Macromolecules
ARTICLE

Macromolecules **2010**, *43*, 5679–5687 **5679**
DOI: 10.1021/ma100894p

Thermo- and pH-Responsive Association Behavior of Dual Hydrophilic Graft Chitosan Terpolymer Synthesized via ATRP and Click Chemistry

Hongqian Bao,[†] Lin Li,*[,†] Leong Huat Gan,[‡] Yuan Ping,[§] Jun Li,[§] and Palaniswamy Ravi[⊥]

[†]*School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798,* [‡]*Natural Sciences and Science Education, National Institute of Education, Nanyang Technological University, 1 Nanyang Walk, Singapore 637616,* [§]*Division of Bioengineering, Faculty of Engineering, National University of Singapore, 7 Engineering Drive 1, Singapore 117574, and* [⊥]*Innovation Centre, 3M Asia Pacific Pte. Ltd., 100 Woodlands Avenue 7, Singapore 738205*

Received April 23, 2010; Revised Manuscript Received May 26, 2010

ABSTRACT: A comb-like dual hydrophilic graft chitosan terpolymer, chitosan grafted with both poly[(2-dimethylamino)ethyl methacrylate] and poly(*N*-isopropylacrylamide) or, simply, CS(-*g*-PDMAEMA)-*g*-PNIPAM, was synthesized by means of atom transfer radical polymerization (ATRP) and click chemistry. At first, PDMAEMA and PNIPAM were synthesized via ATRP, followed by substituting the halide end groups with azido groups. After converting CS into *alkynyl*-CS via amidation, PDMAEMA-N_3 and PNIPAM-N_3 side chains were successfully grafted onto the CS backbone via click reaction, leading to the well-defined graft terpolymer. Thermo- and pH-responsive micellization behavior of the graft terpolymer in aqueous solutions was investigated by proton nuclear magnetic resonance (^{1}H NMR), laser light scattering (LLS), surface tensiometry, zeta potential, and transmission electron microscopy (TEM). The core−shell structured micelles with PNIAPM as a core and CS/PDMAEMA as a shell were formed in acidic environment (pH < 4) at elevated temperature (> 38 °C), whereas the unimers turned into the micelles with CS/PDMAEMA cores in alkaline solutions (pH > 7) at room temperature.

Introduction

Chitosan (CS), a naturally occurring linear cationic polysaccharide, has gained a great deal of attention in the past decades because of its applications in drug and gene delivery,[1,2] in tissue engineering,[3,4] and as a pharmaceutical ingredient.[5] Recent interest has turned to enhancing the properties of a parent polymer by chemical modification to obtain new derivatives for specific applications.[6,7] Grafting of CS is an efficient way to improve properties of CS such as chelating or complexation,[8] bacteriostatic effect,[9] or biosorption.[10] Although grafting of CS modifies its properties, some important intrinsic characteristics such as biocompatibility, biodegradability, and mucoadhesivity are still maintained as shown in many investigations.[11−13]

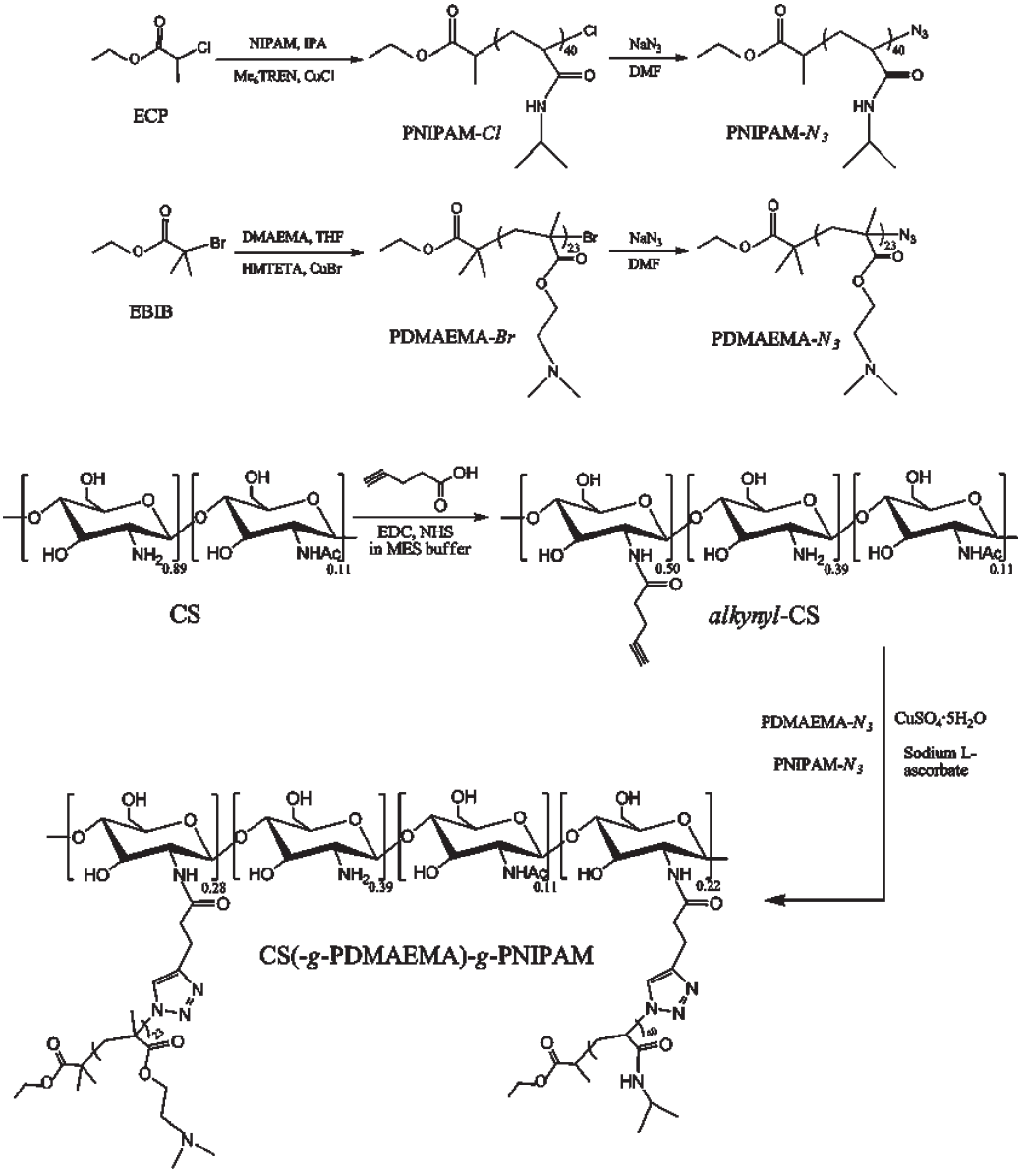
Graft copolymerization of vinyl monomers onto CS and other polysaccharides using atom transfer radical polymerization (ATRP)[14,15] have recently attracted wide interests. The use of a "living" polymerization technique has led to better control of the formation of graft compolymers with well-defined structures, thus benefiting understanding of structure−property relationships. For example, methoxy-capped (PEG 350) methacrylate was polymerized from a CS macroinitiator that was prepared by modifying CS with 2-bromoisobutylryl bromide (2-BIB).[16] Polyacrylamide and polystyrene were successfully grafted to CS particles using heterogeneously surface-initiated ATRP.[17,18] However, most of reported polymerizations took place heterogeneously due to the poor solubility of CS in traditional ATRP solvents, which resulted in a low degree of substitution (DS). Other kinds of polysaccharides, such as cellulose, pullulan, dextran and β-cyclodextrin (β-CD) have also been modified via ATRP.[19−22] Sui et al. recently reported that the homogeneous graft polymerization via ATRP from cellulose was accomplished by preparing a macroinitiator in an ionic liquid,[22] but an ionic liquid suitable for modification of CS via ATRP still remains unavailable. A "grafting onto" approach seems more promising under such a background and several water-soluble graft CS copolymers have been synthesized using different coupling techniques.[23,24] In particular, the active ester conjugation method is of great vitality and has triggered more and more applications to synthesize graft CS copolymers. When combining with an ATRP method, the advantage of using an active ester conjugation method that one precursor can lead to many functional polymers varying in chemical functionality will be achieved. Under this circumstances, we have successfully synthesized a pH-sensitive PDMAEMA grafted CS copolymer and evaluated its special association behavior.[25] Interestingly, Munro et al. also reported the synthesis of CS-*g*-poly(oligoethylene glycol methacrylate) (OEGMA) copolymers using ATRP and active esters, and concluded that the "grafting onto" synthetic route was preferable to the "grafting from" route due to the higher purity and more defined structure.[26]

In the past few years, "click chemistry", as termed by Sharpless et al.,[27−29] has been proven to be a versatile method to boost the development of polymer chemistry due to its high specificity, nearly quantitative yield, compatibility to a variety of functional groups, solvent insensitivity (also highly active in water), and applicability under mild conditions. More recently, the combination of click reactions with ATRP has aroused enormous interest and generated hundreds of research papers.[30−33] In principle, three main strategies, which were summarized by Binder and Sachsenhofer, can be applied: the initiator approach, the Br^-/N_3^- approach and side-chain modifications.[33] Especially for the last route, well-defined brush copolymers with a high grafting efficiency have been synthesized by coupling ATRP "grafting from" and click "grafting onto" techniques.[34,35] There are already a few publications utilizing click chemistry in CS modifications, but further combination with ATRP still remains blank. Kulbokaite et al. synthesized CS-methoxy poly(ethylene glycol) (MPEG) derivatives containing

*Corresponding author. E-mail: mlli@ntu.edu.sg.

