Supplementary Material (ESI) for *Soft Matter*

Supplementary Information

Fabrication of Microgels via Supramolecular Assembly of Cyclodextrin-Containing Star Polycations and Oppositely Charged Linear Polyanions

Yaojuan Wu, Peihong Ni, Mingzu Zhang, Xiulin Zhu**

Key Laboratory of Organic Chemistry of Jiangsu Province, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, China

* To whom correspondence should be addressed. E-mail: phni@suda.edu.cn and xlzhu@suda.edu.cn

Synthesis of ATRP initiator 20Cl-β-CD and polycation β-CD-*g*-PDMA

The initiator, chloroacetyl-terminated β-cyclodextrin (20Cl-β-CD), with about twenty initiation sites, was synthesized using the following procedures: β-CD (5 g, 4.5 mmol) was dissolved completely in 30 mL of anhydrous *N, N*-dimethylformamide (DMF) with magnetic stirring in a three-necked round-bottom flask. Chloroacetyl chloride (25 g, 0.22 mol) in anhydrous *N, N*-dimethylformamide (10 mL) was added dropwise to the β-CD solution over a period of 5 h at room temperature under a nitrogen atmosphere. After this addition, the reaction mixture was kept at 80 °C with refluxing for 24 h. The final reaction mixture was precipitated in a great amount of deionized water. The precipitate was collected by centrifugation and dried in a vacuum oven at 60 °C overnight to get the milk white powder.

The β-CD-*g*-PDMA star polymers were synthesized using a molar feed ratio [DMA]/[20Cl-β-CD (0.1 g)]/[CuBr]/[bpy] of 630: 1.0: 21.0: 42.0, unless otherwise mentioned. The reaction was performed in a 50 mL flask equipped with a magnetic stirrer and under the typical conditions for ATRP.[1] The monomer 2-(dimethylamino)ethyl methacrylate (DMA) was introduced into the flask containing β-CD-Cl_{21} and 2, 2′-dipyridyl (bpy) under stirring. After the ATRP initiator 20Cl-β-CD had dissolved completely, the solvent deionized water (8 mL) was injected into the flask, and then the reaction mixture was degassed by an exhausting-refilling step at least 3 times. When the reaction solution turns to transparent, CuBr was added into the mixture under an inert atmosphere and purged with nitrogen for 10 min. The polymerization was allowed to proceed under continuous stirring at 45 °C for 12 h. The reaction was stopped by exposure to atmosphere. The final reaction product was precipitated in excess sodium hydroxide solution (with a concentration of 5 *wt*%). The crude product was dissolved in dilute hydrochloride acid solution (5 *wt*%) and precipitated in the NaOH solution (5 *wt*%). The precipitating-dissolving step must be repeated at least twice to remove the reactant residues completely. The yield of the β-CD-*g*-PDMA is about 70 %.

Synthesis of Polyanion $PMAA_{30}$-*b*-PEG-*b*-$PMAA_{30}$ (or MePEG-*b*-$PMAA_{60}$)

The P*t*BMA_{30}-*b*-PEG-*b*-P*t*BMA_{30} was prepared by oxyanion-initiated polymerization, which has been described in previous literature.[2,3] A representative synthesis procedure is described as follows: all flasks and magnetic stirring bars used in the experiments were heated overnight at 120 °C, and then an exhausting-refilling argon process was operated several times until room temperature. Potassium hydride, stored in mineral oil, was washed with anhydrous THF three times under an argon atmosphere in a round-bottom flask, and then anhydrous THF was injected into the flask with KH powder. A specified weight of dry poly (ethylene glycol) (HO-PEG-OH) was dissolved in anhydrous THF in a round-bottom flask with an argon atmosphere previously. The HO-PEG-OH/THF solution was then transferred via a cannula into the flask containing KH to produce a telechelic oxyanion K^+O^--PEG-O^-K^+ as the macroinitiator. After the complete reaction, the upper clear liquid was transferred into the other dry flask with the same method. A certain amount of *t*BMA monomer was then added into the initiator solution by syringe, and the reaction was carried out at 25 °C with stirring for 1 h. The living polymer chains were terminated with methanol, and the solvent was afterward removed with a rotary vacuum distillatory. The resulting product P*t*BMA$_{30}$-*b*-PEG-*b*-P*t*BMA$_{30}$ was purified through repeated precipitation into cold n-hexane and dried in a vacuum oven at 30 °C for at least 12 h. The diblock copolymer MePEG-*b*-P*t*BMA$_{60}$ was prepared according to the similar method using polyethylene glycol 2000 monomethyl ether (designated MePEG) as an initiator precursor.

