Organic Nanospheres with Internal Bicontinuous Structure and Their Responsive Phase Inversion

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Supplementary Information

EXPERIMENTAL SECTION

Materials. Styrene (National Pharmaceutical, ≥99%), Isoprene (TCI, ≥99%), diphenylethylene (DPE) (Alfa Aesar, 98%) and *tert*-butyl methacrylate (*t*-BMA) (TCI, ≥99%) were distilled over CaH_2. Styrene and isoprene were redistilled over di-*n*-butylmagnesium ($MgBu_2$) (Aldrich 1.0 M in heptane) and *t*-BMA were redistilled over trioctylaluminum (Aldrich, 25 wt% in hexane) before use. *n*-Butyllithium (*n*-BuLi) (Aldrich, 2.5 M solution in cyclohexane/heptane), *sec*-Butyllithium (*s*-BuLi) (Aldrich, 1.3 M solution in cyclohexane), methyllithium (Acros, 1.0 M solution in diethyl ether) were used as received. The concentration of *s*-BuLi was analyzed by double titration. Cyclohexane and tetrahydrofuran (THF) were refluxed over sodium in the presence of benzophenone until a blue colour appeared. These solvent were redistilled over 1,1-diphenylhexyllithium (DPHLi, adduct of *s*-BuLi and DPE) before use. Methyltriphenylphosphonium bromide (Lancaster, ≥98%) and 1,3-dibenzoylbenzene (Aldrich, 98%) were vacuum dried before use. 1,3-Bis(1-

phenylvinyl)benzene (MDDPE) was synthesized according to literature.[1] All other reagents were used as received unless otherwise indicated.

Measurement. Gel permeation chromatography (GPC) was carried out on a Waters system equipped with three TSK-GEL H-type columns (particle size: 5.0 μm, M_w ranges: 0−1000, 0−2×10^4 and 0−4×10^5 g/mol) and a TSK-GEL guard column, Waters 515 pump, Waters 2414 RI detector, and Waters 2487 UV detector, using THF as the eluent at a flow rate of 1.0 mL/min at 40 °C. The columns were calibrated by polystyrene standards (M_w range: 682−1.4×10^6 g/mol). All samples were also measured by the same GPC system but switching the six-port valve to a multi-angle (14°−145°) laser light scattering (MALS) detector (Wyatt Technology, DAWN HELLOS) with the He-Ne light wavelength at 658.0 nm and a RI detector (λ = 658 nm, Wyatt Technology, OptilabrEX). THF was used as the eluent at a flow rate of 1.0 mL/min at 35 °C. The refractive index increment dn/dc was measured off-line by OptilabrEX at 25 °C. ^{1}H NMR spectra in $CDCl_3$ were recorded on a Bruker 500 MHz instrument with tetramethylsilane (TMS) as internal standard. Dynamic light scattering (DLS) was carried out on ALV/CGS-3 DLS/SLS laser light scattering Goniometer system equipped with a multi-τ digital correlator (ALV-7004) and a He-Ne laser light source (λ = 632.8 nm) at a fixed scattering angle of 90°. The refraction index n of the mixed solvent was measured by Abbe refractometer. The viscosity η of the mixed solvent was calculated by Grunberg-Nissan equation.[2] The mass spectrum was acquired using a Voyager DE-STR matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI TOF-MS) equipped with a 337 nm nitrogen laser. THF solutions of samples, dithranol (matrix), and silver trifluoroacetate with concentrations of 10, 20, and 10 mg/mL, respectively, were mixed in the volume ratio of matrix/polymer/salt = 10/2/1. Mass spectra were acquired in positive reflective mode using an acceleration voltage of 2 kV. External mass calibration was performed using a standard peptide mixture (insulin, thioredoxin). Transmission electron microscopy (TEM) images were collected on a Tecnai G^2 20 Twin TEM (FEI). The specimens for TEM observation were prepared by dipping a carbon-coated copper grid into sample solution for 1 s. For staining, the TEM specimens were exposed to OsO_4 or RuO_4 vapor for 1 h. Cryogenic Transmission Electron Microscopy (cryoTEM) was carried out using a Gatan cryo-holder operating at ~ –170 °C and FEI Tecnai G^2 20 Twin TEM at 200 kV. The vitrification of the sample was carried out using a vitrification robot (FEI Vitrobot™ Mark IV) at 100% humidity and 20 °C. A drop of sample (2 μL) was applied to the grid and blotted with filter paper and then the grid was plunged into liquid ethane at ~ –170 °C. The electron dose to obtain a single image is 0.17 $e^{-}.Å^{-2}$. Tilt series were recorded under low dose conditions (total dose 19.6 $e^{-}.Å^{-2}$ for cryoTEM and 22.7 $e^{-}.Å^{-2}$ for conventional TEM) with 1° tilt increment by the FEI explore 3D software ($I/I_{60} = 1.6$; Δfocus = −1 μm) between −60° and +53° for cryogenic sample and −63° and +68° for conventional sample, respectively. The 3D reconstructions were performed using the Inspect 3D software. Segmentation and visualization were performed with Amira Version 5.0. Scanning electron microscopy (SEM) experiments were carried out on a Hitachi S-4800 operating at 1 kV.