Published on Web 06/04/2010

Scheme 1. Synthetic Route for Preparation of CS(-*g*-PDMAEMA)-*g*-PNIPAM Graft Terpolymer



triazolyl moiety via 1,3-dipolar cycloaddition between pendant azide and end alkyne groups of CS and MPEG, respectively.[36] By introducing a cationic group on CS, Gao et al. obtained water-soluble 6-*N,N,N*-trimethyltriazole-CS (TCS) that showed strong DNA binding ability and high protection of DNA against nuclease degradation.[37] In a more detailed manner, Lallana et al. achieved the functionalizations of CS-*g*-PEG-N_3 via Cu(I)-catalyzed and strain-promoted azide−alkne [3 + 2] cycloadditions (CuAAC and SPAAC), respectively.[38]

Dual hydrophilic copolymers, which consist of two different hydrophilic blocks, can self-assemble into one or more types of micelles in aqueous media, when the hydrophilicity of one block changes differently from other blocks, corresponding to a change in temperature, pH, or ionic strength, and this kind of association behavior was often metaphorized into "schizophrenic" micellizaition.[39−41] It has also been found that the architecture of a dual hydrophilic copolymer can play an important role in determining its association behavior.[42,43] Some novel dual hydrophilic copolymers with different architectures have been developed and their association properties were investigated.[44−48] Liu and co-workers have made significant contributions in this area. Specifically, they synthesized the linear and miktoarm star diblock copolymers poly(*N*-isopropylacrylamide)-*b*-poly[(2-diethylamino)ethyl methacrylate] (PNIPAM-*b*-PDEAEMA and PNIPAM-*b*-PDEAEMA$_4$),[44] the miktoarm star terpolymer PEO(-*b*-PDEAEMA)-*b*-PNIPAM,[45] and the Janus-type heteroarm star copolymer PDEAEMA-*CD*-PNIPAM.[46] They also fabricated the temperature- and pH-responsive four-layered nanoparticles from a poly[oligo(ethylene glycol) monomethyl ether methacrylate]-*b*-poly[(2-dimethylamino)-ethyl methacrylate]-*b*-poly[(2-diethylamino)ethyl methacrylate] (POEGMA-*b*-PDMAEMA-*b*-PDEAEMA) triblock copolymer and PNIPAM.[47] Feng et al. recently synthesized a well-defined graft copolymer, poly(*N*-isopropylacrylamide)-*b*-[poly(ethyl acrylate)-*g*-poly(2-vinylpyridine)] [PNIPAM-*b*-(PEA-*g*-P2VP)] and its micellization behavior was studied using ^{1}H NMR, fluorescence spectroscopy, dynamic light scattering and transmission electron microscopy.[48]

In this paper, we report the synthesis of a comb-like graft CS copolymer via ATRP and click chemistry (the synthesis route is described in Scheme 1), and its association behavior in aqueous solutions. To the best of our knowledge, this work represents the first time of synthesis of a dual hydrophilic graft CS terpolymer

and study of its thermo- and pH-responsive association behaviors. By self-assembling into nanospheres or micelles, graft CS copolymers are finding extensive applications in the field of biomedical science and engineering.[49,50] This unique dual hydrophilic graft CS copolymer is believed to have a great potential in application for gene/drug delivery and controlled release.

Experimental Section

Materials. Chitoan (M_n = 10 kDa) with ~89% degree of deacetylation (DD, determined by ^{1}H NMR) was obtained from Haidebei Marline Bioengineering Co., Ltd., China. It was purified according to the method reported in the literature.[51,52] *N*-Isopropylacrylamide (NIPAM, 97%, Aldrich) was recrystallized from a mixture of *n*-hexane and benzene (3:1, v/v). 2-(Dimethylamino)ethyl methacrylate (DMAEMA, 98%, Aldrich) was purified by passing through a column filled with activated basic alumina and distilled under calcium hydride (CaH_2) to remove inhibitors. Tetrahydrofuran (THF), isopropanol (IPA) and *N*,*N*′-dimethylformamide (DMF) were dried and distilled prior to use. Ethyl 2-chloropropionate (ECP, 97%), Ethyl 2-bromoisobutyrate (EBIB, 98%), 1,1,4,7,10,10-hexamethyl triethylenetetramine (HMTETA, 97%), copper(I) bromide (CuBr, 99.999%), copper(I) chloride (CuCl, 99.995%), sodium azide (NaN_3, 99%), 2-(*N*-morpholino)ethanesulfonic acid (MES, 99%), sodium L-ascorbate (98%), copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, 98%), 4-pentynoic acid (95%), *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl, 99%), *N*-hydroxysuccinimide (NHS, 97%) were purchased from Aldrich and used as received. Tris(2-(dimethlyamino)ethyl)amine (Me_6TREN, 98%) was obtained from ATRP Solutions Inc. and directly used.

Synthesis of Azido-Terminated PDMAEMA Homopolymer (PDMAEMA-N_3). The typical synthesis of linear PDMAEMA capped with an azide group is described as follows. A 25 mL Schlenk flask equipped with a magnetic stirrer was connected to a vacuum line, and the moisture was removed by heating under vacuum. When the flask was cooled down, dry argon was filled in. Under an argon atmosphere, DMAEMA (2.52 mL, 15 mmol), THF (2.5 mL), and CuBr (0.072 g, 0.5 mmol) were charged into the flask that was tightly sealed with a rubber septum. The reaction mixture was degassed by three freeze−vacuum−thaw cycles. Thereafter, HMTETA (0.27 mL, 1.0 mmol) was injected into the system at room temperature and further degassed by three freeze-vacuum-thaw cycles. Using an argon purged syringe, the initiator EBIB (0.074 mL, 0.5 mmol) was introduced into the flask to start the ATRP. The reaction was allowed to proceed with constant stirring at 40 °C for 6 h. The polymerization was then terminated and the solution was passed through a basic alumina column and eluted with additional 100 mL of THF. After being condensed in a rotary evaporator, the solution was poured into excess amounts of cold hexane to precipitate the polymer. The above dissolution−precipitation cycle was repeated for two times to remove the residual monomer. The final viscous product was isolated and dried under vacuum. The yield was equivalent to 76% conversion of the monomer (1.79 g), $M_{n,GPC}$ = 4600 g/mol and M_w/M_n = 1.22. The actual degree of polymerization (DP) of PDMAEMA was obtained from the relative integration of characteristic protons of chain ends to protons of main chains by ^{1}H NMR analysis. Thus, the homopolymer obtained was denoted as $PDMAEMA_{23}$-*Br*.

The resulting homopolymer $PDMAEMA_{23}$-*Br* (1.72 g, 0.45 mmol) and NaN_3 (0.058 g, 0.9 mmol) were dissolved into 25 mL DMF in a 50 mL round-bottomed flask. The mixture was allowed to react under stirring at room temperature for 24 h. After removing the DMF solvent under vacuum, the residue was redissolved in 50 mL THF and passed through a basic alumina column to remove sodium salt and excess NaN_3. The filtrate, after condensed to a volume of ~2 mL by rotary evaporation, was precipitated in 50 mL cold hexane. The precipitate was filtered, washed by hexane and vacuum-dried. The yield was 96% (1.65 g), $M_{n,GPC}$ = 4600 g/mol and M_w/M_n = 1.22.

Synthesis of Azido-Terminated PNIPAM Homopolymer (PNIPAM-N_3). The experiment procedure of ATRP for the synthesis of PNIPAM-N_3 was similar to the preparation of PDMAEMA. A typical mixture containing CuCl (0.05 g, 0.5 mmol), NIPAM (2.82 g, 25 mmol), degassed IPA (7.2 mL), Me_6TREN (0.116 g, 0.5 mmol), and ECP (0.064 mL, 0.5 mmol) reacted at room temperature for 10 h. The flask was quenched into liquid nitrogen to terminate the polymerization and exposed to air. After most of the IPA was evaporated under a reduced pressure, the residue was diluted with THF and passed through a neutral alumina column to remove the copper catalysts. Then, the solution was concentrated into around 3 mL and PNIPAM-*Cl* was precipitated against an excess of diethyl ether. The obtained solid was filtered, washed and dried under vacuum to give a yield of 78% (2.2 g). The GPC analysis revealed M_n = 5600 g/mol and M_w/M_n = 1.12. The actual DP of the PNIPAM block was determined to be 40 by ^{1}H NMR analysis in D_2O (comparing the peak area for NIPAM isopropyl at 3.9 ppm with that for the ethoxy at 4.1 ppm). The homopolymer obtained was denoted as $PNIPAM_{40}$-*Cl*.

