Macromolecules

ARTICLE

pubs.acs.org/Macromolecules

Synthesis and Aggregation Behaviors of Nonlinear Multiresponsive, Multihydrophilic Block Copolymers

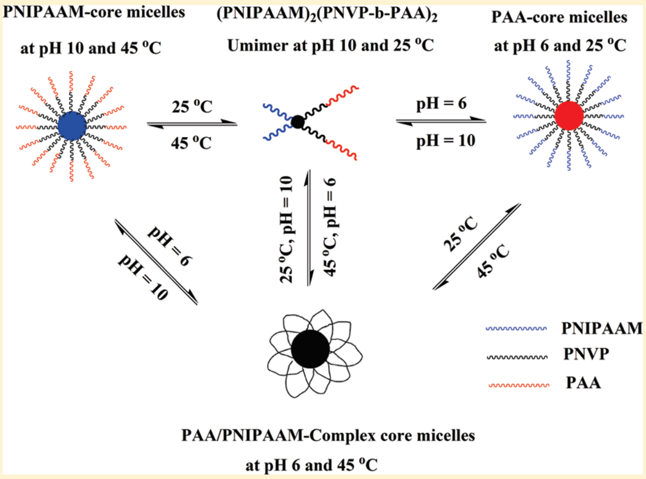
Weidong Zhang,[†,‡,§] Wei Zhang,[†,§] Zhenping Cheng,[†] Nianchen Zhou,[†] Jian Zhu,[†] Zhengbiao Zhang,[†] Gaojian Chen, and Xiulin Zhu*[,†]

[†]Jiangsu Key Laboratory of Advanced Functional Polymer Design and Application, Department of Polymer Science and Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, P. R. China

[‡]Center for Soft Condensed Matter Physics and Interdisciplinary Research, Soochow University, Suzhou 215006, P. R. China

S *Supporting Information*

ABSTRACT: The well-defined nonlinear multiresponsive, multihydrophilic block copolymers (MHBCs), poly(*N*-isopropylacrylamide)$_2$−[poly(*N*-vinylpyrrolidone)-*b*-poly(acrylic acid)]$_2$ ((PNIPAAM)$_2$(PNVP-*b*-PAA)$_2$) and poly[*N*-isopropylacrylamide-*b*-(acrylic acid)]$_2$−poly(*N*-vinylpyrrolidone)$_2$ ((PNIPAAM-*b*-PAA)$_2$(PNVP)$_2$), were successfully synthesized via a combination of single-electron transfer living radical polymerization (SET-LRP) and reversible addition−fragmentation chain transfer (RAFT) polymerization techniques. All the final miktoarm star block copolymers were characterized by GPC and ^{1}H NMR spectra. Interestingly, the hydrogen bonding interactions have a great effect on the water solubility of PAA chains, and the nonlinear MHBCs solutions showed sharp phase separation at pH = 7.0. The novel water-soluble nonlinear MHBCs consisting of pH-responsive PAA segments, thermoresponsive PNIPAAM segments, and hydrophilic, noncharged and biocompatible PNVP segments tend to self-assemble into three different types of micellar aggregates and umimers, which can be reversibly controlled by pH value and temperature of aqueous solution of these copolymers.



INTRODUCTION

In the past decade, stimuli-responsive double hydrophilic block copolymers (DHBCs) have received ever-increasing interest due to the fact that DHBCs can exhibit the so-called "schizophrenic" aggregation behavior in aqueous solution controlled by the solution external environment, such as pH, temperature, and light irradiation.[1−9] External stimulus renders the high-order aggregates in aqueous solution to reversible change in properties, dimensions, structure, and interactions. Many different kinds of DHBCs have been investigated for their potential use as a platform to deliver a drug in a highly efficient and a well-controlled behavior. As for linear DHBCs, previous studies showed that the characteristic parameters of the micelles are influenced by many factors, such as chemical nature, molecular weights of each block, concentration, and solution conditions.[10−19] Recent advances in polymer synthesis techniques facilitated the preparation of the well-defined stimuli-responsive polymers with complex architectures. Most recently, some nonlinear DHBCs with more complex chain architectures have been successfully achieved. The results indicated that the chain architectures (topology) of block copolymers had great effects on their self-assembling behaviors in both organic solvents and aqueous solution.[9,13,14,20−22] Hammond et al.[23] successfully prepared the amphiphilic linear−dendritic block copolymer consisting of poly(amidoamine) and poly(propylene oxide), and the solution-phase behavior of the block copolymer can be tuned by changing genetations, pH, or ionic strengths. Liu et al. also reported the synthesis and self-assembly of nonlinear DHBCs with more complicated topological structures, such as AB$_4$,[9] A$_2$BA$_2$,[24] A$_4$BA$_4$,[24] and ABC types.[6,7] It is valuable to note that the special alkylnyl- or azide-functional resin should be used to remove the remnant linear polymers to obtain the high-purity miktoarm copolymers in some cases. To the best of our knowledge, the fabrication and "schizophrenic" micellization behavior of multiresponsive, nonlinear multihydrophilic block copolymers (MHBCs) has been quite limitedly studied because the synthesis of these well-defined complex copolymers is relatively difficult.

Poly(*N*-isopropylacrylamide) (PNIPAAM) is a typical reversible temperature-responsive polymer, which has a lower critical solution temperature (LCST) at around 32 °C.[25] It is well-known

Received: January 13, 2011
Revised: March 27, 2011
Published: April 08, 2011

that the tunable phase transition behavior of PNIPAAM-containing copolymer has potential application in biomaterials. On the other hand, poly(acrylic acid) (PAA) is one of the typical reversible pH-responsive polymers. PAA becomes soluble in water when pH values are above the pK_a (4.7). However, the previous studies indicated high water solubility of PAA critically restricts its application as a mucoadhesive drug carrier, since it may be dissolved before the drug is delivered across the membrane.[26] Solving this problem is highly desirable in drug delivery systems. In addition, poly(*N*-vinylpyrrolidone) (PNVP) is a water-soluble polymer, which hold great promise due to its unique properties, such as the well solubility in water and organic solvents, very low toxicity, good biocompatibility, and high complexation ability.[27−29] However, the synthesis of the well-designed PNVP-containing block copolymers and application of these block copolymers were rarely reported because the polymerization of NVP is relatively difficult to be well controlled. Up to date, the polymerization of NVP was easy to be controlled by the RAFT technique using xanthate as RAFT agent.[30−33] Recently, the hydrogen bonding between interpolymer, interchain, and intermolecular has a very important effect on the formation of the uncertain aggregates, which has attracted more interest.[34] On the other hand, the results of our previous research indicated that the acid aqueous solution can decrease the hydrophilicity of PNIPAAM chain resulted from the destruction of the hydrogen bonding between the PNIPAAM chains and water molecules.[35] In current research, we believe that fabrication and controlled self-assembly of the water-soluble nonlinear MHBCs, possessing tunable phase transition behavior of PNIPAAM chain, pH-responsive, relatively low water solubility of PAA chain, and good biocompatibility of PNVP chain, would be much more interesting due to the complicated competition of interchain hydrogen bonding (hydrophilic chains and hydrophilic chains) and intermolecular hydrogen bonding (hydrophilic chains and water).

