

Supporting Information for

**Polymer Cubosomes of Block Copolymers Having Cross-linkable
Soft Hydrophobic Blocks**

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1. Materials and methods

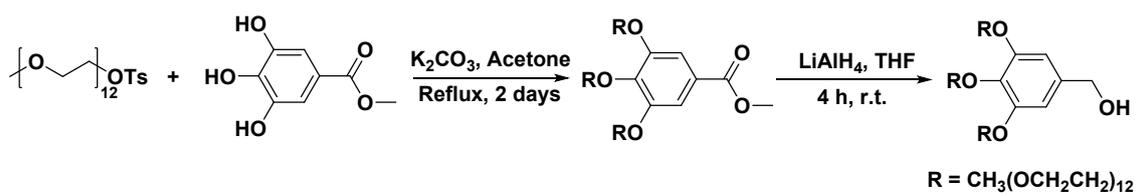
General. Unless otherwise noted, all reagents and chemicals were purchased from Sigma Aldrich, Alfa Aesar, and TCI and used as received. Isoprene was distilled prior to polymerization over CaH₂. Dimethylformamide (DMF), dichloromethane (CH₂Cl₂) and cyclohexane were distilled over CaH₂ under N₂. Tetrahydrofuran (THF) was refluxed over a mixture of Na and benzophenone under N₂ atmosphere and distilled before use. All reactions were performed in an inert atmosphere unless otherwise noted.

Methods. ¹H and ¹³C NMR spectra were recorded on Agilent 500-MR DD2 Magnetic Resonance System and Varian/Oxford AS-500 using CD₂Cl₂ and CDCl₃ as solvents and internal standards. Molecular weights and dispersity D of polymers and block copolymers were measured by Agilent 1260 Infinity gel permeation chromatography (GPC) system equipped with a PL gel 5 μm MiniMIX-D column (Agilent Technologies) and differential refractive index detectors. THF was used as an eluent with a flow rate of 0.3 mL min⁻¹ at 35 °C. A PS standard kit (Agilent Technologies) was used for calibration. Cryogenic transmission electron microscopy (cryo-TEM) images were taken from JEM-1400 (JEOL) operating at 120 kV. The cryo-TEM experiments were performed with a thin film of aqueous sample solution (5 μL) transferred to a lacey supported grid (copper, 200 mesh, EM science) by the plunge-dipping method. The thin aqueous films were prepared at ambient temperature and with a humidity of 97–99% within custom-built environmental chamber in order to prevent water evaporation from the sample solution. The excess liquid was blotted with filter paper for 1–2 s, and the thin aqueous films were rapidly vitrified by plunging them into liquid ethane (cooled by liquid nitrogen) at its freezing point. Conventional TEM was performed on a Hitachi 7600 operating at 100 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 dried overnight. Scanning electron microscopy (SEM) was performed on a Hitachi S-4300 operating at 15 kV. Suspension was cast and dried on a slide glass, and coated with Pt by using a Hitachi E-1030 ion sputter. Dynamic light scattering (DLS) was performed at a Malvern Zetasizer Nano-S. Fourier transform infrared spectrophotometer (FT-IR) was measured on SHIMADZU IR Tracer-100 equipped with MIRacle 10 single reflection ATR accessory. Differential scanning calorimetry (DSC) was carried out under N₂ gas at a scan rate of 5 °C min⁻¹ with TA Instruments Q10.

2. Synthesis of hydrophilic structural modules

Branched hydrophilic block, PEG550₃-N₃ was synthesized by following the previously reported procedure (Scheme S1).^{1,2} PEG550₃-(3,5)-N₃ was synthesized by coupling the corresponding benzyl alcohol and methyl 3,5-bis(azidomethyl)benzoic acid.



Scheme S1. Synthesis of PEG550₃-OH

PEG550₃-OH. It is synthesized in multi-gram quantity by following the literature methods.^{1,2}

¹H NMR (400 MHz, CDCl₃) 6.64(s, 2H), 4.58(d, *J*=5.6 Hz, 2H), 4.18(t, *J*=5.2 Hz, 4H), 4.14(t, *J*=5.2 Hz, 2H), 3.93-3.45 (m, -CH₂CH₂O-), 3.39(s, 9H), 2.52(t, *J*=5.6 Hz, 1H) ppm;
¹³C NMR (400 MHz, CDCl₃) 152.60, 137.49, 137.22, 106.50, 72.29, 71.95, 70.77-70.57,

69.83, 68.79, 64.86, 59.07 ppm; GPC $M_n = 2590 \text{ g mol}^{-1}$, $D = 1.03$.

