Macromolecules **1992,** *25,* 5353–5361

Hydrophobically-Modified Poly(*N*-isopropylacrylamides) in Water: Probing of the Microdomain Composition by Nonradiative Energy Transfer

H. Ringsdorf and J. Simon

Institut für Organische Chemie, Johannes Gutenberg-Universität Mainz,
J.-J. Becherweg 18-20, D-6500 Mainz, FRG

F. M. Winnik*

Xerox Research Centre of Canada, 2660 Speakman Drive, Mississauga, Ontario,
Canada L5K 2L1

Received February 18, 1992; Revised Manuscript Received June 15, 1992

ABSTRACT: Fluorescently labeled copolymers have been prepared by free-radical copolymerization in dioxane of *N*-isopropylacrylamide (NIPAM) and (1) *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide (4) in 100:1, 200:1, and 400:1 molar ratios and (2) mixtures of the two labeled monomers 4 and *N*-[4-(1-pyrenyl)butyl]-*N*-*n*-octadecylacrylamide in 2000:6:1 and 1800:3:1 molar ratios. In aqueous solutions the copolymers form polymeric micellar structures ranging in diameter from 50 to 110 nm (25 °C, 1 g L^{-1}), as determined by quasi-elastic light scattering. Experiments based on nonradiative energy transfer between excited naphthalene (Na) and pyrene (Py) in solutions of the doubly labeled copolymers and in mixtures of the singly labeled copolymers carrying either the Na chromophore or the Py chromophore reveal the existence of interpolymeric micelles (critical aggregation concentration ≤4 ppm). The data are discussed in light of previous fluorescence probe experiments on solutions of copolymers of NIPAM and *n*-alkylacrylamides. The results described here highlight the power of the nonradiative energy transfer technique to detect interpolymeric aggregation.

Introduction

Water-soluble polymers are added to many commercial products, such as paints, inks, cosmetics, foodstuff, and water-borne coatings.[1] In recent years interest in these polymers has been rejuvenated, as a consequence of the growing concerns about the safety of more traditional organic-based fluids. In most applications the polymers act as viscosity and rheology modifiers.[2] Amphiphilic copolymers which contain hydrophobic and hydrophilic segments in the same molecule are particularly effective in this role. The macroscopic properties of the aqueous solutions can be traced to the existence of organized hydrophobic microdomains created by interactions between the hydrophobic segments. The formation, structure, and stability of these microdomains can be controlled through the use of additives or by external stimuli, such as temperature or shear. To the polymer chemist the synthesis of amphiphilic macromolecules offers the challenge of creating structures matched to their intended function. Experimental studies[3] and computer simulations[4] have pointed to the key importance of two factors: (1) the structure and amount of hydrophobic substituents and (2) the architecture of the macromolecule, namely, whether the hydrophobic groups are added at random along the chain or appended to the end of the chain.

The chemical structure of the polymers is a determining factor in directing the formation of microdomains either within a single macromolecule or among several chains. For some polymers there is a clear preference for one of the two micellar structures. For example, polysoaps, which are alternating copolymers of maleic anhydride with either *n*-alkyl vinyl ether or *n*-alkylethylene, form predominantly unimolecular polymeric micelles.[5] In contrast, hydrophobically-modified cellulose ethers tend to undergo interpolymeric association via interaction between alkyl substituents carried by different chains, as shown, for example, in the case of hydrophobically-modified (hydroxyethyl)-cellulose.[6] For other polymers the distinction is not as clear cut. Sensitive analytical techniques are required to establish the composition of the microdomains.

The synthesis of hydrophobically-modified poly(*N*-isopropylacrylamides) was reported by Ringsdorf et al.[7] and by Schild and D. Tirrell.[8] Both studies yielded clear evidence that aqueous solutions of these polymers are not homogeneous: even with polymer concentrations as low as 10 ppm, it was possible to detect the presence of hydrophobic microdomains. Whether these micellar structures are unimolecular or multimolecular has not been clearly established. It is the objective of this study to address this question. The specific technique used here relies on the photophysical process of direct nonradiative energy transfer (NRET) between two chromophores.[9,10] The process originates in dipole–dipole interactions between an energy donor in its excited state and an energy acceptor in its ground state. The probability of energy transfer between two chromophores depends sensitively on their separation distance and to a lesser extent on their relative orientation. Therefore, in mixed solutions of polymers carrying either donor chromophore or acceptor chromophore the extent of energy transfer between the two labels can be related to the extent of interpolymeric association. The application of NRET to probe interpolymeric interactions was pioneered by Nagata and Morawetz.[11] It has proven to be powerful in probing detailed features of polymeric mixtures in solutions[12,13] or in the bulk.[14,15]

The structure of labeled polymers suitable for this type of experiments had to be chosen with care. Since the aim of the study was to probe the composition of hydrophobic microdomains, it was crucial to attach each chromophore in close spatial proximity to a hydrophobic substituent. The design was guided also by other factors such as the spectral characteristics of the donor and acceptor and the amount of label incorporation. On the basis of our previous experience with hydrophobically-modified poly(*N*-isopropylacrylamides), we synthesized the naphthyl (Na)- and

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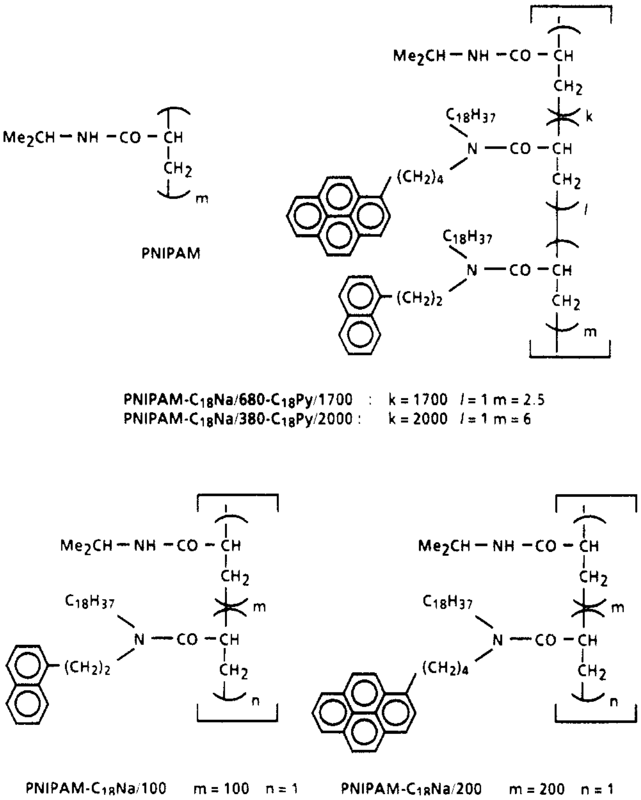

Figure 1. Chemical structures of the polymers described in this study.

pyrenyl (Py)-labeled polymers shown in Figure 1. They are copolymers of *N*-isopropylacrylamide (NIPAM) and either *N*-[4-(1-pyrenyl)butyl]-*N*-*n*-octadecylacrylamide (PNIPAM–C_{18}Py) or *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide (PNIPAM–C_{18}Na). The copolymers differ not only in the chemical structure of the fluorescent label but also, for each chromophore, in the level of label incorporation. In the case of naphthyl-labeled copolymers the ratios of chromophore to NIPAM units were 1:100, 1:200, and 1:400, in molar units. Doubly labeled copolymers (PNIPAM–C_{18}Na–C_{18}Py) were also prepared to allow control experiments and to investigate the occurrence of *intrapolymeric* nonradiative energy transfer between pyrene and naphthalene.

