Synthesis and Aggregation Behaviors of Well-Defined Thermoresponsive Pentablock Terpolymers With Tunable LCST

Yufeng Wu, Xingli Liu, Yu Wang, Zanru Guo, Yujun Feng*

A series of thermoresponsive pentablock terpolymers, poly(N-isopropylacrylamide)-b-poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide)-b-poly(N-isopropylacrylamide), is prepared by reversible addition–fragmentation chain transfer (RAFT) polymerization. The effect of NIPAM and PPO block lengths on lower critical solution temperature (LCST), critical micelle concentration (cmc), and aggregation number (N_{agg}) is investigated via UV–Vis spectroscopy and steady-state fluorescence spectroscopy. The results show that upon increasing the block lengths, LCST and cmc decrease, while N_{agg} increases. TEM observation shows that associated spherical-like particles are evidenced below the LCST of the terpolymers, and regular or irregular spherical micelles or even intermicellar aggregates are observed above the LCST.

1. Introduction

Over the past decades, amphiphilic block copolymers have attracted a noticeable attention due to their unique associative solution properties and great potential in versatile applications such as controlled release, gene delivery, capillary electrophoresis, and surface modification. Thermoresponsive block copolymers whose physicochemical properties change abruptly upon minor stimuli of temperature have been widely investigated and show promising applications in biomedicines and intelligent materials. It is well recognized that N-isopropylacrylamide (NIPAM) is the most frequently employed comonomer to prepare thermosensitive polymers since the lower critical solution temperature (LCST) of its homopolymer is close to human body temperature. Additionally, the LCST or relevant properties of NIPAM-based polymers can be further regulated by incorporating other hydrophilic or hydrophobic comonomers to form diblock or triblock copolymers. For example, Zhuo and co-workers observed reversible dispersion/aggregation of the micelles in response to cyclic change of temperature when introducing methyl methacrylate block onto the poly(N-isopropylacrylamide) (PNIPAM) skeleton. Pispas and co-workers found that the copolymer composition, concentration, and heating rate have pivotal effects on the micelle size and aggregate morphology above LCST of the diblock copolymer, poly(ethylene oxide)-b-poly(N-isopropylacrylamide) (PEO-b-PNIPAM). Dehghani et al. prepared a thermosensitive triblock copolymer, poly(ethylene glycol)-b-poly(methyl methacrylate-co-3-(trimethoxysilyl)propyl methacrylate)-b-poly(N-isopropylacrylamide) (PEG-b-P(MMA-co-MPMA)-b-PNIPAM), and observed micelles or vesicles below and above LCST, respectively. Most of these previous studies showed that thermosensitive triblock copolymer has a richer responsive behavior than their homopolymer or diblock
radii of gyration, size, and aggregation numbers of the micelles. They concluded that the number of blocks and chain architectures has crucial effects on the chain conformation and promising applications. More recently, Du et al.\cite{26} developed a thermosensitive pentablock terpolymer, PNIPAM$_{110}$-PEO$_{100}$-PPO$_{65}$-PEO$_{100}$-PNIPAM$_{110}$, by using ATRP procedure, and examined its chain conformation and solution properties. They observed that loose “associates” and single coil chains coexisted below LCST, and large stable core-shell micelles above LCST. These studies on multiblock copolymers showed that abundant responses and solution properties as well as more complex self-assembly morphologies could be attained by “tailoring” the architecture and functionality of the copolymers. Nevertheless, the overwhelming majority of reports to date have been focused on the sensitivity, micellization or sol–gel transition, less attention has been paid to the interrelated effect of different block lengths on the chain conformation and solution properties of the multiblock copolymers. An attempt has been made in our laboratory to correlate block lengths and LCST, as well as aggregation solution properties of pentablock terpolymers.

Here in this work, a series of pentablock terpolymers, PNIPAM$_n$-PEO-PPO-PEO-PNIPAM$_n$ (PNIPAM$_n$-Pluronic-PNIPAM$_n$), was prepared by RAFT polymerization with NIPAM as comonomer and Pluronic as macroRAFT agents (Scheme 1). We mainly focused on the effect of different block lengths of NIPAM and PPO on LCST, critical micelle concentration (cmc) and aggregation number ($N_{agg}$), and also studied the self-assembly morphology below and above LCST of the terpolymers.

![Scheme 1. Synthesis strategy for PNIPAM$_n$-Pluronic-PNIPAM$_n$ pentablock terpolymers.](image-url)
2. Experimental Section

2.1. Materials
Pluronic F108 (PEO₃₅-PPO₄₅-PEO₃₅, Mₙ = 14 600 g mol⁻¹), F68 (PEO₇₅-PPO₄₀-PEO₇₅, Mₙ = 8400 g mol⁻¹) and F127 (PEO₂₀₀-PPO₆₅-PEO₂₀₀, Mₙ = 12 600 g mol⁻¹) were purchased from Sigma–Aldrich and used without further purification. NIPAM (99%; Tokyo Chemical Industry Co., Ltd.) was recrystallized three times from benzene/n-hexane mixture (65/35, v/v). α′-Azobisisobutyronitrile (AIBN, 98%; Aldrich) and benzophenone (BP, 99%; Shanghai Chemical Reagent Co., Ltd.) were recrystallized from methanol and used without any further treatment.

