Electronic Supplementary Information for:

Thermo- and glucose-responsive micelles self-assembled from phenylborate ester-containing brush block copolymer for controlled release of insulin at physiological pH

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1 Materials

S-dodecyl-S'-(α,α'-dimethyl-α''-acetic acid) trithiocarbonate (DDMAT) was synthesized according to the literature.[1] 2,2'-Azobisisobutyronitrile (AIBN, Aldrich, 98%) was recrystallized from ethanol. *p*-Chloromethylstyrene (CMS) (Aldrich, 90%), anisole, Triethylamine (TEA), methylene dichloride (CH_2Cl_2) were purified by vacuum distillation from CaH_2. *N,N,N',N'',N''*-Pentamethyl diethylenetriamine (PMDETA) (Acros Oganic, 99%), sodium azide (NaN_3, Alfa Aesar) were used as received. Copper bromide (CuBr, Alfa Aesar, 99%) was treated by stirring in glacial acetic acid and washed with ethanol several times. 2-(2-Methoxyethoxy)ethyl methacrylate (MEO_2MA) and oligo(ethylene glycol) methacrylate (OEGMA, M_n=475 g/mol) was purchased from Aldrich and passed through a column of activated basic alumina to remove inhibitors. Phenylboronic acid (PBA), acryloyl chloride, 1,1,1-tris(hydroxymethyl) propane, merrifield resin, propargyl alcohol and 2-bromoisobutyryl bromide was purchase from Aldrich and used as received.

2 Characterization

Attenuated total internal reflectance fourier transform infrared (ATR FT-IR). ATR FT-IR spectra of samples were recorded on an EQUINOSS/HYPERION2000 spectrometer (Bruker, Germany).

Nuclear Magnetic Resonance (NMR). ^{1}H NMR spectra of samples were obtained from a Bruker AVANCE-400MHz NMR spectrometer with DMSO-d_6, $CDCl_3$ or D_2O as solvent. 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. PS calibration kit was used as the calibration standard.

Optical Transmittances. The optical transmittances of copolymer micelles aqueous solution (3 mg/mL) at various temperatures were measured at a wavelength of 500 nm on a UV-visible spectrophotometer (Lambda 35, PerkinElmer). The temperature of the sample cell was thermostatically controlled using an external superconstant temperature bath. The solutions were equilibrated for 10 min at each measuring temperature. The lower critical solution temperature (LCST) values of the copolymer micelles solutions were defined as the temperature producing a 50% decrease in optical transmittance.

Dynamic Light Scattering (DLS). Morphology of the micelles/aggregates of brush block polymers in water was investigated using DLS techniques. The experiments were performed on a Malvern Autosizer 4700 DLS spectrometer. DLS was performed at a scattering angle 90°. The R_h was obtained by a cumulant analysis.

Transmission Electron Microscopy (TEM). The morphology of brush block polymers micelles/aggregates 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 the vesicular or micellar solutions on copper grids coated with thin films and carbon.

Critical Micelle Concentration (CMC) Measurement. CMC was determined using pyrene as a fluorescence probe. 10 μL of pyrene (0.45 mg/mL) in acetone was added to a series of 10.0 mL volumetric flasks. After the acetone evaporated, a measured amount of P(St-*g*-PBDEMA)-*b*-P(MEO_2MA-*co*-OEGMA) solution was added to each flask, then added followed by doubly distilled water. The flasks were kept for 12 h to equilibrate the pyrene and the micelles. The fluorescence spectra were recorded using

a Hitachi F2500 luminescence spectrometer (Hitachi, Ltd.) with an excitation wavelength of 335 nm. The emission wavelengths at 381 nm and 377 nm were monitored. The CMC value was chosen as the concentration when pyrene exhibited an apparent decrease in the I_{381}/I_{377} ratio with an increasing concentration of the copolymer, indicating that the aggregation of the copolymer occurred.

