

Supplementary Information

Effect of the Molecular Weight Distribution of the Hydrophobic Block on the Formation of Bicontinuous Cubic Mesophases of Block Copolymers with a Discrete Branched Hydrophilic Block

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Materials

Unless otherwise stated, all reactions were carried out under N₂ atmosphere. All reagents and chemicals were purchased from Sigma Aldrich, Alfa Aesar and TCI and used as received. Styrene (99+%) was purchased from Sigma Aldrich and filtered over a column of basic alumina before use. Dry solvents were obtained via distillation using Na and benzophenone as drying agents for tetrahydrofuran (THF), and using CaH₂ for dichloromethane (DCM).

Experimental Procedures

General Information

¹H NMR spectra were recorded on Agilent 400-MR DD2 magnetic resonance system and Varian/Oxford As-500 using CD₂Cl₂ or CDCl₃ as solvents. Molecular weights of polymers were measured on Agilent 1260 infinity gel permeation chromatography (GPC) system equipped with a PL gel 5 μm mixed D column and differential refractive index detectors. DMF was used as an eluent with a flow rate of 1 mL min⁻¹ at 35 °C. A PS standard kit (Agilent Technologies) was used for calibration. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was performed on Bruker Ultraflex II TOF/TOF mass spectrometer equipped with a nitrogen laser (335 nm). The analytical sample was prepared by mixing a THF solution of an analyte with a THF solution of sinapic acid.

Scanning electron microscopy (SEM) was performed on Hitachi S-4300 at an acceleration voltage of 15 kV. Typically, dried samples were placed on a conductive carbon tape and then sputtered with Pt with a thickness of 3 nm by using Hitachi E-1030 ion sputter. Transmission electron microscopy (TEM) was performed on JEOL JEM-2100 microscope at 200 kV.

Specimens were prepared by placing a drop of the sample solution on a carbon-coated Cu grid (200 mesh, EM science) and then air-drying the grid.

Synchrotron small angle X-ray scattering (SAXS) data were obtained on 6D SAXS beam line at Pohang acceleration laboratory in Korea (PLS-II, 3.0 GeV). The sample-to-detector distance was 3.5 m. The suspension of cubosomes was dried for 24 h in a freeze-dryer and the resulting cubosome powder was placed on a customized SAXS holder. Ti-SBA-15 was used as standard sample and scattering spectra were taken in a transmission mode at room temperature (11.6 keV). ImageJ software (<https://imagej.nih.gov/ij/>) was used to conduct 2D fast Fourier transform (FFT) on TEM images. Lattice constants of polymer cubosomes were calculated from the FFT images.

Synthesis of a discrete heterobifunctional PEG (Trt-PEG₁₂-Ts) (1)

The following procedure was adapted from the previously published literature.^{1,2} Compound **1** was obtained as a mixture with ditrityl by-products. The mixture was used in the next step without further purification as the by-products are unreacting. The amount of compound **1** in the mixture was determined from ¹H NMR analysis (Fig. S1).

