Protein Adsorption on Poly(N-isopropylacrylamide) Brushes: Dependence on Grafting Density and Chain Collapse

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ABSTRACT: The protein resistance of poly(N-isopropylacrylamide) brushes grafted from silicon wafers was investigated as a function of the chain molecular weight, grafting density, and temperature. Above the lower critical solution temperature (LCST) of 32 °C, the collapse of the water-swollen chains, determined by ellipsometry, depends on the grafting density and molecular weight. Ellipsometry, radio assay, and fluorescence imaging demonstrated that, below the lower critical solution temperature, the brushes repel protein as effectively as oligoethylene oxide-terminated monolayers. Above 32 °C, very low levels of protein adsorb on densely grafted brushes, and the amounts of adsorbed protein increase with decreasing brush-grafting-densities. Brushes that do not exhibit a collapse transition also bind protein, even though the chains remain extended above the LCST. These findings suggest possible mechanisms underlying protein interactions with end-grafted poly(N-isopropyl acrylamide) brushes.

INTRODUCTION

Stimulus-responsive materials1,2 such as temperature-responsive polymer poly(N-isopropylacrylamide) (PNIPAM) are used in numerous applications, including biosensors,3 protein purification,4–11 drug delivery,12,13 and tissue engineering.1 PNIPAM, in particular, undergoes a solubility switch under mild conditions at its lower critical solution temperature (LCST) of 32 °C such that the polymer swells in water below the LCST but becomes less soluble and collapses above the LCST. With a transition near physiological temperature and at neutral pH, PNIPAM has broad potential for applications in biotechnology and the clinic.

Cell-sheet engineering is a notable example.14 In this case, cells adhere to PNIPAM at 37 °C to form confluent cell sheets that can be detached intact simply by decreasing the temperature and without compromising the monolayer integrity.14–24 The assumption is that above the LCST, cells attach to protein adsorbed on the presumably collapsed, hydrophobic polymer. Below the LCST, the polymer is predicted to be more hydrophilic and to swell in water, causing protein lift-off and cell detachment.

Several applications are founded on the assumption that substantial changes in the interfacial properties of PNIPAM coatings associated with this collapse transition promote reversible protein adsorption. The collapsed chains presumably adsorb protein, whereas the water-swollen state below the LCST is assumed to repel protein. Nevertheless, an increasing number of reports suggest that PNIPAM brushes do not always exhibit a collapse transition above the LCST and that proteins do not adsorb to all PNIPAM coatings. For example, PNIPAM spin-coated onto preconditioned silicon wafers did not adsorb human serum albumin either above or below the LCST.25 Cell attachment on PNIPAM hydrogels polymerized by electron beam irradiation on tissue culture polystyrene or on glass depends on the gel thickness, with thicker gels repelling cells above and below the LCST.26–28 Cell adhesion requires adsorbed protein. In the latter example, fibronectin adsorbed on thin (15–20 nm) PNIPAM gels but not on thick (>30 nm) coatings, despite the 10° increase in the water contact angle on the dry, thick gels above the LCST.27,28 However, plasma-deposited PNIPAM coatings reversibly adsorb protein above the 32 °C, regardless of the film thickness.26,29,30

Defining design rules for thermostressive coatings requires identifying key parameters governing the interfacial properties of PNIPAM and its interaction with proteins. This is challenging with some fabrication methods. However, chains grafted from immobilized initiators using atom-transfer radical polymerization (ATRP) enable good control of grafting densities and molecular weights. This in turn facilitates investigations of the impact
of these parameters on the thermally driven collapse transition and on the mechanism and extent of protein adsorption. Prior studies with PNIPAM grafted from alkanethiols on gold showed that their temperature-dependent collapse transition depends on the polymer molecular weight and grafting density. This intriguing finding suggested that some PNIPAM brushes might remain protein-resistant above the LCST and motivated the present investigation of the impact of grafting parameters on protein adsorption onto PNIPAM brushes.

