Both Core- and Shell-Cross-Linked Nanogels: Photoinduced Size Change, Intraparticle LCST, and Interparticle UCST Thermal Behaviors

Jie He,† Bin Yan,† Luc Tremblay,‡ and Yue Zhao*†

†Département de chimie and ‡Département de médecine nucléaire et de radiobiologie and Centre d’imagerie moléculaire de Sherbrooke, Université de Sherbrooke, Sherbrooke, Québec, Canada J1K 2R1

Received October 7, 2010. Revised Manuscript Received November 18, 2010

New thermal- and photoresponsive core−shell nanogel particles were obtained from self-assembly in aqueous solution of a double-hydrophilic block copolymer (DHBCP) of which the two blocks could be photo-cross-linked via the reversible photodimerization and photocleavage of coumarin moieties. The diblock copolymer, consisting of poly[N,N-dimethylacrylamide-co-4-methyl-[7-(methacryloyloxyethyl)oxy]coumarin] and poly[N-isopropylacrylamide-co-4-methyl-[7-(methacryloyloxyethyl)oxy]coumarin] (P(DMA-co-CMA)-b-P(NIPAM-co-CMA)), was synthesized by using reversible addition−fragmentation chain transfer (RAFT) polymerization. At \( T \) > LCST of the P(NIPAM-co-CMA) block, core−shell micelles were formed and UV light irradiation at \( \lambda > 310 \) nm resulted in cross-linking of both the micelle core of P(NIPAM-co-CMA) and the micelle shell of P(DMA-co-CMA); subsequent cooling of the solution to \( T < \) LCST gave rise to water-soluble, swollen nanogel particles. Upon UV light irradiation at \( \lambda < 260 \) nm, the decrease of cross-linking density could increase the swelling of nanogel particles by ~23% in diameter. By alternating irradiation with different wavelengths, the average hydrodynamic diameter of nanogel particles was tunable between ~58 and ~47 nm. Interestingly, upon further cooling of the solution, aggregation occurred for nanogel particles with a moderate cross-linking density (10%−40% dimerization of coumarin moieties). Therefore, such core- and shell-cross-linked nanogel could display both “intraparticle” LCST (solubility of polymer chains forming the core) and “interparticle” UCST (solubility of particles). The possible mechanism and the effect of dimerization degree on the UCST behavior were discussed.

Introduction

Double-hydrophilic block copolymers (DHBCPs) have attracted much attention in the past years due to the facile preparation from living radical polymerizations and their unique self-assembly behaviors under various chemical and physical stimuli (pH, temperature, light and redox, etc.).1–6 A general design of DHBCPs is to combine one “permanently” hydrophilic block with one “smart” block that is able to switch between a hydrophilic and a hydrophobic state under the effect of external stimuli.1 Like amphiphilic BCPs, DHBCPs can self-assemble into various forms of micellar aggregates that can be exploited for such applications as nanocarriers for drug delivery and nanoreactors for chemical reactions.7–14 To prevent self-assembled micellar aggregates from being dissolved, a number of cross-linking strategies of polymer chains have been developed.8,9,15–21

Nanogels, defined as chemically or physically cross-linked hydrogel particles with a nanoscale size, represent one of very interesting stimuli-responsive nanomaterials. Being able to uptake a large amount of water and with relatively low surface tension, nanogel particles may have excellent biocompatibility and could potentially be developed as intravascular injections for intracellular drug delivery.22–25 The general method for preparing nanogels is based on the traditional emulsion or dispersion polymerization.26 Among the reported stimuli-responsive nanogels, those using poly(N-isopropylacrylamide) (PNIPAM) have been most studied.26–30 For instance, Gota et al. showed the application of PNIPAM fluorescent nanogel particles as the intracellular thermometer.24 In contrast to micelles of amphiphilic BCPs whose core is

*Corresponding author. E-mail: yue.zhao@usherbrooke.ca.
(9) Jiang, X. Z.; Ge, Z. S.; Xu, J.; Liu, H.; Liu, S. Y. Biomacromolecules 2007, 8, 3184.
hydrophobic, nanogel particles are made with water-soluble polymers, and their highly hydrophilic nature is necessary to avoid the precipitation at high ionic strength. Cross-linking micelles of DHBCPs is an effective way to prepare nanogel particles. Generally, micelles are first formed under a certain condition (e.g., at temperatures above the lower critical solution temperature (LCST) for one block), and then they are cross-linked (either core- or shell-cross-linking) to have structural integrity; afterward, the condition is changed to allow the whole DHBCP to be soluble in water (T < LCST of the micelle core-forming block). The resulting swollen micelles are nanogel particles. As compared with the traditional preparation methods, nanogels obtained from DHBCPs own smaller and uniform particle sizes. On the other hand, photo-cross-linking has the advantages to be nontoxic (no low molecular weight additives) and easily controllable as compared with other chemical cross-linking methods. 

Recently, we reported the preparation of photoresponsive nanogel particles from a DHBCP composed of poly(ethylene oxide) and poly[2-(2-methoxyethoxy)ethyl methacrylate-co-4-methyl-[7- (methacryloyloxy)ethyl]oxy]coumarin (PEO-b-P(MEOMA-co-CMA)). The core-forming block PMEOMA was a thermo-responsive polymer with a LCST at ∼25 °C, and it was randomly copolymerized with a number of coumarin methacrylate units. By heating the solution to T > LCST, the micellar aggregates were preserved by photo-cross-linking via dimerization of coumarin under UV light λ > 310 nm. After cooling the solution to T < LCST, the obtained nanogel particles could undergo a reverse photocleavage reaction under λ < 260 nm UV light, leading to swelling of nanogel particles with a volume increase of 90% as a result of increased cross-linking density. The size of nanogel particles was photocontrollable through the reversible photo-cross-linking and de-cross-linking reaction of coumarin. In another report, photo- and thermo-responsive microgels from large DHBCP vesicles were also obtained. The DHBCP vesicles were composed of a PNIPAM membrane and a poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA) corona bearing a few coumarin units. By slightly cross-linking the corona-forming block, microgel particles were obtained and they could display a large and reversible temperature-controlled size change (∼700% in volume).

