Facile Preparation of Core-Crosslinked Micelles from Azide-Containing Thermoresponsive Double Hydrophilic Diblock Copolymer via Click Chemistry

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ABSTRACT: Double hydrophilic diblock copolymer, poly(*N*,*N*-dimethylacrylamide)-*b*-poly(*N*-isopropylacrylamide-*co*-3-azidopropylacrylamide) (PDMA-*b*-P(NIPAM-*co*-AzPAM), containing azide moieties in one of the blocks was synthesized via consecutive reversible addition-fragmentation chain transfer polymerization. The obtained diblock copolymer molecularly dissolves in aqueous solution at room temperature, and can further supramolecularly self-assemble into core-shell nanoparticles consisting of thermoresponsive P(NIPAM-*co*-AzPAM) cores and water-soluble PDMA coronas above the lower critical solution temperature of P(NIPAM-*co*-AzPAM) block. As the micelle cores contain reactive azide residues, core crosslinking can be facilely achieved upon addition of difunctional propargyl ether via click chemistry. In an alternate approach in which the PDMA-*b*-P(NIPAM-*co*-AzPAM) diblock copolymer was dissolved in a common organic solvent (DMF), the core-crosslinked (CCL) micelles can be fabricated via "click" crosslinking upon addition of propargyl ether and subsequent dialysis against water. CCL micelles prepared by the latter approach typically possess larger sizes and broader size distributions, compared with that obtained by the former one. In both cases, the obtained (CCL) micelles possess thermoresponsive cores, and the swelling/shrinking of which can be finely tuned with temperature, rendering them as excellent candidates as intelligent drug nanocarriers. Because of the high efficiency and quite mild conditions of click reactions, we expect that this strategy can be generalized for the structural fixation of other self-assembled nanostructures. © 2007 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 46: 860–871, 2008

Keywords: block copolymers; reversible addition fragmentation chain transfer (RAFT); self-assembly; stimuli-sensitive polymers; supramolecular structures

INTRODUCTION

In selective solvents, block copolymers can self-assemble into various mesophases with diverse morphologies.[1–6] To enhance the structural integrity of these supramolecular nanostructures such as block copolymer micelles, the methodology of covalent stabilization via core crosslinking[7–12] or shell crosslinking[13–20] have been extensively explored. The preparation of core-crosslinked (CCL) micelles can be further categorized into two main strategies.

In the first strategy, block copolymers containing polymerizable or crosslinkable groups were synthesized using controlled radical or

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living anionic polymerizations. After micellization in selective solvents, the crosslinking was achieved via subsequent polymerization or crosslinking within the micelle cores. Liu and coworkers reported the first example of CCL micelles,[10] starting from polystyrene-*b*-poly(2-cinnamoylethyl methacrylate) (PS-*b*-PCEMA) diblock copolymer. It forms PCEMA-core micelles in THF/cyclohexane mixtures; upon UV irradiation, photocrosslinking of micelle cores can be successfully achieved. Kataoka and coworkers[21] synthesized poly(ethylene glycol)-*b*-poly(lactic acid) (PEO-*b*-PLA) bearing a methacryloyl group at the hydrophobic PLA chain end, and fabricated structurally stable CCL micelles via free radical polymerization of vinyl groups within micelle cores. Recently, Stenzel and coworkers[22] synthesized poly(2-hydroxyethyl acrylate)-*b*-poly(*n*-butyl acrylate) (PHEA-*b*-PBA) diblock copolymer via reversible addition-fragmentation chain transfer (RAFT) polymerization. The PBA cores of block copolymer micelles self-assembled in aqueous solution was solubilized with hydrophobic difunctional monomer, 1,6-hexanediol diacrylate (HDA). Subsequent RAFT polymerization of HDA within the micelle cores leads to the facile preparation of CCL micelles. Fukuda and coworkers[23] synthesized monodispersed silica nanoparticles surface grafted with poly(3-ethyl-3-(methacryloyloxy)methyloxetane)-*b*-poly(methyl methacrylate) (PEMO-*b*-PMMA) diblock copolymer brushes. The middle PEMO layer was crosslinked by cationic ring-open polymerization of oxetane residues to further stabilize the hybrid nanostructures.

The second strategy employed for the preparation of core-stabilized nanoparticles relies on directly crosslinking one of the blocks in the common solvents of block copolymers or during block copolymerization. Chen et al.[24] reported the synthesis of CCL micelles by directly crosslinking P4VP blocks of polystyrene-*b*-poly(4-vinylpyridine) (PS-*b*-P4VP) diblock copolymers with 1,4-dibromobutane in the common solvent, *N*,*N*-dimethylformamide (DMF). Pan and coworkers[25,26] reported the one-pot synthesis of CCL micelles by block copolymerization of 4-vinyl pyridine and divinyl benzene via the RAFT process using polystyrene-based macro-RAFT agent. It should be noted that CCL micelles can be fabricated in high efficiency at quite high concentrations (up to 10 wt %) using this strategy.

Recently, click chemistry has emerged to be a highly efficient technique, possessing advantages such as high specificity, facile purification, and quantitative yield in both protic and aprotic media.[27–36] Just recently, Hawker and coworkers[37] reported the preparation of CCL micelles from poly(styrene)-*b*-poly(acrylic acid) (PS-*b*-PAA), in which the PS block was selectively incorporated with small amounts of alkynyl residues. In aqueous solution, the self-assembled PS-core micelles can be facilely core crosslinked via click chemistry upon addition of dendrimers surface functionalized with azide groups. However, protecting the group chemistry was necessary to generate alkynyl functionalities in the PS block, because the presence of large amounts of alkynyl residues can result in gelation during the RAFT block copolymerization.[31,38,39] On the other hand, Matyjaszewski and coworkers[29] and Sumerlin and coworkers[30] reported that azide moieties are compatible with controlled radical polymerizations such as RAFT or atom transfer radical polymerization (ATRP).