In order to prepare the azido-terminated PNIPAM homopolymer, the resulting $PNIPAM_{40}$-*Cl* (2.12 g, 0.47 mmol) and NaN_3 (0.062 g, 0.94 mmol) were dissolved into 10 mL DMF in a 25 mL round-bottomed flask. The mixture was allowed to react under stirring at 45 °C for 48 h. After removing DMF under vacuum, the residue was redissolved in 50 mL of THF and passed through a neutral alumina column to remove sodium salt and excess NaN_3. The filtrate was concentrated by rotary evaporation, and precipitated in 50 mL of diethyl ether. The precipitate was filtered, washed by diethyl ether and vacuum-dried. The yield was 86% (1.83 g), $M_{n,GPC}$ = 5600 g/mol and M_w/M_n = 1.12.

Synthesis of Alkynyl-Pendant CS (*alkynyl*-CS). The precursor for the click reaction, *alkynyl*-CS, was prepared by the amidation of CS with 4-pentynoic acid in the presence of EDC/NHS. In a typical procedure, CS (0.5 g, 2.73 mmol) and 4-pentynoic acid (0. 268 g, 2.73 mmol) were dissolved in 50 mL of MES buffer (0.1 M, pH adjusted to 5) and degassed. Being protected by argon, EDC (1.57 g, 8.19 mmol) and NHS (2.825 g, 24.57 mmol) were gradually charged into the flask within 20 min. The reaction was conducted at room temperature under stirring for 16 h. To remove impurities, the polymer solution was dialyzed (MWCO = 3 kDa) against distilled water for 3 days at 4 °C and freeze-dried. The yield was 87% (0.55 g), $M_{n,GPC}$ = 13800 g/mol and M_w/M_n = 1.64.

Synthesis of CS(-*g*-PDMAEMA)-*g*-PNIPAM Terpolymer. To a 50 mL Schlenk flask, *alkynl*-CS (0.25 g, 0.6 mmol), PDMAEMA-N_3 (1.52 g, 0.4 mmol) and PNIPAM-N_3 (1.8 g, 0.4 mmol) was dissolved in 20 mL 0.1 M HCl solution and degassed. Under an argon atmosphere, $CuSO_4 \cdot 5H_2O$ (0.3 g, 1.2 mmol, in 3 mL of water) and sodium L-ascorbate (1.19 g, 6 mmol, in 5 mL of water) were successively added. Then, the pH value of the solution was adjusted to about 6.5 (aq NaOH). The yellow-colored mixture was stirred for 24 h at room temperature. The product was dialyzed (MWCO = 8 kDa) against 0.1% acetic acid for 1 day and against distilled water for 3 days at 4 °C, and then lyophilized into dry powder. The residual homopolymers PDMAEMA and PNIPAM were removed by extraction with isobutyl alcohol for 24 h in a Soxhlet extractor, and the final product was isolated by filtration and dried under vacuum (1.92 g, overall yield: 77%).

Characterization. The ^{1}H NMR spectra were recorded using a Bruker DMX-400 spectrometer with $CDCl_3$, D_2O or D_2O/DCl/NaOD as the solvent. Fourier transformed infrared (FTIR) transmission spectra were obtained on a Perkin-Elmer Spectrum 100 spectrometer by accumulation of 32 scans, with a resolution of 4 cm^{-1}. The samples for FTIR were prepared in KBr pellets.

Molecular weight and polydispersity of the polymers were determined using gel permeation chromatography (GPC) at 25 °C. For PDMAEMA and PNIPAM homopolymers, an Agilent 1100 series GPC system equipped with a LC pump, PLgel columns, and RI detector was used. The columns were calibrated with polystyrene standards (Agilent EasiCal). The HPLC grade THF containing 1% (v/v) triethylamine (TEA) and 0.25% (w/v) tetrabutylammonium bromide (TBABr) were used as a mobile phase for PDMAEMA and PNIPAM, respectively. For CS and its derivatives, the GPC column was replaced by PL aquagel−OH Mixed, which was calibrated with pullulan standards. An acetic acid/sodium acetate buffer solution (0.2 M, pH 2.7) was used as eluent. The flow rate was fixed at 1.0 mL/min.

The potentiometric and conductometric titrations were carried out on a Metrohm Titrando system equipped with a Conductometer 712. The water jacketed titration vessel was maintained at a constant temperature using a circulating water bath. A 30 mL volume of 0.1 wt % polymer solution was prepared with an excess of 0.05 M HCl and titrated by 0.1 M NaOH under stirring.

Static light scattering (SLS) and dynamic light scattering (DLS) experiments were performed by means of a laser light scattering spectrometer (Brookhaven BI-200SM) equipped with an argon ion laser operating at $\lambda = 488$ nm and a BI9000AT multi-τ digital time-correlator. For SLS, the instrument was calibrated with toluene to ensure that the scattering intensity from toluene had no angular dependence in the testing angular range. For DLS, 90° measurement angle and 3 min correlation measurement time were used as standard parameters. Distribution averages and particle size distributions were computed using cumulant analysis and CONTIN routes. All data were averaged over three measurements. The bulk polymer solution of 0.05 wt % was prepared and filtered with 0.8 and 0.2 μm filters in tandem prior to the light scattering experiment.

The zeta potentials of the colloidal systems with varying pH were characterized by a Brookhaven ZetaPALS analyzer. The measurements were preformed at 25 °C under the Smoluchowski approximation, and 3 runs of 20 cycles were chosen for a good reproducibility.

The critical micelle concentration (cmc) of the CS(-*g*-PDMAEMA)-*g*-PNIPAM terpolymer was determined by measuring the surface tension using a Dataphysics DCAT 21 tensiometer equipped with a Wilhelmy plate under thermostatic condition. A 0.2 wt % terpolymer bulk solution was gradually titrated into 50 mL acetic acid buffer with the same pH value and ionic strength as the titrant, and the surface tension values were recorded and plotted against the polymer concentration.

The micrographs of the polymer aggregates were taken using a JEOL JEM-2010 transmission electron microscope (TEM) operating at an accelerating voltage of 200 kV. The TEM samples were prepared by dripping a polymer solution onto 400-mesh copper grids precoated with Formvar and stained by 0.2 wt % phosphotungstic acid prior to freeze-drying them.

Results and Discussion

Synthesis of CS(-*g*-PDMAEMA)-*g*-PNIPAM Terpolymer. As shown in Scheme 1, a three-step approach was employed in the terpolymer synthesis. It proceeded first with the synthesis of homopolymers of DMAEMA and NIPAM via ATRP, followed by substituting the halide end groups with azido groups, and subsequently grafting PDMAEMA-N_3 and PNIPAM-N_3 side chains onto the alkyne-containing CS backbone via click chemistry, resulting in the target terpolymer, CS(-*g*-PDMAEMA)-*g*-PNIPAM.

ATRP has been proved to be a versatile technique for the controlled polymerization of many hydrophilic monomers, and the ATRP of DMAEMA in protic and aprotic solvents have been well documented.[25,53,54] The synthesis of PNIPAM via ATRP basically followed the similar procedures initiated by Stöver et al.[55] The subsequent azidation of homopolymers with NaN_3 leading to PDMAEMA-N_3 and PNIPAM-N_3 was carried out by referring to the previous reports.[47,56] The FTIR spectra of PDMAEMA and PNIPAM after azidation (Supporting Information, Figure S1 and S3, respectively) clearly reveal the new appearance of an absorbance peak around 2100 cm^{-1}, which is characteristic of a terminal azido group.[45,57] Nevertheless, the azido group substitution did not affect the molecular weights and distributions of homopolymers, which can be easily judged from the GPC traces shown in Figure S2 and S4, Supporting Information. It should be noted that, the different small molecules indicated in the Experimental Section were added in the THF mobile phase for GPC characterizations in order to minimize the column absorption and polymer aggregation phenomena.