2.2. Synthesis of S-1-Dodecyl-S′-(α,α′-dimethyl-α″-aceticacid) trithiocarbonate
A typical procedure for synthesizing S-1-Dodecyl-S′-(α,α′-dimethyl-α″-aceticacid) trithiocarbonate (DDAT), was synthesized via a previously reported procedure,[40] and further purified by recrystallization from n-hexane and dried under vacuum at room temperature for 24 h. ¹H NMR (CDCl₃, δ ppm): 0.88 (3H, −CH₃), 1.24–1.37 (m, 18H, −(CH₃)₂), 1.61–1.71 (m, 8H, −C(CH₃)₂) overlapped with −CH₂−, 3.24 (t, 2H, −CH₂−).

2.3. Synthesis of Macro-CTAs DDAT-Pluronic-DDAT
A typical macro-CTA, DDAT-F108-DDAT, was prepared as follows: F108 (7.31 g, 0.5 mmol), DDAT (0.437 g, 1.2 mmol), EDC · HCl (0.473 g, 2.4 mmol), and DMAP (0.156 g, 1.2 mmol) were dissolved in 50 mL CH₂Cl₂ and added into a 100 mL one-neck round-bottom flask equipped with a magnetic stirrer. When the solution was homogenized by stirring, it was degassed by three times and sealed under argon. After 48 h of agitation at 25 °C, the precipitated solid was filtered off. The macro-CTA, DDAT-F108-DDAT, was obtained as yellow solid by precipitating in −72 °C n-hexane twice and dried under vacuum at room temperature for 24 h and then stored away from light at 4 °C in a sealed bottle. The other two macro-CTAs, DDAT-F68-DDAT and DDAT-F127-DDAT, were prepared by the similar procedure, and the detailed characterization results were given in Figure S1 and S2 (Supporting Information).[41] ¹H NMR for DDAT-F108-DDAT (D₂O, δ ppm): 0.88 (6H, −CH₃), 1.10 (135H, −CH₃), 1.25 (36H, −CH₂−), 1.60–1.79 (16H, −C(CH₃)₂) overlapped with −CH₂−, 3.11–3.22 (4H, −CH₂−), 3.42–3.56 (135H, −CH₂− overlapped with −CH(CH₃)₂−), 3.61 (1080H, −CH₂−, −CH₂−).

2.4. Synthesis of PNIPAMₓ–Pluronic–PNIPAMₓ, Pentablock Terpolymers
A standard procedure for the synthesis of PNIPAM₁₀₀-F108-PNIPAM₁₀₀ by RAFT polymerization as follows: DDAT-F108-DDAT (1.51 g, 0.1 mmol), NIPAM (2.36 g, 20.9 mmol), AIBN (3.3 mg, 0.02 mmol), and THF (10 mL) were introduced into a 50 mL reaction tube, which was sealed under argon after three freeze-pump-thaw cycles and placed in oil bath (80 °C) for 24 h, and then quenched by liquid nitrogen. The product was precipitated out from excessive −72 °C n-hexane and dried under vacuum. To remove the impurities such as the unreacted monomers and low-molecular-weight product, the dried solid product was dissolved in distilled water and transferred into a dialysis tube (Mₙ cutoff = 8000–14000) and dialyzed against distilled water for 2 d at room temperature. After dialysis, the product was concentrated with a rotary evaporator and dried under vacuum. Finally, white solid product of PNIPAM₁₀₀-F108-PNIPAM₁₀₀ was obtained, and the yield was 96%. The other four pentablock terpolymers were prepared by a similar procedure, and detailed characterization results were given in Figure S3–S6 (Supporting Information).[42] ¹H NMR for PNIPAM₁₀₀-F108-PNIPAM₁₀₀ (D₂O, δ ppm): 0.90–1.08 (1335H, −NHHCH(CH₃)₂), 1.37–2.12 (600H, −CH₂CH₂CO–), 3.48–3.57 (195H, −CH₂CH₂CH(CH₃)₂), 3.67 (1088H, −CH₂−, −CH₂−), 3.90 (200H, −NHHCH(CH₃)₂), 5.72–6.22 (200H, −NHH(CH₂)₂).

3. Characterization and Measurements

3.1. Nuclear Magnetic Resonance Spectroscopy
¹H NMR spectroscopy was recorded on a Bruker Avance 300 nuclear magnetic resonance spectrometer at 300 MHz and 25 °C, using deuterated chloroform (CDCl₃) or deuterated water (D₂O) as the solvent, and tetramethylsilane as the internal standard.

3.2. Gel Permeation Chromatography
The molecular weight distribution of all the pentablock terpolymers was determined by GPC, which is equipped with a waters model 1515 pump, a model 2410 refractive index detector, and an OH-pak KB-803 column operated at 25 °C. THF or water was used as the eluent at a flow rate of 1.0 mL min⁻¹, and the column was calibrated using monodisperse polystyrene as standards.

3.3. UV–Vis Spectrophotometry
LCST or the cloud points of the pentablock terpolymers were determined by a UNICO UV–Vis 4802 spectrophotometer (Shanghai, China) with double beam of light. The sample cells were thermostatted with a refrigerated circulating bath for 15 min before measurements, and a temperature probe was directly inserted into the measuring
cell to record the temperature instantaneously. The optical transmittance of the terpolymer aqueous solutions was recorded in the range of wavelength from 900 to 300 nm with a step of 1 nm. The transmittance of the aqueous solution at 500 nm was selected to plot as the function of measuring temperature.

