Use of Nonradiative Energy Transfer To Explore Interpolymer and Polymer-Solute Interactions in Aqueous Solutions of Poly(*N*-isopropylacrylamide)

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ABSTRACT: *N*-Isopropylacrylamide (NIPAAM) was copolymerized with small quantities of 9-fluorenylmethyl acrylate to yield labeled poly(*N*-isopropylacrylamide) (PNIPAAM) for use in nonradiative energy transfer (NRET) experiments with various pyrene derivatives in aqueous solution. Microcalorimetry and cloud point measurements of the lower critical solution temperatures (LCST) of these copolymers showed very slight depressions in the LCST as compared to that of the homopolymer. NRET investigations showed no energy transfer from fluorene-labeled PNIPAAM to free pyrene or to PNIPAAM-bound pyrene in mixed aqueous solutions at 24.5 °C in contrast to literature reports of similar work done with (hydroxypropyl)-cellulose. NRET was observed between fluorene-labeled PNIPAAM and amphiphiles with pyrene terminally bound to their hydrocarbon tails. NRET was much more efficient for a quaternary ammonium amphiphile than for a zwitterionic sulfobetaine-based surfactant.

Introduction

The use of nonradiative energy transfer (NRET)[1] to study synthetic polymers has been reviewed by Morawetz;[2] more recent reports[3-12] of such applications are available. This method allows estimation of distances within[4,9] or between polymers[5,6,8-10] or between a polymer and a cosolute[3,7,8] through measurement of energy transfer between donors and acceptors attached to the sites of interest. By definition, the emission spectrum of a donor overlaps the absorption spectrum of the corresponding acceptor, and excitation of the donor fluorophore leads to energy transfer and subsequent emission of fluorescence from the corresponding acceptor if it is nearby. Each donor-acceptor pair can be assigned a characteristic Förster distance (R_0) at which there is a 50% probability for NRET versus other routes for deactivation of the excited donor state.

We have investigated the interaction of poly(*N*-isopropylacrylamide) (PNIPAAM) with a variety of cosolutes in aqueous solution.[13-18] Complexation of PNIPAAM with surfactants was shown[15-17] to be very sensitive to aggregate morphology, head group identity, and length of the hydrophobic tail, as shown by changes in the lower critical solution temperature (LCST) of PNIPAAM and in surfactant association. Fluorescence techniques were used to monitor changes in environmental micropolarity using free[16] and covalently-bound[17] probes. We have now extended such investigations to involve NRET. As a number of our previous studies utilized covalently-bound pyrene derivatives, we chose fluorene as the corresponding donor.[8,10,11] This acceptor-donor pair has an R_0 of 37 Å.[10,11]

We have synthesized and characterized PNIPAAM derivatives with pendant fluorene and pyrene moieties. The first NRET study we discuss involves the fluorene-labeled polymer and free pyrene, a probe employed in much of our earlier work.[13-16,18] Subsequently, NRET experiments using PNIPAAM chains separately labeled with pyrene and with fluorene are presented. This examination of intermolecular association will be compared to similar investigations using the same donor-acceptor pair for (hydroxypropyl)cellulose (HPC),[8] which also exhibits an LCST in aqueous solution, and using the naphthalene/pyrene pair for PNIPAAM.[9] Finally, the interaction of fluorene-labeled PNIPAAM with two pyrene-tailed amphiphiles will be explored. The amphiphiles selected for this study are structural analogs of Zwittergent 3-12 and dodecyltrimethylammonium bromide (DTAB), respectively. We have previously studied[17] the interactions of these surfactants with PNIPAAM in aqueous solution by a combination of fluorescence emission and microcalorimetric techniques.

Experimental Section

Materials. 9-Fluorenemethanol (99%), tetrahydrofuran (THF, HPLC grade), triethylamine (Gold Label), acryloyl chloride (98%), benzene (spectrophotometric grade), pyrene, chloroform (HPLC grade), hexane (HPLC grade), *p*-methoxyphenol, and methanol (HPLC grade) were obtained from Aldrich Chemical Co. Acetone, sodium carbonate, and hydrochloric acid (HCl) were products of Fisher Chemical Co. Magnesium sulfate and ethyl ether were purchased from Mallinckrodt. The sources and purifications of *N*-isopropylacrylamide (NIPAAM) and AIBN were previously given.[13] (1-Pyrenylundecyl)trimethylammonium iodide (C11PN+) and the inner salt 3-(dimethyl(1-pyrenylundecyl)ammonium)propanesulfonate (ZwPy), and 1-pyrenemethanol were used as received from Molecular Probes, Inc.