The second step related to the synthesis of PMAA$_{30}$-*b*-PEG-*b*-PMAA$_{30}$ via the hydrolysis of P*t*BMA$_{30}$-*b*-PEG-*b*-P*t*BMA$_{30}$. To a CH_2Cl_2 solution of P*t*BMA$_{30}$-*b*-PEG-*b*-P*t*BMA$_{30}$ (0.8333 g), trifluoroacetic acid (TFA, 3.4 mL) was slowly added at 0 °C under magnetic stirring. After the addition was completed, the reaction mixture was kept at room temperature for 12 h. The reaction mixture was concentrated, precipitated into 50 mL of cold diethyl ether, filtered, dissolved in anhydrous ethanol, and repeated the precipitation-dissolving step at least twice. Finally, the product was dried in a vacuum oven to give an off white solid product PMAA$_{30}$-*b*-PEG-*b*-PMAA$_{30}$ (0.5819 g). Yield: 93.2%. The diblock copolymer MePEG-*b*-PMAA$_{60}$ had yield as 90.8%.

Measurements

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR)

^{1}H NMR spectrum of PMAA-*b*-PEG-*b*-PMAA was recorded on a 300-MHz NMR instrument (INOVA-300) using d_6-DMSO as solvent and tetramethylsilane (TMS) as the internal standard. For P*t*BMA-*b*-PEG-*b*-P*t*BMA, $CDCl_3$ was used as the solvent and tetramethylsilane (TMS) as the internal standard.

Gel Permeation Chromatography (GPC)

GPC measurements were conducted on a Waters 1515 HPLC (Waters Chromatography, Inc.) equipped with a differential refractive-index detector and a three-column series, a PLgel 5.0-μm bead-size guard column (50×7.5 mm^2), followed by two linear PLgel columns (500 Å and Mixed-C). The system was equilibrated at 30 °C in THF, which served as the polymer solvent and eluent with a flow rate of 1.0 mL/min. Molecular weight and molecular weight distributions were determined based on calibration of the GPC system with a series of poly(methyl methacrylate) standards.

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra of the copolymers were recorded on a Necolet Avatar 360.The samples were prepared by grinding the copolymers with spectroscopic grade KBr and then compressing the mixture of samples and KBr into transparent tablets.

Steady-State Fluorescence Spectroscopy

To estimate the critical aggregation concentration (cac) of the β-CD-*g*-PDMA, steady-state fluorescence spectrum was recorded at a FLS920 spectrofluorometer (Edinburgh Co. UK) with a slit of 1 nm for both excitation and emission. The excitation wavelength was 335 nm, and pyrene was used as the probe. The intensity ratio of the third band to the first band of the pyrene emission spectrum, I_3/I_1, was used to indicate the polarity of the pyrene environment.

Characterizations

The chemical structures of the ATRP initiator 20Cl-β-CD and resultant star polymer β-CD-*g*-PDMA were confirmed by FT-IR analysis as shown in Figure S1. Comparing the FT-IR spectra of the original β-CD, the ATRP inititor 20Cl-β-CD and the star polymer β-CD-*g*-PDMA (Figure S1(a, b, c)), we can find that the symmetrical and asymmetrical stretching of the methyl and methylene group can be observed at 2990-2850 cm^{-1}. The stretch vibration for C=O in 20Cl-β-CD (Figure S1(b)) and β-CD-*g*-PDMA (Figure S1(c)) appears at 1754 cm^{-1}. Beside this, it has been found that the absorbance at 3350-3450 cm^{-1}, which is ascribed to hydroxyl groups of β-CD, has disappeared in Figure S1(b), indicating the formation of ATRP initiator 20Cl-β-CD. While the stronger band displays at 3350-3450 cm^{-1} again in Figure S1(c) may be attributed to the highly hydrophilic property of β-CD-*g*-PDMA star polymers.

