Supporting Information for

Temperature Response of Aqueous Solutions of a Series of Pyrene End-Labeled Poly(*N*-isopropylacrylamide)s Probed by Turbidimetry, Light Scattering, and Fluorescence

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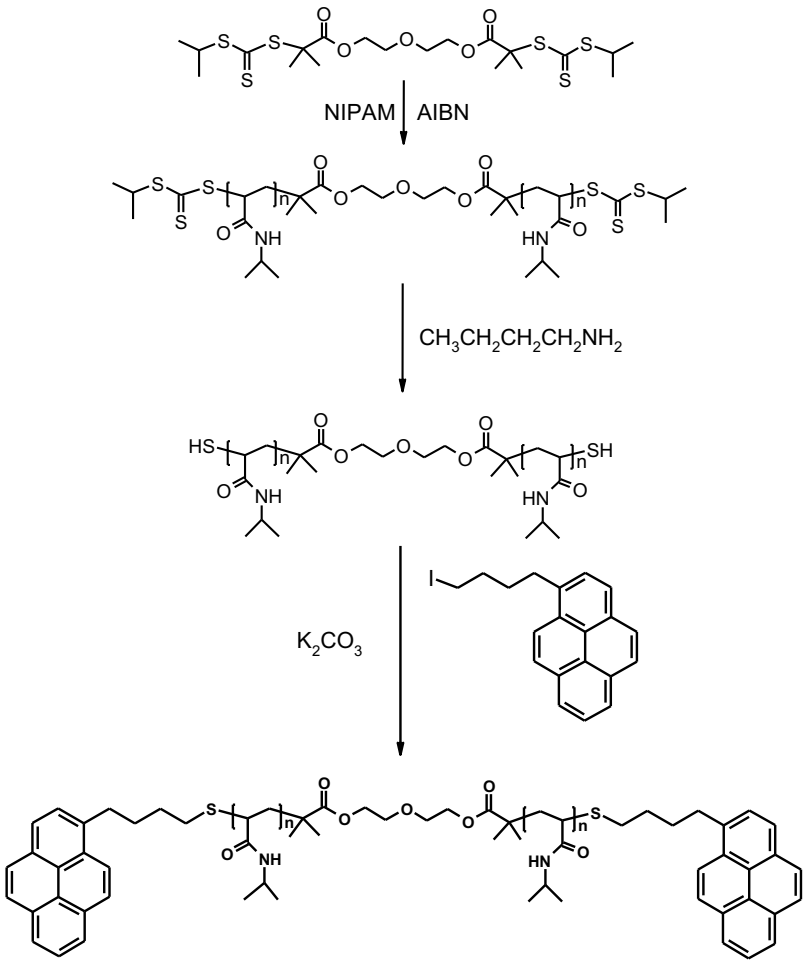
Synthesis of the Py_n-PNIPAM

The preparation and characterization of the Py_n-PNIPAM samples with n =1 or 2 have been described in detail earlier.[1,2] The synthesis was carried out according to Scheme S1 for the telechelic Py_2-PNIPAM samples. The main difference for the synthetic protocol of the telechelic and semi-telechelic Py_n-PNIPAM samples was the chain transfer agent (CTA). Whereas diethylene glycol di(2-(1-isobutyl)sulfanylthiocarbonyl-2-methylpropionate) (DEGDIM) was employed as a bifunctional CTA for the synthesis of the Py_2-PNIPAM samples as shown in Scheme S1, the monofunctional CTA ethyl 2-(1-isobutyl)sulfanylthiocarbonyl-2-methylpropionate) was used for the synthesis of the Py_1-PNIPAM samples.



Scheme S1. Synthetic procedure for telechelic Py_2-PNIPAM.

The distribution of absolute molecular weight of the Py_n-PNIPAM samples was determined with a GPC instrument equipped with a multi angle laser light scattering (MALLS) detector. Table S1 presents the number average molecular weight (M_n) and polydispersity index (M_w/M_w) where M_w is the weight average molecular weight of the polymer. Figure S1 shows all the GPC traces obtained with the polymers.

Table S1. GPC-MALLS characterization of the telechelic α,ω-di-[4-(1-pyrenyl)butyl] poly(*N*-isopropylacrylamides) (Py_2-PNIPAM) and semi-telechelic α-ethyl-ω-[4-(1-pyrenyl)butyl] poly(*N*-isopropylacrylamides) (Py_1-PNIPAM) samples

Polymer Sample	M_n (kDa)	M_w/M_n
Py_2-PNIPAM(7K)	7.6	1.08
Py_2-PNIPAM(14K)	13.7	1.10
Py_2-PNIPAM(25K)	25.4	1.07
Py_2-PNIPAM(45K)	44.5	1.10
Py_1-PNIPAM(7K)	7.7	1.02
Py_1-PNIPAM(12K)	12.3	1.02
Py_1-PNIPAM(25K)	23.5	1.09

