

Collapse and Swelling of Poly(*N*-isopropylacrylamide-*co*-sodium acrylate) Copolymer Brushes Grafted on a Flat SiO₂ Surface

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ABSTRACT: Poly(*N*-isopropylacrylamide-*co*-sodium acrylate) copolymer brushes grafted on SiO₂-coated quartz crystal surface were prepared with a surface-immobilized initiator. The collapse and swelling of the thermally sensitive copolymer brushes in water were studied with quartz crystal microbalance *in situ*. The frequency and dissipation changes with the temperature increasing in the range 20–38 °C indicate that the brushes undergo a continuous collapse transition. Our results show that the copolymer brushes collapse to a state where the brushes were prepared. A hysteresis was observed in the cooling process. Fourier transform infrared (FTIR) results revealed that the formation of some additional hydrogen bonds within the copolymer chains at their collapsed state is responsible for the hysteresis. ©2006 Wiley Periodicals, Inc. *J Polym Sci Part B: Polym Phys* 44: 770–778, 2006

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INTRODUCTION

It is known that protein chains can normally fold into their defined tertiary structure via a specific pathway,^{1,2} which might be determined by their primary structure.^{3,4} Protein chains generally contain hydrophobic, hydrophilic, and charged amino acid residues. The cooperation of the intra- and interchain hydrogen bonding, hydrophobic attraction, and electrostatic interaction leads to some complicated bioactive structures.¹ Because of the complexity in structure, protein folding as a fundamental issue in life science has long been a challenge. Different the-

ories have been developed to explain various properties of proteins from a biological point of view.^{2,5–7} Considering the macromolecular nature of protein chains, it is helpful to simplify the problem by using a synthetic amphiphilic copolymer as a model system to understand the folding.

Recent computer simulations on the coil-to-globule transition of amphiphilic copolymer chains composed of hydrophobic and hydrophilic units have demonstrated the effects of comonomer distribution on the folding of a single copolymer chain in dilute solution.^{8–11} Khokhlov et al. showed that for a chain with a globular protein-like structure where the soluble comonomer B was incorporated on the periphery of a collapsed A chain backbone, the chain would fold more easily than a copolymer with a random sequence.¹⁰ Moreover, the protein-like chain forms

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a more stable globule with higher chain density. The simulation suggested that such a chain could “memorize” or “inherit” some special functional properties of the parent structure in collapsed state where it was prepared. Timoshenko et al. also showed that a AB copolymer chain with a segmented comonomer distribution folds easier than a random copolymer chain with the same degree of amphiphilicity, and the former forms a more stable mesoglobules.¹¹

However, it is rather difficult to prepare a pair of AB copolymers with the same composition and same chain length but different comonomer distributions on the chain backbone. Using poly(*N*-isopropylacrylamide) (PNIPAM) with a lower critical solution temperature (LCST~32 °C), Tenhu et al. prepared PNIPAM-*g*-PEO copolymers at temperatures below and near the LCST of PNIPAM.¹² Their results showed that the copolymer chains prepared at different temperatures exhibited different LCST, indicating that the copolymer chains have quite different comonomer distributions. Following the same procedure, other protein-like copolymers were also prepared.^{13–17} All the experimental studies on the coil-to-globule transition of the copolymers support the results of computer simulation.

On the other hand, it is known that the interior of a biological cell where protein folding occurs is extraordinarily crowded. Studies on the coil-to-globule transition of amphiphilic polymer chains in a similar environment are much more important. In the present study, we directly grew poly(*N*-isopropylacrylamide-*co*-sodium acrylate) (PNIPAM-*co*-PNaA) on the sensor surface of quartz crystal microbalance (QCM) using a so-called “grafting from” strategy at a temperature much higher than the LCST of PNIPAM. Such grafted copolymer chains are expected to be protein-like and crowded on the surface. The collapse and swelling of such crowded chains were investigated *in situ* using QCM. Note that macroscopic phase separation, which usually occurs for the chains free in solution at a temperature above the LCST, can be avoided for the grafted chains. Meanwhile, the origin of the hysteresis occurring in the cooling process was studied with FTIR.