As a continuing effort, in the present study, we synthesized a new series of DHBCPs that consist of poly[N,N-dimethylacrylamide-co-4-methyl-[7-(methacryloyloxy)ethyl]oxy]coumarin and poly[N-isopropylacrylamide-co-4-methyl-[7-(methacryloyloxy)ethyl]oxy]coumarin (denoted as P(DMA-co-CMA)-b-P(NIPAM-co-CMA), hereafter). The novelty of this system is that a number of photo-cross-linkers (coumarin) were randomly incorporated into the two constituting blocks. As schematically illustrated in Figure 1, this DHBCP design allows both core and shell of the micellar aggregates self-assembled at T > LCST of P(NIPAM-co-CMA) to be photo-cross-linked. The objective of this study was twofold. First, we wanted to know how the double cross-linking could influence the photoinduced size change of nanogel particles at a given temperature as compared to only core- or only shell-cross-linked nanogel particles. Can a cross-linked shell also contribute to a size change in response to changing cross-linking density? Second, we wanted to know if the size of such highly cross-linked nanogel particles can change in response to the thermal phase transition of the core-forming block. As reported in this paper, we found that this type of photo- and thermo-responsive nanogels exhibits interesting and intriguing behaviors. In addition to photoinduced size change of highly cross-linked nanogel particles as a result of photocontrollable dimerization degree of coumarin, moderately cross-linked nanogel particles not only undergo size change resulting from the LCST-determined water solubility of core-forming P(NIPAM-co-CMA) chains but also exhibit a reversible interparticle aggregation characterized by an upper critical solution temperature (UCST). This unusual UCST thermal transition is schematically illustrated in Figure 1, and a possible underlying mechanism is proposed.

**Experimental Section**

1. **Materials.** All chemicals were purchased from Aldrich and used as received unless otherwise noted. 2,2′-Azobisisobutyronitrile (AIBN) was recrystallized twice from ethanol. N,N-Dimethylacrylamide (DMA, 99%) was passed through a basic aluminum oxide column to remove the inhibitor. N-Isopropylacrylamide (NIPAM, 97%) was recrystallized from hexane. 4-Methyl-[7-(methacryloyloxy)ethyl]oxy]coumarin (coumarin methacrylate, CMA) was synthesized using a previously reported method. The chain transfer agent (CTA), 5-1-dodecyl-S-(α,α′-dimethyl-α′-acetic acid)trithiocarbonate, was prepared using a literature method.

2. **Synthesis of P(DMA-co-CMA) Macro-Chain-Transfer Agent.** The synthetic route to the diblock copolymer is shown in Scheme 1. The general procedure for RAFT polymerization followed the previous report. Using the sample of P(DMA-co-CMA), in Table 1 as example, the synthetic procedure is as

---

**Figure 1.** Schematic illustration of the preparation of both core- and shell-cross-linked nanogel particles and their thermal transitions in aqueous solution.
follows. DMA (3 g, 30.3 mmol), CMA (0.4 g, 1.5 mmol), CTA (0.21 g, 0.58 mmol), and AIBN (20 mg, 0.12 mmol) were dissolved in 3 mL of anisole (99%, anhydride) in a 10 mL flask. The reaction mixture was purged by nitrogen for 10 min under stirring and then placed in a preheated oil bath at 70°C for 1 h. After polymerization, the polymer was reprecipitated in ethyl ether three times and dried under vacuum for 24 h. From GPC measurements using polystyrene (PS) standards, the polymer sample has a $M_n$ of 5400 g/mol and a polydispersity index ($M_w / M_n$) PDI = 1.12. By comparing the integral of the side methylene groups of CMA at ∼4.3 ppm, the side methyl groups of DMA at ∼3.0 ppm, and the methylene group from RFAT agents at ∼3.4 ppm in the $^1$H NMR spectrum (in CDCl₃), the NMR-based molecular weight is ∼7100 g/mol and the mole fraction of CMA is ∼4.7%.


Using the sample of P(DMA 64-co-CMA 3)-b-P(NIPAM 182-co-CMA 8) in Table 1 as example, the diblock copolymer synthesis was carried out by using the following procedure. Macro-CTA of P(DMA 64-co-CMA 3) (210 mg, 0.03 mmol), NIPAM (820 mg, 7.3 mmol), CMA (97 mg, 0.36 mmol), and AIBN (1.2 mg, 0.007 mmol) were dissolved in 3 mL of dioxane (99%, anhydride) in a 10 mL flask. After being purged by nitrogen for 10 min under stirring, the flask was placed in a preheated oil bath at 70°C for 1 h. The polymer was purified by reprecipitation in ethyl ether three times and dried under vacuum for 24 h. From GPC, the diblock copolymer has a $M_n$ of 22 200 g/mol and PDI = 1.20. The diblock copolymer composition was determined from the $^1$HNMR spectrum (in CDCl₃), and the estimated numbers of NIPAM and CMA units are 182 and 8, respectively, by using the side methylene group of PNIPAM at ∼3.9 ppm and side methylene groups of CMA at ∼4.3 ppm.