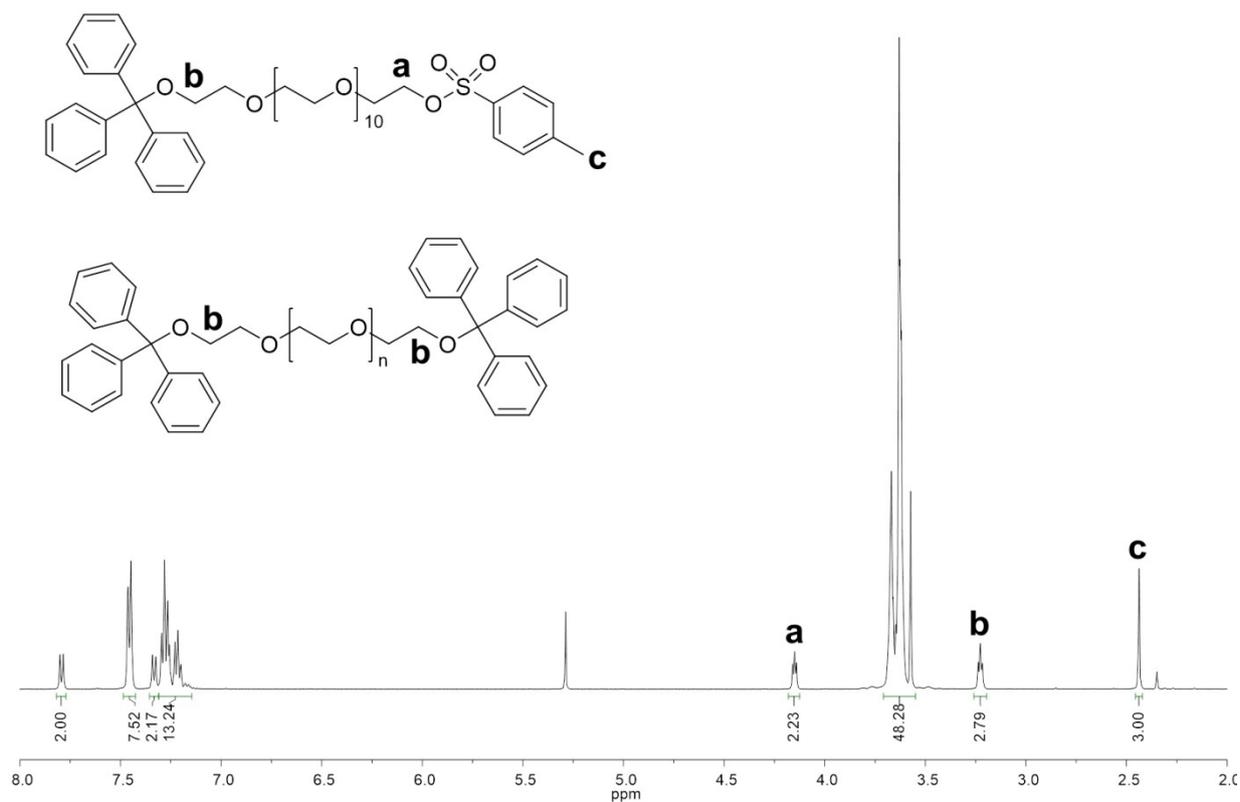


Fig. S1 ^1H NMR spectrum (400 MHz, CDCl_3) of Trt-PEG₁₂-Ts (crude).

Synthesis of a discrete hydrophilic PEG block (3)

Methyl gallate (0.195 g, 1.06 mmol) and K_2CO_3 (1.17 g, 8.48 mmol) were added to the 500 mL two-neck flask charged with acetone under N_2 . The crude mixture from the previous step (6.93 g) containing compound **1** (less than 3.30 g, 3.50 mmol) was added to the flask, and the solution was refluxed at 75 °C for 12 h. The reaction was monitored by MALDI-TOF. Additional amount of the polyethylene glycol reactant mixture was added until the reaction was complete. After the complete consumption of the methyl gallate was confirmed on MALDI-TOF, the reaction was cooled at room temperature. The crude mixture was filtered through a filter paper and the solvent was evaporated under reduced pressure. The residue

was extracted with DCM and washed with brine three times. The organic phase was dried over anhydrous Na₂SO₄ and the solvent was removed under reduced pressure.

Without further purification, the crude mixture containing **2** (less than 1.06 mmol) was reduced with LiAlH₄ (80 mg, 2.12 mmol) in THF under N₂ atmosphere for 3 h. The reduction yielded crude product which was purified by column chromatography on silica (DCM/MeOH, 20/1 to 10/1, v/v) to obtain compound **3** as a pale yellow liquid (2.30 g, 0.932 mmol, 88% over 2 steps). ¹H NMR (δ = ppm, 400 MHz, CDCl₃) 7.44 (d, 15H, *J* = 8 Hz), 7.30-7.17 (m, 36H), 6.61 (s, 2H), 4.54 (d, 2H, *J* = 8 Hz), 4.14 (t, 4H, 8 Hz), 4.10 (t, 2H, 4 Hz), 3.81 (t, 4H, *J* = 8 Hz), 3.75 (t, 2H, *J* = 4 Hz), 3.70-3.57 (m, -CH₂CH₂O-), 3.21 (t, 6H, *J* = 4 Hz).

Compound **3** (2.30 g, 0.932 mmol) was dissolved in dry THF (25 mL) and the solution was added dropwise to a suspension of NaH (60% suspension in oil, 44.7 mg, 1.86 mmol) in dry THF at 0 °C under N₂ atmosphere, and the mixture was stirred from 0 °C to room temperature for 2 h. Propargyl bromide (0.166 g, 1.40 mmol) was added to the reaction mixture and stirred at room temperature for 12 h. The reaction mixture was cooled to 0 °C and saturated NH₄Cl solution was added. After evaporating THF under reduced pressure, the mixture was extracted with DCM, washed with brine and dried over anhydrous Na₂SO₄. The crude product was used in the next step without further purification.