Most recently, an approach of combinations of various mature techniques, such as controlled free radical polymerization (CRP), ring-opening polymerization (ROP), click chemistry, and living anionic polymerization (LAP), have been successfully employed to synthesize the miktoarm star block copolymers.[36−39] Our previous results indicated that combination of single-electron-transfer living radical polymerization (SET-LRP) and reversible addition−fragmentation chain transfer (RAFT) polymerization techniques is a simple, efficient, and versatile strategy to prepare various kinds of miktoarm star smart copolymers.[40,41] Herein, we further reported the design and synthesis of the well-defined nonlinear multiresponsive MHBCs consisting of acid-responsive PAA, temperature-responsive PNIPAAM, and biocompatible and water-soluble PNVP chains via combination of SET-LRP and RAFT polymerization techniques. This polymer tends to self-assemble into three different types of micellar aggregates and unimers reversibly controlled by controlling pH value and temperature of aqueous solution.

EXPERIMENTAL SECTION

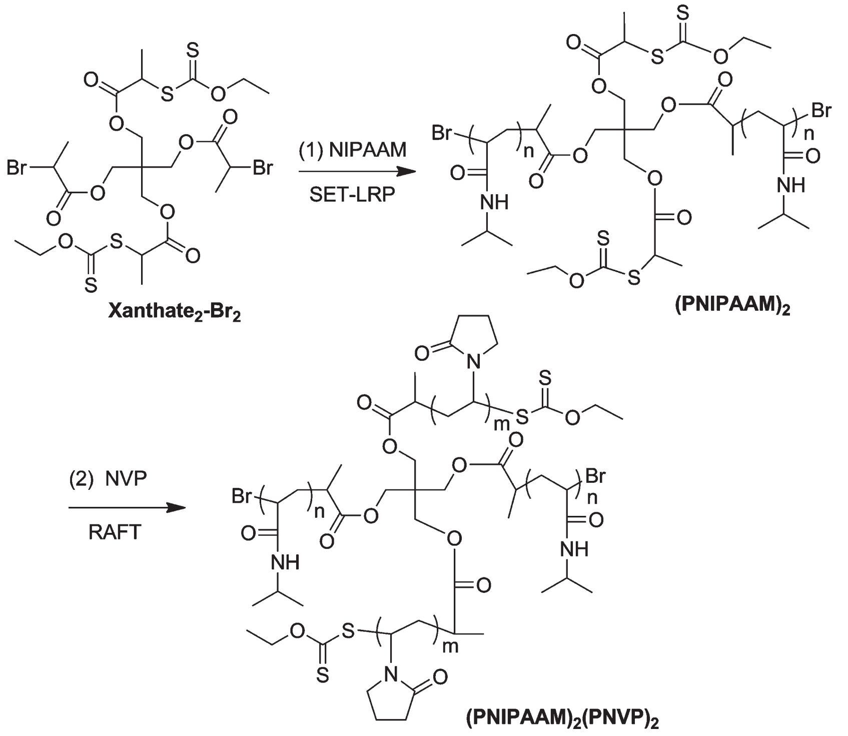
Materials. *N*-Isopropylacrylamide (NIPAAM, TCI Co., 97%) was purified by recrystallization from a mixture of toluene and hexane. *N*-Vinylpyrrolidone (NVP, Alfa Aesar Co., 99%) and *tert*-butyl acrylate (*t*BA, Alfa Aesar, 99%) were distilled under a reduced pressure and kept in a refrigerator at −15 °C for short-time store. 2,2′-Azobis(isobutyronitrile) (AIBN) (Shanghai Chemical Reagent Co. Ltd., China, 99%) was recrystallized three times from ethanol. *N,N,N′,N′′,N′′*-Pentamethyldiethylenetriamine (PMDETA) (Jiangsu Liyang Jiangdian Chemical Factory, Liyang, China, 98%) was dried with 4 Å molecular sieve and distilled in vacuum. Trifluoroacetic acid (Shanghai Chemical Reagent Co. Ltd., China, 99%), pentaerythritol (Shanghai Chemical Reagent Co. Ltd., China, 98%), potassium ethyl xanthate (Alfa Aesar Co., 98%), and copper powder (Cu(0), <75 μm, Aldrich, 98%) were used as received. Tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF), and acetone (analytical reagent, Shanghai Chemical Reagent Co. Ltd., China) were dried by 4 Å molecular sieve. The initiator (xanthate$_2$-Br$_2$) was synthesized according to our reported method.[41,42] Unless otherwise specified, all other chemicals were purchased from Shanghai Chemical Reagents Co. Ltd., China, and used as received without further purification.

Preparation of Two-Armed (PNIPAAM)$_2$ by SET-LRP. A representative example for the preparation of the two-armed poly(*N*-isopropylacrylamide) is described below. NIPAAM monomer (2 g, 17.68 mmol), xanthate$_2$-Br$_2$ (67.0 mg, 0.0884 mmol), Cu(0) (16.98 mg, 0.264 mmol), and acetone (4 mL) were placed in a dry glass ampule equipped with a magnetic stirring bar. The reaction mixture was bubbled with argon for ∼10 min to eliminate the oxygen. In succession, PMDETA (55.2 μL, 0.264 mmol) was added to the above mixture under an argon atmosphere. Then the ampule was flame-sealed under an argon atmosphere and placed in water bath at 25 °C. After 60 min, the ampule was opened, and the contents were diluted with 10 mL of THF. The solution was allowed to pass through neutral Al_2O_3 column to remove the copper salt and then precipitated into 600 mL petroleum ether solution. The resulting product was dried under vacuum at room temperature to a constant weight. This purification process was repeated twice. The conversion of the monomer was determined by gravimetry. The obtained two-armed polymer was denoted as (PNIPAAM)$_2$.

Synthesis of Nonlinear DHBCs (PNIPAAM)$_2$(PNVP)$_2$. The obtained two-armed poly(*N*-isopropylacrylamide) (PNIPAAM)$_2$ ($M_{n(GPC)}$ = 25 100 g mol^{-1}, $M_{n(th)}$ = 11 400 g mol^{-1}, M_w/M_n = 1.27) was selected as macro-RAFT agent to mediate the polymerization of *N*-vinylpyrrolidone (NVP) to prepare (PNIPAAM)$_2$(PVP)$_2$. A typical procedure of RAFT polymerization of NVP is as follows. NVP (2 g, 18.0 mmol), (PNIPAAM)$_2$ (1.026 g, 0.090 mmol), and AIBN (7.38 mg, 0.045 mmol) were dissolved in 3.0 mL of tetrahydrofuran (THF). The above solution was purged with argon for 10 min to eliminate the oxygen. Then the ampule was flame-sealed and placed in an oil bath held by a thermostat at 70 °C to carry out the polymerization. After 3 h, the ampule was cooled with ice−water and opened. The reaction mixture was diluted with 5 mL of THF and precipitated into an excess of petroleum ether (∼300 mL). The above dissolution−precipitation cycle was repeated twice. The polymer was obtained by filtration and dried at room temperature in vacuum to a constant weight. The conversion of polymerization was determined gravimetrically. The obtained nonlinear DHBCs was denoted as (PNIPAAM)$_2$(PVP)$_2$.