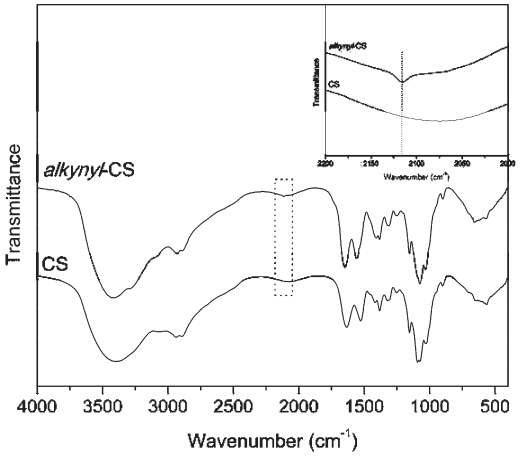


Figure 1. FTIR spectra of CS and *alkynyl*-CS. The inset represents the magnified spectrograms in the specific wavenumber range.

Being utilized as a carboxyl activating reagent, EDC can initiate the formation of an amide linkage between 4-pentynoic acid and CS by forming an active intermediate, and NHS can increase the stability of the active intermediate.[23,58] In the FTIR spectrum of *alkynyl*-CS precursor shown at Figure 1, the characteristic absorbance peak at ~2120 cm^{-1} suggests the successful introduction of alkynyl group on CS backbone.[59,60] As compared with the CS spectrum, other newly appeared or strengthened absorbance peaks around 3250, 1400, and 630 cm^{-1} represent the alkyne C−H stretching, alkane C−H bending and alkyne C−H bending, respectively. The ^{1}H NMR spectra of CS and *alkynyl*-CS (2% DCl in D_2O) are shown Figure 2. The new signals appearing at $\delta = 2.2-2.5$ ppm can be ascribed to methylene protons (4H, $-CH_2-CH_2-$), and the alkynyl proton (1H, $-C\equiv CH$) signal at $\delta = 1.9$ ppm is overlapped with that of CS acetyl groups.[34,61] The degree of substitution (DS = 0.5) of *alkynyl*-CS was determined by the peak integral ratio of resonance signals in the range of 2.2−2.5 ppm to that at 2.9 ppm (H2 of CS). Serving as an alternative method, potentiometric titration also can help to get DS by gradually neutralizing protonated amino groups along the *alkynyl*-CS backbone. As shown in Figure 3, the consumption of titrant NaOH between two inflection points for *alkynyl*-CS is smaller than that of CS, which means the *alkynyl*-CS has a lower content of amino group due to the amidation. The experiments were conducted by titrating 30 mL 0.1 wt % polymer solutions (dissolved in 0.05 M HCl) with 0.1 M NaOH, and the molar amount of amino groups within the copolymer is equal to that of titrated base. Thus, the concrete calculation is written as:

$$\frac{30 \times (0.89 - x)}{[244x + 162 \times (0.89 - x) + 202 \times 0.11]} = 0.07 \qquad (1)$$

where x is the DS; 162, 202, and 244 are the unit molecular weights for glucosamine, acetylglucosamine and alkynylglucosamine, respectively. Thus, we get $x = 0.42$, which is very close to

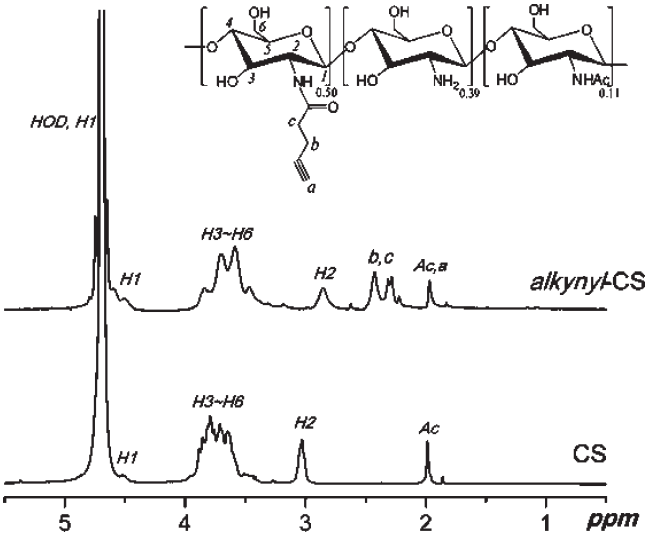


Figure 2. ^{1}H NMR spectra recorded in D_2O/DCl for CS and *alkynyl*-CS.

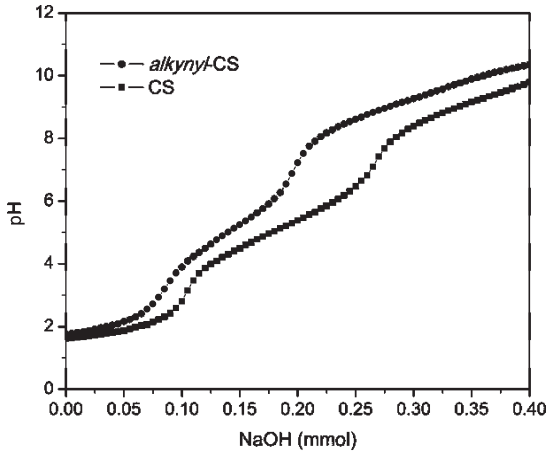


Figure 3. Potentiometric titration curves for 0.1 wt % CS and *alkynyl*-CS in an excess of 0.05 M HCl solution.

the DS obtained from ^{1}H NMR. The aqueous GPC traces for CS and *alkynyl*-CS are displayed in Figure 6, revealing a similar peak shape with M_n of 10200 (M_w/M_n of 1.71) and 13800 g/mol (M_w/M_n of 1.64) correspondingly. The GPC results not only reconfirm the successful incorporation of alkynyl groups, but also coincide with the NMR result, $M_{n,NMR}$ = 12600 g/mol, for *alkynyl*-CS.

In the final step, the synthesis of the CS graft terpolymer was accomplished by the click reaction of *alkynyl*-CS with PDMAEMA-N_3 and PNIPAM-N_3 under mild conditions. In order to increase the grafting efficiency and obtain a balanced dual-responsive copolymer during the "grafting onto" process, the lengths of the linear side chains were deliberatively manipulated to reduce the steric congestion.[34] Meanwhile, the pH value was adjusted to ~6.5 to minimize the electrostatic repulsion between PDMAEMA and CS blocks, and the solubility of CS can also be maintained in this range because the incorporation of alkynyl groups destroyed the strong inter- and intramacromolecular hydrogen bonds. An excess of azido-terminated homopolymers was used to ensure the complete consumption of alkynyl moieties, and the removal of excess homopolymers was achieved by dialysis and extraction. The FTIR spectrum of the purified product clearly reveals the presence of absorbance peaks of all three components

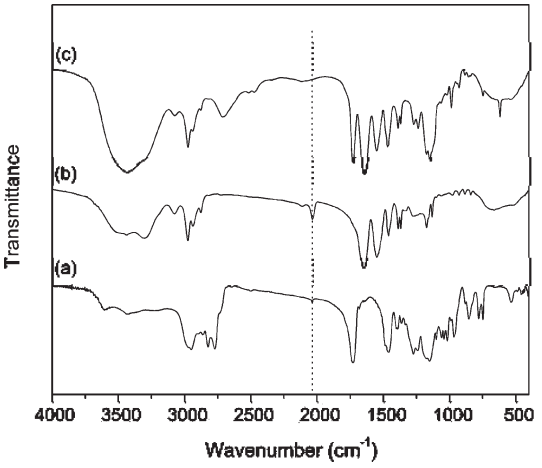


Figure 4. FTIR spectra of (a) PDMAEMA-N_3, (b) PNIPAM-N_3, and (c) CS(-*g*-PDMAEMA)-*g*-PNIPAM.