4. Preparation of Nanogel Particles.

BCP (30 mg) was dissolved in 15 mL of distilled water with 50 mM of NaCl below 10°C overnight (the same concentrations of polymer and NaCl were used in all preparations unless otherwise stated). The polymer solution was filtrated with a cellulose filter (200 nm pore size, from Whatman) and then set in a preheated water bath at 45°C (>LCST of P(NIPAM-co-CMA)). With the small solution volume, no change in light scattering was observed after 10 min, suggesting that core-shell micelles were formed and the thermal equilibrium in the solution was achieved. We then exposed the solution to $\lambda > 310$ nm UV light (intensity at 320 nm was ∼35 mW cm⁻²) for the photo-cross-linking of coumarin side groups, giving rise to both core- and shell-cross-linked micelles.

Table 1. Characteristics of Synthesized Block Copolymers

<table>
<thead>
<tr>
<th>sample</th>
<th>$M_n$,GPC × 10⁻³ (g/mol)</th>
<th>$M_n$,NMR × 10⁻³ (g/mol)</th>
<th>PDI</th>
<th>mol % of CMA in PDMA</th>
<th>mol % of CMA in PNIPAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(DMA 77)</td>
<td>4.8</td>
<td>7.6</td>
<td>1.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P(DMA 77)-b-P(NIPAM 118)</td>
<td>11.4</td>
<td>20.9</td>
<td>1.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P(DMA 77)-b-P(NIPAM 142-co-CMA 10)</td>
<td>14.7</td>
<td>21.8</td>
<td>1.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P(DMA 64-co-CMA 3)</td>
<td>21.1</td>
<td>35.1</td>
<td>1.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P(DMA 64-co-CMA 3)-b-P(NIPAM 256)</td>
<td>24.0</td>
<td>36.1</td>
<td>1.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P(DMA 64-co-CMA 3)-b-P(NIPAM 76-co-CMA 5)</td>
<td>22.2</td>
<td>29.9</td>
<td>1.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Determined by GPC using polystyrene standards for calibration. Molecular weight calculated from $^1$H NMR spectra in CDCl₃. The mole fraction of CMA in each block determined from the integral area of $^1$H NMR spectra in CDCl₃.

Nanogel particles were obtained by cooling the solution from 45 °C to room temperature (< LCST, see Figure 1). The dimerization degree of coumarin was monitored by UV–vis spectra and calculated with the equation $1 - A / A_1 (A_1$ and $A$ are the initial absorbance and the absorbance at irradiation time $t$ at 320 nm, respectively). For each measurement, 100 μL of polymer solution was further diluted in 2 mL of H₂O to get an appropriate absorbance. At a certain dimerization degree, ~2 mL of nanogel solution was used to characterize the scattering intensity and the hydrodynamic diameter. We mention here that at a given polymer concentration the UV irradiation intensity (for both photodimerization and photocleavage of coumarin groups) can affect the time required to reach the photostationary state, but once the maximum photodimerization degree (or minimum degree in the case of photocleavage) is obtained, a longer irradiation time, i.e., a greater exposure dose, makes little difference on the data (e.g., Figure S4).

5. Characterizations. Generally, ¹H NMR spectra (in CDCl₃, unless otherwise stated) were obtained with a Bruker spectrometer (300 MHz, AC 300). For the variable-temperature measurements (in D₂O), the spectra were recorded on a Varian spectrometer (600 MHz, INOVA system). Before each measurement, the solution was held at a given temperature for 10 min for equilibrium. Gel permeation chromatography (GPC) measurements were performed on a Waters system equipped with a refractive index detector (RI 410) and a photodiode array detector (PDA 996). THF was used as the eluent at an elution rate of 1 mL/min, while PS standards were used for calibration. The UV light at $\lambda > 310$ nm for the photodimerization of coumarin (photo-cross-linking reaction) was generated from a UV–vis spot-curing system (Novacure) with a 320–500 nm filter and the intensity at 320 nm is ~35 mW cm⁻², while the photo-photocleavage of cyclobutane rings (photo-de-cross-linking) was achieved by using a UV-C air sterilizer lamp (1.25 W) peaked at $\lambda = 254$ nm at a distance of 5 cm to the nanogel solution. During the photo-cross-linking and photo-de-cross-linking, the dimerization degree of coumarin was recorded on a Varian 50 Bio UV–vis spectrophotometer. Using a thermostat sample holder (Varian SPVF), change in transmittance (500 nm) as a function of temperature was measured by using the UV–vis spectrophotometer. LCST and UCST were taken as temperatures where the transmittance changed abruptly. The measurements were carried out using a solution of cooling or heating rate of 0.5 °C/min. Dynamic light scattering (DLS) experiments were carried out on a Brookhaven goniometer (BI-200) equipped with a highly sensitive avalanche photodiode detector (Brookhaven, BI-APD), a digital correlator (Brookhaven, TurboCorr) that calculates the photon intensity autocorrelation function $g^o(t)$, a helium–argon laser (wavelength $\lambda = 632.8$ nm), and a thermostat sample holder. The volume-averaged hydrodynamic diameter ($D_H$) of nanogels was obtained by a CONTIN analysis, and the polydispersity of nanogel sizes was obtained by a cumulant analysis. The change in scattered light intensity was measured at 90°. $D_H$ reported in the paper is the average value with at least three measurements. The sizes of nanogel particles were also examined using a Hitachi S-4700 field-emission-gun scanning electron microscope (SEM) operating at 3 kV. Samples for SEM were prepared by casting 3–5 μL of nanogel solution on silicon wafers and dried at a given temperature.