The synthesis, characterization, and solution properties of the copolymers are described in this paper. Evidence is given for the formation of polymeric micellar structures in aqueous solutions of the copolymers prepared for this study, highlighting the generality of the aggregation phenomenon for hydrophobically-modified PNIPAM. The composition of the microdomains is established firmly on the basis of NRET experiments carried out with solutions of the double labeled copolymers and with solutions containing mixtures of PNIPAM–C_{18}Na and PNIPAM–C_{18}Py.

Experimental Section

Materials. Analytical grade solvents (Merck or Riedel de Haen, distributed by A. E. Fisher, Wiesbaden, Germany) were used without purification. Silica gel (40 μm) for chromatography was purchased from Baker. Thin-layer chromatography (TLC) was done with silica plates (Merck). Spots were visualized with Hanes–Isherwood solution.[16] Water was deionized with a Millipore Milli-Q water purification system. NIPAM (Eastman Kodak Chemicals) was purified by two recrystallizations from benzene/hexane. Octadecylamine was purchased from Riedel de Haen; triethylamine, acryloyl chloride, thionyl chloride, and AIBN were purchased from Merck; BH_3-Me_2S, 1-naphthaleneacetic acid, and di-*tert*-butylcresol were purchased from Aldrich Chemicals. *N*-[4-(1-Pyrenyl)butyl]-*N*-*n*-octadecylamine and PNIPAM–C_{18}Py/200 were prepared as described previously.[7]

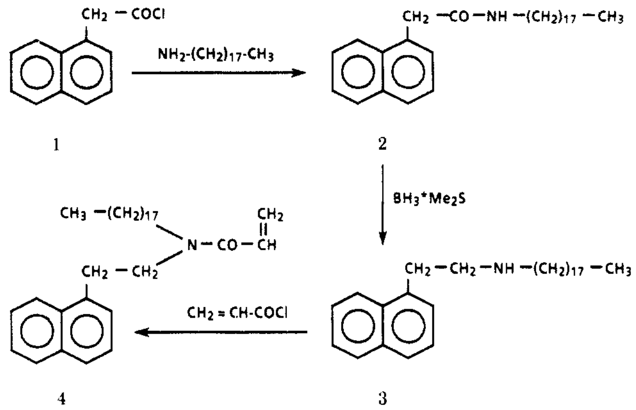

Figure 2. Synthetic scheme for the preparation of *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide.

Synthesis. *N*-[2-(1-Naphthyl)ethyl]-*N*-*n*-octadecylacrylamide (NaODAA, Figure 2). A solution of 1-naphthaleneacetic acid (10 g, 53.7 mmol) in thionyl chloride (15 g, 126 mmol) was heated at reflux for 2 h. After this time residual thionyl chloride was removed by vacuum distillation. The residue was dissolved twice in benzene followed by evaporation of the benzene to yield 1-naphthaleneacetyl chloride[17] (9.8 g, 90%) as a reddish oil which was used without further purification. The crude acid chloride (9.8 g, 47.8 mmol) was dissolved in dichloromethane (100 mL). To the solution cooled to 0 °C under nitrogen was added dropwise a solution of octadecylamine (12.9 g, 47.8 mmol) and triethylamine (7.5 mL, 58 mmol) in CH_2Cl_2 (200 mL). The reaction mixture was stirred overnight at room temperature. Evaporation of the solvent and crystallization from 2-propanol gave the *N*-[2-(1-naphthyl)ethyl]-*N*-octadecylacetamide (15.6 g, 75%) as a white solid (mp 102 °C). This material (9.0 g, 20.5 mmol) was dissolved under nitrogen in refluxing THF (200 mL). To the solution was added dropwise a 2 M solution of BH_3·Me_2S in THF (40.2 mmol). At the end of the addition the reaction was heated at reflux for 1 h and kept overnight at room temperature. To the concentrated solution (60 mL) was added a solution in methanol of concentrated HCl (2.3 mL). After neutralization with NaOH the solution was diluted with CH_2Cl_2, dried over Na_2SO_4, and filtered. Evaporation of the solvent and crystallization from methanol yielded *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylamine (6.9 g, 76%) as a white solid (mp 47–48 °C). This material (2.0 g, 4.7 mmol), triethylamine (1 mL), and 2,6-di-*tert*-butylcresol (20 mg) were dissolved in CH_2Cl_2. Acryloyl chloride (0.46 mL, 0.51 g, 5.6 mmol) was added dropwise to the solution kept under nitrogen. The mixture was stirred at room temperature for 2.5 h, until complete disappearance of starting material (TLC, $CHCl_3$/MeOH, 10/1 v/v). It was washed with 0.1 N HCl (30 mL), water (30 mL), and a saturated aqueous $NaHCO_3$ solution (30 mL) and dried over Na_2SO_4. Evaporation of the solvent yielded *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide (NaODAA) as an oil, which was purified by chromatography (silica gel eluted with petroleum ether/ethyl acetate, 3/1 v/v) (1.6 g, 71%): UV (MeOH) λ_{max} = 290 nm (ϵ = 5100). NMR, IR, and mass spectroscopy confirmed the structural assignment. Anal. Calcd (MW 477.4): C, 82.95; H, 10.76; N, 3.34. Found: C, 82.87; H, 10.87; N, 2.98.

***N*-Isopropylacrylamide–*N*-[2-(1-Naphthyl)ethyl]-*N*-*n*-octadecylacrylamide Copolymers: General Procedure.** NIPAM (20 mmol) and *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide (varying amounts, see Table I) were dissolved in dioxane (40 mL, freshly distilled from sodium under nitrogen). The solution was degassed with nitrogen for 20 min. AIBN (0.1 mmol) was added. The solution was heated at 60 °C for 2 h, a time period for which polymerization occurred to approximately 50% monomer conversion; then it was cooled in an ice bath. The polymers were isolated by precipitation into diethyl ether (600 mL). They were purified further by precipitations from dioxane into diethyl ether. They were dissolved in water. The polymers were isolated by lyophilization of the filtered solution. They were analyzed and used without further purification.