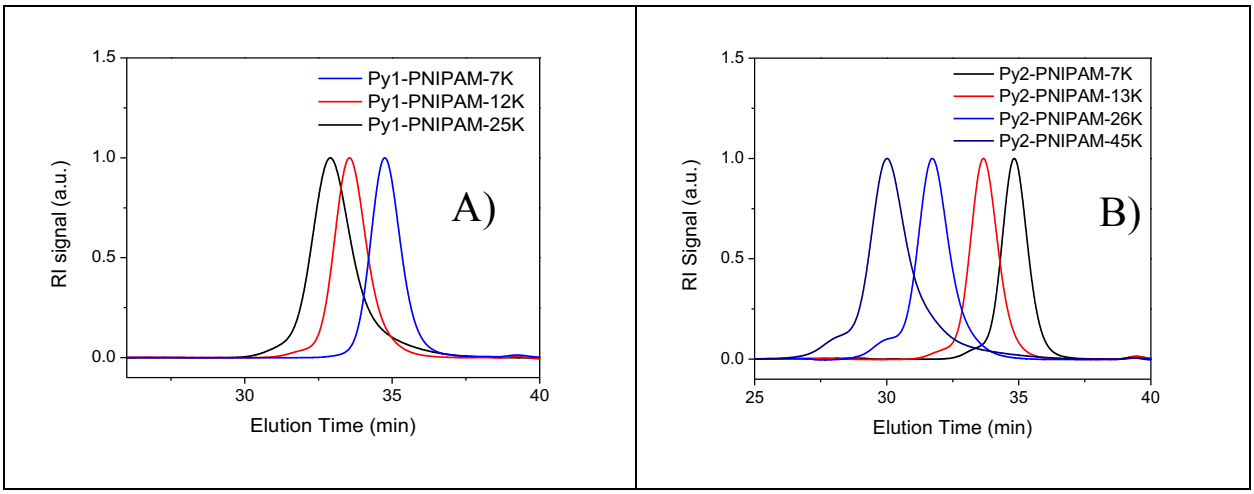


Figure S1. GPC-traces of A) semi-telechelic Py_1-PNIPAM(7K), Py_1-PNIPAM(12K) and Py_1-PNIPAM(25K) and B) Py_2-PNIPAM(7K), Py_2-PNIPAM(13K), Py_2-PNIPAM(25K) and Py_2-PNIPAM(45K) in DMF at 40 °C.

Determination of T_c by Turbidimitry

The % transmittance of a 0.5 g/L solution of Py_2-PNIPAM(14K) was obtained as a function of temperature with a heating rate of 0.5 ^oC/min, as shown in Figure S2A. The derivative of the % transmittance was taken, which is shown in Figure S2B.