Here, ATRP was used to generate controlled PNIPAM brushes. The results document the influence of brush architecture on (i) the temperature-dependent collapse of PNIPAM brushes grafted from silicon wafers and (ii) temperature-dependent protein adsorption onto those brushes. Ellipsometry, the use of radio-isotope-labeled proteins, and fluorescence measurements in turn determined protein adsorption as a function of the brush architecture and temperature.

**MATERIALS AND METHODS**

**Chemicals.** N-Isopropylacrylamide (NIPAM) and 1,1,4,7,7-pentaalkyl-2,5-dihydro-3H-diethylenetriamine (PMDETA) were purchased from Acros. NIPAM monomer was recrystallized from hexane. 2-[Methoxy(polyethylenoxy)propyl]-trimethoxysilane (OEG) was from Gelest Inc. CuBr, methanol, and anhydrous toluene were purchased from Aldrich. The initiator, 11-(2-bromo-2-methyl)-propionyl undecyl trichlorosilane, was synthesized as described. Chicken egg white lysozyme and human serum albumin (HSA) were purchased from Sigma-Aldrich. All aqueous solutions were prepared with Milli-Q purified water (Millipore, Bedford, MA) with a resistivity of 18.2 MΩ·cm.

**Surface-Initiated Polymerization of N-Isopropylacrylamide Brushes.** The poly(N-isopropylacrylamide) (PNIPAM) brush was synthesized by the surface-initiated atom-transfer radical polymerization (ATRP) of N-isopropylacrylamide from initiator-functionalized silicon substrates according to published procedures. First, silicon wafers were cleaned in piranha solution (25% v/v hydrogen peroxide and 75% v/v sulfuric acid) at 60 °C for 1 h. The catalyst, CuBr, methanol, and anhydrous toluene were purchased from Aldrich. The initiator, 11-(2-bromo-2-methyl)-propionyl undecyl trichlorosilane, was synthesized as described. Chicken egg white lysozyme and human serum albumin (HSA) were purchased from Sigma-Aldrich. All aqueous solutions were prepared with Milli-Q purified water (Millipore, Bedford, MA) with a resistivity of 18.2 MΩ·cm.

**X-ray Photoelectron Spectroscopy (XPS).** The surface chemical composition was determined by XPS using a Kratos Axis Ultra spectrometer with monochromatized Al Kα radiation at 1486.6 eV (223 W, 40 eV pass energy) at a pressure of 10⁻⁹ Torr. The samples were loaded onto a rectangular metal support using double-sided adhesive tape. The C 1s hydrocarbon peak at 285.0 eV was used as the reference peak for all measurements. Surface chemical compositions were obtained from the integrated area under the peaks in the XPS spectra, normalized by the appropriate sensitivity factors.

**Ellipsometric Determination of Adsorbed Protein on PNIPAM Brushes.** The proteins investigated were chicken egg white lysozyme (Sigma-Aldrich L8766) and human serum albumin (HSA, Sigma-Aldrich A9511). Lysozyme is small and positively charged at neutral pH (pI = 10.9), and HSA is a larger negatively charged protein (pI = 4.4) and the predominant globular protein in serum. The proteins were dissolved in phosphate-buffered saline (pH 7.0, ionic strength 0.1).

The instrument used for these studies is a Horiba Jobin-Yvon MM-16 spectroscopic phase-modulated ellipsometer. The light sources are halogen and LED lamps with a wavelength range of 430–850 nm. Measurements were performed near the Brewster angle at an incident angle of 70°. All measurements were obtained over the entire spectral range. The samples were contained in a home-built cell used for in situ studies of protein adsorption at solid/liquid interfaces. The incident light enters the liquid via a thin glass window, reflects from the solid/liquid interface, and is detected after passing through another thin glass window. The temperature is regulated by a thermostatic water bath that circulates water at the base of the cell. The lid consists of poly(tetrafluoroethylene).