Synthesis. 9-Fluorenylmethyl Acrylate. Nitrogen was bubbled through a solution of 9-fluorenemethanol (9.81 g, 50 mmol) in THF (350 mL) at 0 °C with stirring. Triethylamine (9.8 mL, 70 mmol) was then added by syringe. Afterward, acryloyl chloride (6.0 mL, 74 mmol) in THF (35 mL) was added dropwise over 10 min with magnetic stirring. Stirring followed for 31 h under nitrogen, allowing the system to gradually reach room temperature. Triethylamine hydrochloride was filtered off, and the solvent was removed on the rotary evaporator. The resulting oil was dissolved in a mixture of water (100 mL), ether (200 mL), and HCl (ca. 2.4 N, 100 mL). The organic layer was washed with 10% w/v sodium carbonate solution (100 mL) and then with water until the pH of the washings was neutral. The ether layer was dried with magnesium sulfate and filtered, and the solvent was removed on the rotary evaporator to leave a crude solid. The product repeatedly polymerized to insoluble particles upon attempted recrystallization even in the dark. Therefore, *p*-methoxyphenol (92 mg) was added as an inhibitor, and the remaining crystals were dissolved in methanol and filtered without polymerization. The filtrate was ultimately vacuum dried to yield ca. 4 g (ca. 33% yield) of crystalline product. Anal. Calcd for $C_{17}H_{14}O_2$: C, 81.6; H, 5.6. Found: C, 81.4; H, 5.7. ^{1}H NMR (300

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MHz, $CDCl_3$): δ 7.8 (2 H, d), 7.6 (2 H, d), 7.4 (4 H, m), 6.5 (1 H, m), 6.3 (1 H, m), 5.9 (1 H, m), 4.5 (2 H, d), 4.3 (1 H, t). Signals were also observed for *p*-methoxyphenol. IR ($CHCl_3$-cast film): 3430 (br), 3070, 3040, 2950, 2900, 1950, 1930, 1730, 1640, 1620, 1520, 1480, 1460, 1420, 1380, 1300, 1280, 1190, 1110, 1060, 990, 920, 820, 740 cm^{-1}.

1-Pyrenylmethyl Acrylate. 1-Pyrenemethanol (0.919 g, 3.96 mmol) was dissolved in THF (150 mL) and cooled to ca. 0 °C while bubbling nitrogen through the solution. Triethylamine (0.69 mL, 4.96 mmol) was added by syringe. Afterward, acryloyl chloride (0.41 mL, 4.96 mmol) in THF (15 mL) was added dropwise over 20 min with magnetic stirring. Stirring followed for 28 h under nitrogen, allowing the system to gradually reach room temperature. The triethylamine hydrochloride was filtered off, and the solvent was removed on the rotary evaporator. The crude solid was vacuum dried, triturated with methanol, filtered, and vacuum dried again. The reaction and workup were done in the absence of light. The off-white crystals (0.18 g, 16% yield) did not melt but polymerized upon heating. ^1H NMR (300 MHz, $CDCl_3$): δ 8.2 (9 H, m), 6.48 (1 H, m), 6.20 (1 H, m), 5.94 (2 H, s), 5.85 (1 H, m). IR ($CHCl_3$-cast film): 3050, 2960, 2930, 2860, 1750, 1660, 1610, 1470, 1415, 1380, 1300, 1270, 1190, 1070, 1050, 970, 850, 820 cm^{-1}.

Polymerizations. Nitrogen was bubbled for 15 min through a solution of recrystallized NIPAAM (ca. 5 g),[13] 9-fluorenylmethyl acrylate (monomer feeds of 10 and 105 mg (respectively 0.08 and 0.93 mol %)), and recrystallized AIBN[13] (ca. 90 mg, ca. 1.2 mol %) in benzene (100 mL). The polymerization was done under nitrogen while stirring the flask in an oil bath at ca. 50 °C for 24 h. The benzene was then removed on the rotary evaporator, and the resulting solid was dissolved in chloroform (100 mL). Upon precipitation in hexane (ca. 1000 mL), the polymer was filtered and vacuum dried (ca. 85% yields). Copolymer compositions were determined by UV spectroscopy on solutions of the copolymers in methanol. The extinction coefficient of the 9-fluorenylmethyl acrylate residues in the copolymer was assumed to be that of 9-fluorenemethanol (ϵ_{300} = 6300).[8] The copolymers respectively contained 0.05 ± 0.01 and 0.42 ± 0.04 mol % of the fluorene-labeled repeating unit.

A similar procedure was followed to synthesize the pyrene-labeled copolymer except that the comonomer 1-pyrenylmethyl acrylate (monomer feed of 22 mg, 0.17 mol %) was used. The extinction coefficient of the 1-pyrenylmethyl acrylate units was assumed to be that of 1-ethylpyrene (log ϵ_{343} = 4.6).[19] UV spectroscopy indicated that the copolymer carried 0.06 ± 0.01 mol % of the pyrene-labeled repeating units.

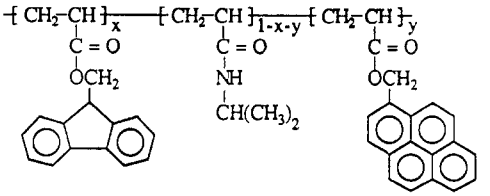
Gel permeation chromatography (DMF) indicated peak molecular weights of ca. 580 000; molecular weight distributions of the homopolymer and all the copolymers were indistinguishable.