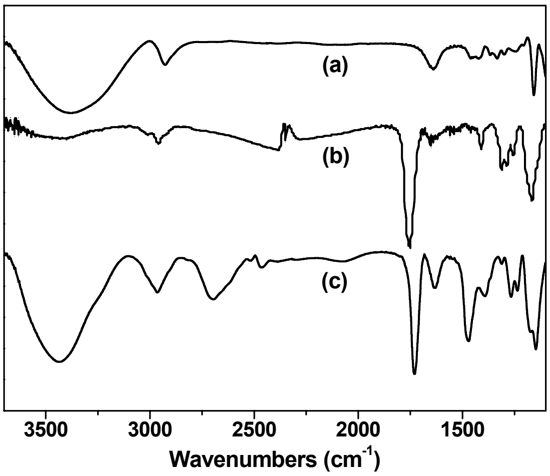


Figure S1. FT-IR spectra of (a) the original β-CD, (b) ATRP initiator 20Cl-β-CD, and (c) β-CD-g-PDMA star polymer.

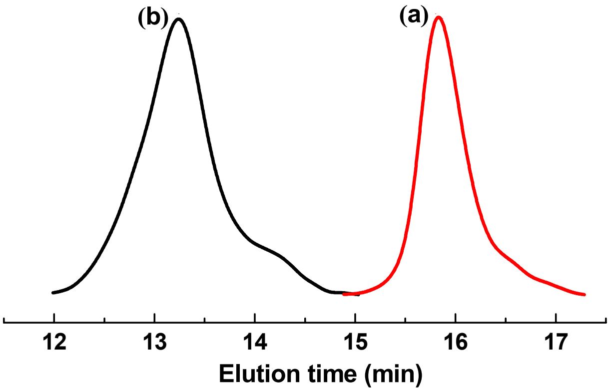


Figure S2. Typical GPC plots showing (a) ATRP initiator 20Cl-β-CD and (b) star polymer β-CD-*g*-PDMA$_{10}$ which was prepared according to a molar feed ratio [DMA]/[20Cl-β-CD (0.1 g)]/[CuBr]/[bpy] of 210: 1.0: 21.0: 42.0.

The number average molecular weights and polydispersity indexes of initiator and star polymers were determined by GPC analysis. Figure S2 depicts the GPC elution process of ATRP initiator 20Cl-β-CD (Figure S2(a)) and star polymer β-CD-*g*-PDMA$_{10}$ (Figure S2(b)). It is obvious that curve b represented β-CD-*g*-PDMA$_{10}$ moves toward higher molecular weight compared with curve a (20Cl-β-CD). Moreover, there is no detectable homopolymer in the star polymer, indicating that DMA monomer has reacted with the halide completely. The molecular weights ($\overline{M}_{n,\mathrm{GPC}}$) and polydispersity index (PDI) measured by GPC, along with theoretical molecular weights ($\overline{M}_{n,\mathrm{th}}$) calculated by the original feed molar ratios, can be shown for 20Cl-β-CD: $\overline{M}_{n,\mathrm{GPC}}$ = 1880 g mol^{-1}, $\overline{M}_{n,\mathrm{th}}$ = 1920 g mol^{-1}, PDI ($\overline{M}_w / \overline{M}_n$) = 1.05; and for β-CD-*g*-PDMA$_{10}$: $\overline{M}_{n,\mathrm{GPC}}$ = 11530 g mol^{-1}, $\overline{M}_{n,\mathrm{th}}$ = 16620 g mol^{-1}, PDI ($\overline{M}_w / \overline{M}_n$) = 1.19.