3 Experimental procedures

3.1 Synthesis of poly(p-chloromethylstyrene) (PCMS) by RAFT

DDMAT (0.476 g, 1.312 mmol), *p*-chloromethylstyrene (4.4044 g, 28.864 mmol) and AIBN (43 mg, 0.262mmol) were dissolved in anisole (2 mL). The mixture was degassed with three freeze-evacuate-thaw cycles. The polymerization reaction was performed at 70℃ for 24 h. Then, the product mixture was diluted by the same volume of dry THF, and the final product was obtained by precipitation in *n*-hexane and drying under vacuum for 24 h. $M_{n,NMR}$=2020 g/mol. ATR FTIR (cm^{-1}): 2820-3046 (ν_{C-H}). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.25-7.51 (arom. C*H* of styrenic ring), 4.67(CH_2Cl), 0.85-1.92 ((CH_3)$_2$C, CH_2CH, $C_{11}H_{23}$).

3.2 Synthesis of poly(p-azidomethylstyrene) (PAMS)

PCMS (2.003 g, 0.54 mmol) was dissolved in dry DMF (30 mL) and then NaN_3 (3.861 g, 65.5 mmol) was added. The reaction mixture was stirred at room temperature for 48 h and then precipitated in excess of water. The obtained crude product was re-dissolved in dichloromethane and re-precipitated in cold methanol. The resultant white solid was collected by filtration and dried in a vacuum oven for 48 h. $M_{n,NMR}$ =2090 g/mol. ATR FTIR (cm^{-1}): 2814-3046 (ν_{C-H}), 2084 ($\nu_{azide\ group}$). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.21-7.51 (arom. C*H* of styrenic ring), 4.35 (CH_2N_3), 0.66-1.92 ((CH_3)$_2$C, CH_2CH, $C_{11}H_{23}$).

3.3 Synthesis of poly(p-azidomethylstyrene)-block-poly(2-(2-methoxyethoxy)ethyl methacrylate-co-oligo(ethylene glycol) methacrylate) (PAMS-b- P(MEO_2MA-co-OEGMA)) copolymers by RAFT

MEO_2MA (8.6480 g, 46mmol), OEGMA (1.900 g, 4 mmol), AIBN (0.0103 mg, 0.0625 mmol) and PAMS (0.9865 g, 0.5 mmol) were dissolved in dry toluene (43

mL). The mixture was degassed with three freeze-evacuate-thaw cycles. The reaction mixture was stirred at 70 °C for 24 h. After removing toluene, the crude product was diluted with DMF and then dialyzed against deionized water for 48 h to remove the unreacted MEO_2MA and OEGMA monomers. Finally, the purified copolymer was obtained by lyophilization of water. $M_{n,NMR}$= 21080 g/mol. ATR FTIR (cm^{-1}): 2736-3028 (ν_{C-H}), 2091 ($\nu_{azide\ group}$), 1730 ($\nu_{C=O}$). ^1H NMR ($CDCl_3$, δ, ppm): 6.28-7.16 (arom. C*H* of styrenic ring), 4.24 (CH_2N_3), 4.10 ($COOCH_2$), 3.52-3.72 ($CH_2OCH_2CH_2O$), 3.40 (CH_3O), 1.62-2.06 ($CHCH_2$, $CH_2C(CH_3)$), 0.74-1.14 ($(CH_3)_2C$, CH_2CH, $CH_2C(CH_3)$, $C_{11}H_{23}$).

3.4 Synthesis of Propargyl 2-bromoisobutyrate

Propargyl 2-bromoisobutyrate was prepared by the esterification reaction of propargyl alcohol with 2-bromoisobutyryl bromide. In a typical example, propargyl alcohol (11.6 mL, 0.2 mol), triethylamine (27.8 mL, 0.2 mol), and 100 mL of CH_2Cl_2 were added into a 250 mL three-neck round-bottom flask. The mixture was cooled to 0 °C and 2-bromoisobutyryl bromide (24.7 mL, 0.2 mol) was added dropwise over 2 h. The reaction mixture was stirred overnight at room temperature. After removing insoluble salts by suction filtration, the filtrate was further purified by passing through a silica gel column using CH_2Cl_2 as the eluent. After removing all the solvents by a rotary evaporator, the obtained residues were distilled under reduced pressure, yielding a colorless liquid (30.8 g, yield: 75%). ^1H NMR ($CDCl_3$, δ, ppm): 4.72 (2H, $-CH_2O$), 2.53 (1H, $CH\equiv C-$), 1.92 (6H, $-C(CH_3)_2Br$)