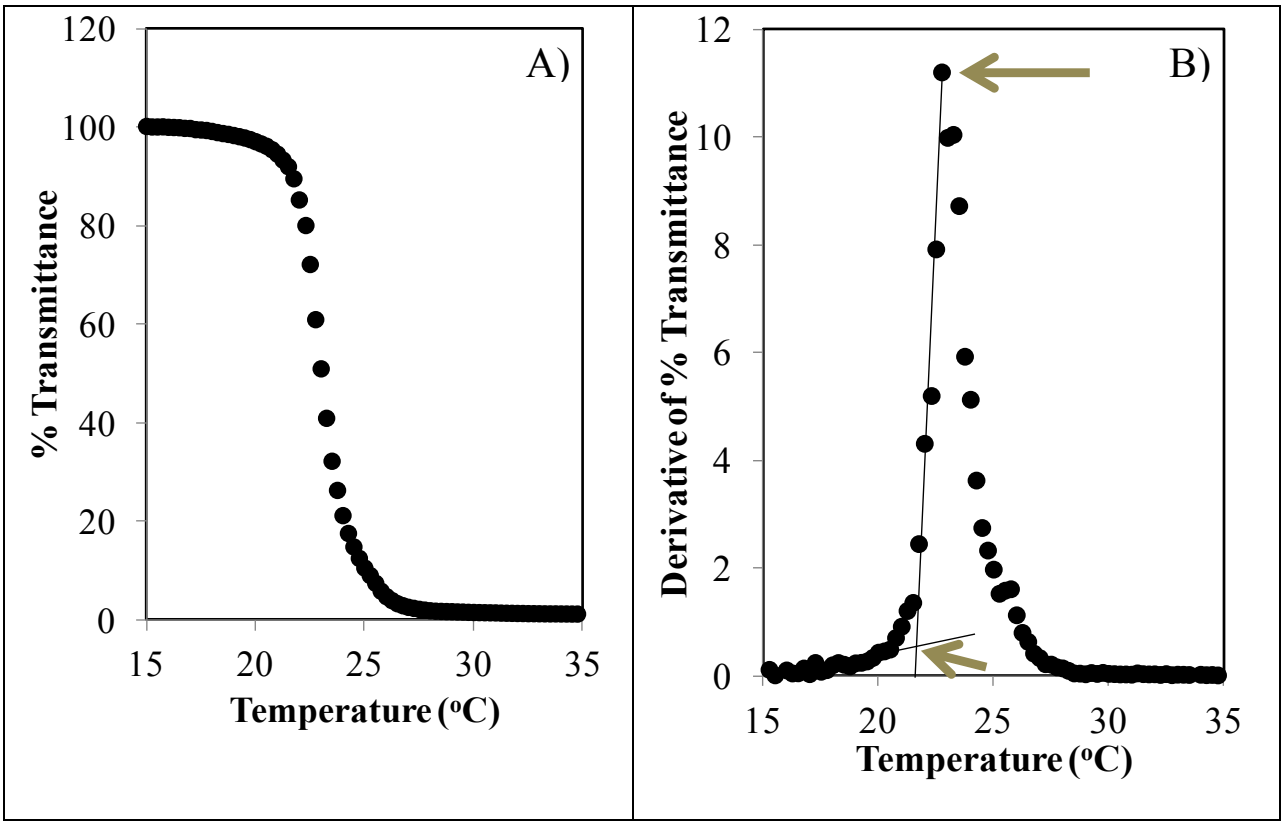


Figure S2: Turbidimetry results obtained for 0.5 g/L Py_2-PNIPAM(14K) with a heating rate of 0.5 °C/min. A) % transmittance curve. B) Derivative curve.

The turbidity curve shown in Figure S3A shows a clear transition from high to low transmittance that occurs over a range of temperature values. When the derivative of the curve is taken, as shown in Figure S2B, the critical temperature could be taken as either the peak in the derivative or the intersection point between the baseline and the rise of the derivative.

The T_c trends observed for 0.5 g/L solutions with a 0.5 °C/min heating rate are reported in the main text in Figure 3B where T_c was taken as the derivative onset. The trends for these same solutions where T_c was taken as the derivative peak are shown in Figure S3.

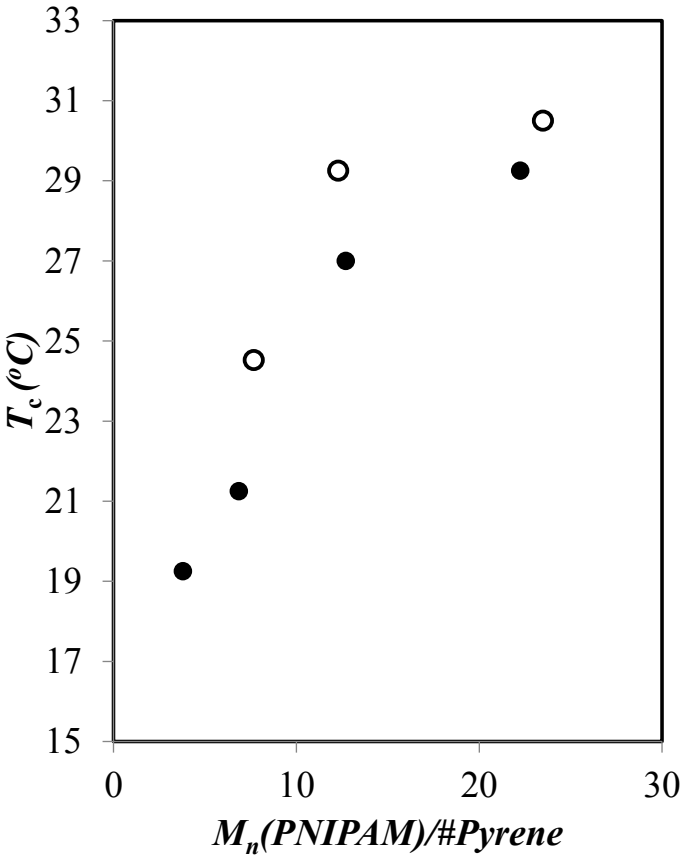


Figure S3: T_c determined from the derivative peak as a function of the ratio of M_n for the Py-PNIPAM samples over their respective number of pyrenes. (●) Py_2-PNIPAM (○) Py_1-PNIPAM

Analysis of the Fluorescence Spectra

Even at the low polymer concentrations used in the fluorescence experiments, the fluorescence spectra of the Py_2-PNIPAM solutions showed some light scattering distortion at 350 nm where pyrene does not emit. This is illustrated in Figure S4A that shows the fluorescence spectrum of Py_2-PNIPAM(14K) at $T = 26\ ^oC$ ($T_c = 24\ ^oC$ for Py_2-PNIPAM(14K) obtained from the I_E/I_M trend in Figure 4. Consequently the analysis of the fluorescence spectra of the doubly labelled PNIPAM samples (Figure S4A) was performed by first applying a light scattering correction. A fluorescence spectrum was acquired for non-fluorescent PNIPAM(22K) above its T_c to obtain the scattering envelop. It was normalized to the scattering intensity observed in the fluorescence spectrum between 350 and 360 nm, and the normalized scattering envelop was subtracted from the fluorescence spectrum of Py_2-PNIPAM(14K) to yield the fluorescence spectrum S3C. Then the fluorescence of the pyrene excimer was determined by subtracting the fluorescence spectrum of Py_1-PNIPAM(25K) at a given temperature normalized to the fluorescence intensity at 375 nm of the Py_2-PNIPAM(14K) sample. Py_1-PNIPAM(25K) showed hardly any excimer over the entire temperature range and was used as a model representative of the pyrene monomer. The spectra of Py_1-PNIPAM(25K) were employed to carry out the subtraction. After subtraction, the fluorescence spectrum shown in Figure S4D was obtained. The "spiky" features observed in the 370 – 390 nm range in Figure S4C are the result of a slight mismatch between the wavelengths in the fluorescence spectra of the pyrene monomer for the Py_1-PNIPAM(25K) and the corresponding Py_2-PNIPAM sample. The fluorescence spectrum of the excimer shown in Figure S4D was then integrated from 420 to 600 to yield I_E in the I_E/I_M calculations.

