Electronic Supplementary Material (ESI) for Polymer Chemistry
This journal is © The Royal Society of Chemistry 2013

Electronic Supplementary Information for:

# Supramolecular micelles with dual temperature and redox responses for multi-controlled drug release

*Weizhong Yuan*[ab], Hui Zou[a], Wen Guo[a],Tianxiang Shen[a] and Jie Ren[ab]*

[a]*Institute of Nano and Bio-polymeric Materials, School of Materials Science and Engineering, Tongji University, 4800Cao'an Road, Shanghai 201804, People's Republic of China. E-mail: yuanwz@tongji.edu.cn; Fax: +86-21-6958-4723; Tel: +86- 21-6958-0234*

[b]*Key Laboratory of Advanced Civil Materials, Ministry of Education, 4800Cao'an Road, Shanghai 201804, People's Republic of China. E-mail: yuanwz@tongji.edu.cn; Fax: +86-21-6958-4723; Tel: +86- 21-6958-0234*

# Contents of supporting information

**1. Materials**
**2. Characterization**
**3. Experimental procedures**
*3.1. Synthesis of 2-Hydroxyethyl 2-Bromoisobutyrate (HEBI)*
*3.2. Synthesis of PEG-SS-COOH*
*3.3. Synthesis of PEG-SS-Br ATRP macroinitiator*
*3.4. Synthesis of PEG-SS-PDMAEMA block copolymer*
*3.5. Preparation of PRX-SS-PDMAEMA*
*3.6. Dox loading and temperature and DTT-mediated in vitro release*
**4. Dynamic Light Scattering (DLS) results**
**References**

## 1. Materials

Poly(ethylene glycol) monomethyl ether (PEG, Fluka) with $M_n$ = 5K was dried by azeotropic distillation in the presence of toluene. 2-(*N*,*N*-dimethylamino) ethyl methacrylate (DMAEMA, Acros Organic, 99%) was dried over $CaH_2$ and distilled under reduced pressure. Ethylene glycol (Aldrich, 99.8%) was dried over CaO and distilled under reduced pressure. Copper bromide (CuBr, Alfa Aesar, 99%) was treated by stirring in glacial acetic acid and washed with ethanol several times. Methylene chloride ($CH_2Cl_2$), tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), and triethylamine ($Et_3N$), were dried over $CaH_2$ and distilled before use. *N,N',N',N'',N''*-Pentamethyldiethylenetriamine (PMDETA, Acros Oganic, 99%), 2-bromoisobutyryl bromide (BiBB, Alfa Aesar, 99%), α-cyclodextrin (α-CD, Aldrich, 98%), 3,3′-dithiodipropionic acid (DTDP, Aldrich, 99%), doxorubicin hydrochloride (Dox·HCl, Aldrich, 98%), DL-dithiothreitol (DTT, Aldrich, 99%), dicyclohexylcarbodiimide (DCC, Alfa Aesar, 99%) and 4-dimethylaminopyridine (DMAP, Fluka, 99%) were used as received.

## 2. Characterization

*Nuclear magnetic resonance (NMR).* $^1$H NMR spectra of samples were obtained from a Bruker DMX 500 NMR spectrometer with $CDCl_3$ and $D_2O$ as solvents. The chemical shifts were relative to tetramethylsilane.

*Gel permeation chromatography (GPC).* GPC analysis was carried out with a HLC-8320 (Tosoh, Japan) analysis system with two columns (TSK gel super AWM-H×2, R0091+R0093), using DMF with 10 mM LiBr as eluents at a flow rate of 0.6 mL min$^{-1}$ at 40$^o$C. PMMA calibration kit was used as the calibration standard.

