

Loading and Distribution of a Model Small Molecule Drug in Poly(*N*-isopropylacrylamide) Brushes: a Neutron Reflectometry and AFM Study

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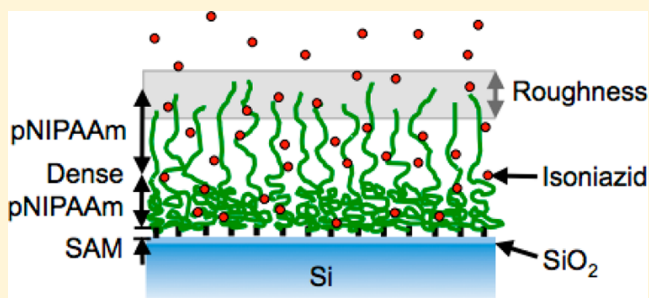
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Supporting Information

ABSTRACT: The structure of a hydrated poly(*N*-isopropylacrylamide) brush loaded with 5 vol % Isoniazid is studied as a function of temperature using neutron reflectometry (NR) and atomic force microscopy (AFM). NR measurements show that Isoniazid increases the thickness of the brush before, during and after the polymer collapse, and it is retained inside the brush at all measured temperatures. The Isoniazid concentration in the expanded brush is ~14% higher than in the bulk solution, and the concentration nearly doubles in the collapsed polymer, suggesting stronger binding between Isoniazid and the polymer compared to water, even at temperatures below the lower critical solution temperature (LCST) where the polymer is hydrophilic. Typically, additives that bind strongly to the polymer backbone and increase the hydrophilicity of the polymer will delay the onset of the LCST, which is suggested by AFM and NR measurements. The extent of small-molecule loading and distribution throughout a thermo-responsive polymer brush, such as pNIPAAm, will have important consequences for applications such as drug delivery and gating.



INTRODUCTION

Poly(*N*-isopropylacrylamide), pNIPAAm, is a thermally sensitive polymer that exhibits a lower critical solution temperature, LCST, above which the hydrated polymer collapses and expels interstitial water molecules.¹ This collapse is accompanied by the reorientation of polymer chains^{2,3} and is induced by a shift in the balance of entropic and enthalpic forces that govern the interaction of water with hydrophobic (isopropyl) and hydrophilic (amide) portions of the polymer (Figure 1a).⁴ The LCST for pNIPAAm (~32 °C) occurs near human body temperature and can be modulated by pH, ionic strength, and the presence of copolymers, making pNIPAAm-coated surfaces attractive candidates for loading and controlled delivery of medication.⁵ In particular, surfaces and particles coated by pNIPAAm brushes are well situated to become

important components in nanoscale devices for drug delivery and gating. There is an ongoing research effort in pursuit of this application;^{6–9} however, little work has been completed on the molecular level effects of additives on surface-tethered pNIPAAm and the implications for loading and release. This is especially important because the magnitude of the thickness change and temperature of the collapse can be modified by additives. The studies that investigate LCST changes due to small molecule additives have focused on pNIPAAm cross-linked gels and linear chains in solution, and do not reveal information about the spatial distribution of the drug on the molecular level.^{10–13} For devices employing brushes, the distribution of the additive throughout the polymer and its effect on the LCST behavior is critical to functionality. However, characterizing the distribution of a small molecule throughout brushes that are often nanometers thick is particularly challenging, considering the small length scales involved. Neutron reflectometry, NR, is well suited for this type of investigation and has been previously used to monitor the

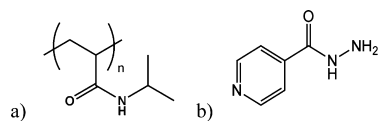


Figure 1. Chemical structures of (a) pNIPAAm and (b) Isoniazid.

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LCST behavior of hydrated pNIPAAm.^{14–18} NR can provide the density profile of the material normal to the surface with Å-scale resolution. Moreover, the contribution from a particular component in the system can be highlighted via deuterium labeling, exploiting the sensitivity of neutrons to the isotopes of hydrogen. The purpose of this study is (1) to evaluate structural changes in a pNIPAAm brush as a function of temperature upon loading with Isoniazid, a small molecule drug (Figure 1b) and (2) to characterize the loading and distribution of Isoniazid before, during, and after the collapse of the brush. We use atomic force microscopy, AFM, to identify the LCST range, and NR to characterize the distribution of polymer and drug normal to the surface within this temperature range.

To understand the interactions of complex molecules with pNIPAAm, we use the well-documented effects of additives that can shift the end points of the pNIPAAm LCST transition to higher or lower temperatures and increase or decrease the magnitude of the thickness change. pNIPAAm in the gel and solution phases shows quantitatively similar behavior, and the influence of surfactants, salts, and solvents on the LCST response of these systems was comprehensively reviewed by Schild.⁵ Generally, an additive can depress the LCST by interrupting the solvent structure or by decreasing hydrophilicity of the chains through direct binding with the polymer, promoting chain collapse. Conversely, an additive can elevate the LCST by strengthening the solvent structure or by direct binding with polymer chains that results in increased hydrophilicity of the polymer, delaying chain collapse. Specifically, salts affect the LCST by interrupting or strengthening the hydrogen bonded water structure, depending on whether the salt tends to disrupt or reinforce the water structure. For example, Eliassaf showed that 6 M LiCl causes pNIPAAm to precipitate from solution at 30 °C thereby decreasing the LCST onset temperature.¹⁹ In contrast, surfactants can increase the LCST through direct interactions by stabilizing the hydrophobic isopropyl group on the amide nitrogen of pNIPAAm (Figure 1a), an example of this being sodium dodecyl sulfate, SDS. At concentrations above ~0.8 mM, SDS increases the LCST transition and expanded volume in proportion to the amount of surfactant added.¹⁹

These general examples form the basis for understanding more complex systems, such as additives with hydrophilic and hydrophobic portions, varying interaction strength with pNIPAAm and water, and different degrees of ionic character (e.g., drug molecules). It has been empirically demonstrated that the drug release rate from hydrogel devices is a function of drug loading,^{10,11,20–22} however, the interplay between drug–polymer and drug–water interactions are particular to each drug and are generally not fully understood. Several groups have investigated the specific additive–polymer interactions between small molecules and pNIPAAm gels and linear chains in solution. Hofmann and co-workers studied the influence of a series of substituted benzaldehydes over a small concentration range (0–0.025 mol/L) in linear pNIPAAm solutions.¹¹ These additives are hydrophobic and therefore only slightly soluble in water (3–28 mg/mL). All additives decreased the LCST, some to a greater extent than others; however, there was no correlation between hydrophobicity or solubility within this small concentration range.