3.4. Steady-State Fluorescence Measurement

Steady-state fluorescence spectra were recorded on a Varian Cary Eclipse spectrometer (Varian Inc., USA), and the temperature of the water-jacketed cell holder was controlled with a Neslab circulating bath. The fluorescence emission spectra were recorded from 350 to 500 nm. The excitation wavelength was set at 335 nm; the excitation and emission slit widths were set at 10 and 1.5 nm, respectively. Pyrene was used as a fluorescence probe due to its low solubility in water, long fluorescence lifetime, and high sensitivity to the polarity of its microenvironment, and preferentially solubilized into the interior of hydrophobic micromdomains.

The terpolymer stock solutions were prepared by dissolving 1 g of terpolymer in 25 mL distilled water, and then a series of terpolymer concentrations ranging from 1 to 30 mg mL$^{-1}$ was prepared by diluting the prepared terpolymer stock solutions with distilled water. To get pyrene–terpolymer solutions, a predetermined amount of pyrene in methanol was added to a series of volumetric flask, and the methanol was then evaporated by gently flowing N$_2$ before adding the terpolymer aqueous solutions with different concentrations to give the final pyrene concentration in the samples $6 \times 10^{-7}$ mol L$^{-1}$, and the pyrene–terpolymer solutions were stirred 48 h at room temperature for solubilization equilibration prior to measurements. The vibronic fluorescence spectrum of pyrene exhibits five peaks, the first at 373 nm ($I_1$) and the third at 384 nm ($I_3$) of the emission intensity were used to calculate the ratio $I_3/I_1$, which correlates with the polarity of the intermediate microenvironment of the pyrene molecules. It is well known that the $I_3/I_1$ values range from about 1.9 in polar solvent (for example 1.87 in water, see Figure S9, Supporting Information) to about 0.6 in hydrocarbon. The cmc can be obtained from measurement of the changes in $I_3/I_1$ as a function of terpolymer concentrations.

The steady-state fluorescence quenching measurement was performed following previously reported procedures. Pyrene was used as the donor with the concentration at $6 \times 10^{-7}$ mol L$^{-1}$, and BP was used as the fluorescence quencher with the concentrations range from $0.2 \times 10^{-5}$ to $2.0 \times 10^{-5}$ mol L$^{-1}$. All the pentablock terpolymer solutions were prepared at 10 mg mL$^{-1}$ because it can sufficiently assure formation of micelles. The sample solutions, containing pyrene, BP, and terpolymer, were stirred 48 h at room temperature for solubilization equilibration prior to measurements. Under assumed circumstances, the emission intensity, $I_0$, of the probe in the presence of quencher, was related by equation:

$$\frac{I_0}{I_0} = \exp \left( \frac{|Q|}{[M]} \right)$$  \hspace{1cm} (1)

where $I_0$ refers to the fluorescence intensity in the absence of quencher, and $[M]$ denotes the concentration of micelles, and can be calculated by

$$[M] = \frac{C - \text{cmc}}{N_{agg}}$$  \hspace{1cm} (2)

where $C$ stands for the total polymer molar concentration, and cmc is used to approximate the free polymer concentration. The combination of the Equations 1 and 2 to yield

$$\ln \left( \frac{I_0}{I_0} \right) = \frac{N_{agg}}{C - \text{cmc}} |Q|$$  \hspace{1cm} (3)

Thus the slope of a plot of $\ln(I_0/I_0)$ as a function of quencher concentrations allows the determination of $N_{agg}$. Each concentration was measured in three times to get the averaged value.

3.5. Transmission Electron Microscopy

Transmission electron microscopy (TEM) observation was conducted on a Hitachi H600 electron microscope instrument operating at an acceleration voltage of 75 kV. Negative staining technique with phosphotungstic acid (PTA) as staining agent was used for TEM sample preparation. A drop of preheated (25 or 50 °C) polymer solutions was placed on a Formvar-coated copper grids and dried in the oven for half an hour. Subsequently, the specimens were stained with PTA, and then again allowed to dry in the oven prior to observation.

4. Results and Discussion

4.1. Synthesis and Characterization of Macro-CTAs and Pentablock Terpolymers

RAFT polymerization has emerged as one of the most powerful synthetic techniques in polymer science due to the claimed advantages of solvent and functionality tolerance and the absence of any metal ions. In addition, RAFT polymerization has higher initiation efficiency under mild reaction conditions. Thus, RAFT polymerization was
DDAT-F108-DDAT was given in Figure 1 in comparison with those of DDAT and F108. When comparing the $^1$H NMR spectra, the signals at 0.88 ppm ($-\text{CH}_3$) and 1.25 ppm ($-\text{CH}_2-$) are characteristics of DDAT, and those at 1.10 ppm ($-\text{CH}_3$) and 3.61 ppm ($-\text{CH}_2-\text{CH}_2-$) are attributed to F108, which confirmed the successful synthesis of DDAT-F108-DDAT. By integrating the peak area of the signals at $\delta=3.61$ ppm and $\delta=0.88$ ppm, the degree of terminal difunctionalization can be calculated,[26] and found it was 91%. The number-average molecular weight ($M_n$) of DDAT-F108-DDAT can also be calculated from its $^1$H NMR spectrum,[49,50] and found it to be $1.52 \times 10^4$ g mol$^{-1}$.

The other two macro-CTAs, DDAT-F68-DDAT (95%) and DDAT-F127-DDAT (93%), were also successfully synthesized and characterized by the similar method (see Figure S1 and S2, Supporting Information), and the $M_n$ were $0.90 \times 10^4$ and $1.33 \times 10^4$ g mol$^{-1}$, respectively.