(Figure 4c): 3420 cm^{-1} (O−H stretching) from CS; 1725 cm^{-1} (ester stretching), 1460 cm^{-1} (CH_2 bending), and 1145 cm^{-1} (C−N stretching) from DMAEMA; and 2970 cm^{-1} (C−H stretching), 1645 cm^{-1} (carbonyl stretching), and 1545 cm^{-1} (N−H bending) from NIPAM. Comparing the FTIR spectra among *alkynyl*-CS, PDMAEMA-N_3, and PNIPAM-N_3, the most noteworthy change is the complete disappearance of the absorbance peaks characteristic of the azido and alkynyl groups at ~2100 cm^{-1}. On the basis of the result, two important conclusions can be drawn: the alkynyl moieties have fully attached with homopolymeric side chains, and excess azido-terminated homopolymers have been thoroughly removed.[45,57,62] From the ^{1}H NMR spectrum of the CS graft terpolymer (Figure S5a), all characteristic signals of PDMAEMA, PNIPAM and CS segments can be discerned, which are accompanied by the appearance of a new resonance peak j at ~8.1 ppm (methine proton of 1,2,3-triazole ring).[63] The average constitution of the terpolymer was determined by calculating the integral ratios between nonoverlapping peaks belonging to each block: H2 at δ = 3.1 ppm from CS (Figure S5b), methylene protons (2H, $-CH_2-CH_2-N-$) at δ = 3.45 ppm from PDMAEMA and tertiary proton (1H, -NH−C*H*-) at δ = 3.75 ppm from PNIPAM, respectively. Assuming that no degradation occurred during the click reaction, the $M_{n,NMR}$ of the terpolymer can reach 130000 g/mol. The GPC eluograms (Figure 6) indeed show that the graft terpolymer has larger M_n (= 54000 g/mol) and smaller M_w/M_n (= 1.33) than these of CS and *alkynyl*-CS, which is inconsistent with the recent reports showing serious CS backbone breakdown under "clicking" in the presence of Cu(II)/ascorbate.[36,38] It has been proposed that the depolymerization of CS was catalyzed by the hydroxyl radicals (•OH), and the amino groups of CS may facilitate the depolymerization by favoring the generation of •OH close to the Cu−NH_2 binding site.[38] This explains why CS with a lower content of amino group was more stable against degradation.[36] Therefore, CS(-*g*-PDMAEMA)-*g*-PNIPAM terpolymer synthesized from *alkynyl*-CS with a real DD of 39% revealed inconspicuous degradation compared to other studies[36,38] and reconfirmed the above proposed mechanism. From another point of view, the disparity between the theoretical and GPC measured number-average molecular weights of terpolymer may be attributed to the more compact nature of comb-like copolymers in good solvents.[64,65] Alfred et al. also reported that the GPC underestimated the molecular weights of polymerized norbornene or oxanorbornene macromonomers grafted with PEO recently.[66] The low M_w/M_n of the terpolymer, indicating a high structural regularity, mainly

benefited from the inclusion of narrow-distributed side chains: PDMAEMA and PNIPAM. Of course, the dialysis process that eliminated the polymer chains with the lowest range of molecular weights also helped to improve the uniformity. On the basis of the above results, we can conclude that obtained graft CS terpolymer has a statistically well-defined structure: PDMAEMA side chains (23 repeating units) and PNIPAM side chains (40 repeating units) were attached to 28% and 22% glucosamine units of CS backbone, respectively, and CS backbone also has 39% glucosamine and 11% acetyl-glucosamine units. In the subsequent section, we further investigated its pH- and thermo-responsive micellization behavior in different aqueous solutions.

Thermo- and pH-Responsive Micellization of the Graft CS Terpolymer. The homopolymers of DMAEMA and NIPAM exhibit typical stimuli-responsive amphiphilicity. PDMAEMA is a weak polybase and its conjugated acid possesses a pK_a of about 7: it is soluble as a weak cationic polyelectrolyte due to the protonation of tertiary amine groups when pH < 6 while it becomes more hydrophobic and even water-insoluble at higher pH.[47,67,68] PNIPAM dissolves in cold aqueous solution but becomes insoluble above 32 °C, which is ascribed to the lower critical solution temperature (LCST) phase transition behavior.[69] As reported previously, CS-*g*-PDMAEMA demonstrated a pH-induced association behavior at pH 5−6, because the CS had a p$K_a \sim 5.5$ and the deprotonated CS moieties formed the cores of micelles due to hydrophobic association and hydrogen-bonding interation.[22,25] In the present case, it is expected that the presence of pH-responsive CS/PDMAEMA segments and thermo-responsive PNIPAM side chains can make the CS(-*g*-PDMAEMA)-*g*-PNIPAM terpolymer exhibit a dually responsive feature. Two typical solution conditions [i.e., (a) pH 4 and 40 °C; (b) pH 10 and 25 °C] were selected to facilitate the micellization of the terpolymer through self-assembly. From the obvious characteristic bluish tinge of the colloidal dispersions under both conditions, the micellization processes could be persuasively evidenced. Subsequently, the critical micellization concentrations (cmc) of CS(-*g*-PDMAEMA)-*g*-PNIPAM under the two conditions were determined by measuring their surface tension variations with polymer concentration (Figure 7). The cmc values of the terpolymer under two conditions are both around 0.01 mg/mL, which are quite consistent with those of another reported Janus-type star copolymer, (PDEAEMA)-*CD*-(PNIPAM).[46]

The dual-responsive formation of different types of aggregates at different solutions has also been supported by the ^{1}H NMR results (Figure 5).[45−48] At 25 °C and pH 4, all the segments of CS(-*g*-PDMAEMA)-*g*-PNIPAM are hydrophilic, so that the comb-like graft terpolymer molecularly dissolves in aqueous solution and the signals of the corresponding protons of PDMAEMA and PNIPAM are distinct in Figure 5a. Upon heating the pH 4 solution to 40 °C, the signals characteristic of the PNIPAM side chains around δ = 1.0, 1.5, 1.9, and 3.8 ppm (peaks f, h, i, and g) thoroughly disappear, while the signals from PDMAEMA and CS are still visible (Figure 5b), indicating the insolubility of PNIPAM segments. At the same time, the characteristic bluish tinge of the colloidal solution suggests the formation of micelles consisting of PNIPAM hydrophobic cores and well-dissolved hybrid CS/PDMAEMA coronas. At 25 °C and pH 6.8 (Figure S5b, Supporting Information), ^{1}H NMR resonance signal of CS at δ = 3.1 ppm completely disappeared, which represents the initial deprotonation and aggregation of CS segments. On the other hand, the characteristic signals of PDMAEMA at δ = 2.9, 3.4, and 4.3 ppm (peaks a, b, and c) also become weak and broad compared with these at pH 4, resulting from the decreased hydrophilicity of PDMAEMA which is attributed to the partial deprotonation

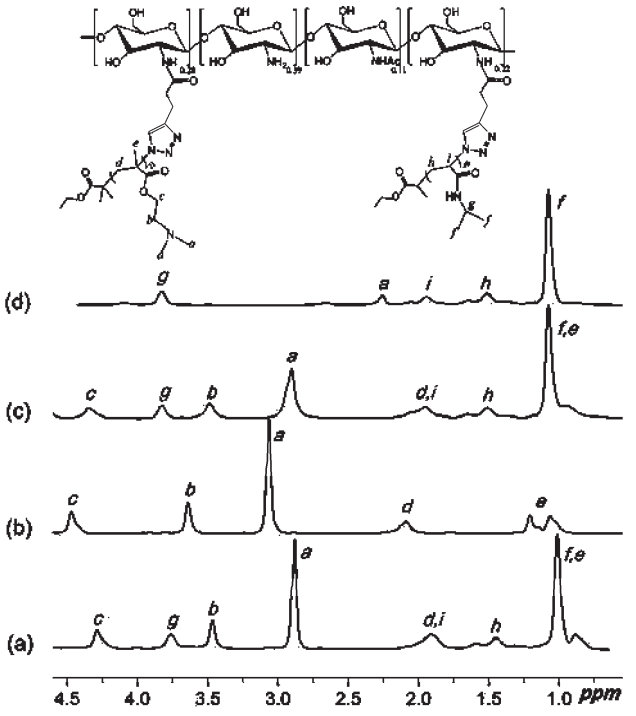


Figure 5. Partial ^{1}H NMR spectra of CS(-*g*-PDMAEMA)-*g*-PNIPAM recorded in D_2O under different conditions: (a) pH 4 and 25 °C, (b) pH 4 and 40 °C, (c) pH 6.8 and 25 °C, and (d) pH 9.6 and 25 °C.

of the tertiary amino groups.[70] Further addition of NaOD gave the final solution a pH of 9.6, and the characteristic NMR signals of PDMAEMA are intensively suppressed (shown at Figure 5d) due to the limited solubility of the fully neutralized PDMAEMA segments. Considering the appearance of bluish tinge of the solution, another type of aggregates consisting of CS/PDMAEMA hydrophobic cores and PNIPAM hydrophilic coronas could be formed. The existence of residual peak a from PDMAEMA segments reveals that this kind of micelle has a relatively less compact hydrophobic core than that PDEAEMA-cored micelles have, because PDMAEMA still has some water solubility at neutral or alkaline pH.[45−47,71]

The following LLS studies provide more detailed insights on the microstructures of the aggregates formed under different conditions. Figure 8a shows the pH dependence of intensity-average hydrodynamic radius (R_h) and the dimensionless ratio of gyration radius (R_g) to R_h for the CS(-*g*-PDMAEMA)-*g*-PNIPAM terpolymer at 25 °C. It is well-known that the theoretical values of R_g/R_h for random coil, core−shell structure and hard sphere are 1.5, 0.9 and 0.77, respectively.[72] Below pH 4, terpolymer chains molecularly disperse in an acidic solution with an almost constant R_h (ca. 60 nm). The ratio of R_g/R_h, ranging between 1.3 and 1.5 which are very close to the theoretical value for Gaussian chains, reveals that the polymer chains exist in random coil conformation owing to the strong electrostatic repulsion. Upon addition of NaOH, significant aggregation occurs over pH 5 to 7, as judged by the dramatic increase in R_h, corresponding to the major deprotonation of CS and PDMAEMA segments. Taking account of the association mechanism of the previously reported CS and PDMAEMA binary system, their pK_a difference leads to the formation of "onion-like" micelles consisting of CS core, PDMAEMA inner shell, and hydrophilic PNIPAM outer corona when pH > 7.[25,73−75] It is notable that using the CS(-*g*-PDMAEMA)-*g*-PNIPAM terpolymer to replace the CS-*g*-PDMAEMA copolymer can increase the micelles' stability in alkaline

