-tethered layer that responds to both pH and temperature. To our knowledge, this is the first molecularly detailed theory of end-tethered polymers that are both pH and temperature responsive.

B. Alternating A-B Polymers ((AB)₂₅)

It is important to note that in both Fig. 1a and Fig. 1b the responsive effects are present, yet the magnitude of these effects is slight. When hydrophobic B monomers are introduced in the copolymer (Fig. 2) the behavior changes significantly. Comparing Fig. 1a to Fig. 2a, the inclusion of hydrophobic monomers causes a two-fold increased temperature-dependent collapse. Hydrophobic Bs do not have the ability to hydrogen bond with water molecules which leads to a decrease in solvent quality and causes greater chain collapse. Not only do the B monomers replace 25 responsive A monomers that would otherwise be able to form hydrogen bonds with water, but they also interfere with the existing A monomers' capacity to form bonds with water. The fewer hydrogen bonds with water that exist, the more the chain is expelled from the surrounding system. At the expense of conformational entropy, the chain is urged to collapse. Thus, the inclusion of hydrophobic monomers in the A-B copolymer causes a decrease in the LCST, which has also been shown experimentally.⁴¹ The pH effects in the (AB)₂₅ case (Fig. 2b) persist and show that at high pH, the addition of electrostatic interactions can overcome the hydrophobic effect of B monomers and lead to brush swelling.

In Figure 3, we expand on the structural information of Figure 2 to show the corresponding thermodynamic properties for the (AB)₂₅ system by presenting the chemical potential and lateral pressure. The chemical potential gives information about the thermodynamic stability of the layer. The lateral pressure validates the chemical potential because it is a direct, experimental measure of the surface forces of the system. Unlike the monotonical isotherm of the A₅₀ system, shown in S2a, the introduction of hydrophobic monomers creates a non-monotonical chemical potential isotherm (Fig. 3a). In other words, a zone appears for a given monomer density and pH where the slope of the chemical potential turns from positive to negative. At surface coverages below the maximum, the slope of the isotherm is positive and above the maximum, the slope is negative. This signals the potential for phase separation. We cannot call this a true Van der Waals loop because the polymers are irreversibly end-grafted to the surface and therefore do not have translational degrees of freedom. However, we do take this to be an indication that alternating A-B brush polymers experience a structural transition that may be further indicative of microphase separation. Only future 3D calculations can conclusively identify what kind of microdomain is potentially formed.

The corresponding lateral pressure isotherm in Fig. 3b echoes the observation of the chemical potential. The lateral pressure is shown as a function of area per polymer chain where $a = 1/\sigma$. Unlike the positive lateral pressure of the A₅₀ case (S2b), the lateral pressure for the (AB)₂₅ case becomes negative at higher surface coverages, or smaller area per

polymer. Negative lateral pressure suggests interpolymer attractions, again indicative of microphase separation. When we broaden the pH range, we see that the purely responsive case has monotonically increasing chemical potential isotherms, indicative of homogeneity at all pHs (S2c). However, for the $(AB)_{25}$ case, the chemical potential varies non-monotonically within the pH range (3c). At pH 4 and below, the brush is simply too collapsed and exhibits negative slope in the chemical potential—the system is unstable. Yet, as the system swells with increasing pH, all isotherms above pH 4 still exhibit this structural transition for a given pH and density. Because our polymers are immobile, we cannot posit that this is phase separation, however, both the chemical potential and the lateral pressure suggest the possibility of lateral aggregation and formation of microdomains. We also note that these calculations are halted at a surface coverage of 0.2 nm^{-2} because above this value, the non-linear equations become difficult to solve due to the electrostatic potential built up from addition of more charged monomers.

To underscore the effect of the competitive relationship of temperature and pH on phase behavior, we can visualize both parameters as a 3D graph of the average height profile of the $(AB)_{25}$ case (Fig. 4a). One of the applications for these responsive polymers is as biomaterials for bone regeneration. During regeneration, the pH of the bone acidifies as bone-resorbing osteoclasts secrete hydrogen ions into the matrix. Thus, we investigate pHs 1 to 6 as a preliminary testing range that corresponds to a biopolymer in this application. As temperature decreases and pH increases the system swells. However, when both temperature and pH increase, the magnitude of the height response is a product of these two conflicting forces. The response arises from the competition between both monomer types, the responsive A monomers which either favour swelling at high pH or collapse at high temperature and hydrophobic Bs which favour collapse in all cases. We demonstrate this for a single surface coverage in Fig. 4a, however, surface coverage is itself a parameter. In Fig. 4b, we can use a given pH (3-6) and temperature (27°C) to predict the structural behavior for a range of surface coverages. To do this, we extract the maxima from Fig. 3c. The maxima in Fig. 3c represent the point between positive and negative isothermal compressibility. When the slope is positive, this signifies stability and the system is swollen. When the slope is negative, this signifies instability and the system is collapsed. Therefore, we use this change from stability to instability as the criteria to distinguish swelling and collapse. By plotting the curve that results from these maxima in Fig. 4b, we can use the output of the theoretical approach to predict structural behavior across parameters. Therefore, the power of the theoretical approach not only lies in the ability to scan over many parameters such as pH and temperature simultaneously, but also to use the results to predict phase behavior for conditions beyond those explicitly tested.