PNIPAM$_n$-Pluronic-PNIPAM$_n$ pentablock terpolymers were obtained by RAFT polymerization of NIPAM and DDAT-Pluronic-DDAT in THF at 80 °C, for which NIPAM was chosen because of its thermosensitivity, and Pluronic possess the characteristics of low toxicity, biocompatibility, and high solubility in water with a wide temperature range. Different feed ratios of NIPAM/macro-CTAs were used in the copolymerization so as to get a series of terpolymers with different block lengths. The $M_n$ and polydispersity index (PDI) of all terpolymers were characterized by $^1$H NMR and GPC, respectively.

Exhibited in Figure 2 is the $^1$H NMR spectrum of PNIPAM$_{100}$-F108-PNIPAM$_{100}$ pentablock terpolymer. The characteristic signals of both F108 and NIPAM can be clearly identified; the signals at 3.90 ppm ($-\text{NHC}H(\text{CH}_3)_2$) and 5.72–6.22 ppm ($-\text{NHCH(\text{CH}_3)_2}$) are attributed to PNIPAM, and that at 3.67 ppm ($-\text{CH}_2-\text{CH}_2-$) is assigned to F108. By integrating the intensity ratio of the signals at $\delta=3.67$ ppm and $\delta=3.90$ ppm,[26,49,50] the polymerization degree of NIPAM calculated is 200, and the $M_n$ is $3.78 \times 10^4$ g mol$^{-1}$, which is close to the theoretical value ($M_n\text{theo}$) $3.88 \times 10^4$ g mol$^{-1}$. As shown in Figure 3, the GPC elution peak of PNIPAM$_{100}$-F108-PNIPAM$_{100}$ is quite symmetric and shows narrow-molecular-weight distribution, and the PDI is as low as around 1.24, which is consistent with
It was found that the $M_{n,\text{nmr}}$ for the other terpolymers are very close to the $M_{n,\text{theo}}$ and all PDIs show low values (1.22–1.26), indicative of the characteristics of RAFT process.

### 4.2. The Effect of Hydrophobic Block Lengths on LCST

LCST is a basic physical property of thermoresponsive block copolymers and can be tuned by regulating copolymer composition, varying copolymer concentration, or adding inorganic salt.\[15,18\] In this study, one of the aims is to examine the effect of NIPAM and PPO block lengths on LCST.

Normally, the LCST value was defined as the midpoint of the temperature–transmission curve.\[53,54\] First, the LCST of the original Pluronic (F68, F108, 127) was determined, the transmittance of Pluronic aqueous solutions was not changed upon increasing temperature from 25 to 80°C (see Figure S8, Supporting Information). Next, when Pluronic solutions were placed in oil bath, which was set at 98°C, they were still transparent, implying that the LCST was not lower than such a temperature, which is good line with the literature reported where the LCST value of Pluronic more than 100°C.\[55\] Figure 4 shows the optical transmittance as a function of temperature at 10 mg mL$^{-1}$ of PNIPAM$\_n$-Pluronic-PNIPAM$\_n$ aqueous solutions. It was found that all the terpolymer solutions exhibit sharp transition when increasing temperature to certain values, and the reduction of transmittance reflected the decrease in the solubility. As a representative example, the transmittance of PNIPAM 50-F108-PNIPAM 50 started to strongly decrease at 37.5°C, and the LCST was determined to be 40°C. The LCST of other pentablock terpolymers was given in Table 2. The LCST of PNIPAM$\_n$-F108-PNIPAM$\_n$ increases from 34.5 to 36.5 and then to 40°C with decreasing the NIPAM block lengths from 150 to 100 and 50; the LCST changes from 33.5 to 36.5 and then to 37 with decreasing the PPO block lengths from 65 to 45 and 30 when the NIPAM block length is fixed at 100. These results showed that the LCST value decreases with increasing the hydrophobic NIPAM and PPO block lengths, which ascribed to the increase characteristics of RAFT polymerization.\[51\] However, the $M_n$ of these pentablock terpolymers cannot be accurately determined by GPC probably because the terpolymer chains aggregated and adsorbed partly in the column pre-filter, and the $M_{n,\text{GPC}}$ were also shown in Table 1. Similar phenomena were observed in other PNIPAM-based copolymers.\[26,52\] So, the GPC results were used here only to estimate the PDIs for all the pentablock terpolymers. In addition, the PDIs and molecular weights were also determined by GPC using water as eluent. The results showed that the PDIs are very low; however, the molecular weights are also not accurately determined (see Figure S7, Supporting Information). $^1$H NMR spectra of the other terpolymers were shown in Figure S3–S6 (Supporting Information), and the detailed results were summarized in Table 1. It was found that the $M_{n,\text{nmr}}$ for the other terpolymers are very close to the $M_{n,\text{theo}}$ and all PDIs show low values (1.22–1.26), indicative of the characteristics of RAFT process.