EXPERIMENTAL

Chemicals

N-isopropylacrylamid (NIPAM) from Aldrich was purified by recrystallization in a mixture of ben-

zene/*n*-hexane. Sodium acrylate (NaA) from Aldrich was used as received. *N,N'*-dimethylformamide (DMF) was dried with anhydrous CaSO₄ and distilled under reduced pressure. 4,4'-azobis(4-cyanovaleric acid) (ABCA), 3-(aminopropyl)-trimethoxysilane (APTMS), and 1,3-dicyclohexylcarbodiimide (DCC) were all from Acros and used as received.

The SiO₂-coated crystal surface was cleaned using Piranha solution composed of H₂O₂:H₂SO₄ (1:3), and blown dry with a stream of nitrogen gas. Several washes/dry cycles were performed until concordant frequencies were achieved. After the crystal was immersed in a 1 mM APTMS solution in ethanol for 40 min, it was washed with ethanol and water, blown with nitrogen, and baked at 120 °C in an oven for 30 min to complete the formation of Si—O bond. The APTMS modified surface was exposed to 40 mL solution in DMF containing 0.208 g of ABCA, 2.120 g of DCC, and 50 μL of pyridine as the catalyst, then the initiator (ABCA) was rooted on the SiO₂ surface. The reaction was carried out at room temperature for 8 h. Then, the crystal was immersed in an aqueous solution of 1.060 g of NIPAM, 0.035 g of NaA with one side covered with a protective casing made of Teflon. The grafting polymerization was conducted at 65 °C for 8 h under a nitrogen blanket. The crystal grafted with PNIPAM-*co*-PNaA was rinsed with Milli-Q water until stable frequencies were obtained, so that the chains physically absorbed were completely removed. Then, it was blown dry with nitrogen, and further dried in a vacuum oven at room temperature. The details can be found elsewhere.¹⁸

Parallely, PNIPAM-*co*-PNaA chains were also grafted on a two-side-polished silicon wafer with a diameter of 3.5 in. on the same conditions. The grafted chains were cleaved by hydrolysis at pH = 12.¹⁹ The apparent molecular mass (M_w) and radius of gyration ($\langle R_g \rangle$) of the grafted copolymer determined by static laser light scattering were 2×10^6 g/mol and 78 nm, respectively. The polydispersity (M_w/M_n) of the copolymer was evaluated to be 2.1 by dynamic laser light scattering.²⁰ The distance between the two anchoring sites was evaluated to be $d \sim 14$ nm. Since $2\langle R_g \rangle$ is much larger than d , the grafted copolymer chains are expected to form brushes. The crystal grafted with PNIPAM brushes had been immersed in water at 20 °C for at least 1 week before the QCM measurements, so that the brushes were sufficiently swollen.²¹

QCM-D Technique

Quartz crystal microbalance with dissipation monitoring (QCM-D) makes the time-resolved measurements of the changes in both frequency (f) and energy dissipation factor (D) possible.^{22–32} The QCM-D having an AT-cut quartz crystal with a fundamental resonant frequency of 5 MHz and a diameter of 14 mm was from Q-sense AB. The quartz crystal was mounted in a fluid cell with one side exposed to the solution. The constant (C) of the crystal used is 17.8 ng/cm² Hz, and the frequency shift is measurable to within ± 1 Hz in aqueous medium.

When the crystal is excited to oscillate in the thickness shear mode at its fundamental resonant frequency f_0 by applying a RF voltage across the electrodes near the resonant frequency, a small layer added to the electrodes induces a decrease in resonant frequency. If the added mass is evenly distributed and much smaller than the mass of the crystal, the shift in resonant frequency (Δf) is proportional to the layer mass (Δm). This relation was the so-called Sauerbrey equation.³³

$$\Delta m = -\frac{\rho l \Delta f}{f_0 n} \quad (1)$$

where ρ and l are the specific density and thickness of the quartz crystal, respectively, and $n = 1, 3, 5, \dots$. The dissipation factor is defined by $\Delta D = E_{\text{dissipated}}/2\pi E_{\text{stored}}$, where $E_{\text{dissipated}}$ and E_{stored} are the dissipated energy and stored energy during one oscillation. The measurement of ΔD is based on the fact that the voltage over the crystal decays exponentially as a damped sinusoidal when the driving power of a piezoelectric oscillator is switched off. By switching the driving voltage on and off periodically, we can simultaneously obtain a series of the changes of the resonant frequency and the dissipation factor.