3.5 Synthesis of (2-phenylboronic esters-1,3-dioxane-5-ethyl) methylacrylate (PBDEMA) monomer

The synthesis of the monomer involving two steps was modified based on reported methods.1 For the first step, the mixture of phenylboronic acid (5.9 g, 48.4 mmol) and 1,1,1-tris(hydroxymethyl) propane (6.5 g, 48.4 mmol) charged in one three-necked round bottle was stirred in toluene (80 mL) in the presence of small amount of molecular sieves at 120 ºC for 4 h. The reaction solution was filtered, and the crude solid precursor 1 was obtained by tolueneremo val in vacuum. Yield: 9.1 g (85.4%).

^{1}H NMR ($CDCl_3$, δ, ppm): 7.79 (2H, o-C_6H_5), 7.42 (1H, p-C_6H_5), 7.32 (2H, m-C_6H_5), 3.84-4.05 (4H, CH_2OBOCH_2), 3.65 (2H, CH_2OH), 1.53 (2H, CH_2CH$_3$), 0.89 (3H, CH$_2$CH_3);

For the second step, the preparation of monomer PBDEMA was carried out by the reaction of precursor **1** (10.0 g, 45.0 mmol), acryloyl chloride (4.8 g, 54.0 mmol) andtriethy lamine (5.4 g, 54.0 mmol) in anhydrous CH_2Cl_2 (40 mL) in an ice bath for 8 h. After filtration, the concentrated filtrate was purified by column chromatography with mixture of petroleum ether and ethyl acetate (v/v=8/1) as eluent to afford colorless liquid. Yield: 9.7 g (78.6%). ^{1}H NMR ($CDCl_3$, δ, ppm): 7.77 (2H, *o*-C6H5), 7.42 (1H, *p*-C6H5), 7.34 (2H, *m*-C_6H_5), 6.40 (1H, *C*H_2=CH), 6.12 (1H, CH$_2$=C*H*), 5.84 (1H, CH_2=CH), 4.19 (2H, CH_2OOCCH=CH$_2$), 4.05 (2H, CH_2OBOCH$_2$), 3.92 (2H, CH$_2$OBOCH_2), 1.50 (2H, CH_2CH$_3$), 0.92 (3H, CH$_2$CH_3);

3.6 Preparation of alkynyl poly((2-phenylboronic esters-1,3-dioxane-5-ethyl) methylacrylate) (Alkynyl PPBDEMA)

Propargyl 2-bromoisobutyrate (0.205 g, 1 mmol), PMDETA (0.2079 g, 1.2 mmol), PBDEMA (2.74 g, 10 mmol) were dissolved in anisole (2.5 mL). The mixture was degassed with three freeze-evacuate-thaw cycles. At the last cycle, CuBr (0.1728 g, 1.2 mmol) was put into the mixture solution, and then immersed in an oil bath at 80 ^{o}C for 44 h under thermostat control. After a predetermined polymerization time, the cooled reaction solution was diluted with THF and passed through a neutral alumina column to remove the catalyst. The concentrated reaction solution was dropwise added into the mixed solvent of hexane/diethyl ether (v/v=4/1). The polymer was dried in vacuum at room temperature for 24 h to obtain (1.02 g) in 35% yield. $M_{n,NMR}$ =4290 g/mol. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.75 (2H, *o*-C_6H_5), 7.37 (m, 1H, *p*-C_6H_5), 7.29 (2H, *m*-C_6H_5), 4.32(2H, CH≡C-CH_2-)3.76-4.19 (6H, CH_2OOC, CH_2OBOCH$_2$ and CH$_2$OBOCH_2), 2.29 (1H, C*H*CH$_2$), 1.85(6H, C(CH_3)$_2$) 1.60-1.81 (2H, CHCH_2), 1.26 (2H, CH_2CH$_3$), 0.88 (3H, CH$_2$CH_3)

3.7 Preparation of polystyrene-graft-poly(2-phenylboronic esters-1,3-dioxane-5-ethyl) methylacrylate)-block-poly(2-(2-methoxyethoxy)ethyl methacrylate-co-oligo(ethyleneglycol) methacrylate)) (P(St-g-PBDEMA)-b-P(MEO$_2$MA-co-OEGMA))