To a solution of crude mixture containing **4a** (less than 0.932 mmol) in DCM/MeOH (1/1, v/v) was added *p*-toluene sulfonic acid monohydrate (0.605 g, 3.18 mmol), and the mixture was stirred at room temperature for 2.5 h. The reaction mixture was concentrated under reduced pressure, and the residue was extracted with DCM. The organic phase was washed with a saturated NaHCO₃ aqueous solution (10 mL) and brine (10 mL), and dried over anhydrous Na₂SO₄. The crude product was purified by column chromatography on silica

(DCM/MeOH, 9/1, v/v) to obtain compound **4b** as a slightly yellow liquid (1.41 g, 0.792 mmol, 85% over 2 steps). ¹H NMR (δ = ppm, 400 MHz, CDCl₃) 6.59 (s, 2H), 4.49 (s, 2H), 4.15 (m, 6H), 4.11 (t, 2H, 4 Hz), 3.83 (t, 4H, *J* = 4 Hz), 3.77 (t, 2H, *J* = 4 Hz), 3.74-3.58 (m, -CH₂CH₂O-), 2.49 (t, 1H, *J* = 4 Hz).

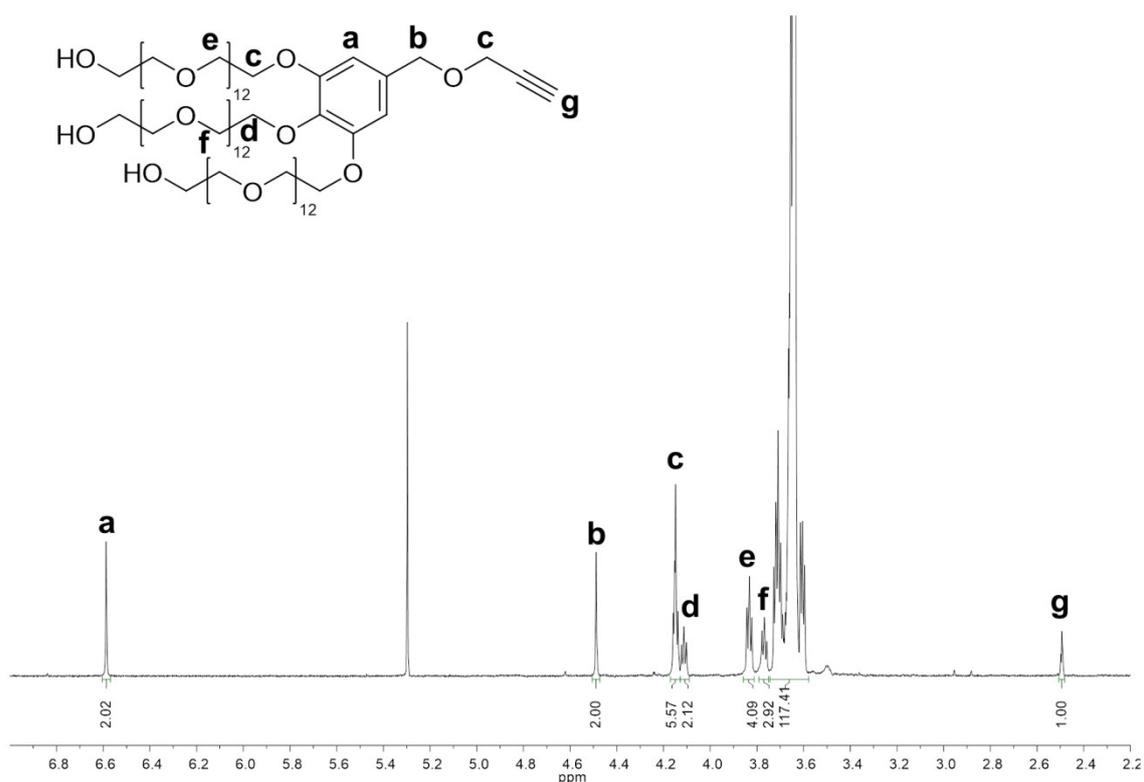


Fig. S2 ¹H NMR spectrum (400 MHz, CDCl₃) of **3**.

General procedure for the synthesis of azido end-functionalized polystyrenes with low \bar{D}

CuBr (313 mg, 2.2 mmol) was charged in a 100 mL Schlenk flask and dried under vacuum for 15 min. *N,N,N',N'',N''*-pentamethyldiethylenetriamine (PMDETA) (0.91 mL, 4.4 mmol) dissolved in anisole (2 mL) was added, and the mixture was gently stirred under N₂ for 10 min. (1-Bromoethyl)benzene (80 mg, 0.44 mmol) in anisole (1 mL) and styrene (50 mL, 440

mol) was added to the mixture and was degassed via bubbling N₂ into the mixture for 15 min. ATRP reaction was carried out at 90 °C. The reaction was monitored by GPC and quenched by exposing the mixture to air and cooling it in liquid N₂ bath. The cooled mixture was filtered through a pad of aluminum oxide (basic) using DCM as an eluent for removal of Cu ions. The filtered solution was concentrated by rotary evaporation, diluted with a small amount of DCM and then precipitated into methanol. Polystyrene end-functionalized with bromine was obtained as white powder by filtration and dried in vacuo.