In all the above examples of CCL micelles, hydrophobic or amphiphilic diblock copolymers were typically employed. Our recent research interests have focused on stimuli-responsive double hydrophilic block copolymers (DHBCs),[40–46] which can dissolve molecularly in aqueous solution, and self-assemble into micelles in water media by a simple adjustment of solution conditions such as pH, ionic strength, or/and temperature. Thus, the micellization of DHBCs can avoid the use of any organic cosolvent during block copolymer micellization; moreover, we can expect that CCL micelles fabricated from DHBCs might also possess stimuli-responsive cores or coronas.

In this article, an azide-containing DHBC, poly(*N*,*N*-dimethylacrylamide)-*b*-poly(*N*-isopropylacrylamide-*co*-3-azidopropylacrylamide) (PDMA-*b*-P(NIPAM-*co*-AzPAM), was synthesized via RAFT technique using PDMA-based macroRAFT agent. This diblock copolymer self-assembles in aqueous solution to form P(NIPAM-*co*-AzPAM)-core micelles above the lower critical solution temperature (LCST) of P(NIPAM-*co*-AzPAM) block. In the presence of copper catalysts, the obtained micelles can be facilely core crosslinked via click chemistry using difunctional crosslinking reagent such as propargyl ether. In an alternate approach in which the PDMA-*b*-P(NIPAM-*co*-AzPAM) diblock copolymer was dissolved in a common organic solvent (DMF), the CCL micelles can also be fabricated via "click" crosslinking

upon addition of propargyl ether, followed by dialysis against water. To the best of our knowledge, this represents the first report concerning the preparation of CCL micelles via click chemistry starting from a well-defined thermoresponsive DHBC.

EXPERIMENTAL

Materials

N,*N*-Dimethylacrylamide (DMA, 99%; Aldrich) was dried over calcium hydride, vacuum-distilled, and then stored at −20 °C prior to use. *N*-Isopropylacrylamide (NIPAM, 97%; Tokyo Kasei Kagyo) was recrystallized from a mixture of benzene and *n*-hexane (1/3, v/v). 1,4-Dioxane and triethylamine (TEA) were distilled over CaH_2. Methylene chloride was distilled from P_2O_5. 4,4′-Azobis(4-cyanopentanoic acid) (V501; Aldrich) was recrystallized from 95% ethanol. 4-Cyanopentanoic acid dithiobenzoate (CPAD) was synthesized according to literature procedures.[47] Sodium azide (NaN_3), copper(I) bromide (CuBr), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA), propargyl ether, and acryloyl chloride were purchased from Aldrich and used as received. 3-Chloropropylamine hydrochloride, hydroquinone, sodium ascorbate, copper sulfate ($CuSO_4$), potassium iodide (KI), and all other chemicals were purchased from Shanghai Chemical Reagent and used as received.

Sample Preparation

Synthesis of 3-Azidopropylamine

3-Chloropropylamine hydrochloride (20.0 g, 0.154 mol) and KI (0.05 g) were dissolved in 200 mL water, NaN_3 (20.0 g, 0.308 mol) was added, and the reaction mixture was stirred at 90 °C for 24 h. The solution was cooled to room temperature and an appropriate amount of NaOH pellets was added to adjust the solution pH to ~10. The reaction mixture was extracted with diethyl ether for three times. The organic fractions were combined and dried over anhydrous $MgSO_4$. After filtration and removing all the solvent under reduced pressure, the residues were purified by vacuum distillation to give 7.7 g (50% yield) of 3-azidopropylamine (b.p. 32 °C/1.7 mbar).

^{1}H NMR in $CDCl_3$, δ (ppm): 1.16 (s, 2H), 1.73 (m, 2H), 2.80 (t, 2H), 3.37 (t, 2H).

Synthesis of AzPAM

3-Azidopropylamine (7.5 g, 0.075 mol), TEA (8.6 mL, 0.062 mol), and hydroquinone (0.05 g) were dissolved in 120 mL methylene chloride. The mixture was cooled to ~3 °C in an ice-water bath. Acryloyl chloride (5.1 mL, 0.062 mol) in 10 mL methylene chloride was then added dropwise within 1 h under nitrogen atmosphere. After stirring for 12 h at room temperature, the mixture was filtrated to remove insoluble salts. The organic layer was thoroughly washed with aqueous solutions of HCl (3 wt %, 2 × 100 mL), NaOH (3 wt %, 2 × 100 mL), and water (3 × 100 mL). After drying over anhydrous $MgSO_4$ and filtration, the solvent was removed under reduced pressure. The product was further purified by column chromatography on silica gel (60–200 mesh) using methylene chloride as the eluent, giving 4.4 g (46% yield) colorless liquid.

^{1}H NMR in $CDCl_3$, δ (ppm): 1.8 (2H, $CH_2CH_2CH_2N_3$), 3.4 (4H, $CH_2CH_2CH_2N_3$), 5.6 (1H, CH—CH_2), 6.2 (2H, CH—CH_2), 6.5 (s, 1H, NH). ^{13}C NMR in $CDCl_3$, δ (ppm): 166.1 (C—O), 130.9 (CH_2—CH), 126.4 (CH_2—CH), 49.3 (CH_2N_3), 37.2 ($CH_2CH_2CH_2N_3$), 28.8 ($CH_2CH_2CH_2N_3$). ESI-MS: calcd. for $(C_6H_{10}N_4O + H)^+$: 155.09; found: 155.11.