PAMS-*b*-P(MEO$_2$MA-*co*-OEGMA) (1.2265 g, 0.07 mmol), PBDEMA (3.3018 g, 0.77 mmol), PMDETA(0.133 g, 0.77 mmol) was dissolved in DMF (10 mL). The mixture was degassed with three freeze-evacuate-thaw cycles. At the last cycle, CuBr (0.11 g, 0.77 mmol) was put into the mixture solution, and then immersed in an oil bath at 60 ^{o}C for 48 h under thermostat control, alkynyl-functionalized Wang resin (0.524 g, 0.294 mmol of alkynyl mioeties) was then added. The suspension was kept stirring for another 8 h at 60 ^{o}C. After suction filtration, the filtrate was diluted with DMF, and passed through a neutral alumia column to remove copper catalysts and then dialyzed against deionized water for 48 h to remove solvents. Finally, the purified copolymer was obtained by lyophilization of water. $M_{n,NMR}$=53280 g/mol. ^{1}H NMR (DMSO-d_6, δ, ppm): 6.52-7.53(C_6H_5), 4.43 ($CH_2OOCCHCH_2$), 4.02 (CH_2OBOCH_2, CH_2OOC), 3.96 (CH_2OBOCH_2), 3.52 ($CH_2OCH_2CH_2OCH_3$), 3.43 ($CH_2OCH_2CH_2OCH_3$) , 1.52-2.02 ($CH_3CCH_2CCH_3$), $BrCHCOOCH_2$), 1.18($CHCH_2C(CH_3)$,$COOCH_2CCH(N)$), 0.92(CH_3CH_2), 0.65-0.82 (CH_3C, $CH_2CC_6H_5$, C(CH3)CH_2CH, $CH_3(CH_2)_{11}$, $BrCOOCH$)

3.8 Self-assembly of copolymer

P(St-*g*-PBDEMA)-*b*-P(MEO$_2$MA-*co*-OEGMA) copolymer (30 mg) was dissolved in 10 mL of THF. Then, deionized water was added at a rate of 1 mL/h under vigorous stirring until the solution was changed from transparent to translucent. Then the solution was subsequently dialyzed against deionized water for 72 h (dialysis membrane, molecular weight cut-off: 14000 Da) at room temperature. After diluted by deionized water and equilibrated at room temperature for 48 h, the resulting micelles solution had a concentration of 2 mg/mL for transmittance, DLS and TEM measurements.