### Table 1. Molecular Characteristics of PNIPAM$_n$-Pluronic-PNIPAM$_n$.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$[M]_0/[I]_0$</th>
<th>Yield [%]</th>
<th>DP$_n$</th>
<th>$M_{n,\text{nmr}}$</th>
<th>$M_{n,\text{theo}}$</th>
<th>$M_{n,\text{GPC}}$</th>
<th>PDI $^{(d)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNIPAM$<em>{150}$-F108-PNIPAM$</em>{150}$</td>
<td>315</td>
<td>95</td>
<td>300</td>
<td>49 100</td>
<td>50 900</td>
<td>30 900</td>
<td>1.23</td>
</tr>
<tr>
<td>PNIPAM$<em>{150}$-F108-PNIPAM$</em>{100}$</td>
<td>207</td>
<td>95</td>
<td>200</td>
<td>31 600</td>
<td>32 400</td>
<td>24 600</td>
<td>1.24</td>
</tr>
<tr>
<td>PNIPAM$<em>{100}$-F68-PNIPAM$</em>{100}$</td>
<td>204</td>
<td>98</td>
<td>200</td>
<td>35 800</td>
<td>36 200</td>
<td>28 800</td>
<td>1.22</td>
</tr>
</tbody>
</table>

$^{(a)}[M]_0/[I]_0$ refers to the mole ratio of NIPAM/macro-CTAs; $^{(b)}$Estimated from $^1$H NMR spectrum; $^{(c)}$Expected number-average molecular weight from polymerization stoichiometry; $^{(d)}$Determined by GPC in tetrahydrofuran at 25°C (PS calibration).
attractive hydrophobic interactions of the pentablock terpolymers.

4.3. The Effect of the Terpolymer Concentrations on LCST

LCST of the pentablock terpolymers is also dependent on its concentrations. As shown in Figure 5, the LCST decreases with increasing terpolymer concentrations, and the decrease mainly takes place at lower concentration region. For instance, the LCST of PNIPAM₅₀-F₁₀₈-PNIPAM₅₀ decreases from 50 to 38 °C when the terpolymer concentration increases from 1 to 20 mg mL⁻¹, and the sharp decrease from 50 to 40.5 °C mainly occurs at lower concentration region (1–7.5 mg mL⁻¹), while LCST only decrease from 40.5 to 38 °C at higher concentration region (7.5–20 mg mL⁻¹). The LCST for other four pentablock terpolymers has similar trend as that of PNIPAM₅₀-F₁₀₈-PNIPAM₅₀. Such a tendency could be attributed to the stronger hydrophobic interactions with increasing the terpolymer concentrations. It was also found that the longer PPO and NIPAM block, the lower LCST can be found at the same terpolymer concentration. For instance, when the concentration of PNIPAM₁₅₀-F₁₀₈-PNIPAM₁₅₀ at 5 mg mL⁻¹, its LCST is 36.5 °C, which is much lower than that of PNIPAM₅₀-F₁₀₈-PNIPAM₅₀.

Table 2. The aggregation properties of PNIPAMₙ-Pluronic-PNIPAMₙ.

<table>
<thead>
<tr>
<th>Sample</th>
<th>LCST (°C)</th>
<th>cmc × 10⁵ (mol L⁻¹)</th>
<th>Nagg</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₁₀₈</td>
<td>&gt; 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₆₈</td>
<td>&gt; 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₁₂₇</td>
<td>&gt; 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PNIPAM₅₀-F₁₀₈-PNIPAM₅₀</td>
<td>40.0</td>
<td>10.9</td>
<td>14</td>
</tr>
<tr>
<td>PNIPAM₁₀₀-F₁₀₈-PNIPAM₁₀₀</td>
<td>36.5</td>
<td>2.65</td>
<td>15</td>
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<tr>
<td>PNIPAM₁₅₀-F₁₀₈-PNIPAM₁₅₀</td>
<td>34.5</td>
<td>1.43</td>
<td>17</td>
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<tr>
<td>PNIPAM₁₀₀-F₆₈-PNIPAM₁₀₀</td>
<td>37.0</td>
<td>3.16</td>
<td>14</td>
</tr>
<tr>
<td>PNIPAM₁₀₀-F₁₂₇-PNIPAM₁₀₀</td>
<td>33.5</td>
<td>1.96</td>
<td>17</td>
</tr>
</tbody>
</table>

4.4. Critical Micelle Concentration of Pentablock Terpolymers

One of the basic parameters of micelles is the cmc, the threshold concentration at which micelle starts to form. Fluorescent probe technique is a simple and widely used tool to determine the cmc of surfactants and block copolymers. The decrease of the intensity ratio of I₃/I₆ with increasing polymer concentrations can be assigned to a partition of the probe from water to hydrophobic microdomains formed by the hydrocarbon groups. Thus, the variation of I₃/I₆ versus terpolymer concentrations was used to determine the cmc and to investigate the self-assembly process of terpolymer. As a typical sample,
Figure 6 displays the variation of $I_1/I_3$ as a function of PNIPAM$_{100}$-F108-PNIPAM$_{100}$ concentrations. At lower terpolymer concentration region, the $I_1/I_3$ value is very close to that of pyrene in pure water. Then, the $I_1/I_3$ gradually decreases with increasing terpolymer concentrations and the decrease stretches over a broad range of concentrations about an order of magnitude, revealing the formation of hydrophobic microdomains or micelles. The cmc is determined at the first fall of $I_1/I_3$ when pyrene starts to include in micelle, as shown by the intersection of the two lines in Figure 6. Thus, the cmc for this polymer is 1 mg mL$^{-1}$ or $2.65 \times 10^{-5}$ mol L$^{-1}$. The cmc of the other pentablock terpolymers can be determined by the similar way (see Figure S10, Supporting Information), and the results are summarized in Table 2. Additionally, the cmc values of PNIPAM$_n$-F108-PNIPAM$_n$ are lower than that (4.5 $\times$ 10$^{-4}$ mol L$^{-1}$) of their precursor F108. This implies that when increasing hydrophobic block lengths, the cmc shifts toward lower concentration.