Sample Preparation. PNIPAAM was studied at a concentration of 0.40 mg/mL unless indicated otherwise. Stock solutions of concentration 4.00 mg/mL were prepared through dissolution of the polymer in distilled water with 0.1% w/v sodium azide for several days. Refrigeration was required to obtain optically clear solutions of the more highly fluorene-labeled polymer. Aliquots (0.20 mL) of these polymer solutions were diluted to 2.00 mL with distilled water. For solutions involving pyrene, microliters of a millimolar solution of the probe in acetone were added to sample vials with subsequent evaporation of the acetone prior to the addition of the polymer solution. For solutions containing C11PN+ and ZwPy, the same procedure was followed, but methanol was substituted for acetone. For polymer–polymer mixtures, aqueous solutions of the polymers were mixed. All samples were vortexed (Genie, Fisher) prior to spectral measurements. Measurements were continued until steady values were obtained.

Measurements. Infrared spectra were obtained on films cast from chloroform on NaCl plates with a Perkin-Elmer 1320 infrared spectrophotometer. NMR spectra were obtained on a Varian XL-300 spectrometer. Gel permeation chromatography was performed with a Waters M45 solvent pump coupled to an R410 differential refractometer and a Hewlett-Packard 3380A recorder. DMP (HPLC grade, Aldrich) was eluted at 1.0 mL/min through three Waters μBondagel columns (E-1000, E-500, E-125). Polystyrene standards (Polysciences) were used for calibration; molecular weights are thus estimated as those of polystyrenes of equivalent elution volume. Copolymer composition and optical density (OD) measurements (500 nm) were acquired on a Beckman DU-7 spectrophometer with a water-jacketed cell holder coupled with a Lauda RM-6 circulating bath, monitored by an Omega 450-ATH thermistor thermometer. LCSTs were measured as previously described.[13]

A Perkin-Elmer MPF-66 fluorescence spectrophotometer was applied for measuring NRET using the temperature control system above. Emission and excitation spectra were obtained at 24.5 ± 0.1 °C using 5-nm slit widths unless otherwise indicated. The wavelengths of excitation and emission are indicated in the text and figure captions.

Chart I



Sample	x	y
A	0	0
B	5×10^{-4}	0
C	4.2×10^{-3}	0
D	0	6×10^{-4}

Results and Discussion

Synthesis and Characterization. Synthesis. 9-Fluorenylmethyl acrylate (9-FA) was synthesized by reaction of 9-fluorenemethanol and acryloyl chloride in THF. Elemental, infrared, and ^1H NMR analyses were consistent with the expected structure (Chart I). Purification of 9-FA was complicated by its apparent facile polymerization. We did not analyze the side product carefully, but its insolubility (in water and a wide range of organic solvents) and the absence of vinyl protons in the NMR spectra of suspensions suggest polymerization, perhaps with cross-linking. We were able to inhibit this reaction by the addition of *p*-methoxyphenol and with the stringent absence of light. 1-Pyrenylmethyl acrylate (1-PA) was constructed by reacting 1-pyrenemethanol and acryloyl chloride in THF; IR and NMR spectra were consistent with the expected structure of this compound.

All polymerizations were done in benzene and initiated by AIBN (Table I). We adjusted the AIBN concentration such that the resulting copolymers had molecular weights similar to that obtained previously with the NIPAAM homopolymer (Table I, sample A). The molecular weight distributions of all of the samples used in this work were indistinguishable. Table I summarizes the characterization of these samples: the fluorene copolymers carry 1 fluorene unit every 2000 and 240 repeating units (samples B and C, respectively), and the pyrene copolymer (sample D) bears approximately 1 fluorophore per 1700 units.

LCST Transition. The LCST observed in aqueous solutions of PNIPAAM results from a balance of hydrogen-bonding and hydrophobic effects.[13,14,17] We have found this transition to be extremely sensitive to polymer structure;[13-18] variations in molecular weight or polydispersity or the incorporation of small amounts (<2 mol %) of *N*-hexadecylacrylamide comonomers[18] has been observed to lead to substantial differences in the LCST behavior of PNIPAAM. With less than one fluorophore per polymer chain for samples B and D (Table I), we hoped to avoid significant changes in the aqueous solution behavior[8,21] of the NIPAAM homopolymer. We examined the behavior of each copolymer sample by microcalorim-

Table I
Characterization of PNIPAAM Samples

sample	fluorene (mol %)[a]	pyrene (mol %)[b]	fluorophores per chain[c]	cloud point (°C)[d]	microcalorimetry[e] LCST (°C)	$\Delta T_{1/2}$ (°C)	ΔH^f	ΔC_p^g
A[h]	0	0	0	32.2	32.4	0.9	13	13
B	0.05	0	0.5	31.4	32.0	1.2	14	9
C	0.42	0	4.2	29.4	30.7/31.7	2.6	15	5
D	0	0.06	0.6	31.5	32.1/32.6	1.5	13	7

