Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supplementary Information for

Doubly Responsive Polymersomes towards Monosaccharides and Temperature under Physiologically Relevant Conditions

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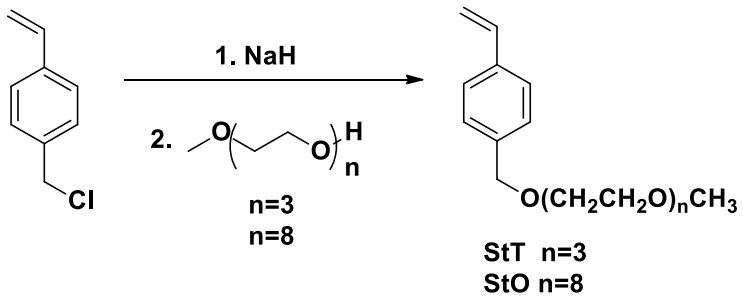
Materials and Methods

General. Unless otherwise noted, all reagents and chemicals were used as received from Sigma Aldrich and TCI. Tetrahydrofuran (THF) was refluxed over a mixture of Na and benzophenone under N_2 and distilled before use. All reactions were performed under N_2 unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VNMRS 600 spectrometer, using DMSO-d_6 and $CDCl_3$ as solvents. Toluene was distilled over CaH_2, and DMF was distilled over CaH_2 under a reduced pressure. Molecular weights of block copolymers were measured on an Agilent 1260 Infinity GPC system equipped with a PL gel 5 μm mixed D column (Polymer Laboratories) and a differential refractive index detector. A DMF was used as an eluent with a flow rate of 1 mL/min. PS standards (Polymer Laboratories) were used for calibration. A Cary Eclipse Fluorescence Spectrophotometer was used for all fluorescence studies. Transmission electron microscopy (TEM) was recorded on a JEOL JEM-2100 microscope at 200 kV. Specimens were prepared by placing a drop of the solution on a carbon-coated Cu grid (200 mesh, EM science). After 30 min, remaining solution on a grid was removed with a filter paper, and the grid was air-dried overnight. Dynamic light scattering (DLS) experiments were carried out on a BI-200SM equipped with a diode laser (637 nm, 4 mW). All DLS data were handled on Dispersion Technology Software (Brookhaven Instruments). Optical transmittance of the solutions was measured on an Agilent 8453 spectrophotometer equipped with a thermostat sample holder with a magnetic stirrer.

Styreneboroxole (1). StB was synthesized by following the literature procedure.[1]

Synthesis of StT and StO



Synthesis of StT.

To a solution of 4-vinylbenzyl chlroride (8.0 g, 52.5 mmol) in dry THF (100 mL) under a N_2 atmosphere was added sodium hydride (3.0 g, 78.8 mmol) in ice bath. The mixture was stirred for 2 hours before the addition of diethylene glycol (13.79 g, 78.8 mmol). The reaction mixture was stirred at room temperature. After 1 days, the solvent was evaporated under reduced pressure (using a high vacuum rotavap). The crude mixture was purified by silica gel column chromatography (ethyl acetate: haxane = 1:3 v/v). A colorless liquid was obtained. That products passed basic aluminum oxide using dichloromethane. Yield 7.0 g (49.8 %). ^{1}H NMR (600 MHz, $CDCl_3$-d6) δ 7.39-7.35 (d, 2H), 7.31-7.27 (d, 2H), 6.70 (dd, 1H ,*J* = 17.6, 10.9 Hz) , 5.73 (dd, 1H, *J* = 17.6, 0.9 Hz) , 5.22 (dd, 1H, *J* = 10.9, 0.9 Hz), 4.54 (s, 2H), 3.69-3.59 (m, 11H), 3.55-3.52 (m, 2H), 3.36 (d, 3H, *J* = 0.9 Hz) HRMS (ESI, *m/z*) Calcd for $C_{16}H_{24}O_4$ 280.17, found 280.07.

Synthesis of StO.

To a solution of 4-vinylbenzyl chlroride (8.0 g, 52.5 mmol) in dry THF (100 mL) under a N_2 atmosphere was added sodium hydride (3.0 g, 78.8 mmol) in ice bath. The mixture was stirred for 2 hours before the addition of oligoethylene glycol (27.58 g, 0.0788 mmol). The reaction mixture was stirred at room temperature. After 1 days, the solvent was evaporated under reduced pressure (using a high vacuum rotavap). The crude mixture was purified by silica gel column chromatography (ethyl acetate: haxane = 1:3 v/v). A colorless liquid was obtained. That products passed basic aluminum oxide using dichloromethane. Yield 5.0 g (20 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39-7.37 (d, 2H, *J* = 8.0 Hz), 7.31-7.29 (d, 2H, *J* = 8.0 Hz), 6.71 (dd, 1H ,*J* = 17.6, 10.9 Hz) , 5.74 (dd, 1H, *J* = 17.6, 0.9 Hz) , 5.23 (dd, 1H, *J* = 10.9, 0.9 Hz), 4.55 (s, 2H), 3.67-3.62 (m, 24H), 3.55-3.54 (m, 2H), 3.37 (d, 3H) , ^{13}C NMR