3.9 Preparation of insulin-loaded P(St-g-PBDEMA)-b-P(MEO$_2$MA-co-OEGMA) micelles

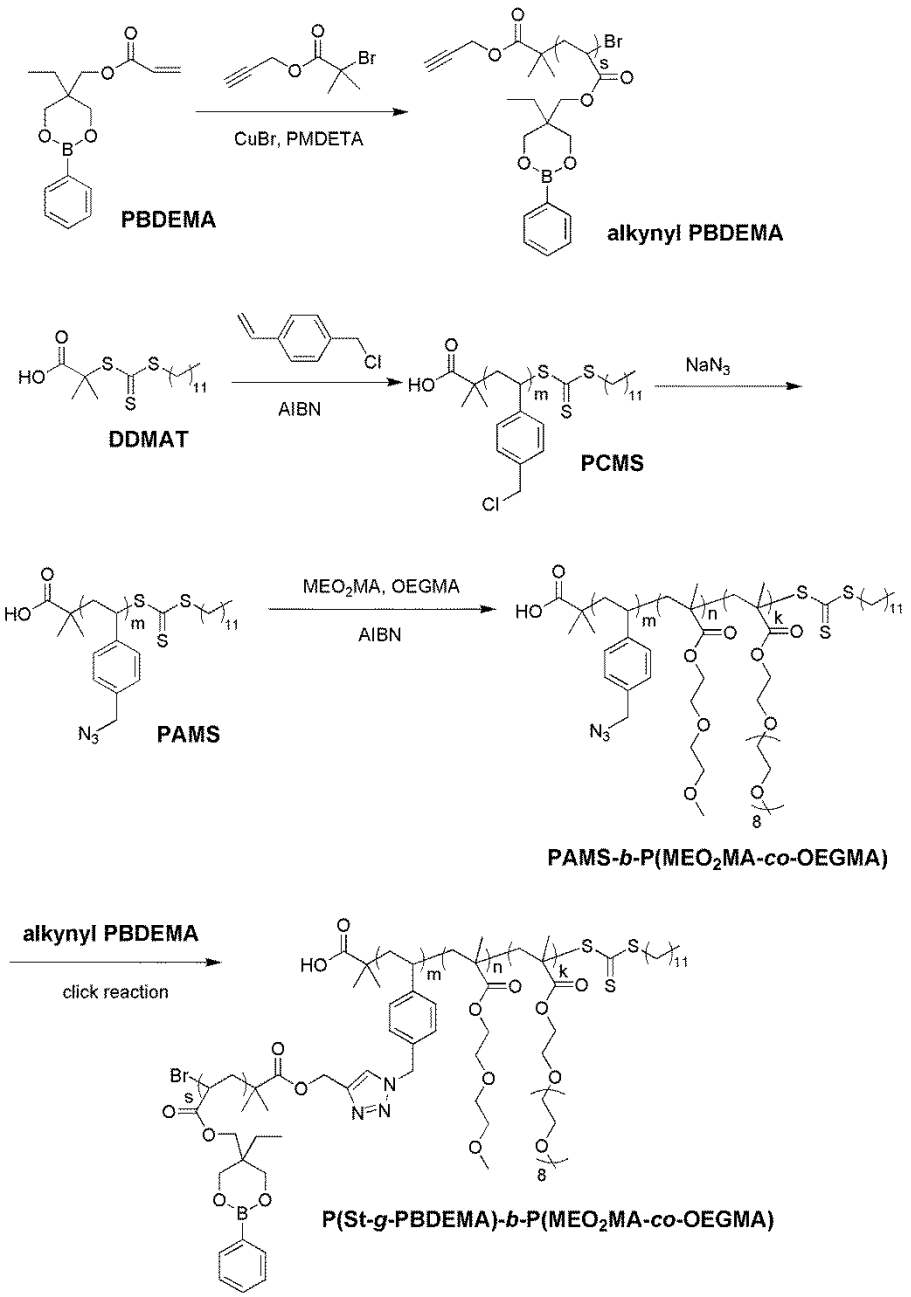
A stock of insulin (10 mg) dissolved in 8 mL of HCl (0.01 mol/L) was adjusted to pH 6.0 using NaOH (0.1 mol/L) and dropwise-added into the copolymer (50 mg) in 1.5 mL of THF with strong stirring in an ice bath overnight. The purification of the polymer micelles encapsulating insulin was performed by dialysis for 24 h and replaced with fresh water at intervals of 6 h. Finally, the mixture was diluted to 2 mg/mL for the next step.

The *in vitro* release test of insulin from the polymeric micelles was evaluated by the dialysis method. A dialysis bag filled with 2 mL of purified insulin-loaded micelles was immersed into 25 mL of PBS (0.05 mol/L) of pH 7.4 at different glucose concentrations. At the set interval, 2 mL of release solution was withdrawn and replaced by 2 mL of fresh glucose PBS.

The cumulative release amount (E) was calculated according to the following formulae:

$$E = \frac{Ve\sum_{1}^{n-1} Ci + V_0 C_n}{m_{insulin}}$$

Here, E was the cumulative release amount. V_e was the replaced PBS volume (2 mL). V_0 was 25 mL. C_n was the concentration of insulin after n times replacements of PBS. $m_{insulin}$ was the content of insulin loading in micelles.



Scheme S1. Synthesis of P(St-*g*-PBDEMA)-*b*-P(MEO_2MA-*co*-OEGMA) Brush Block Polymer.

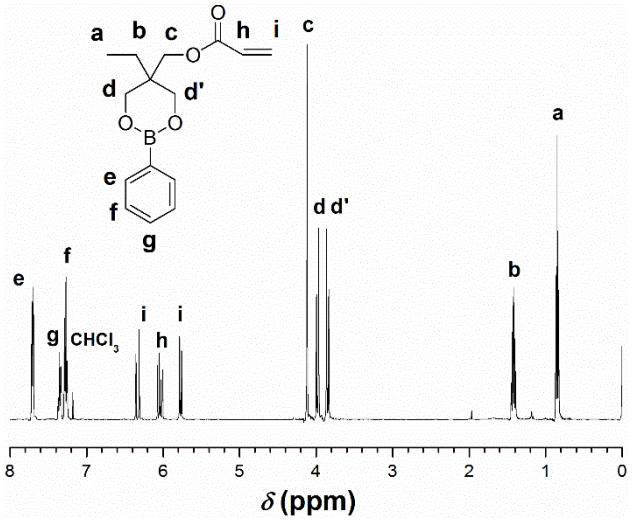


Fig. S1 ^{1}H NMR Spectrum of PBDEMA.