Preparation of PDMA MacroRAFT Agent

RAFT polymerizations were employed to prepare PDMA macroRAFT agent and PDMA-*b*-P(NIPAM-*co*-AzPAM) diblock copolymer (Fig. 1). In a typical example, DMA (3.57 g, 36 mmol), CPAD (101 mg, 0.36 mmol), and V501 (20 mg, 0.072 mmol) were added into a glass ampoule containing 1,4-dioxane (6 mL). The mixture was degassed via three freeze–pump–thaw cycles, flame-sealed under vacuum, and then immersed into an oil bath preheated at 70 °C to conduct the polymerization. After 14 h, the ampoule was plunged into liquid nitrogen to terminate the polymerization. The mixture was diluted with 4 mL of 1,4-dioxane and then precipitated into an excess of diethyl ether; this purification cycle was repeated for three times to remove residual DMA monomer. The obtained slightly pink powder was dried in a vacuum oven at room temperature for 12 h. The overall yield was 62%. The molecular weight and molecular weight distribu-

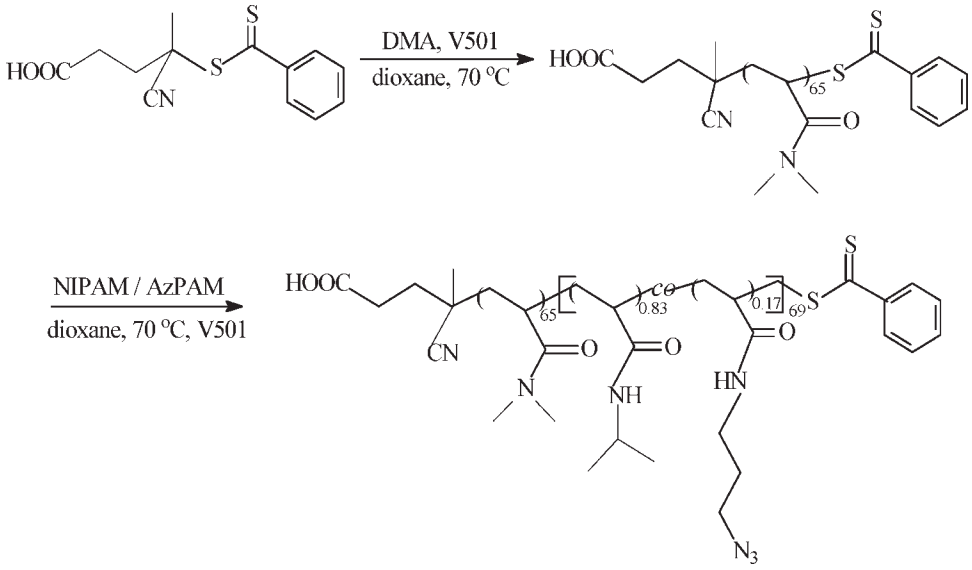


Figure 1. Schematic illustration of the synthesis of the $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ diblock copolymer.

tion of PDMA homopolymer were determined by DMF GPC: M_n = 5800, M_w/M_n = 1.13. The actual degree of polymerization (DP) of PDMA homopolymer was determined to be 65 by ^1H NMR in D_2O.

Preparation of PDMA-b-P(NIPAM-co-AzPAM) Diblock Copolymer

In a typical example, NIPAM (1.13 g, 10 mmol), AzPAM (0.40 g, 2.6 mmol), PDMA macroRAFT agent (0.64 g, 0.1 mmol), and V501 (6 mg, 0.02 mmol) were charged into a glass ampoule containing 1,4-dioxane (4 mL). The mixture was degassed through three freeze–pump–thaw cycles. The ampoule was then flame-sealed under vacuum, and immersed into an oil bath thermostated at 70 °C to start the polymerization. After 12 h, the ampoule was put into liquid nitrogen to stop the polymerization. The mixture was diluted with 1,4-dioxane, and then precipitated into an excess of diethyl ether. This purification cycle was repeated twice. The obtained slightly pink powder (1.35 g, 62% yield) was dried in a vacuum oven overnight at room temperature. The molecular weight and molecular weight distribution of PDMA-*b*-P(NIPAM-*co*-AzPAM) diblock copolymer was determined by DMF GPC: M_n = 14,700, M_w/M_n = 1.23. The overall DP of P(NIPAM-*co*-AzPAM) block and the AzPAM content were determined to be 69 and 17 mol %, respectively, by ^1H NMR analysis in D_2O. The obtained diblock copolymer was thus denoted as $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$.

Thermoresponsive Micellization and Preparation of CCL Micelles (Method 1)

At ambient temperature, $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ diblock copolymer was dissolved in water at a concentration of 10.0 g/L. The aqueous solution (24 mL) was then heated to 45 °C in a water bath. Upon heating, micellization occurred immediately as indicated by the bluish tinge characteristic of the colloidal aggregates. The solution was equilibrated at 45 °C for 30 min. $CuSO_4$ (3 mg, 0.02 mmol), propargyl ether (10 μL, 0.1 mmol), and sodium ascorbate (8 mg, 0.04 mmol) were added to the solution. The molar ratio of propargyl ether and AzPAM residues was kept constant at 1:2. The reaction mixture was stirred at 45 °C for 2 days, and then dialyzed (MW cutoff, 14,000 Da) against deionized water for 2 days to remove copper catalysts. The final dispersion exhibits the bluish tinge characteristic of the micelle aggregates even at 25 °C, indicating successful core-crosslinking reactions.

One-Pot Synthesis of CCL Micelles (Method 2)

The $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ diblock copolymer was dissolved in DMF at a concentration of 10.0 g/L (25 °C). CuBr (28 mg,

0.196 mmol), PMDETA (41 μL, 0.196 mmol), and propargyl ether (10 μL, 0.098 mmol) were added into degassed DMF solution (24 mL) of the diblock copolymer and the mixture was stirred at 25 °C for 4 days. The reaction mixture was dialyzed (MW cutoff, 14,000 Da) against deionized water for 2 days to remove copper catalysts and DMF.

Characterization

Gel Permeation Chromatography

Molecular weights and molecular weight distributions were determined by gel permeation chromatography (GPC) equipped with Waters 1515 pump and Waters 2414 differential refractive index detector (set at 30 °C). It used a series of three linear Styragel columns HT2, HT4, and HT5 at an oven temperature of 45 °C. The eluent was DMF at a flow rate of 1.0 mL/min. A series of low polydispersity PEO standards were employed for the GPC calibration.

Nuclear Magnetic Resonance Spectroscopy

All ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or D_2O at 25 °C on a Bruker AV300 Nuclear Magnetic Resonance (NMR) spectrometer (resonance frequency of 300 MHz for ^{1}H and 75 MHz for ^{13}C) operating in the Fourier transform mode.