(150 MHz, $CDCl_3$) δ = 137.845, 136.885, 136.494, 127.877, 127.135, 126.143, 113.679, 77.445, 77.126, 76.807, 72.879, 71.874, 70.585, 70.585, 70.542, 70.515, 70.446, 69.357, 58.956 HRMS (ESI, *m/z*) Calcd for $C_{26}H_{44}O_9$ 500.30 found 500.258.

Synthesis of PEG-chain transfer agent (PEG-CTA).

2-(dodecylthiocarbonothiolylthio)-2-methylpropanoic acid (7.2 g, 20 mmol) and oxalyl chloride (8.6 mL, 100 mmol) were added into 40 mL dry CH_2Cl_2. This solution was stirred at room temperature until the evolution of gas stopped. The solvent and excess reagents were then removed under vacuum, and the residue was redissolved in 80 mL of dry CH_2Cl_2. To this solution was added monomethoxy PEG (8 g, 4 mmol, M_n = 2000 g/mol, Aldrich) at once. After stirring this solution for 24 h at room temperature, the solution was concentrated on a rotavap, and the concentrated solution was precipitated into cold diethyl ether twice. Yellow powder was obtained after filtration and vacuum drying. Yield 62 %. ^1H NMR (600 MHz, $CDCl_3$) δ 4.25 (t, 2H), 3.64(m, 178H), 3.38 (s, 3H), 3.26 (t, 2H), 1.70 (s, 6H), 1.23-1.40 (m, 20H), 0.88 (t, 3H).

RAFT Polymerization of StB, StT and StO.

Synthesis of homopolymers of PSt(T) and PSt(O)

A representative procedure is described: **StT** (700 mg, 2.498 mmol) and CTA (4.55 mg, 1.2 x 10^{-2} mmol) were charged in a Schlenk tube with a magnetic stir bar. This tube was vacuumed and charged with N_2 three times. To this mixture was added 5 mL of dry THF. The solution was degassed by bubbling N_2 for 15 min. A THF solution of AIBN (1.02 mg, 6.2 x 10^{-3} mmol in 0.10 mL) was added at once to this solution, which, then, was immersed into a preheated oil bath (70 °C). The Schlenk tube was sealed, and the polymerization was performed at 70 °C. After polymerization, the reaction mixture was exposed to air and immersed in an ice-water bath. The solution was precipitated into cold diethyl ether. The precipitates were collected by centrifugation.

Synthesis of random copolymers PSt(T-*r*-O).

A representative procedure is described: **StT** (400 mg, 1.43 mmol) and **StO** (64.24mg, 0.1427mmol) and CTA (5.21 mg, 1.43 x 10^{-2} mmol) were charged in a Schlenk tube with a magnetic stir bar. This tube was vacuumed and charged with N_2 three times. To this mixture

was added 5 mL of dry THF. The solution was degassed by bubbling N_2 for 15 min. A THF solution of AIBN (0.66 mg, 2.86 x 10^{-3} mmol in 0.10 mL) was added at once to this solution, which, then, was immersed into a preheated oil bath (70 °C). The Schlenk tube was sealed, and the polymerization was performed at 70 °C. After polymerization, the reaction mixture was exposed to air and immersed in an ice-water bath. The solution was precipitated into cold diethyl ether. To remove unreacted monomer, the crude compound was further purified by silica gel column chromatography (dichloromethane: methanol = 90: 10) and then filtered using methanol.

Synthesis of block copolymers PEG_{45}-*b*-PSt(T-*r*-O-*r*-B).

A representative procedure is described: **StB** (65.68 mg, 0.41 mmol), **StT** (200 mg, 0.72 mmol) and **StO** (48.6mg, 0.108mmol) and PEG-CTA (14.4 mg, 7.2 x 10^{-3} mmol) were charged in a Schlenk tube with a magnetic stir bar. This tube was vacuumed and charged with N_2 three times. To this mixture was added 5 mL of dry THF. The solution was degassed by bubbling N_2 for 15 min. A THF solution of AIBN (0.66 mg, 7.2 x 10^{-4} mmol in 0.10 mL) was added at once to this solution, which, then, was immersed into a preheated oil bath (70 °C). The Schlenk tube was sealed, and the polymerization was performed at 70 °C. After polymerization, the reaction mixture was exposed to air and immersed in an ice-water bath. The solution was precipitated into cold diethyl ether. The precipitates were collected by centrifugation.