In a Newtonian liquid, the frequency response of a quartz resonator can be quantitatively described by the Kanazawa-Gordon relation.³⁴

$$\Delta f = -n^{0.5} f_0^{1.5} (\eta_l \rho_l / \pi \mu_q \rho_q)^{0.5} \quad (2)$$

where f_0 is the fundamental frequency, n is the harmonic number, ρ_q and μ_q are the density and shear modulus of quartz, ρ_l and η_l are the density and viscosity of the liquid medium,

respectively. The dissipation response is given by,^{35,36}

$$\Delta D = 2(f_0/n)^{0.5} (\eta_l \rho_l / \pi \mu_q \rho_q)^{0.5} \quad (3)$$

The frequency and dissipation responses due to the grafting of the polymer chains on the surface of the quartz resonator can be obtained by removing the effects of the viscosity and density of water at different temperatures based on eqs 2 and 3. All the measurements were conducted with the uncertainty of solution temperature within ± 0.02 °C. Δf and ΔD values from the fundamental were not used because they were usually noisy due to the insufficient energy trapping.³⁷

FTIR Measurements

FTIR spectra were measured on a Nicolet Magna 750 spectrometer with a resolution of 4 cm⁻¹. In a typical experiment, 6 μ L of polymer solution in D₂O with a concentration of 0.055 g/mL was added to a cell between two KRS-5 crystals with a diameter of 32 mm and thickness of 3.5 mm. The space between the crystals is 20 μ m. The temperature of the cell holder was measured continuously by an electronic thermometer.

RESULTS AND DISCUSSION

Figure 1 shows the temperature dependence of frequency shift ($-\Delta f$) at $n = 3, 5, 7$ for PNIPAM-co-PNaA brushes in one heating-and-cooling cycle, which were obtained by subtracting the values for the bare crystal resonator from the corresponding values measured for the crystal grafted with the polymer chains. It is known that there are two kinds of water in the system: namely, the bound water and free water.^{38,39} The former is related to the conformation of the polymer chains. For a viscoelastic polymer layer on a quartz crystal, Δf is influenced by its thickness, density, storage and loss moduli.³² In the present study, the layer is much thinner than the quartz crystal, and the density of the layer slightly fluctuates since the polymer has a density close to water. Accordingly, the thickness and the loss modulus due to hydration or dehydration should have dominant effects on the frequency and dissipation. In the heating process, as temperature increases, $-\Delta f$ continuously decreases, indicating that the copolymer chains