Methyl 3,5-dimethylbenzoate. A few drops of H_2SO_4 (95%) were added into methyl 3,5-dimethylbenzoic acid (10 g, 66 mmol) in methanol (100 mL). The solution was refluxed at 70 °C for 10 h. The reaction mixture was quenched with ice and extracted with ether. The organic layer was washed with Na_2CO_3 saturated solution, dried over MgSO_4 , and removed under reduced pressure. The crude product was purified by column chromatography using *n*-hexane as an eluent. The compound was obtained as colourless oil. Yield 9.8 g (89.63%) $^1\text{H NMR}$ (400 MHz, CDCl_3) 7.66 (s, 2H), 7.19 (s, 1H), 3.90 (s, 3H), 2.36 (s, 6H).

Methyl 3,5-bis(bromomethyl)benzoate. Methyl 3,5-dimethylbenzoate (5.8 g, 35.3 mmol) and *N*-bromosuccinimide (13.83 g, 77.70 mmol) were added into benzene (250 mL). The solution was degassed by bubbling N_2 for 15 min. Azobisisobutyronitrile (0.58 g, 3.53 mmol) was added into the solution and stirred at 95 °C for 12 h. The precipitate was filtered off and washed with hot benzene. The combined filtrate was washed with a saturated NaHCO_3 solution and brine. The organic layer was dried with MgSO_4 and removed under reduced pressure. The crude product was purified by column chromatography using hexane as an eluent. Yield 3.33 g (30.61%) $^1\text{H NMR}$ (400 MHz, CDCl_3) 8.00 (s, 2H), 7.62 (s, 1H), 4.50 (s, 4H), 3.93 (s, 3H).

Methyl 3,5-bis(azidomethyl)benzoate. Methyl 3,5-bis(bromomethyl)benzoate (3.33 g, 10.34 mmol) and NaN_3 (2.68 g, 41.37 mmol) were added into DMF (50 mL). The solution was stirred at 65 °C for 3 h. It was diluted with water and then extracted with ethyl acetate (EA). The organic layer was dried with MgSO_4 . The solvents were removed under reduced pressure. The crude product was purified by column

chromatography using hexane as an eluent. Recrystallization in hexane gave a white crystalline solid. Yield 2.23g (87.57%) ^1H NMR (400 MHz, CDCl_3) 7.97 (s, 2H), 7.49 (s, 1H), 4.49 (s, 4H), 3.95 (s, 3H).

Methyl 3,5-bis(azidomethyl)benzoic acid. A solution of 2M LiOH (10ml) was added into methyl 3,5-bis(azidomethyl)benzoate (1g, 4.06 mmol) in methanol (70 mL). The reaction was stirred at room temperature for 12 h. The solvents were removed under reduced pressure and monitored by thin layer chromatography (TLC). Then, 2M HCl solution was added into the mixture until pH was dropped to pH 5~6. Mixture was extracted with ethyl acetate and brine, and then dried with MgSO_4 . The solvents were removed under reduced pressure. A white solid was collected. Yield 0.91 g (71.95%) ^1H NMR (400 MHz, CDCl_3) 8.04 (s, 2H), 7.56 (s, 1H), 4.49 (s, 4H).

PEG550₃-(3,5)-N₃ (3). Methyl 3,5-bis(azidomethyl)benzoic acid (0.475 g, 1.54 mmol), *N,N'*-dicyclohexylcarbodiimide (0.367 g, 1.78 mmol), and 4-dimethylaminopyridine (0.007 g, 0.059 mmol) were dissolved in dry CH_2Cl_2 (50 mL) at 0 °C. The mixture was slowly added to the CH_2Cl_2 solution (50 mL) of PEG550₃-OH (0.7 g, 0.297 mmol) at 0 °C. The resulting mixture was gradually warmed to room temperature. After 24 h, the crude mixture was cooled, and urea was removed by repeated filtration with cold EA. Then mixture was purified by flash column chromatography using silica gel (CH_2Cl_2 :MeOH = 95:5 v/v). ^1H NMR (400 MHz, CDCl_3) 8.00 (s, 2H), 7.50 (s, 1H), 6.68 (s, 2H), 5.25 (s, 2H), 4.45 (s, 4H), 3.93–3.45 (m, $-\text{CH}_2\text{CH}_2\text{O}-$), 3.38 (s, 9H). GPC $M_n = 3060 \text{ g mol}^{-1}$, $D = 1.04$.