Fluoresce in labelling of insulin (F-Insulin). The stock solution of insulin was obtained by dissolving 1 mg of insulin (Human insulin, Sigma) in 0.1 mL of 1 % acetate buffer and the working solution of insulin (0.1 mg/mL) was obtained by diluting the stock solution by 100 folds into reaction buffer containing 50 mM NaH_2PO_4, 100 mM NaCl (pH 7.5). The working solution of insulin was incubated with 10 molar equivalents of fluorescein isothiocyanate (FITC) at room temperature with vigorous shaking for one and half hours. Reactions were implemented to dialysis with sterile deionized water to remove unreacted FITC overnight.

Fluorescein-labelled Insulin (F-insulin) encapsulation in the polymersome of PEG_{45}-*b*-PSt(T_{47}-*r*-O_5-*r*-B_{10}). PEG_{45}-*b*-PSt(T_{47}-*r*-O_5-*r*-B_{10}) (5 mg) was dissolved in THF (1 mL) in a 15 mL capped vial with a magnetic stirrer. The solution was stirred for 3 h at room temperature. A syringe pump was calibrated to deliver Fluorescein-labeled Insulin (F-insulin) in HEPES buffer (1.0 mg/mL) a speed of 2.5 mL/h. The vial cap was replaced by a rubber

septum. 5 mL of F-Insulin was added to the organic solution with vigorous stirring (850 rpm) by a syringe pump with a 5 mL syringe equipped with a steel needle. The remaining solution was subjected to dialysis (SpectraPor, molecular weight cut-off: 12,000−14,000 Da) against HEPES buffer for 2 day with a frequent change of HEPES buffer. The resulting suspension was collected from a dialysis bag, and the suspension was then purified by size exclusion column chromatography (Sephadex G-50), which showed two green bands. The first band was collected and combined.

Release of F-insulin from polymersome of PEG_{45}-*b*-PSt(T_{47}-*r*-O_5-*r*-B_{10}). The polymersome sample (2 mL) encapsulating fluorescein-labelled insulin was subjected to a dialysis bag (SpectraPor, molecular weight cut-off: 12,000 Da), which was dialyzed against 100 mL of HEPES buffer with glucose (0.1 M) at pH 7.5. The buffer outside the dialysis bag was taken at regular intervals to check the fluorescence intensity (excitation wavelength of 495 nm). Without glucose in buffer, no fluorescence was observed from the buffer solution for a 24 h period.