Preparation of Amphiphilic Miktoarm Star Terpolymer (PNIPAAM-*b*-P*t*BA)$_2$(PNVP)$_2$. The amphiphilic miktoarm star block copolymer, (PNIPAAM-*b*-P*t*BA)$_2$(PVP)$_2$, was synthesized by SET-LRP of *t*BA using (PNIPAAM)$_2$(PVP)$_2$ ($M_{n(GPC)}$ = 29 100 g mol^{-1}, $M_{n(NMR)}$ = 19 800 g mol^{-1}, M_w/M_n = 1.21) as macroinitiator. In a typical example, *t*BA monomer (1 mL, 6.95 mmol), (PNIPAAM)$_2$(PVP)$_2$ (0.68 g, 0.0347 mmol), Cu(0) (6.50 mg, 0.102 mmol), DMF (1 mL), and THF (1 mL) were placed in a dry glass ampule equipped with a magnetic stirring bar. The reaction mixture was bubbled with argon for ∼10 min to eliminate the oxygen. Then PMDETA (27.6 μL, 0.132 mmol) was added to the above mixture. The ampule was flame-sealed under argon atmosphere and placed in the water bath at 25 °C. After 4 h, the ampule was opened and the contents were diluted with 5 mL of THF, and the solution was allowed to pass through neutral Al_2O_3

Scheme 1. Synthetic Routes of Nonlinear DHBCs $(PNIPAAM)_2(PNVP)_2$



column to remove copper catalysts. After that, the solution was precipitated into 300 mL of petroleum ether. The above dissolution—precipitation cycle was repeated twice. The polymer was dried at room temperature in vacuum to a constant weight. The conversion of the monomer was determined by gravimetry. The obtained miktoarm star polymer was denoted as (PNIPAAM-*b*-P*t*BA)$_2$(PNVP)$_2$.

Hydrolysis of Miktoarm Star Terpolymer (PNIPAAM-*b*-P*t*BA)$_2$(PVP)$_2$. The miktoarm star block copolymer (PNIPAAM-*b*-P*t*BA)$_2$(PVP)$_2$ (1.0 g, $M_{n(GPC)}$ = 36 000 g mol^{-1}, $M_{n(NMR)}$ = 34 600 g mol^{-1}, M_w/M_n = 1.27) was dissolved in the mixture of dichloromethane (100 mL) and trifluoroacetic acid (3.0 g). The above solution was stirred for 1 day at room temperature. After evaporating all solvent, the hydrolyzed copolymer was dissolved in about 5 mL of THF and precipitated in a large amount of hexane (300 mL). The above dissolution—precipitation cycle was repeated twice. The final nonlinear MHBCs, (PNIPAAM-*b*-PAA)$_2$(PVP)$_2$, was dried at room temperature in vacuum to a constant weight.

Preparation of Nonlinear MHBCs (PNIPAAM)$_2$(PNVP-*b*-PAA)$_2$. The nonlinear MHBCs, (PNIPAAM)$_2$(PNVP-*b*-PAA)$_2$, was synthesized by RAFT polymerization of acrylic acid (AA) employing (PNIPAAM)$_2$(PVP)$_2$ ($M_{n(GPC)}$ = 29 100 g mol^{-1}, $M_{n(NMR)}$ = 19 800 g mol^{-1}, M_w/M_n = 1.21) as macro-RAFT agent. A detailed procedure is as follows. AA (0.5 g, 6.95 mmol), (PNIPAAM)$_2$(PVP)$_2$ (0.688 g, 0.0347 mmol), AIBN (5.7 mg, 0.0347 mmol), and methanol (1.5 mL) were carefully added to one dry ampule, which was purged with argon for 10 min to eliminate the oxygen. Polymerization was performed at 70 °C for 3 h. After repeated purification by dissolving in THF and precipitating into petroleum ether for two times, the resulting copolymer (PNIPAAM)$_2$(PNVP-*b*-PAA)$_2$ was obtained.

Preparation of the Micelle Solutions. The multiresponsive and multihydrophilic miktoarm star terpolymer (PNIPAAM)$_2$(PNVP-*b*-AA)$_2$ (5 mg, $M_{n(GPC)}$ = 28 100 g mol^{-1}, $M_{n(NMR)}$ = 26 300 g mol^{-1}, M_w/M_n = 1.22) was directly dissolved in deionized water (10 mL) or (50 mL) (pH = 10) at the room temperature under vigorous stirring. The pH values of solution were adjusted by the addition of the HCl or NaOH solution. The dust particles were removed by filtering the polymer solutions through 0.45 μm pore-size membrane filters (Millipore). The micelle solutions of (PNIPAAM-*b*-PAA)$_2$(PVP)$_2$ were prepared in a similar way.

Characterization. The molecular weights and molecular weight distributions of the polymers were measured on a Waters 1515 gel permeation chromatography (GPC) equipped with refractive index detector (RI 2414), using a 500 Å, 10 × 10^3 Å, 10 × 10^4 Å column with PS standard samples, and 0.05 mol L^{-1} lithium bromide solution in DMF was used as the eluent at a flow rate of 0.8 mL min^{-1} operated at 30 °C. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on an Inova nuclear magnetic resonance instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C) with chloroform ($CDCl_3$) or dimethyl-d_6 sulfoxide (DMSO-d_6) as the solvent and tetramethylsilane (TMS) as the internal standard at room temperature. The aggregate size analysis was performed using dynamic light scattering (DLS) measurements (Zetasizer Nano ZS: Malvern Instrument Ltd. UK) at different temperatures. Ultraviolet—visible (UV—vis) absorption spectra of micelle solutions in aqueous solutions were performed on a Shimadzu (Kyoto, Japan) UV-240. The LCST was defined as the temperature of the onset of decrease in transmittance. Transmission electron microscopy (TEM) was recorded on a Tecnai G^2-20 TEM at a 200 kV accelerating voltage.

■ RESULTS AND DISCUSSION

Preparation of Nonlinear DHBCs (PNIPAAM)$_2$(PNVP)$_2$. The general synthetic routes of (PNIPAAM)$_2$(PNVP)$_2$ are described in Scheme 1. First, the well-defined two-armed PNIPAAM ((PNIPAAM)$_2$) functionalized with xanthate groups was successfully prepared by SET-LRP employing multifunctional xanthate agent (xanthate$_2$-Br$_2$) as an initiator. The SET-LRP of NIPAAM was carried out in acetone at 25 °C with the

Table 1. Summary of Structural Parameters of Polymers Synthesized in This Work

entry	monomer conversion (%)	$M_{n(GPC)}$ (g mol^{-1})	$M_{n(th)}$ (g mol^{-1})	$M_{n(NMR)}$ (g mol^{-1})	M_w/M_n
$(PNIPAAM_{47})_2$	59.0	25 100	13 700	11 400	1.27
$(PNIPAAM_{47})_2(PNVP_{38})_2$	41.7	28 100	23 000	19 800	1.21
$(PNIPAAM_{47}$-*b*-$PtBA_{58})_2$-$(PNVP_{38})_2$	63.0	36 000	35 900	34 600	1.27
$(PNIPAAM_{47}$-*b*-$PAA_{58})_2$-$(PNVP_{38})_2$		30 300	28 900	28 100	1.23
$(PNIPAAM_{47})_2$-$(PNVP_{38}$-*b*-$PAA_{45})_2$	56.2	29 100	27 900	26 300	1.22