Results and Discussion

1. Synthesis, Characterizations, and Thermoresponsive Behaviors. The synthetic route to the diblock copolymer of P(DMA₆₄-CO-CMA)₃-b-P(NIPAM₁₈₂-CO-CMA₈) is shown in Scheme 1. The target polymer was synthesized by a two-step RAFT polymerization, in which the functional monomer CMA was randomly copolymerized in each block. A series of diblock copolymers with various molecular weights and compositions were successfully prepared and characterized by GPC and NMR spectra. The molecular weights, polydispersity, and the mole fraction of CMA in each block (with respect to DMA or NIPAM units) are summarized in Table 1. The typical ¹H NMR spectra of a macro-CTA, PDMA₆₄(CO-CMA)₃, and a resulting diblock copolymer P(DMA₆₄(CO-CMA)₃-b-P(NIPAM₁₈₂(CO-CMA)₈) are presented in Figure S1. The NMR-based molecular weight of the macro-CTA can be determined by comparing the integrals of the peaks from DMA and the RAFT agent. The number of DMA units in the P(DMA₆₄(CO-CMA)₃) block was estimated to be 64 by comparing the peak $g$ from methyl side group at 2.8–3.1 ppm and the methylene group from RAFT agent at 3.4 ppm, while the number of CMA units was ~3, obtained by using the methylene group $e$ at ~4.2 ppm.³³ For the BCP, knowing the molecular weight of P(DMA₆₄-co-CMA)₃ block, the relative amount of PNIIPAM could be estimated by its side methylene group $g$ at ~3.9 ppm. The mole fraction of CMA in the second block was calculated from the integral of the peak $e$ while subtracting the contribution from the P(DMA₆₄(CO-CMA)₃) block.

As for the choice of the constituting blocks, PDMA is a hydrophilic polymer at the entire temperature range of liquid water (0–100 °C), being wildly employed in amphiphilic BCPs as a hydrophilic block.²²,Because of the highly hydrophilic nature, PDMA bearing ~5 mol% of hydrophobic CMA units remains water-soluble at $T < 80$ °C. On the other hand, PNIIPAM is one of the most studied thermoresponsive polymers.²³–³⁰ It can be dissolved in water at low temperature and dilute concentration, but becomes insoluble at $T > ∼32$ °C (LCST), as a result of breaking hydrogen bonding between amide groups and water molecules. With the BCP design of P(DMA-co-CMA)₃-b-PNIIPAM-co-CMA)₈, we expected the formation of micelles at $T > LCST$ of the P(NIIPAM-co-CMA) block. Knowing that the actual LCST should be influenced by the amount of CMA, thermally induced micellization behaviors of diblock copolymers were first studied by DLS. By slowly increasing the solution temperature (~0.3 °C/min), the scattering intensity and $D_H$ of various BCPs were recorded. The temperature, at which abrupt increase of scattering intensity is observed, is taken as the LCST. Figure 2a shows the change in scattering intensity vs temperature for three BCP samples differing in the CMA location. The apparent LCSTs at 18, 19, and 33 °C were obtained for PDMA₇₉-b-PNIIPAM₁₃₈-CMA₈, PDMA₆₄(CO-CMA)₈-b-PNIIPAM₁₈₂-CMA₈, and P(DMA₆₄-CO-CMA)₃-b-PNIIPAM₁₆₈, respectively, indicating micellization at a lower temperature with increasing the amount of CMA in the PNIIPAM block. The hydration to dehydration transition of PNIIPAM block was further investigated by recording variable-temperature ¹H NMR spectra. Using P(DMA₆₄(CO-CMA)₃-b-PNIIPAM₁₈₂(CO-CMA)₈) as an example, the ¹H NMR spectra in D₂O at different temperature are presented in Figure 2b. At 10 °C (blow the LCST), all characteristic signals of PDMA and PNIIPAM block are visible, indicating the molecularly dissolved state blow the LCST. Upon increasing the temperature to 40 °C (above the LCST), while the peaks of the P(DMA-co-CMA) block are essentially unchanged, those of the isopropyl group of the P(NIIPAM-co-CMA) block at 3.9 and 0.9 ppm are basically disappeared. This indicates the hydration to dehydration transition of P(NIIPAM-co-CMA) chains and the formation of micelles. The values of LCST and $D_H$ at 15 and 45 °C obtained for various BCPs are summarized in Table 2. Two conclusions can be made. First, the

References:

The copolymerization of hydrophobic monomer CMA in PNIPAM can significantly bring down the LCST. With 5 mol% of CMA, the decrease of the LCST of PNIPAM is more than 10 °C. This is consistent with our finding in a previous study. The incorporation of hydrophobic CMA comonomer units in a water-soluble polymer brings down its LCST. In the present case, the LCST of PNIPAM can effectively be tuned by the CMA content. However, it is noted that the CMA content is not the only factor that determines the LCST of PNIPAM. The interactions with the hydrophilic PDMA block also play a role. This can be seen by comparing the LCSTs of P(DMA64-co-CMA3)-b-P(NIPAM182-co-CMA8) and P(DMA64-co-CMA3)-b-P(NIPAM182-co-CMA8). While the former has a higher CMA/NIPAM ratio than the latter, its shorter P(NIPAM-co-CMA) block is subjected to a greater influence of the P(DMA-co-CMA) block, which results in a slightly higher LCST. Second, a higher molecular weight of the PNIPAM or P(NIPAM-co-CMA) block gives rise to larger micellar aggregates due to bigger hydrophobic core, which is also in good agreement with previously reported results. Another observation is worth a discussion. The first two samples in Table 2 have essentially the same degree of polymerization. In the water-soluble state at 15 °C, P(DMA77)-b-P(NIPAM77-co-CMA8) has a larger D₄ than that of P(DMA77)-b-P(NIPAM77-co-CMA8), implying that the presence of a small number of CMA units in the PNIPAM block (~4 mol%) could change significantly the chain conformation. In the aggregated state at 45 °C, however, their micelles have basically the same D₄. This suggests two possibilities: (1) they have a similar aggregation number, and (2) they do not have the same aggregation number but the difference is compensated by different chain conformations. More investigations are needed to understand the effect of the CMA content on the chain conformation and aggregation number in the micelles.