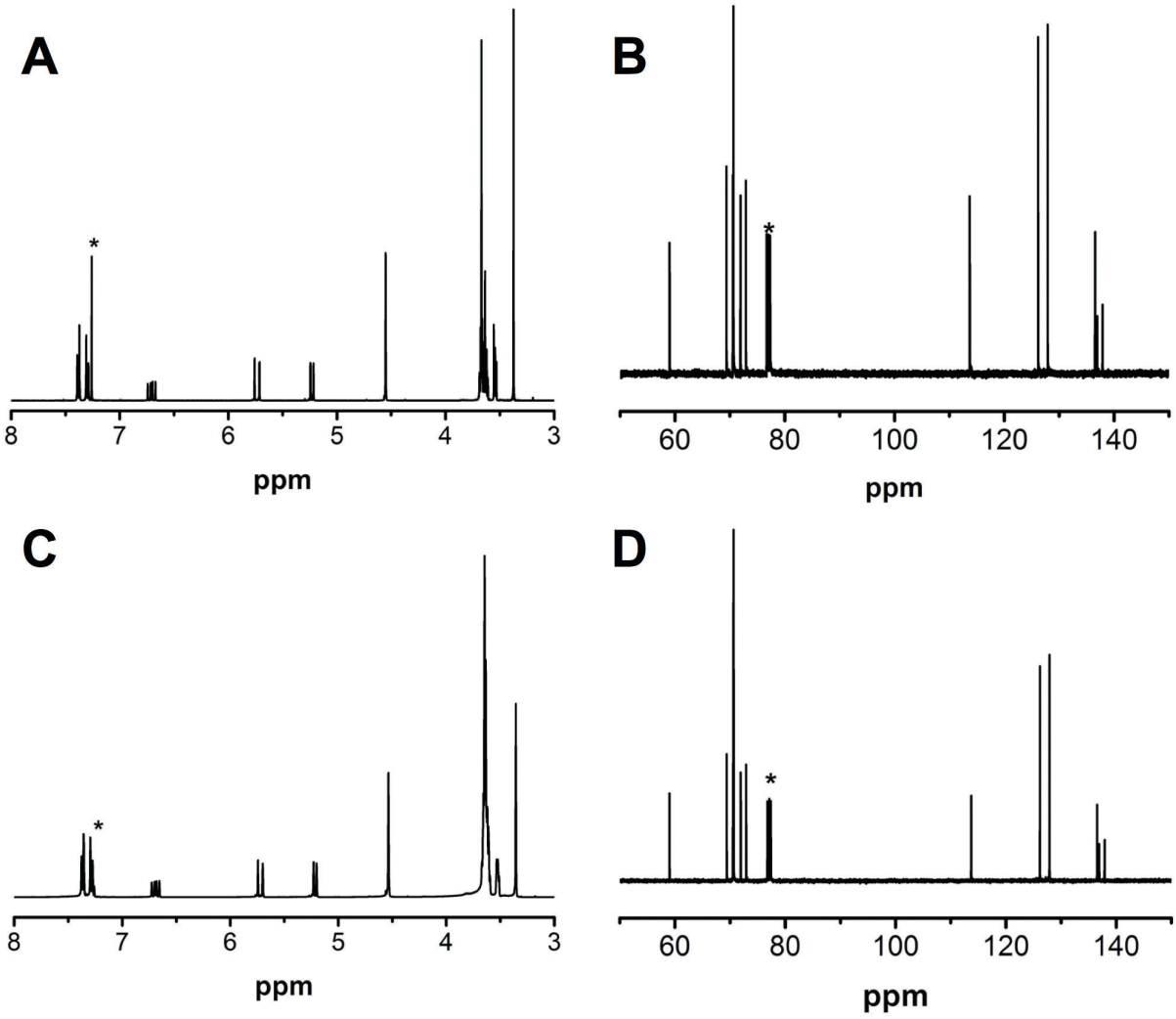


Figure S1. ^{1}H NMR spectra of (A) **StT**, (C) **StO** and ^{13}C NMR spectra of (B) **StT**, (D) **StO**. Asterisks indicate NMR solvents (7.26 ppm $CDCl_3$).

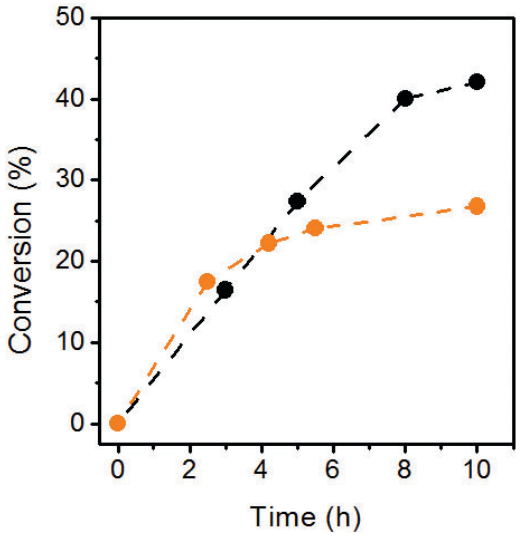


Figure S2. Time-conversion plot for polymerization of **PSt(T)** (black) and **PSt(O)** (orange) under RAFT condition.