Fourier Transform Infrared Spectroscopy

All spectra were recorded on a Bruker VECTOR-22 IR spectrometer. The spectra were collected at 64 scans with a spectral resolution of 4 cm^{-1}.

Temperature-Dependent Turbidimetry

The optical transmittance of the aqueous solution was acquired on a Unico UV/vis 2802PCS spectrophotometer and measured at a wavelength of 500 nm using a thermostatically controlled cuvette.

Dynamic Laser Light Scattering

A commercial spectrometer (ALV/DLS/SLS-5022F) equipped with a multi-tau digital time correlator (ALV5000) and a cylindrical 22 mW UNIPHASE He-Ne laser (λ_0 = 632 nm) as the light source was employed for dynamic laser light scattering (LLS) measurements. Scattered light was collected at a fixed angle of 90° for duration of ~10 min. Distribution averages and particle size distributions were computed using cumulant analysis and CONTIN routines. All data were averaged over three measurements.

Transmission Electron Microscopy

All images were recorded using a Philips CM120 electron microscope at an accelerating voltage of 200 KV. Transmission electron microscopy (TEM) sample was prepared by placing 10 μL aqueous solutions of CCL micelles (0.2 g/L) on copper grids coated with thin films of Formvar and carbon. No staining was required.

RESULTS AND DISCUSSION

Synthesis of PDMA MacroRAFT Agent and PDMA-*b*-P(NIPAM-*co*-AzPAM) Diblock Copolymer

The controlled RAFT polymerizations of DMA and NIPAM monomers in organic or aqueous media have been well documented.[48–56] The general approach for the preparation of PDMA macroRAFT agent and PDMA-*b*-P(NIPAM-*co*-AzPAM) diblock copolymer was shown in Figure 1. The target diblock copolymer was synthesized by statistical RAFT copolymerization of NIPAM and AzPAM comonomers using PDMA as the macroRAFT agent.

First, DMA was polymerized at 70 °C using CPAD as the chain transfer agent. ^{1}H NMR analysis in D_2O revealed the presence of characteristic PDMA signals at δ = 2.7–3.2 ppm [Fig. 2 (a)]. Signals at δ = 7.9, 7.6, and 7.4 ppm were ascribed to protons of dithiobenzoyl group located at the PDMA chain end. DMF GPC analysis revealed a mono-modal and symmetric peak with an M_n of 5800 and a polydispersity, M_w/M_n, of 1.13 [Fig. 3 (a)]. The actual DP of PDMA was determined to be 65 by ^{1}H NMR.

A novel azide-containing monomer, AzPAM, was prepared by the reaction of 3-azidopropylamine with acryloyl chloride. It was reported in the literature[29,30] that azide groups are relatively inert and compatible with RAFT processes. In preliminary experiments, RAFT polymerization of AzPAM was attempted in 1,4-dioxane at 70 °C using CPAD as the chain trans-

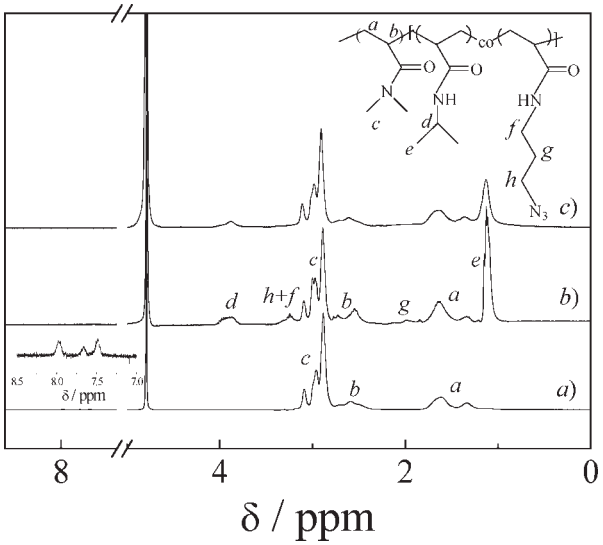


Figure 2. ^{1}H NMR spectra of (a) $PDMA_{65}$ macroRAFT agent and (b) $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ diblock copolymer in D_2O at 25 °C; (c) CCL micelles (prepared via Method 1) in D_2O at 25 °C.

fer agent. Relatively good control was achieved, as revealed by the narrow and symmetric GPC trace (data not shown). The polydispersity of PAzPAM was typically less than 1.2, and M_n was quite close to the theoretical one. As AzPAM was structurally similar to NIPAM, the statistical RAFT copolymerization of AzPAM and NIPAM using PDMA macroRAFT agent was quite expected, leading to the formation of PDMA-*b*-P(NIPAM-*co*-AzPAM) diblock copolymer (Fig. 1).

^{1}H NMR spectrum of the obtained diblock copolymer in D_2O was shown in Figure 2(b). Besides signals characteristic of PDMA block, the NMR spectrum also revealed the presence of signals characteristic of NIPAM residues at 3.9 ppm (*d*), and AzPAM residues at 3.2–3.4 ppm (*h*, *f*). Most importantly, DMF GPC analysis revealed that the elution peak of PDMA-*b*-P(NIPAM-*co*-AzPAM) shifted to higher molecular weight, compared with that of the PDMA precursor. The elution peak of the diblock copolymer was also symmetric and showed no tailing at the lower molecular weight side, indicating a complete consumption of PDMA macroRAFT agent and a successful preparation of the target diblock copolymer (Fig. 3). GPC analysis revealed an M_n of 14,700 and an M_w/M_n of 1.23. The AzPAM content and the overall DP of P(NIPAM-*co*-AzPAM) block were determined to be 17 and 69 mol %, respectively, by ^{1}H NMR. Thus, the obtained diblock copolymer was denoted as $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$.