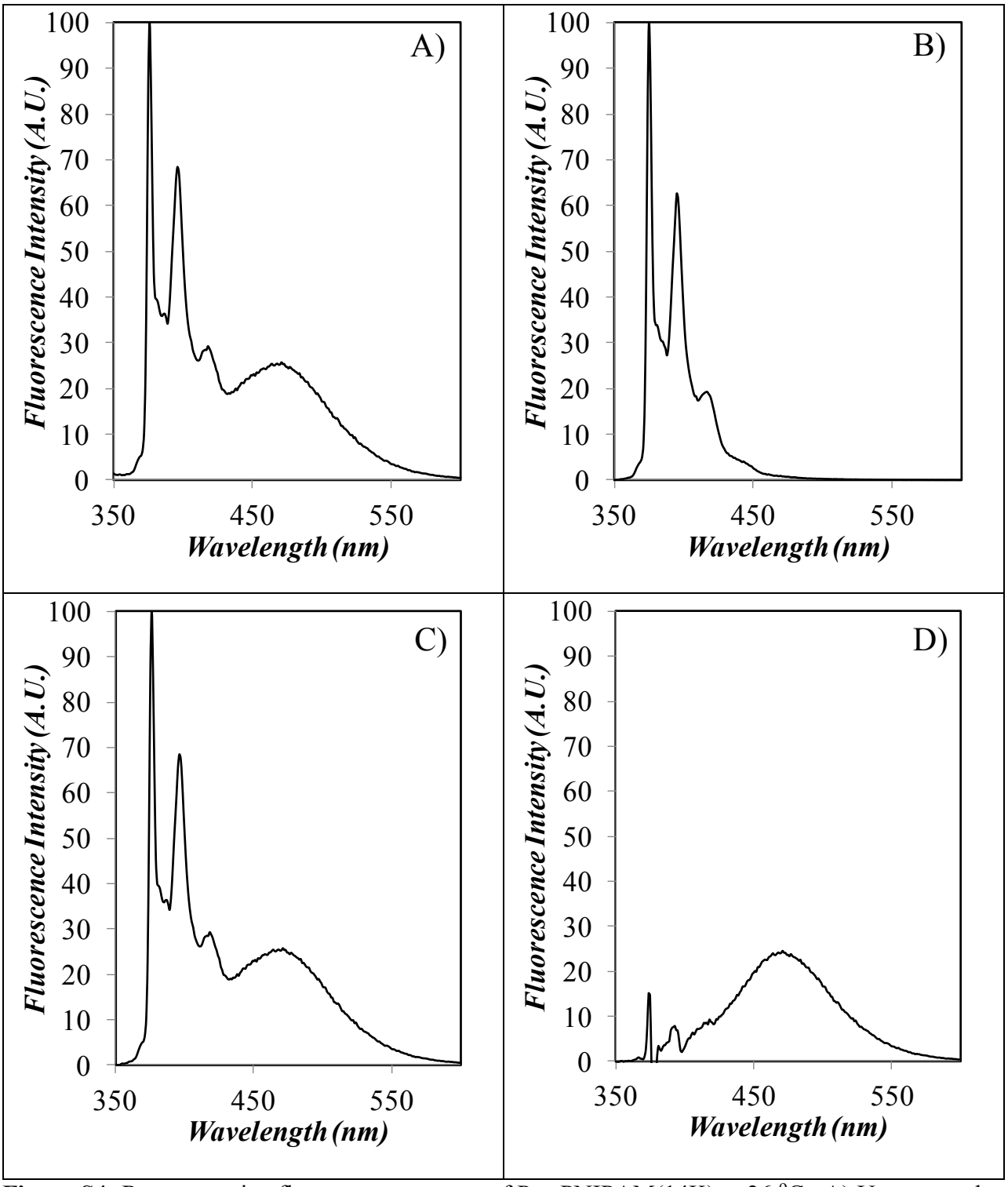


Figure S4: Representative fluorescence spectra of Py_2-PNIPAM(14K) at 26 ^{o}C. A) Uncorrected fluorescence spectrum. B) Monomer fluorescence spectrum. C) Spectrum after the application of a light scattering correction. D) Spectrum after subtraction of the monomer fluorescence.

Example Fluorescence spectra and decays

Time resolved fluorescence decays and steady-state fluorescence spectra were acquired for the aqueous Py_n-PNIPAM solutions, examples of which are shown in Figure S5.