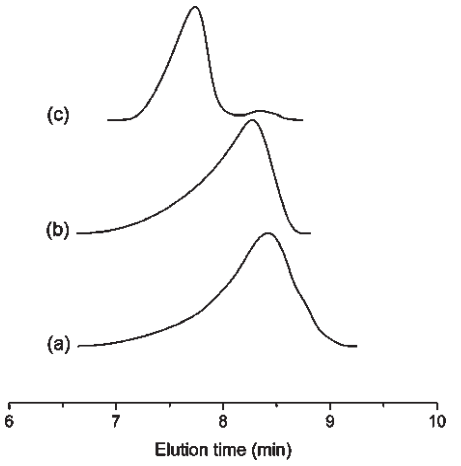


Figure 6. GPC traces for (a) CS, (b) *alkynyl*-CS, and (c) CS(-*g*-PDMAEMA)-*g*-PNIPAM in acetic acid buffers.

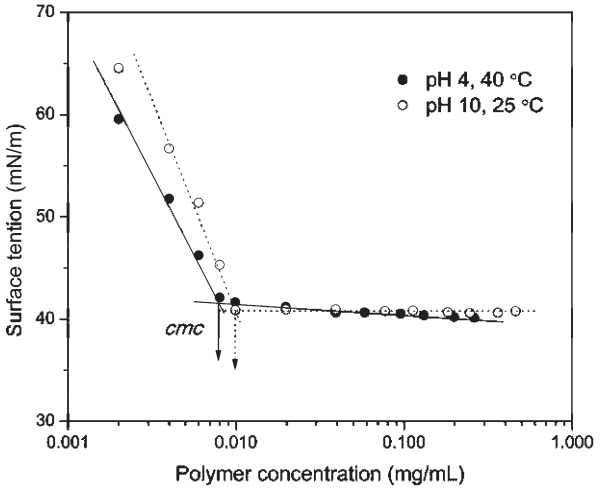


Figure 7. Surface tension versus concentration of CS(-*g*-PDMAEMA)-*g*-PNIPAM under two representative conditions: (a) pH 4 and 40 °C; (b) pH 10 and 25 °C.

media due to the steric stabilization imparted by the hydrophilic PNIPAM segments. Above pH 8, the sizes of aggregates remain almost constant at R_h ~150 nm, and the R_g/R_h values are about 0.85−0.9, indicating the formation of a core−shell structure.[76] The low polydispersity index (μ_2/Γ^2~0.1) of aggregates (as shown in Figure 8b) shall be ascribed to the narrow dispersity of the terpolymer endowed by the ATRP-synthesized side chains. In addition, the change in zeta potential according to pH variation was summarized in Figure 8c. The zeta potential is more than +25 mV at pH < 4, and it decreases continuously to +3.8 mV when pH is increased to 8, which reflects the ongoing deprotonation process of the terpolymer. Finally, the averaged zeta potential approaching to 0 mV at pH > 9 means the vanishing of electrostatic charge on the surface of micelles, but the obtained transparent colloidal solution rather than cloudy precipitation further confirms that the stability of micelles roots in the incorporation of PNIPAM segments.

Starting from the unimer state of the terpolymer in an aqueous solution at pH 4 and 25 °C, micelles consisting of PNIPAM cores and CS/PDMAEMA hybrid coronas can also be fabricated via heating. Figure 9 shows the temperature dependence of R_h and R_g/R_h for the CS(-*g*-PDMAEMA)-*g*-PNIPAM terpolymer at pH 4. Below 33 °C, the graft terpolymer molecularly dissolves with R_h varying between 62 and 66 nm. Aggregation

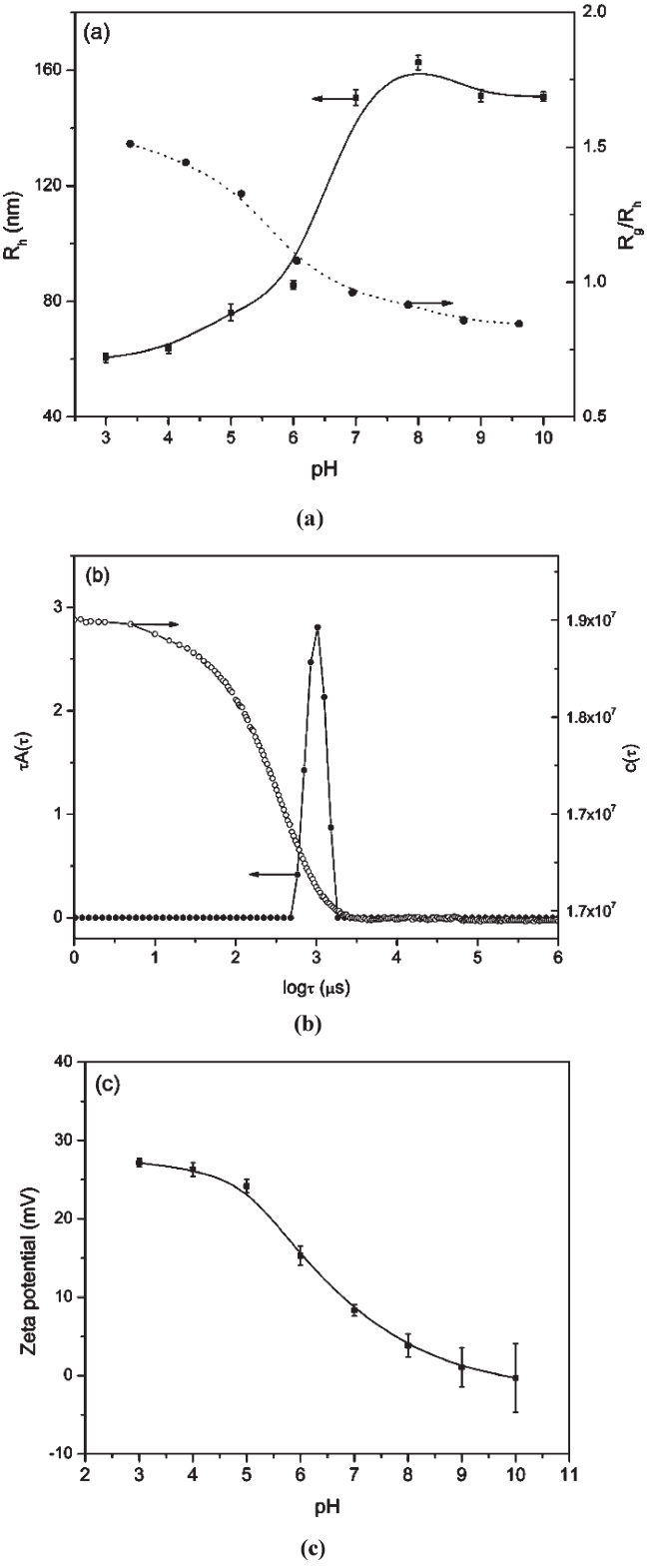


Figure 8. (a) Variations of hydrodynamic radius (R_h) and the raio of R_g/R_h as a function of pH at 25 °C for CS(-*g*-PDMAEMA)-*g*-PNIPAM in aqueous solution. (b) Intensity autocorrelation function and decay time distribution function for CS(-*g*-PDMAEMA)-*g*-PNIPAM at pH 10 and 25 °C. (c) Zeta potential of CS(-*g*-PDMAEMA)-*g*-PNIPAM terpolymer at different pH values.

starts to occur when temperature exceeds 33 °C, which is accompanied by a sharp increase in R_h. Above 40 °C, the sizes of the micelles remain almost constant at D_h ~ 200 nm. Synchronously, the value of R_g/R_h decreases from 1.5 to 0.9,

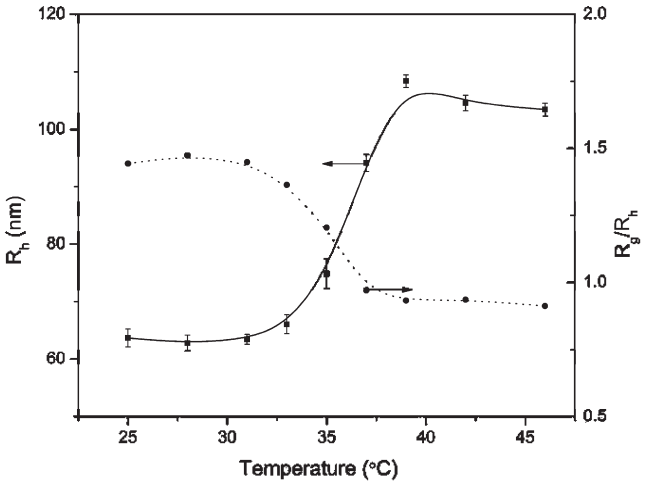


Figure 9. Temperature-dependences of R_h and R_g/R_h in aqueous solutions at pH 4 for CS(-*g*-PDMAEMA)-*g*-PNIPAM.