Thermoresponsive Micellization and Core Crosslinking via Method 1

Poly(*N*-isopropylacrylamide) (PNIPAM) is the most extensively studied thermoresponsive polymer that exhibits a LCST in water at about 32 °C.[41,42] PDMA homopolymer is water soluble up to 100 °C.[48] McCormick and coworkers[52] reported the thermoresponsive micellization behavior of PDMA-*b*-PNIPAM, forming PNIPAM-core micelles stabilized by well-solvated PDMA coronas.

To incorporate "click" crosslinkable moieties, AzPAM comonomer need to be statistically copolymerized with NIPAM. However, AzPAM monomer and its homopolymer are basically hydrophobic. Based on previous reports of the water solubility of poly(ethylene oxide)-*b*-poly(*N*,*N*-dimethylacrylamide-*co*-*N*-acryloxysuccinimide) [PEO-*b*-P(DMA-*co*-NAS)][57] and poly(glycerol monomethacrylate) (PGMA) partially esterified with cinnamoyl chloride,[58] this dilemma can be solved by the incorporation of relatively low contents of AzPAM into the PNIPAM block. Preliminary experiments revealed that P(NIPAM-*co*-AzPAM) remains water soluble when the AzPAM content was $\leq$20 mol %. Thus, we expect that $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ might

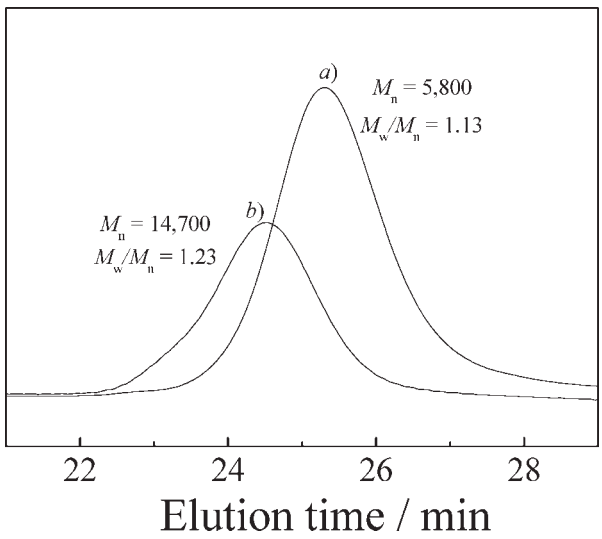
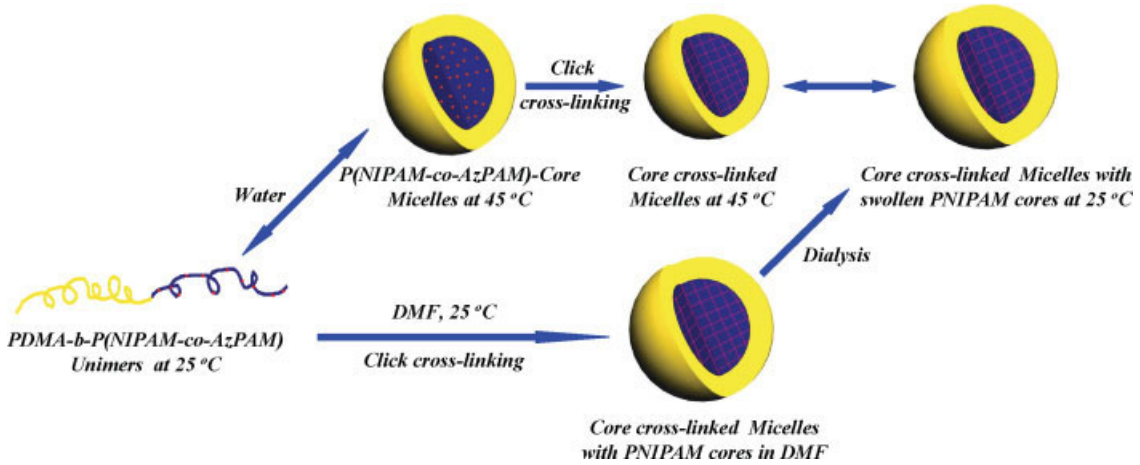


Figure 3. DMF GPC traces of (a) $PDMA_{65}$ macroRAFT agent and (b) $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ diblock copolymer.



Scheme 1. Schematic illustrations of the preparation of core-crosslinked (CCL) micelles from PDMA-*b*-P(NIPAM-*co*-AzPAM) diblock copolymer via two alternate approaches: Method 1, thermoresponsive micellization in aqueous solution followed by core crosslinking via click chemistry; Method 2, one-pot synthesis of CCL micelles from PDMA-*b*-P(NIPAM-*co*-AzPAM) in a common organic solvent (DMF) via click chemistry.

exhibit thermoresponsive micellization behavior in aqueous solution and the formed micelles can be further covalently stabilized via "click" core crosslinking (Scheme 1).

$PDMA_{65}$-*b*-P($NIPAM_{0.83}$-*co*-$AzPAM_{0.17}$)$_{69}$ diblock copolymer is directly soluble in water at 25 °C. Upon heating to 45 °C, the bluish tinge characteristic of the micellar solutions appears immediately. The temperature-dependent transmittance obtained for 10.0 g/L aqueous solution of $PDMA_{65}$-*b*-P($NIPAM_{0.83}$-*co*-$AzPAM_{0.17}$)$_{69}$ was shown in Figure 4. The transmittance starts to decrease dramatically above 35 °C, indicating that the P(NIPAM-*co*-AzPAM) block is getting water insoluble due to its thermal phase transition behavior. Above 40 °C, the transmittance stabilizes out, suggesting the complete thermoinduced micellization. Based on chemical intuition, the formed micelles should possess a core consisting of hydrophobic P(NIPAM-*co*-AzPAM) and a well-solvated PDMA corona (Scheme 1). It should be noted that the micellar solution is at pH 7–8, and thus the terminal carboxyl group resulting from the RAFT agent are ionized and its hydrogen bonding interaction with NIPAM moiety can be safely excluded.