Synthesis of dendritic block terpolymer. Isoprene (2.7 g, 39 mmol) was dissolved in THF (35 mL) and initiated by *s*-BuLi (0.96 mL, 1.25 mmol) at 0 °C. After 1 h, the PILi solution was added into a THF solution (120 mL) of MDDPE (0.36 g, 1.25 mmol) at –78 °C. The mixture was stirred at –78 °C for 3.5 h. Then the solution was distilled to dryness and the solid residue was dissolved in cyclohexane (120 mL). An aliquot of solution was sampled and terminated by methanol for characterization, and the remaining solution was used to initiate polymerization of styrene (10.3 g, 99.3 mmol). After stirring at 40 °C for 5 h, the living polymer was end-capped with DPE (0.5 g, 2.7 mmol) in mixture of cylohexane/THF (1/2, v/v) at –78 °C for 30 min. A small amount of solution was sampled and terminated by methanol, and the residual solution was divided into 2 portions in equivalent volume and each part was mixed with LiCl (0.125 g, 2.9 mmol) in THF (20 mL). *t*-BMA of calculated weights for predetermined lengths of P*t*BMA segments were injected into each solution and all reaction systems were stirred at –78 °C for 1 h and terminated by methanol.

Synthesis of linear triblock terpolymer. Isoprene (1.6 g, 24 mmol) was dissolved in THF (30 mL) and initiated by *s*-BuLi (0.66 mL, 1.25 mmol) at 0 °C. After 1 h, 60 mL toluene and styrene (7.4 g, 71 mmol) were added subsequently at –40 °C and the reaction stood for 1 h. The resulting living polymer was end-capped with DPE (0.5 g, 2.7 mmol) and mixed with LiCl (0.38 g, 8.8 mmol) in a mixture of cylohexane/THF (1/2, v/v) at –78 °C for 30 min. *t*-BMA (2.0 g, 14 mmol) was injected into solution and reaction system was stirred at –78 °C for 1 h and terminated by methanol. The terpolymer was obtained after precipitation in methanol.

Self-assembly of dendritic block terpolymer. Dendri(PI_{14}-*b*-PS_{64}-*b*-P*t*BMA_{22}) (10 mg) was dissolved in THF (10 mL). Then ethanol (ethanol/THF = 10/1, v/v) was added dropwise using a syringe pump under stirring within 2 h. For staining, samples were exposed to OsO_4 or RuO_4 vapor for 1 h. To switch the solvent, *n*-heptane (*n*-heptane/ethanol = 10/1, v/v) was added dropwise using a syringe pump under stirring within 2 h. To switch the solvent back to ethanol, THF (THF/*n*-heptane = 1/10, v/v) was first added into the solution and the system was balanced for 2 h before subsequent addition of ethanol (ethanol/*n*-heptane = 10/1, v/v). Samples were stirred for 3 days before TEM observation.