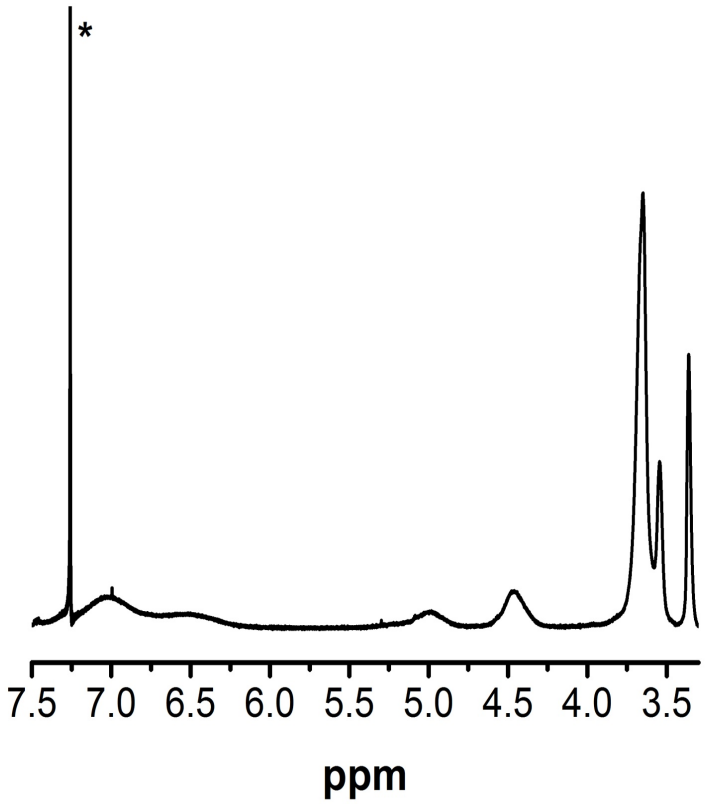


Figure S3. ^{1}H NMR spectra of **PSt(T_{39}-*r*-O_5-*r*-B_{22}).** The degree of polymerization of the **PSt(T_x-*r*-O_y-*r*-B_z)** was calculated by comparing the integration of the benzyl signal of the **StB** (5.1 ppm) and ethylene glycol signal of **PSt(T-*r*-O)** (4.4 ppm). Asterisk indicates NMR solvents (7.26 ppm $CDCl_3$).