2. Preparations of Nanogels. As described above, coumarin side groups can be used for reversible photo-cross-linking and photo-de-cross-linking of BCP chains under UV irradiation at different wavelengths. Using this photoreaction, we can prepare photoresponsive nanogel particles via the self-assembly of DBCPCPs. In the present study, the use of P(DMA-co-CMA)-b-P(NIPAM-co-CMA) allowed us to prepare nanogels of which both core and shell were cross-linked. For comparison purposes, P(DMA)-b-P(NIPAM-co-CMA) and P(DMA-co-CMA)-b-P(NIPAM), for only cross-linkable core or shell, were also prepared.

We utilized an aqueous solution of P(DMA64-co-CMA3)-b-P(NIPAM182-co-CMA8) (2 mg/mL) to investigate the photo-cross-linking reaction in the micellar aggregates and its effect on the obtained nanogels. To this end, a micellar solution equilibrated at 45 °C was exposed to UV light at λ > 310 nm, and UV-Vis spectra at various irradiation times were recorded by diluting a small aliquot of the solution (diluted to 0.1 mg/mL) after a given irradiation time (see Figure S2). The absorption of coumarin centered at ~320 nm decreased over irradiation time, indicating the dimerization of coumarin groups. From the UV-Vis spectra, the dimerization degree (DD) of coumarin could be calculated from DD = 1 - Aᵣ/A₀, where A₀ and Aᵣ are the initial absorbance and the absorbance at irradiation time t, respectively. The increase in DD over time is plotted in Figure 3.

BCP micelles photo-cross-linked at T > LCST became core- and shell-cross-linked nanogel upon cooling of the solution to T < LCST. We investigated the effect of DD, which determines the cross-linking density, on the size of nanogel particles. The results are shown in Figure 4 by plotting D₀ and the scattering intensity vs DD for a nanogel solution at 15 °C. It can be seen that the scattering intensity increases continuously until DD reaches 40% and then remains almost unchanged upon further increase in DD. This result suggests that at DD < 40% micelles are not fully cross-linked, and consequently, a number of free polymer chains are dissociated from the aggregate once in the swollen nanogel state. This would account for the reduced scattering intensity. At DD > 40%, micelles are fully cross-linked, and the resulting nanogel particles could preserve polymer chains. This partial dissociation of un-cross-linked polymer chains at lower DD may also explain the dependence of the measured average D₀ as shown in Figure 4. Below 40% of dimerization degree, coexisted nanogel particles and free polymer chains may account for the increase of D₀ over dimerization degree. After DD reaches 40% and higher, there is no more dissociation of free polymer chains as the cross-linking density is high enough to lock-in all the polymer chains within the particles. In this regime, the size of nanogel particles decreases with increasing the cross-linking density due to restricted swelling by water molecules. The size of nanogel particles with the highest DD = 72% is ~47 nm. From Figure 4, it could be expected that a cross-linking density dependent size change of stable nanogel particles would occur with DD between ~72 and 30% (no severe dissociation of polymer chains). This was indeed observed as shown below.

Compared to both core- and shell-cross-linked nanogels prepared with P(DMA-co-CMA)-b-P(NIPAM-co-CMA), nanogels with only cross-linked core or shell, prepared with P(DMA)-b(P(NIPAM-co-CMA) and P(DMA-co-CMA)-b-P(NIPAM), respectively, were different (see Supporting Information). In particular, no stabilization of scattering intensity upon cross-linking.

was observed even at high dimerization degree, which indicates the absence of stable nanogel particles by using only core- or shell-cross-linking, likely due to the low mole fraction of coumarin moieties in each block.

3. Photoinduced Size Change of Nanogel Particles. Being aware of the reversible photo-cross-linking and photo-de-cross-linking reaction and the dependence of swelling degree of nanogel on the cross-linking density, we investigate the photoresponsive behaviors of core- and shell-cross-linked nanogels. The highly cross-linked \( \text{P(DMA}_{64\text{-co-CMA}_3}\text{-b-P(NIPAM}_{182\text{-co-CMA}_8}) \) nanogel (2 mg/mL) was exposed to UV light at \( \lambda < 260 \text{ nm} \). The photocleavage of cyclobutane bridges back to monomeric coumarin could be observed from UV–vis spectra (see Figure S3). The recovery of absorption of coumarin at \( \sim 320 \text{ nm} \) indicates the photocleavage of coumarin dimers, and the dimerization degree could be reduced to \( \sim 27\% \) after 1800 s. During the photo-de-cross-linking at 15 °C, the size of nanogel particles and the solution scattering intensity were measured by DLS at \( T < \text{LCST} \). The results in Figure 5a show that the average \( D_H \) of nanogel particles displayed a significant increase from \( \sim 47 \) to \( \sim 58 \text{ nm} \) (corresponding to a volume increase of \( \sim 90\% \)) with the decrease of dimerization degree from \( \sim 72\% \) to \( \sim 27\% \). It is understandable that the swelling of nanogel is dependent on the cross-linking density of inside networks. At a highly cross-linked state, the network of polymer chains will confine the swelling by the restricted effect of cross-linkers (coumarin dimers). After de-cross-linking, the decrease in the number of cross-linking point would weaken this constraint and facilitate the particle swelling. The results also suggest that the cross-linking density could be easily controlled not only in the photo-cross-linking step but also using the photo-de-cross-linking in solution and that tunable size change of nanogel particles could be achieved by controlling the dimerization degree. The results also suggest that the cross-linking density could be easily controlled not only in the photo-cross-linking step but also using the photo-de-cross-linking in solution.