Dhara and co-workers studied the effects of hydroxybenzenes, hydroxybenzoic acids, and hydroxybenzoates on the LCST of linear and cross-linked pNIPAAm.^{12,23} The hydrophobic hydroxybenzenes, and hydroxybenzoic acids were

measured over a small concentration range (0.001–0.1 mol/L), while the hydrophilic anionic hydrotropes were measured over a larger range (0.1–2 mol/L). With one exception, all additives decreased the LCST, and the authors suggested that hydrophobicity and structure/substitution patterns induced the LCST decrease.

While the interactions between small and large molecules with bulk pNIPAAm have been studied by several groups, little is known about how small molecules interact with and are distributed through surface-bound pNIPAAm brushes. Only within the past decade has the distribution of the polymer itself been characterized within a hydrated pNIPAAm brush. Yim and co-workers used NR to study pNIPAAm brushes without additives over a large range of molecular weights and grafting densities.^{14–18} At low grafting densities (<0.02 chains/nm²), strong segmental absorption of the polymer is detected on SiO₂ within a short distance of the polymer/substrate interface.¹⁴ The authors attribute this to hydrophobic interactions between the isopropyl groups of NIPAAm and the exposed self-assembled monolayer at low grafting density. As a consequence of weak attractive interactions between polymer chains at low grafting density, a subtle coil-to-globule transition was detected at a molecular weight of 209 kg/mol, which was nonexistent at 33 kg/mol.¹⁴ At high grafting densities (>0.2 chains/nm²), and high molecular weight (>209 kg/mol) on SiO₂, vertical phase separation is detected at temperatures near the LCST,^{16,18} as theoretically predicted by Baulin and Halperin.²⁴ Here, the scattering length density, SLD, profile must be fit to a bilayer model, where a layer of higher polymer density exists near the substrate. Surprisingly, at grafting densities greater than 0.1 chains/nm² and molecular weights lower than 100 kg/mol, the authors observe that the magnitude of the collapse on both SiO₂ and gold surfaces is larger than predicted by theory. Originally, they attributed this to lateral heterogeneity within the polymer brush,¹⁵ but later explained that this could result from vertical phase separation if it occurs at lower temperature for lower molecular weight.¹⁶ It remains unclear why vertical phase separation would occur below the LCST at lower molecular weight.

In the present study, we use NR and AFM to investigate the distribution and effect of a model drug, Isoniazid, in a pNIPAAm brush. Isoniazid is an antitubercular drug that is hydrophilic as indicated by a high water solubility of 140 mg/mL.²⁵ Based on the acid dissociation constants of the conjugate acids of the amide, pyridine, and hydrazide nitrogens, some of the drug is positively charged at our experimental pH of 6.85.²⁶ Two groups have studied Isoniazid loading and interactions in microcapsules comprising a shell of pNIPAAm and poly(acrylic acid), pAA, with an empty cavity.^{27,28} Using FTIR on dry samples, both groups detect hydrogen bonding between the pNIPAAm amide and the Isoniazid hydrazide groups. While these studies focused on the effect of Isoniazid in pNIPAAm/AA microcapsules, here we investigate the influence on the LCST of a pNIPAAm brush, and quantify the loading and distribution of the drug throughout the hydrated brush.

■ EXPERIMENTAL DETAILS

Materials. Unless otherwise noted, reagents and solvents were obtained from Sigma Aldrich.²⁹ Silane initiator, 11-trichlorosilylundecyl-2'-bromo-2-isobutyrate (95+%, ATRP Solutions),²⁹ ethyl 2-bromoisobutyrate, hexanes (ACS reagent, 99.9%, Fisher),²⁹ dichloromethane (DCM, Chromasol Plus),²⁹ ethanol (EtOH, denatured for HPLC, Acros),²⁹ cuprous

bromide (CuBr, 99.999%), ethanol (EtOH, 99%, Fisher),²⁹ toluene (ACS spectrophotometric), hydrogen peroxide (H₂O₂, 30%, Certified ACS, Fisher Chemical),²⁹ sulfuric acid (H₂SO₄, 95–98%, c.p., Acros Chemicals),²⁹ deuterium oxide (D₂O, 99.9%), and Isoniazid (>99%) were used as received.

N-Isopropylacrylamide (NIPAAm, 97%) was purified by running a saturated solution of 1:1 hexanes/DCM by volume through a 2.5 cm basic alumina column, removing the solvent by reduced-pressure evaporation, recrystallizing the remaining solid in hot hexanes at <50 °C, rinsing with minimal ice cold hexanes, and removing the solvent by reduced pressure evaporation. Methanol (MeOH, ACS reagent, 99.9%, Fisher),²⁹ pentamethyldiethylenetriamine (PMDETA, 99%), and deionized (DI) water ($r = 18 \text{ M}\Omega \text{ cm}$, Millipore Corp.)²⁹ in Schlenk flasks and toluene (anhydrous, 99.8%) and triethylamine (TEA, $\geq 99.5\%$) in SureSeal bottles were degassed by bubbling nitrogen for 5–10 min, and then were immediately transferred into a controlled atmosphere box.

pNIPAAm brushes of equivalent thicknesses were prepared on two Si wafers: (1) a 4 in. diameter, 5 mm thick, n-type wafer (P dopant, [111], 20–40 $\Omega \text{ cm}$, El-Cat, Inc.)²⁹ for NR and (2) a 4 in. diameter, 525 μm thick, p-type (B dopant, [100], El-Cat, Inc.)²⁹ for AFM and ellipsometry measurements. For NR, the substrate must be flat over the entire footprint of the neutron beam, thus a thick silicon wafer was chosen for its low bow and warp properties. A thin silicon wafer was chosen for the remaining experiments, because it is easy to cleave the sample into small pieces.