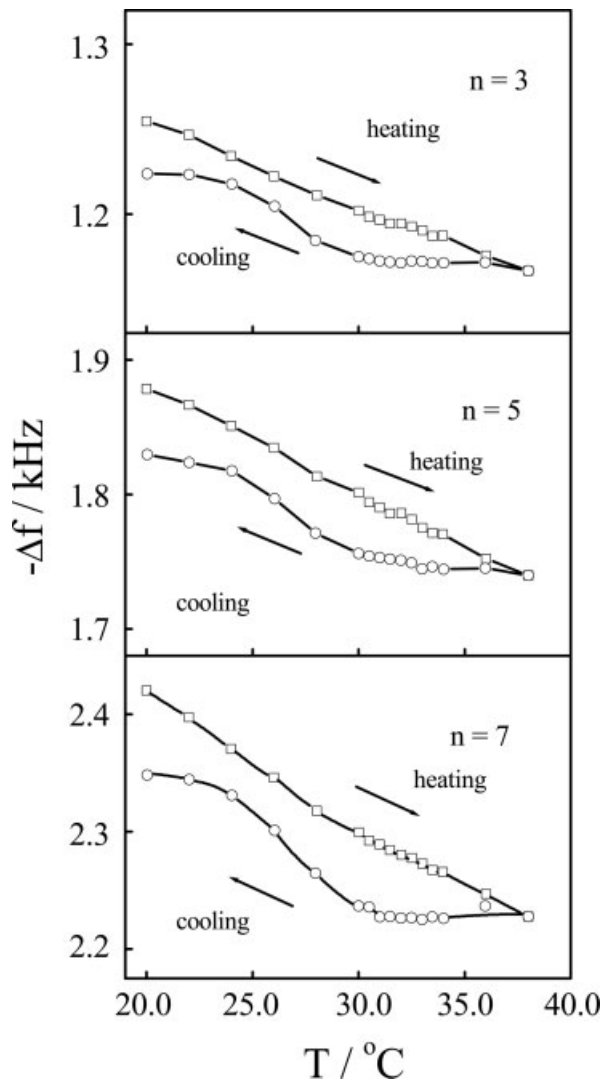


Figure 1. Temperature dependence of frequency shift ($-\Delta f$) of PNIPAM-co-PNAA brushes at $n = 3, 5, 7$.

gradually dehydrate. In the cooling process, $-\Delta f$ slowly increases with the temperature decreasing at a temperature above ~ 30 °C, indicating a gradual hydration. Particularly, at a temperature below ~ 30 °C, the sharp increase in $-\Delta f$ reveals that the chains are hydrated quickly at low temperatures. In the cooling process, the transition occurring at ~ 30 °C clearly indicates that the copolymer has a segmented structure, since the transition would happen at a temperature much higher than the LCST (~ 31 °C) of PNIPAM if the copolymer chain has a random structure.¹² On the other hand, the frequency shift at lower harmonics is larger than that at higher harmonics; this is because the sensing range of higher overtones is even closer to the

surface.^{36,37} Figure 1 also shows a hysteresis in the cooling process, and this will be discussed later.

Figure 2 shows the temperature dependence of the dissipation change (ΔD) at $n = 3, 5, 7$ in one heating-and-cooling circle, which were also obtained by subtracting the dissipation factors for the bare crystal resonator from the corresponding values measured for the crystal grafted with the polymer chains. It is known that the dissipation of a polymer layer on quartz resonator depends upon its structure. A dense and thin layer has a small dissipation of energy, a looser and thicker layer leads to a larger dissipation.⁴⁰ At lower temperatures, the copolymer chains are stretched with a larger dissipation factor. As temperature increases, the layer shrinks