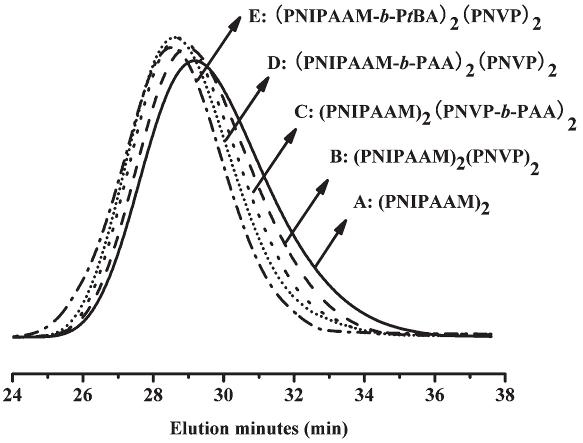


Figure 1. GPC traces recorded for two-armed $(PNIPAAM)_2$ (A), nonlinear DHBCs $(PNIPAAM)_2(PNVP)_2$ (B), nonlinear MHBCs $(PNIPAAM)_2$(PNVP-*b*-PAA$)_2$ (C), (PNIPAAM-*b*-PAA$)_2(PNVP)_2$ (D), and amphiphilic miktoarm star terpolymer (PNIPAAM-*b*-PtBA$)_2$-$(PNVP)_2$ (E).

ratio of NIPAAM/xanthate$_2$-Br$_2$/Cu(0)/PMDETA = 200/1/3/3. The polymerization results are summarized in Table 1. As presented in Table 1 (entry 1), the well-controlled $(PNIPAAM)_2$ ($M_{n(GPC)}$ = 25 100 g mol^{-1}, M_w/M_n = 1.27) was successfully obtained as evident by its sharp and symmetric shape of GPC elution peak (Figure 1A). However, the M_n value determined by GPC was only an apparent value because PS was used as calibration standards in GPC measurement. The actual M_n value of $(PNIPAAM)_2$ ($M_{n(NMR)}$ = 11 400 g mol^{-1}) was determined by the ^{1}H NMR spectrum (Figure S1). Our previous results confirmed that RAFT polymerization of NIPAAM (or *t*BA) did not occur during the SET-LRP of NIPAAM (or *t*BA) in the presence of the similar bromoxanthate iniferter.[40,41] Here, the polymerization behavior (Figures S2−S4) is similar to that of polymerization of NIPAAM with tetrafunctional bromoxanthate iniferter (xanthate-Br$_3$) as an initiator and copper(0)/PMDETA as the catalyst system. As shown in Figures S2−S4, the first kinetic plot, the linear increase of molecular weight of PNIPAAM with monomer conversion, and a monomodal and symmetric distribution of GPC curves of PNIPAAM, indicated that no obvious cross-linking was detected. The results confirmed that the RAFT agent group (xanthate) has little effect on the SET-LRP of NIPAAM due to the decreasing chance of chain transfer reaction at room temperature.

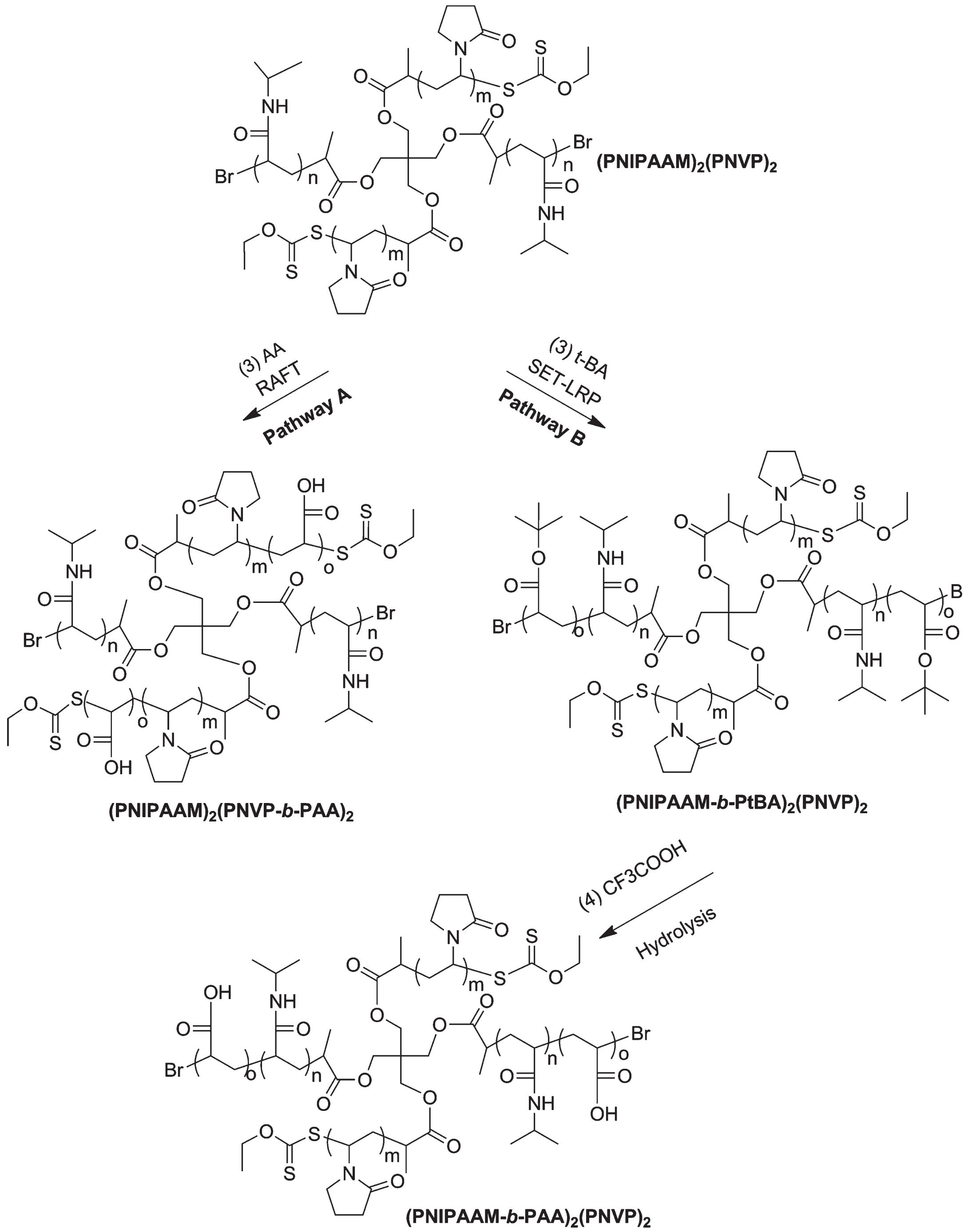
The double hydrophilic miktoarm star block copolymer $(PNIPAAM)_2(PNVP)_2$ was synthesized by the following RAFT polymerization of NVP using $(PNIPAAM)_2$ as the macro-RFAT agent. The results are listed in the Table 1 (entry 2) and Figure 1. The narrow molecular weight distribution (($M_{n(GPC)}$ = 28 100 g mol^{-1}, M_w/M_n = 1.21) and an unimodal and symmetrical GPC curve in Figure 1B indicated that the $(PNIPAAM)_2$ was an effective macro-RAFT agent for RAFT polymerization of NVP. The above results are further supported by ^{1}H NMR analysis (Figure S5), which clearly revealed the actual degrees of polymerization (DP) of NVP and the actual molecular weight of $(PNIPAAM_{47})_2(PNVP_{38})_2$ ($M_{n(NMR)}$ = 19 800 g mol^{-1}).