The adsorbed amount of protein \( \Gamma \) (mg/m²) was determined from

\[
\Gamma = \frac{h (n - n_0)}{10 \frac{dn}{dc}}
\]

where \( h \) is the thickness (in Å) of the layer (box) in which the protein is adsorbed, \( n_0 \) is the refractive index of the medium in the thin layer before protein insertion, \( n \) is the refractive index of the medium with adsorbed protein, and \( \frac{dn}{dc} \) is the refractive index increment of the protein (mL/g). In the case of protein insertion into the brush, \( h \) is the brush thickness, \( n_0 \) is the refractive index of the brush without protein, and \( n \) is the refraction of the brush with adsorbed protein. Both for the lysozyme and HSA, \( \frac{dn}{dc} \) is about 0.19 mL/g.
A 1 or 3.5 mg/mL solution of lysozyme was injected into the cell containing the PNIPAM brush. The PNIPAM samples were immersed in the protein solution in order to avoid flushing, which could disturb the brush and prevent weak protein adsorption. The PNIPAM brushes were first incubated with the protein solution at a given temperature (25 or 37 °C) for up to 5 h to equilibrate the system, and then the solution was heated to 37 °C or cooled to 25 °C, respectively. The protein absorption onto the PNIPAM brushes was monitored as a function of the temperature change.

Radio Assays of Adsorbed Protein. To detect protein levels below the detection limit of ellipsometry, BSA adsorption on PNIPAM brushes was quantified with \(^{125}\)I-labeled protein. BSA was iodinated with iodobeads (Pierce) and carrier-free Na\(^{125}\)I (Perkin-Elmer). The labeled protein was desalted with a PD-10 column (GE Healthcare Bio-science) to remove unbound \(^{125}\)I, and the determined specific activity for 1 h. After being rinsed with excess ultrapure water (18.2 MΩ-cm), the samples were maintained at 37 °C during all rinsing steps to prevent brush reswelling. Substrates were placed in vials containing 5 mL of the scintillation cocktail, and the counts were recorded with an LS 6500 scintillation counter (Beckman Instruments) with specified settings for \(^{125}\)I detection. Control measurements were made with oligoethylene oxide-terminated silane monolayers. Each set of measurements was repeated to ensure reproducibility.

Patterned PNIPAM Brush Formation. To visualize temperature-dependent differences in protein adsorption on the PNIPAM brushes above and below the LCST, patterns of the initiator were microcontact printed on clean glass slides. For comparison, we similarly patterned OEG-SAMs. A second, positive control used octadecyl trimethoxy silane (OTS) patterns with the nonpatterned region back-filled with OEG-SAM. Stamps were fabricated by casting a mixture of poly(dimethyl siloxane) (PDMS) prepolymer and curing agent (10/1 v/v) on the silicon master that was photolithographically patterned. PDMS was cured overnight at 65 °C.

Silicon (111) wafers and glass substrates were cleaned in piranha solution for 1 h. After being rinsed with excess ultrapure water (18.2 MΩ-cm), substrates were dried with a steam of nitrogen. Silicon wafers were used for reference thickness measurements of patterned PNIPAM brushes. Glass slides were used to obtain fluorescence microscopy images of PNIPAM patterns after incubation with fluorescein isothiocyanate (FITC)-labeled albumin. Images were obtained with a Zeiss Axiovert 200 M fluorescence microscope (Thornwood, NY).

The PDMS stamp was inked with a 10 mM solution of the initiator in hexane and dried with a stream of nitrogen for 3 s. The inked PDMS was brought into contact with a clean silicon wafer (or glass) for 10 s. Subsequently, the initiator-printed surface was washed with ethanol and dried with nitrogen. For backfilled samples, the unprinted regions were coated with OEG silane by immersing the substrates in a 2 mM toluene solution of OEG silane for 1 h at room temperature. Finally, surfaces were washed with toluene, ethanol, and then deionized water before drying with nitrogen. Patterned PNIPAM brushes were grown from patterns of SAM-Br by following the same procedure as for the non-patterned PNIPAM brushes except that the polymerization took place for 2 h.

Statistical Analyses. The student’s t test was used for pairwise comparisons of data. The difference between two measurements was considered to be statistically significant if \(p < 0.05\).