pNIPAAm Brush Synthesis. Silanization and polymerization methods are described in detail elsewhere³⁰ and were adapted from published procedures.^{31,32} Briefly, substrates were cleaned in piranha solution (1:3 H₂O₂/H₂SO₄) and then transferred to a nitrogen-filled glovebox where α -bromide terminated silane monolayers were deposited by liquid silanization. Subsequently surface-tethered pNIPAAm brushes were grown by atom transfer radical polymerization, ATRP. Adapted from published procedures,³³ liquid silanization was carried out by immersing the substrate for 15 min in 80 mL of toluene, 0.6 mL of TEA, and 120 μL of silane initiator at room temperature. Stock solutions of CuBr/PMDETA/MeOH were prepared in the glovebox and diluted for each reaction. This approach reduced the error associated with weighing small quantities of CuBr powder in a glovebox for each synthesis. To prepare the stock solution, 1 g of CuBr (entire contents of ampule) was dissolved in 4.38 mL of PMDETA, and 37.5 mL of MeOH. When running individual reactions, 4.2 g of NIPAAm was dissolved in 20 mL of MeOH, followed by addition of 2 mL CuBr/PMDETA/MeOH stock solution. After stirring, 20 mL of DI water was added and a transparent pale green solution was obtained (the order of addition is important to avoid phase separation of NIPAAm, evident by a cloudy solution). The molar ratios corresponding to the masses given above were 100:1 NIPAAm/CuBr and 1:3 CuBr/PMDETA. The reaction time was 60 s at room temperature. pNIPAAm brush samples of equivalent thickness were synthesized simultaneously on the thick and the thin Si wafer. After polymerization, the wafers were sonicated in methanol for 5 min. A solution polymerization was also performed under the same reaction conditions and precursor concentrations described above, using a free initiator, ethyl 2-bromoisobutyrate, to characterize the molecular weight and grafting density of the pNIPAAm chains. A total of 2 mL of a hydroquinone/methanol solution (1 g/mL) was used to terminate the reaction

after 60 s. The free chains in solution were filtered with methanol and run through a basic alumina column to remove copper catalyst prior to GPC (PEG standard).

After sample preparation, the samples were stored in a nitrogen drybox for two months prior to the scheduled neutron beamtime. Some of the characterization measurements were performed several months after the synthesis; however, polymer thickness and LCST response, as measured by AFM and ellipsometry, remained constant under storage in a nitrogen drybox during this time period.

Preparation of Small-Molecule Drug Solutions. An Isoniazid concentration of 5 vol % (0.43 M) was chosen, because (1) the polymer brush LCST response (magnitude and temperature of collapse) was affected at this drug concentration and (2) this is the minimum concentration required to make a significant contribution to the scattered intensity. We measured two solutions containing Isoniazid. The first contained 5 vol % Isoniazid in D₂O to achieve maximum contrast between water and pNIPAAm (56.6 mg of Isoniazid per 1 g of D₂O; final pH 6.85; Isoniazid density of 1.244 g/mL, verified experimentally.) The second contained a mixture of H₂O and D₂O contrast matched to the polymer such that the contribution of Isoniazid could be isolated (61 mg of Isoniazid per 1 g of a H₂O/D₂O solution with H₂O mass fraction of 0.81; final pH 6.85). Hydrogen–deuterium (H-D) exchange was accounted for by considering the pK_a values of pNIPAAm and the Isoniazid. With a pK_a of 15 on the amide, essentially none of the pNIPAAm can undergo H-D exchange. The same is true for Isoniazid at a pH of 6.8 because the amide pK_a is ~ 15 , and the primary amine pK_a is greater than 27.³⁴ The neutron scattering length densities of D₂O, pNIPAAm, and Isoniazid are 6.33×10^{-6} , 7.4×10^{-7} , and $2.6 \times 10^{-6} \text{ \AA}^{-2}$ at mass densities of 1.1, 1.0, and 1.244 g/cm³ respectively.

Ellipsometry. Thicknesses of the ATRP initiator monolayer and pNIPAAm brushes (dry and hydrated) were measured on a Gaertner L116C Fixed Wavelength Variable Angle Stokes Ellipsometer (632.8 nm HeNe laser).²⁹ Dry ellipsometry measurements (15 measurements on a 4 in. wafer) were used to characterize the unhydrated thickness at room temperature. The hydrated brush thickness was measured with ellipsometry immediately after synthesis, and seven months later to verify sample integrity. The sample was immersed in a homemade cell with polystyrene windows at 70° angles (i.e., normal to incident and reflected laser light.)

AFM Measurements. Temperature-dependent brush thickness measurements were made using tapping-mode AFM (Multimode, Nanoscope IV controller, Veeco)²⁹ in the temperature range of 19.5 to 46 °C with a temperature-controlled fluid cell (Veeco).²⁹ pNIPAAm brushes on one piece of a thin Si wafer immersed in D₂O with or without added Isoniazid was used for all AFM measurements. After sealing the sample with a glass probe holder (MTFML, Veeco)²⁹ and an O-ring, solution was injected into the fluid cell. The AFM tip was silicon nitride (NP, Veeco),²⁹ which is hydrophilic and has negative surface charge.³⁵ Measurements were made on one sample immersed in pure D₂O and 5 vol % Isoniazid in D₂O, and the liquid cell was flushed with 5 mL of the drug solution between measurements. The temperature was controlled by a Nanoscope Heater Controller (HS-1, Veeco)²⁹ and maintained to within ± 0.1 °C at each temperature. AFM data were collected every 2 °C, and the sample temperature never exceeded the set point temperature. The samples were equilibrated at each temperature for 4–5 min prior to the

scan, which is sufficient time for the 200 μL fluid cell to reach thermal equilibrium. Thus, at these long equilibration times, we expect the thermal treatment to have no effect on the LCST. In order to measure polymer thickness, a step between pNIPAAm and the Si substrate was created by manually scratching the surface with a razor blade immediately prior to the measurement.³⁶ The step height values are calculated by averaging five randomly chosen step heights on a single image, and the error bars are obtained from the standard deviation of step height data.

Several sets of data were required to obtain high quality AFM scans in the presence of the drug. The force between the tip and sample is manually set, and needed to be reset several times between scans to correct for sticking and dragging caused by the tip–polymer and tip–drug interactions. These difficulties are common for probe-based measurements of soft matter, and limit our quantitative accuracy of thickness, especially at low temperature where the extremely soft brush with low modulus is easily compressed by the AFM tip with tapping force at the nN level.^{37,38}

Reflectometry. Neutron reflectometry, NR, measurements were performed on the NG7 Horizontal Neutron Reflectometer at the NIST Center for Neutron Research, NCNR, in Gaithersburg, MD, USA. NR measures the reflected neutron intensity as a function of incident angle, providing information about the atomic composition normal to the reflecting surface. Specifically, the reflectivity, R , is the ratio of reflected to incident intensity, measured as a function of wave vector, q ($q = 4\pi/\lambda \sin \theta$, where λ is the neutron wavelength and θ is the angle of incidence.) The dependence of R on q is determined by the SLD of the material normal to the reflecting surface. The SLD represents the sum of the coherent neutron scattering lengths of the constituent atoms divided by the molecular volume.