4.5. Aggregation Number of Pentablock Terpolymers

The fluorescence intensity ratio $I_1/I_0$ plotted against fluorescence quencher concentrations for PNIPAM$_{100}$-F108-PNIPAM$_{100}$. Figure 7 depicts the fluorescence intensity ratio $\ln(I_0/I_Q)$ versus quencher concentrations for PNIPAM$_{100}$-F108-PNIPAM$_{100}$. The $N_{agg}$ calculated from the slope of the line in Figure 7 using Equation (3) is 15, suggesting average 15 hydrophobic groups come together to form a micellar core. The $N_{agg}$ of the other four pentablock terpolymers can be obtained in a similar procedure (see Figure S11, Supporting Information), and the results are reported in Table 2. The $N_{agg}$ increases from 14 to 15 and then to 17 with increasing the NIPAM block lengths for PNIPAM$_n$-F108-PNIPAM$_n$, and the $N_{agg}$ increases from 14 to 17 with increasing the PPO block lengths when keeping NIPAM block constant. Furthermore, all the $N_{agg}$ values of PNIPAM$_n$-Pluronic-PNIPAM$_n$ are higher than that of Pluronic. It is worth noting that the possession of longer hydrophobic NIPAM and PPO blocks is supposed to have a better intermolecular hydrophobic group packing, and therefore, it showed a larger $N_{agg}$. This is, in good agreement, with the theoretical models for amphiphilic diblock copolymers that was also predicted an increase of $N_{agg}$ with increasing the hydrophobic chain lengths.

4.6. Self-Assembly in Aqueous Solution

TEM observation was performed to visualize the self-assembly morphologies below and above LCST of PNIPAM$_{100}$-F108-PNIPAM$_{100}$.
PNIPAM<sub>n</sub>-Pluronic-PNIPAM<sub>n</sub> in aqueous solutions. Figure 8 displays the morphologies of PNIPAM<sub>n</sub>-F108-PNIPAM<sub>n</sub> aqueous solutions at 25 and 50 °C. At the temperatures below their LCST, water was a good solvent for PEO, PPO, and PNIPAM. PNIPAM-PEO-PPO-PEO-PNIPAM should exist as a random coil conformation. However, due to the hydrophobic C<sub>12</sub> chain from CTA at the end of the pentablock terpolymer (see Scheme 1) and incompatibility of PEO, PPO and PNIPAM, some of the terpolymer chains associated together with hydrophobic C<sub>12</sub> chain to form associated spherical-like particle structures, as shown in Figure 8(A–C). The size of the particles was calculated 28 ± 3 nm. When the temperatures above their LCST, the PPO and PNIPAM blocks underwent a coil-globule transition and become insoluble in water, thus the hydrophobic blocks collapsed to form the spherical micelle with PPO and PNIPAM as the core and PEO as the shell. Furthermore, the various self-assembly morphologies with irregular micelles or even intermicellar aggregates were observed [see Figure 8(D–F) and Figure S12, Supporting Information]. For instance, the spherical micelle aggregates and two or more layers overlapping micelles were evidenced in Figure 8D. Figure 8E shows the ellipsoidal or even cylindrical shape micelles. Moreover, the self-assembly morphologies can undergo reversible transitions below and above LCST of the terpolymers (see Figure S13, Supporting Information). On the basis of the above observation, the various self-assembly morphologies were observed above the LCST. It was acceptable because the hydrophobic blocks would be dissociating and shrinking to form the micellar core, the PPO and PNIPAM blocks could be forming the cores, and the solely PPO or PNIPAM or even hydrophobic C<sub>12</sub> chains could also be forming the cores (Scheme 2).

5. Conclusions

A series of well-defined thermoresponsive PNIPAM<sub>n</sub>-Pluronic-PNIPAM<sub>n</sub> pentablock terpolymers with low PDIs and controlled molecular architectures was prepared by RAFT polymerization. Their LCST, cmc, and N<sub>agg</sub> values could be readily regulated by adjusting NIPAM and PPO block lengths. In the dilute aqueous solution, the terpolymer chains associated together with hydrophobic C<sub>12</sub> chain to form associated spherical-like particle structures below LCST, whereas the morphologies exhibited regular spherical micelles or irregular micelles or even intermicellar aggregates above LCST. From a practical point of view, these thermoresponsive pentablock terpolymers have wider LCST range and intricate morphologies, which in turn could be promising in pharmaceutical applications.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Supporting Information

Synthesis and Aggregation Behaviors of Well-Defined Thermo-Responsive Pentablock Terpolymers with Tunable LCST

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$^1$H NMR spectra of macro-CTAs and pentablock terpolymers

By integrating the intensity ratio of the signals at $\delta = 3.62$ ppm and $\delta = 0.88$ ppm, the degree of terminal difunctionalization can be calculated and found it is 95%. The $M_n$ of DDAT-F68-DDAT was calculated from the $^1$H NMR spectrum to be $0.90 \times 10^4$ g mol$^{-1}$.

