

Temperature-Responsive Phase Transition of Polymer Vesicles: Real-Time Morphology Observation and Molecular Mechanism

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Supporting Information

S1. Experimental section

Synthesis and Self-Assembly. The hyperbranched multi-arm copolymer, HBPO-star-PEO, was synthesized by two-step cationic ring-opening polymerization as reported previously.¹ At the first step, 3-ethyl-3-oxetanemethanol was polymerized in the solution of $\text{BF}_3 \cdot \text{OEt}_2$ and CH_2Cl_2 under argon atmosphere (the molar ratio of 3-ethyl-3-oxetanemethanol to $\text{BF}_3 \cdot \text{OEt}_2$ is 2:1) to obtain the hyperbranched poly[3-ethyl-3-(hydroxymethyl)oxetane] (HBPO, the degree of the branching is 0.4) precursor. At the second step, the HBPO precursor was carefully divided into several parts (the active species was still living in each part), and different amounts of ethylene oxide (EO) were dropwise added into each part of the precursor carefully to

produce the multi-arm copolymers denoted as HBPO-star-PEOs (samples HB1-6). The molecular weight of the obtained polymers relative to polystyrene standards were determined by the size exclusive chromatography (SEC, Perkin-Elmer series 200) with a refraction index detector in *N,N*-dimethylformamide (DMF). Every HBPO-star-PEO molecule has a HBPO core and many PEO arms (Scheme 1 in main text).

The self-assembly process involved the direct addition of synthesized HB1-6 samples into the deionized water under stirring with a magnetic bar in room temperature. Water is the good solvent for PEO arms and the non-solvent for HBPO cores. The appearance of turbidity in the solution indicated the formation of the aggregations. HB1-3 samples can self-assemble into giant polymer vesicles with the average diameter of 112.8 μm , 22.6 μm and 4.0 μm , respectively.²

LCST Measurements: The optical transmittance of the polymer solutions at various temperatures was measured at 500 nm with a UV-VIS spectrophotometer (GBC Cintra 100, Aus.) equipped with a thermo cell at a rate of 0.5 °C/min in heating and cooling scans between 4 and 90 °C. The temperature at which the heating curve begins to decrease is defined as the LCST. To observe the reversible thermo-responsibility, optical transmittance was evaluated by temperature fluctuation between 25°C and 55°C for the HB3 vesicle solution. In addition, the concentration dependence of the LCST was also studied by performing the transmittance measurements for the HB3 vesicle solution with different polymer concentration from 0.1, 1, 5 and 10 to 20 mg/ml.

Critical Aggregation Concentration (CAC) Measurements: The CAC of the HBPO-star-PEOs was determined by using pyrene as a fluorescent probe.³ Solutions of HBPO-star-PEO with different concentrations were prepared with a constant pyrene concentration of 4×10^{-7} M. Both the excitation and emission spectra were recorded under LS-50B luminescence spectrometer (Perkin Elmer Co., U.S.A.). The emission wavelength was set at 375 nm, which was chosen according to the maximum intensity obtained in the excitation spectra. The fluorescence excitation spectra were recorded between 300 nm~360 nm.

Dynamic Light Scattering (DLS) Measurements. The measurements were performed in aqueous solution using a Malvern Zetasizer 3000 HS apparatus (Malvern Instruments Ltd.) equipped with a 125 MW laser operating at $\lambda = 633$ nm. The DLS measurements were conducted at room temperature and at a scattering angle of 90°.

¹H Nuclear Magnetic Resonance Spectroscopy (¹H NMR) Measurements: HBPO-star-PEO polymers were dissolved in D₂O and measured under a Varian Mercuryplus 400 MHz spectrometer with TMS as the inner standard. All solution-state ¹H NMR spectra in the temperature of 35 °C, 40 °C, 45 °C, 50 °C, 60 °C and 70 °C were recorded.