Table I
Physical Properties of the Polymers

polymer	monomer feed (molar fraction) NIPAM	 NaODAA	 PyODAA	$[\eta]^a$ (mL g^{-1})	$M_v{}^b$	$M_n{}^c$	$M_w{}^c$	$M_w/M_n{}^c$	LCST (°C)
PNIPAM–C_{18}Na/100	100	1	0	34.8	301 000	38 000	69 000	1.81	29.5
PNIPAM–C_{18}Na/200	200	1	0	33.3	279 000	36 000	69 000	1.92	30.4
PNIPAM–C_{18}Na/400	400	1	0	35.2	305 000	34 000	66 000	1.97	30.7
PNIPAM–C_{18}Py/1000	1000	0	1	38.8	354 000	45 000	84 000	1.87	31.8
PNIPAM–C_{18}Py/200	200	0	1	41.3	390 000	23 000	38 000	1.66	30.6
PNIPAM–C_{18}Na/360–C_{18}Py/2040	1940	6.0	1	28.9	235 000	28 000	62 000	2.24	30.8
PNIPAM–C_{18}Na/680–C_{18}Py/1700	1870	2.8	1	31.9	263 000	31 000	67 000	2.18	30.7

[a] Polymer solutions in THF. [b] From $[\eta] = 9.59 \times 10^{-3} M_v{}^{0.65}$; see ref 22. [c] GPC determination.

Table II
Chemical Composition of the Copolymers

polymer	composition (unit mol) NIPAM: C_{18}Na:C_{18}Py	[Na] (mol g^{-1})	[Py] (mol g^{-1})
PNIPAM–C_{18}Na/100	$115^a(108)^b$:1	7.6×10^{-5}	
PNIPAM–C_{18}Na/200	$230^a(205)^b$:1	4.0×10^{-5}	
PNIPAM–C_{18}Na/400	$476^a(386)^b$:1	2.0×10^{-5}	
PNIPAM–C_{18}Py/1000	1015^b:1		0.86×10^{-5}
PNIPAM–C_{18}Py/200	$206^a(181)^b$:1		4.4×10^{-5}
PNIPAM–C_{18}Na/360–C_{18}Py/2000	2040^b:5.6:1 340:1(C_{18})^c	2.4×10^{-5} [Na]:[Py] = 5.6	0.42×10^{-5}
PNIPAM–C_{18}Na/680–C_{18}Py/1700	1704^b:2.5:1 530:1 (C_{18})^c	1.3×10^{-5} [Na]:[Py] = 2.5	0.51×10^{-5}

[a] By ^{1}H NMR. [b] From UV measurements. [c] Total $C_{18}H_{37}$ content, determined by ^{1}H NMR (see text).

Doubly Labeled Polymers: General Procedure. They were prepared following the procedure described for the preparation of the naphthyl-labeled copolymers, starting from NIPAM (20 mmol), and a mixture of *N*-[2-(1-naphthyl)ethyl]-*N*-*n*-octadecylacrylamide and *N*-[4-(1-pyrenyl)butyl]-*N*-*n*-octadecylacrylamide (PyODAA) in varying amounts (see Table I).

Pyrene-Labeled Polymer (PNIPAM–C_{18}Py/1000). The polymer was prepared following the general procedure, starting with NIPAM (20 mmol) and *N*-[4-(1-pyrenyl)butyl]-*N*-*n*-octadecylarylamide (11.6 mg, 0.02 mmol). Physical properties and compositions of the samples are compiled in Tables I and II. Compositions based on UV absorption spectroscopy were calculated from the absorbances of solutions of the polymers in methanol, using as model compounds NaODAA (ϵ = 5100 at 290 nm) and PyODAA (ϵ = 34 900 at 341 nm and ϵ = 1300 at 290 nm).[7]

Instrumentation. UV spectra were recorded with a Varian UV–vis–near-IR Cary-5 spectrophotometer. Solution viscosities of THF solutions (3 g L^{-1}) were determined with a 53810/I Ubbelohde microviscometer linked to an AVS 300/P22 Schott automatic viscometer. GPC measurements were performed with a Waters WISP 700 system equipped with a Waters RI 410 refractive index detector. Four ultrastyragel columns (10 000, 5000, 500 and 100 Å) were used. They were calibrated for molecular weight with polystyrene standards obtained from Polysciences Inc. The eluent (THF) was used at a flow rate of 0.8 mL min^{-1}. Polymer micelle sizes were determined at 25 °C by quasi-elastic light scattering (QELS) with a fixed 90° scattering angle, using a Brookhaven Instrument Corp. particle sizer, model BI-90, equipped with a He/Ne laser. In a typical measurement values were determined over 2000 cycles with a count rate lower than 50 kcps. The software provided by the manufacturer was employed to determine the particle sizes. An average value over 10 consecutive measurements is reported. Proton NMR spectra were recorded for $CDCl_3$ solutions of the polymers at ambient temperature on a Bruker AMX400 spectrometer operating at a ^{1}H frequency of 400.14 MHz. Cloud points were determined by spectrophotometric detection of changes in turbidity of solutions heated at a constant rate (0.2 °C min^{-1}) in a magnetically stirred UV cell, as described previously.[18]

Fluorescence Measurements. Steady-state fluorescence spectra were recorded on a SPEX Fluorolog 212 spectrometer equipped with a DM3000F data system. The temperature of the water-jacketed cell holder was controlled with a Neslab circulating bath connected to a Neslab MTP-6 programmer. The temperature of the sample fluid was measured with a thermocouple immersed in the sample. Excitation spectra were measured in the ratio mode. Emission spectra were not corrected, except for fluorescence quantum yield measurements. They were recorded with an excitation wavelength of 330 nm (pyrene) and 290 nm (naphthalene), unless otherwise specified.

Fluorescence lifetimes were measured with a LS-1 instrument from Photon Technology International, Inc. (London, Ontario, Canada), equipped with a thyratron-gated N_2 lamp and a proprietary analog stroboscopic optical boxcar detection system. Samples were excited at 337 nm (pyrene) or 294 nm (naphthalene). Detection wavelengths were set at 380 and 480 nm for the pyrene monomer and excimer emissions, respectively, and at 340 nm for the naphthalene monomer emission. The temperature of the water-jacketed cell holder was controlled with a Neslab circulating bath. The data analysis was performed with proprietary software from Photon Technology International, Inc.

Samples for spectroscopic analysis were prepared from stock solutions (5 or 1 g L^{-1}) kept at 5 °C to ensure complete dissolution of the polymers. Aliquots of the stock solutions were diluted to the desired concentration. They were kept at room temperature for at least 2 h before spectroscopic measurements (Table III) were taken. Solutions in water were not degassed. Solutions in methanol were degassed by a 1-min vigorous bubbling of methanol-saturated argon through the solutions.

Determination of the Spectroscopic Parameters. Quantum yields were calculated by integration of peak areas of corrected spectra in wavenumber units, using as standards either 2-aminopyridine in 0.1 N H_2SO_4 (Φ = 0.60, λ_{exc} = 290 nm, 25 °C)[19] or quinine sulfate in 1 N H_2SO_4 (Φ = 0.56, λ_{exc} = 328 nm, 25 °C).[20] Beer's law corrections were applied for optical density changes at the excitation wavelengths. Corrections were made for refractive index differences between methanol and water. The fraction of the total light absorbed by naphthalene in the presence of pyrene at an excitation wavelength of 290 nm was calculated at the excitation wavelength λ_1 (λ_1 = 290 nm) using eq 1,[21] where $A_N(\lambda_1)$ and $A_{Py}(\lambda_1)$ are the absorbances at λ_1 of naphthalene and pyrene, respectively.

$$P = \frac{1 - 10^{-A_N(\lambda_1)}}{2 - 10^{-A_N(\lambda_1)} - 10^{-A_{Py}(\lambda_1)}} \qquad (1)$$

The pyrene excimer to monomer ratios $[I_E/I_M]^{Py}$ were calculated by taking the ratio of the intensity (peak height) at 480 nm to the half-sum of the intensities at 379 and 399 nm. In the case of naphthalene, the excimer to monomer intensity ratios $[I_E/I_M]^{Na}$ were determined by taking the ratio of the intensity (peak height) at 399 nm to the intensity at 343 nm, after correcting for residual monomer emission at 399 nm. The monomer emission was determined in each case by subtracting a normalized (at 343 nm) spectrum.