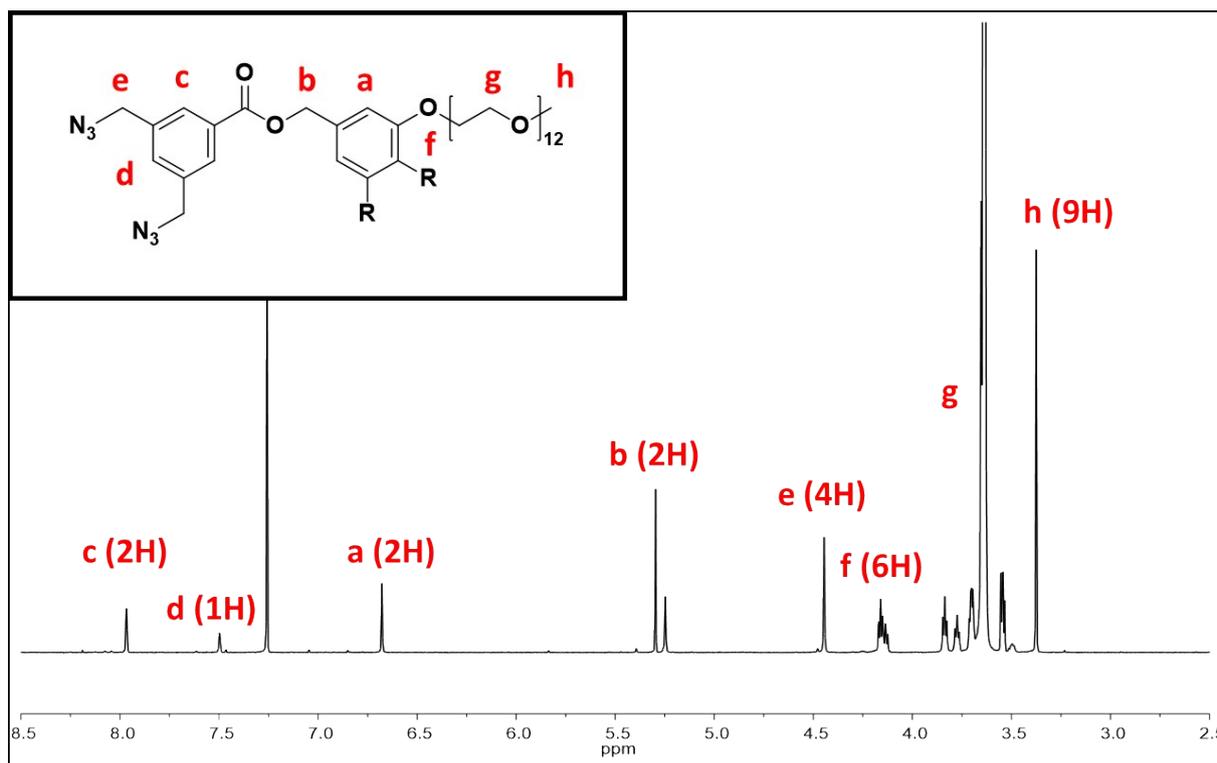


Fig. S1 ^1H NMR (500 MHz, CDCl_3) spectrum of branched hydrophilic block

3. Synthesis of hydrophobic structural modules

5-triethylsilyl-4-pentynyllithium (TESP-Li).³ A 10-fold excess of lithium (0.8 g, 0.11 mol) over the (5-chloro-1-pentynyl)triethylsilane (TESP-Cl) (2.5g, 0.011 mol) was used. Dry cyclohexane (50 mL) was added to the lithium placed in a 100 mL two neck flask with a reflux condenser under an argon atmosphere. The reaction mixture was vigorously stirred for 30 min at 50 °C. Then, TESP-Cl was added dropwise to this solution, and the solution was stirred for 4h at 50 °C and overnight at room temperature. The reaction mixture was then filtered through a fritted Schlenk filter to afford a clear red-orange solution. ^1H NMR (400 MHz, CDCl_3) 2.15 (t, 2H), 1.45(m, 2H), 0.97–0.82 (m, 12H), 0.49 (m, 6H).