Dynamic LLS was then employed to characterize the thermoresponsive micellization behavior. Figure 5 shows typical hydrodynamic diameter distributions, $f(D_h)$, obtained at different temperatures for aqueous solutions of $PDMA_{65}$-*b*-P($NIPAM_{0.83}$-*co*-$AzPAM_{0.17}$)$_{69}$. At 25 °C, the diblock copolymer directly dissolves in aqueous solution, exhibiting an average hydrodynamic diameter, $\langle D_h \rangle$, of ~9 nm and quite low scattered light intensity. Although AzPAM content of P(NIPAM-*co*-AzPAM) block is quite low (17 mol %), we cannot exclude the possible presence of loose aggregates resulting from the hydrophobic association of AzPAM residues, as revealed by the relatively broad size distribution curve

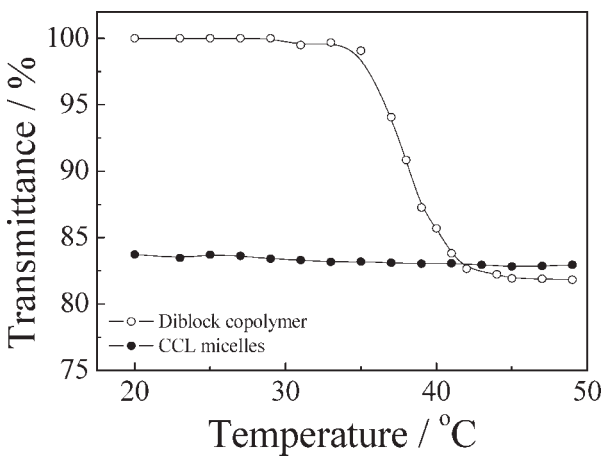


Figure 4. Temperature dependence of optical transmittance at 500 nm obtained for 10.0 g/L aqueous solutions of $PDMA_{65}$-*b*-P($NIPAM_{0.83}$-*co*-$AzPAM_{0.17}$)$_{69}$ and CCL micelles prepared via Method 1.

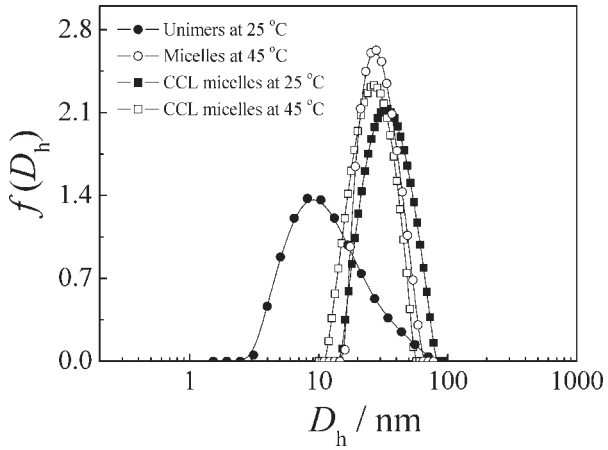


Figure 5. Typical hydrodynamic diameter distributions, $f(D_h)$, obtained at different temperatures for 1.0 g/L aqueous solutions of unimers, micelles, and CCL micelles prepared from $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ diblock copolymer via Method 1.

(Fig. 5). On the other hand, dynamic LLS of the aqueous solution at 45 °C reveals relatively narrow size distribution, with an intensity-average hydrodynamic diameter, $\langle D_h \rangle$, of 28 nm and a polydispersity (μ_2/Γ^2) of 0.09.

Figure 6 shows the temperature dependences of $\langle D_h \rangle$ and scattered light intensity obtained for 1.0 g/L aqueous solution of $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$. Above 34 °C, micellization starts to take place, as revealed by a dramatic increase of $\langle D_h \rangle$ and scattered light intensity. Dynamic LLS only reveals one population corresponding to micelles above 40 °C, and the micelle size remains almost constant at ~28 nm in diameter. This clearly indicates the thermoinduced formation of core-shell nanoparticles from $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ (Scheme 1).

Hawker and coworkers[37] prepared amphiphilic PS-*b*-PAA diblock copolymer with the core-forming PS block derivatized with alkynyl groups. "Click" core crosslinking was achieved in the presence of dendrimers surface functionalized with azide groups. However, the core of CCL micelles was hydrophobic and does not exhibit any stimuli-responsiveness. In this study, the cores of thermoinduced micelles of $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ contain reactive azide groups. Thus, click reactions in the presence of difunctional alkynyl compounds, propargyl ether, should lead to the facile preparation of stimuli-responsive CCL micelles (Scheme 1).

Propargyl ether is hydrophobic, and it will be solubilized into the hydrophobic P(NIPAM-*co*-AzPAM) cores upon addition. The molar ratio of propargyl ether to that of azide residues was kept constant at 1:2, targeting for a theoretical degree of crosslinking of 100%. Fourier transform infrared (FTIR) spectra of noncrosslinked and CCL micelles after freeze drying were shown in Figure 7. We can clearly see that the

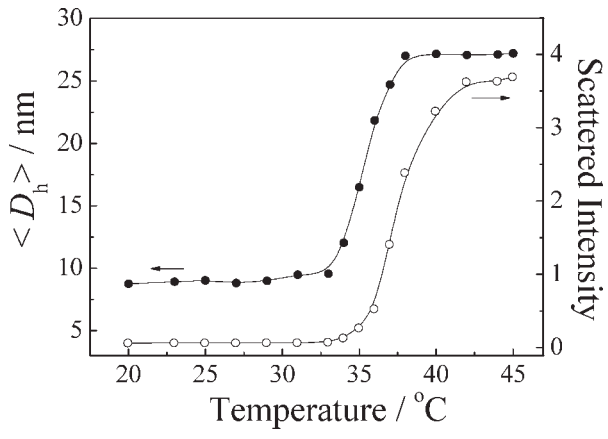


Figure 6. Variation of intensity-average hydrodynamic diameters, $\langle D_h \rangle$, and scattered light intensity with temperature obtained for 1.0 g/L aqueous solution of $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ diblock copolymer.