$^1$H NMR for DDAT-F68-DDAT (D$_2$O, $\delta$, ppm): 0.88 (6H, -CH$_3$), 1.10 (90H, -CH$_3$), 1.25 (36H, -CH$_2$), 1.62–1.81 (16H, -C(CH$_3$)$_2$ overlapped with -CH$_2$-), 3.01–3.20 (4H, -CH$_2$-), 3.42–3.56 (90H, -CH$_2$- overlapped with –CH(CH$_3$)-), 3.62 (592H, -CH$_2$-CH$_2$-), 4.20 (8H, -OCH$_2$CH$_2$OOC-).
Figure S1. $^1$H NMR spectrum of DDAT-F68-DDAT

By integrating the intensity ratio of the signals at $\delta = 3.59$ ppm and $\delta = 0.82$ ppm, the degree of terminal difunctionalization can be calculated and found it is 93%. The $M_n$ of DDAT-F127-DDAT was calculated from the $^1$H NMR spectrum to be $1.33 \times 10^4$ g mol$^{-1}$.

$^1$H NMR for DDAT-F127-DDAT (D$_2$O, $\delta$, ppm): 0.82 (6H, -CH$_3$), 1.05 (195H, -CH$_3$), 1.20 (36H, -CH$_2$), 1.60–1.80 (16H, -C(CH$_3$)$_2$ overlapped with -CH$_2$), 2.96–3.15 (4H, -CH$_2$), 3.34–3.46 (195H, -CH$_2$ overlapped with -CH(CH$_3$)

Figure S2. $^1$H NMR spectrum of DDAT-F127-DDAT
By integrating the intensity ratio of the signals at $\delta = 3.68$ ppm and $\delta = 3.90$ ppm, the polymerization degree of NIPAM calculated is 100, and the $M_n$ was $2.65 \times 10^4$ g mol$^{-1}$.

$^1$H NMR for PNIPAM$_{50}$-F108-PNIPAM$_{50}$ (D$_2$O, $\delta$, ppm): 0.90-1.08 (735H, -NHCH(CH$_3$)$_2$ overlapped with -CHCH$_3$), 1.37–2.12 (300H, -CH$_2$CHCO-), 3.48–3.57 (135H, -CH$_2$CH(CH$_3$)), 3.68 (1088H, -CH$_2$-CH$_2$), 3.90 (100H, -NHCH(CH$_3$)$_2$), 5.72-6.22 (100H, -NICH(CH$_3$)$_2$).

Figure S3. $^1$H NMR spectrum of PNIPAM$_{50}$-F108-PNIPAM$_{50}$ terpolymer

By integrating the intensity ratio of the signals at $\delta = 3.61$ ppm and $\delta = 3.84$ ppm, the polymerization degree of NIPAM calculated is 300, and the $M_n$ was $4.91 \times 10^4$ g mol$^{-1}$.

$^1$H NMR for PNIPAM$_{150}$-F108-PNIPAM$_{150}$ (D$_2$O, $\delta$, ppm): 0.91-1.10 (1935H, -NHCH(CH$_3$)$_2$ overlapped with -CHCH$_3$), 1.22–2.08 (900H, -CH$_2$CHCO-), 3.42–3.58 (135H, -CH$_2$CH(CH$_3$)), 3.61 (1088H, -CH$_2$-CH$_2$), 3.84 (300H, -NHCH(CH$_3$)$_2$), 5.66–6.21 (300H, -NICH(CH$_3$)$_2$).
By integrating the intensity ratio of the signals at $\delta = 3.69$ ppm and $\delta = 3.80$ ppm, the polymerization degree of NIPAM calculated is 200, and the $M_n$ was $3.16 \times 10^4$ g mol$^{-1}$.

$^1$H NMR for PNIPAM$_{100}$-F68-PNIPAM$_{100}$ (D$_2$O, $\delta$, ppm): 0.90–1.08 (1290H, -NHCH(CH$_3$)$_2$ overlapped with -CHCH$_3$), 1.35–2.10 (600H, -CH$_2$CHCO-), 3.44–3.52 (90H, -CH$_2$CH(CH$_3$)_), 3.69 (600H, -CH$_2$-CH$_2$-), 3.80 (200H, -NHCH(CH$_3$)$_2$), 5.60–6.10 (200H, -NHCH(CH$_3$)$_2$).
By integrating the intensity ratio of the signals at \( \delta = 3.66 \text{ ppm} \) and \( \delta = 3.90 \text{ ppm} \), the polymerization degree of NIPAM calculated is 200, and the \( M_n \) was \( 3.58 \times 10^4 \text{ g mol}^{-1} \).

\(^1\text{H NMR for PNIPAM}_{100}-\text{F127}-\text{PNIPAM}_{100} (\text{D}_2\text{O}, \delta, \text{ ppm}): 1.01–1.13 (1395\text{H}, -\text{NHCH(CH}_3\text{)}_2 \text{overlapped with -CHCH}_3), 1.42–2.18 (600\text{H}, -\text{CH}_2\text{CHCO}-), 3.45–3.60 (195\text{H}, -\text{CH}_2\text{CH(CH}_3\text{)}), 3.66 (800\text{H}, -\text{CH}_2\text{-CH}_2-), 3.90 (200\text{H}, -\text{NHCH(CH}_3\text{)}_2), 5.80–6.10 (200\text{H}, -\text{NHCH(CH}_3\text{)}_2).

Figure S6. \(^1\text{H NMR spectrum of PNIPAM}_{100}-\text{F127}-\text{PNIPAM}_{100} \text{ terpolymer}

**Gel Permeation Chromatography (GPC)**

The results showed that the GPC elution peaks are symmetric and show narrow molecular weight distribution. However, the molecular weight results are probably not so accurate because they associate into micellar structures that are adsorbed partly onto the column in aqueous solvent, and the micellar structures are also confirmed by Figures 8(A-C).