Synthesis of Nonlinear MHBCs, (PNIPAAM-*b*-PAA)$_2$-(PNVP)$_2$ and (PNIPAAM)$_2$(PNVP-*b*-PAA)$_2$. The multiresponsive nonlinear MHBCs, $(PNIPAAM)_2$(PNVP-*b*-PAA$)_2$, was prepared via RAFT chain extension experiment (Scheme 2, pathway A) using nonlinear $(PNIPAAM_{47})_2(PNVP_{38})_2$ as the macro-RAFT agent and acrylic acid (AA) as the monomer. As shown in Figure 2, the chemical shifts at δ = 3.8−4.2, 6.8−7.8, and 12.1−13.1 ppm are the characteristic peaks of the NIPAAM, NVP, and AA units, respectively. Based on the corresponding integration values of *b* and *c*, the molecular weight of $(PNIPAAM_{47})_2(PNVP_{38}$-*b*-$PAA_{45})_2$ ($M_{n(NMR)}$ = 26 300 g mol^{-1}) was calculated, which is close to the theoretical value ($M_{n(th)}$ = 27 900 g mol^{-1}). As evident from Figure 1C, the GPC elution peak of $(PNIPAAM_{47})_2(PNVP_{38}$-*b*-$PAA_{45})_2$ is quite symmetric and showed narrow molecular weight distribution (M_w/M_n = 1.22), which demonstrated that the original nonlinear $(PNIPAAM_{47})_2$-$(PNVP_{38})_2$ was still "living".

As shown in Scheme 2 (pathway B), the miktoarm star terpolymer (PNIPAAM-*b*-PtBA$)_2(PNVP)_2$ could be prepared via SET-LRP method employing $(PNIPAAM_{47})_2(PNVP_{38})_2$ as macroinitiator and *tert*-butyl acrylate (*t*BA) as the monomer. Subsequently, the target multiresponsive nonlinear MHBCs, (PNIPAAM-*b*-PAA$)_2(PNVP)_2$, could be obtained by selectively hydrolysis of the P*t*BA block. As shown in Figure 1B,E,D, all the polymers ($(PNIPAAM_{47})_2(PNVP_{38})_2$ (M_w/M_n = 1.21), (PNIPAAM-*b*-PtBA$)_2(PNVP)_2$ (M_w/M_n = 1.27), and ((PNIPAAM-*b*-PAA$)_2(PNVP)_2$) (M_w/M_n = 1.23) showed the narrow molecular distributions and quite symmetric GPC curves. ^{1}H NMR analysis (Figure S6) proved that the degree of the hydrolysis reaction was complete and the successful preparation of the targeted polymers with determined structures. The above results demonstrated that pathway B is an efficient way to prepare the well-designed $(PNIPAAM_{47}$-*b*-$PAA_{58})_2(PNVP_{38})_2$ ($M_{n(NMR)}$ = 28 100 g mol^{-1}, $M_w/M_n \leq 1.23$).

Optical Transmittance. PNIPAAM is one of the extensively studied thermoresponsive polymers; the balance between segment−segment interactions and segment−water intermolecular interactions can be shifted by temperature change.[25] PNIPAAM possessed reversible thermal phase separation above a critical temperature due to a lower critical solution temperature (LCST) at about 32 °C. The well-adjusted LCST of PNIPAAM offers new appealing opportunities for biomaterials. In this work, the LCST of aqueous solutions of the resulting nonlinear MHBCs, $(PNIPAAM_{47})_2(PNVP_{38}$-*b*-$PAA_{45})_2$ and $(PNIPAAM_{47}$-*b*-$PAA_{58})_2(PNVP_{38})_2$, were also investigated as presented in Figure 3. Figure 3 shows the temperature-dependent optical

Scheme 2. Synthetic Routes of Nonlinear MHBCs, $(PNIPAAM)_2(PNVP\text{-}b\text{-}PAA)_2$ (Pathway A) and $(PNIPAAM\text{-}b\text{-}PAA)_2(PNVP)_2$ (Pathway B)



transmittance at 700 nm obtained for 0.5 mg mL^{-1} micelle solution. For $(PNIPAAM_{47})_2(PNVP_{38}\text{-}b\text{-}PAA_{45})_2$, the transmittance of aqueous solutions remained almost constant below 34 °C; however, the transmittance of aqueous solutions slowly decreased with the temperature above 34 °C. It is known that the copolymer of NIPAAM containing hydrophilic unit would increase the LCST of PNIPAAM. So the relatively high LCST value of the nonlinear MHBCs, $(PNIPAAM_{47})_2(PNVP_{38}\text{-}b\text{-}PAA_{45})_2$ (34 °C), should be ascribed to the existence of hydrophilic segments of both PAA and PNVP chains. Compared with the $(PNIPAAM_{47})_2(PNVP_{38}\text{-}b\text{-}PAA_{45})_2$, the other nonlinear MHBCs, $(PNIPAAM_{47}\text{-}b\text{-}PAA_{58})_2(PNVP_{38})_2$, have possessed relatively high LCST (36 °C) due to the existence of relatively much PAA chains.

Poly(acrylic acid) exhibits good pH-sensitive water solubility properties.[26] When pH value is below 4.0, precipitation will occur in aqueous solutions due to protonations of the carboxylate groups, which renders the polymer sparsely soluble in water. In this case, pH-responsive optical transmittance at 700 nm obtained for 0.1 mg mL^{-1} micelle solution of the nonlinear MHBCs, $(PNIPAAM)_2(PNVP\text{-}b\text{-}PAA)_2$ and $(PNIPAAM\text{-}b\text{-}PAA)_2(PNVP)_2$,

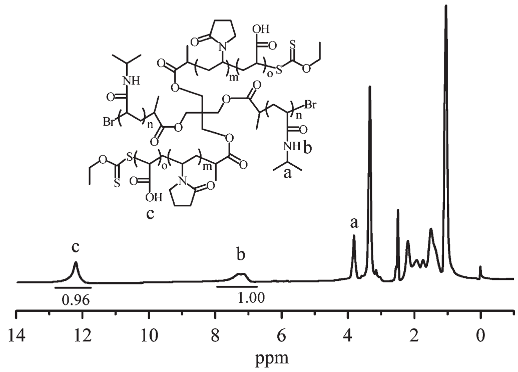


Figure 2. ^{1}H NMR spectrum of nonlinear (PNIPAAM)$_2$(PNVP-*b*-PAA)$_2$ ($M_{n(GPC)}$ = 28 100 g mol^{-1}, M_w/M_n = 1.23) in DMSO-d_6 with tetramethylsilane (TMS) as the internal standard.