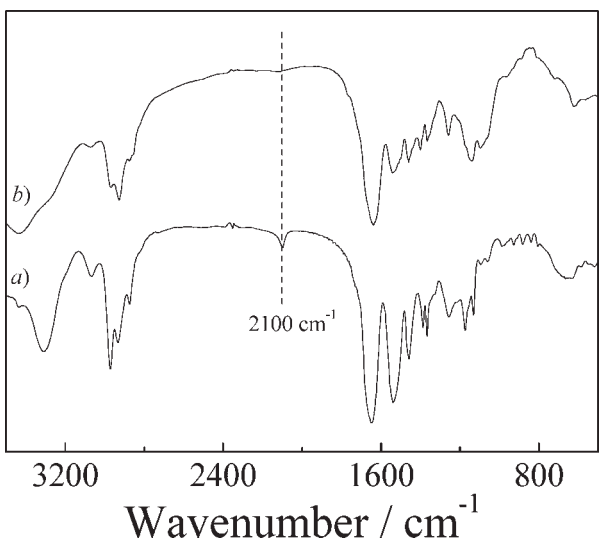


Figure 7. FTIR spectra of (a) $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ diblock copolymer and (b) core-crosslinked (CCL) micelles prepared via Method 1 after freeze-drying.

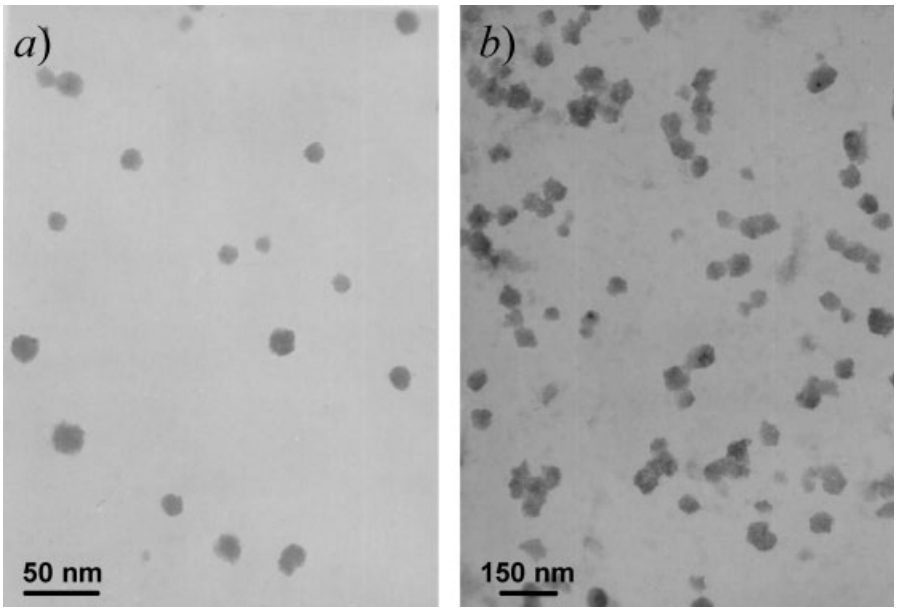


Figure 8. Typical TEM images of CCL micelles at 25 °C prepared from $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ diblock copolymer via (a) Method 1 and (b) Method 2.

absorbance peak characteristic of azide groups at ~2100 cm^{-1} reduced to a large extent after core crosslinking. A closer examination of Figure 7(b) revealed that there still exist a weak absorbance peak of residual azide groups, which might be further utilized for core functionalization, such as attaching with fluorescent probes and other bioactive species.

After freeze-drying the CCL micellar solution prepared via Method 1, the obtained powder was redispersed in D_2O and its ^1H NMR spectrum (25 °C) is shown in Figure 2(c). We can clearly discern characteristic signals of NIPAM residues at $\delta = 1.1$ and 3.9 ppm; however, their intensities decreased ~60% relative to that of PDMA signals if the NMR spectrum of $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ in D_2O [25°C, Fig. 2(b)] was employed as the reference. The dramatic decrease of NIPAM signal intensities should be ascribed to the crosslinked nature of micellar cores (Scheme 1), which greatly reduces the mobility of core chain segments. Figure 2(c) shows that signals characteristic of AzPAM residues are barely discernible after core crosslinking, which is reasonable considering the low AzPAM molar content and reduced signal intensities due to crosslinking. Thus, the accurate quantification of the degree of "click" core crosslinking and the amount of residual azido or alkynyl groups was not possible.

After removing copper catalysts via dialysis, the dispersion exhibits a bluish tinge even at 25 °C, apparently suggesting successful core crosslinking. Otherwise, the micelles will dissociate into unimers after cooling to 25 °C and clear solution should be obtained. Figure 4 also shows the temperature-dependent optical transmittance obtained for aqueous solution of CCL micelles. We can clearly see that the transmittance of CCL micelles is almost independent of temperatures in the range of 20–48 °C, which is drastically different from that of noncrosslinked micelles. This further confirms the successful core crosslinking.

Dynamic LLS was utilized to study the thermoresponsive behavior of structurally stable CCL micelles (Fig. 5). The swelling of CCL micelles at room temperature can be clearly interpreted in terms of the shift of hydrodynamic diameter distributions. At 45 °C, D_h of CCL micelles ranges from 10 to 65 nm with a $\langle D_h \rangle$ of 26 nm. Upon decreasing to 25 °C, D_h is in the range of 15–85 nm with a $\langle D_h \rangle$ of 33 nm. This reflects that upon cooling from 45 to 25 °C, the swelling of P(NIPAM-*co*-AzPAM) cores leads to approximately twofold increase of the hydrodynamic volume of CCL micelles (Scheme 1). Figure 8 (a) shows the TEM image of CCL micelles prepared via Method 1, which reveals the presence of spherical nanoparticles with an average diameter of ~15–20 nm.