RESULTS

Initiator SAMs and PNIPAM Brush Synthesis. Self-assembled monolayers of the initiator (SAM-Br) and OEG (SAM-OEG) were first characterized by ellipsometry. The measured values for the SAM-Br and SAM-OEG monolayer are 18 ± 2 and 12 ± 1 Å respectively and compare well with prior studies. To control the initiator coverage, SAMs were prepared from mixtures of initiator silane and OEG silane. The surface composition of the mixed initiator/OEG monolayers was determined by XPS (Figure 1). Table 1 shows the surface densities of the initiator (SAM-Br) and OEG (SAM-OEG) monolayers of the initiator (SAM-Br) and OEG (SAM-OEG) monolayers for \(^{125}\)I detection. Control measurements were made with oligoethylene oxide-terminated silane monolayers. Each set of measurements was repeated to ensure reproducibility.

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thicknesses of dry and water-swollen PNIPAM brushes. The wet brush thicknesses were determined at 23 and 37 °C. The swelling ratio, which is the ratio of the hydrated PNIPAM thickness to the dry PNIPAM brush thickness at 23 °C, increases with decreasing grafting density (Table 2). This trend might be expected because the chains in more densely packed brushes are already highly stretched and should swell less upon full hydration. The decrease in the refractive index (Figure S1a) with decreasing initiator density also agrees with the expected increase in the water content of the more dilute chains. In addition, from the estimated initiator densities and dry film thicknesses, the molecular weight of grafted PNIPAM was estimated with the equation $\sigma$ of grafted PNIPAM was estimated with the equation

$$
\text{degree of polymerization} = \frac{\text{MW}}{\text{MW}_{\text{PS}}}
$$

where $\text{MW}$ is the molecular weight of the polymer and $\text{MW}_{\text{PS}}$ is the molecular weight of polystyrene. The grafting density $\sigma$ was calculated as

$$
\sigma = \frac{\text{MW}}{\text{MW}_{\text{PS}}} \times \frac{1}{\text{MW}_{\text{PS}}} \times \frac{1}{\text{MW}_{\text{PS}}}
$$

Table 1. Surface Chemical Composition of Self-Assembled Silane Monolayers on Silicon Wafers, Determined by XPS

<table>
<thead>
<tr>
<th>mole% of SAM-Br in the solution of SAM-Br and SAM-OEG</th>
<th>O (%)</th>
<th>C (%)</th>
<th>Si (%)</th>
<th>Br (%)</th>
<th>Br/Si</th>
<th>SAM-Br coverage (%)</th>
<th>$\sigma$ (no./nm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>22.37</td>
<td>30.52</td>
<td>44.6</td>
<td>2.51</td>
<td>0.056</td>
<td>100</td>
<td>2.1</td>
</tr>
<tr>
<td>80</td>
<td>26.24</td>
<td>20.93</td>
<td>51.19</td>
<td>1.64</td>
<td>0.032</td>
<td>57.1</td>
<td>1.1</td>
</tr>
<tr>
<td>50</td>
<td>26.14</td>
<td>20.28</td>
<td>52.22</td>
<td>1.35</td>
<td>0.0259</td>
<td>46.3</td>
<td>0.9</td>
</tr>
<tr>
<td>20</td>
<td>26.28</td>
<td>23.46</td>
<td>49.21</td>
<td>1.06</td>
<td>0.0215</td>
<td>38.4</td>
<td>0.8</td>
</tr>
</tbody>
</table>

$\sigma$ is The initiator coverage was estimated from the Br/Si ratios.

Table 2. Determined Thicknesses of Dry and Water Swollen PNIPAM Brushes after Subtracting the Alkane Monolayer Thickness