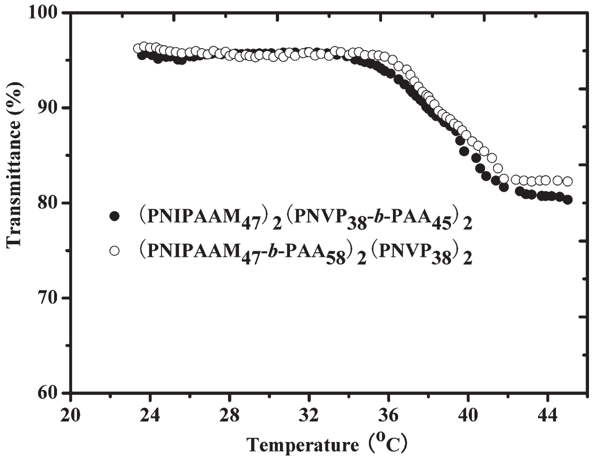


Figure 3. Transmittance versus temperature plots for nonlinear MHBCs, (PNIPAAM)$_2$(PNVP-*b*-PAA)$_2$ and (PNIPAAM-*b*-PAA)$_2$(PNVP)$_2$, in aqueous solution. The copolymer concentration is 0.5 mg mL^{-1}.

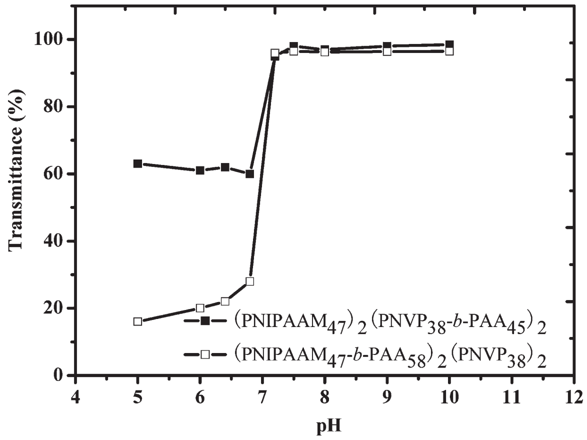


Figure 4. Transmittance versus temperature plots for nonlinear MHBCs, (PNIPAAM$_{47}$)$_2$(PNVP$_{38}$-*b*-PAA$_{45}$) and (PNIPAAM$_{47}$-*b*-PAA$_{58}$)$_2$-(PNVP$_{38}$)$_2$, in aqueous solution. The concentration is 0.1 mg mL^{-1}.

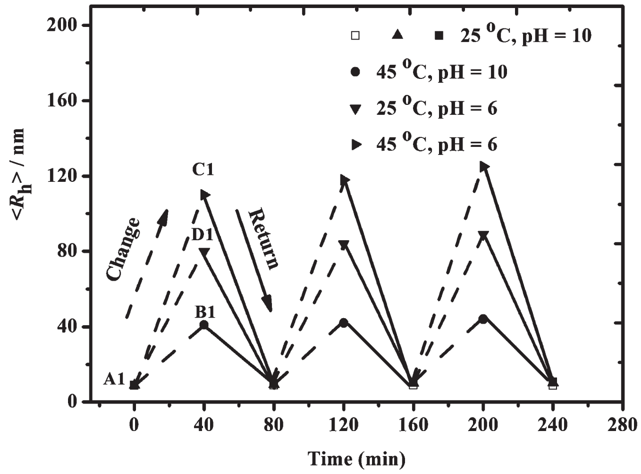


Figure 5. Temperature-induced and pH-induced reversible association of the nonlinear MHBCs (PNIPAAM$_{47}$)$_2$(PNVP$_{38}$-*b*-PAA$_{45}$) measured by DLS upon cycling between 25 and 45 °C or pH = 10 and pH = 6 at 40 min intervals. The copolymer concentration is 0.1 mg mL^{-1}.

were observed (Figure 4). From Figure 4, we can see that pH = 7 is an inflection point value. In case of pH > 7, PAA chain is water-soluble and the transmittance is close to 100%. On the contrary, the micelle solution immediately become turbid for (PNIPAAM$_{47}$)$_2$(PNVP$_{38}$-*b*-PAA$_{45}$), and the transmittance of micellar solution is near 63% in the case of pH < 7. However, compared with the polymer (PNIPAAM$_{47}$)$_2$(PNVP$_{38}$-*b*-PAA$_{45}$), the PAA content is much higher in (PNIPAAM$_{47}$-*b*-PAA$_{58}$)$_2$(PNVP$_{38}$)$_2$, and interchain hydrogen bonding between the amide groups in PNIPAAM chain and carboxyl groups in PAA chains,[4] or the carboxyl groups in PAA chains and the carbonyl groups in PVP chains,[43,44] increased significantly, which resulted in the rapid precipitating at relatively low concentration in the case of pH < 7. Moreover, it should be pointed out that the precipitation phenomenon of (PNIPAAM$_{47}$)$_2$(PNVP$_{38}$-*b*-PAA$_{45}$) solution will be observed at relatively high concentration due to the much stronger interpolymer, interchain, and intermolecular interaction. A similar phenomenon can be observed in poly(acrylic acid)-*g*-poly(ethylene oxide) system.[34]

Aggregation Behavior of the Nonlinear Multiresponsive MHBCs, (PNIPAAM)$_2$(PNVP-*b*-PAA)$_2$ and (PNIPAAM-*b*-PAA)$_2$-(PNVP)$_2$. The purpose of our design is to provide well-controlled smart biomaterials triggered by environmental stimulis. It is quite expected that these novel nonlinear MHBCs containing pH-responsive PAA chain, thermoresponsive PNIPAAM chain, and water-soluble PNVP chain can show multiresponsive "schizophrenic" aggregation behaviors. By tuning the temperature or pH value of the aqueous solution of (PNIPAAM$_{47}$)$_2$(PNVP$_{38}$-*b*-PAA$_{45}$) (balance of the hydrophilic/hydrophobic), three distinct types of micellar aggregates and unimer state can be reversible obtained. Here, four characteristic solution conditions (A1: 25 °C, pH = 10; B1: 45 °C, pH = 10; C1: 45 °C, pH = 6; D1: 25 °C, pH = 6) were selected to investigate the behaviors of micellar aggregates. The DLS measurement was employed to monitor the "schizophrenic" aggregation behaviors. In the experiment, the hydrodynamic radius (R_h) of aggregates increased with increasing of the temperature, or decreasing of pH value, or both increasing of the temperature and decreasing of pH value. Furthermore, the similar size of hydrodynamic radius can be reobtained by reversibly returning to initial state after 40 min intervals. So three symbols (□, ▲, ■) in Figure 5 were overlapped. As shown in Figure 5 (A1), at 25 °C and pH = 10, PNIPAAM, PNVP, and PAA chain are hydrophilic; thus, miktoarm copolymers (PNIPAAM$_{47}$)$_2$-(PNVP$_{38}$-*b*-PAA$_{45}$)$_2$ molecularly dissolves in aqueous solution as a unimer, the average hydrodynamic radius is 8.7 nm, and the solution is