Optical Microscopy Measurements: The morphology of the aggregates of the HBPO-star-PEO polymer solution was observed by fluorescence microscopy (Leica DM 4500 B) equipped with a color video recorder (leica DFC 300FX, 3.15 million pels) and a 100-watt mercury lamp. The observation was conducted in a bright field

pattern using transmission light. The magnification of the eyepiece used is 10 \times , and the objective lens are 40 \times (working distance is 3.3 mm) and 63 \times (water lens), respectively. For the measurement, the sample (polymer vesicle solution in water, typically 10 mg/ml) was transferred immediately into a culture dish for the direct observation by optical microscopy.

The fluorescence-labeled vesicles were observed by confocal laser scanning microscope (LSM 510 META). The aqueous polymer vesicle suspension was directly used as the samples for the observation.

Transmission Electron Microscopy (TEM) Measurements: The aggregation morphology was also observed by TEM. For TEM measurement, a drop of the polymer solution was placed onto 400 mesh copper grids coated with parlodion film stabilized with vacuum evaporated carbon. The excess fluid was drained off with filter paper after five minutes. Then a drop of negative stain of 2% aqueous uranyl acetate solution was added to stain the samples for four minutes. The excess stain was removed with filter paper. The samples were examined in an electron microscope (JEOL JEM-2010), operating at 200 kv.

S2. Supramolecular self-assembly of polymer vesicles

HBPO-star-PEO has several structure features, which makes it a good precursor for supramolecular self-assembly. First, HBPO-star-PEO is one of the amphiphilic block copolymers that possesses a hydrophobic HBPO core and many hydrophilic PEO arms; Second, HBPO-star-PEO is flexible due to its polyether skeleton structure, and

the conformational freedom may allow the polymer molecules to adapt to the specific dynamic and geometric requirements for the self-assembly process. Third, as a hyperbranched macromolecule, HBPO-star-PEO has many terminal functional groups (hydroxyl groups), which facilitates the formation of hydrogen bonds in the self-assembly process. In fact, regardless of the hyperbranched architecture, HBPO-star-PEO is similar to the famous linear block copolymer from Poly(propylene oxide) (PPO) and PEO, such as PEO-PPO-PEO triblock copolymers (Pluronics).¹ So, the self-assembly of HBPO-star-PEO molecules is promising.

CAC measurement: The self-assembly ability of the HB polymers in water was proved by the fluorescence spectrometry using pyrene as the probe. The fluorescence excitation spectra (emission wavelength is set at 375 nm) of the polymer solution containing pyrene were recorded (the inset in Figure S1). A red shift of the pyrene (0,0) band from 335 to 338.5 nm was observed on increasing the copolymer concentration. This change reflects the formation of the supramolecular aggregates with increasing the polymer concentration and the pyrene was transferred from water into the hydrophobic part of the aggregates. A plot of the ratio of the intensity of the signal at 338.5 nm to that at 335 nm ($I_{338.5}/I_{335}$) versus the logarithm of the block copolymer concentration (Figure S1) results in a CAC value. The CAC was obtained from the intersection of two straight lines (as shown in the arrow). All the HBPO-star-PEO polymer samples (HB1-6) exhibit the CAC values (Table 1 in main text), it can also be found that the more the hydrophilic PEO contents (the higher the $R_{A/C}$), the higher the CAC.