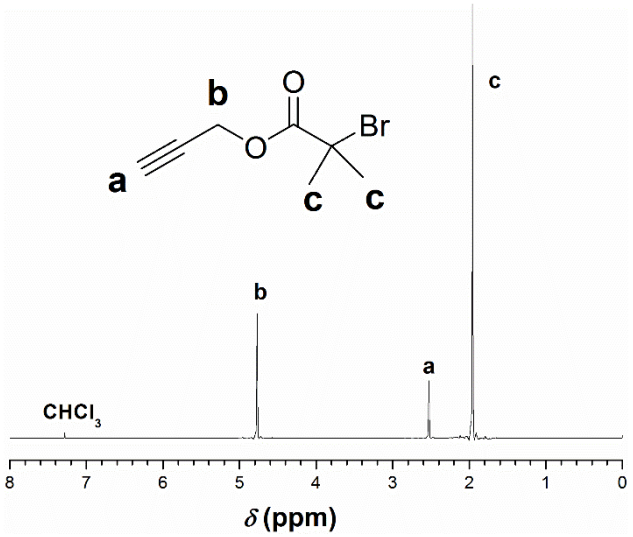


Fig. S2 ^{1}H NMR spectrum of Propargyl 2-Bromoisobutyrate.

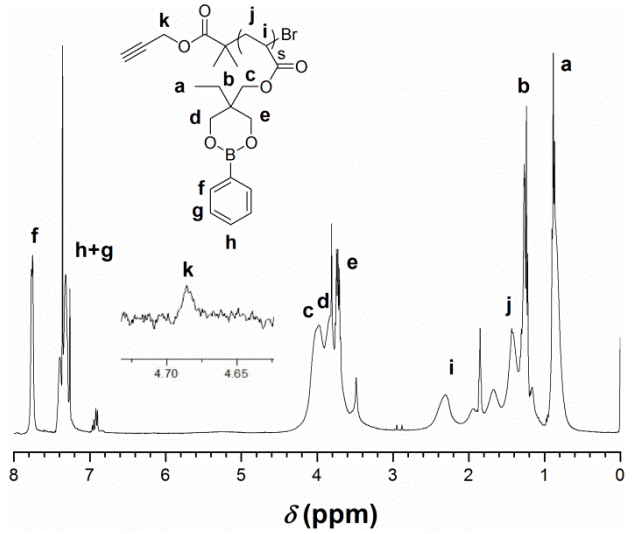


Fig. S3 ^{1}H NMR spectrum of PPBDEMA.

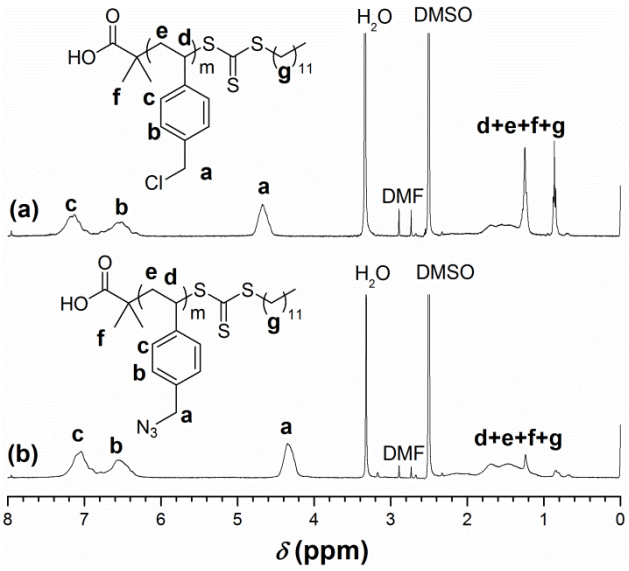


Fig. S4 ^{1}H NMR of (a) PCMS and (b) PAMS.