The obtained bromo end-functionalized polystyrene (10 g, ca. 0.44 mmol, 1 eq.) and sodium azide (0.283 g, 10 eq.) were dissolved in DMF (250 mL) and stirred at room temperature under N₂ for 12 h. DMF was removed by rotary evaporation and azido end-functionalized polystyrene was precipitated into methanol.

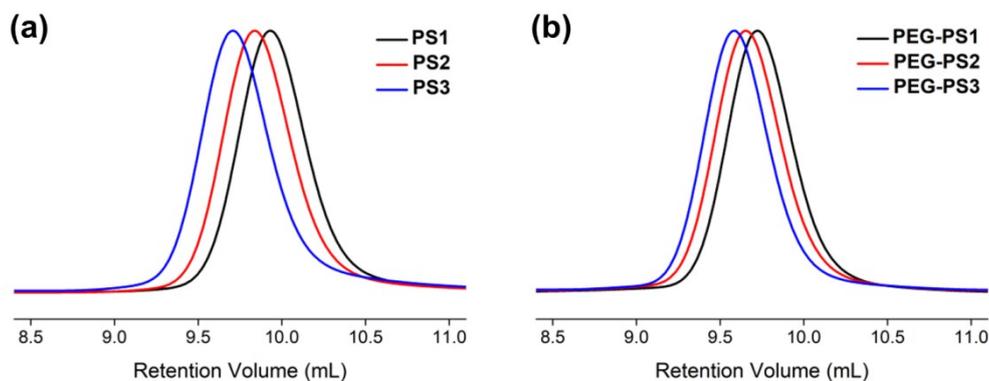


Fig. S3 GPC (DMF) profiles of (a) PS1, PS2, and PS3; (b) bPEG-PS1, bPEG-PS2, and bPEG-PS3.

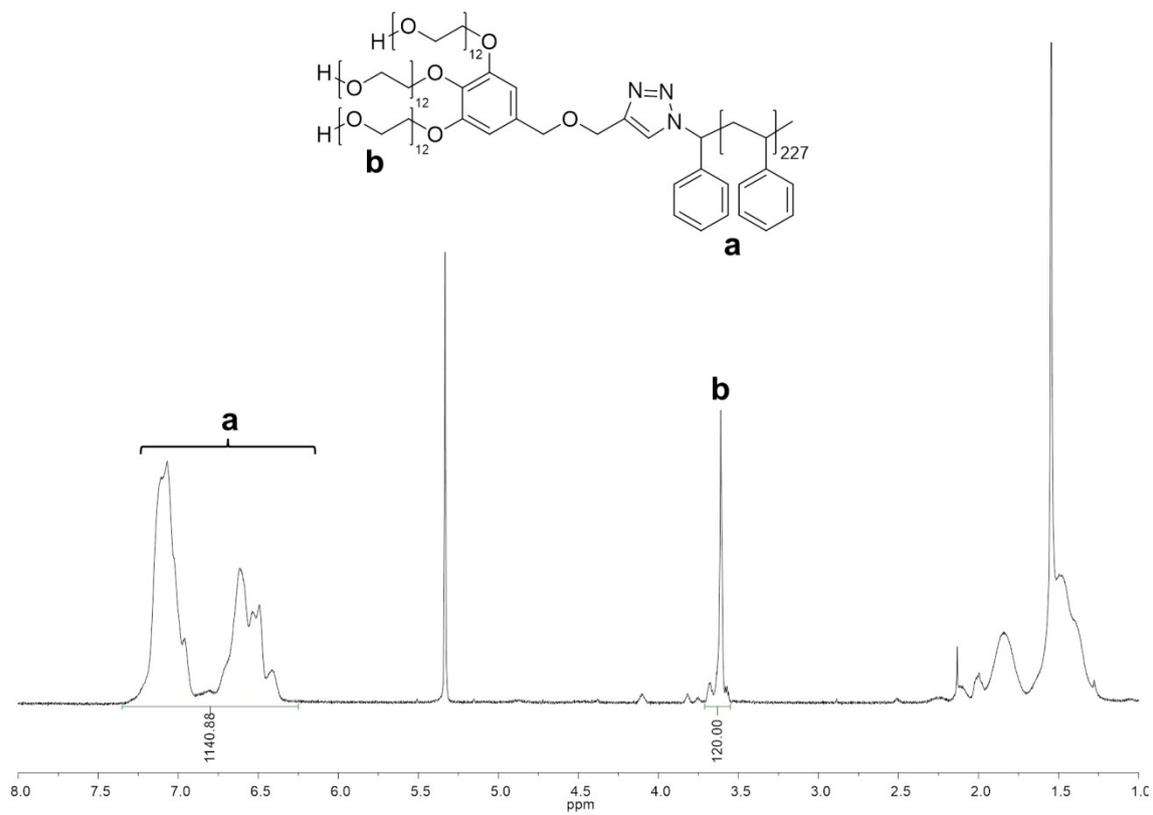


Fig. S4 ¹H NMR spectrum (400 MHz, CDCl₃) of bPEG-PS1.