*Dynamic light scattering spectrophotometer (DLS).* The hydrodynamic radius ($R_h$) of the copolymer micelles was investigated using DLS techniques. The experiments

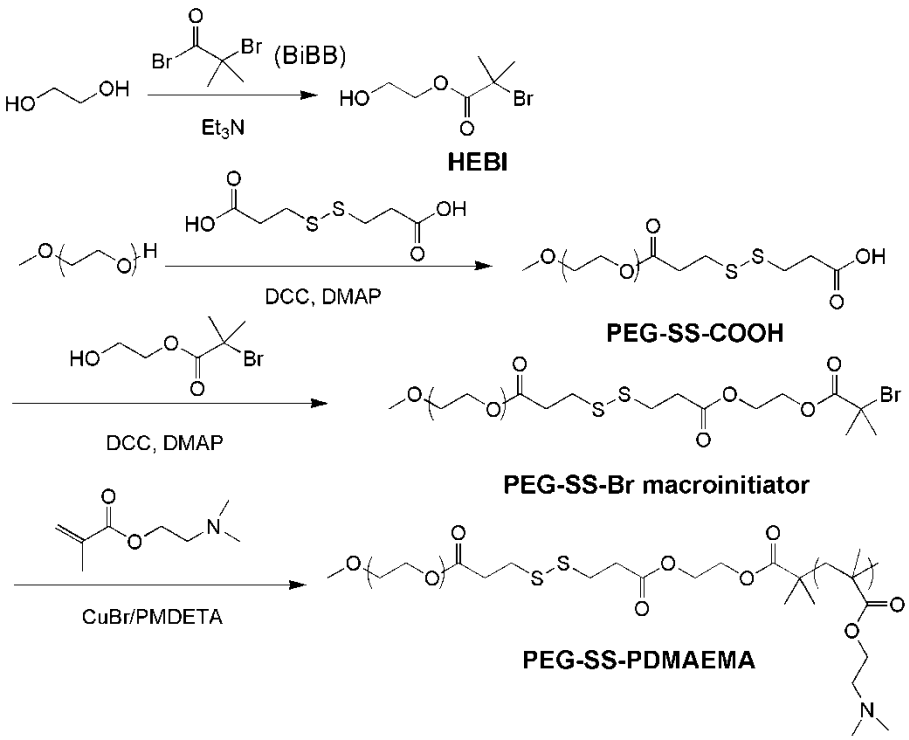
were performed on a Malven Autosizer 4700 DLS spectrometer. DLS was performed at a scattering angle 90°. The $R_h$ was obtained by a cumulant analysis.

*X-ray Diffraction (XRD).* XRD patterns were recorded with a Rigaku D/max 2500 X-ray powder diffractometer using Cu K$\alpha$ (1.54 Å ) radiation (50 kV, 250 mA). All samples were scanned from $2\theta = 3^\circ$ to $50^\circ$ at a speed of $5^\circ$ min$^{-1}$.

*Transmission electron microscopy (TEM).* The morphology of copolymer micelles was observed with a JEOL JEM-2010 TEM at an accelerating voltage of 120 kV. The samples for TEM observation were prepared by placing 10 μL of copolymer micelles solution on copper grids coated with thin films and carbon.

*Fluorescence.* Fluorescence spectra were performed on a Fluorolog-2 spectrofluorometer (Spex Industries, Edison, NJ) under the control of the dedicated SPEX DM3000F software. Fluorescence scans were performed at room temperature in the range of 400-800 nm using increment of 1 nm, and an excitation wavelength of 470 nm. The slit widths were set at 10 nm for both the excitation and the emission.

## 3. Experimental Procedures




**Scheme S1** Synthesis of PEG-SS-PDMAEMA copolymer

*3.1. Synthesis of 2-Hydroxyethyl 2-Bromoisobutyrate (HEBI)*

HEBI was synthesized by the reaction of BiBB with excess anhydrous ethylene glycol in the presence of $Et_3N$ according to the literature.[1,2] A typical reaction was as follows: BiBB (7.497 g, 32.7 mmol) was added dropwise to the THF solution of ethylene

glycol (46.138 g, 74.4 mmol) and triethylamine (3.303 g, 32.7 mmol) at 0°C for 2 h under vigorous stirring. The reaction was continued for another 2 h at 0°C and then heated to 50°C for 5 h. The reaction mixture was concentrated by rotary evaporator and then added to 500 mL of deionized water. The aqueous solution was extracted with chloroform for three times, and then the chloroform layer was washed successively with diluted HCl, saturated $NaHCO_3$, and deionized water. After removal the solvent by evaporating through rotary evaporator, the crude product was obtained. The purified product was collected through distillation under vacuum (yield: 65%). $^1$H NMR (δ, ppm, $CDCl_3$): 1.98 (s, 6H, $(C\boldsymbol{H_3})_2$-CBr-), 3.90 (t, 2H, $-CH_2-C\boldsymbol{H_2}-OH$), 2H), 4.34 (t, 2H, $-C\boldsymbol{H_2}-CH_2-OH$) (Fig. S1).