Thermogravity Analysis (TGA)

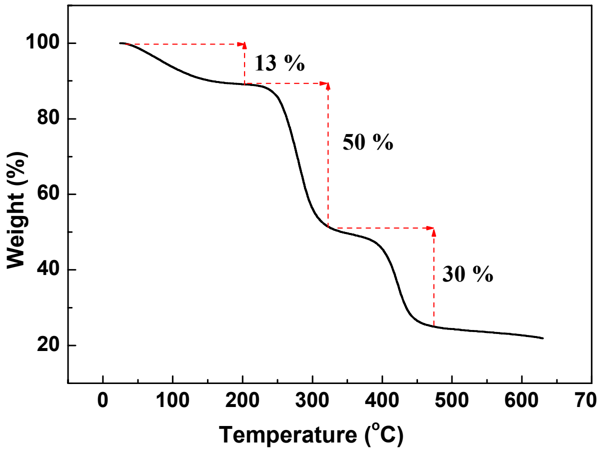


Figure S3. TGA curve of the star polycation β-CD-*g*-PDMA. The heating rate was 10 °C/min during the analysis under a nitrogen atmosphere over a temperature range of 25-600 °C. The second heating cycle was recorded.

It is worth noting that not all the twenty-one alkyl chloride initiating sites were capable of chain extension as a result of the steric effect. Thus, the reaction efficiency of the trigger points is only about 50%. Evidence for the polymer structure was obtained by TGA analysis. The first weight loss is about 13% in the temperature range (25 to 200 °C) corresponding to that ATRP initiating sites chloride while the second one is about 50%. This second weight loss in the temperature range (200-330 °C) is possibly due to the growth of some PDMA chains outside far away from the β-CD. However, the growth of some PDMA chains could occur inside the cavity of the β-CD so the third weight loss 30% can be explained in the temperature range (350-450 °C). In the elemental analysis (N: 7.124%; C: 52.77%; H: 8.239), we can easily found that the content of chloride and PDMA in the β-CD-*g*-PDMA star polymer were 13.647% and 79.865%, respectively, through a simple calculate, which is correspond with the DSC results (Cl: 13%; DMA: 80%).

Fluorescence Spectra of Star Polycation β-CD-*g*-PDMA$_{30}$

To understand better the amphiphilic behavior of the star polymer β-CD-*g*-PDMA$_{30}$, we selected a sample with the arm length of 30 DMA repeating units to investigate its critical aggregation concentration (cac). Fluorescence probe is a powerful method to study cac values of amphiphilic copolymer solutions. A higher peak ratio of the intensity of the third band (383 nm, I_3) to the first band (373 nm, I_1) of pyrene, which was obtained from the emission spectra of pyrene, was observed when pyrene was located in a more hydrophobic environment. Figure S4 shows the intensity ratio I_3/I_1 versus the polymer concentrations of β-CD-*g*-PDMA$_{30}$ star polymers in PBS 7.4 buffer solution, from which we can obtain cac value of about 0.9 g L^{-1}.

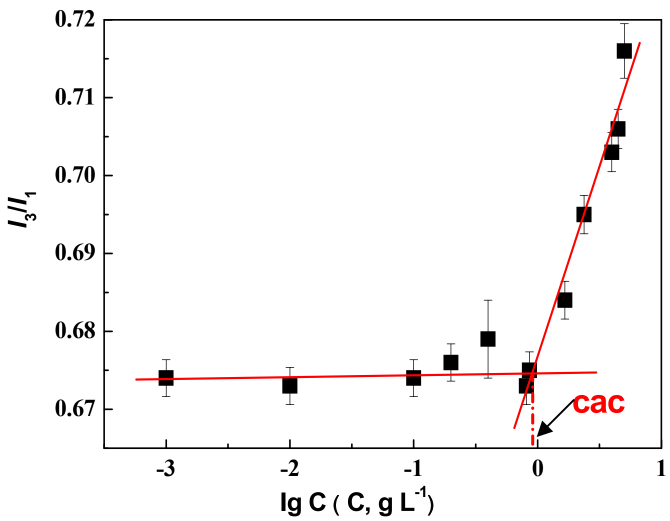


Figure S4. Intensity ratio I_3/I_1 obtained from the fluorescence excitation spectra of pyrene plotted versus the concentrations of β-CD-*g*-PDMA in PBS 7.4 buffer solution measured at 25 °C.