The neutron beam width was 35 mm, and the height was varied to maximize intensity while retaining a constant beam footprint and relative q resolution of $\delta q/q = 0.04$ throughout the measurement. Reflectivity data were collected at a fixed wavelength of 4.75 Å, within a q range of 0.008 to 0.161 Å^{−1} for the dry polymer brush, and within a q range of 0.008 to 0.125 Å^{−1} for the brush immersed in liquid. The dry pNIPAAm brush was measured at room temperature, and the brush immersed in D₂O/drug solution was measured at 23, 34, and 46 °C in a liquid cell using a Neslab circulating bath. The cell was equilibrated for 40 min at each temperature prior to the measurement. After measuring the brush in pure D₂O, the cell was flushed three times with solutions containing Isoniazid (i.e., D₂O/Isoniazid and D₂O/H₂O/Isoniazid).

The neutron data were reduced and analyzed using programs from the REFLPAK suite.³⁹ Data were corrected for background scattering and normalized against the main beam using Refred, and data were fit via Refit using the Parratt formalism.⁴⁰ SLD profiles were converted to pNIPAAm and Isoniazid volume fractions assuming additivity of volumes from the pNIPAAm/D₂O and Isoniazid/pNIPAAm/D₂O/H₂O measurements respectively.

X-ray reflectivity, XR, was used to confirm the dry brush thickness and contrast matching conditions for the H₂O/D₂O solution, by measuring the electron density profile of the dry polymer brush normal to the substrate. There exists a large electron density contrast between air and pNIPAAm, making XR a more accurate way to measure the dry pNIPAAm mass density than NR. XR measurements were collected at the NCNR on a Bruker D8 Advance reflectometer employing

CuK α radiation. The copper tube was operated at 1.6 kW, and the wavelength was 0.154 nm. There were two entrance slits before the sample with 0.1 mm height and 10 mm width. The beam height and width were fixed during the measurements. The XR data were reduced and analyzed using the REFLPAK suite.³⁹ The best fit to the dry polymer sample gave an X-ray SLD of $9.9 \times 10^{-6} \text{ Å}^{-2}$, corresponding to a polymer density of 1.03 g/cm³. The corresponding neutron SLD based on this mass density is $7.62 \times 10^{-7} \text{ Å}^{-2}$, yielding a contrast match concentration of 81% H₂O and 19% D₂O.

RESULTS AND DISCUSSION

Dry pNIPAAm Brush. The dry pNIPAAm brush consists of two, well-defined layers: a self-assembled monolayer (SAM) of initiator beneath a layer of pNIPAAm. In order to improve the quality of our NR fits of this multilayer system, we first characterized the thickness of our SAM [Cl₃Si(CH₂)₁₁OCOC-(CH₃)Br] using XR (Figure 2a.) The fit yields a thickness of 2.06 nm, consistent with the expected length of a densely packed linear molecule with 16 covalent bonds, and previous XR measurements on the same initiator molecule.⁴¹ The X-ray and neutron data for the dry pNIPAAm brush are illustrated in Figure 2a,b along with the corresponding fits. Based on an

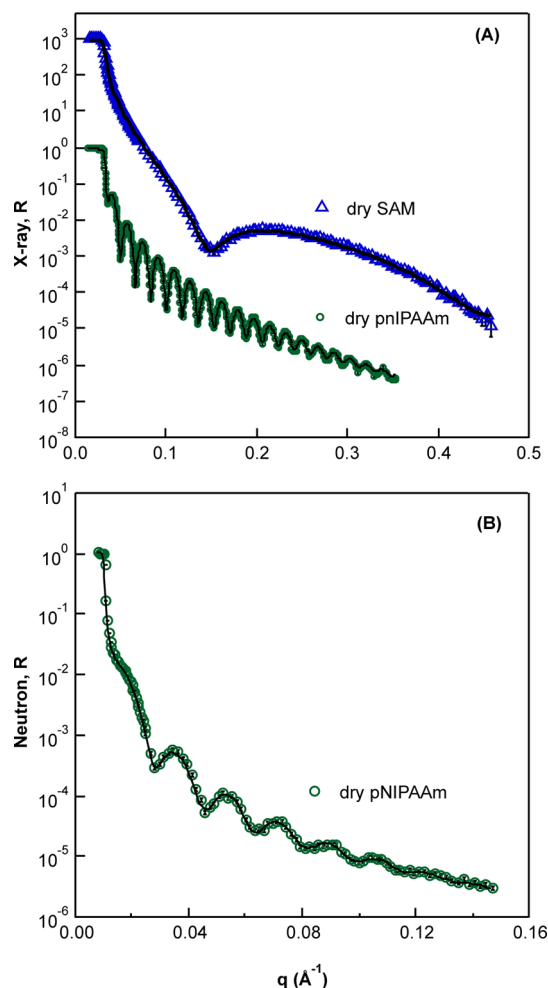


Figure 2. (A) Dry pNIPAAm and SAM X-ray reflectivity data and (B) dry pNIPAAm neutron reflectivity data. The SAM X-ray data are shifted on the Y axis for clarity. The error bars represent $\pm 1\sigma$ and are smaller than the size of the symbol for most data points.

average of the two measurements, the dry pNIPAAm brush is 35.4 nm thick. The thicknesses, root mean squared (RMS) roughness, and mass densities of the SAM and pNIPAAm layers are provided in Table 1. The mass densities of the SAM

Table 1. Fit Parameters for the Dry SAM and pNIPAAm Brush^a

	thickness (nm)	RMS roughness (nm)	density (g/cm ³)
SAM (XR)	2.06 ± 0.01	0.42 ± 0.01	1.30 ± 0.01
SAM + pNIPAAm (XR)	35.2 ± 0.1	0.72 ± 0.02	1.03 ± 0.01
SAM + pNIPAAm (NR)	35.6 ± 0.3	1.5 ± 0.2	0.9 ± 0.03

^aTo achieve the fits illustrated in Figure 2, the thickness of the SiO₂ layer varied from 0.5–1.2 nm with RMS roughness of 0.26–0.32 nm.

and pNIPAAm were calculated based on the SLDs returned by the fits. The NR roughness is twice as large as the XR roughness, and the value from XR is likely more accurate due to the larger q range of this measurement.