[a] Content of 9-FA as determined by ultraviolet spectroscopy. [b] Content of 1-PA as determined by ultraviolet spectroscopy. [c] Number-average value based on an assumed number-average dp of 1000. [d] Obtained from the temperature of the onset of increased optical density.[13] [e] Technique discussed elsewhere.[13] [f] Calorimetric enthalpy of endotherm (cal (g of polymer)$^{-1}$). [g] Peak height cal °C^{-1} (g of polymer)$^{-1}$. [h] Synthesis and characterization reported previously.[13]

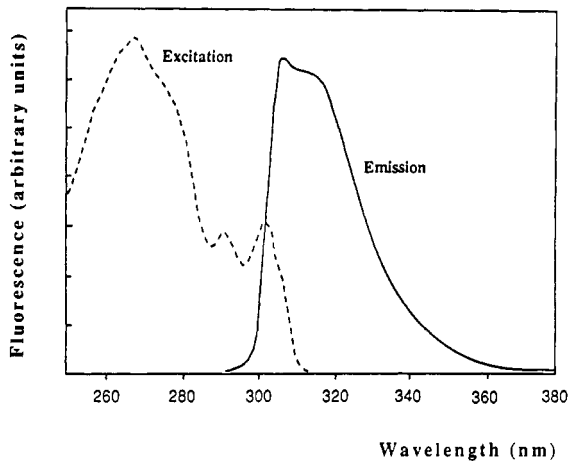


Figure 1. Emission (—) and excitation (- - -) spectra for an aqueous solution of PNIPAAM-C (0.40 mg/mL, 24.5 °C). Excitation was done at 269.2 nm, and emission was monitored at 305 nm.

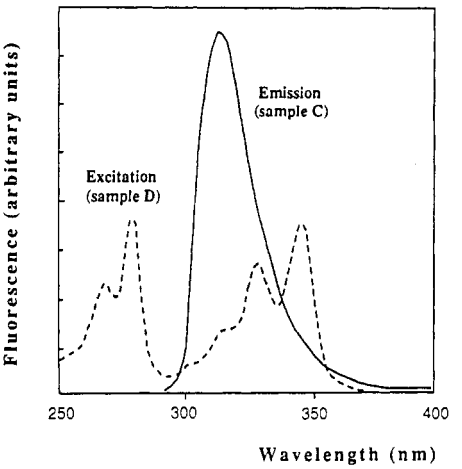


Figure 2. Overlap between the emission spectrum of PNIPAAM-C (—) (3.6 mg/mL in water) and the excitation spectrum of PNIPAAM-D (- - -) (0.40 mg/mL in water) at 24.5 °C.

etry and cloud point measurements. As Table I indicates, the LCST is slightly depressed upon copolymerization of NIPAAM with 9-FA or 1-PA; moreover, the LCST transition width increases and the calorimetric peak height decreases as the content of the comonomer is raised.

Fluorescence. Figure 1 shows the excitation and emission spectra for fluorene-labeled PNIPAAM-C (0.40 mg/mL) in aqueous solution; the spectra for copolymer B are qualitatively similar, but of lower intensity. An excitation wavelength of 269.2 nm was chosen to maximize the emission intensity monitored at 305 nm. Only monomer emission was observed; no red-shifted broad emission ascribed to excimers was seen. Furthermore, no excimer formation was observed in solutions of the pyrene-labeled PNIPAAM-D. Thus the level of labeling we introduced is low enough that we find no evidence of intrachain chromophore interactions.

Figure 2 illustrates the emission spectrum of PNIPAAM-C along with the excitation spectrum of PNIPAAM-D, the pyrene-labeled polymer; the excitation spectra of the other pyrene derivatives used in this work (pyrene, C11PN+, and ZwPy) are qualitatively equivalent. Overlap between the spectra suggests that energy transfer from fluorene should excite emission from pyrene, and both Winnik[8] and Watanabe and Matsuda[10,11] have demonstrated the utility of polymer-bound fluorene and pyrene derivatives as NRET donors and acceptors, respectively. The excitation wavelength chosen for donor excitation ideally would not result in any direct excitation of the acceptor; however, at even the best value for selective excitation of fluorene (289.7 nm), pyrene is directly excited. We chose the more highly labeled sample C for subsequent experiments to ensure preferential excitation of fluorene.

No hydrolysis of bound pyrene or fluorene chromophores was observed in any of the investigations reported below; no changes in fluorescence, microcalorimetry, or cloud points were detected over the course of our experiments.

Nonradiative Energy Transfer from Fluorene-Labeled PNIPAAM. The experimental approach for observation of NRET entailed measurement of spectra of three aqueous solutions: those of the fluorene-labeled polymer, the pyrene derivative, and a mixture of the two at the same concentrations. Each solution was excited at 289.7 nm, and the extent of NRET was estimated in terms of the ratio (I_P/I_F) of the pyrene emission intensity at 377 nm to that of fluorene at 305 nm. We also used excitation at ca. 340 nm to excite pyrene directly in order to observe pyrene emission independent of NRET.