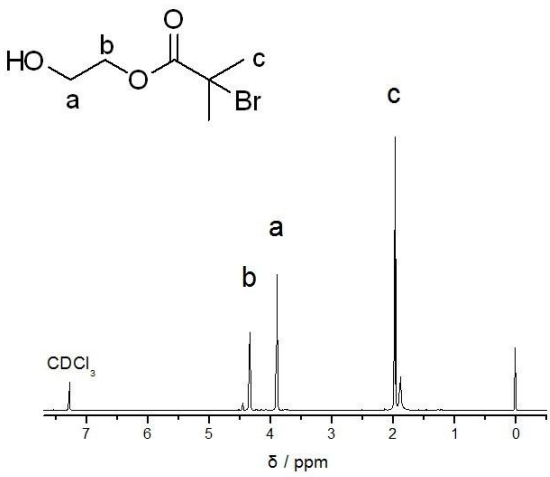



**Fig. S1** $^1$H NMR spectrum of HEBI

*3.2. Synthesis of PEG-SS-COOH*

PEG-SS-COOH was obtained by the reaction of PEG with excess DTDP in the presence of DCC and DMAP. The PEG ($M_n$ = 5000 kg mol$^{-1}$, 8.000 g, 1.6 mmol), DTDP (0.630 g, 3.0 mmol), DMAP (0.195 g, 1.6 mmol), DCC (0.640 g, 3.2 mmol), and $Et_3N$ (0.162 g, 1.6 mmol) were dissolved in 50 mL of anhydrous THF. And the reaction was carried out at room temperature for 48 h under vigorous stirring. The reaction byproduct dicyclohexylcarbodiurea (DCU) was removed by filtration. The final product was collected by precipitation into an excess of cold diethyl ether and dried in vacuum at room temperature until constant weight (yield: 84%). $^1$H NMR (δ, ppm, $CDCl_3$): 3.66 (s, $-C\boldsymbol{H_2}C\boldsymbol{H_2}O-$), 3.40 (s, $-OC\boldsymbol{H_3}$), 2.91-2.99 (m, $-CH_2-C\boldsymbol{H_2}-SS-C\boldsymbol{H_2}-CH_2-$), 2.73-2.81 (m, $-C\boldsymbol{H_2}-CH_2-SS-CH_2-C\boldsymbol{H_2}-$) (Fig. S2).