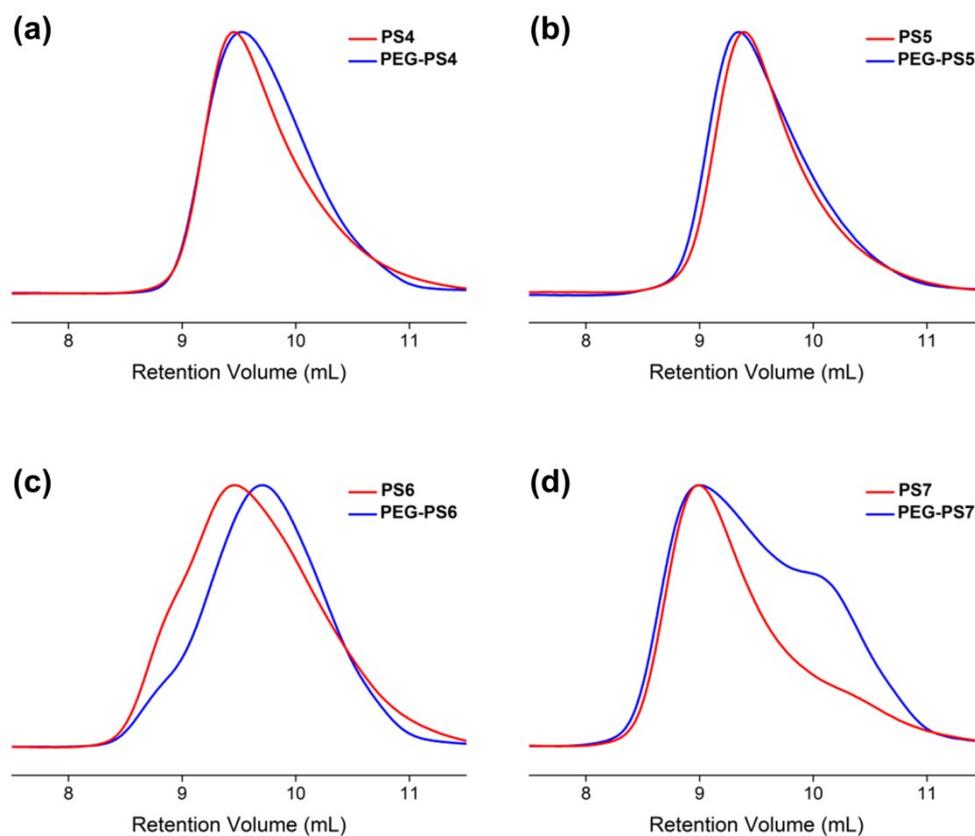


Fig. S5 GPC (DMF) profiles of (a) PS4 (red) and PEG-PS4 (blue); (b) PS5 (red) and bPEG-PS5 (blue); (c) PS6 (red) and PEG-PS6 (blue); (d) PS7 (red) and PEG-PS7 (blue).

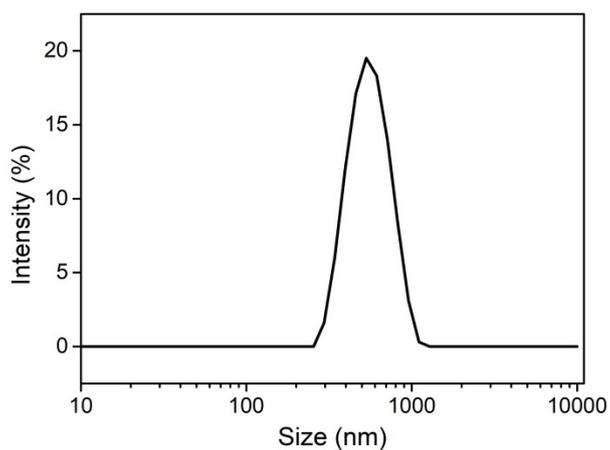


Fig. S6 DLS spectrum of the self-assembled vesicles of bPEG-PS1 (average diameter = 530 nm) using dioxane as the common solvent.