Pyrene. We have used pyrene as a probe in our studies of the interaction of PNIPAAM with surfactants in aqueous solution.[16-18] The micropolarity-sensitive vibronic band structure of the pyrene emission in aqueous solution is independent of the presence of PNIPAAM, and pyrene has no effect on the LCST of PNIPAAM as monitored by cloud point and microcalorimetric measurements. Thus interactions between these solutes appear weak or absent in aqueous solutions. The NRET experiments summarized in Table II and Figure 3 confirm this conclusion: the emission spectra of pyrene and fluorene-labeled PNIPAAM are simply additive upon excitation at 289.7 nm. The emission intensity at 377 nm measured in the mixture of the fluorene-labeled polymer and pyrene is the sum of the intensities emitted by their separate, directly excited solutions; no NRET is observed. Furthermore, direct excitation (at 337 nm) of pyrene in the mixture yields an emission spectrum equivalent to that observed for pyrene in water or in aqueous solutions of the NIPAAM homopolymer (sample A). Thus the labeled polymer does not affect the spectroscopic behavior of pyrene in aqueous solution. In contrast, Winnik[8] has reported enhancement of pyrene emission intensity in

Table II
NRET Systems

sample	I_F(305 nm)	I_P(377 nm)
pyrene (1 mM)[a]	0.00	0.03
PNIPAAM-C (0.40 mg/mL)[a]	9.63	0.13
mixture[b]	9.63	0.16
PNIPAAM-D (0.40 mg/mL)[a]	0.01	0.77
PNIPAAM-C (3.60 mg/mL)[a]	16.87	0.32
mixture[b]	13.62	0.67

[a] Intensities I_F and I_P are those observed upon excitation of donor or acceptor species separately. [b] Intensities observed upon excitation of donor–acceptor mixture.

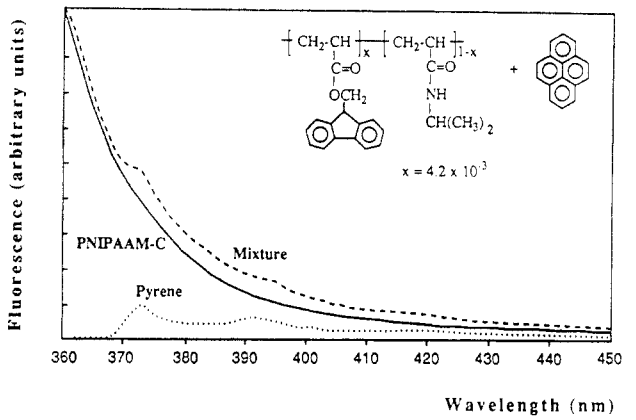


Figure 3. Emission spectra for aqueous solutions of pyrene (· · ·) (1 μM), 0.40 mg/mL PNIPAAM-C (—), and their mixture (- - -) at 24.5 °C. Excitation at 289.7 nm.

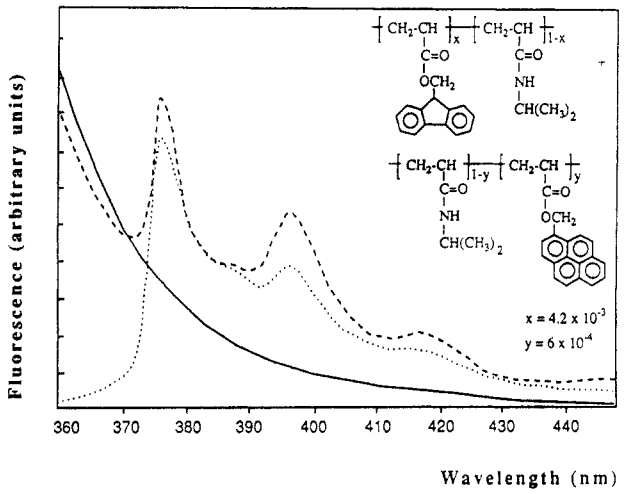


Figure 4. Emission spectra for aqueous solutions of PNIPAAM-C (—) (3.60 mg/mL), PNIPAAM-D (· · ·) (0.40 mg/mL), and their mixture (- - -) at 24.5 °C. Excitation at 289.7 nm.

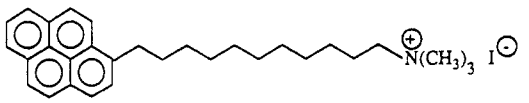
solutions of fluorene-labeled HPC as a result of NRET and notes some perturbation of the fine structure of the pyrene emission. In making this comparison, we note that the fluorene-labeled HPC used in Winnik's experiments carried a significantly higher concentration of the chromophore (1 donor per 33 chain units) than does the PNIPAAM used herein (1 chromophore per 240 repeating units).

Although we have demonstrated[17,18] that pyrene can serve as an indicator of the PNIPAAM LCST through its solubilization in the precipitated polymer phase above the transition temperature, we did not attempt to do NRET experiments at temperatures above the LCST. The contribution from scattering from these turbid solutions was overwhelming compared to the observed fluorescence.