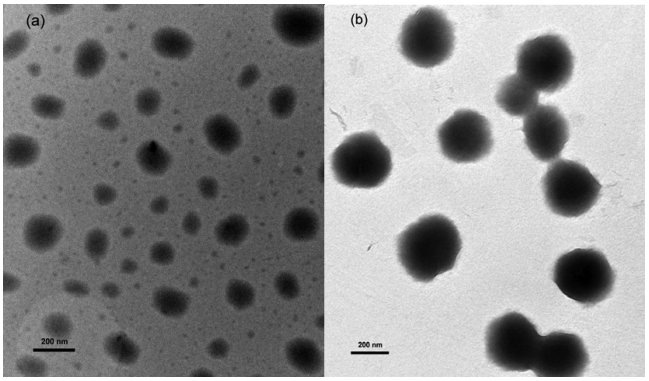
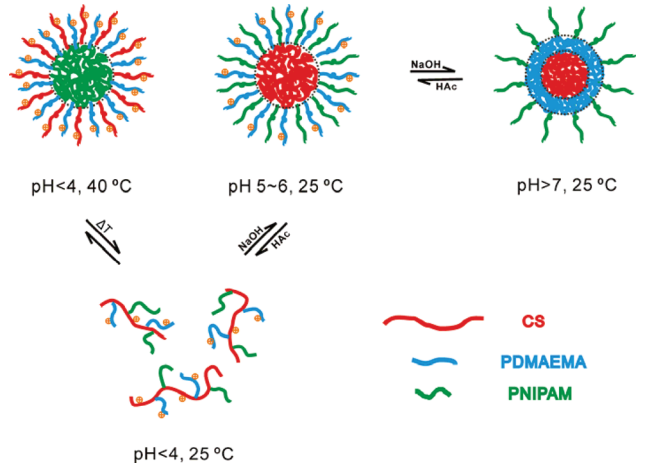


Figure 10. TEM micrographs for self-assembled micelles obtained from 0.05 wt % CS(-*g*-PDMAEMA)-*g*-PNIPAM terpolymer solutions at (a) pH 4 and 40 °C and (b) pH 10 and 25 °C.

Scheme 2. Schematic Description for the Magic Association Behavior of the Dual Hydrophilic CS(-*g*-PDMAEMA)-*g*-PNIPAM Terpolymer



demonstrating that the terpolymer has changed from random coils to core−shell structured micelles. It should be pointed out that the self-assembled aggregates under both conditions are completely reversible as evidenced by LLS studies. The tuning of solution conditions can switch the terpolymer in aqueous solution to form microstructures between different types of aggregates and umimers.

The actual morphologies of the aggregates formed under two representative conditions were further testified by TEM micrographs shown in Figure 10. At pH 10 and 25 °C, spherical micelles with diameters in the range from 260 to 280 nm can be identified, which is in good agreement with that determined by DLS considering the hydration effect. At the same time, the uneven darkness distribution in the particles corroborates the formation of core−shell structured micelles. At pH 4 and 40 °C, nearly spherical micelles consisting of PNIPAM hydrophobic cores and CS/PDMAEMA hydrophilic coronas can be observed and the mean diameter of them is around 170 nm. On the basis of the above presented ^{1}H NMR, LLS and TEM results, the conformational changes of CS(-*g*-PDMAEMA)-*g*-PNIPAM at different solution conditions were schematically shown in Scheme 2.

Conclusions

To sum up, a novel dually stimuli-responsive graft terpolymer, CS(-*g*-PDMAEMA)-*g*-PNIPAM, based on the CS derivative, has been synthesized via the combination of ATRP and click reaction. The utilization of ATRP not only allowed us to modulate the side chains flexibly but also guaranteed their narrow molecular weight distribution, which further brought on a low polydispersity of the terpolymer. The click reaction taking place in the mild and homogeneous solution was proved to be an efficient coupling method to obtain a high degree of grafting on CS. The graft terpolymer could aggregate into three-layer "onion-like" micelles at 25 °C when pH is above 7. Moreover, spherical micelles with PNIPAM cores were obtained around 40 °C in acidic solution (pH $<$ 4). Most importantly, switching between different types of aggregates and umimers can be reversibly achieved by properly tuning pH and temperature of the aqueous solutions. This work represents the first example of nonlinear dual hydrophilic CS copolymer showing the magic micellization behavior.

Supporting Information Available: Figures showing additional FTIR, GPC, and ^{1}H NMR characterization results of PDMAEMA, PNIPAM, and CS derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Kim, J.-H.; Kim, Y.-S.; Park, K.; Kang, E.; Lee, S.; Nam, H. Y.; Kim, K.; Park, J. H.; Chi, D. Y.; Park, R.-W.; Kim, I.-S.; Choi, K.; Chan Kwon, I. *Biomaterials* **2008**, *29*, 1920–1930.
(2) Mao, H.-Q.; Roy, K.; Troung-Le, V. L.; Janes, K. A.; Lin, K. Y.; Wang, Y.; August, J. T.; Leong, K. W. *J. Controlled Release* **2001**, *70*, 399–421.
(3) Kim, I.-Y.; Seo, S.-J.; Moon, H.-S.; Yoo, M.-K.; Park, I.-Y.; Kim, B.-C.; Cho, C.-S. *Biotechnol. Adv.* **2008**, *26*, 1–21.
(4) Di Martino, A.; Sittinger, M.; Risbud, M. V. *Biomaterials* **2005**, *26*, 5983–5990.
(5) Säkkinen, M.; Linna, A.; Ojala, S.; Jürjenson, H.; Veski, P.; Marvola, M. *Int. J. Pharm.* **2003**, *250*, 227–237.
(6) Kim, T.-H.; Jiang, H.-L.; Jere, D.; Park, I.-K.; Cho, M.-H.; Nah, J.-W.; Choi, Y.-J.; Akaike, T.; Cho, C.-S. *Prog. Polym. Sci.* **2007**, *32*, 726–753.
(7) Mourya, V. K.; Inamdar, N. N. *React. Funct. Polym.* **2008**, *68*, 1013–1051.
(8) Chen, S.; Wang, Y. *J. Appl. Polym. Sci.* **2001**, *82*, 2414–2421.
(9) Jung, B.-O.; Kim, C.-H.; Choi, K.-S.; Lee, Y. M.; Kim, J.-J. *J. Appl. Polym. Sci.* **1999**, *72*, 1713–1719.
(10) Thanou, M.; Verhoef, J. C.; Junginger, H. E. *Adv. Drug Delivery Rev.* **2001**, *52*, 117–126.
(11) Ono, K.; Saito, Y.; Yura, H.; Ishikawa, K.; Kurita, A.; Akaike, T.; Ishihara, M. *J. Biomed. Mater. Res.* **2000**, *49*, 289–295.
(12) Singh, D. K.; Ray, A. R. *Carbohydr. Polym.* **1998**, *36*, 251–255.