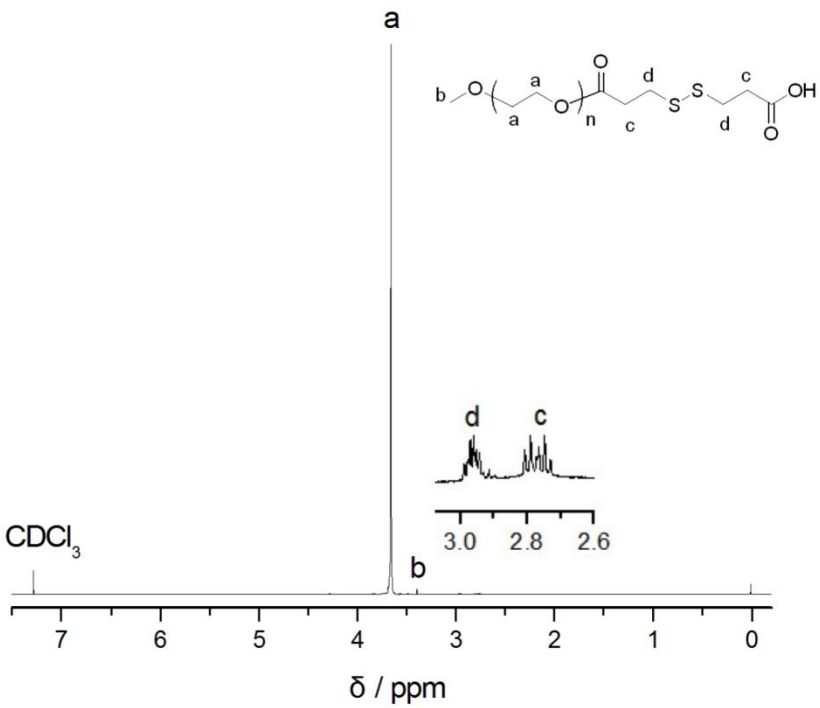



**Fig. S2** $^{1}$H NMR spectrum of PEG-SS-COOH

*3.3. Synthesis of PEG-SS-Br ATRP macroinitiator*

The PEG-SS-Br macroinitiator was synthesized by the reaction between PEG-SS-COOH and HEBI. A 50 mL round-bottom flask was charged with anhydrous $CH_2Cl_2$ (15 mL), PEG-SS-COOH (4.154 g, 0.8 mmol), DMAP (0.098 g, 0.8 mmol), DCC (0.320 g, 1.6 mmol), and excess HEBI (0.34 g, 1.6 mmol). The solution was stirred at room temperature for 48 h. The insoluble byproduct DCU was removed by suction filtration. The filtrate was concentrated by rotary evaporator and precipitated into an excess of cold diethyl ether. The final product was dried in vacuum at room temperature over night (yield: 86%).$^{1}$H NMR (δ, ppm, $CDCl_3$): 4.38 (d, -COO-C$\boldsymbol{H_2}$C$\boldsymbol{H_2}$COO-), 3.65 (s, -C$\boldsymbol{H_2}$C$\boldsymbol{H_2}$O-), 3.38 (s, -OC$\boldsymbol{H_3}$), 2.91-2.94 (t, -$CH_2$-C$\boldsymbol{H_2}$-SS-C$\boldsymbol{H_2}$-$CH_2$-), 2.75-2.79 (t, -C$\boldsymbol{H_2}$-$CH_2$-SS-$CH_2$-C$\boldsymbol{H_2}$-), 1.94 (s, -C(C$\boldsymbol{H_3}$)$_2$Br) (Fig. S3a).