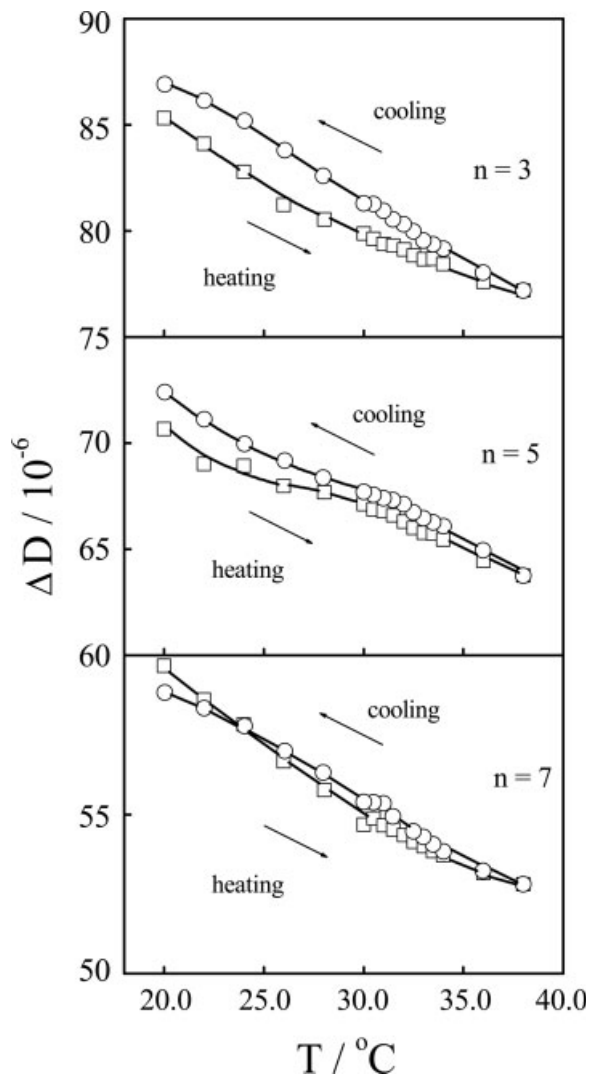


Figure 2. Temperature dependence of dissipation factor change (ΔD) of PNIPAM-co-PNAA brushes at $n = 3, 5, 7$.

because of the collapse of PNIPAM segments, leading to a smaller dissipation. Like the frequency shift, the energy dissipation also exhibits a continuous transition. The continuous transition of conformational change of polymer brushes was theoretically predicted in previous studies,^{41,42} and the results were supported by some experiments.^{22,43} The continuity was attributed to the nonuniformity of the densely grafted chains and the surface constraint. However, it should be noted that the collapse of PNIPAM-co-PNaA is more continuous than that of PNIPAM homopolymer brushes on a flat surface.¹⁸ This certainly arises from the structure of the copolymer. Khokhlov et al. predicted that when NIPAM monomers copolymerize with hydrophilic monomers at a temperature above the LCST of PNIPAM, they form a core-shell structure with the collapsed PNIPAM segments inside and the hydrophilic comonomers on the periphery of PNIPAM segments.¹⁰ Such segmented copolymer chains would unfold in a cool aqueous solution. However, they would readily refold into the original core-shell structure once the temperature is above the LCST of PNIPAM, which leads the collapse transition to be more continuous. This was experimentally confirmed by Siu et al. for poly(*N*-isopropylacrylamid-*co*-vinyl-pyrrolidone) copolymers.⁴⁴ In the present study, PNIPAM-co-PNaA was synthesized at 65 °C, much higher than the LCST of PNIPAM, the copolymer is expected to have a segmented structure. That's why the copolymer chains exhibit a smooth collapse transition.

In the cooling process, ΔD slowly increases as the temperature decreases, indicating a gradual swelling of the chains. In addition, ΔD value in the cooling process is always larger than that at the same temperature in the heating process. The same phenomenon was also observed in the case of PNIPAM brushes before,¹⁸ and it was attributed to the effect of tails. In the heating process, the tails of the chains are collapsed and folded inside. However, in the cooling process, the outer surface of the collapsed brushes would first swell leading to many flexible and swollen tails. Such tails would increase the energy dissipation of the layer. The conformational change of the brushes can be better viewed in terms of ΔD versus $-\Delta f$.

Figure 3 shows a typical Δf dependence of ΔD at $n = 3$. It clearly demonstrates that ΔD almost linearly decreases with $-\Delta f$ decreasing in the heating process, suggesting only one kinetic dur-

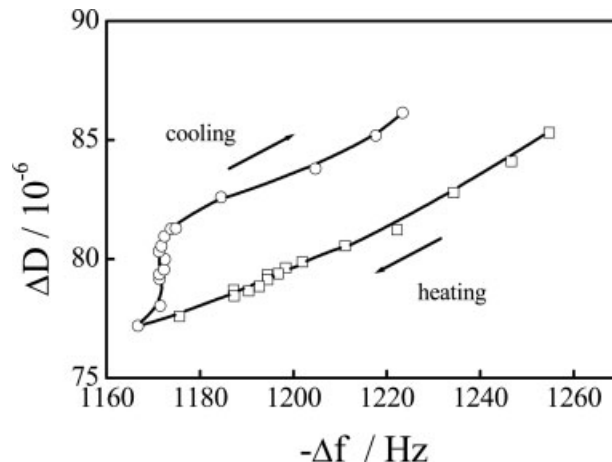


Figure 3. Typical frequency shift ($-\Delta f$) dependence of dissipation factor change (ΔD) of a PNIPAM-co-PNaA brushes at $n = 3$.