Polymerization of α -acetylene-functionalized polyisoprene. All polymerization was performed in a dry condition under inert atmosphere in Glove box. Isoprene was pre-dried over CaH_2 for 24 h, which was distilled prior to use. A desired amount of isoprene was dissolved in dry cyclohexane and THF in a vial. The prepared TESP-Li was introduced to the solution at once. The polymerization was monitored by GPC at 30 min intervals. After 2h, the reaction was quenched by injecting degassed methanol. The solution was evaporated under reduced pressure and precipitated into methanol for 3 times. Colourless viscous product was obtained after drying. Then, the product and tetrabutylammonium fluoride trihydrate (TBAF) (5 eq. to polyisoprene) were dissolved in THF and stirred at room temperature for 4 h. Then, the solution was precipitated in methanol and dried. Colourless viscous product was obtained. In case of 1,2-addition, polymerization was going under $-78\text{ }^\circ\text{C}$. $^1\text{H NMR}$ (400 MHz, CDCl_3) 6.1–5.5 (1,2, -CH-), 5.10 (1,4, -CH-), 4.8–1.6 (1,2, - CH_2), 4.75 (3,4, - CH_2), 2.03 (1,4, - CH_2 -), 2.0 (3,4, -CH-), 2.0–1.8 (1,4, - CH_2 -), 1.8–1.2 (1,2, - CH_2), 1.65 (1,4, - CH_3), 1.64 (3,4, - CH_3), 1.36 (3,4, - CH_2 -), 1.15-1.0 (1,2, - CH_3).³

Table S1. Characterization of polyisoprene

Entry	M_n (g mol ⁻¹) ^a	D^a	DP_n^b	1,4:3,4:1,2 ^c	Extended length (nm) ^d
PI(1,4)(10K)	9780	1.20	143	94:0:6	60
PI(1,4)(13K)	12880	1.17	189	94:0:6	80
PI(1,4)(15K)	14600	1.08	214	94:0:6	90
PI(1,4)(33K)	33500	1.32	492	94:0:6	209
PI(1,2)(10K)	10500	1.09	154	0:38:62	29

^a Number-average molecular weight and molecular weight distribution determined by GPC. ^b Number-average degree of polymerization estimated by the molecular weight. ^c Ratio between microstructures of the repeating unit of the PI chain calculated by $^1\text{H NMR}$ integration. ^d Fully-extended chain length calculated based on the microstructure of the repeating unit.

4. Synthesis of block copolymers by click chemistry

CuBr(I) (40 mg) was dried in vacuum for 15 min. *N,N,N',N'',N'''*-pentamethyldiethylenetriamine (PMDETA) (80 mg) mixed with THF (1.5 mL) was added and the mixture was stirred in N₂ for 15 min. To this solution, a solution of the hydrophilic PEG block and deprotected polyisoprene (4 eq. to the hydrophilic module) in THF (5 mL) was added. The mixture was degassed by bubbling N₂ for 15 min. After degassing, the click reaction was proceeded at 40 °C until completion. The extent of the reaction was monitored by GPC. The reaction was quenched by exposing the solution to air, followed by dilution with chloroform. The cooled solution was filtered through aluminum oxide (basic) with CHCl₃ to remove the Cu catalyst. The filtered solution was concentrated on a rotary evaporator, and then crude products were filtered through a pack of silica with CH₂Cl₂ to remove the excess homo PI. To collect the click product, a mixed eluent (dichloromethane:methanol = 90:10 v/v) was used. The filtered solution was concentrated on a rotary evaporator to afford a pure block copolymer as a pale yellow gummy product. If necessary, the resulting block copolymer was purified by preparatory size exclusion chromatography.

