Electronic Supplementary Information

High-genus multicompartment vesicles evolved from large compound micelles

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1 Materials

Poly(ethylene oxide) (PEO_{43}, M_n = 1900) was purchased from Alfa Aesar. Ethyl 4-aminobenzene carboxylate, 4-aminobenzyl alcohol, $KHSO_4$, dibutyltin dilaurate (DBTL), 2-isocyanatoethyl methacrylate (IEM), AIBN, anhydrous dichloromethane (DCM) and *N*, *N*'-dimethylformamide (DMF) were obtained from Aladdin Chemistry, Co. Ltd. Hydrochloric acid, $NaHCO_3$, NaCl, anhydrous $MgSO_4$, anhydrous Na_2SO_4, NaOH, 4-dimethylaminopyridine (DMAP), *N*, *N*'-dicyclohexylcarbodiimide (DCC), *N*, *N*'-dimethylpyridin-4-amine 4-methylbenzenesulfonate (DPTS) and organic solvents containing tetrahydrofuran (THF), ethyl acetate (EtOAc), acetic acid (AcOH), *n*-hexane, ethyl ether and toluene were obtained from Sinopharm Chemical Reagent Co., Ltd. (SCRC, Shanghai, China). DMSO-d_6 was purchased from J&K Scientific Ltd.

2 Experimental section

2.1 Synthesis of PEO_{43}-DDMAT

The synthesis of macromolecular chain transfer agent PEO_{43}-DDMAT refers to literature methods.[1] PEO_{43} (20.0 g, 0.0100 mol) and DMAP (0.244 g, 2.00 mmol) were dissolved in toluene (150 mL) and stirred at 145 ºC. The reaction temperature was decreased to room temperature when around 120 mL toluene was evaporated. Then anhydrous DCM (50.0 mL), DDMAT (7.30 g, 0.0200 mol), DCC (0.350 g, 0.0500 mol) and DPTS (1.48 g, 5.00 mmol) were added to the above solution, and the mixture was stirred at room temperature for 20 h. Afterwards, this reaction solution was filtered to remove the solids, and precipitated in *n*-hexane for three times. The solid was dried in a vacuum oven to obtain the final product.

2.2 Synthesis of monomer ACEMA

Ethyl 4-aminobenzene carboxylate (1.00 g, 6.05 mmol) and $KHSO_4$ (7.44 g, 12.1 mmol) were dissolved in deionized water (50.0 mL) and stirred at 25 ºC. After 2 h, the mixture was washed with 1.0 M HCl (50.0 mL × 2), saturated $NaHCO_3$ (50.0 mL × 2) and saturated NaCl (50.0 mL × 2). After dried over anhydrous $MgSO_4$, the solution was evaporated by rotary evaporator to yield mediate product of ethyl 4-cyanobenzoate (0.930 g, yield: ~97%).

The whole of this mediate product (0.930 g, 5.19 mmol) and 4-aminobenzyl alcohol (360 mg, 2.93 mmol) was dissolved in the mixture of anhydrous DCM (50.0 mL) and AcOH (50.0 mL). Then the mixture was stirred at room temperature for 24 h and monitored by thin-layer chromatography (TLC). The solution was evaporated by rotary evaporator to obtain crude product. Then the crude product was dissolved in EtOAc (50.0 mL). Then this solution was washed by saturated $NaHCO_3$ (100 mL × 3) and saturated NaCl (100 mL × 3). After dried over anhydrous Na_2SO_4, the organic phase was evaporated by rotary evaporator to remove EtOAc. The crude product was purified by column chromatography (*n*-hexane/EtOAc = 2/1, v/v) to yield preliminary product of ethyl 4-hydroxyazobenzoate (0.550 g, 2.16 mmol).