<table>
<thead>
<tr>
<th>mole% initiator in the mixture of OEG and initiator (polymerization time)</th>
<th>initiator monolayer (Å)</th>
<th>dry film thickness (Å) at 23 °C</th>
<th>wet film thickness (Å) at 23 °C</th>
<th>wet film thickness (Å) at 37 °C</th>
<th>swelling ratio at 23 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 (2 h)</td>
<td>18 ± 1</td>
<td>707 ± 4</td>
<td>1043 ± 4</td>
<td>876 ± 16</td>
<td>1.5</td>
</tr>
<tr>
<td>100 (10 min)</td>
<td>18 ± 1</td>
<td>352 ± 2</td>
<td>668 ± 9</td>
<td>519 ± 3</td>
<td>1.9</td>
</tr>
<tr>
<td>80 (10 min)</td>
<td>16 ± 1</td>
<td>243 ± 1</td>
<td>652 ± 3</td>
<td>516 ± 3</td>
<td>2.7</td>
</tr>
<tr>
<td>50 (10 min)</td>
<td>16 ± 1</td>
<td>134 ± 1</td>
<td>594 ± 11</td>
<td>484 ± 3</td>
<td>4.4</td>
</tr>
<tr>
<td>50 (4 min)</td>
<td>16 ± 1</td>
<td>76 ± 5</td>
<td>490 ± 20</td>
<td>491 ± 7</td>
<td>6.5</td>
</tr>
<tr>
<td>20 (10 min)</td>
<td>12 ± 1</td>
<td>62 ± 2</td>
<td>490 ± 40</td>
<td>488 ± 8</td>
<td>7.9</td>
</tr>
</tbody>
</table>

Table 3. Summary of PNIPAM Brush Parameters

<table>
<thead>
<tr>
<th>mole% initiator in the mixture of OEG and initiator (reaction time)</th>
<th>estimated grafting density (no./nm$^2$)</th>
<th>estimated molecular weight (g/mol)</th>
<th>degree of polymerization</th>
<th>$R_F$ (Å)</th>
<th>$s$ (Å)</th>
<th>$s/(2R_F)$</th>
<th>configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 (2 h)</td>
<td>0.21</td>
<td>202 000</td>
<td>1789</td>
<td>268</td>
<td>22</td>
<td>0.04</td>
<td>brush</td>
</tr>
<tr>
<td>100 (10 min)</td>
<td>0.21</td>
<td>101 000</td>
<td>891</td>
<td>176</td>
<td>22</td>
<td>0.06</td>
<td>brush</td>
</tr>
<tr>
<td>80 (10 min)</td>
<td>0.11</td>
<td>126 000</td>
<td>1118</td>
<td>202</td>
<td>30</td>
<td>0.07</td>
<td>brush</td>
</tr>
<tr>
<td>50 (10 min)</td>
<td>0.09</td>
<td>85 000</td>
<td>753</td>
<td>159</td>
<td>33</td>
<td>0.10</td>
<td>brush</td>
</tr>
<tr>
<td>50 (4 min)</td>
<td>0.09</td>
<td>48 000</td>
<td>427</td>
<td>113</td>
<td>33</td>
<td>0.15</td>
<td>brush</td>
</tr>
<tr>
<td>20 (10 min)</td>
<td>0.08</td>
<td>44 000</td>
<td>392</td>
<td>107</td>
<td>35</td>
<td>0.16</td>
<td>brush</td>
</tr>
</tbody>
</table>

To determine whether the grafted polymers are in the brush regime, we calculated the distance between grafting sites $s$ relative to the Flory radius $R_F = L_n n^{3/5}$, assuming that water at 23 °C is a good solvent for PNIPAM. Here, $L$ is the estimated monomer size (≈3 Å) and $n$ is the degree of polymerization. If $s < 2R_F$, then the chains form stretched brushes. The values of $R_F$ and distances between grafting sites $s = \sigma^{-1/2}$ are in Table 3. On the basis of these calculations, all PNIPAM preparations used produced brushes.

A comparison of the distance between grafting sites relative to the protein dimensions also determined the feasibility of primary protein adsorption by penetrating the brush to adsorb on the underlying substrate. The dimensions of lysozyme are 45 Å × 35 × 35 Å, and those of HSA are 40 × 40 × 40 Å such that even at the lowest grafting density the chain density and molecular weight should be sufficient to repress primary adsorption. Proteins could still penetrate the brush (tertiary adsorption), but they would not likely adsorb to the underlying surface.