Synthesis and Characterization of Di- and Tri- Block Polyanions

Table S1 lists the number-average molecular weights and PDI of the copolymers measured by GPC. The anionic polyelectrolyte $PMAA_{30}$-*b*-PEG-*b*-$PMAA_{30}$ (or MePEG-*b*-$PMAA_{60}$) was then obtained by the hydrolysis of P*t*BMA_{30}-*b*-PEG-*b*-P*t*BMA_{30} (or MePEG-*b*-P*t*BMA_{60}).

Table S1. Molecular Parameters of MePEG-*b*-P*t*BMA_{60} Diblock Copolymers and P*t*BMA_{30}-*b*-PEG-*b*-P*t*BMA_{30} Triblock Copolymers.

Sample ID	Polymer chemical formula (theor.)	$\overline{M}_{n,\mathrm{th}}$	$\overline{M}_{n,\mathrm{GPC}}$	$\overline{M}_w / \overline{M}_n$
1	MePEG-*b*-P*t*BMA_{30}	6260	6350	1.31
2	MePEG-*b*-P*t*BMA_{60}	10500	9300	1.18
3	MePEG-*b*-P*t*BMA_{60}	10500	9900	1.21
4	MePEG-*b*-P*t*BMA_{60}	10500	10680	1.23

5	P*t*BMA$_{30}$-*b*-PEG-*b*-P*t*BMA$_{30}$	10500	11960	1.21
6	P*t*BMA$_{30}$-*b*-PEG-*b*-P*t*BMA$_{30}$	10500	11150	1.21
7	P*t*BMA$_{30}$-*b*-PEG-*b*-P*t*BMA$_{30}$	10500	9020	1.26
8	P*t*BMA$_{30}$-*b*-PEG-*b*-P*t*BMA$_{30}$	10500	10580	1.16

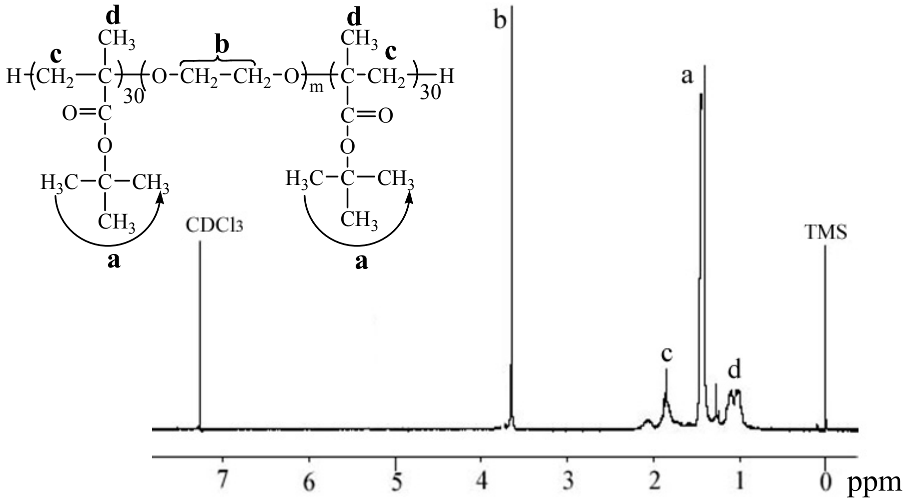


Figure S5. ^{1}H NMR (300 MHz) spectrum of amphiphilic triblock copolymer P*t*BMA$_{30}$-*b*-PEG-*b*-P*t*BMA$_{30}$ in CDCl$_3$.

The chemical structure of P*t*BMA$_{30}$-*b*-PEG-*b*-P*t*BMA$_{30}$ was verified with ^{1}H NMR measurement. Figure S5 shows the ^{1}H NMR spectrum of P*t*BMA$_{30}$-*b*-PEG-*b*-P*t*BMA$_{30}$ and the characteristic signals are identified as follows: ~1.4 ppm (signal a, H of -O-C(C***H***$_3$)$_3$ from P*t*BMA), ~3.6 ppm (signal b, H of -O-C***H***$_2$-C***H***$_2$- from HO-PEG-OH), 1.8-2.0 ppm (signal c, H of -C***H***$_2$- from P*t*BMA), ~1.0 ppm (signal d, H of -C***H***$_3$ from P*t*BMA). After hydrolysis reaction in CH$_2$Cl$_2$, the triblock polyanion PMAA$_{30}$-*b*-PEG-*b*-PMAA$_{30}$ was acquired. From ^{1}H NMR spectrum (Figure S6), we can confirm its chemical structure. The characteristic signal appeared at 12.2 ppm can be ascribed to the proton of carboxyl group (–COO***H***) in PMAA block, indicating the successful conversion of P*t*BMA to PMAA.