General procedure for the synthesis of azido end-functionalized polystyrenes with high \bar{D}

CuBr (194 mg, 1.4 mmol) was charged in a 50 mL Schlenk flask and dried under vacuum for 1 h. *N,N,N',N'',N'''*-pentamethyldiethylenetriamine (PMDETA) (0.42 mL, 2.0 mmol) was dissolved in anisole (2 mL) in a sealed vial under N₂, degassed via bubbling N₂ into the mixture for 15 min, and the solution was transferred to the Schlenk flask containing CuBr under N₂. Styrene (47 mL, 409 mmol) was charged in a 50 mL Schlenk flask under N₂, degassed via bubbling N₂ for 15 min and was added to the mixture of CuBr and PMDETA. The initiator, (1-Bromoethyl)benzene (62.5 mg, 1.7 mmol), was dissolved in anisole (5 mL) in a sealed vial under N₂, degassed via bubbling N₂ for 15 min, and was filled into a 6 mL syringe. The initiator was added to the flask containing CuBr, PMDETA, and styrene during ATRP reaction via syringe pump at 90–100 °C. The addition rate of the initiator was controlled while monitoring the progress of the reaction by GPC. When the growing polymer reached the desired M_n and \bar{D} , the polymerization was quenched by exposing the mixture to air and cooling it in liquid N₂ bath. The cooled mixture was filtered through a pad of aluminum oxide (basic) using DCM as an eluent for removal of Cu ions. The filtered solution was concentrated by rotary evaporation, diluted with a small amount of DCM and then precipitated into methanol. Polystyrene end-functionalized with bromine was obtained as white powder by filtration and dried in vacuo. Azido end-functionalized polystyrene was obtained following the same procedure as described above.

The obtained bromo end-functionalized polystyrene (10 g, ca. 0.435 mmol, 1 eq.) and sodium azide (0.283 g, 10 eq.) were dissolved in DMF (250 mL) and stirred at room temperature under N₂ for 12 h. DMF was removed by rotary evaporation and azido end-functionalized polystyrene was precipitated into methanol.

Table S1. GPC (DMF) profiles of PS4 at each reaction time during ATRP.

Time (h)	M_n (g/mol) ^a	\mathcal{D}^a	Addition Rate (mL/h) ^b
2	8100	1.24	0.5
3	15620	1.32	0.6
4	20000	1.39	0.7
5	23370	1.40	0.8

Table S2. GPC (DMF) profiles of PS5 at each reaction time during ATRP.

Time (h)	M_n (g/mol) ^a	\mathcal{D}^a	Addition Rate (mL/h) ^b
3	7160	1.23	0.5
5	13460	1.30	0.5
6	16790	1.33	0.5
8	24110	1.37	0.5
9	25870	1.38	0.5

Table S3. GPC (DMF) profiles of PS6 for different ATRP reaction time.

Time (h)	M_n (g/mol) ^a	\mathcal{D}^a	Addition Rate (mL/h) ^b
19	9930	1.42	0.05
27	11080	1.50	0.05
42	11690	1.56	0.05
66	18600	1.63	0.05
77	19670	1.70	0.05
97	23870	1.75	0.05

Table S4. GPC (DMF) profiles of PS7 at each reaction time during ATRP.

Time (h)	M_n (g/mol) ^a	\mathcal{D} ^a	Addition Rate (mL/h) ^b
16	28680	1.34	0.1
19	30760	1.49	0.3
22	29520	1.76	0.5
23	28400	1.86	0.7

^a M_n and \mathcal{D} were determined by GPC (DMF, 35 °C, 1 mL/min) using PS standards. ^bThe volume refers to the volume of the initiator solution in anisole.

General procedure for the conjugation of the hydrophilic block and the polystyrenes

The discrete hydrophilic block and the polystyrenes were coupled via Cu-catalyzed azide-alkyne Huisgen cycloaddition (CuAAC). Compound **4** (30 mg, 0.017 mmol), azido end-functionalized polystyrene (2.5 equivalents of compound **4**), CuSO₄·5H₂O (13 mg, 0.052 mmol), and sodium ascorbate (7 mg, 0.035 mmol) were dissolved in dry DMF under N₂ atmosphere. The mixture was stirred for 3 h at room temperature. After confirming via GPC that compound **4** were exhausted, DMF was removed by rotary evaporation. The excess unreacted polystyrene was removed by column chromatography on silica (EA/Hexane 1/1, v/v) and the desired product, bPEG-PS, was collected (DCM/MeOH, 9/1, v/v). The product was precipitated into methanol to obtain pure bPEG-PS in white powder.

General procedure for the self-assembly of bPEG-PS in solution

The synthesized bPEG-PS was self-assembled following the method used in our previous studies.³⁻⁷ 5 mg of bPEG-PS was dissolved in 1 mL of acetone/dioxane solvent mixture followed by the addition of water at the rate of 0.25 mL/h for 4 h. The organic solvent was removed by subsequent dialysis against water for 24 h.