The dry samples described above were prepared on a thick silicon wafer. As described in the Experimental Details, we simultaneously prepared a polymer brush on a thin silicon wafer, and used this sample for AFM measurements. The AFM measurements returned a dry pNIPAAm thickness of ~30 nm, which is 5 nm thinner than the brush thickness measured by XR and NR. It is reasonable to expect the AFM measurement to give thicknesses lower than the actual brush thickness, because the scratched area may not be completely free of polymer and initiator. The RMS roughness of the dry polymer measured by AFM is ~0.43 nm, only ~0.3 nm smaller than the roughness measured by XR. Ellipsometry measurements on the two samples yielded equivalent Δ and ψ values, further confirming equivalent thicknesses (see the SI for additional information).

pNIPAAm Brush in D₂O and Isoniazid. Temperature-dependent AFM thickness measurements for the hydrated pNIPAAm brush are illustrated in Figure 3. We first consider the brush collapse in pure D₂O and then in a mixture of D₂O and Isoniazid. In pure D₂O, the brush collapses to approximately one-half its original thickness, and the collapse occurs between 30 and 38 °C. Previous studies of cross-linked gels and linear pNIPAAm chains in solution show that, when H₂O is replaced with D₂O, the offset of the LCST will increase by approximately 2 °C.^{42–44} Although chemically identical, D₂O and H₂O have different physical properties. Due to the larger mass of deuterium, the intermolecular vibrational bond energies are lowered, providing a stronger and therefore more stable network of hydrogen bonds.⁴⁵ This network results in a stiffening of pNIPAAm chains⁴³ that increases the LCST by several degrees. Because the LCST shift results from stronger intermolecular interactions between pNIPAAm and D₂O, we expect this behavior to qualitatively persist for pNIPAAm brushes, suggesting that the LCST range detected in Figure 3 occurs at higher temperature than would be expected in pure H₂O.

Next, we consider the effect of 5 vol % Isoniazid on the pNIPAAm brush collapse as measured by AFM. At low temperature, the presence of Isoniazid appears to decrease the thickness of the sample and increase the onset temperature of the transition. The ending temperature of the transition and the thickness at this temperature remain unaffected by the drug

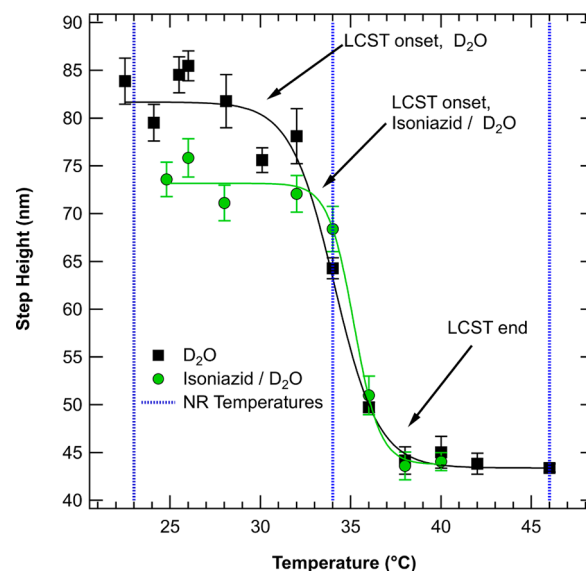


Figure 3. Hydrated AFM measurements for pNIPAAm immersed in D₂O and 5 vol % Isoniazid. Sigmoid best-fit lines are plotted to guide the eye. Because tip–pNIPAAm interactions vary between solutions, step heights cannot be compared between samples in D₂O and D₂O/Isoniazid, especially at low temperature. The error bars on the data represent $\pm 1\sigma$.

within the error of the measurement. As mentioned in the Experimental Details, the quantitative accuracy of the thickness at $T < \text{LCST}$ where the brush is soft is limited. In addition, the silicon nitride tip is hydrophilic and will therefore have stronger interactions with the polymer chains at $T < \text{LCST}$ and will exhibit decreasing attraction to the surface with increasing temperature. Although the thickness varied between the scans, the onset and end of the transition were reproducible over multiple scans despite varying force on the sample.

Using the AFM results to guide our choice of NR measurement temperatures, we measure hydrated samples with and without Isoniazid before (23 °C), during (34 °C) and after (46 °C) the brush collapse. The reflectivity data, R , are illustrated in Figure 4, where R is multiplied by q^4 to compensate for the q^{-4} decay (Fresnel law). SLD profiles with and without the drug are illustrated in Figure 5; the initiator layer is included as an independent layer in all the fits. As the polymer collapses with increasing temperature, features begin to emerge in the reflectivity data indicating the presence of well-defined interfaces (Figure 4). At 46 °C, both samples can be fit with a model that includes the initiator layer, and one layer of polymer. The only difference at this temperature between the sample with and without drug is thickness: the sample containing 5 vol % Isoniazid is ~10% thicker. According to the AFM data provided in Figure 3, the polymer has completely collapsed at this temperature, and therefore any variation in thickness cannot be attributed to a shift in the LCST caused by the additive. The total thicknesses (initiator and brush) returned by the fit are 41 ± 0.5 and 45 ± 0.5 nm without and with the drug, respectively. These thicknesses are within several nanometers of the AFM step height data in Figure 3.

Midway through the collapse (at 34 °C), the polymer cannot be fit with one layer. Instead, two layers are required in addition to the initiator layer: one layer of denser polymer brush near the substrate interface and one layer of less dense polymer

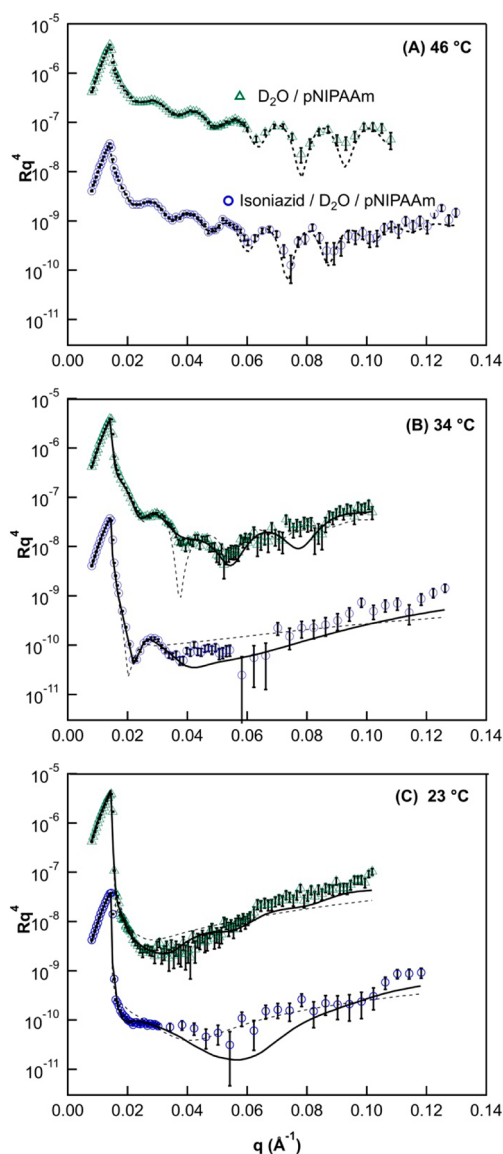


Figure 4. Neutron reflectivity $\times q^4$ as a function of q for pNIPAAm brush samples without and with 5 vol % Isoniazid at (a) 46, (b) 34, and (c) 23 °C. The data without Isoniazid are shifted vertically by a factor of 100 for clarity. Lines indicate fits using one layer (dashed) and two layers (solid) to describe the polymer brush. The error bars on the data represent $\pm 1\sigma$.

brush further away from the interface. This result is consistent with that of Yim and co-workers, who were the first to detect this vertical phase separation during the collapse of a pNIPAAm brush in D_2O .¹⁸ It is also consistent with predictions for pNIPAAm brushes made by Laloyaux and co-workers.⁴⁶ We detect vertical phase separation (i.e., bilayer collapse) in samples with and without Isoniazid; however, the layer of dense polymer near the substrate is thinner in the presence of Isoniazid. This could simply be the result of the additive increasing the overall thickness of the brush, or it could indicate that the polymer collapse is shifted to higher temperature in the presence of the drug.