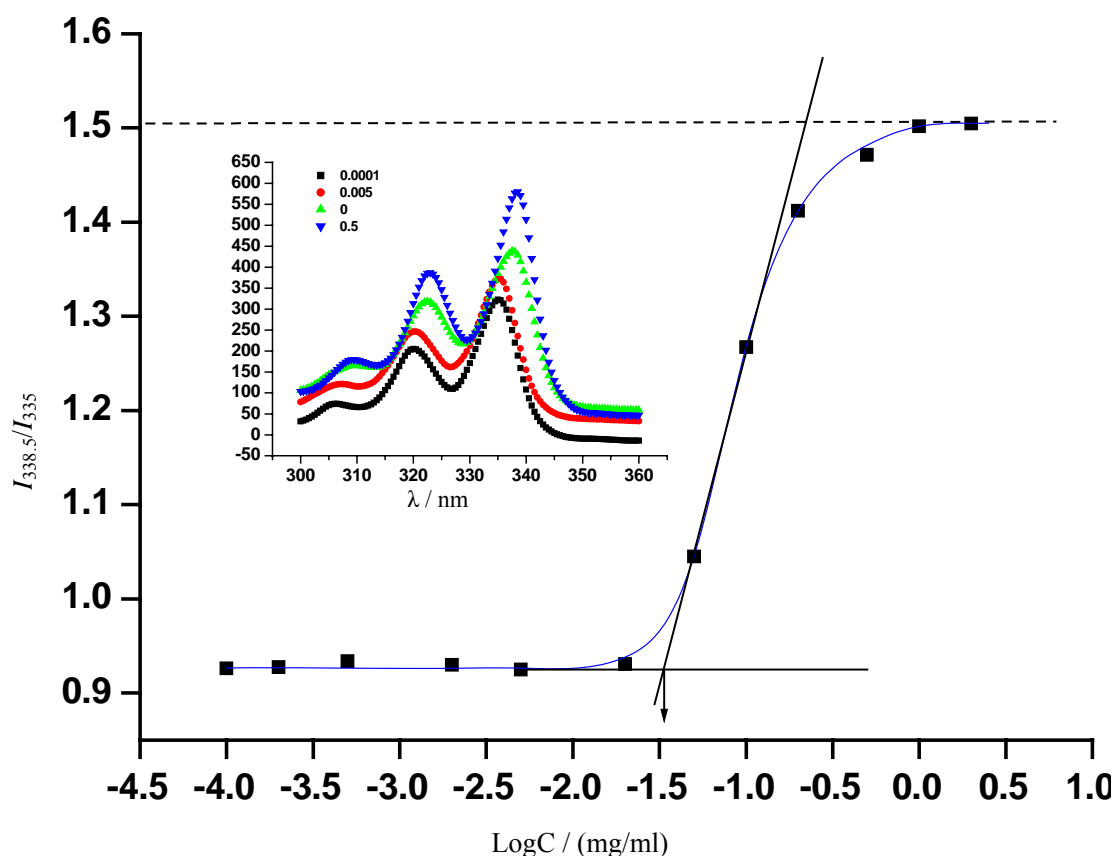


Figure S1 Intensity ratio $I_{338.5}/I_{335}$ obtained from the fluorescence excitation spectra of pyrene plotted versus HBPO-star-PEO concentration. Inset: fluorescence excitation spectra of pyrene solution below and above the CAC.

Vesicle structures: In our previous work, we² have already shown that the HB1-3 copolymer samples can self-assemble into a novel polymer vesicles coined as branched-polymersomes in water. As shown in Scheme 1 of main text, branched-polymersomes have a sandwich-like structure with an internal hydrophobic layer of HBPO cores and outer hydrophilic shells of PEO arms.² Similar to the phase

separation of hydrophilic dendrimer decorated with hydrophobic side chains as reported by Meijer and coworkers⁴, in branched-polymersomes each HBPO-star-PEO molecule spontaneously segregates into a hydrophilic PEO part and a hydrophobic HBPO part (similar to AB block copolymer) to form the bilayer structure or segregates into a molecular sandwich-like structure with an internal layer consisting of the HBPO core and outer shells of PEO arms (similar to ABA block copolymer) to form monolayer structure. Branched-polymersome possesses two special characteristics that distinguish it from other polymer vesicles. One is that it is the first demonstration of the polymer vesicle derived from the molecular self-assembly of hyperbranched polymers; the other is that the vesicle-forming polymers for branched-polymersome have a high hydrophilic fraction (the volume fraction of the hydrophilic PEO components, more than 60%).

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