Then ethyl 4-hydroxyazobenzoate (350 mg, 1.37 mmol) was dissolved in anhydrous THF, and catalyst DBTL (0.100 mL, 3.50 mmol) was added. IEM (300 mg, 1.94 mmol) was dissolved in anhydrous THF and added in the flask with argon bubbling under stirring for 15 minutes. Then the flask sealed with argon was placed in an oil bath at room temperature and monitored by TLC. The solution was evaporated by rotary evaporator to obtain solid. This solid was dissolved in DCM (50.0 mL), and washed by 1.0 M NaOH (50.0 mL), 1.0 M HCl (50.0 mL) and saturated NaCl (50.0 mL). After dried over anhydrous $MgSO_4$, the organic phase was evaporated by rotary evaporator to remove THF. The crude product was purified by column chromatography (200-300 mesh, *n*-hexane/EtOAc = 1/1, v/v) to yield ACEMA monomer. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.17 (d, 2H), 8.01 (m, 4H), 7.95 (d, 2H), 6.11 (s, H), 6.05 (s, H), 5.68 (s, H), 5.15 (s, 2H), 4.35 (m, 2H), 4.07 (dd, 2H), 3.28 (m, 2H), 1.87 (m, 3H), 1.36 (m, 3H), 1.26 (m, 3H), 1.15 (s, 2H).

2.3 Synthesis of copolymer PEO_{43}-*b*-$PACEMA_{10}$

The PEO_{43}-*b*-$PACEMA_{10}$ copolymer was synthesized via RAFT polymerization. PEO-DDMAT (100 mg, 0.440 mmol) and ACEMA monomer (300 mg, 0.680 mmol) were added to a round bottom flask. Then oxygen was removed by purging with argon for 15 minutes. AIBN (2.0 mg, 0.010 mmol) was added into the flask with argon bubbling for additional 5 minutes. Then the flask sealed with argon was placed in an oil bath at 70 °C. After 20 h, the reaction was terminated by cooling to room temperature and exposure to the air. The mixture was evaporated under vacuum, then dissolved in DCM and precipitated in diethyl ether for 3 times. After filtration, the filter residue was dried under vacuum for 24 h to obtain the solid of PEO_{43}-*b*-$PACEMA_{10}$.

2.4 Preparation of high-genus multicompartment vesicles (HGMVs)

The HGMVs were self-assembled from PEO_{43}-*b*-$PACEMA_{10}$ copolymer by solvent exchange method. This copolymer was dissolved in THF with an initial concentration (C_{ini}) of 1.0 mg mL^{-1}. Then deionized water was added into the solution dropwise under slow stirring (rotation speed: 300 rpm; dropping rate: 5.0 μL s^{-1}; THF/water = 1/4, v/v). The mixture was still stirred for 30 min. Then the THF was removed by dialyzing against deionized water for 2 days (using the dialysis tube with molecular weight cutoff 3500 kDa). After dialysis, the concentration of the HGMVs aqueous solution was calculated to be 0.5 mg mL^{-1}.

2.5 Effect of water content during self-assembly

The PEO_{43}-*b*-$PACEMA_{10}$ copolymers were dissolved in THF with a C_{ini} of 1.0 mg mL^{-1}. Then 5.0 mL of this solution was taken out, and deionized water was added into the solution dropwise under slow stirring (rotation speed: 300 rpm; dropping rate: 5.0 μL s^{-1}). When 0.5 mL deionized water was added every time, the transmittance and the fluorescence intensity of the solution were measured to monitor the variation of the solution.

Additionally, 5.0 mL of the above solution was taken out as 9 groups. Then 2.0, 4.0, 4.5, 5.0, 5.5, 7.5, 10.0, 15.0 and 20.0 mL of deionized water was added into the 9 groups of the solution dropwise under slowly stirring. After stirring for another 30 min, the mixture was dialyzed against deionized water to remove THF. Then the aqueous solutions were characterized by DLS and TEM to observe the change of the size and the morphology of nanostructures during self-assembly.

2.6 Photo-responsive behaviour of HGMVs

After the HGMVs aqueous solution was diluted to 0.1 mg mL^{-1}, the absorbance of this solution was examined by UV-Vis spectroscopy. Then this aqueous solution was irradiated by UV light of 8000 W cm^{-2} power for 90 min. The solution was characterized by UV-Vis spectroscopy when the irradiation time was 2, 8, 30, 60 and 90 min, respectively. The nanostructure of the last group (UV-irradiated for 90 min) was filmed by TEM to observe the effect of photo-isomerization on the membrane structure of HGMVs. Afterwards, the solution was exposed to visible light and characterized by DLS to monitor the variation of the vesicle size.