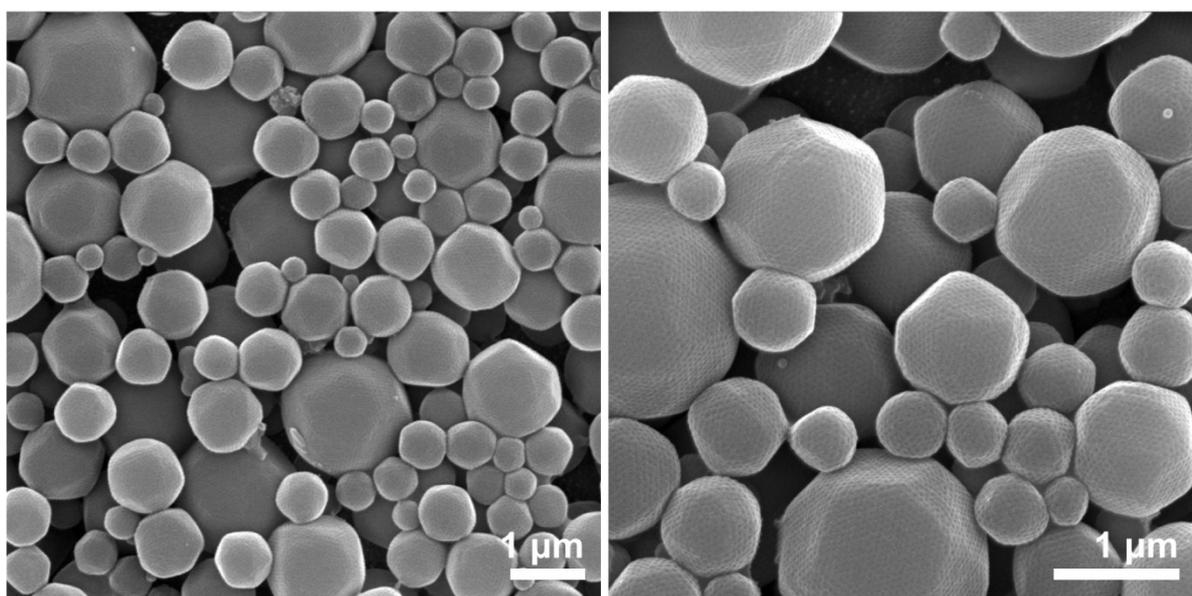


Fig. S7 SEM images of cubosomes of bPEG-PS1 ($D = 1.09$, acetone content = 85 vol%).