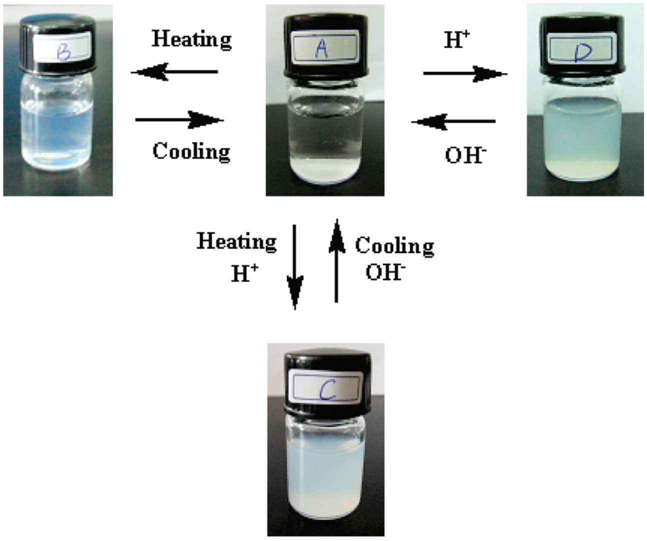
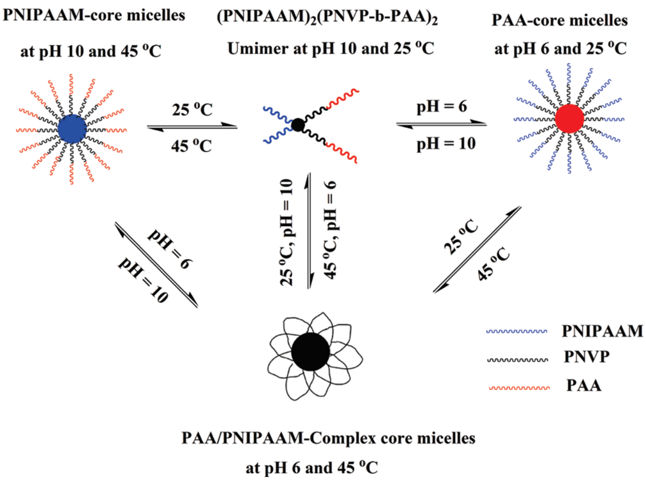


Figure 6. Photos of the original micelle solutions (A: 25 °C, pH = 10; B: 45 °C, pH = 10; C: 45 °C, pH = 6; D: 25 °C, pH = 6). The copolymer concentration is 0.1 mg mL^{-1}.

Scheme 3. Schematic Illustration of Multiresponsive "Schizophrenic" Micellization Behaviors of the Nonlinear MHBCs, $(PNIPAAM)_2(PNVP\text{-}b\text{-}PAA)_2$, in Aqueous Solution



transparent. Keeping pH value of the solution constant, when the temperature of the solution increased to 45 °C (B1, 45 °C, pH = 10), both PNVP and PAA chain are hydrophilic, but PNIPAAM chain is insoluble in water; the miktoarm copolymers $(PNIPAAM_{47})_2$-$(PNVP_{38}$-b-$PAA_{45})_2$ can self-assembly into micelles with PNIPAAM-core and PNVP/PAA-corona. The aggregate sizes increased to 42 nm, and the micellar solution became light blue and slightly turbid. At 25 °C and pH = 6 (keeping the temperature constant, decreasing pH value) (Figure 5, D1), both PNVP and PNIPAAM chains are in soluble states; however, PAA block is insoluble in water, and the miktoarm copolymers $(PNIPAAM_{47})_2(PNVP_{38}$-$b$-$PAA_{45})_2$ can aggregate into micelles with a PAA-core and PNIPAAM/PNVP-corona. The sizes of aggregates increased to 84 nm, and the micelle solution became turbid. When both tuning the temperature and pH values (45 °C and pH = 6, Figure 5, C1), the new aggregates composed of a hydrophobic core (PNIAAM and PAA) and a hydrophilic corona (PNVP) were successfully formed with 116 nm along with the more turbid micelle solution. In addition, the results of TEM (Figure S7) also indicated that the size and the sphere-shaped morphology can be well controlled by precisely adjusting the solution condition. It should be noted that three types of aggregates formed at different temperature and pH values were completely reversible, and the repeat experiments were measured at different time as shown in Figure 5 (80−160 and 160−240 min). The tuning of external conditions can reversibly switch the three types of aggregates and the unimer states, and every micelle corona all possesses the biocompatible PNVP chain. We believe that this smart polymer is well suited for potential applications in biology and drug delivery area. Figure 6 and Scheme 3 dramatically illustrated the process of the multiresponsive "schizophrenic" micellization behaviors. Interestingly, the similarly revisible phase transfer between unimer, micellar aggregates, and precipitation was also observed in the case of $(PNIPAAM_{47}$-b-$PAA_{58})_2(PNVP_{38})_2$ (Figure S8). Similarly, PNIPAAM, PNVP, and PAA chain are water-soluble, and the unimer can be obtained at 25 °C and pH = 10. When increasing the temperature of the solution to 45 °C (pH = 10), the thermo-tunable PNIPAAM-core micelles could be observed. However, when adjusting the pH value to 6 (45 °C), the precipitation appeared. The reason may be ascribed to the different polymer structure and the different hydrogen bonding interactions between the amide groups in PNIPAAM chain and carboxyl groups in PAA chains or the carboxyl groups in PAA chains and the carbonyl groups in PVP chains.

■ CONCLUSIONS

In summary, the novel water-soluble stimuli-responsive nonlinear MHBCs, $(PNIPAAM)_2(PNVP$-b-$PAA)_2$ and $(PNIPAAM$-b-$PAA)_2(PNVP)_2$, were successfully prepared via a combination of SET-LRP and RAFT polymerization techniques, respectively. By precisely tuning pH values and temperatures of aqueous solution of these two copolymers, three types of micellar aggregates and the unimer state can undergo reversible switch on and off in size and morphology. Moreover, hydrogen bonding interactions have a great effect on the water solubility of PAA chain.

■ ASSOCIATED CONTENT

Ⓢ **Supporting Information.** ^{1}H NMR spectra of $(PNIPAAM)_2$, $(PNIPAAM)_2(PNVP)_2$, $(PNIPAAM$-b-$PtBA)_2$-$(PNVP)_2$, $(PNIPAAM$-b-$PAA)_2(PNVP)_2$, photos of the original micellar solution of $(PNIPAAM_{47}$-b-$PAA_{58})_2(PNVP_{38})_2$ at different temperature and pH values, results of SET-RAFT of NIPAAM, and TEM images of the nonlinear MHBCs, $(PNIPAAM_{47})_2(PNVP_{38}$-$b$-$PAA_{45})$. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Fax 86-512-65112796; e-mail xlzhu@suda.edu.cn.

Author Contributions

[§]Weidong Zhang and Wei Zhang contributed equally to this paper.

■ ACKNOWLEDGMENT

The financial support from the National Natural Science Foundation of China (Nos. 20874069, 50803044, 20974071, 20904036, 21074080, and 21074082), the Specialized Research

Fund for the Doctoral Program of Higher Education contract grant (Nos. 200802850005 and 20103201110005), the Qing Lan Project, the Program of Innovative Research Team of Soochow University, and a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) is gratefully acknowledged.