Figure S7. GPC traces of PNIPAM\(_n\)-Pluronic-PNIPAM\(_n\) terpolymers using water as eluent
UV-vis spectrophotoscopy

As shown in Figure S8, the transmittance of Pluronics aqueous solutions was not changed upon increasing temperature from 25 to 80 °C. Since the temperature of the measured cell regulated by a water bath cannot be increased anymore, the transmittance of parent Pluronics solutions could not be determined higher than 80 °C. We put Pluronics solutions in oil bath which was set at 98 °C, they were still transparent, implying that the cloud points of Pluronics were not lower than such a temperature.

![Figure S8. Temperature dependence of optical transmittance of F68 (a), F108 (b) and F127 (c) at 10 mg mL⁻¹ aqueous solutions.](image)

Steady-state Fluorescence Measurement

The vibronic fluorescence spectrum of pyrene exhibits five peaks, the first at 373 nm ($I_1$) and the third at 384 nm ($I_3$) vibronic peaks were used to calculate the ratio $I_1/I_3$. The $I_1/I_3$ value of pyrene in pure water was 1.87 at 25 °C as shown in Figure S9.

![Figure S9. A typical fluorescence spectrum of pyrene in pure water at 25 °C.](image)
Figure S10 displays the variation of $I_1/I_3$ as a function of terpolymer concentrations of PNI\textsubscript{m}A\textsubscript{n}-Pluronic-PNI\textsubscript{m}A\textsubscript{n}, the cmc is determined at the first fall of $I_1/I_3$.

The cmc of PNI\textsubscript{50}A-F108-PNI\textsubscript{50}A is 2.9 mg mL\textsuperscript{-1} or $1.09\times10^{-4}$ mol L\textsuperscript{-1}.

The cmc of PNI\textsubscript{150}A-F108-PNI\textsubscript{150}A is 0.7 mg mL\textsuperscript{-1} or $1.43\times10^{-5}$ mol L\textsuperscript{-1}.

The cmc of PNI\textsubscript{100}A-F68-PNI\textsubscript{100}A is 1 mg mL\textsuperscript{-1} or $3.16\times10^{-5}$ mol L\textsuperscript{-1}.

The cmc of PNI\textsubscript{100}A-F127-PNI\textsubscript{100}A is 0.7 mg mL\textsuperscript{-1} or $1.96\times10^{-5}$ mol L\textsuperscript{-1}.

Figure S10. The $I_1/I_3$ values versus terpolymer concentration of PNI\textsubscript{50}A-F108-PNI\textsubscript{50}A (A), PNI\textsubscript{150}A-F108-PNI\textsubscript{150}A (B), PNI\textsubscript{100}A-F68-PNI\textsubscript{100}A (C), and PNI\textsubscript{100}A-F127-PNI\textsubscript{100}A (D) at 25 °C.

Figure S11 shows the slope of the fit to the ln($I_0/I_Q$) versus different fluorescence quencher benzophenone concentrations at 25 °C for PNI\textsubscript{m}A-Pluronic-PNI\textsubscript{m}A. The $N_{agg}$ is calculated from the slope of the line in Figure S9 using Equation (3).
The slope of the fit for PNIPAM\textsubscript{50}-F108-PNIPAM\textsubscript{50} is 54,000, the cmc is $1.09 \times 10^{-4}$ mol L\textsuperscript{-1}, and so the $N_{\text{agg}}$ is 14.

The slope of the fit for PNIPAM\textsubscript{150}-F108-PNIPAM\textsubscript{150} is 90,460, the cmc is $1.43 \times 10^{-5}$ mol L\textsuperscript{-1}, and so the $N_{\text{agg}}$ is 17.

The slope of the fit for PNIPAM\textsubscript{100}-F68-PNIPAM\textsubscript{100} is 50,090, the cmc is $3.16 \times 10^{-5}$ mol L\textsuperscript{-1}, and so the $N_{\text{agg}}$ is 14.

The slope of the fit for PNIPAM\textsubscript{100}-F127-PNIPAM\textsubscript{100} is 65,780, the cmc is $1.96 \times 10^{-5}$ mol L\textsuperscript{-1}, and so the $N_{\text{agg}}$ is 17.

Figure S11. The fluorescence intensity ratio $I_0/I_Q$ versus quencher concentrations at 10 mg mL\textsuperscript{-1} of PNIPAM\textsubscript{50}-F108-PNIPAM\textsubscript{50} (A), PNIPAM\textsubscript{150}-F108-PNIPAM\textsubscript{150} (B), PNIPAM\textsubscript{100}-F68- PNIPAM\textsubscript{100} (C), and PNIPAM\textsubscript{100}-F127-PNIPAM\textsubscript{100} (D) at 25 °C.
Transmission Electron Microscopy (TEM)

**Figure S12.** TEM images of the morphologies of PNIPAM\textsubscript{100}-F68-PNIPAM\textsubscript{100} at 25 (A) and 50 °C (B), and PNIPAM\textsubscript{100}-F127-PNIPAM\textsubscript{100} at 25 (C) and 50 °C (D) at 3 mg mL\textsuperscript{-1} aqueous solutions.

**Figure S13.** TEM images of the morphologies of PNIPAM\textsubscript{50}-F108-PNIPAM\textsubscript{50} at 25 (A), 50 (B) and again 25 °C (C) at 3 mg mL\textsuperscript{-1} aqueous solutions.