Pyrene-Labeled PNIPAAM. Winnik[8] has used the NRET technique to assemble strong evidence for the intermolecular aggregation of HPC chains in aqueous solution below the LCST. Heskins and Guillet have interpreted viscometric data for PNIPAAM similarly.[24] However, Pelton's study[25] of PNIPAAM latexes argues against aggregation, since the latex particles were observed to shrink—but not to flocculate—below the LCST. Recent light scattering studies of the coil–globule transition of PNIPAAM[26,27] also support an intramolecular collapse prior to aggregation *above* the LCST.

Our NRET experiment is summarized in Table II and illustrated in Figure 4. We used a total concentration of 4.00 mg/mL of polymer (3.60 mg/mL of fluorene-labeled PNIPAAM-C and 0.40 mg/mL of lightly pyrene-labeled PNIPAAM-D, Figure 1). The fluorene:pyrene ratio is thus ca. 63:1. Nevertheless, despite a small decrease in the donor emission intensity, no enhancement of pyrene intensity was observed. We interpret these results as indicating simply that pyrene and fluorene compete independently for the energy put into the system; no energy transfer is observed. Our results thus support the recent reports[9,25-27] that PNIPAAM does not aggregate below the LCST.

Pyrene-Labeled Cationic Amphiphile. In previous experiments using free pyrene as probe, we found a critical aggregate concentration (cac) of ca. 13 mm for the formation of PNIPAAM-bound dodecyltrimethylammonium bromide (DTAB) micelles; elevation of the LCST of PNIPAAM was also observed to occur at this concentration.[17] The same value of the cac was determined in fluorescence experiments employing a pyrene-substituted analogue of DTAB (C11PN+, 1) or pyrene-labeled PNIPAAM-D.[17] In each case, the fluorescence spectrum



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of the pyrene probe was abruptly perturbed at the cac. In the present work, we have attempted to use the NRET experiment to observe directly the binding of PNIPAAM and C11PN+ at micromolar concentrations.

At room temperature, C11PN+ is below its Krafft point[29] and does not form micelles in free solution. At a fixed concentration of 14 μM C11PN+, addition of 0.4 mg/mL PNIPAAM-C increases the emission intensity (at 377 nm) by almost an order of magnitude (Figure 5 and Table III). The fluorene emission is quenched in the mixture, as compared to the emission observed from the surfactant-free solution, and pyrene dimers (showing the characteristic red-shifted emission maximum[20,21] at ca. 485 nm) are observed (Figure 5). No such emission is detected in the spectrum of the polymer-free C11PN+ solution. Excitation spectra (Figure 6) show that the excitation of the dimer (485 nm emission) is red shifted relative to that of isolated pyrene (377 nm emission). This is evidence of two distinct absorbing species[8] and thus for the formation of ground-state pyrene dimers in solutions of PNIPAAM-C and C11PN+. Direct excitation of C11PN+ at 340 nm (Figure 7) also clearly indicates that surfactant aggregation is induced by PNIPAAM-C, as shown by an increase in dimer emission. We conclude that the enhanced emission intensities at 377 and 485 nm in Figure 5 result from formation of C11PN+ aggregates (at least dimers) attached to the PNIPAAM chain and from energy transfer from polymer-bound fluorene donors. We believe this to be the first example of the use of the NRET technique to detect polymer–surfactant binding in aqueous solution.

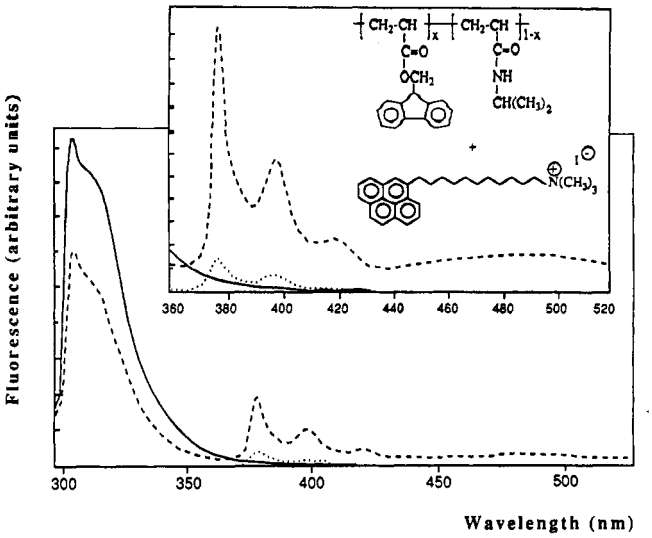


Figure 5. Emission spectra for aqueous solutions of C11PN+ (···) (14 μM), PNIPAAM-C (—) (0.40 mg/mL), and their mixture (- - -) at 24.5 °C. Excitation at 289.7 nm.