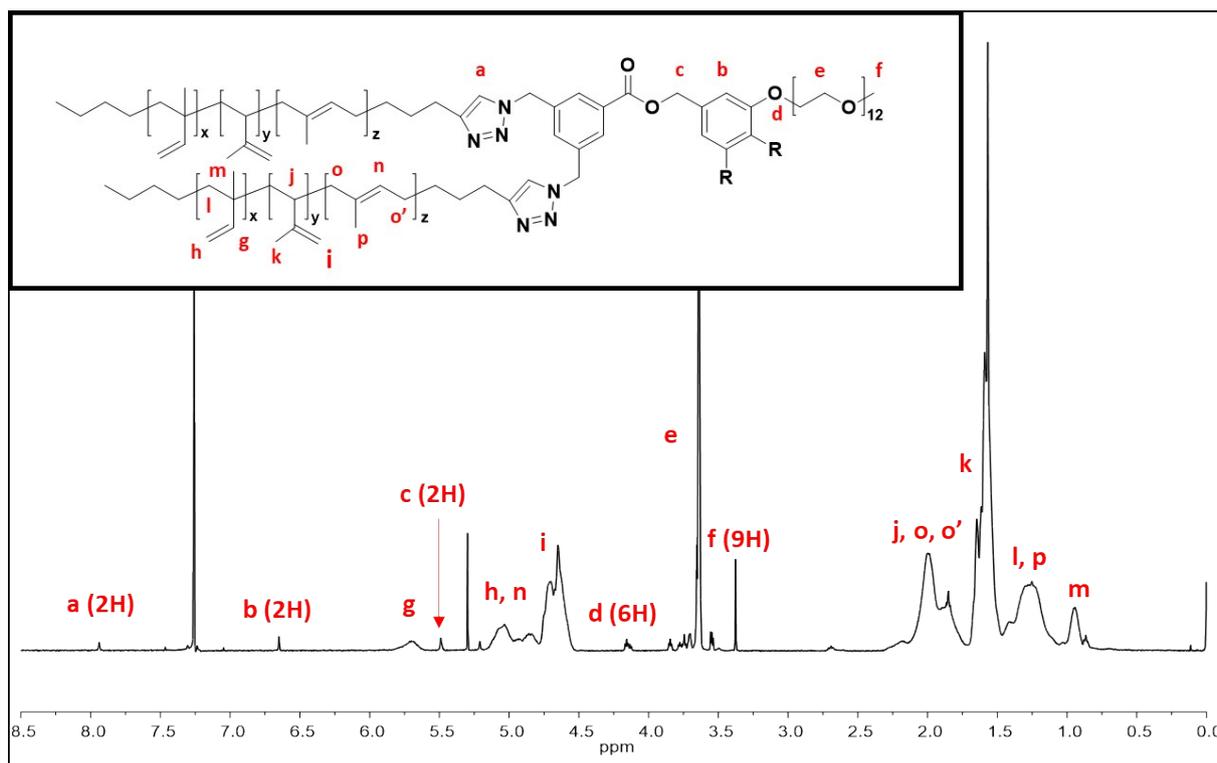


Fig. S2 ^1H NMR (500 MHz, CDCl_3) spectrum of PEG550₃-*b*-PI₂

5. Solution self-assembly and cross-linking of block copolymers

Typically, BCPs (10 mg) was dissolved in 1,4-dioxane (1 ml) in a 20 mL capped vial with a magnetic stirrer. The solution was stirred for two hours at room temperature (800 revolutions per minute). To this solution, 1 mL of water was slowly added at a rate of 2 mL h⁻¹ by using a syringe pump. To the solution (dioxane:water = 1:1 for volume ratio), 5 mg of 2-Hydroxy-4'- (2-hydroxyethoxy)-2-methylprophenone (Igarcure 2959) was added for cross-linking. The suspension was stirred for 30 min and exposed to UV light ($\lambda = 365$ nm, 15 W) for 5 h with mild stirring, followed by dialysis against water. All BCPs were self-assembled and cross-linked under identical conditions for comparison. The cross-linking of PI chains was confirmed by changing the suspension medium from water to THF.

Cross-linking experiment. The cross-linking of vinyl group in PI chain was confirmed with polymersomes of PEG550₃-PI(33K) which is the simplest self-assembled structures in this study. In the presence of photoradical generator, the formation of covalent bonds between PI blocks could maintain the structures upon exchanging the solvent from water to THF, of which the average diameter was increased from 455.2 nm in water to 610.1 nm in THF. Cross-linked polymersomes could also be observed with conventional TEM due to their enhanced physical stability. It indicates that the membrane of the polymersomes showing swelling behaviour was covalently stabilized by cross-linking of polyisoprene domains in the bilayer membrane.