The extent of pyrene emission due to NRET from naphthalene was determined by integration of spectra from 378 to 500 nm, after correcting for emission due to direct pyrene excitation. In this case the corrections were performed by subtracting from each spectrum the emission spectrum of PNIPAM–C_{18}Py/200 (λ_{exc} = 290 nm) of identical pyrene concentration.

Results and Discussion

Synthesis and Characterization of the Copolymers. Poly(*N*-isopropylacrylamide) and its *N*-octadecyl copol-

Table III
Spectroscopic Characteristics of the Polymers[a]

(a) Pyrene Chromophore

	$[I_E/I_M]^{Py\ b}$		$\Phi_{Py}{}^{b}$		τ_{Py} (ns)	
polymer	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O
PNIPAM–C_{18}Py/1000	0.02	0.43	0.88	0.36		27 (0.54), 109 (0.45) $\langle\tau\rangle = 90$
PNIPAM–C_{18}Py/200	0.02	1.10			44 (0.15), 129 (0.85) $\langle\tau\rangle = 124$	22 (0.54), 94 (0.46) $\langle\tau\rangle = 78$
PNIPAM–C_{18}Na/360–C_{18}Py/2000	0.02	0.03	0.87	0.21	direct[d] 210 (1.0)	101 (0.34), 213 (0.66) $\langle\tau\rangle = 175$
					energy transfer[e]	3.0 (−2.0), 194 (1.0)
PNIPAM–C_{18}Na/680–C_{18}Py/1700	0.01	0.16	0.82	0.22	direct[d] 177 (1.0)	132 (0.68), 273 (0.32) $\langle\tau\rangle = 201$
					energy transfer[e]	1.0 (−0.4), 156 (1.0)

(b) Naphthalene Chromophore[c]

	$[I_E/I_M]^{Na}$		$\Phi_{Na}{}^{f}$		τ_{Na} (ns)	
polymer	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O
PNIPAM–C_{18}Na/100	<0.01	0.15	0.13	0.18	5 (0.71), 67 (0.29) $\langle\tau\rangle = 23$ ns	58 (1.0)
PNIPAM–C_{18}Na/200	<0.01	0.12	0.14	0.11		65 (1.0)
PNIPAM–C_{18}Na/400	<0.01	0.10	0.15	0.05		46 (1.0)
PNIPAM–C_{18}Na/360–C_{18}Py/2000			0.11	0.02		3 (0.98), 45 (0.02) $\langle\tau\rangle = 4.2$ ns
PNIPAM–C_{18}Na/680–C_{18}Py/1700			0.13	0.03		1 (0.87), 15 (0.13) $\langle\tau\rangle = 2.6$ ns

[a] Temperature 25 °C. [b] $\lambda_{exc} = 328$ nm. [c] $\lambda_{exc} = 290$ nm. [d] $\lambda_{exc} = 337$ nm. [e] $\lambda_{exc} = 294$ nm. [f] Values calculated assuming that the naphthalene groups are responsible for 77% of the light absorbed by the solution at 290 nm.

ymers were prepared by free-radical polymerization in dioxane to an approximate conversion of 50%. The polymers were purified by repeated precipitations of dioxane solutions into diethyl ether, a nonsolvent for PNIPAM but a good solvent for all the monomers. The purity of the polymers was assessed by TLC and GPC. Four singly labeled polymers were prepared: PNIPAM–C_{18}Na/100, PNIPAM–C_{18}Na/200, PNIPAM–C_{18}Na/400, and PNIPAM–C_{18}Py/1000. In addition two doubly labeled polymers were also synthesized: PNIPAM–C_{18}Na/360–C_{18}Py/2000 and PNIPAM–C_{18}Na/680–C_{18}Py/1700. The digits in the polymer designations refer to the average number of *N*-isopropyl units per chromophore; e.g., PNIPAM–C_{18}Na/200 has on average one naphthyl and one octadecyl substituent per 200 NIPAM units (see below).

Molecular weights were estimated from the intrinsic viscosities of polymer solutions in THF, using the relationship $[\eta] = 9.59 \times 10^{-3}\bar{M}_v{}^{0.65}$ cm^3 g^{-1} established by Fujishige for PNIPAM.[22] Here we assume this expression to be valid also for the NIPAM–*n*-octadecyl copolymers in THF where perturbations due to the hydrocarbon substituents should be minimal (Table I). Molecular weights and molecular weight distributions were calculated also from GPC measurements run in THF and calibrated against polystyrene standards. The data obtained by the two techniques establish that the molecular weight characteristics of the copolymers are independent of their chemical compositions at this low level of substitution. The absence of residual unattached chromophores was confirmed by the GPC traces.

The chemical composition of the copolymers was determined from their ^{1}H NMR spectra in $CDCl_3$ and from their UV absorption spectra (Table II). Analysis by NMR gave the ratios of isopropyl to *n*-octadecyl groups, calculated from the area of the singlet at 4.01 ppm due to the resonance of the methine proton of the isopropyl groups and the area of the triplet centered at 0.9 ppm, attributed to the terminal methyl protons of the octadecyl chains.[7] Compositions from UV absorption spectra were calculated from the absorbance of polymer solutions in methanol, assuming that the extinction coefficients of naphthalene (5100 cm^{-1} mol^{-1} L at 290 nm) and pyrene (34 900 cm^{-1} mol^{-1} L at 341 nm) in the polymers are identical to those of the corresponding monomers. In the case of the doubly labeled copolymers the naphthalene content was determined from the solution absorbance at 290 nm, after subtraction of the pyrene contribution to the absorbance at this wavelength. For these copolymers, NMR analysis yielded only the total *n*-octadecyl chain content. Satisfactory agreement (within 15%) between the two technques was achieved in all cases. The compositions also correspond closely to the polymerization monomer feed ratios. Assuming a degree of polymerization of about 3000, there are on average 30, 15, 7, and 3 octadecyl groups in the copolymers with NIPAM to octadecyl groups molar ratios of 100:1, 200:1, 400:1, and 1000:1, respectively. The doubly labeled polymers carry about 6–10 octadecyl groups per macromolecule.