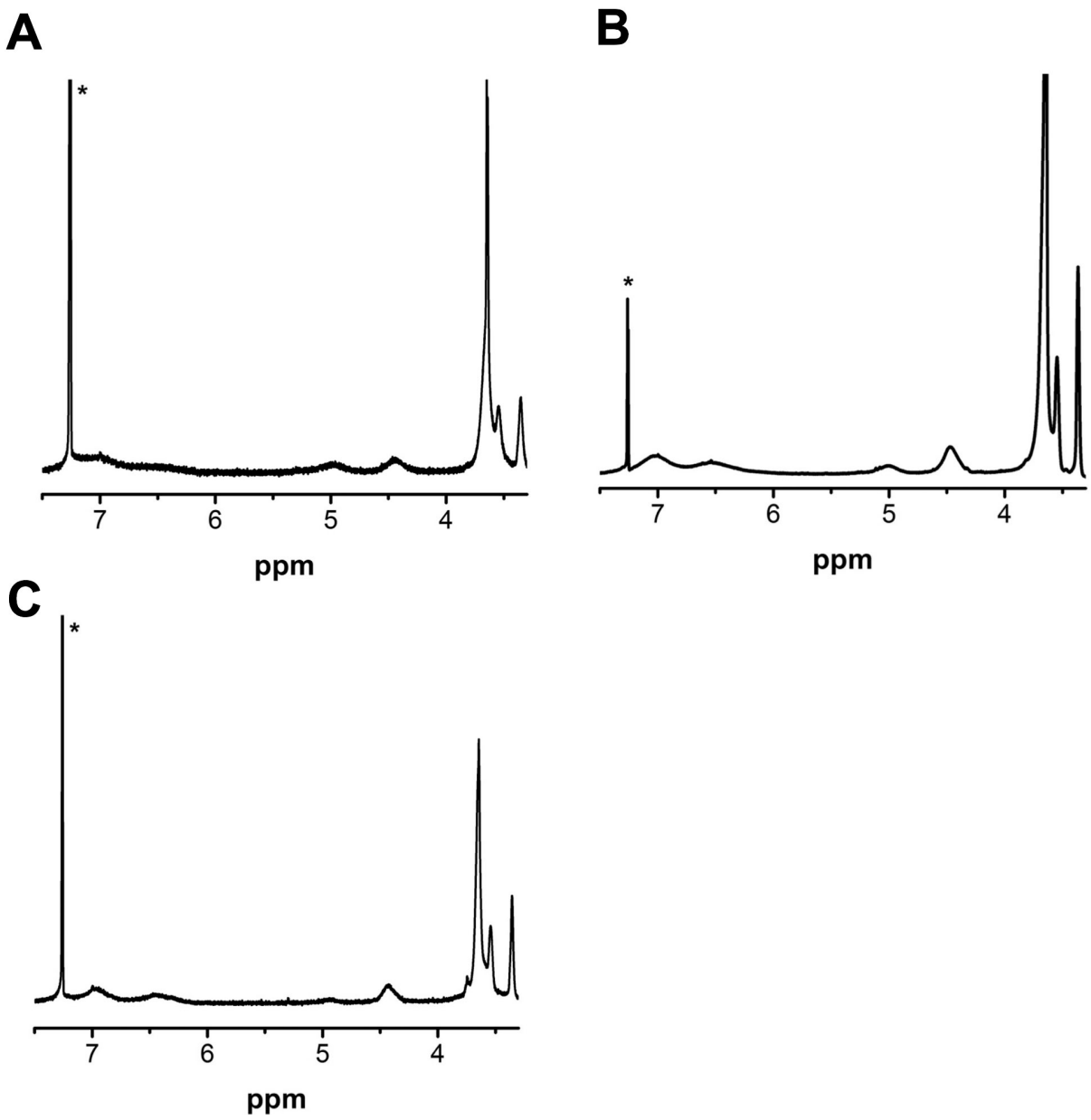


Figure S4. ^{1}H NMR spectra of (A) **PEG$_{45}$-*b*-PSt(T$_{36}$-*r*-B$_{24}$)**, (B) **PEG$_{45}$-*b*-PSt(T$_{19}$-*r*-O$_3$-*r*-B$_{12}$)**, (C) **PEG$_{45}$-*b*-PSt(T$_{47}$-*r*-O$_5$-*r*-B$_{10}$)**. The degree of polymerization of the **PEG$_{45}$-*b*-PSt(T$_x$-*r*-O$_y$-*r*-B$_z$)** block was calculated by comparing the integration of the benzyl signal of the **StB** (5.1 ppm) and ethylene glycol signal of **PSt(T-*r*-O)** (4.4 ppm) and the methylene signals of the PEG (3.6 ppm). Asterisks indicate NMR solvents (7.26 ppm $CDCl_3$).

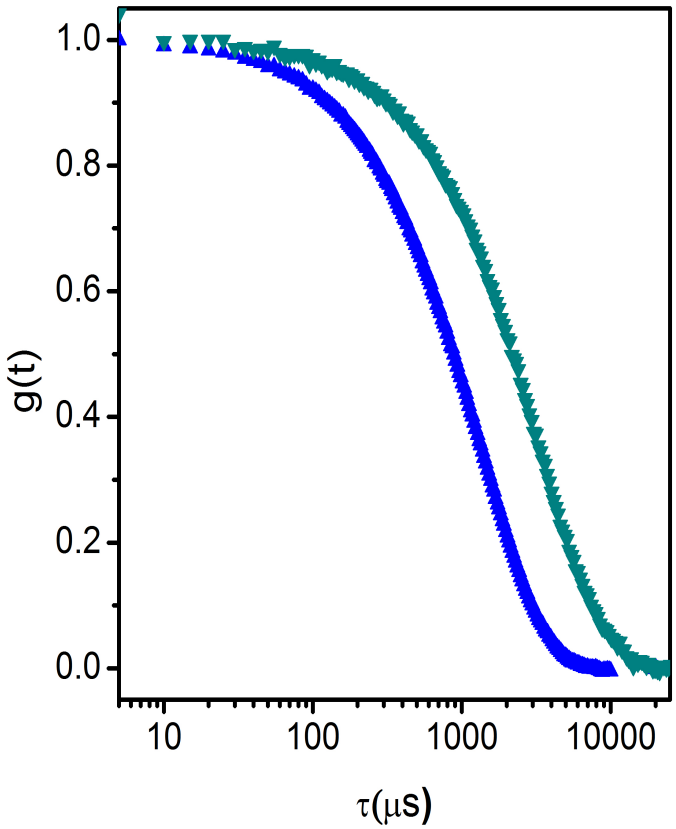


Figure S5. Autocorrelation functions of polymersomes of **PEG$_{45}$-*b*-PSt(T$_{36}$-*r*-B$_{24}$)** (blue) and **PEG$_{45}$-*b*-PSt(T$_{47}$-*r*-O$_5$-*r*-B$_{10}$)** (green) in water.