ing the collapse, that is the dehydration and collapse occur cooperatively. It has been shown that PNIPAM brushes exhibit a three-regime-kinetics collapse because of a poor cooperation between the dehydration and collapse.¹⁸ As we discussed earlier, PNIPAM-co-PNaA has a segmented structure where PNIPAM and PNaA segments distribute alternatively. As temperature increases, PNIPAM segments gradually collapse to the surface, while the hydrophilic PNaA segments tend to stay on the periphery of the collapsed PNIPAM segments, forming a core-shell structure. Since the strong hydrophilic interaction from the PNaA shell tends to pull the chains to water, the copolymer brushes are much less compact than collapsed PNIPAM homopolymer chains. The loose structure allows the detached water molecules to more quickly move into the bulk solution, so that the collapse and dehydration occur simultaneously. Therefore, ΔD is almost linearly related to Δf in the heating process.

In the cooling process, ΔD slightly changes with $-\Delta f$ at a temperature above ~ 30 °C, because the layer does not begin to swell. As the temperature decreases to ~ 30 °C, ΔD sharply increases with a slight increase in $-\Delta f$. As discussed earlier, this is due to the swelling of the PNIPAM segments near PNaA shell, forming a number of flexible and swollen tails that have a critical effect on ΔD . Further decrease of the temperature leads to a gradual increase of ΔD with the frequency shift, because the solvated chains stretch out.

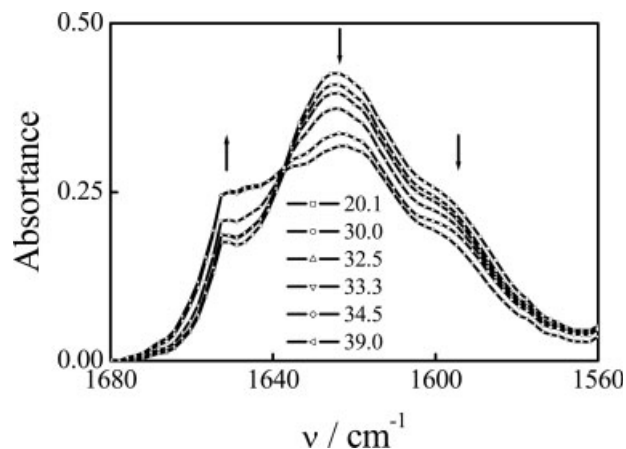


Figure 4. Temperature dependence of FTIR spectra of PNIPAM-*co*-PNaA in D₂O in the amide I region.

Figure 3 also shows the hysteresis in the cooling processes. Such a hysteresis was previously observed in the swelling of PNIPAM brushes¹⁸ and globule-to-coil transition of PNIPAM chains free in solution.⁴⁵ It has been attributed to the formation of additional hydrogen bonds formed within PNIPAM chains in their collapsed state, which cannot be completely destroyed in the cooling process, because water is not a good solvent yet when the temperature is near the LCST. Such additional hydrogen bonds were actually studied by Maeda et al. though they did not correlate it to the hysteresis.^{46–48} After measuring the IR spectral changes of PNIPAM and NIPAM-*co*-AA chains in aqueous solution during the phase transition, Maeda et al. found that as the temperature increases over the LCST, the intensities of the bands at 1650 cm⁻¹ assigned to the C=O group bound to H–N group through hydrogen bonding increase, while the intensity of the band assigned to the C=O group bound to water (1625 cm⁻¹) decreases. The facts suggest that the contact and overlapping of the polymer chains in the collapsed state result in some “new” hydrogen bonds or the so-called additional hydrogen bonds. In the present study, the FTIR spectra of PNIPAM-*co*-PNaA chains in water as a function of temperature were also examined. Following a similar procedure described earlier,^{46–48} D₂O was used instead of H₂O in the experiments, so that the O–H bending band of water around 1640 cm⁻¹, which overlaps with the amide I band of PNIPAM moiety, was masked. Another reason for using D₂O instead of H₂O is that a hydrogen bond in D₂O is stronger than that in H₂O though their lengths are close.⁴⁹