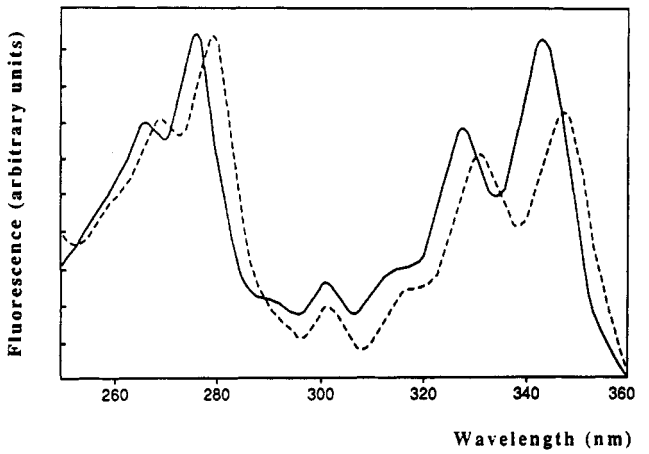


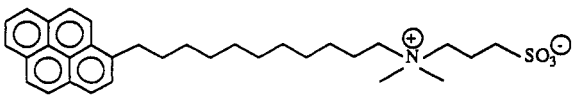
Figure 6. Excitation spectra for an aqueous solution of C11PN+ (14 μM) mixed with PNIPAAM-C (0.40 mg/mL) at 24.5 °C, monitored at 377 (—) and 485 (- - -) nm.

Table III
NRET Systems with Excimers

sample	I_F(305 nm)	I_P(377 nm)	$I_{excimer}$
C11PN+ (14 mM)[a]	0.00	0.26	0.01
PNIPAAM-C (0.40 mg/mL)[a]	9.77	0.10	0.01
mixture[b]	6.39	2.07	0.32[c]
ZwPy (14 mM)[a]	0.0	0.04	0.06
PNIPAAM-C (0.40 mg/mL)[a]	9.4	0.10	0.01
mixture[b]	9.9	0.27	0.07[d]
error	0.6	0.03	0.03

[a] Intensities I_F and I_P are those observed upon excitation of donor or acceptor species separately. [b] Intensities observed upon excitation of donor–acceptor mixture. [c] λ_{max} for excimers = 485 nm. [d] λ_{max} for excimers = 468 nm.

Pyrene-Labeled Zwitterionic Amphiphile. We performed experiments similar to those described above, using ZwPy (2) as a fluorescent analogue of the commonly used



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zwitterionic detergent Zwittergent 3-12.[17] Previous experiments[17] led us to believe that detergents of this head group type do not bind strongly to PNIPAAM. For

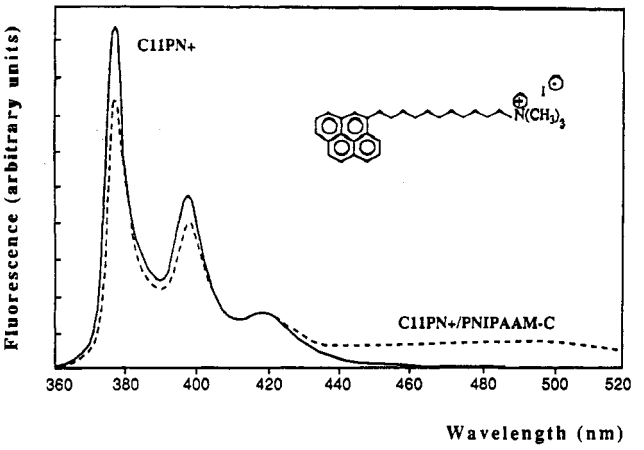

Figure 7. Emission spectrum for an aqueous solution of C11PN+ (—) (14 μM) and an emission spectrum of its mixture with PNIPAAM-C (- - -) (0.40 mg/mL) at 24.5 °C, both excited at 340 nm.

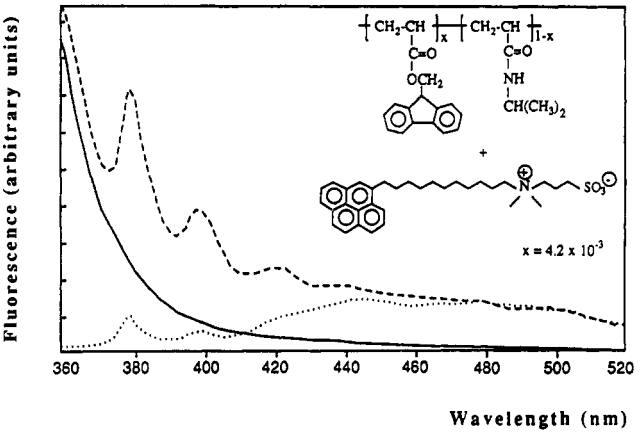

Figure 8. Emission spectra for aqueous solutions of ZwPy (···) (14 μM), PNIPAAM-C (—) (0.40 mg/mL), and their mixture (- - -) at 24.5 °C. Excitation at 289.7 nm.

example, we found that aggregation of Zwittergent 3-12 occurs at a cmc of 2.8 mM, independent of the addition of PNIPAAM, and that such aggregation causes no change in the LCST of PNIPAAM solutions. Instead, addition of increasing amounts of surfactant causes a monotonic decrease in the LCST, much in the manner of simple salts.[13] As shown in Figure 8 and Table III, conditions that yield efficient energy transfer from fluorene-labeled PNIPAAM to the cationic curfactant C11PN+ produce weaker emission from ZwPy. The fluorene intensity appears unchanged within experimental error, and although the pyrene intensity does rise, the increment in intensity is much less than that observed with C11PN+. There is no enhancement of the pyrene dimer emission at 485 nm. Thus the NRET method is consistent with the fluorescence probe and calorimetric techniques in reporting an absence of strong binding of zwitterionic surfactants and PNIPAAM in aqueous solution.