Solution Properties of the Copolymers in Water. Evidence of Micellar Structure Formation. Simple quasi-elastic light scattering (QELS) experiments performed at fixed angle gave no signal with PNIPAM solutions in water (5 g L^{-1}), in agreement with previous reports of the absence of interchain aggregation in PNIPAM solutions below their lower critical solution temperature (LCST).[23] By contrast similar QELS experiments on the labeled copolymers (5 g L^{-1}, 25 °C) in water showed a strong signal, indicating the presence of micellar structures. The effective hydrodynamic diameter of the aggregates, determined by a cumulant analysis of the data, was ~50 nm in the case of copolymers carrying fewer than 30 hydrophobic substituents per macromolecule and ~110 nm in the case of PNIPAM–C_{18}Na/100. The polydisper-

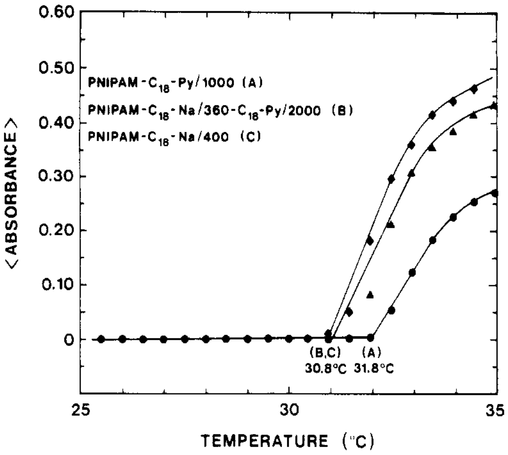

Figure 3. Absorbance changes with temperature (400–800 nm) for aqueous polymer solutions (0.1 g L^{-1}). Samples were heated at 0.2 °C min^{-1}.

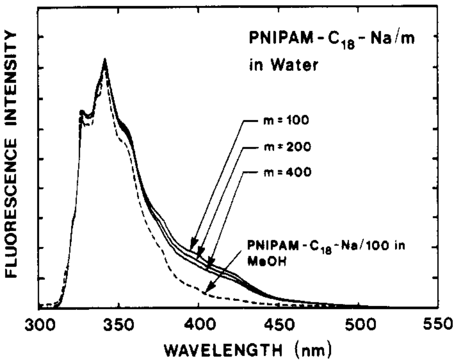

Figure 4. Fluorescence spectra of PNIPAM-C_{18}Na/*m* (*m* = 100, 200, 400) in water and of PNIPAM–C_{18}Na/100 in methanol (polymer concentration 250 ppm; λ_{exc} = 290 nm).

sity values (~0.22) point to a broad distribution in size of the micellar structures. The effective diameters did not vary with polymer concentration within the concentration range ($\geq$1 g L^{-1}) for which the polymeric micelles were detectable with the BI 90 particle sizer used for the measurements. Note that QELS measurements performed under the same conditions on solutions of the copolymers in THF or methanol gave no signal, thus confirming the absence of polymeric micellar structures in these solvents.

Temperature Effects. Aqueous polymer solutions became turbid when heated, signaling the occurrence of a lower critical solution temperature (LCST), as in the case of solutions of PNIPAM (32 °C).[24] Typical cloud point data are shown in Figure 3 for solutions in water of PNIPAM–C_{18}Py/1000, PNIPAM–C_{18}Na/360–C_{18}Py/2000, and PNIPAM–C_{18}Na/400. Increasing the octadecyl chain content of the polymer depresses slightly its LCST (see also Table I). A similar phenomenon was reported in the case of hexadecyl-substituted PNIPAM derivatives.[8] The effect of substitution is small in all cases. It is much less pronounced than is usual in polymeric systems of increasing hydrophobicity, as discussed by Taylor and Cerankowski in their classic work on LCST phenomena.[25] A typical example is that of the poly(vinyl alcohol)–poly(vinyl acetate) copolymers: as the degree of hydrolysis of poly(vinyl acetate) increases, insolubility vanishes, cloud point temperatures increase, and after almost complete hydrolysis of the acetate groups the copolymers are soluble in water at all temperatures.[26] The rather minor effect played by the alkyl chains in the case of the PNIPAM copolymers studied here is another indication that the alkyl substituents associate to form polymeric micellar structures consisting of a hydrophobic core surrounded by a more hydrophilic layer formed by the polymer backbone. The hydrophobic groups within the micellar aggregates are removed from the water but are interconnected by the PNIPAM chains. In this way they do not make a significant hydrophobic contribution to the LCST transition.

Spectroscopic Properties of the Copolymers in Solution. Pyrene-Labeled Copolymers. The spectroscopy of the PNIPAM–C_{18}Py copolymers in methanolic and aqueous solutions has been described previously.[7] In summary, it was determined that (1) in methanol the copolymers exhibit an emission due to locally isolated excited pyrene chromophores (intensity I_M, pyrene "monomer" emission) with the [0,0] band at 376 nm, and (2) in water, at 20 °C, they feature a strong and broad emission centered at 480 nm, in addition to the pyrene monomer emission. This emission (intensity I_E) originates from pyrene excimers. The dynamic nature of the excimer was vouched for (1) by the matching of the excitation spectra obtained for emissions monitored at 380 and 480 nm and (2) by the time-resolved profiles of the excimer emission which presented a growing-in component ($\tau \approx 20$ ns) and a decaying component (Table IIIa). Note that even in the case of aqueous solutions of a lightly labeled polymer, PNIPAM–C_{18}Py/1000 (0.1 mol % octadecyl chains), synthesized for the present study, the excimer emission still accounts for a large fraction of the total pyrene emission (Table IIIa).

Naphthalene-Labeled Copolymers. Steady-state spectra and time-resolved spectra were measured for polymer solutions in methanol and in water. In methanolic solution all polymers exhibited a structured spectrum with a maximum at 340 nm characteristic of the emission of locally isolated excited naphthalene (see Figure 4 for the spectrum of PNIPAM–C_{18}Na/100). The quantum yields of fluorescence were independent of the label content of the polymers (Φ = 0.14). The fluorescence decay profiles monitored at 340 nm were nonexponential. They were analyzed as a sum of two exponential functions of the following form:

$$I(t) = \sum a_i \exp(-t/\tau_i) \qquad (2)$$

The values of the preexponential factors a_i and the lifetimes τ_i are listed in Table IIIb together with the average lifetime, $\langle\tau\rangle$, defined by

$$\langle\tau\rangle = \sum a_i \tau_i^2 / \sum a_i \tau_i \qquad (3)$$

Acceptable fits of the experimental decay curves to a double exponential function were obtained in all cases ($0.95 < \chi^2 < 1.2$).

From the overall spectroscopic data one can conclude that, in methanol, a good solvent for PNIPAM, the copolymers adopt an open coil conformation. No interaction between the chromophores can be detected. The naphthalene groups are kept apart from each other by the polymer chain. The average separation distance of the chromophores is too large to allow the diffusion-controlled formation of excimers during the lifetime of Na* (23 ns). This implies that (1) the naphthyl groups attached to the same polymer chain are distributed at random, rather than in blocky segments and (2) there are no interpolymeric aggregates in methanol, in agreement with the QELS data. The fact that their decay profiles are not exponential implies some degree of heterogeneity in the environment of the chromophores. Similar effects have been reported previously for other naphthyl-labeled polymers, such as, for example, a naphthyl-labeled poly(vinyl acetate) (τ_1 = 9.6 ns, τ_2 = 31.1 ns, and a_1/a_2 = 0.526 in methanol at 6

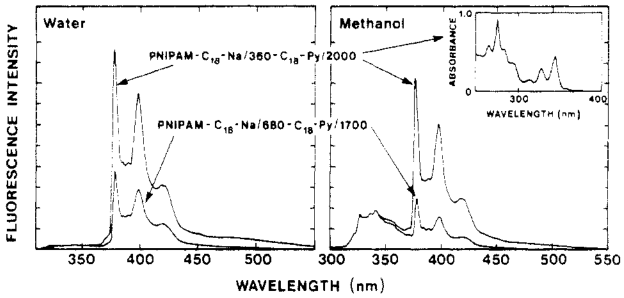

Figure 5. Fluorescence spectra of the terpolymers in water and in methanol (polymer concentration 120 ppm, λ_{exc} = 290 nm). The inset shows the UV absorption spectrum of PNIPAM–C_{18}-Na/360–C_{18}Py/2000 in methanol).