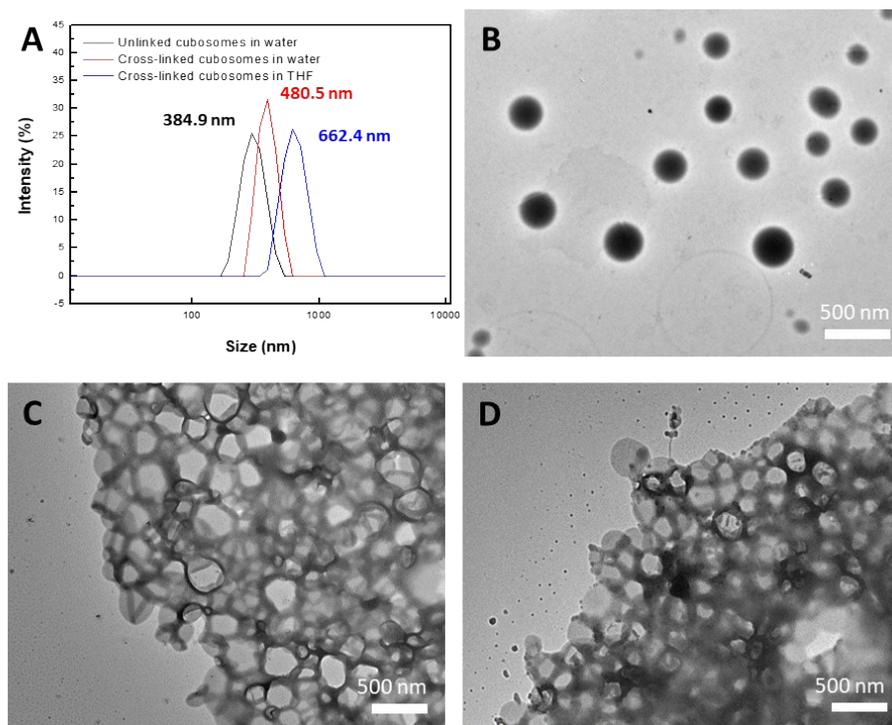


Fig. S3 (A) Dynamic light scattering (DLS) size plots of the uncross-linked (black) and cross-linked (red) polymersomes in aqueous and THF (blue). TEM images of (B) uncross-linked vesicles in water, and cross-linked vesicles (C) in water and (D) in THF.

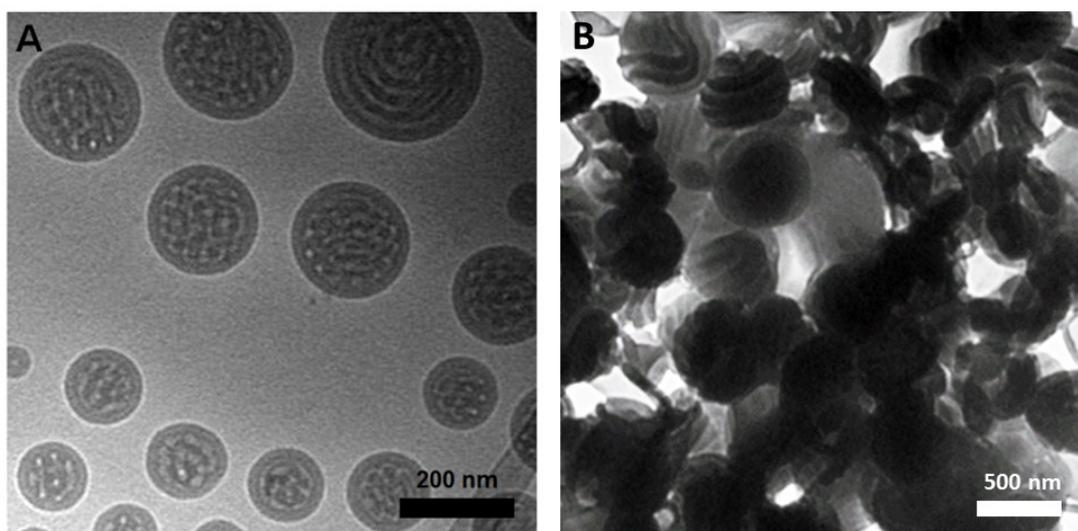


Fig. S4 (A) Cryo-TEM and **(B)** TEM images of mixed structures of hexagonal structures of PEG550₃-PI(1,4)(13K)₂

References

- 1 M. G. Jeong, J. C. M. van Hest, K. T. Kim, *Chem. Commun.*, 2012, **48**, 3590–3592.
- 2 V. Percec, M. Peterca, T. Tadjiev, X. Zeng, G. Ungar, P. Leowanawat, E. Aqad, M. R. Imam, B. M. Rosen, U. Akbey, R. Graf, S. Sekharan, D. Sebastiani, H. W. Spiess, P. A. Heiney, S. D. Hudson, *J. Am. Chem. Soc.*, 2011, **133**, 12197–12219.
- 3 A. Touris, J. W. Mays, N. Hadjichristidis, *Macromolecules*, 2011, **44**, 1886–1893.