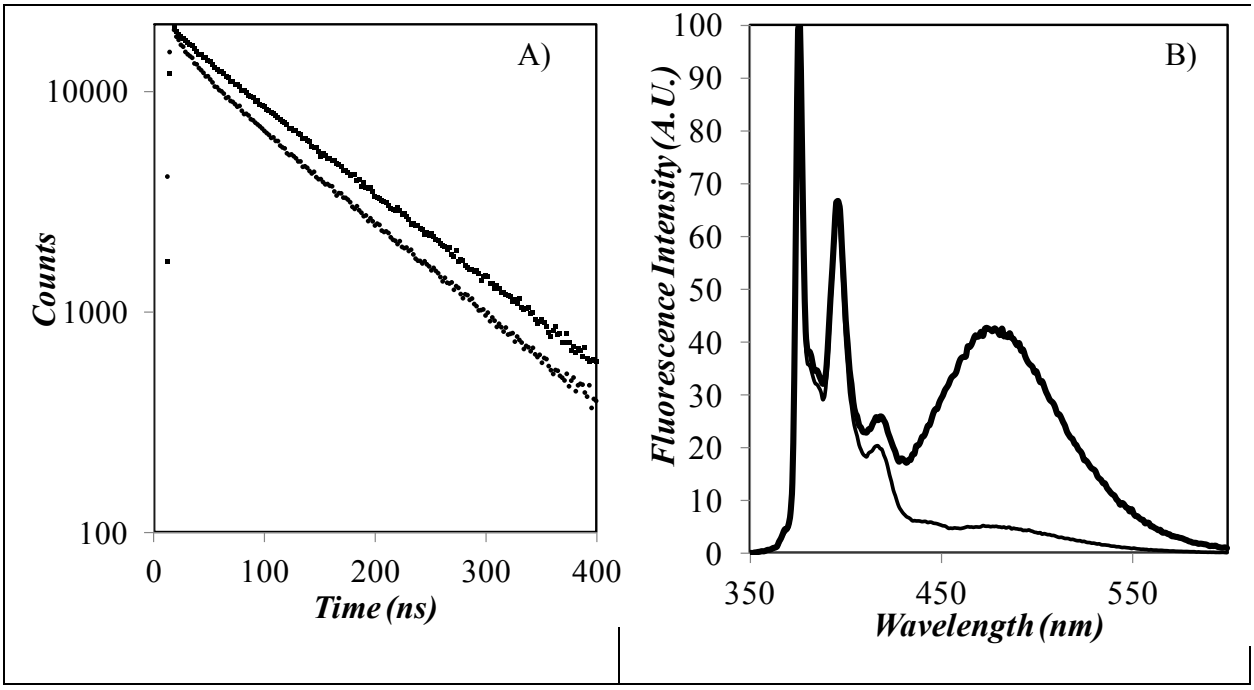


Figure S5. Pyrene monomer fluorescence decays and steady-state emission spectra. A) Upper decay: Py_2-PNIPAM(14K). Lower decay: Py_2-PNIPAM(25K) B) Upper Spectrum: Py_2-PNIPAM(14K). Lower spectrum: Py_2-PNIPAM(25K)

T_c measured by DLS.

The increase in turbidity of the aqueous Py_n-PNIPAM solutions observed as a function of temperature was due to an increase in the amount of light being scattered by the solution as a result of an increase in the size of the particles being generated in solution. To determine the exact nature of the changes in particle size, dynamic light scattering (DLS) measurements were carried out as a function of temperature for the Py_n-PNIPAM aqueous solutions, the results of which are shown in Figure S6.