3 Characterization

3.1 Proton nuclear magnetic resonance (^{1}H NMR)

^{1}H NMR spectra were recorded using a Bruker AV 400 MHz spectrometer at room temperature using DMSO-d_6 as solvent and tetramethylsilane (TMS) as the standard.

3.2 Size exclusion chromatography (SEC)

An Agilent 1260 Infinity SEC analysis system was used to measure the molecular weights (M_n) and polydispersity ($Đ$) of the copolymer. The SEC set-up comprised an Agilent 1260 Infinity series degasser and pump, two Agilent PLgel 5 mm Mixed-C columns in series and a refractive index detector. THF was used as an eluent at 40 °C at a fixed flow rate of 0.8 mL min^{-1}. A series of ten PEO standards (M_p values ranging from 430 to 26000) were used for calibration.

3.3 Dynamic light scattering (DLS)

The hydrodynamic diameter (D_h) and particle dispersion (PD) index of the nanostructures were determined using a ZETASIZER Nano series instrument (Malvern Instruments ZS 90) at a fixed scattering angle of 90°. Each reported measurement is an average from three times that consists of 11 runs (10 seconds for each run).

3.4 Ultraviolet-visible spectroscopy (UV-Vis)

The UV-Vis spectra of the solution at different water content were acquired at the scanning speed of 300 nm min^{-1} using a UV759S UV-Vis spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd.). All the samples were analysed using quartz cuvettes. When UV-Vis analysis was conducted, THF and water were used as background, respectively.

3.5 Fluorescence spectroscopy

The fluorescence spectra of the solution at different water content were acquired using an OLYMPUS IX73 Fluorescence Microscope (New York Thermo Fisher Scientific). The fluorescence intensity of the characteristic peaks of 320 nm and 440 nm for each group were recorded to analyse the formation procedure of HGMVs.

3.6 Transmission electron microscopy (TEM)

The aqueous solutions of the nanostructures (8.0 μL) were dropped onto a copper grid and dried at room temperature. The samples were viewed without staining. Images were recorded on a JEOL JEM-2100F TEM instrument at 200 kV equipped with a Gatan 894 Ultrascan 1k CCD camera.

3.7 Scanning electron microscopy (SEM)

To obtain SEM images, a drop of the HGMVs solution (0.2 mg mL^{-1} in deionized water) was spread on a silicon wafer and dried at ambient temperature. Then the sample on the wafer was coated with gold and viewed using an FEI Quanta FEG 250 electron microscope equipped with a digital camera at 10 kV.

4 Supplemental figures

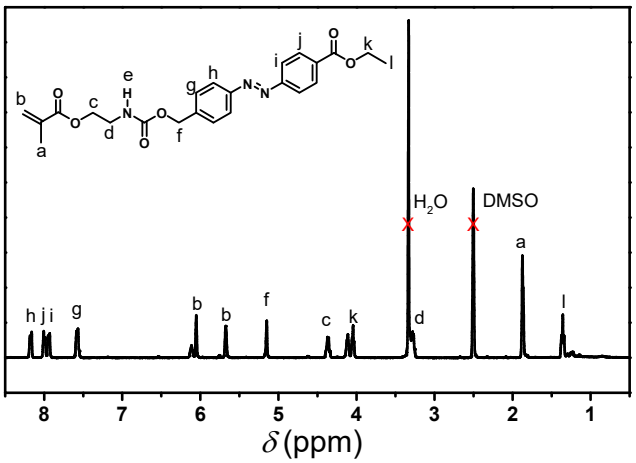

Fig. S1 ^{1}H NMR spectrum of ACEMA monomer in DMSO-d_6.