Temperature Dependence of Advancing Water Contact Angles. The dry PNIPAM brushes were characterized with advancing water contact angle measurements at 37 and 23 °C (Figure 2). Advancing water contact angles generally tracked with the extent of the brush collapse above 32 °C. The difference in contact angles above and below the LCST was greatest with the PNIPAM brush at the highest grafting density, and then decreased somewhat with decreasing grafting density. However, no change was observed with the most dilute brush (0.08/nm$^2$) or with the lower-molecular-weight PNIPAM synthesized at an intermediate initiator density (0.09/nm$^2$, Table 3).

Temperature Dependence of the Hydrated PNIPAM Brush Thickness. Figure 3 shows the measured thickness of the
water-swollen PNIPAM brushes at increasing temperature. Two observations can be made. First, changes in the hydrated thicknesses similarly depended on the chain-grafting density, when PNIPAM was synthesized under otherwise identical conditions. Different from the swelling ratios at 23 °C, chains at the higher grafting densities exhibited the greatest decrease in brush thickness above 32 °C. The thickness changes in the two most densely packed brushes were similar, and refractive index changes (Figure S1b) suggest a decrease in the water content of the brushes. There was no apparent change in the thickness of the swollen chains grafted from the least dense initiator monolayers (0.08/nm²).

The initial thickness of PNIPAM at the intermediate grafting density (0.09/nm²) was less than that of brushes grafted from initiator densities of 0.11/nm² and 0.21/nm², as expected, and the brush similarly collapsed above the LCST. PNIPAM grafted from SAM-Br at 0.09/nm² appears to lie at a crossover between two regimes: namely, higher-molecular-weight chains (~85 kDa) collapsed above the LCST but the thinner brushes (~48 kDa) remained swollen above 32 °C. This agrees with a prior report in which the collapse of PNIPAM grafted from alkanethiols on gold depends on the grafting density and on the polymer molecular weight.31 Second, for the dense PNIPAM brush, the chain collapse was not abrupt, but exhibited a more gradual decrease over the temperature range of ~26 to 32 °C.

Ellipsometry Measurements of Adsorbed Protein on PNIPAM Brushes. Next, protein adsorption measurements determined whether the grafting density and molecular weight dependence of the chain collapse generate corresponding differences in protein adsorption. Control measurements with 100 mole% SAM-OEGs first confirmed that the underlying OEG monolayer is protein-resistant. The SAM-OEG beneath the brushes should therefore repel the adsorption of both protein and NIPAM. A total of 14 measurements were then carried out with PNIPAM brushes.

Figure 4 shows the difference in ellipsometric angles ψ and Δ at 546 nm before and after incubation with the protein solution. The difference is maximal in this wavelength region. In all cases, there was no significant change in the ψ and Δ spectra before and after incubation with 1 mg/mL lysozyme solution. Contrary to expectations,8,10,30 there was no detectable protein adsorption on any of the PNIPAM brushes studied at either 25 or 37 °C. Even with a higher lysozyme concentration (3.5 mg/mL), there were no significant changes in Ψ and Δ. Identical results were obtained with a 1 mg/mL solution of HSA. Two measurements with PNIPAM brushes grafted from dense initiator monolayers (0.11/nm²) were carried out at initial temperatures of 25 and at 37 °C. In both cases, the thicknesses, refractive indices, and percentages of solvent in the brush were similar to those of the polymers in the absence of protein.

Simulations determined the instrument sensitivity to protein adsorption. Changes in ψ and Δ spectra were simulated for adsorbed protein amounts of 0, 0.5, 1.5, 2.5, and 3.5 mg/m² in the PNIPAM brush (Figure S2a). Spectral shifts at Γ = 0.5 mg/m² were barely detectable. However, the spectra are particularly sensitive to protein at Γ > 1 mg/m². At 1 mg/m², ψ and Δ shift by, respectively, 0.269 and 1.016° at 546 nm. These differences could be easily detected by ellipsometry. A comparison of these simulations with experimental data in Figure 4 indicates that any adsorbed protein is below 1 mg/m², supporting the experimental finding of negligible protein adsorption on both swollen and collapsed PNIPAM brushes under the experimental conditions investigated.