°C).[27] Clearly a more sophisticated multiexponential fit is required to account in detail for the complexity of the fluoresence decays.

The spectroscopic characteristics of the polymers in water differed from those of the polymers in methanol in two major aspects. First, in the steady spectra a significant contribution of the excimer emission (λ_{max} = 400 nm) to the total emission was observed in all cases (Table IIIb). The excimer emission persists in solutions of concentration as low as 50 ppm, and its intensity decreases only slightly with decreasing label incorporation (Figure 4). Second, the fluorescence quantum yield of the naphthalene monomer emission is sensitive to the level of label incorporation: it decreases with decreasing label content. This sensitivity of Φ_{Na} to the composition of the copolymers is puzzling, and its origin will be investigated further.[28]

Doubly Labeled Copolymers. The two doubly labeled polymers described here are substituted with octadecyl and naphthyl, and octadecyl and pyrenyl, groups attached at random along the polymer chain. The two polymers differ not only in the level of total chromophore incorporation (on average 1 chromophore per 530 and 340 NIPAM units) but also in the ratio of pyrene to naphthalene (ca. 2.5 and 5.6 in PNIPAM–C_{18}Na/680–C_{18}Py/1700 and PNIPAM–C_{18}Na/360–C_{18}Py/2000, respectively). With these polymers it is best to record two emission spectra. Excitation at 330 nm detects the emission from directly excited pyrene with no contribution from naphthalene, since only Py absorbs light at this wavelength. With λ_{exc} = 290 nm, one monitors both the direct emission from excited naphthalene and the emission of pyrene excited through nonradiative energy transfer from naphthalene. Therefore, in polymer systems where NRET takes place excitation at 290 nm will result in a complex emission consisting of the emission from Na* (310–400 nm) and of the emission of Py* excited by energy transfer from Na*. These two chromophores are known to interact as energy donor (naphthalene) and energy acceptor (pyrene) by nonradiative energy transfer[29] with a characteristic distance, R_o, of 29 Å.[30] Note that in addition there is a weak emission from directly excited pyrene, since this chromophore has a weak absorbance at 290 nm. This contribution to the spectrum will be taken into account where required.

Turning our attention first to the polymeric solutions in methanol, we observe that the emission spectra, upon excitation at 290 nm, consist of a contribution from Na* and some contribution from Py* (Figure 5, right-hand side). The pyrene emission (exclusively monomer emission) is stronger in the case of the polymer with the highest total labeled content, PNIPAM–C_{18}Na/360–C_{18}Py/2000, even though the pyrene concentrations were almost identical in the two solutions (~(5–6) × 10^{-7} mol L^{-1}, see Table IV). These data provide evidence of the occurrence of some NRET between the two chromophores. The extent of energy transfer is higher in the more densely labeled copolymer or, in other words, the polymer for which the average distance between pendent chromophores is shorter. This observation gives strong indication that the NRET occurs exclusively between chromophores attached to the same chain. The energy transfer occurs through space and is probably assisted by local internal motion of the polymer chain.

Figure 5 (left-hand side) shows the emission spectra of the two doubly labeled polymers in water (λ_{exc} = 290 nm). Note the key difference between the spectra recorded from aqueous solutions and from methanolic solutions. In the spectra from aqueous solutions the pyrene monomer emission is much stronger, with respect to the Na emission. The naphthalene emission itself is quenched considerably in comparison with the spectra from methanolic solutions. Other features of the naphthalene spectroscopy confirm the occurrence of a severe quenching of Na* emission: (1) the fluorescence quantum yields of naphthalene are reduced by a factor of 5 in water solutions compared to methanol solutions (see Table IIIb), and (2) the decays of fluorescence are much faster and they do not follow a single exponential decay function. They can be analyzed as a sum of two exponential components with predominant contribution from a short-lived species ($\tau \approx 1$ ns).

Emission spectra resulting from direct pyrene excitation (330 nm) were recorded as well for aqueous polymer solutions. The contribution of the excimer emission is larger than in methanol, but still significantly lower than in the case of singly labeled polymers (see Table IIIa). It is worth noting that the ratio $[I_E/I_M]^{Py}$ depends on the concentration of naphthyl groups attached to the polymer: the polymer with the higher [Na]:[Py] molar ratio exhibits a weaker excimer emission.

In summary, the spectroscopy of the doubly labeled polymers corroborates the existence of hydrophobic microdomains in water. First, the increase in the extent of NRET from Na* to Py in aqueous solutions relative to methanolic solutions reflects a drastic decrease of the average separation distance between the two chromophores brought in close proximity in the hydrophobic microdomains. Second, the decrease of the ratio $[I_E/I_M]^{Py}$ with increasing Na content of the copolymer corresponds to a localized dilution of pyrene in the domains: as the naphthalene content increases, the encounter probability of two pyrenes decreases accordingly. Whether the hydrophobic groups brought together are attached to a single polymer or whether they belong to different molecules cannot be determined from the data presented so far. But the stage is set for the key experiments which will allow unambigous distinction between intrapolymeric and interpolymeric interactions. These will be described next.

Properties of Mixed Copolymer Solutions. The composition of the hydrophobic microdomains was scrutinized by a series of energy transfer experiments between chromophores attached to the singly-labeled polymers PNIPAM–C_{18}Na/200 and PNIPAM–C_{18}Py/200. In all experiments the samples were prepared by mixing equilibrated solutions of each copolymer (see Experimental Section). Their compositions are listed in Table IV. In a first experiment the two solutions were mixed in relative amounts such that the [Na]:[Py] molar ratio matched that of a doubly labeled polymer (5:1). Emission spectra of the mixed solution were monitored upon excitation at 290 and 330 nm, together with those of solutions of PNIPAM–C_{18}Py/200 and PNIPAM–C_{18}Na/200 of corre-

Table IV
Composition of the Solutions Used in the Energy Transfer Experiments

polymer	solvent	[Py] (mol L^{-1})	[Na] (mol L^{-1})	[$C_{18}H_{37}$] (mol L^{-1})
terpolymer experiments				
PNIPAM–C_{18}Na/360–C_{18}Py/2000 (100 ppm)	H_2O, MeOH	5.16×10^{-7}	2.92×10^{-6}	3.44×10^{-6}
PNIPAM–C_{18}Na/680–C_{18}Py/1700 (100 ppm)	H_2O, MeOH	6.24×10^{-7}	1.56×10^{-6}	2.19×10^{-6}
mixed polymer experiment				
PNIPAM–C_{18}Na/200 (330 ppm) and PNIPAM–C_{18}Py/200 (70 ppm)[a]	H_2O	2.83×10^{-6}	1.42×10^{-5}	1.70×10^{-5}

[a] This solution was diluted further, keeping [Na]:[Py] = 5 (see text).