Table 2. Thermoresponsive Behaviors of Block Copolymers

<table>
<thead>
<tr>
<th>sample</th>
<th>( D_H ) (nm) at 15 °C</th>
<th>( D_H ) (nm) at 45 °C</th>
<th>LCST(^a) (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{P(DMA}<em>{77\text{-b-P(NIPAM}</em>{118})} )</td>
<td>6.2 ± 2.4</td>
<td>28.3 ± 1.3</td>
<td>37</td>
</tr>
<tr>
<td>( \text{P(DMA}<em>{77\text{-b-P(NIPAM}</em>{114\text{-co-CMA}_3})} )</td>
<td>11.7 ± 2.5</td>
<td>27.3 ± 0.9</td>
<td>24</td>
</tr>
<tr>
<td>( \text{P(DMA}<em>{77\text{-b-P(NIPAM}</em>{324})} )</td>
<td>15.9 ± 2.6</td>
<td>34.9 ± 0.7</td>
<td>34</td>
</tr>
<tr>
<td>( \text{P(DMA}<em>{77\text{-b-P(NIPAM}</em>{142\text{-co-CMA}_10})} )</td>
<td>8.3 ± 3.0</td>
<td>26.1 ± 0.8</td>
<td>18</td>
</tr>
<tr>
<td>( \text{P(DMA}_{64\text{-co-CMA}<em>3\text{-b-P(NIPAM}</em>{138})} )</td>
<td>10.3 ± 0.9</td>
<td>21.9 ± 1.0</td>
<td>37</td>
</tr>
<tr>
<td>( \text{P(DMA}_{64\text{-co-CMA}<em>3\text{-b-P(NIPAM}</em>{182\text{-co-CMA}_3})} )</td>
<td>6.9 ± 3.1</td>
<td>23.3 ± 1.0</td>
<td>21</td>
</tr>
<tr>
<td>( \text{P(DMA}_{64\text{-co-CMA}<em>3\text{-b-P(NIPAM}</em>{124})} )</td>
<td>18.9 ± 1.7</td>
<td>42.2 ± 0.8</td>
<td>33</td>
</tr>
<tr>
<td>( \text{P(DMA}_{64\text{-co-CMA}<em>3\text{-b-P(NIPAM}</em>{182\text{-co-CMA}_8})} )</td>
<td>15.1 ± 2.3</td>
<td>35.2 ± 0.3</td>
<td>19</td>
</tr>
</tbody>
</table>

\(^a\) LCST determined as the onset of the abrupt increase of scattering intensity by DLS.

Figure 3. Increase of dimerization degree of \( \text{P(DMA}_{64\text{-co-CMA}_3\text{-b-P(NIPAM}_{182\text{-co-CMA}_8})} \) (2 mg/mL) during the photo-cross-linking at above LCST (45 °C) vs the irradiation time.

Figure 4. Average hydrodynamic diameter of \( \text{P(DMA}_{64\text{-co-CMA}_3\text{-b-P(NIPAM}_{182\text{-co-CMA}_8})} \) nanogel particles and the solution scattering intensity as a function of dimerization degree at 15 °C.

Figure 5. DLS results obtained with an aqueous solution of \( \text{P(DMA}_{64\text{-co-CMA}_3\text{-b-P(NIPAM}_{182\text{-co-CMA}_8})} \) nanogel (2 mg/mL) during the photo-de-cross-linking under UV light \( \lambda < 260 \text{ nm} \): (a) average hydrodynamic diameter and scattering intensity as a function of dimerization degree at 15 °C; (b) autocorrelation functions and relaxation time distributions (by CONTIN) for micelle (40 °C), cross-linked, and de-cross-linked nanogel particles (15 °C).
change of nanogel particles could be achieved by adjusting the cross-linking density. The concomitant decrease of scattering intensity during the photo-de-cross-linking is noteworthy at the dimerization degree lower than ∼50%, which is attributed to partial dissociation of polymer chains and the reduced contrast of refractive index between water and nanogel particles after full swelling by water. Figure 5b shows typical autocorrelation functions and relaxation time distributions of DLS, indicating the thermally and photoinduced size changes between core- and shell-cross-linked micelles at 40 °C (> LCST), the corresponding nanogel, and the de-cross-linked nanogel at 15 °C. The size distribution is narrower for highly cross-linked particles (micelle and nanogel); nevertheless, after photo-de-cross-linking of nanogel, the polydispersity increased only from 0.03 to 0.12, indicating a quite uniform size distribution even in the de-cross-linked state. The different hydration and swelling degree for the nanogel before and after de-cross-linking can also be observed from 1H NMR spectra (see Figure S3). The spectra were recorded from the highly cross-linked (∼80%) and de-cross-linked (∼27%) nanogel solution in D2O at 10 °C. In both cases, the clear signal of isopropyl group at 3.6 and 0.9 ppm indicates that the cross-linked core of the nanogel, formed by P(NIPAM-co-CMA), is hydrated, as it should be. However, upon closer inspecting, the integral ratio of peaks at 3.6 ppm (methylene on isopropyl group from PNIPAM in the core) and 2.6 ppm (methyl group from PDMA in the shell) changes from 0.17 to 0.29 after photo-de-cross-linking of the nanogel. Assuming that the hydration state of the shell remained essentially the same, this result reveals a more swollen and hydrated state of the core of nanogel after photo-de-cross-linking.