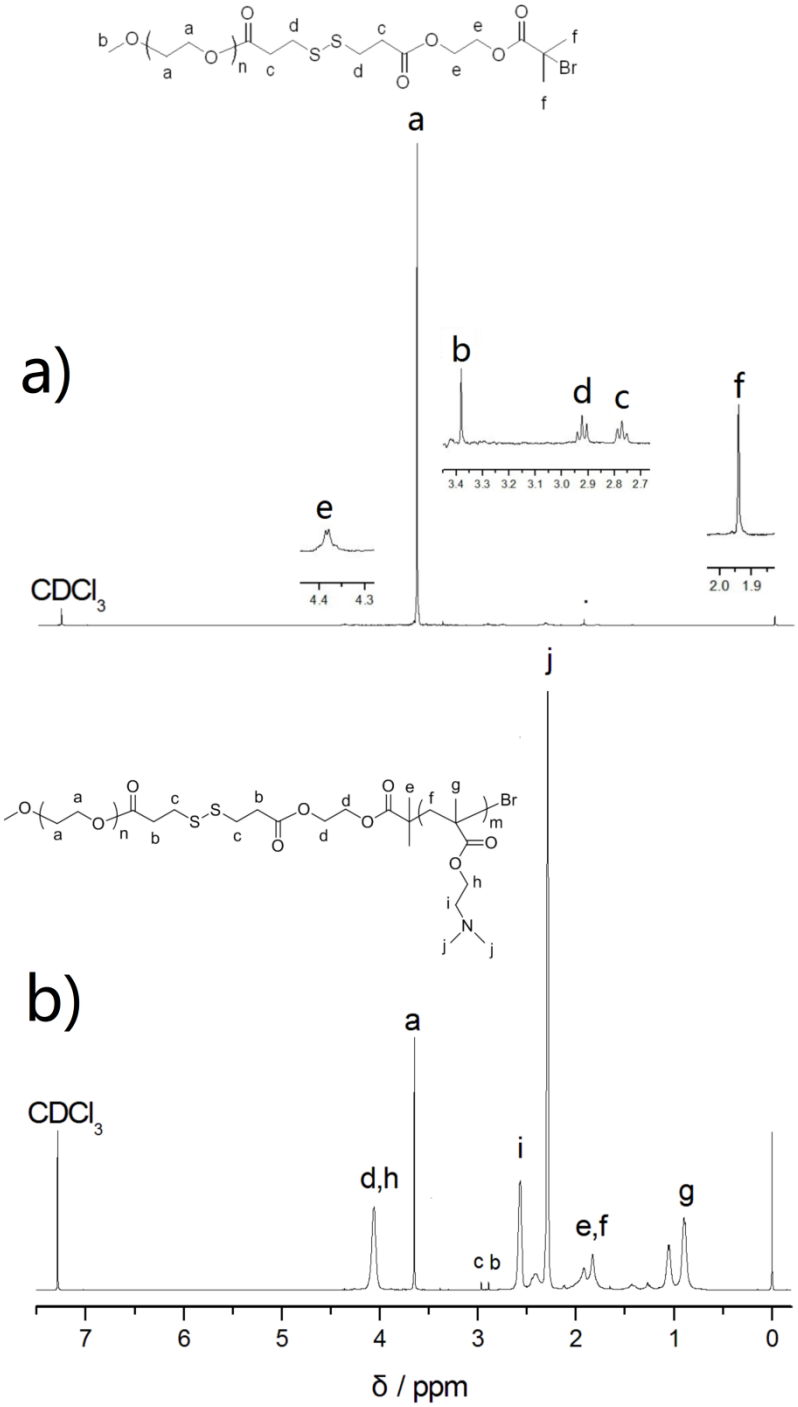



**Fig. S3** $^{1}$H NMR spectra of (a) PEG-SS-Br macroinitiator and (b) PEG-SS-PDMAEMA copolymer.

*3.4. Synthesis of PEG-SS-PDMAEMA block copolymer*

The double hydrophilic PEG-SS-PDMAEMA copolymer was prepared by ATRP of DMAEMA with PEG-SS-Br as the macroinitiator. The general procedure was as follows. A dried 25 mL round-bottom flask with a magnetic stirrer was charged with CuBr (0.012 g, 0.086 mmol), PEG-SS-Br macroinitiator (0.463 g, 0.086 mmol),

DMAEMA (4.3 g, 27.388 mmol), PMDETA (18 μL, 0.086 mmol), and anhydrous DMF (2 mL). The flask was degassed with three freeze-evacuate-thaw cycles. Then, the polymerization was performed at 60°C. After 4 h, the reaction system was exposed to air to stop the polymerization. The mixture was diluted with THF and passed through a neutral alumina column to remove the copper catalysts. The eluent was concentrated by rotary evaporator and then precipitated with an excess of *n*-hexane. The product was dried in vacuum at room temperature until constant weight (yield: 95%). $^1$H NMR (δ, ppm, $CDCl_3$): 4.05 (s, -COO-C***H₂***$CH_2N(CH_3)_2$), 3.65 (s, -C***H₂***C***H₂***O-), 2.91-2.94 (t, -$CH_2$-C***H₂***-SS-C***H₂***-$CH_2$-), 2.75-2.79 (t, -C***H₂***-$CH_2$-SS-$CH_2$-C***H₂***-), 2.56 (s, -COO-$CH_2$C***H₂***$N(CH_3)_2$), 2.28 (s, -N(C***H₃***)$_2$), 1.68-2.10 (m, -$C(CH_3)_2$-C***H₂***-$C(CH_3)$(Br)-), 0.75-1.17 (m, -$CH_2$-C(C***H₃***)(Br)-) (Fig. S3b).