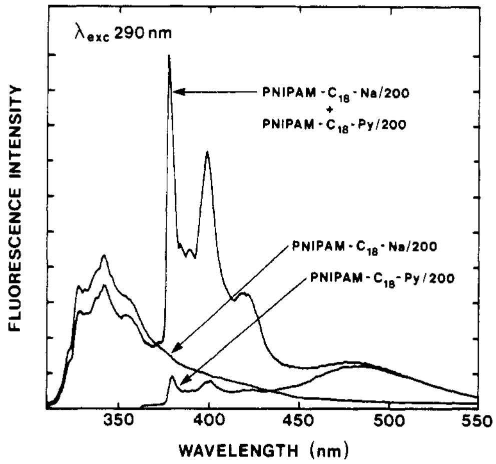

Figure 6. Fluorescence spectra of solutions in water of PNIPAM–C_{18}Na/200 and PNIPAM–C_{18}Py/200 and of a mixture of the two polymers (total polymer concentration 395 ppm; see Table IV for the relative polymer concentration in the mixture; λ_{exc} = 290 nm).

spondingly identical pyrene and naphthalene content. Excitation of the mixed solution at 290 nm (Figure 6) resulted in an emission spectrum with a very strong contribution from pyrene monomer emission, a weak pyrene excimer emission (maximum at 480 nm), and a contribution from excited naphthalene (maximum at 340 nm). By comparing this emission to the spectra of the solutions of each polymer, one observes a significant quenching of the naphthalene emission and a dramatic enhancement of the pyrene monomer emission, clear evidence of the occurrence of NRET between the two chromophores. Control experiments carried out with methanolic solutions showed that no energy transfer occurs in this solvent. Excitation of the mixed aqueous solution at 330 nm (spectrum not shown) resulted in pyrene monomer and excimer emission, with a weaker excimer emission, compared to the solution of PNIPAM–C_{18}Py/200 in the absence of PNIPAM–C_{18}Na/200: the ratio $[I_E/I_M]^{Py}$ decreased from a value of 1.10 in the solution of PNIPAM–C_{18}Py/200 to 0.5 in the mixed solution. The observation in the mixed solution of the occurrence of energy transfer between Na* and Py and of a decrease in $[I_E/I_M]^{Py}$ implies that Py and Na are brought into close proximity as the polymers intermix. A schematic view of the microstructures formed in the solutions before and after mixing is given in Figure 7. Our intent here is to provide a pictorial description of the phenomena and distances involved, rather than to draw a realistic representation of the polymeric micelles.

Effects of NRET of the Relative Chromophore Concentrations in the Mixed Solutions. The relative Na to Py molar ratio was varied from 1 to 6, keeping the concentration of PNIPAM–C_{18}Py/200 constant (13 ppm). The ratio $[I_E/I_M]^{Py}$ was affected by the addition of very small amounts of PNIPAM–C_{18}Na/200: it reached a constant value after addition of ca. 20 ppm of the naphthyl-labeled polymer (Figure 8, top). The increase in energy transfer efficiencies followed a more complex function of the [Na]:[Py] upon addition of the second polymer: at first a rapid enhancement of the pyrene emission took place, up to a [Na]:[Py] ratio of 2.5, and then a much smaller, continuous increase occurred with increasing [Na] concentration (Figure 8, bottom).

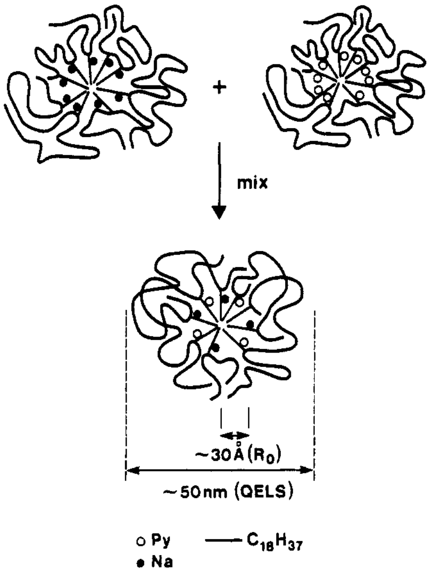

Figure 7. Pictorial representation of the polymeric microstructures formed upon mixing aqueous solutions of the pyrene-labeled polymer (PNIPAM–C_{18}Py/200) and the naphthalene-labeled polymer (PNIPAM–C_{18}Na/200). Also shown are the distances (not to scale) probed by the NRET and the QELS measurements.

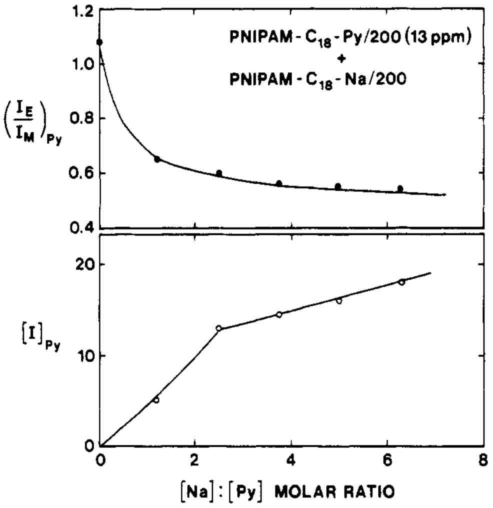

Figure 8. Changes in $[I_E/I_M]^{Py}$ (top) and in pyrene emission due to energy transfer (bottom see Experimental Section) as a function of the molar ratio of Na and Py for solution mixtures of PNIPAM–C_{18}Na/200 and PNIPAM–C_{18}Py/200 (13 ppm) in water.

Effect of the Total Polymer Concentration. The changes in energy transfer efficiency in mixed polymer solutions as a function of total polymer concentration were monitored in mixtures of increasing polymer concentration (4–150 ppm) for a constant chromophore molar ratio ([Na]:

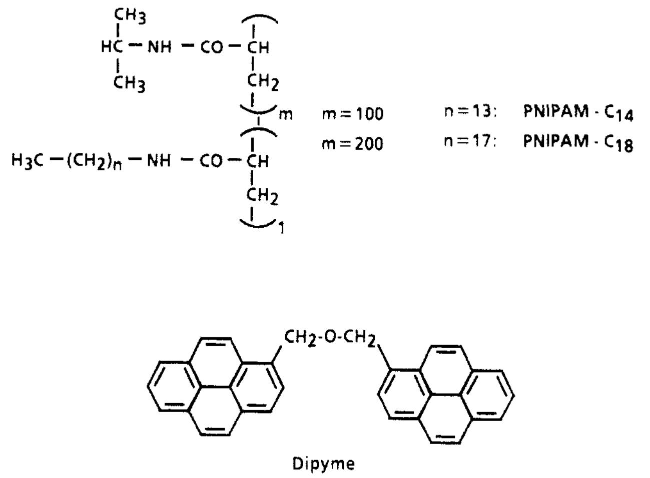

Figure 9. Chemical structures of the polymers PNIPAM–C_n and of dipyme.