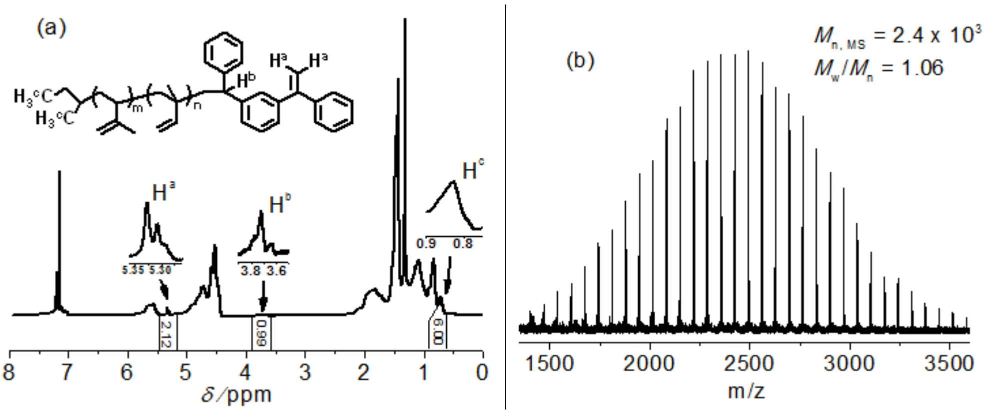

Figure S1. ^{1}H NMR (in CDCl$_3$) (a) and MALDI−TOF MS (b) of PI macroinimer.

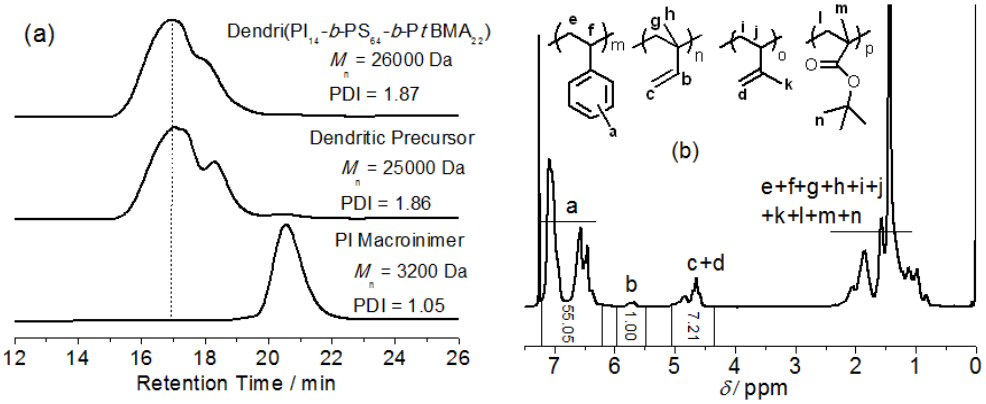

Figure S2. Results of GPC (a) and ^{1}H NMR (in CDCl$_3$) (b) of dendritic block terpolymer, Dendri(PI$_{14}$-*b*-PS$_{64}$-*b*-P*t*BMA$_{22}$).