C. Diblock Polymers ($A_{25}B_{25}/B_{25}A_{25}$)

In the previous section, we considered a polymer sequence of alternating hydrophobic and pH/T responsive monomers. Here, we consider a diblock sequence where one of the blocks is hydrophobic and the other is pH/T responsive. We place the hydrophobic block either at the beginning ($B_{25}A_{25}$) or at the end of the chain ($A_{25}B_{25}$). We examine both structural and thermodynamic properties of this system in Figure 5. Figures 5a and 5b show the average polymer volume fractions for these cases at constant pH 5 and increasing surface coverages.

At pH 5, the volume fraction for the B₂₅A₂₅ case is more collapsed toward the surface as expected with a large hydrophobic block. However, interestingly, when the blocks are flipped the profile is much different. Instead of a uniform collapse toward the surface, the profile shows the collapse shifted outward. This suggests the hydrophobic block is collapsing separately away from the rest of the responsive chain. The collapse of the hydrophobic block also affects the responsive block. Comparing the corresponding Figs. 5c and 5d, when the block is farther from the surface, the A monomers near the surface collapse more than their flipped counterparts. This profile is highly unusual and has never before been detected in a brush system using this molecular theory.

In Figures S3a and S3b, the corresponding chemical potentials are shown. For both cases, for pH values below 4, the chemical potential monotonically decreases, suggesting instability drawn from the highly collapsed state. In this case, the amount of charged groups are insufficient to overcome the hydrophobic repulsion of B monomers. Higher pH ranges permit swelling and allow for greater thermodynamic stability. When the hydrophobic block is closer to the surface, the chemical potential maxima are at higher surface coverages. This potentially suggests the system can stably afford the addition of more monomers before it becomes thermodynamically unstable. We take it to underscore the effect of sequence modification on stability and structural behavior—namely that placing the hydrophobic block at the beginning of a polymer chain is more thermodynamically stable than at the end. Thus, the repulsive interactions of the hydrophobic block behave in a pH, temperature, and sequence-dependent manner.

Finally, we have also investigated reducing the length of the hydrophobic block to model a hydrophobic linker. In doing so, we found that when the length of the hydrophobic block was reduced from 50 to 10 monomers, the structural response mimics that of the diblocks case—collapsed near the surface for B₁₀A₄₀ and outward for A₄₀B₁₀. However, the A₄₀B₁₀ case also shows an interesting sudden collapse at higher surface coverage (0.29 nm⁻²) that is absent in the diblocks case. We hypothesize that a short linker away from the surface may form a sub-domain, akin to a micelle, that is absent in the diblocks case because the loss in conformational entropy would be too high for a longer hydrophobic chain. These results are not shown, yet we present them as yet another example of the flexibility of the theoretical approach in predicting results of sequence modification.

Discussion

We present a novel molecular theory to describe pH and temperature responsive brush layers. We successfully develop a model to study the degree of brush swelling, finding that it depends on the interplay between electrostatics, conformational entropy, the acid-base equilibrium, hydrogen bonding, hydrophobicity and sequence. By scanning over temperature, pH, and surface coverage we are able to detect rich varieties of phase behavior for different sequences—purely responsive polymers, alternating A-B polymers and diblocks.

The structural response arises from the competition between both monomer types, the responsive A monomers which either favour swelling at high pH or collapse at high

temperature and the hydrophobic Bs which favour collapse in all cases. Simultaneously there is the interplay between pH and temperature effects for A monomers alone. At room temperature at $pK_a \sim pH = 5$, the pH effect dominates. However, as temperature is increased at constant pH, the swelling due to charge repulsion is reduced by the decrease in hydrogen bonding which forces the brush to collapse instead. Thus, with increasing temperature the Van der Waals loop in the chemical potential narrows, suggesting that it takes addition of fewer monomers to promote the structural transition (Fig. 6). By scanning the parameter space, we can use thermodynamic information such as the chemical potential to identify regions of stability and instability which we to explore in the future.