The reversibility of phototunable size change of nanogel particles was further studied. Similar to nanogel of PEO-b-P(MEOMA-co-CMA) reported previously, photo-de-cross-linked nanogel could not recover to the initial highly cross-linked state by directly re-photo-cross-linking swollen nanogel at T < LCST. To recover highly cross-linked nanogel of P(DMA-co-CMA)-b-P(NIPAM-co-CMA), photo-cross-linking was performed after heating the solution to T > LCST (45 °C) for re-formation of micelles, followed by cooling back to T < LCST. We have carried out three cycles of reversible photorelease and measured the size change of nanogel particles (see Figure S4). By alternating irradiations with the different wavelengths of UV light (λ > 310 nm and λ < 260 nm), the dimerization degree could be switched between ∼28% and ∼80%, and the corresponding average hydrodynamic diameter of nanogel was tunable between ∼58 and ∼47 nm (∼23% increase of diameter). Moreover, core- and shell-cross-linked nanogel also exhibited size change in response to temperature switching between below and above LCST of the P(NIPAM-co-CMA) core. Upon heating the nanogel solution from 15 to 40 °C, the average size of particles contracts from ∼47 to ∼38 nm due to the thermal phase transition of P(NIPAM-co-CMA) (Figure 5b). The combined size change of nanoparticles, i.e., cooling the micelle solution from T > LCST to T < LCST followed by photo-de-cross-linking at T < LCST, is as ∼60% in diameter, which corresponds to a volume increase of ∼300%. A schematic illustration recapitulating the thermal- and photo-induced size change of both core- and shell-cross-linked nanogel is presented in Supporting Information (Figure S5).

By contrast, for nanogel particles of P(DMA7)-b-P(NIPAM17-CMA30) with only cross-linked core, photo-de-cross-linking results in an average diameter change from ∼37 to ∼40 nm, while for nanogel of P(DMA54-CMA3)-b-P(NIPAM52) with only cross-linked shell, no photoinduced size change and no reversible change in of dimerization degree of coumarin were observed (see Figure S6). The much larger photocontrollable size change observed for core- and shell-cross-linked nanogel is likely contributed by the two layers of the core–shell nanogel. The phototunable size change of nanogel particles was also observable with SEM, as shown in Figure 6. At highly cross-linked state, uniform nanogel particles were present, and the average size is ∼30 nm, which is slightly smaller than that in aqueous solution due to contraction of dried particles. After exposed to UV light λ < 260 nm, a larger size distribution and some larger particle appeared in SEM images, indicating photoinduced swelling of nanogel particles in solution. This observation is consistent with the DLS (Figure 5b) and 1H NMR results (Figure S6).

4. UCST-Type Thermosensitivity of Nanogel Particles.

In the course of the study, we found an interesting and apparently intriguing phenomenon. Upon cooling, which normally enhances the water solubility of the whole DHBCP, solution of moderately core- and shell-cross-linked nanogel (DD 10%−40%) could display an abrupt increase of scattering intensity at a certain temperature. By further decreasing temperature, the solution could turn opaque, visually observable. These observations indicate a thermally induced water solubility change characterized by an upper critical solution temperature (UCST); that is, the same solution has a LCST at higher temperatures and a UCST at lower temperatures. This peculiar thermoresponsive behavior is shown in Figure 7 where the results obtained with P(DMA45-co-CMA3)-b-P(NIPAM152-co-CMA8) nanogel solution (DD ∼19%) as example. The scattering intensity was recorded during cooling from 45 to 15 °C. At elevated temperatures, the scattering intensity is about 6−7 kcps due to scattering by BCP micelles with an insoluble P(NIPAM-co-CMA) core. When the solution is cooled to near 25 °C, the scattering intensity drops due to the swelling of nanogels. The LCST comes from the solubility change of P(NIPAM-co-CMA) chains within a particle (intraparticle). Upon further cooling of the solution, an abrupt increase in scattering intensity is observed at ∼17 °C, indicating a UCST thermal transition. The size of nanogel particle at 17 °C rises to ∼240 nm with a high polydispersity 0.3. Further decrease of the temperature results in micrometer-sized particles with light scattering intensity out of the measurable limit. The large particles are
Figure 7. Change in scattering intensity upon cooling from 45 to 15 °C (cooling rate ~0.3 °C/min) for a solution of moderately cross-linked P(DMA<sub>64</sub>-co-CMA<sub>3</sub>)-b-P(NIPAM<sub>182</sub>-co-CMA<sub>3</sub>) (~19% dimerization, 2 mg/mL). The inset shows pictures of the solution equilibrated at three temperatures.

Figure 8. Change of scattering intensity over time for P(DMA<sub>64</sub>-co-CMA<sub>3</sub>)-b-P(NIPAM<sub>182</sub>-co-CMA<sub>3</sub>) solution (2 mg/mL), with two different dimerization degrees, during cooling from 45 to 10 °C. instable, and their precipitation is observed after 2–3 h. Visually observable changes of the solution can also be seen from the pictures at three temperatures in Figure 7. The coexistence of two phase transitions, one LCST and one UCST, is visible.

Figure 8 shows the kinetics of scattering intensity change upon fast cooling of the solution from T > LCST to T < UCST. Two nanogel solutions with dimerization degree of 12% and 19%, respectively, were utilized in this experiment. Each solution was first equilibrated at 45 °C (T > LCST) and then quickly transferred to the sample holder of DLS preset at 10 °C (T < UCST); the change in scattering intensity over time was recorded. For both solutions, during the change of solution temperature from 45 to 10 °C, the scattering intensity decreases first before rising again, reflecting the LCST followed by the UCST.