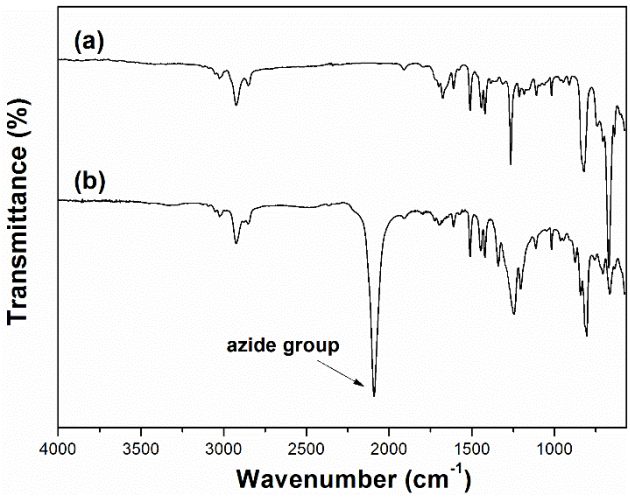


Fig. S5 ATR FTIR spectra of (a) PCMS and (b) PAMS.

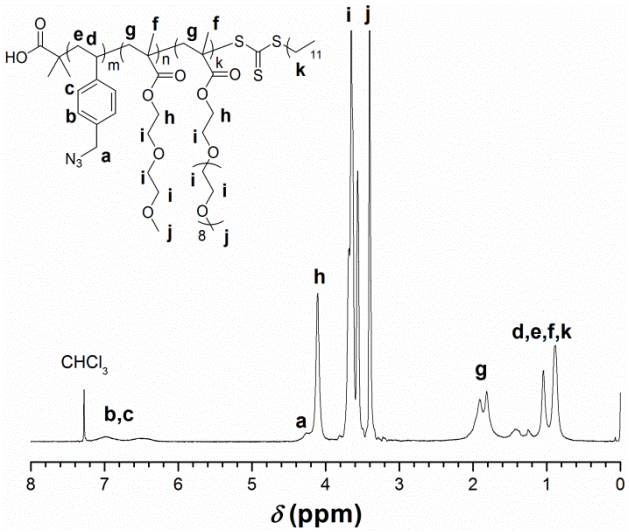


Fig. S6 ^{1}H NMR spectrum of PAMS-*b*-P(MEO_2MA-*co*-OEGMA).

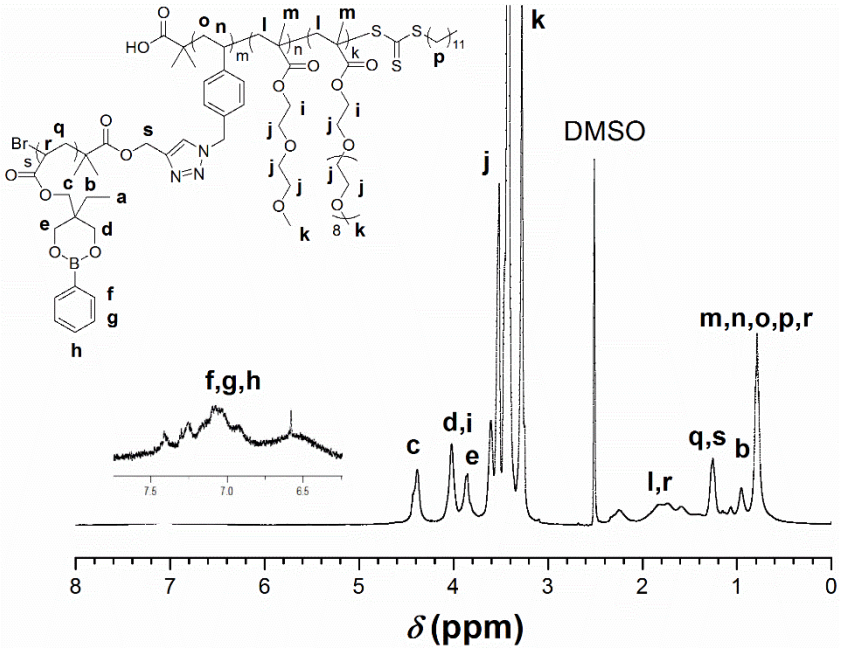


Fig. S7 ^{1}H NMR of P(St-*g*-PBDEMA)-*b*-P(MEO_2MA-*co*-OEGMA).