This model also enables us to detect the variation in pH responsivity across sequence. Fig. 7 shows the average degree of dissociation ($\langle f_d \rangle$) at room temperature for the purely responsive, alternating, and diblock systems for different physiological salt concentrations. The average degree of dissociation is the spatial average of the local degrees of dissociation, weighted by the polymer volume fraction. Our results compare the bulk degree of dissociation (f_{bulk}) to that of the tethered layer. f_{bulk} represents an ideal solution where dissociation depends only on the bulk pH. However, the average local degree of dissociation also depends on the electrostatic potential which follows from the Poisson equation, which is a result of the interplay of many aforementioned forces such as the chemical equilibrium, the local polymer and counter-ion density, and the distribution of ionic species. To corroborate data seen before on polymer gels and brushes, these data demonstrate that, at constant pH, high salt increases the degree of dissociation.¹⁹ Conversely, decreasing the salt concentration leads to reduced screening of repulsions between monomers, which in turn decreases the degree of dissociation. A more collapsed layer will try to mitigate high electrostatic repulsion. Yet, looking at degree of dissociation between sequences is not sufficient to truly understand the pH-dependent dissociation. In Fig. 8, we show that the pH inside the layer actually differs from the bulk pH by approximately 2 pH units, a significant logarithmic difference. This signifies that the concentration of protons is higher in the layer than in surrounding solution. A higher proton concentration inside the layer can mitigate electrostatic repulsion by shifting the chemical equilibrium toward the protonated, uncharged state. Also, from Fig. 7 it appears the degree of dissociation is similar in all three sequences.

However, Fig. 8 actually elucidates that there is a 0.5 greater pH difference for the purely responsive system. This is because each purely responsive monomer, A_{50} , can become charged. In this case, the pH inside the layer differs the most from bulk pH. However, with the addition of hydrophobic monomers, the layer pH more closely matches the bulk pH. This is because reducing the number of responsive A monomers, the charge density in the layer drops which reduces the shift toward protonation of the local acid-base equilibrium.

With increasingly complex biomaterials, there is a growing need for increasingly detailed theory to emerge to predict how systems will behave in response to changing variables—which is not feasible to test experimentally. To date, a molecular theory had not been developed to model a complex system that contains hydrophobic and hydrophilic interactions, hydrogen bonding, chargeable groups and LCST-governed collapse. Notably, this theory is specific to PEG because we use the energy of hydrogen bond formation of

PEG as input. However, in the future we aim to broaden the scope of this work by applying this brush theory to NIPAAm and beyond this, to a 3D gel system where we can make predictions about microphase separation. We propose this work as a preliminary step in achieving these goals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Average polymer volume fractions shown as a function of distance from the surface for purely responsive polymers (A_{50}). Temperature response (a) is shown at pH 1 and pH response (b) is shown at 27°C . For both cases the polymers are 50 segments in length. The surface coverage is $\sigma = 0.1$ chains/ nm^2 and the salt concentration is 0.1 M.

Figure 2.

Average polymer volume fractions shown as a function of distance from the surface for polymers with an alternating sequence of responsive and neutral, hydrophobic monomers $(AB)_{25}$. Temperature response (a) is shown at pH 1 and pH response (b) is shown at 27°C . For both cases the polymers are 50 segments in length. The surface coverage is $\sigma 0.1$ chains/ nm^2 and the salt concentration is 0.1 M.

Figure 3.

Thermodynamic properties of $(AB)_{25}$ system. Chemical potential of polymers shown as a function of surface coverage for alternating A-B polymers for pH 5 at 27°C (3a). The osmotic pressure associated with pH 5 is shown (3b). The chemical potential is also shown for wider pH range (3c). The polymers are 50 segments in length. The surface coverage is σ 0.1 chains/ nm^2 and the salt concentration is 0.1 M.

Figure 4. 3D plot of average height of polymer layer as a function of both pH and temperature (4a). In 4b, maxima were extracted from the chemical potential profiles for the $(AB)_{25}$ system that exhibited loop behavior. They are plotted here to show predictive potential of theory in determining the zone where the system will collapse for a given pH and surface coverage. Results are shown for room temperature ($T = 27^\circ\text{C}$).

Figure 5.

Average polymer volume fractions are shown as a function of distance from the surface for diblock cases with the hydrophobic block closer to the surface (a,c; $B_{25}A_{25}$) or away from the surface (b,d; $A_{25}B_{25}$). Figs. 5c and 5d are the average polymer volume fractions of A and B blocks separately at $\sigma = 0.1 \text{ nm}^{-2}$. Volume fractions were splined through the points.

Figure 6. Chemical potential shown as a function of surface coverage for alternating A-B polymers for pH 5. With increasing temperature at constant pH, the p-w hydrogen bonds decrease favouring brush collapse.

Figure 7.

Average degree of dissociation, f_d , as a function of pH for different salt concentrations and different sequences (a, b, c).

Figure 8.

The pH inside the tethered layer for a given bulk pH is shown for corresponding sequences (a, b, c) and different salt concentrations.