[Py] = 5). The intensity of the pyrene emission due to NRET from Na*, I_{Py}, was determined for each sample, after correcting for the emission from directly excited pyrene (see Experimental Section). It increased linearly with total polymer concentration. To account for changes in pyrene concentration, we calculated also a "concentration-reduced" pyrene emission intensity (I_{Py}/[Py]). This parameter remained constant, within experimental errors, over the entire polymer concentration range. Therefore, the observed increase in NRET is simply a result of the increase in total chromophore concentration. It is independent of polymer concentration. The polymers intermix even at concentrations as low as a few parts per million. The onset of intermixing cannot be detected by this technique.

Influence of Sample Preparation. It is worth noting that in the preceding experiments identical spectra were obtained from mixed solutions prepared either by diluting a "mother" mixed solution or by mixing solutions of each polymer of various concentrations. Moreover, spectra recorded at various time intervals after mixing of the polymers were identical in all features, over time periods ranging from a few minutes to several days for solutions kept at either 5 or 25 °C. This apparent absence of aging effects is atypical. It is in contrast with the well-known time-dependent behavior of related associative polymers, such as the acrylamide–*N*-alkylacrylamide copolymers studied by McCormick and co-workers.[31]

Critical Aggregation Concentration. In solutions of hydrophobically modified polymers it is usually possible to determine experimentally a concentration below which the polymers do not aggregate but rather form unimolecular polymeric micelles. This concentration, referred to as the critical aggregation concentration (cac) by analogy with the critical micelle concentration (cmc) of surfactants, varies greatly with polymer structure. Different techniques have different sensitivity. Fluorescence techniques reveal phenomena on the angström distance scale from 4 to 5 Å in the case of pyrene excimer emission and up to several tens of angströms in the case of NRET phenomena. We compare here our results on energy transfer measurements with experiments carried out previously with PNIPAM–C_{18}Py/200[7] and with a series of the closely related unlabeled hydrophobically-modified PNIPAM–C_n (Figure 9). The pyrene label was the intrinsic probe in the case of PNIPAM–C_{18}Py/200. In the solutions of the unlabeled polymers an extrinsic probe, dipyme (bis(1-pyrenylmethyl) ether), was employed (Figure 9).[32]

Spectra of PNIPAM–C_{18}Py/200 in water were recorded for polymer concentrations ranging from 5 to 250 ppm. The ratio $[I_E/I_M]^{Py}$ was insensitive to changes in polymer concentration for polymer solutions of concentration exceeding ~20 ppm. Below this concentration a small decrease in $[I_E/I_M]^{Py}$ was detected, from 1.10 (20 ppm) to 0.90 (5 ppm). It was not possible to ascertain whether this decrease is a true indication of a change in micellar structure or whether it is due to a poor signal at these low levels of fluorescence intensity, a common occurrence when fluorescence measurements are carried out with extremely dilute polymer solutions.

The experiments with PNIPAM–C_n relied on data generated by a low molecular weight probe solubilized exclusively in the hydrophobic microdomains. In this situation the probe concentration can be kept constant in solutions of polymers of varying concentrations or structures. The polymer concentration range that can be investigated with a probe can be extended to concentrations much higher than with solutions of labeled polymers. In the latter case fluorescence experiments cannot be relied upon at high concentrations because of excessive chromophore content.[33] The fluorescent probe was dipyme, a dichromophoric molecule which forms an intramolecular excimer. Excimer formation depends upon a change in the conformation of the molecule. The rate of this motion is resisted by the local viscosity of the environment. As a consequence the excimer-to-monomer intensity ratio $[I_E/I_M]^{DP}$ provides a measure of the microviscosity sensed by the probe.[34] The solubility of dipyme in water is extremely low. Therefore, in solutions of hydrophobically-modified polymers it resides in the hydrophobic microdomains and reports exclusively on the microviscosity of this medium. The ratio $[I_E/I_M]^{DP}$ of dipyme dissolved in solutions of a series of hydrophobically-modifed PNIPAM was monitored as a function of polymer concentration from 0.01 g L^{-1} (10 ppm) to 1.7 g L^{-1}.[32] These experiments also showed an insensitivity of the ratio to changes in polymer concentrations. The only change in $[I_E/I_M]^{DP}$ occurred at the lowest concentrations of probe and polymer, where spectra are quite noisy. Hence, the microviscosity of the probe medium does not vary, an observation pointing again toward the remarkable stability of the hydrophobic microdomains upon dilution of the polymer solutions.

The occurrence of interpolymeric aggregation at extremely low polymer concentrations has been observed with other nonionic hydrophobically-modified polymers. Akiyoshi et al. examined by a fluorescence probe technique the solution properties of hydrophobic polysaccharides, pullulans (MW 50 000) substituted either with cholesteryl or palmitoyl (C_{15}) moieties (ca. 5 hydrophobic substituents per 100 glucose units).[35] They reported a cac value of a few parts per million ($(1-4) \times 10^{-7}$ mol L^{-1}). Wilhelm et al. investigated the formation of aggregates in solutions of polystyrene–poly(ethylene oxide) diblock and triblock copolymers (M_n 8500–29000). Using pyrene as a fluorescent probe, they were able to detect the onset of interpolymeric aggregation.[36] They calculated cac values ranging between 1 and 5 ppm. In a recent preliminary communication, Nomura et al. reported interpolymeric micelle formation in solutions of amphiphilic poly(sodium 2-acrylamido-2-methylpropanesulfonates) containing different hydrophobic groups and chromophores.[37] The polymers studied carry a large fraction of hydrophobic substituents (30–50 mol %) and a low fraction of chromophores (1–4 mol %). Polymeric solutions of concentration higher than 100 ppm were monitored. In all cases the presence of micellar structures was detected. Unfortunately, experiments with solutions of lower concentration have not yet been reported.

Conclusion

The experiments reported here give convincing evidence that in water hydrophobically-modified poly(*N*-isopropylacrylamides) carrying as few as 6–10 octadecyl groups per macromolecule form interpolymeric micellar structures in extremely dilute solutions. Aggregation occurs at temperatures well below the LCST, in contrast to PNIPAM which does not self-aggregate in water below its LCST. The results lead the way to new experiments directed toward a deeper understanding of the molecular phenomena occurring in aqueous solutions of hydrophobic polymers. Of particular importance are studies probing the sensitivity of polymeric micelle formation to the nature of the hydrophobic substituents, to details of sample preparation, and to the temperature-induced perturbations of the microdomain structures.

Acknowledgments. It is a pleasure to acknowledge the competent help of Dr. G. Hamer (XRCC) in measuring and interpreting the NMR spectra and that of Mr. A. Davidson (XRCC) for carrying out molecular weight determinations by GPC. We also thank Professor M. A. Winnik (University of Toronto) for many stimulating discussions and for his helpful comments on this paper. Financial support for this work was provided in part (H.R. and J.S.) by the Bundesministerium für Forschung und Technologie (BMFT) and by the Deutsches Forschungsgemeinschaft (DFG).

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