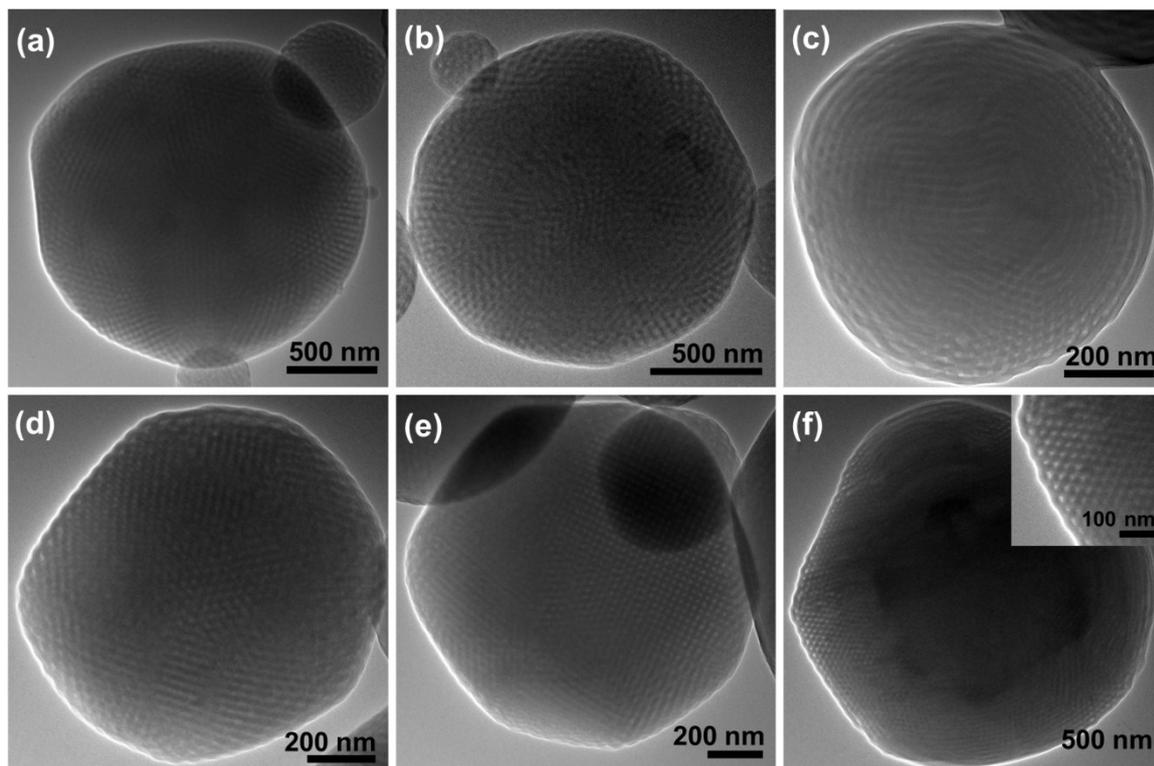


Fig. S8 TEM images of the self-assembled morphologies of (a–c) bPEG-PS1 and (d–f) bPEG-PS3. (a) A cubosome (acetone content = 85 vol%), (b) a cubosome (acetone content = 90 vol%), and (c) a hexosome (acetone content = 95 vol%) of bPEG-PS1. (d) A cubosome (acetone content = 45 vol%), (e) a cubosome (acetone content = 75 vol%), and (f) a hexosome (acetone content = 45 vol%) of bPEG-PS3. The inset is a magnified view of the internal lattice.

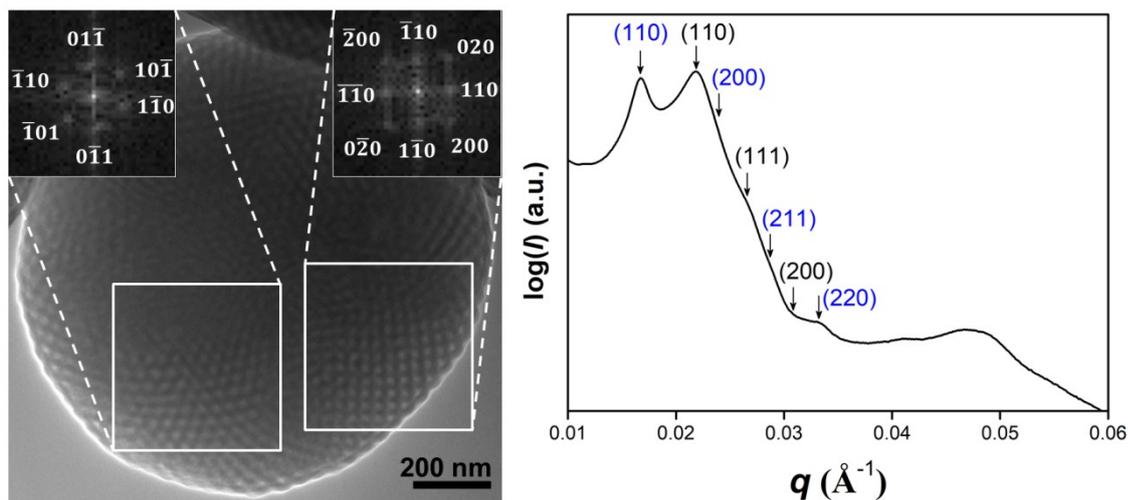


Fig. S9 TEM image (left) and SAXS spectrum (right) of the self-assembled cubosome of bPEG-PS1 (acetone content = 82 vol%). Miller indices in the SAXS spectrum correspond to expected Bragg peak positional ratios (blue: $Im\bar{3}m$, black: $Pn\bar{3}m$).

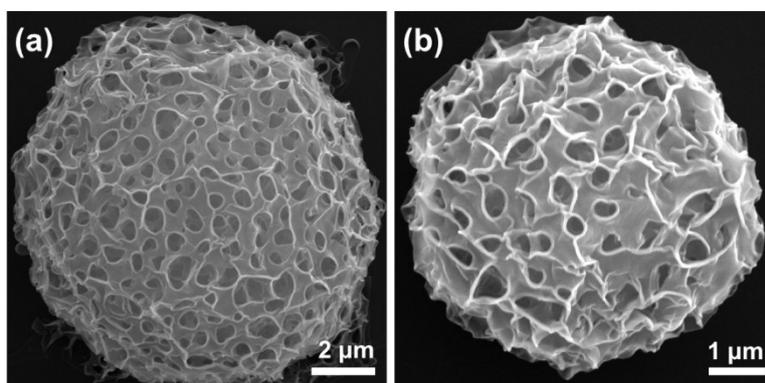


Fig. S10 SEM images of sponge phases self-assembled from (a) bPEG-PS4 ($D = 1.29$, acetone content = 80 vol%) and (b) bPEG-PS5 ($D = 1.32$, acetone content = 60 vol%).

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