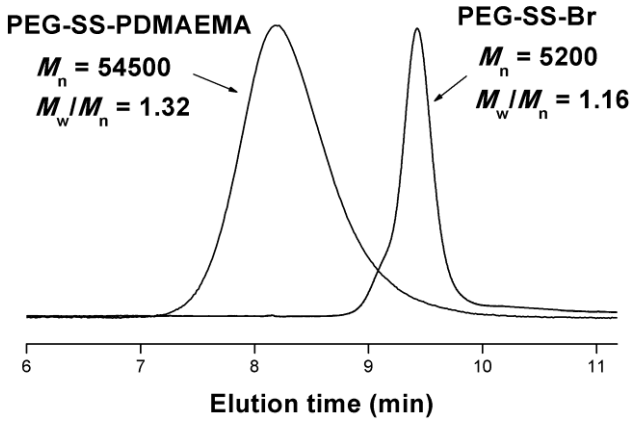



**Fig. S4** GPC traces of PEG-SS-Br macroinitiator and PEG-SS-PDMAEMA copolymer.

*3.5. Preparation of PRX-SS-PDMAEMA*

PRX-SS-PDMAEMA was prepared via the inclusion complexation between α-CD and PEG chains of PEG-SS-PDMAEMA copolymer. A typical procedure is as follows: 1.0 g of PEG-SS-PDMAEMA was added into the saturated aqueous solution of α-CD. The reaction carried out for 48 h under stirring at room temperature. The free α-CD was removed by dialysis against deionized water for 48 h (molecular weight cut-off: 3000 Da). The resulting product was obtained by freeze-drying of the dialysis solution. XRD patterns of PEG-SS-PDMAEMA/α-CD, PEG-SS-PDMA and α-CD were shown in Fig. S5.

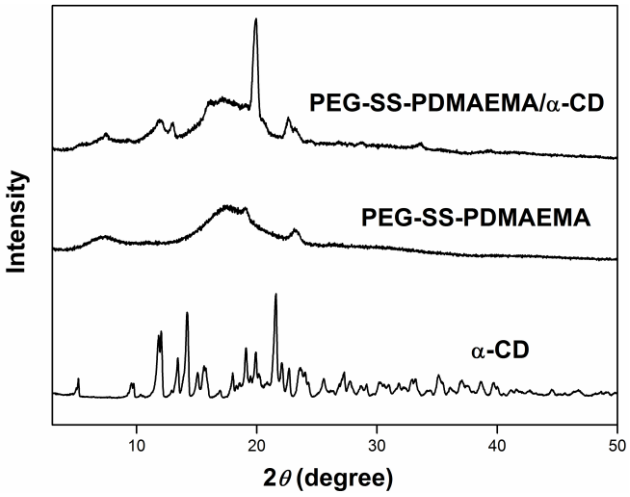



**Fig. S5** XRD patterns of PEG-SS-PDMAEMA/α-CD, PEG-SS-PDMA and α-CD.

*3.6. Dox loading and temperature and DTT-mediated in vitro release*

Dox·HCl (8 mg) and $Et_3N$ (3 mL) were dissolved in DMSO (15 mL), and the mixture was stirred overnight. Then PRX-SS-PDMAEMA copolymer (40 mg) was added. The solution was subjected to dialysis against 1 L of PBS (pH 7.4, 10 mM) using a dialysis bag with Mw cut-off of 8000-14000 Da at room temperature for 24 h. The copolymer concentration of the drug-encapsulated micellar solution was adjusted to 1.0 mg $mL^{-1}$. A typical *in vitro* drug release procedure was as follows: 2.0 mL of drug loaded micellar solution was transferred into a dialysis bag with Mw cut-off of 8000-14000 Da and then immersed in 50 mL of PBS (pH 7.4, 10 mM). The Dox release profile was determined by fluorescence measurement (excitation at 470 nm and emission at 559 nm, respectively). Different media was used in studying the release profile, i.e. PBS ( pH 7.4, 10 mM) at room temperature, PBS (pH 7.4, 10 mM) at 40 $^{o}$C, PBS (pH 7.4, 10 mM) at 50 $^{o}$C, PBS (pH 7.4, 10 mM) with 1 mM DTT at 25$^{o}$C, PBS (pH 7.4, 10 mM) with 5 mM DTT at 25$^{o}$C, PBS (pH 7.4, 10 mM) with 10 mM DTT at 25$^{o}$C, and PBS (pH 7.4, 10 mM) with 10 mM DTT at 50$^{o}$C. At desired intervals, 2 mL of release media was taken out and the equal volume of fresh media was added into the drug-release system. The amount of Dox released from the drug-loaded micelles was determined by fluorescence measurement (excitation of 470 nm and emission of 559 nm, respectively).