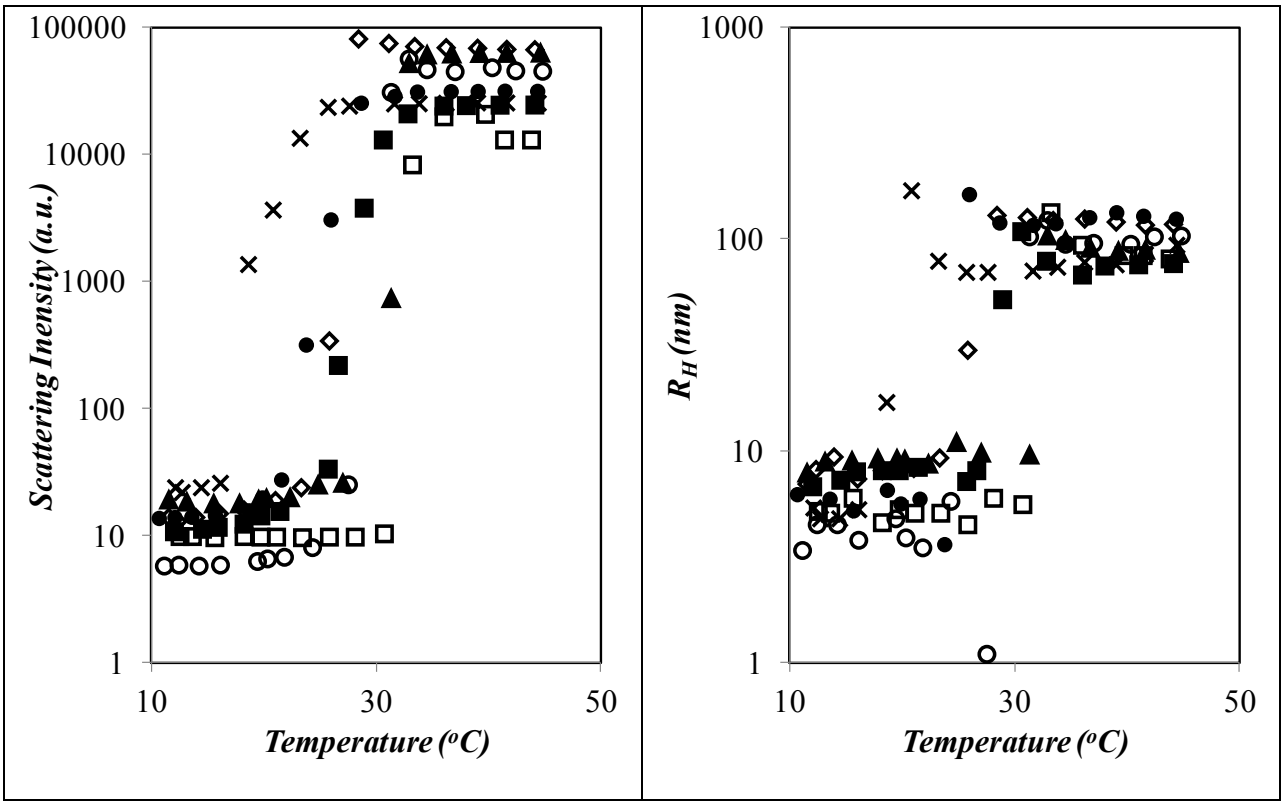


Figure S7: DLS measurements for Py-PNIPAM as a function of temperature A) Intensity of scattered light. B) Hydrodynamic radius. (×) Py_2-PNIPAM(7K), (●) Py_2-PNIPAM(14K), (■) Py_2-PNIPAM(25K), (▲) Py_2-PNIPAM(45K), (◊) Py_1-PNIPAM(7K), (○) Py_1-PNIPAM(12K), and (□) Py_1-PNIPAM(25K).

The light scattering intensity profiles shown in Figure S6A undergo a sharp transition at temperatures corresponding to the cloud point of the respective Py_n-PNIPAM solutions. The size of the objects present in each solution increased significantly at T_c, indicating the formation of mesoglobules (Figure S6B). Interestingly, the temperature at which this size transition took place for the Py_1-PNIPAM solutions matched the temperature of the Py_2-PNIPAM solutions with the same pyrene content and polymer concentration, and both trends agreed remarkably well with the values of T_c determined by turbidimetry (Figure 1B). The polymer coil size of the Py_n-PNIPAM

samples in solution, as determined from the DLS measurements, was then compared to the theoretically derived hydrodynamic radii for single chains in Table 2 (see Grosberg, A. Y.; Kokhlov, A. R. *Statistical Physics of Macromolecules*. American Institute of Physics Press: New York, 1994).

While the theoretical determination neglected the presence of the pyrene moieties, the theoretical hydrodynamic radius (R_H) for the longer semi-telechelic Py_1-PNIPAM(12K) and Py_1-PNIPAM(25K) chains showed a relatively good agreement with the experimental R_H values measured by DLS. This observation led to the conclusion that the longer, and thus more hydrophilic, semi-telechelic chains exist primarily as unimers in solution. In contrast, the theoretical R_H values for the Py_2-PNIPAM samples were all significantly smaller than the R_H values calculated for unimers, leading to the conclusion that these chains exist primarily as micelles in solution.

Effect of Polymer Concentration and Heating Rate on T_c

The difficulty in determining T_c at low concentration and for short chains was due in part to a broadening of the transition whose half point in the transmission profile shifted to higher temperature. This broadening is reflected by the full-width at half-maximum values (FWHM) reported in Tables S2 and S3, which are distinctly larger for the shorter chains and lower polymer concentrations. This represents a problem when conducting turbidimetry measurements with a spectrophotometer to assess the exact temperature of a cloud point as the transition occurring at T_c decreases in intensity and broadens substantially with decreasing polymer concentration. The breadth of the transition is also significant as it determines the difference between the onset and the mid-point of the change in turbidimetry, as reflected by the onset and peak in the derivative of

the % transmittance. The onset of the change in the derivative of the turbidity of the solution will reflect the behaviour of the least soluble chains; in the case of Py_n-PNIPAM these would be the shortest chains. The peak in the derivative will reflect the point at which aggregation is 50% complete, so long as the turbidity above T_c does not reach zero. It is therefore possible that while the peak in derivative best reflects the behaviour of the solution as a whole, the onset will show the best agreement with light scattering techniques that also are most sensitive to the least-soluble chains.