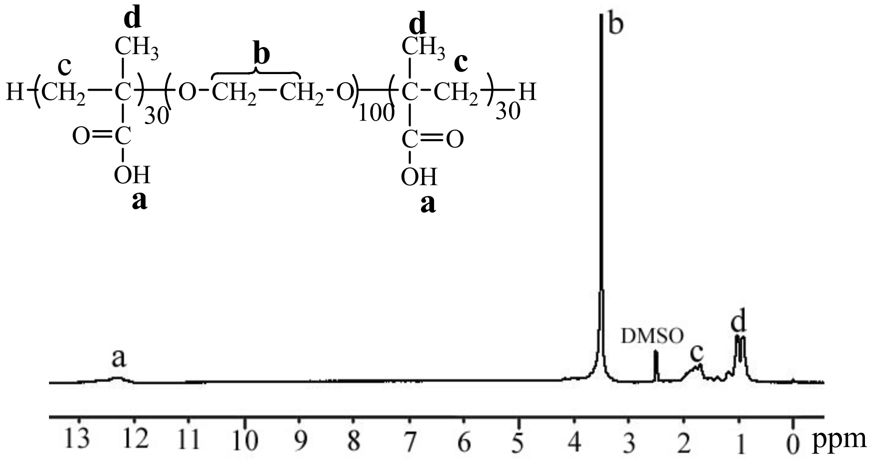
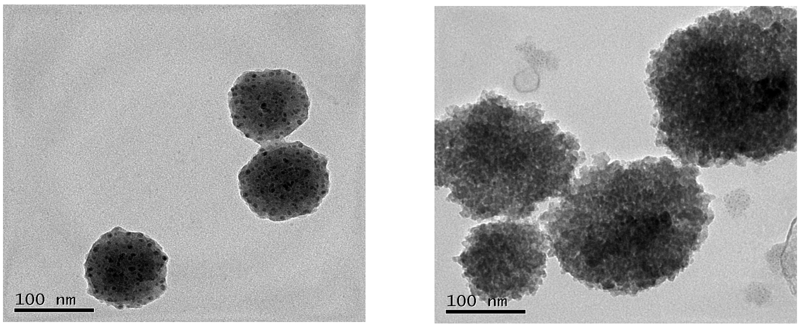


Figure S6. ^{1}H NMR (300 MHz) spectrum of triblock polyanion PMAA$_{30}$-*b*-PEG-*b*-PMAA$_{30}$ in *d*$_6$-DMSO.

TEM images of polyanion (diblock copolymer MePEG-*b*-PMAA$_{60}$ and triblock copolymer PMAA$_{30}$-*b*-PEG-*b*-PMAA$_{30}$)

Figure S7 shows the TEM images of micelles formed from (A) MePEG-*b*-PMAA$_{60}$ and (B) PMAA$_{30}$-*b*-PEG-*b*-PMAA$_{30}$ (bar=100 nm in the two images).



A **B**

Figure S7. TEM images of micelles formed from (A) MePEG-*b*-PMAA$_{60}$ and (B) PMAA$_{30}$-*b*-PEG-*b*-PMAA$_{30}$ (bar=200 nm in the two images).

References

(1) Xu, F. J.; Kang, E. T.; Neoh, K. G. *Biomaterials*. **2006**, *27*, 2787-2796.

(2) Xu, J.; Ni, P. H.; Mao, J. *e-Polymers*. **2006**, 015.

(3) He, J. L.; Ni, P. H.; Liu, C. C. *J. Polym. Sci., Part A: Polym. Chem*. **2008**, *46*, 3029-3041.