Below the LCST (Figure 4c), the brush is fully extended, and the data are essentially featureless with and without Isoniazid. This indicates an extremely rough interface, resulting either from polydispersity of the polymer brush or variations in

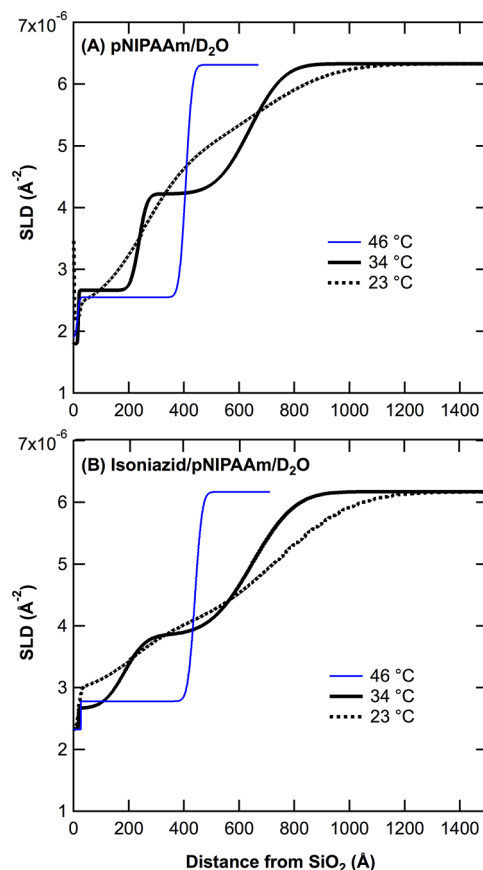


Figure 5. SLD profiles for (a) pNIPAAm/ D_2O and (b) Isoniazid/pNIPAAm/ D_2O at each temperature.

thickness that occurs over large lateral areas. We use conservation of mass to constrain the fit at 23 °C based on fits to the dry sample and the hydrated sample at high temperatures where the features are well-defined. We discovered that the quality of the fit at 23 °C can be significantly improved by including a layer of dense polymer at the substrate interface. This suggests that vertical phase separation persists at temperatures below the LCST, with or without the drug, an observation not predicted by theory.

As discussed in the introduction, Yim and co-workers showed that the magnitude of the collapse depends strongly on molecular weight and grafting density.¹⁶ According to NR, our sample collapses by a factor of ~ 1.6 , corresponding to intermediate grafting densities (~ 0.1 chains/ nm^2) and molecular weights (~ 100 kg/mol).¹⁶ Because of the small mass of pNIPAAm on a 4" wafer (~ 0.1 mg), it is difficult to measure the molecular weight of this sample directly. Therefore, we performed the identical polymerization in solution, yielding a M_w of 167 kg/mol with GPC, and a calculated grafting density of 0.12 chains/ nm^2 . For this calculation, we used a dry polymer density of 0.95 g/ cm^3 averaged from the XR and NR measurements reported in Table 1. We also use scaling theory to estimate the M_w and grafting density as 102 kg/mol and 0.17 chains/ nm^2 based on the hydrated and unhydrated thicknesses at room temperature and dry polymer density.³⁶ The calculated and experimental values correspond well to the expected values of Yim and co-workers for a pNIPAAm brush that collapses by a factor of nearly two.¹⁶ They also observe vertical phase separation in a pNIPAAm sample with similar molecular weight and grafting density on

SiO₂ and for pNIPAAm on gold substrates at the highest grafting density and lowest molecular weight.¹⁶ This cannot be attributed to polymer segmental adsorption, especially at such high grafting densities, and it remains unclear why vertical phase separation would persist at low temperatures where the brush is fully extended.

In order to determine the location and volume fraction profile of the Isoniazid, we measured a sample for which the water is contrast-matched to the polymer. The data, illustrated in Figure 6, are practically featureless, indicating that the drug is

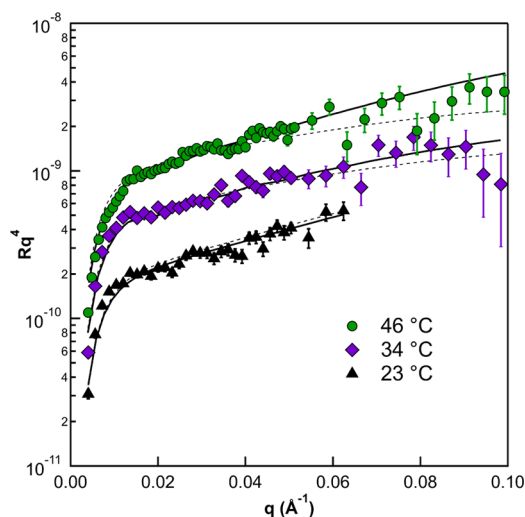


Figure 6. Neutron reflectivity $\times q^4$ as a function of q for Isoniazid/D₂O/H₂O/pNIPAAm, where the D₂O/H₂O is contrast matched to the polymer. The 34 and 46 °C data are shifted $\times 2.5$ and $\times 5$ on the y axis, respectively. Dashed lines indicate fits to an infinite layer of D₂O, H₂O, and 5 vol % Isoniazid, and solid lines indicate fits using one layer with thickness equal to the total thickness of the polymer. The error bars on the data represent $\pm 1\sigma$.

not strongly partitioned into any one section of the polymer brush. Even though the polymer density within several nanometers of the substrate has increased, the concentration of Isoniazid has not increased in this region to an extent detectable by the NR measurement. Moreover, the critical edge is absent because the critical q is lower than the lowest measured q value. However, we can use these data in combination with data collected from previous measurements to extract information about the volume fraction of the drug. Based on previous fits to this sample, we know the SLDs and thicknesses of the SiO₂, SAM and hydrated pNIPAAm layers with and without Isoniazid. To estimate the volume fraction of the drug, we start with a model that includes Si, SiO₂, SAM, and an infinite layer of D₂O/H₂O containing 5 vol % Isoniazid. Fits using this model are illustrated in Figure 6 by the dashed lines, and they do not accurately fit the data. At all temperatures, the models that best describe the data include either one layer with Isoniazid distributed homogeneously or a gradient model in which the Isoniazid concentration decreases toward the brush-water interface. In all cases, the fits were constrained to obey conservation of mass and to meet a fit quality criteria of $\chi^2 < 3.0$. We checked for concentration gradients in both directions (i.e., a higher concentration of Isoniazid near the substrate, or near the brush-water interface.) A one-layer model represents the minimum Isoniazid concentration gradient whereas a five-layer model represents the maximum gradient (Figure 7b).