UCST observed for some hydrophilic polymers is usually attributed to ionic interactions or hydrogen-bonding competition with an organic solvent. This is not the case with P(DMA-co-CMA)-b-P(NIPAM-co-CMA) in water. To understand this phase transition, 1H NMR spectra were recorded with P(DMA<sub>64</sub>-co-CMA<sub>3</sub>)-b-P(NIPAM<sub>182</sub>-co-CMA<sub>3</sub>) nanogel solution (DD 19%) in D<sub>2</sub>O at various temperature. If the observed UCST is caused by water insolubility of the BCP, polymer interchain interactions should dominate over polymer–water interactions, and as a result, significant differences of polymer resonance peaks below and above the UCST should be observed. The 1H NMR spectra show that, surprisingly, resonance signals of both blocks are well present at temperatures below UCST with similar relative intensity near the LCST (19 °C) (see Figure S7). The well-dissolved state of BCP chains at T < UCST suggests that there is no hydration to dehydration transition or polymer chain solubility change across the apparent UCST. Therefore, the solubility change (Figures 7 and 8) should come from aggregation of nanogel particles. This would also explain the DLS result indicating a rapid increase of the average hydrodynamic diameter of particles at T < UCST.

In order to confirm this, SEM was used to reveal the morphology change of particles at T < UCST. To prepare the sample, 3–5 μL of the solution in Figure 7 was cast on silicon a wafer and cold-dried at 10 °C (T < UCST). For comparison, the same solution was cast and dried at 40 °C (T > LCST). Figure 9 shows the SEM images. Large aggregates with hundreds of nanometers in diameter can be seen in the sample cast at T < UCST, although there are still many small particles. By contrast, for the sample cast at T > LCST, mostly small particles with diameters around 50 nm are visible. The same experiments were also conducted by using TEM, and large aggregates were observed at T < UCST as well. These observations are consistent with the DLS and 1H NMR measurements, suggesting the occurrence of nanogel particles at T < UCST, resulting in large cluster of nanogel particles.

This phenomenon is reminiscent of the cluster of micelles formed by interpolymer chain bridges, especially in flowerlike micelle aggregation from telechelic amphiphilic polymers. François et al. reported that flowerlike micelles from co-functional PEO with long hydrophobic alkyl chain tend to gather into larger aggregation or form the dense phase (minimizing the interface energy) even at T < LCST and that the intermicelle bridging attraction could form via the dynamic exchange of hydrophobic end segments between the micelles and free polymer chains. In the present case, the aggregation of nanogel particles at T < UCST may also be caused by a kind of internanogel bridging attraction that, we believe, is related to free polymer chains present in the solution. As mentioned above, the UCST behavior was only observed for moderately core- and shell-cross-linked nanogel particles. For them, a partial dissociation of nanogel particles at T < LCST can occur; i.e., a certain amount of BCP chains may detach from the particles. It is reasonable to assume that those free chains would be able to get in and out of nanogel particles. A dynamic exchange of the free polymer chains may create a

bridging effect on the nanogel particles and lead to their clustering. Coumarin moieties, which are highly hydrophobic, may play a similar role as the hydrophobic units in telechelic amphiphilic polymers in inducing the bridging effect.\textsuperscript{50–52} As temperature is lowered, stronger intermolecular interactions between the coumarin groups (hydrophobic and possibly $\pi-\pi$ interactions) on free chains could strengthen their packing tendency and lead to aggregation of nanogel particles. Understandably, with highly cross-linked particles, the bridging effect is weak because of the absence of a sufficient amount of free chains. On the other hand, if nanogel particles are only slightly cross-linked, they are basically dissolved at $T < \text{LCST}$, and no cross-linking of particles can be formed.

The effect of dimerization degree on the UCST was further studied by transmittance measurements. The results in Figure 10 show that with an increase of dimerization degree from 19% to 35% the UCST decreases from 16.5 t0 8.5 °C. UCST could not be detected at higher dimerization degrees. This result may be explained by the proposed mechanism of nanogel cluster formation. As the dimerization degree (cross-linking density) increases, there are fewer free polymer chains to exchange between nanogel particles, which reduce the bridging effect. Consequently, a lower temperature (lower UCST) is needed to better swell nanogel particles so that available free polymer chains could exchange between them more effectively. The phenomenon of clustering of fully water-soluble nanogel particles characterized by a UCST, observed for the first time to our knowledge, is schematically recapitulated in Figure 1.

**Conclusion**

In summary, a new series of coumarin-containing DHBCPs of $\text{P(NIPAM-co-CMA)}_b\text{-P(DMA-co-CMA)}_n$ were synthesized and characterized. Their formation of core–shell micelles in aqueous solution at $T > \text{LCST}$ of $\text{P(NIPAM-co-CMA)}$ was used to prepare both core- and shell-cross-linked nanogel through photodimerization of coumarin under UV light $\lambda > 310 \text{ nm}$ followed by cooling to $T < \text{LCST}$. Photoinduced size change of nanogel was observed upon the reverse photocleavage of coumarin dimers under UV light $\lambda < 260 \text{ nm}$. The average size of core- and shell-cross-linked nanogel particles could be changed between $\sim 58$ and $\sim 47 \text{ nm}$ by optically controlling the dimerization degree between $\sim 28\%$ and $\sim 80\%$. With this BCP, the photoinduced size change was found to be more important for both core- and shell-cross-linked nanogel than nanogel gel with only cross-linked core or only cross-linked shell. In addition to the LCST of $\text{P(NIPAM-co-CMA)}$ that determines the micelle-to-nanogel transition, a UCST thermal phase transition was observed at lower temperatures for moderately cross-linked nanogel. Experiments found that this UCST is related to aggregation or clustering of nanogel particles. A mechanism based on dynamic exchange of dissolved free polymer chains between nanogel particles was proposed to explain this phenomenon observed for the first time.

**Acknowledgment.** We acknowledge financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC) and le Fonds québécois de la recherche sur la nature et les technologies of Québec (FQRNT). Y.Z. is a member of the FQRNT-funded Center for Self-Assembled Chemical Structures (CSACS).

**Supporting Information Available:** UV-vis, $^1\text{H}$ NMR, and DLS results for only core- or only shell-cross-linked and for both core- and shell-cross-linked nanogels. This material is available free of charge via the Internet at http://pubs.acs.org.