And the encapsulation efficiency (EE%) of Dox was calculated as:

EE [%] = (mass of Dox in micelles/mass of total Dox added) ×100 %

The cumulative amount of Dox released from the micelles was calculated as:

Cumulative Dox release [%] = ($M_t/M_0$) ×100 %

where $M_t$ is the total amount of Dox released from the micelles at time t, and $M_0$ is the amount of Dox initially loaded into the micelles.

## 4. Dynamic Light Scattering (DLS) results

PEG-SS-PDMAEMA is a double hydrophilic copolymer at room temperature and can easily dissolve in water to form copolymer solution. However, PDMAEMA is a typical thermoresponsive polymer and exhibits a lower critical solution temperature (LCST) in water and the LCST of PDMAEMA homopolymer is in the range of 32-46$^{o}$C depending on its molar mass.[3,4] When the temperatures are higher than the LCST, the hydrophilic PDMAEMA chains become hydrophobic. The disulfide bond linking PEG and PDMAEMA blocks as a junction has the responsive behavior to external reducing agents (such as DTT). Due to temperature and redox responsive behaviors, PEG-SS-PDMAEMA copolymer can be considered as a multi-stimuli responsive copolymer.

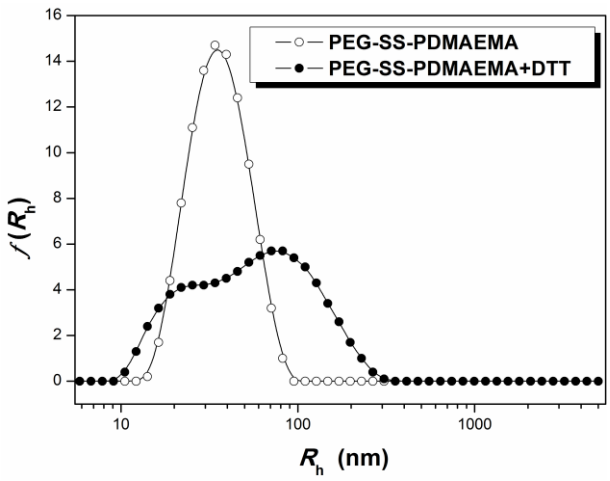



**Fig. S6** Micellar hydrodynamic radius ($R_h$) distributions at 45 $^{o}$C and at 45 $^{o}$C with10 mM DTT for PEG-SS-PDMAEMA aqueous solutions (copolymer concentration: 5 mg mL$^{-1}$).

Fig. S6 shows the micellar hydrodynamic radius ($R_h$) distributions at 45 $^{o}$C and at 45 $^{o}$C with 10 mM DTT for PEG-SS-PDMAEMA aqueous solutions. From the $R_h$ distribution, it can be seen that the PEG-SS-PDMAEMA copolymer can self-assemble into micelles at 45 $^{o}$C ($R_h$ = 33.14 nm, PDI = 0.118), which indicates PEG-SS-PDMAEMA became amphiphilic copolymer. At this temperature, hydrophobic PDMAEMA chains are mainly in the core and permanently hydrophilic PEG chains are mainly in the shell of the micelles. In order to investigate the redox responsive behavior of PEG-SS-PDMAEMA copolymer micelles, DTT solution (10 mM) was added into the PEG-SS-PDMAEMA micelle solution at 45 $^{o}$C, and the mixture was stirred vigorously in argon atmosphere at 45$^{o}$C for 48 h. As shown in Fig. S5, the $R_h$ distribution of micelle solution became much wider (PDI = 1.000) than that

without adding DTT, indicating that the micelles were destroyed seriously after adding DTT with high concentration. It is because the disulfide bonds were broken by the reaction with DTT. The result suggests that the PEG-SS-PDMAEMA has excellent sensitivity to reducing agent.