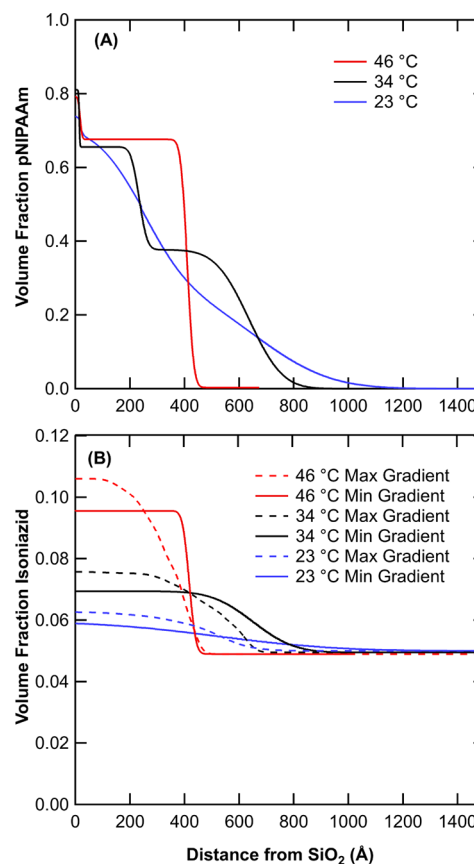


Figure 7. Volume fraction profiles for (A) pNIPAAm and (B) Isoniazid. The profiles in (A) and (B) are based on data from pNIPAAm/D₂O and pNIPAAm/D₂O/H₂O/Isoniazid respectively. Dashed lines represent the minimum concentration gradient (1 layer model), and solid lines represent the maximum gradient (5 layer model).

Regardless of the temperature or the number of layers, the fits return a SLD larger than that of the bulk 5 vol% Isoniazid solution, and we therefore interpret that the Isoniazid concentration is higher in the region occupied by the brush. The volume fraction profiles for pNIPAAm and Isoniazid are given in Figure 7.

At 23 °C where the brush is fully extended and rough, the reflectivity data are best described by models with an average Isoniazid concentration of 5.7 ± 0.5 vol %. The volume fraction profiles at 23 °C suggest that the polymer has a stronger affinity for Isoniazid than for water, even at $T < \text{LCST}$ where the polymer is hydrophilic. At the intermediate temperature of 34 °C, the vol % increases to 6.7 ± 0.8 and to 9.6 ± 1 at high temperature, 46 °C. (The average Isoniazid vol % is the concentration at 20 nm from the substrate interface in the minimum gradient profile, and the error bars are the deviation from this average in the maximum gradient profile.)

Together, these NR results indicate that (1) Isoniazid is not partitioned into well-defined layers in the polymer brush, (2) the concentration could be higher near the substrate than near the brush-water interface, and (3) the drug is not expelled from the polymer after collapse. In fact, the volume fraction of drug increases by a factor of ~ 1.6 while the volume occupied by the brush decreases by a factor of ~ 1.6 , implying that the total amount of drug inside the polymer is unchanged before and after collapse. This further suggests that, as the polymer chains

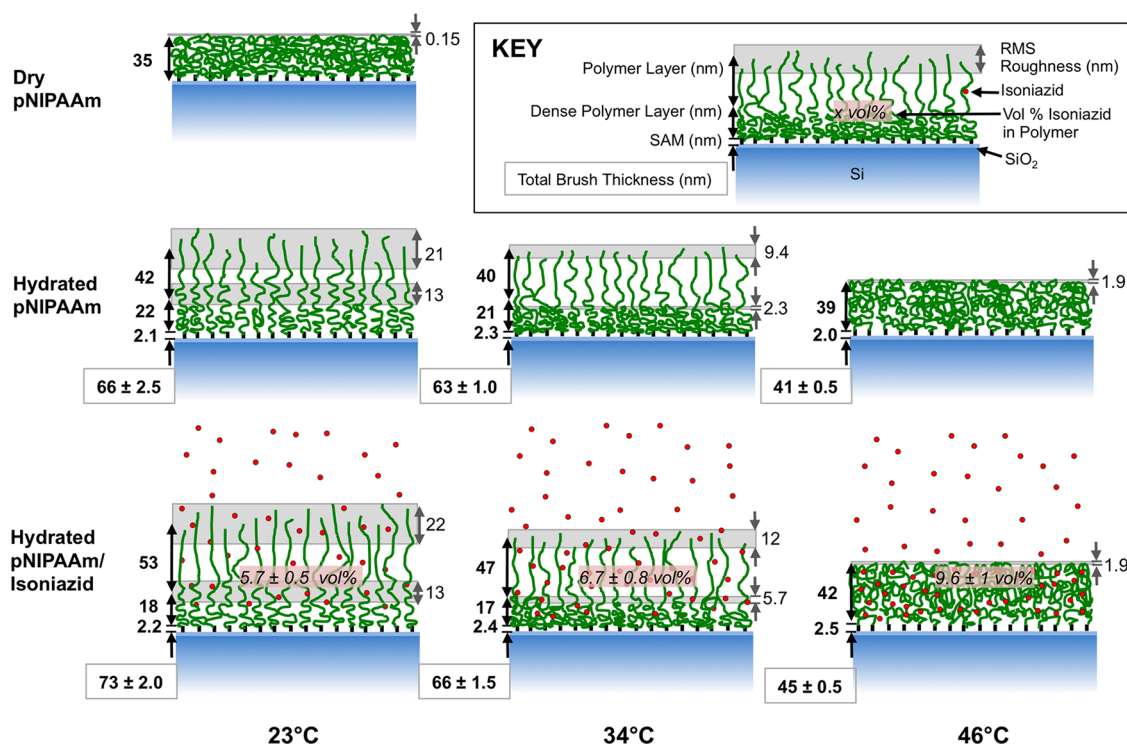


Figure 8. Schematic illustration of the samples investigated in this study. The polymer brush thickness and RMS roughness from NR are reported on the left and right side of each sample schematic. Isoniazid molecules are depicted by red circles, and the volume percent of Isoniazid inside the brush is provided at each temperature.

are collapsing and the polymer–water interactions are becoming weaker, the polymer–drug interactions remain strong enough that the drug stays in the polymer region. The increased drug concentration shows that some fraction of the hydrogen-bound water is replaced by hydrogen-bound Isoniazid. A summary of the brush collapse, including thickness and RMS roughness values returned by the fits, is provided in Figure 8.