■ REFERENCES

(1) Rao, J. Y.; Luo, Z. F.; Ge, Z. S.; Liu, H.; Liu, S. Y. *Biomacromolecules* **2007**, *8*, 3871–3878.

(2) Cai, Y. L.; Tang, Y, Q.; Armes, S. P. *Macromolecules* **2004**, *37*, 9728–9737.

(3) Li, J. G.; Wang, T.; Wu, D. L.; Zhang, X. Q.; Yan, J. T.; Du, S.; Guo, Y. F.; Wang, J. T.; Zhang, A. *Biomacromolecules* **2008**, *9*, 2670–2676.

(4) Schilli, C. M.; Zhang, M. F.; Rizzardo, E.; Thang, S. H.; Chong, Y. K.; Edwards, K.; Karlsson, G.; Muller, A. H. E. *Macromolecules* **2004**, *37*, 7861–7866.

(5) Andre, X.; Zhang, M. F.; Muller, A. H. E. *Macromol. Rapid Commun.* **2005**, *26*, 558–563.

(6) Zhang, Y. F.; Liu, H.; Hu, J. M.; Li, C. H.; Liu, S. Y. *Macromol. Rapid Commun.* **2009**, *30*, 941–947.

(7) Liu, H.; Li, C. H.; Liu, H. W.; Liu, S. Y. *Langmuir* **2009**, *25*, 4724–4734.

(8) Ge, Z. S.; Liu, S. Y. *Macromol. Rapid Commun.* **2009**, *30*, 1523–1532.

(9) Ge, Z. S.; Cai, Y. L.; Zhu, Z. Y.; Rao, J. Y.; Liu, S. Y. *Langmuir* **2007**, *23*, 1114–1122.

(10) Dimitrov, I.; Trzebick, B.; Muller, A. H. E.; Dworak, A.; Tsvetanov, C. B. *Prog. Polym. Sci.* **2007**, *32*, 1275–1343.

(11) Rodriguez-Hernandez, J.; Checot, F.; Gnanou, Y.; Lecommandoux, S. *Prog. Polym. Sci.* **2005**, *30*, 691–724.

(12) Gohy, J. F. *Adv. Polym. Sci.* **2005**, *190*, 65–136.

(13) Riess, G. *Prog. Polym. Sci.* **2003**, *28*, 1107–1170.

(14) Gil, E. S.; Hudson, S. M. *Prog. Polym. Sci.* **2004**, *29*, 1173–1222.

(15) Thurmond, K. B.; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **1997**, *119*, 6656–6665.

(16) Cameron, N. S.; Corbierre, M. K.; Eisenberg, A. *Can. J. Chem.* **1999**, *77*, 1311–1326.

(17) Zhu, Z. Y.; Armes, S. P.; Liu, S. Y. *Macromolecules* **2005**, *38*, 9803–9812.

(18) Wang, D.; Yin, J.; Zhu, Z. Y.; Ge, Z. S.; Liu, H. W.; Armes, S. P.; Liu, S. Y. *Macromolecules* **2006**, *39*, 7378–7385.

(19) Zhu, Z. Y.; Xu, J.; Zhou, Y. M.; Jiang, X. Z.; Armes, S. P.; Liu, S. Y. *Macromolecules* **2007**, *40*, 6393–6398.

(20) Hadjichristidis, N.; Iatrou, H.; Pitsikalis, M.; Pispas, S.; Avgeropoulos, A. *Prog. Polym. Sci.* **2005**, *30*, 725–782.

(21) Hadjichristidis, N.; Pispas, S. *Adv. Polym. Sci.* **2006**, *200*, 37–55.

(22) Cai, Y. L.; Burguiere, C.; Armes, S. P. *Chem. Commun.* **2004**, 802–803.

(23) Nguyen, P. M.; Hammond, P. T. *Langmuir* **2006**, *22*, 7825–7832.

(24) Xu, J.; Ge, Z. S.; Zhu, Z. Y.; Luo, S. Z.; Liu, H. W.; Liu, S. Y. *Macromolecules* **2006**, *39*, 8178–8185.

(25) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163–249.

(26) Needleman, I. G.; Smales, F. C. *Biomaterials* **1995**, *16*, 617–624.

(27) Luo, L.; Ranger, M.; Lessard, D. G.; Garrec, D. L.; Gori, S.; Leroux, J. C.; Rimmer, S.; Smith, D. *Macromolecules* **2004**, *37*, 4008–4013.

(28) Dsouza, A. J. M.; Schowen, R. L.; Topp, E. M. *J. Controlled Release* **2004**, *94*, 91–100.

(29) Lou, X.; Panaro, N. J.; Wilding, P.; Fortina, P.; Kricka, L. J. *Biotechniques* **2004**, *36*, 248–252.

(30) Wan, D. C.; Satoh, K.; Kamigaito, M.; Okamoto, Y. *Macromolecules* **2005**, *38*, 10397–10405.

(31) Postma, A.; Davis, T. P.; Evans, R. A.; Li, G.; Moad, G.; OShea, M. *Macromolecules* **2006**, *39*, 5293–5306.

(32) Nguyen, L. T. U.; Eagles, K.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 4372–4383.

(33) McDowall, L.; Chen, G. J.; Stenzel, M. H. *Macromol. Rapid Commun.* **2008**, *29*, 1666–1671.

(34) Hao, J. K.; Yuan, G. C.; He, W. D.; Cheng, H.; Han, C. C.; Wu, C. *Macromolecules* **2010**, *43*, 2002–2008.

(35) Zhang, W. D.; Zhang, W.; Zhou, N. C.; Zhu, J.; Cheng, Z. P.; Zhu, X. L. *J. Polym. Sci.; Part A: Polym. Chem.* **2009**, *47*, 6304–6315.

(36) Luan, B.; Zhang, B. Q.; Pan, C. Y. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 549–560.

(37) Durmaz, H.; Karatas, F.; Tunca, U.; Hizal, G. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 499–509.

(38) Glaied, O.; Delaite, C.; Dumas, P. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 1159–1169.

(39) Zhu, J.; Zhu, X. L.; Kang, E. T.; Neoh, K. G. *Polymer* **2007**, *48*, 6992–6999.

(40) Zhang, W. D.; Zhang, W.; Zhu, J.; Zhang, Z. B.; Zhu, X. L. *J. Polym. Sci.; Part A: Polym. Chem.* **2009**, *47*, 6908–6918.

(41) Zhang, W. D.; Zhang, W.; Zhang, Z. B.; Cheng, Z. P.; Tu, Y. F.; Qiu, Y. S.; Zhu, X. L. *J. Polym. Sci., Part A: Polym. Chem.* **2010**, *48*, 4268–4278.

(42) Qiu, Y. S.; Zhang, W.; Yan, Y. F.; Zhu, J.; Zhang, Z. B.; Zhu, X. L. *J. Polym. Sci., Part A: Polym. Chem.* **2010**, *48*, 5180–5188.

(43) Lau, C. L.; Mi, Y. L. *Polymer* **2002**, *43*, 823–929.

(44) Chun, M. K.; Cho, C. S.; Choi, H. K. *J. Controlled Release* **2002**, *81*, 327–334.