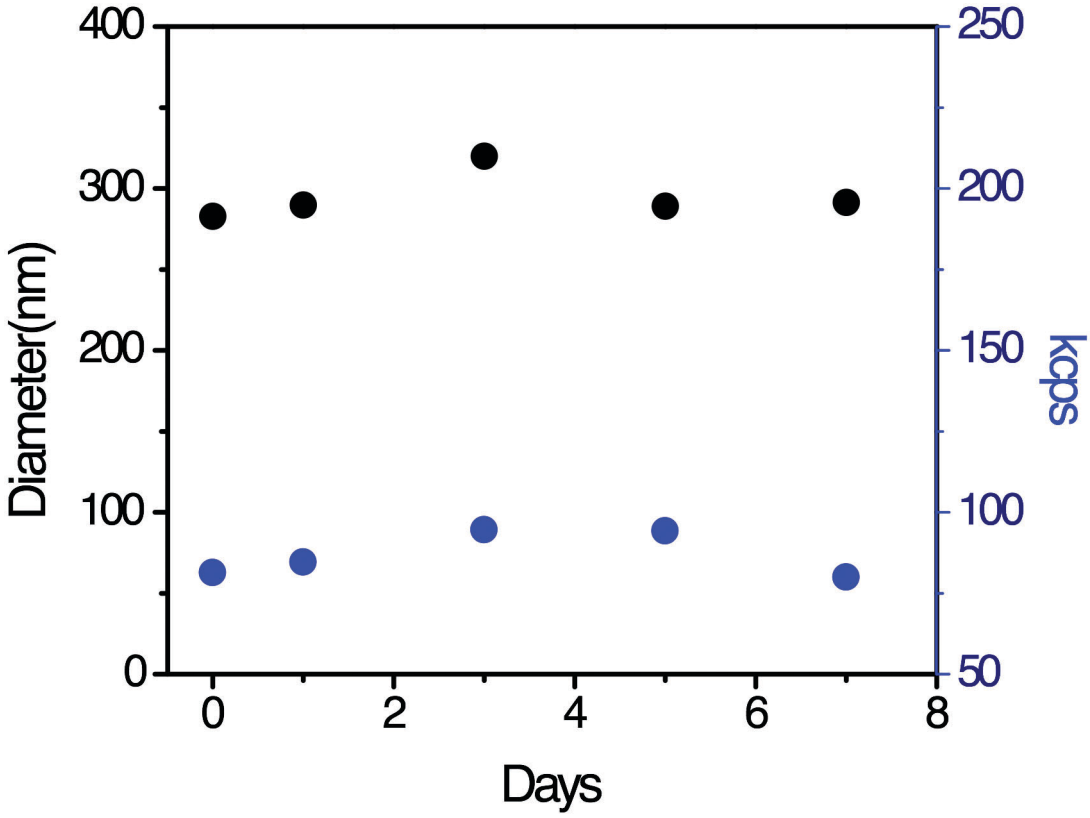


Figure S6. Time-course analysis of diameter change and scattered light intensity of **PEG_{45}-*b*-PSt(T_{36}-*r*-B_{24})** solution in the HEPES buffer (pH = 7.5).

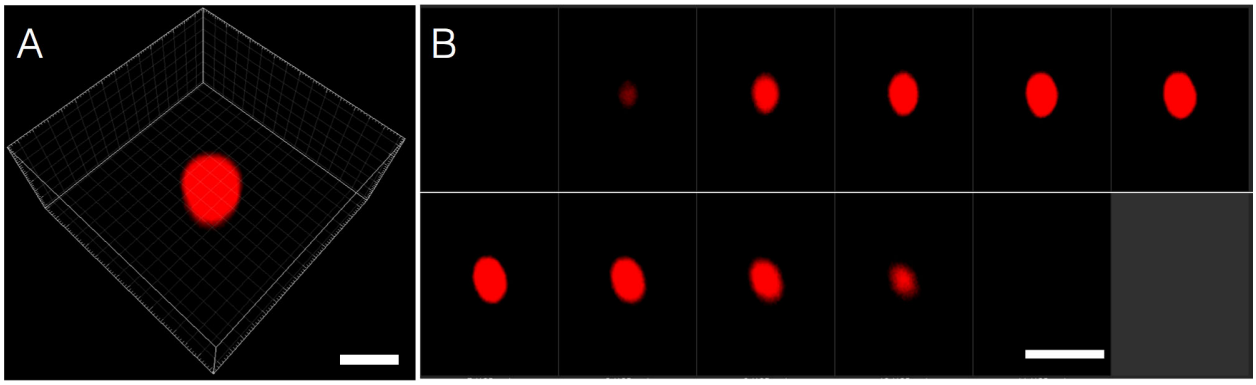


Figure S7. (A) 3D reconstructed CLSM image of the polymersome of **PEG_{45}-*b*-PSt(T_{47}-*r*-O_5-*r*-B_{10})** encapsulating Rhodamine B. Scale bar: 700 nm. (B) CLSM images of the polymersome of **PEG_{45}-*b*-PSt(T_{47}-*r*-O_5-*r*-B_{10})** encapsulating Rhodamine B at 11 different focal planes. Scale bar: 2 μm.