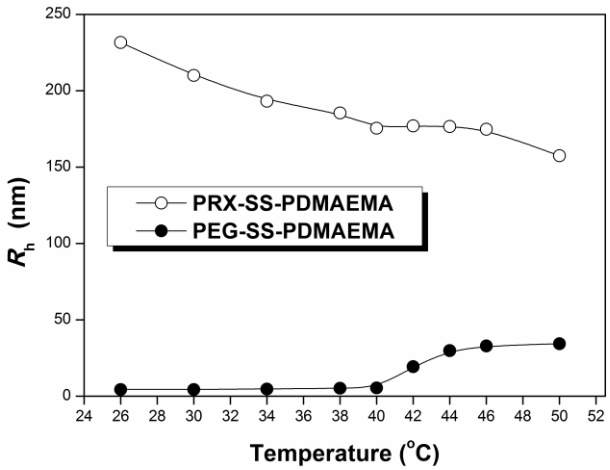



**Fig. S7** Temperature dependence of hydrodynamic radius ($R_h$) for PEG-SS-PDMAEMA and PRX-SS-PDMAEMA micelle solutions (concentration: 5 mg mL$^{-1}$)

Fig. S7 shows the temperature dependence of hydrodynamic radius ($R_h$) for PEG-SS-PDMAEMA aqueous solution and PRX-SS-PDMAEMA micelle solution. For PEG-SS-PDMAEMA, when the temperature is below 40 $^{o}$C, the average $R_h$ was about 5 nm. This indicated that PEG-SS-PDMAEMA copolymer is molecularly dissolved in water.[5] When the temperature is above 40 $^{o}$C, micellization starts to occur, accompanied with a dramatic increase of $R_h$. The bluish tinge phenomenon for micellar solutions occurred immediately during heating process. The result indicates that the self-assembly behavior occurs and the self-assembled micelles are formed when the temperature is above 40 $^{o}$C. For PRX-SS-PDMAEMA, at low temperature range (such as 26 $^{o}$C), the micelle size is large because the micelles are composed of hydrophilic PDMAEMA shell and hydrophobic stiff PRX core. With the increase of temperature, some α-CD molecules are dissociated from PDMAEMA chains, and the PRX became more flexible. Therefore, the core size of the micelles became smaller, which led to the micelle size became smaller accordingly. When the temperature increases to 40-46 $^{o}$C (above the LCST of PDMAEMA), the micelle size is smaller than that at lower temperatures but changed slightly, because the PDMAEMA shell became hydrophobic and collapsed onto the core at this stage. When the temperature reaches 50 $^{o}$C, PDMAEMA shell is still hydrophobic but more α-CD molecules are dissociated from PDMAEMA chains, which led to the PRX further became more flexible and more naked PEG segments transferred to shell and the micelles became more stable and smaller in size.

# References

1 K. N. Jayachandran, A. Takacs-Cox and D. E. Brooks, *Macromolecules*, 2002, **35**, 4247–4257.

2 D. M. Haddleton, C. Waterson, P. J. Derrick, C. B. Jasieczek and A. J. Shooter, *Chem. Commun.*, 1997, **7**, 683-684.

3 V. Butun, S. P. Armes and N. C. Billingham, *Polymer*, 2001, **42**, 5993-6008.

4 F. A. Plamper, M. Ruppel, A. Schmalz, O. Borisov, M. Ballauff and A. H. E. Müller, *Macromolecules*, 2007, **40**, 8361-8366.

5 Y. M. Zhou, K. Q. Jiang, Q. L. Song and S. Y. Liu, *Langmuir*, 2007, **23**, 13076-13084.