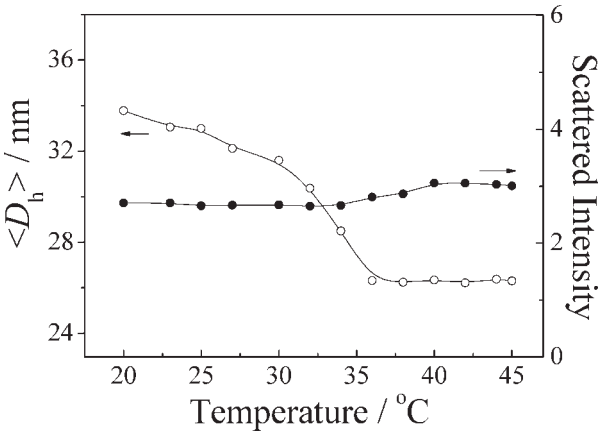


Figure 9. Variation of intensity-average hydrodynamic diameter, $\langle D_h \rangle$, and scattered light intensity with temperature obtained for the 1.0 g/L aqueous solution of CCL micelles prepared from $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ via Method 1.

The temperature dependence of $\langle D_h \rangle$ for the obtained CCL micelles is illustrated in Figure 9. We can see that the most dramatic increase of micelle sizes occurs within the temperature range of 30–35 °C, while scattered light intensity remains almost constant in this temperature range. This again confirms the successful core crosslinking, and the size increase of CCL micelles upon cooling is due to the solvation and swelling of P(NIPAM-*co*-AzPAM) cores. This augurs well for the potential applications of this novel type of CCL micelles as nanosized drug-delivery vehicles because the changes in hydrophilicity of P(NIPAM-*co*-AzPAM) cores should also lead to the "triggered release" of encapsulated guest molecules.

Core Crosslinking in Common Organic Solvent via Method 2

The one-pot preparation of CCL micelles was originally reported by Chen et al.,[24] starting from PS-*b*-P4VP diblock copolymer in a common solvent, DMF. The addition of a difunctional reagent, 1,4-dibromobutane, leads to the crosslinking of P4VP block, whereas the noncrosslinkable PS block can stabilize the crosslinked P4VP core and effectively prevent gelation. However, the obtained CCL micelles cannot be dispersed in water, as the micelle coronas consist of hydrophobic PS blocks.

Following similar principle, we further attempted the synthesis of CCL micelles from $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ in DMF, in which both blocks are soluble. Upon addition of propargyl ether, the "click" crosslinking was conducted at 25 °C in the presence of copper catalysts. After 4 days, the dispersion was dialyzed against deionized water to remove DMF and copper catalysts (Scheme 1). Dynamic LLS and TEM were then employed to characterize the obtained CCL micelles.

Figure 10 shows the typical hydrodynamic diameter distributions, $f(D_h)$, obtained at different temperatures for aqueous solution of CCL micelles prepared via Method 2. The swelling of the CCL micelles could be obviously observed after cooling from 45 to 25 °C. At 45 °C, D_h of CCL micelles ranges from 5 to 430 nm with a $\langle D_h \rangle$ of 50 nm; whereas at 25 °C, D_h is in the range of 10–590 nm with a $\langle D_h \rangle$ of 68 nm. Thus, the swelling of CCL micelles upon cooling is clearly evident.

Compared with that obtained via Method 1, CCL micelles prepared by Method 2 exhibit broader size distributions and larger sizes, as revealed by dynamic LLS results (Figs. 5 and 10); however, Method 2 provides a more convenient and versatile approach for the fabrication of CCL micelles. TEM image obtained for CCL micelles prepared via Method 2 clearly reveals the presence of robust and nearly spherical nanoparticles [Fig. 8(b)]. The sizes of these nanoparticles vary in the range of 20–50 nm in diameter.

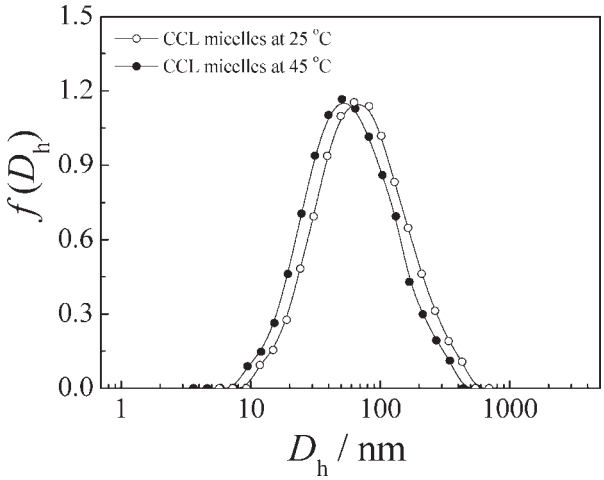


Figure 10. Typical hydrodynamic diameter distributions, $f(D_h)$, obtained at different temperatures for 1.0 g/L aqueous solutions of CCL micelles prepared from $PDMA_{65}$-*b*-$P(NIPAM_{0.83}$-*co*-$AzPAM_{0.17})_{69}$ via Method 2.

CONCLUSIONS

In summary, well-defined PDMA-*b*-(PNIPAM-*co*-PAzPAM) diblock copolymer containing azide moieties in one of the blocks was synthesized via consecutive RAFT polymerization. The obtained double hydrophilic diblock copolymer molecularly dissolves in aqueous solution at room temperature, and self-assembles into micelles consisting of PNIPAM-*co*-PAzPAM cores and PDMA coronas at elevated temperatures. CCL micelles were facilely fabricated via click chemistry upon addition of propargyl ether in the presence of copper catalysts. In an alternate approach, CCL micelles can also be conveniently prepared by directly "click" crosslinking the azide-containing block in DMF, a common solvent for the diblock copolymer. The obtained CCL micelles in both approaches exhibit thermotunable core swellability. This augurs well for their potential application as nanosized drug-delivery vehicles because the changes in core hydrophilicity should also lead to "triggered release" of guest molecules. Because of the high efficiency and quite mild conditions of click reactions, we expect that this strategy can be generalized for the structural fixation of other self-assembled nanostructures of DHBCs.

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