Table S1. Characterization Results of Dendritic and Linear Block Terpolymers

	Macroinimers			Dendritic Precursors			Dendritic Block Copolymers			
Sample	$M_{\mathrm{n,GPC}}$ (g/mol)	$M_{\mathrm{w,MALS}}$ (g/mol)	$M_{\mathrm{w}}/M_{\mathrm{n}}$ (GPC)	$M_{\mathrm{n,GPC}}$ (10^4 g/mol)	$M_{\mathrm{w,MALS}}$ (10^4 g/mol)	$M_{\mathrm{w}}/M_{\mathrm{n}}$ (GPC)	$M_{\mathrm{n,GPC}}$ (10^4 g/mol)	$M_{\mathrm{w,MALS}}$ (10^4 g/mol)	$M_{\mathrm{w}}/M_{\mathrm{n}}$ (GPC)	PI/PS/P*t*BMA[b] (wt/wt/wt)
Dendri(PI$_{14}$-*b*-PS$_{64}$-*b*-P*t*BMA$_{22}$)	3200	2500	1.05	2.6	6.6	1.86	2.6	7.2	1.87	14/64/22
Dendri(PI$_{6}$-*b*-PS$_{28}$-*b*-P*t*BMA$_{66}$)	3200	2500	1.05	2.6	6.6	1.86	5.1	15.9	2.00	6/28/66
Linear(PI$_{13}$-*b*-PS$_{66}$-*b*-P*t*BMA$_{21}$)[a]	-	-	-	-	-	-	1.5	2.2	1.19	13/66/21
Dendri(PS$_{72}$-*b*-P*t*BMA$_{28}$)	2100	2500	1.06	2.0	4.9	1.63	2.3	5.9	1.70	−/72/28

[a] Linear triblock terpolymer. [b] Determined by ^{1}H NMR.

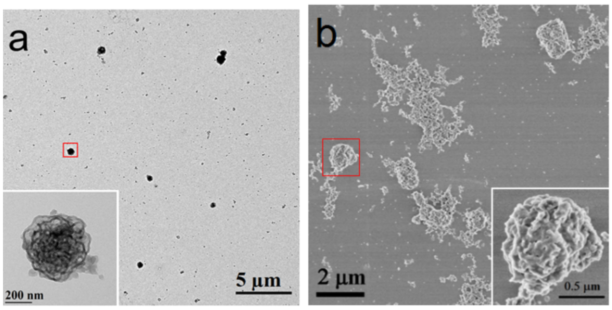

Figure S3. Conventional TEM (a) and SEM (b) images of aggregates from self-assembly of Dendri(PI_{14}-*b*-PS_{64}-*b*- P*t*BMA$_{22}$) in a mixture of ethanol/THF (10/1, v/v).

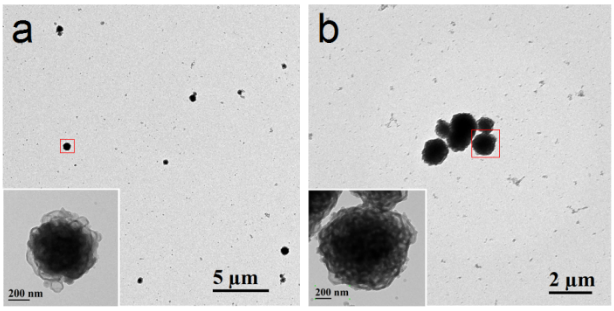

Figure S4. Conventional TEM images of aggregates from self-assembly of Dendri(PI_{14}-*b*-PS_{64}-*b*-P*t*BMA$_{22}$) after stained by OsO_4 (a) and RuO_4 (b).

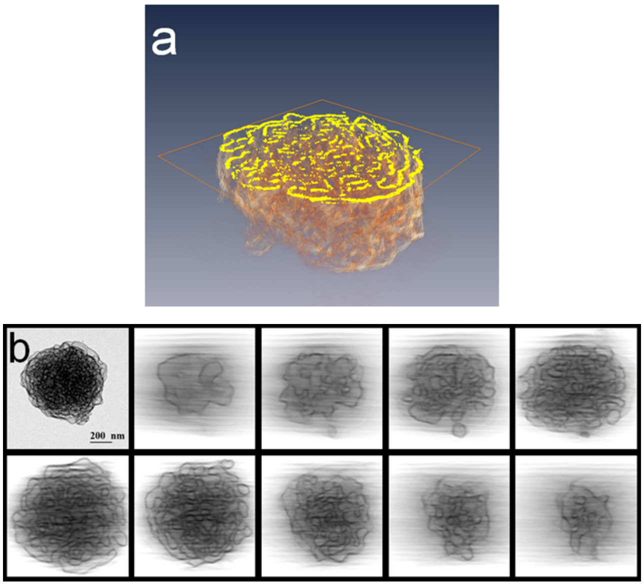

Figure S5. Conventional TEM image of the nanosphere assembled from Dendri(PI_{14}-*b*-PS_{64}-*b*- P*t*BMA$_{22}$). (a) 3D reconstruction of the segmented volume; (b) tomographic slices of different cross-sections along *z*-axis.