Figure 4 shows the FTIR spectra of PNIPAM-*co*-PNaA in the range 1680–1560 cm⁻¹ at 20.1, 30.0, 32.5, 33.3, 34.5 and 39.0 °C in the heating process. The bands centered at 1625 and 1650 cm⁻¹ are assigned to the C=O groups in >C=O···D–O and >C=O···H–N< species,^{46–48} respectively. The band at 1590 cm⁻¹ is ascribed to the C=O stretching of carboxylate. As temperature increases, PNIPAM segments become hydrophobic, the number of hydrogen bonds between water and C=O decreases,^{38,39} leading to the decreases in the intensities of the bands at 1625 and 1590 cm⁻¹. On the other hand, the collapse makes the formation of additional hydrogen bonds between H–N< and O=C< possible because they are closer to each other, so that the intensity of the band at 1650 cm⁻¹ increases with the temperature.

Assuming that each C=O species have an equal molar fraction, and using a nonlinear curve fitting after separating the amide I region into three Gaussian peaks, we evaluated the molar fraction of the additional hydrogen bonds. Figure 5 shows that the temperature dependence of the molar fraction (f_A) of the additional hydrogen bonds in the heating and cooling processes. f_A slightly decreases at a temperature below the LCST of PNIPAM in either heating or cooling process. It is known that the concentration of the copolymer solution used for FTIR measurements was high as 5.5×10^{-2} g/mL. The copolymer chains already overlap at such a concentration, and some additional hydrogen bonds are expected to form at a temperature below the LCST. As the temperature increases,

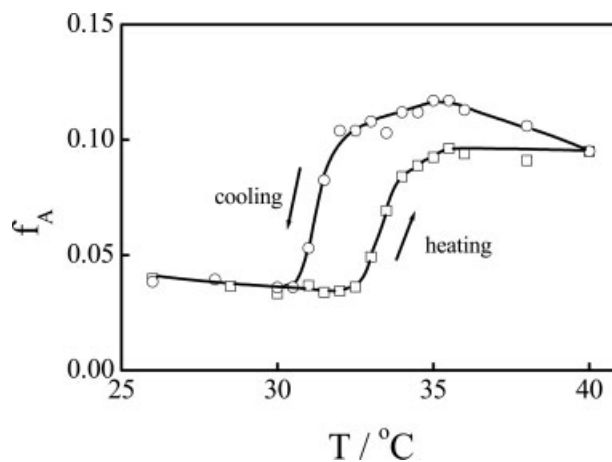


Figure 5. Temperature dependence of the molar fraction of the additional hydrogen bonds in the heating and cooling processes.