The effect of the heating rate on T_c is shown in Figure S7B where T_c is found to increase with increasing heating rate. This trend reflects the time needed to develop a sufficient number of sticky contacts between different PNIPAM chains to induce mesoglobule formation. By decreasing the heating rate to afford a longer time for interpolymeric contacts to occur, the cloud point of the solution is being detected at a lower temperature. The Py_n-PNIPAM(7K) samples also showed distinctly larger FWHM values, regardless of heating rate. The observations made for Figures S7A and B illustrate the difficulty associated with the comparison of trends obtained under different experimental conditions. The T_c values obtained as a function of heating rate and polymer concentrations have been listed in Tables S4-7.

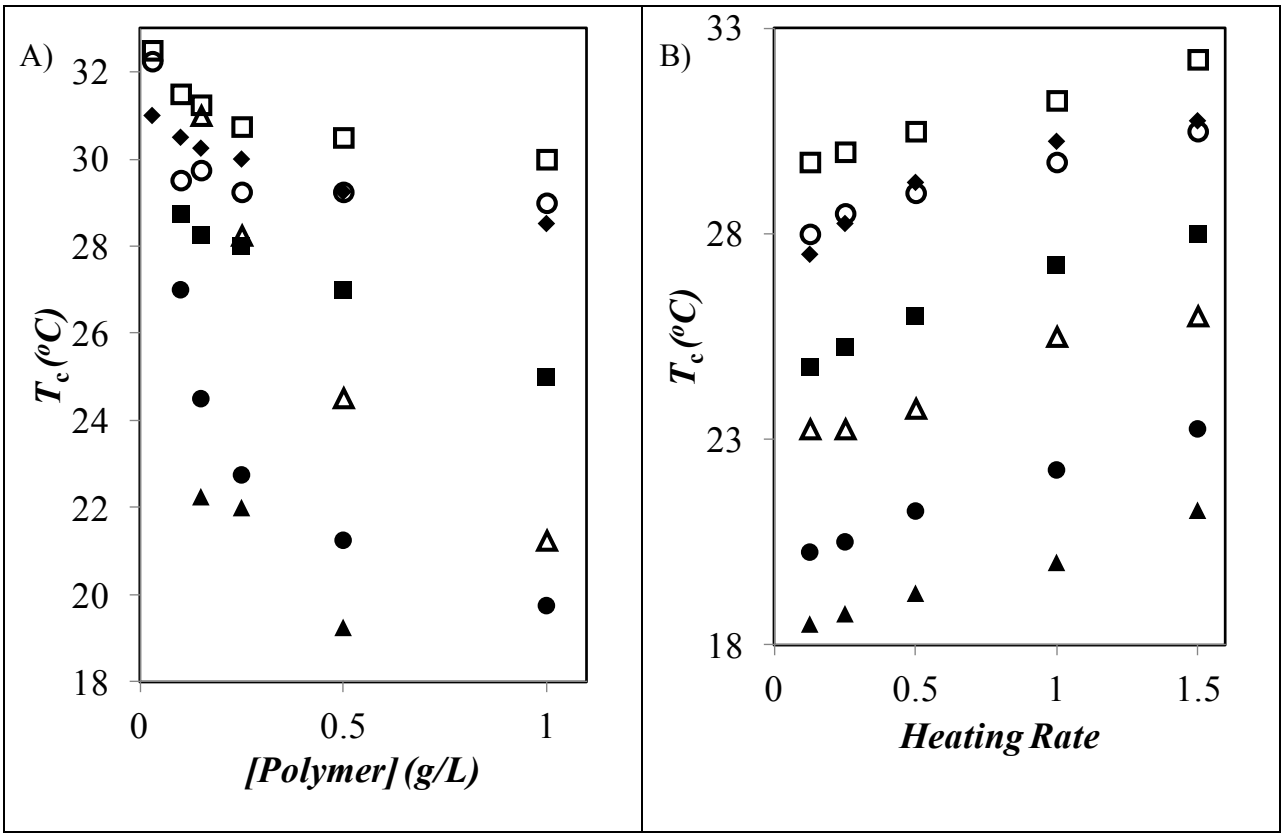


Figure S7: Turbidimetry measurements for pyrene-labeled PNIPAM samples as a function of A) polymer concentration with a heating rate of 0.5 ^{o}C/min and B) heating rate with a polymer concentration of 0.5 g/L. (▲) Py_2-PNIPAM(7K), (●) Py_2-PNIPAM(14K), (■) Py_2-PNIPAM(25K), (♦) Py_2-PNIPAM(45K), (Δ) Py_1-PNIPAM(7K), (○) Py_1-PNIPAM(14K), and (□) Py_1-PNIPAM(25K).

Turbidimetry Full-Width Half-Max

The Full-Width at Half-Maximum (FWHM) values obtained from the derivative of the transmittance for the Py_n-PNIPAM solutions are shown in Table S2 and Table S3 for varying concentration and heating-rate, respectively.

Table S2: FWHM (^oC) of the derivative of the transmittance for Py_n-PNIPAM solutions with a heating rate of 0.5 ^oC/min.