(13) Jayakumar, R.; Prabaharan, M.; Reis, R. L.; Mano, J. F. *Carbohydr. Polym.* **2005**, *62*, 142–158.
(14) Wang, J.-S.; Matyjaszewski, K. *J. Am. Chem. Soc.* **1995**, *117*, 5614–5615.
(15) Kato, M.; Kamigaito, M.; Sawamoto, M.; Higashimura, T. *Macromolecules* **1995**, *28*, 1721–1723.
(16) El Tahlawy, K.; Hudson, S. M. *J. Appl. Polym. Sci.* **2003**, *89*, 901–912.
(17) Li, N.; Bai, R.; Liu, C. *Langmuir* **2005**, *21*, 11780–11787.
(18) Liu, P.; Su, Z. *Mater. Lett.* **2006**, *60*, 1137–1139.
(19) Carlmark, A.; Malmstrom, E. E. *Biomacromolecules* **2003**, *4*, 1740–1745.
(20) Bontempo, D.; Masci, G.; DeLeonardis, P.; Mannina, L.; Capitani, D.; Crescenzi, V. *Biomacromolecules* **2006**, *7*, 2154–2161.
(21) Xu, F. J.; Zhang, Z. X.; Ping, Y.; Li, J.; Kang, E. T.; Neoh, K. G. *Biomacromolecules* **2009**, *10*, 285–293.
(22) Sui, X.; Yuan, J.; Zhou, M.; Zhang, J.; Yang, H.; Yuan, W.; Wei, Y.; Pan, C. *Biomacromolecules* **2008**, *9*, 2615–2620.
(23) Sun, S.; Liu, W.; Cheng, N.; Zhang, B.; Cao, Z.; Yao, K.; Liang, D.; Zuo, A.; Guo, G.; Zhang, J. *Bioconjugate Chem.* **2005**, *16*, 972–980.
(24) Chan, P.; Kurisawa, M.; Chung, J. E.; Yang, Y.-Y. *Biomaterials* **2007**, *28*, 540–549.
(25) Bao, H.; Hu, J.; Gan, L. H.; Li, L. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 6682–6692.
(26) Munro, N. H.; Hanton, L. R.; Moratti, S. C.; Robinson, B. H. *Carbohydr. Polym.* **2009**, *77*, 496–505.
(27) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.
(28) Wu, P.; Feldman, A. K.; Nugent, A. K.; Hawker, C. J.; Scheel, A.; Voit, B.; Pyun, J.; Fréchet, J. M. J.; Sharpless, K. B.; Fokin, V. V. *Angew. Chem., Int. Ed.* **2004**, *43*, 3928–3932.
(29) Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192–3193.
(30) Gao, H.; Matyjaszewski, K. *Prog. Polym. Sci.* **2009**, *34*, 317–350.
(31) Lodge, T. P. *Macromolecules* **2009**, *42*, 3827–3829.
(32) Binder, W. H.; Sachsenhofer, R. *Macromol. Rapid Commun.* **2007**, *28*, 15–54.
(33) Binder, W. H.; Sachsenhofer, R. *Macromol. Rapid Commun.* **2008**, *29*, 952–981.
(34) Gao, H.; Matyjaszewski, K. *J. Am. Chem. Soc.* **2007**, *129*, 6633–6639.
(35) Jiang, X.; Lok, M. C.; Hennink, W. E. *Bioconjugate Chem.* **2007**, *18*, 2077–2084.
(36) Kulbokaite, R.; Ciuta, G.; Netopilik, M.; Makuska, R. *React. Funct. Polym.* **2009**, *69*, 771–778.
(37) Gao, Y.; Zhang, Z.; Chen, L.; Gu, W.; Li, Y. *Biomacromolecules* **2009**, *10*, 2175–2182.
(38) Lallana, E.; Fernandez-Megia, E.; Riguera, R. *J. Am. Chem. Soc.* **2009**, *131*, 5748–5750.
(39) Gil, E. S.; Hudson, S. M. *Prog. Polym. Sci.* **2004**, *29*, 1173–1222.
(40) Liu, S.; Armes, S. P. *Angew. Chem., Int. Ed.* **2002**, *41*, 1413–1416.
(41) Chang, C.; Wei, H.; Feng, J.; Wang, Z.-C.; Wu, X.-J.; Wu, D.-Q.; Cheng, S.-X.; Zhang, X.-Z.; Zhuo, R.-X. *Macromolecules* **2009**, *42*, 4838–4844.
(42) Cai, Y.; Armes, S. P. *Macromolecules* **2004**, *38*, 271–279.
(43) Tezuka, Y.; Oike, H. *J. Am. Chem. Soc.* **2001**, *123*, 11570–11576.
(44) Ge, Z.; Cai, Y.; Yin, J.; Zhu, Z.; Rao, J.; Liu, S. *Langmuir* **2007**, *23*, 1114–1122.
(45) Zhang, Y.; Liu, H.; Hu, J.; Li, C.; Liu, S. *Macromol. Rapid Commun.* **2009**, *30*, 941–947.
(46) Ge, Z.; Xu, J.; Hu, J.; Zhang, Y.; Liu, S. *Soft Matter* **2009**, *5*, 3932–3939.
(47) Jiang, X.; Zhang, G.; Narain, R.; Liu, S. *Soft Matter* **2009**, *5*, 1530–1538.
(48) Feng, C.; Shen, Z.; Yang, D.; Li, Y.; Hu, J.; Lu, G.; Huang, X. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 4346–4357.
(49) Cai, G.; Jiang, H.; Chen, Z.; Tu, K.; Wang, L.; Zhu, K. *Eur. Polym. J.* **2009**, *45*, 1674–1680.
(50) Zhang, Y.; Huo, M.; Zhou, J.; Yu, D.; Wu, Y. *Carbohydr. Polym.* **2009**, *77*, 231–238.
(51) Bao, H.; Li, L.; Zhang, H. *J. Colloid Interface Sci.* **2008**, *328*, 270–277.
(52) Bao, H.; Li, L.; Gan, L. H.; Zhang, H. *Macromolecules* **2008**, *41*, 9406–9412.
(53) Mao, B.; Gan, L.-H.; Gan, Y.-Y.; Li, X.; Ravi, P.; Tam, K.-C. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 5161–5169.
(54) Agut, W.; Taton, D.; Lecommandoux, S. *Macromolecules* **2007**, *40*, 5653–5661.
(55) Xia, Y.; Yin, X.; Burke, N. A. D.; Stöver, H. D. H. *Macromolecules* **2005**, *38*, 5937–5943.
(56) Yu, H.; Gan, L. H.; Hu, X.; Gan, Y. Y. *Polymer* **2007**, *48*, 2312–2321.
(57) Liu, H.; Li, C.; Liu, H.; Liu, S. *Langmuir* **2009**, *25*, 4724–4734.
(58) Chen, J.-P.; Cheng, T.-H. *Macromol. Biosci.* **2006**, *6*, 1026–1039.
(59) Xu, X.-D.; Chen, C.-S.; Wang, Z.-C.; Wang, G.-R.; Cheng, S.-X.; Zhang, X.-Z.; Zhuo, R.-X. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 5263–5277.
(60) Xu, X.-D.; Chen, C.-S.; Lu, B.; Wang, Z.-C.; Cheng, S.-X.; Zhang, X.-Z.; Zhuo, R.-X. *Macromol. Rapid Commun.* **2009**, *30*, 157–164.
(61) Mespouille, L.; Vachaudez, M.; Suriano, F.; Gerbaux, P.; Van Camp, W.; Coulembier, O.; Degée, P.; Flammang, R.; Du Prez, F.; Dubois, P. *React. Funct. Polym.* **2008**, *68*, 990–1003.
(62) Zhang, J.; Zhou, Y.; Zhu, Z.; Ge, Z.; Liu, S. *Macromolecules* **2008**, *41*, 1444–1454.
(63) Liu, H.; Zhang, Y.; Hu, J.; Li, C.; Liu, S. *Macromol. Chem. Phys.* **2009**, *210*, 2125–2137.
(64) Nakamura, Y.; Wan, Y.; Mays, J. W.; Iatrou, H.; Hadjichristidis, N. *Macromolecules* **2000**, *33*, 8323–8328.
(65) Sun, T.; Chance, R. R.; Graessley, W. W.; Lohse, D. J. *Macromolecules* **2004**, *37*, 4304–4312.
(66) Alfred, S. F.; Al-Badri, Z. M.; Madkour, A. E.; Lienkamp, K.; Tew, G. N. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 2640–2648.
(67) Vamvakaki, M.; Unali, G. F.; Butun, V.; Boucher, S.; Robinson, K. L.; Billingham, N. C.; Armes, S. P. *Macromolecules* **2001**, *34*, 6839–6841.
(68) Tan, B. H.; Gudipati, C. S.; Hussain, H.; He, C.; Liu, Y.; Davis, T. P. *Macromol. Rapid Commun.* **2009**, *30*, 1002–1008.
(69) Yoshida, R.; Uchida, K.; Kaneko, Y.; Sakai, K.; Kikuchi, A.; Sakurai, Y.; Okano, T. *Nature* **1995**, *374*, 240–242.
(70) Bütün, V.; Armes, S. P.; Billingham, N. C. *Polymer* **2001**, *42*, 5993–6008.
(71) Smith, A. E.; Xu, X.; Kirkland-York, S. E.; Savin, D. A.; McCormick, C. L. *Macromolecules* **2010**, *43*, 1210–1217.
(72) Rubinstein, M.; Colby, R. H. *Polymer Physics*; Oxford University Press: Oxford, U.K., 2003.
(73) Liu, L.; Xu, X.; Guo, S.; Han, W. *Carbohydr. Polym.* **2009**, *75*, 401–407.
(74) Jiang, X.; Zhang, G.; Narain, R.; Liu, S. *Langmuir* **2009**, *25*, 2046–2054.
(75) Liu, C.; Hillmyer, M. A.; Lodge, T. P. *Langmuir* **2009**, *25*, 13718–13725.
(76) Ravi, P.; Wang, C.; Tam, K. C.; Gan, L. H. *Macromolecules* **2003**, *36*, 173–179.