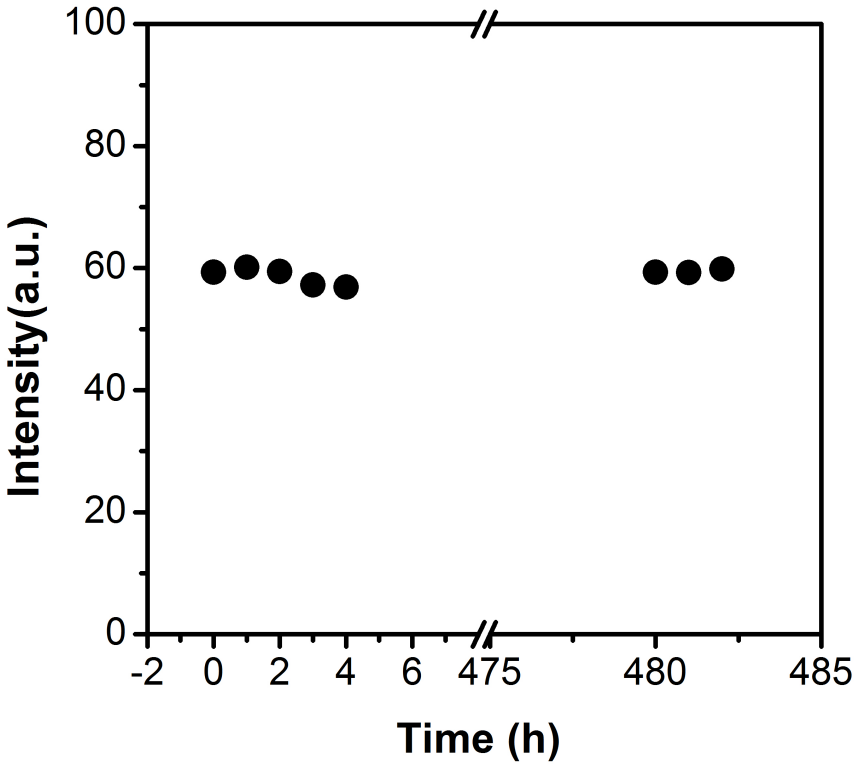


Figure S8. Time-course analysis of fluorescence intensity of the polymersome solution of **PEG_{45}-*b*-PSt(T_{47}-*r*-O_5-*r*-B_{10})** encapsulating Rhodamine B, indicating no leakage of the dyes from the interior of the polymersome over 20 days.

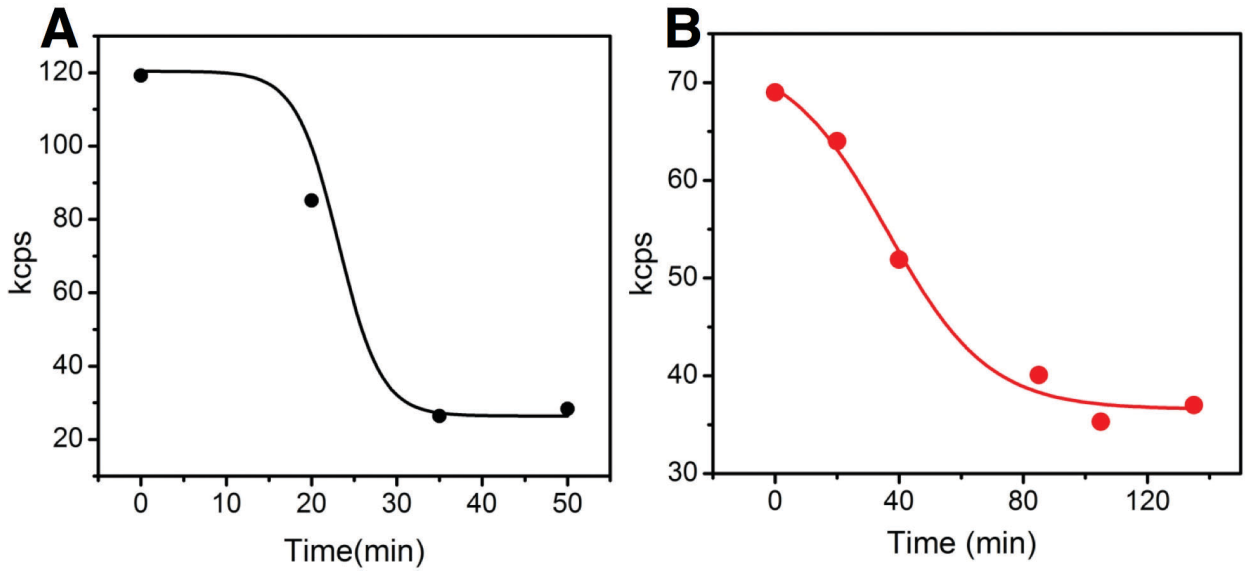


Figure S9. Scattered light intensity changes of polymersome solutions of **PEG_{45}-*b*-PSt(T_{47}-*r*-O_5-*r*-B_{10})** in the presence of glucose (0.1 M) at 20 ° C (A) and 37 ° C (B) (HPES pH = 7.5).

References

1. Kim, H.; Kang, Y. J.; Kang, S.; Kim, K. T. *J. Am. Chem. Soc.* **2012**, *134*, 4030.