[Polymer] (g/L)	Py_1-PNIPAM (7K)	Py_1-PNIPAM (12K)	Py_1-PNIPAM (25K)	Py_2-PNIPAM (7K)	Py_2-PNIPAM (14K)	Py_2-PNIPAM (26K)	Py_2-PNIPAM (45K)
1.0		1	0.75		2.5	2	1
0.5	7	1	1	1.25	1.75	1.5	1
0.25	5.25	2.25	1.5	3	2.75	1.25	1.5
0.15	4.25	2.75	1.25	4.25	1.5	1.25	1.5
0.1		2.5	1.5			1.25	1.5
0.03		2.5	2.75				

Table S3: FWHM (^oC) of the derivative of the transmittance for Py_n-PNIPAM solutions with a concentration of 0.5 g/L.

Heating Rate (^oC/min)	Py_1-PNIPAM (7K)	Py_1-PNIPAM (12K)	Py_1-PNIPAM (25K)	Py_2-PNIPAM (7K)	Py_2-PNIPAM (14K)	Py_2-PNIPAM (26K)	Py_2-PNIPAM (45K)
1.5	5.5	1.5	1	4	1.75	1.75	1
1	7	1.25	0.75	3.5	1.5	1.75	1
0.5	6.75	1.25	1	1.75	1.5	1.75	0.75
0.25	6.25	1.5	1.25	3	1.5	1.25	1

0.125	7.75	1	1	2.25	1.5	2	1.25

Turbidimetry T_c Results:

Table S4: T_c as determined by the peak in the derivative of % Transmitance for Py_n-PNIPAM solutions with a heating rate of 0.5 ^oC/min.

[Polymer] (g/L)	Py_1-PNIPAM (7K)	Py_1-PNIPAM (12K)	Py_1-PNIPAM (25K)	Py_2-PNIPAM (7K)	Py_2-PNIPAM (14K)	Py_2-PNIPAM (26K)	Py_2-PNIPAM (45K)
1.0		29.75	30.75		21.25	27.25	29.75
0.5	30.25	30.25	31.5	21.5	23	28	30.25
0.25	32.25	30.75	32.25	23	24.25	29	30.75
0.15	34.5	31.75	32.5	24.25	25.75	29.5	31.25
0.1		32.25	33			29.75	31.5
0.03		33.75	34.5				

Table S5: T_c as determined by the peak in the derivative of % Transmitance for Py_n-PNIPAM solutions with a concentration of 0.5 g/L.

Heating Rate (^oC/min)	Py_1-PNIPAM (7K)	Py_1-PNIPAM (12K)	Py_1-PNIPAM (25K)	Py_2-PNIPAM (7K)	Py_2-PNIPAM (14K)	Py_2-PNIPAM (26K)	Py_2-PNIPAM (45K)
1.5	32.25	31.75	33.25	24	25	30	32
1	31.5	31	32.5	23.5	24.5	29	31.25
0.5	30.25	30.25	31.5	21.5	22.75	28	30.25
0.25	30.25	29.75	30.75	20.5	22.5	27.75	29.75
0.125	29	29.5	30.75	20	22.25	27.25	29.5

Table S6: T_c as determined by the intersection of the baseline and rise in the derivative of % Transmitance for Py_n-PNIPAM solutions with a heating rate of 0.5 ^oC/min.

[Polymer] (g/L)	Py_1-PNIPAM (7K)	Py_1-PNIPAM (12K)	Py_1-PNIPAM (25K)	Py_2-PNIPAM (7K)	Py_2-PNIPAM (14K)	Py_2-PNIPAM (26K)	Py_2-PNIPAM (45K)
1.0	21.25	29	30		19.75	25	28.52
0.5	24.52	29.25	30.5	19.25	21.25	27	29.25
0.25	28.25	29.25	30.75	22	22.75	28	30
0.15	31	29.75	31.25	22.25	24.5	28.25	30.25
0.1		29.52	31.5		27	28.75	30.5
0.03		32.25	32.5				31

Table S7: T_c as determined by the intersection of the baseline and rise in the derivative of % Transmitance for Py_n-PNIPAM solutions with a concentration of 0.5 g/L.

Heating Rate (^oC/min)	Py_1-PNIPAM (7K)	Py_1-PNIPAM (12K)	Py_1-PNIPAM (25K)	Py_2-PNIPAM (7K)	Py_2-PNIPAM (14K)	Py_2-PNIPAM (26K)	Py_2-PNIPAM (45K)
1.5	26	30.5	32.25	21.27	23.25	28	30.75
1	25.5	29.75	31.25	20	22.25	27.25	30.25
0.5	23.75	29	30.5	19.25	21.25	26	29.25
0.25	23.25	28.5	30	18.75	20.5	25.25	28.25
0.125	23.25	28	29.75	18.5	20.25	24.75	27.5

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1. Yip, J. Duhamel, J.; Qiu, X.-P. ; Winnik, F. M. Fluorescence Study of a Series of Monodisperse Telechelic a,w-Dipyrenyl Poly(N-isopropylacrylamide)s in Ethanol and Water. *Can. J. Chem.* **2011**, *89*, 163-172.
2. Yip, J. Duhamel, J.; Qiu, X.-P. ; Winnik, F. M. Long-Range Polymer Chain Dynamics of Pyrene-Labelled Poly(*N*-isopropylacrylamide)s Studied by Fluorescence. *Macromolecules* **2011**, *44*, 5363-5372.