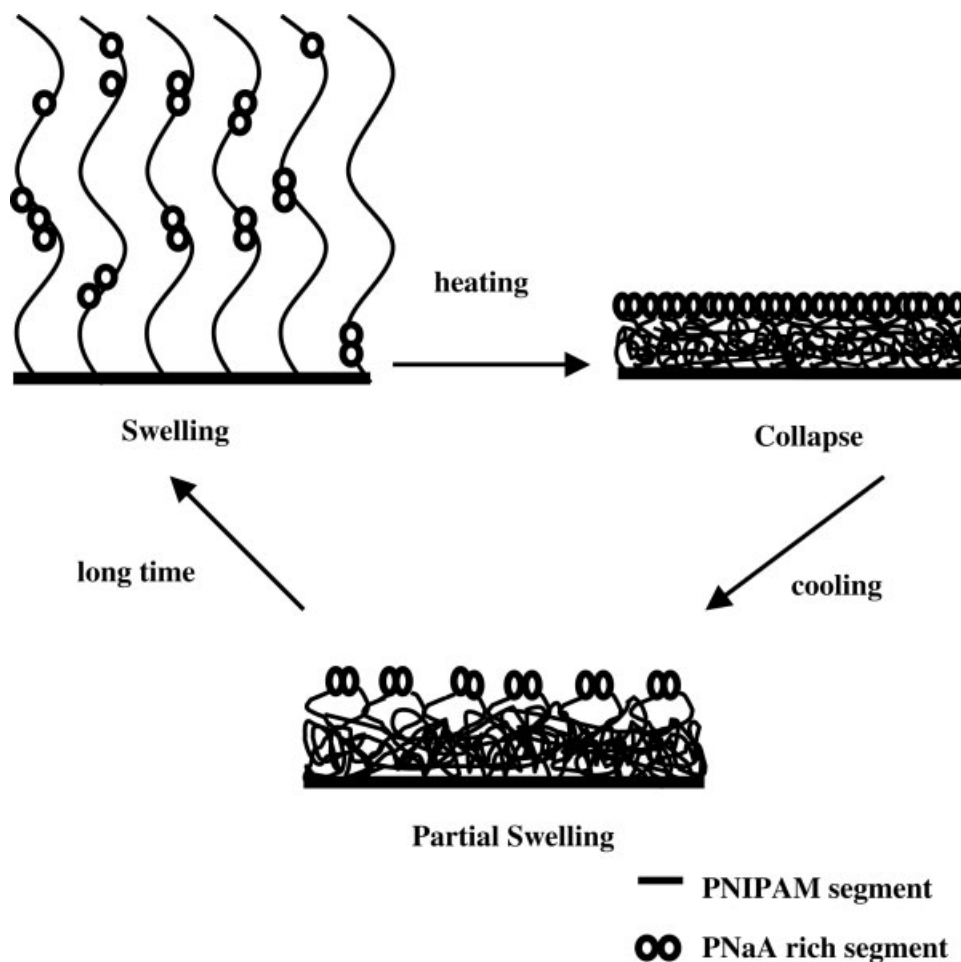


Figure 6. Schematic representation of the collapse and swelling of PNIPAM-co-PNaA brushes in water.

the chains would shrink reducing the overlapping. Thus, the fraction of additional hydrogen bonds decreases.⁵⁰ When the temperature reaches the LCST, the aggregation of the collapsing chains leads to more intra- and interchain overlapping, and the population of the additional hydrogen bonds increases. At a temperature above the LCST, the collapsed chains are more “frozen,” further overlapping almost stops, so that f_A only slightly increases. However, even in the fully collapsed state, f_A is less than $\sim 12\%$, that is most C=O groups are free from the formation of the additional hydrogen bonds. That’s why the chains can reversibly unfold into coils when the solution is cooled down.

Figure 5 also shows a hysteresis in the cooling process. It is known that f_A value in the cooling process is generally larger than that in the heating process at the same temperature unless the temperature is lower than ~ 30 °C, clearly

indicates that the hysteresis is due to the additional hydrogen bonds formed in the heating process. Particularly, even at a temperature above the LCST, the cooling leads to a f_A higher than that in the heating process. It is known that the collapsed chains have already overlapped at a temperature above the LCST. A collapsed chain is not swollen as whole until the temperature is decreased to ~ 30 °C. However, the segments of the chains between two knotting sites become movable, leading to more overlapping. Accordingly, more additional hydrogen bonds form in the collapsed chains. In short, the FTIR spectra clearly indicate that the hysteresis in the cooling process is due to the incomplete removal of the additional hydrogen bonds. The formation of the additional hydrogen bonding can also explain the hysteresis in the globule-to-coil transition of individual PNIPAM homopolymer chains⁴⁵ and that in the dissolution of

aggregated PNIPAM chains in aqueous solutions.⁵¹ The collapse and swelling of the grafted PNIPAM-co-PNAA chains are schematically illustrated in Figure 6.

CONCLUSIONS

We prepared thermally-sensitive PNIPAM-co-PNAA copolymer brushes on SiO₂-coated sensor surface of a QCM, and investigated the collapse and swelling of the brushes *in situ*. The brushes can refold to the state where the chains were prepared. This is consistent with the theoretical prediction for the folding of protein-like polymers. On the other hand, FTIR studies reveal the formation of additional hydrogen bonds in the collapsed state of the copolymer chains, indicating that the hysteresis in the cooling process is due to the incomplete removal of additional hydrogen bonds.

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