Simulated ellipsometry data for protein adsorption onto the OEG monolayer at 0, 0.5, 1.5, and 2.5 mg/m² (Figure S2b) also confirmed that such measurements would be sensitive to protein adsorption such that 0.1 mg/m² would generate detectable shifts in ψ and Δ of 0.011 and 0.567°, respectively, at 546 nm (Figure S2b). At 0.1 mg/m², the change in ψ is insignificant, but the change in Δ could be detected easily. The comparison with ellipsometry data indicates that any adsorbed protein on OEG must be Γ < 0.1 mg/m².

**[I]-BSA Adsorption on PNIPAM Brushes.** More sensitive radioisotope assays revealed subtle differences in the low protein adsorption on the different brushes. The results are summarized in Figure 5. At 23 °C, 20 ± 3 ng/cm² on OEG-terminated SAMs is slightly higher than reported by Yu et al.,46 it.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Advancing water contact angle on dry PNIPAM brushes at 23 and 37 °C. The numbers below the graph indicate the estimated chain grafting densities and corresponding molecular weights. Here * indicates a significant difference between the values measured at 23 and 37 °C for each sample (p < 0.001), and ** indicates a significant difference between the water contact angle at 37 °C on the low-density brush (0.08 chains/nm² at 44 kD or 0.09 chains/nm² at 48 kD) relative to the other samples (p < 0.01).

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Measured changes in the ellipsometric thickness of PNIPAM brushes in pure water as a function of temperature. The curves correspond to different brush preparations: ■ (0.08/nm², 44 kDa), □ (0.09/nm², 48 kDa), ○ (0.09/nm², 85 kDa), ● (0.11/nm², 126 kDa), and Δ (0.21/nm², 101 kDa).
Protein Adsorption on Patterned PNIPAM Brushes. We further visualized the adsorption of fluorescently tagged BSA onto patterned PNIPAM above and below the LCST. Patterned PNIPAM brushes do not have the identical grafting density as nonpatterned PNIPAM brushes because of the difference in the SAM-Br preparation methods. Nevertheless, our measurement shows that the PNIPAM brush prepared on the microcontact-printed SAM-Br is around 30 nm.

Images of printed OEG-SAM at 23 and 37 °C are shown for comparison (Figure 6a,b). The black regions are the patterned OEG, and the fluorescence is from protein adsorbed on the uncoated glass. The resulting images obtained with the PNIPAM brush are consistent with the ellipsometry studies of protein adsorption; namely, the polymer patches appear black in the fluorescent images (Figure 6c,d) at both 23 and 37 °C. The background fluorescence shows adsorbed protein on the unmodified glass slide, whereas there is no detectable fluorescence on the PNIPAM pattern at either temperature, similar to patterned SAM-OEG on glass (cf. Figure 6a,b). If the regions surrounding the SAM-Br pattern were instead backfilled with SAM-OEG prior to polymerization, then there is little detectable fluorescence on the patterns and no contrast between the PNIPAM pattern and SAM-OEG background (not shown). However, fluorescent images obtained with the positive control, comprising printed SAM-OTS with unprinted regions backfilled with SAM-OEG, show clear fluorescence on the OTS patterns but no protein in SAM-OEG regions (Figure 6e,f). These results validate the ellipsometry data and show that the PNIPAM brushes repressed protein adsorption both above and below the LCST.

**DISCUSSION**

Prior findings by us and by others showed that the thermally driven collapse of PNIPAM brushes depends on the grafting density and molecular weight. These results confirm prior findings but also show that this transition (or its absence) does not correlate with protein adsorption under the conditions investigated. These results instead support an emerging view that the reversible, temperature-dependent adsorption and
lift-off of proteins and cells from PNIPAM coatings depend on the film architecture.\textsuperscript{11,25,50}

With grafted polymers, the chain densities and molecular weights (inferred from thicknesses) affect protein adsorption modes, which may include primary adsorption to the underlying surface, secondary adsorption at the outer edge of the brush, or ternary adsorption involving brush penetration.\textsuperscript{45} In this study, the high grafting densities of the brushes would prevent primary adsorption. The most densely packed brushes also appear to suppress secondary and ternary adsorption. At lower grafting densities where BSA could penetrate the brush (ternary adsorption), adsorption increases above the LCST. This suggests the formation of more extensive, attractive protein-segment interactions, which would support increased adsorption.