Conclusions

We have applied NRET techniques to the study of interpolymer and polymer–solute interactions in aqueous solutions of PNIPAAM. No energy transfer was observed from fluorene-labeled PNIPAAM to free pyrene or to PNIPAAM-bound pyrene. These results contrast with those of similar experiments reported for HPC,[8] though the comparison must be made with caution, owing to large differences in the extent of labeling used in this work and in that reported in ref 8. The absence of energy transfer

to free pyrene supports the validity of the use of pyrene as a nonperturbing probe in PNIPAAM solutions.[16,17] The efficiency of energy transfer from fluorene-labeled PNIPAAM was found to be higher for the cationic surfactant C11PN+ than for a zwitterionic surfactant (ZwPy) of identical tail structure, consistent with earlier results on the relative binding of cationic and zwitterionic surfactants by PNIPAAM in aqueous solution.

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References and Notes

(1) Pesce, A. J.; Rosén, C.-L.; Pasby, T. L. *Fluorescence Spectroscopy: An Introduction for Biology and Medicine*; Marcel Dekker Inc.: New York, 1971.
(2) Morawetz, H. *Science* **1988**, *240*, 172.
(3) Sutton, R. C.; Thai, L.; Hewitt, J. M.; Voycheck, C. L.; Tan, J. S. *Macromolecules* **1988**, *21*, 2432.
(4) Horský, J.; Morawetz, H. *Makromol. Chem.* **1988**, *189*, 2475.
(5) Chen, C.-T.; Morawetz, H. *Macromolecules* **1989**, *22*, 159.
(6) Horský, J.; Morawetz, H. *Macromolecules* **1989**, *22*, 1622.
(7) Borden, K. A. Ph.D. Dissertation, University of Massachusetts at Amherst, 1989.
(8) Winnik, F. M. *Macromolecules* **1989**, *22*, 734.
(9) Winnik, F. M. *Polymer* **1990**, *31*, 2125.
(10) Watanabe, A.; Matsuda, M. *Macromolecules* **1986**, *19*, 2253.
(11) Watanabe, A.; Matsuda, M. *Macromolecules* **1985**, *18*, 273.
(12) Nagata, I.; Morawetz, H. *Macromolecules* **1981**, *14*, 87.
(13) Schild, H. G.; Tirrell, D. A. *J. Phys. Chem.* **1990**, *94*, 4352.
(14) Schild, H. G.; Muthukumar, M.; Tirrell, D. A. *Macromolecules* **1991**, *24*, 948.
(15) Schild, H. G.; Tirrell, D. A. *Langmuir* **1990**, *6*, 1676.
(16) Schild, H. G.; Tirrell, D. A. *Langmuir* **1991**, *7*, 665.
(17) Schild, H. G. Ph.D. Dissertation, University of Massachusetts at Amherst, 1990.
(18) Schild, H. G.; Tirrell, D. A. *Langmuir* **1991**, *7*, 1319.
(19) *Sadtler Standard Ultra Violet Spectra*; Sadtler Research Laboratories, Inc.: Philadelphia, PA, 1968; p 14499.
(20) Winnik, F. M. *Macromolecules* **1987**, *20*, 2745.
(21) Chu, D.-Y.; Thomas, J. K. *Macromolecules* **1984**, *17*, 2142.
(22) Winnik, F. M.; Winnik, M. A.; Tazuke, S. *J. Phys. Chem.* **1987**, *91*, 594.
(23) Chandar, P.; Somasundaran, P.; Turro, N. J. *Macromolecules* **1988**, *21*, 2346.
(24) Heskins, M.; Guillet, J. E. *J. Macromol. Sci., Chem.* **1968**, *A2* (8), 1441.
(25) Pelton, R. H.; Pelton, H. M.; Morphesis, A.; Rowell, R. L. *Langmuir* **1989**, *5*, 816.
(26) Yamamoto, I.; Iwasaki, K.; Hirotsu, S. *J. Phys. Soc. Jpn.* **1989**, *58*, 210.
(27) Fujishige, S.; Kubota, K.; Ando, I. *J. Phys. Chem.* **1989**, *93*, 3311.
(28) Winnik, F. M. *Macromolecules* **1990**, *23*, 233.
(29) Schwuger, M. J. In *Structure/Performance Relationships in Surfactants*; Rosen, M. J., Ed.; American Chemical Society: Washington, DC, 1984.