The data show that the concentration of Isoniazid in the polymer is larger than the bulk at all measured temperatures. The fact that the drug remains inside the polymer region during collapse even though water is expelled, suggests relatively strong binding between the polymer and the drug. As discussed in the introduction, Gu and co-workers, and Xing and co-workers show FTIR evidence for hydrogen bonding between the pNIPAAm amide and the Isoniazid carbonyl on dry samples.^{27,28} The presence of bound drug molecules could increase the thickness, as seen at all temperatures, by increasing the persistence length of the chains, similar to the effect of replacing H₂O with D₂O, where the chains are stiffened due to stronger hydrogen bonds. This effect will also raise the LCST, as suggested by our AFM measurements.

The effect of additives that increase the hydrophilicity and ionic character of the system should also be considered. For example, hydrophilic and ionic copolymers have been shown to increase the LCST by increasing hydrophilicity.⁴⁷ In the case of copolymers, where the NIPAAm monomer is covalently bound to other monomers, some of the hydrophobic groups are directly replaced with hydrophilic groups, increasing the number of polymer–water interactions and delaying the LCST onset. Although a copolymer is not a small molecule additive, it could shed light on the current study. While Isoniazid is not replacing hydrophobic groups on the polymer

backbone like a copolymer, it is adding hydrophilic groups to the polymer chain by virtue of its tendency to bind to pNIPAAm. As mentioned in the introduction, hydrophilic ionic additives have been shown to increase the LCST.^{12,23} Based on the pH of our solution (6.85) and the pK_a values of the conjugate acids (1.8 for the amide, 3.5 for the pyridine, and 10.8 for the primary amine),²⁶ we expect some fraction of the Isoniazid to protonate at the primary amine and therefore to carry a single positive charge. It is therefore likely that electrostatic interactions will affect the LCST by changing the hydration sphere around pNIPAAm and the thickness by a “salting in” effect, pulling more water molecules into the polymer layer.

We also consider the possibility of binding between the hydrophobic isopropyl groups on pNIPAAm and the pyridine functional group of Isoniazid through hydrophobic interactions. It has been demonstrated that some surfactants (molecules with hydrophobic and hydrophilic groups) can increase the LCST by stabilizing the hydrophobic isopropyl group of pNIPAAm.^{19,48} They can also increase the expanded volume of pNIPAAm, similar to what we observe with Isoniazid at all temperatures, also by a “salting in” effect.⁴⁹ Although it is not a surfactant, the Isoniazid has hydrophobic and hydrophilic/ionic moieties, and it is therefore possible that it could stabilize the hydrophobic group of pNIPAAm, contributing to the observed increases in the LCST and the thickness. Coughlan and co-workers used FTIR to detect peak frequency shifts in coprecipitated mixtures of pNIPAAm and with various benzoate derivatives that suggested hydrophobic interactions between the pNIPAAm isopropyl group and the phenyl group of their additives.^{10,21}

We have compared our results to previous studies of pNIPAAm containing various additives; however, it is

important to note two key differences between our study and those described above. First, our experiments were performed on brushes as opposed to gels and linear chains in solution. Although pNIPAAm brushes, cross-linked gels and linear chains all undergo the same hydrophilic to hydrophobic transition at the LCST, the local environment is significantly different in the case of a brush. Chain stretching, a consequence of osmotic pressure, depends on grafting density and is a feature of the brush that sets it apart from gels and chains in solution. Second, because we are studying drug-loaded brushes instead of bulk polymer in solution, we use different characterization techniques. The LCST transition for gels and linear chains in solution is often monitored by differential scanning calorimetry, DSC, and cloud point measurements, which measure changes in enthalpy and optical transmittance, respectively. In contrast, we use AFM and NR, which probe tip–sample interactions and variations in neutron scattering length, respectively, to monitor spatial variations on the nanometer length scale.

CONCLUSIONS

We conclude that 5 vol % Isoniazid (0.43 mol/L) increases the thickness of a hydrated pNIPAAm brush before, during, and after collapse, and it is retained inside the brush at all measured temperatures. Based on NR measurements where water is contrast matched to the polymer brush, we find that the concentration of Isoniazid in the fully extended brush is ~14% larger than in the bulk solution, and the concentration in the collapsed brush is doubled compared to the bulk, even though water is expelled. These results show that the affinity of pNIPAAm for Isoniazid is larger than for water, even at $T < \text{LCST}$ where the brush is hydrophilic. Previous FTIR evidence from Gu and co-workers and Xing and co-workers on Isoniazid loaded in a copolymer of pNIPAAm and pAA reveals H-bonding between the Isoniazid and the polymer.^{27,28}

Consistent with previous NR measurements on pNIPAAm brushes without additives by Yim and co-workers, we detect vertical phase separation within the brush before and during the collapse.¹⁸ This vertical phase separation persists even when the drug is present; however, midway through the polymer collapse, the thickness of the collapsed polymer next to the substrate is smaller than the collapsed polymer in pure water. In combination with our AFM results, this could suggest that the Isoniazid delays the onset of the LCST. Based on previous studies of additives on pNIPAAm, an increased LCST would be expected for an additive that forms hydrogen bonds with pNIPAAm and increases the hydrophilicity of the polymer. For example, salicylate, which is structurally similar to Isoniazid, also increases the LCST of pNIPAAm in solution by 6–7 °C at 1 mol/L.²³

The extent of drug loading and distribution within the polymer, along with the effect on the LCST behavior, could have important implications for devices coated with this stimulus responsive polymer for applications in drug loading or gating. Based on the results of this study and previous results on pNIPAAm loaded with Isoniazid, diffusion out of a collapsed pNIPAAm brush would be inhibited by the interactions between Isoniazid and pNIPAAm. Thus, pNIPAAm might be more useful as a gating tool for Isoniazid and other drugs with similar chemical characteristics.

ASSOCIATED CONTENT

Supporting Information

Details on the ellipsometry measurements. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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