These results and postulated adsorption mechanisms are consistent with reported cell attachment studies. Other studies did not focus on the impact of chain density or molecular weight on the polymer collapse, as this study does. However, enhanced cell attachment on dilute (low grafting density) or thin (e.g., low-molecular-weight) brushes above the LCST is consistent with greater ternary or even primary protein adsorption.\textsuperscript{45}

These studies used model protein BSA to probe qualitative changes in the interfacial properties of PNIPAM brushes. Altered surface properties, reflected in altered BSA adsorption, would similarly impact the adsorption of other proteins, including extracellular matrix proteins that support cell adhesion. Changes in the PNIPAM surface properties will necessarily affect all protein adsorption, although the adsorbed amounts of different proteins will differ quantitatively on account of the variety of shapes, sizes, charge, and melting temperatures that also contribute to protein—surface interactions.

The low protein adsorption on dense brushes differs from the increased protein adsorption on thick films reported by Yu et al.\textsuperscript{46} However, these results agree with other reports showing negligible protein deposition and cell attachment on dense, high-molecular-weight PNIPAM brushes.\textsuperscript{50} The reason for the discrepancy with

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**Figure 6.** Fluorescent images of adsorbed FITC-BSA (1 mg/mL in 0.01 M PBS buffer) on different micropatterned surfaces at two different temperatures. Microcontact-printed OEG silane (dark zigzag region) on a clean glass substrate at (a) 37 and (b) 23 °C. Microcontact-printed PNIPAM brush (dark zigzag region) on a clean glass substrate at (c) 37 and (d) 23 °C. Microcontact-printed OTS on clean glass substrate (bright zigzag), which was backfilled with OEG silane (dark region) at (e) 37 and (f) 23 °C.
Yu et al. 46 is presently unclear, but direct comparisons are hindered by the absence of molecular weight or grafting density information in the latter report.

Brushes with high PNIPAM grafting densities and molecular weights do not support reversible cell attachment. 27-50,52-56 The cell resistance and protein resistance of dense, high-molecular-weight brushes above the LCST, 1,5,10,11,22,52,56 partitioning into PNIPAM-coated chromatographic packing S1a) indicates a less polar core, as does steroid and peptide adsorption is also consistent with the solution stability of proteins adsorb to more dilute brushes that allow proteins to penetrate the layer and interact more extensively with the hydrated outer chains. Here, the resistance to protein resistance of dense, high-molecular-weight brushes above the LCST is determined by the capacity of proteins to adsorb to the outer edge of dense brushes. Howev-er, proteins adsorb to more dilute brushes that allow proteins to penetrate the layer and interact more extensively with the chains, rather than on the collapsed configuration of chains.

[CONCLUSIONS]

These results demonstrate the ability to control the collapse of PNIPAM brushes above the lower critical solution temperature of 32 °C. They also provide a plausible mechanistic understanding of protein interactions with end-grafted PNIPAM films. Adsorbed protein levels were below the detection limit of ellipsometry and required the use of isotope-labeled protein for quantification. Differences in the low levels of adsorbed protein depended on the grafting density and, in part, on the molecular weight. Dense brushes prevent protein penetration, and protein adsorption does not adsorb at the outer edge of dense brushes. However, proteins adsorb to more dilute brushes that allow proteins to penetrate the layer and interact more extensively with the grafted chains. These findings suggest that protein adsorption above the LCST is determined by the capacity of proteins to penetrate PNIPAM brush layers and interact extensively with the chains, rather than on the collapsed configuration of chains.

[ASSOCIATED CONTENT]

Supporting Information. PNIPAM brush refractive index change and protein adsorption measurements on PNIPAM and OEG samples using ellipsometry. This material is available free of charge via the Internet at http://pubs.acs.org.

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