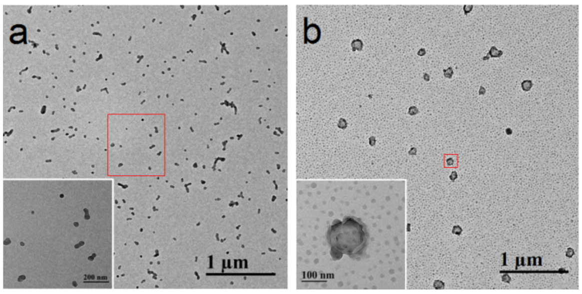

Figure S6. Conventional TEM images for self-assembly of Dendri(PS_{72}-*b*-P*t*BMA_{28}) (replacing PI by PS segments in the synthesis starting from a PS macroinimer) (a) and Dendri(PI_6-*b*-PS_{28}-*b*-P*t*BMA_{66}) (b) in a mixture of ethanol/THF (10/1, v/v).

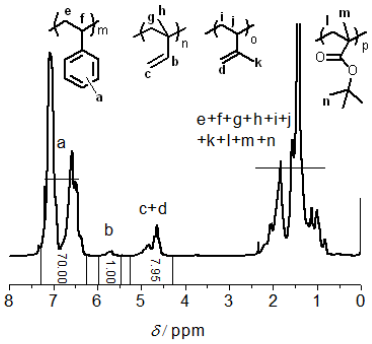

Figure S7. ^1H NMR (in $CDCl_3$) for linear triblock terpolymer, PI_{13}-*b*-PS_{66}-*b*-P*t*BMA_{21}.

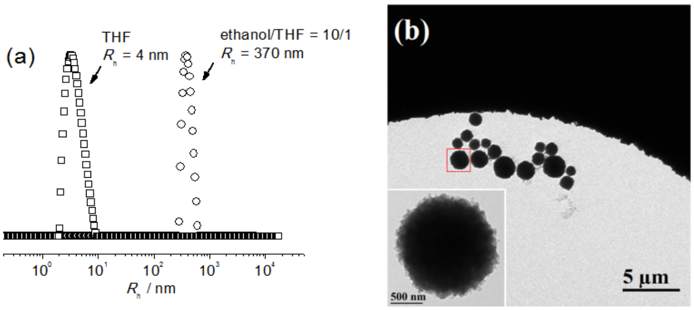

Figure S8 DLS result (a) and Conventional TEM image (b) of aggregates from self-assembly of linear PI_{13}-*b*-PS_{66}-*b*- P*t*BMA_{21} in a mixture of ethanol/THF (10/1, v/v).

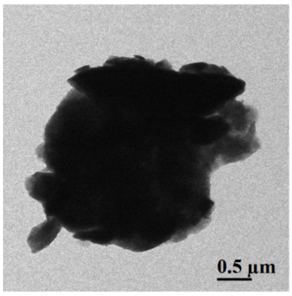

Figure S9 Conventional TEM image of aggregates formed from Dendri(PI_{14}-*b*-PS_{64}-*b*-P*t*BMA_{22}) by adding ethanol (ten-fold in volume) to the sample of Figure 4b without aid of THF.

REFERENCE

[1] G. G. H. Schulz, H. Höcker, *Makromol. Chem.* 1977, **178**, 2589−2594.

[2] L. Grunberg, A. H. Nissan, *Nature* 1949, 764.