Table S1 The data of precursors

Precursors	$M_{n,NMR}$ (g/mol)	DP
Alkynyl PPBDEMA	4290	15
PCMS	2020	11
PAMS	2090	11
PAMS-*b*-P(MEO_2MA-*co*-OEGMA)	PAMS: 2090	11
	P(MEO_2MA-*co*-OEGMA): 18990	90
	[MEO_2MA]:[OEGMA]=92%:8%	

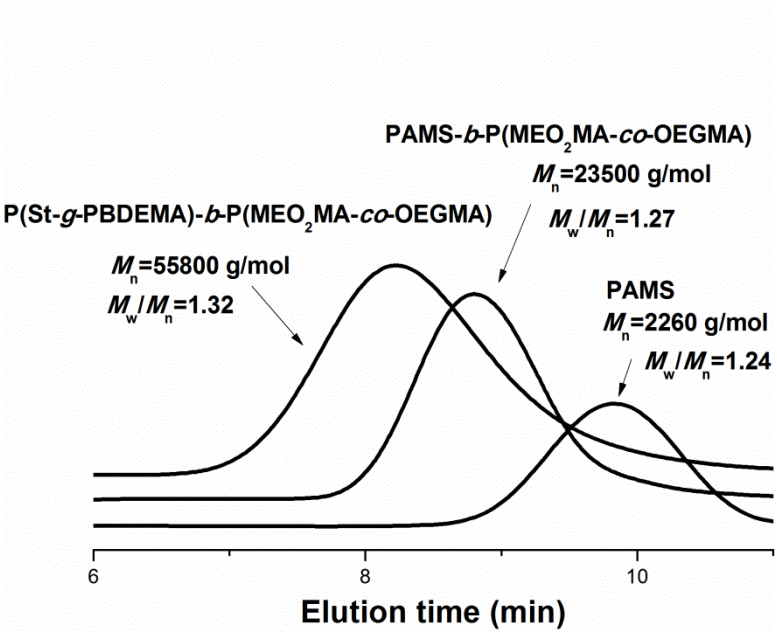


Fig. S8 GPC traces of PAMS, PAMS-*b*-P(MEO_2MA-*co*-OEGMA) and P(St-*g*-PBDEMA)-*b*-P(MEO_2MA-*co*-OEGMA).

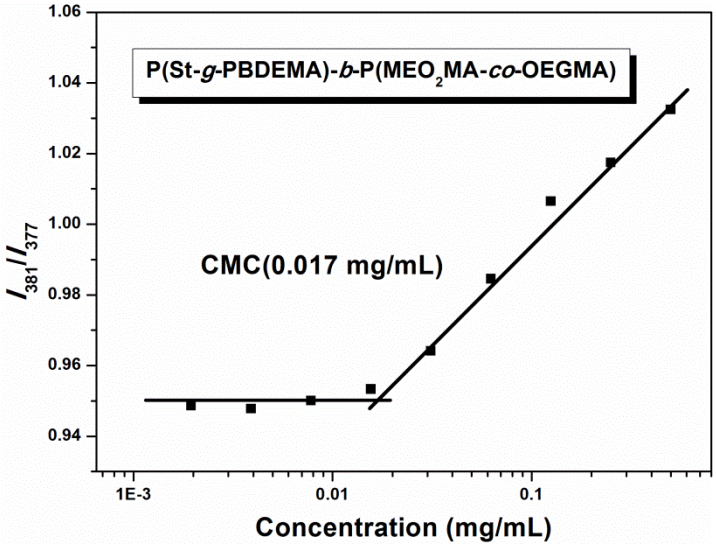


Fig. S9 Critical micelle concentration (CMC) of P(St-*g*-PBDEMA)-*b*-P(MEO_2MA-*co*-OEGMA) brush block copolymer.

References

1 J. T. Lai, D. Filla and R. Shea. *Macromolecules* 2002, **35**, 6754-6756.