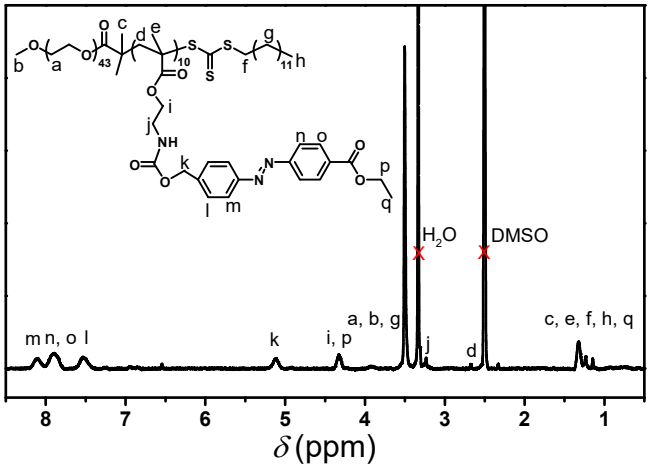

Fig. S2 ^{1}H NMR spectrum of PEO_{43}-*b*-$PACEMA_{10}$ copolymer in DMSO-d_6.

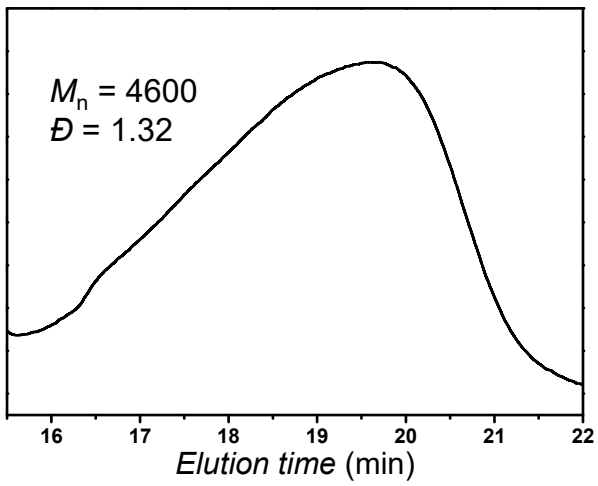

Fig. S3 THF SEC trace of a PEO_{43}-*b*-$PACEMA_{10}$ copolymer.

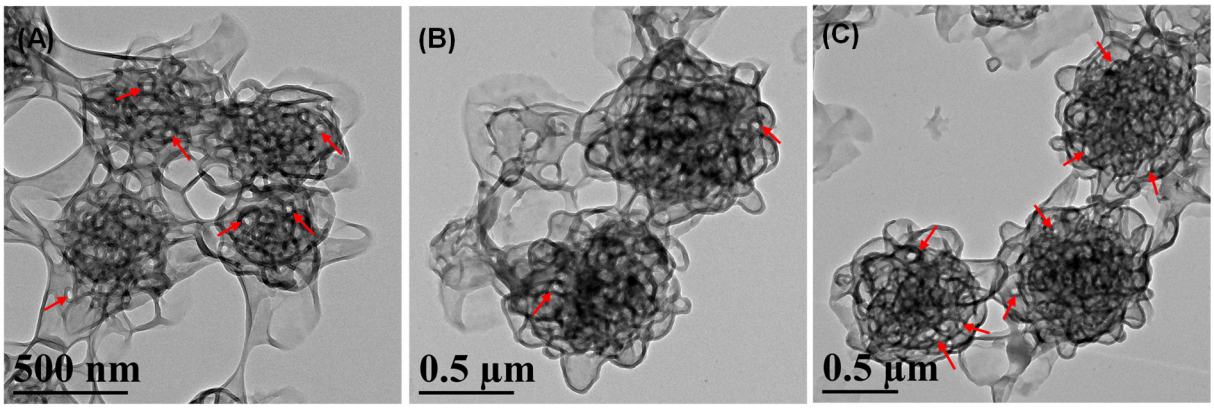

Fig. S4 TEM images of high-genus multicompartment vesicles (HGMVs) self-assembled from PEO_{43}-*b*-$PACEMA_{10}$ copolymer at C_{ini} = 1.0 mg mL^{-1} in THF/water (1/4, v/v); the red arrows point to the perforated holes on the surface.

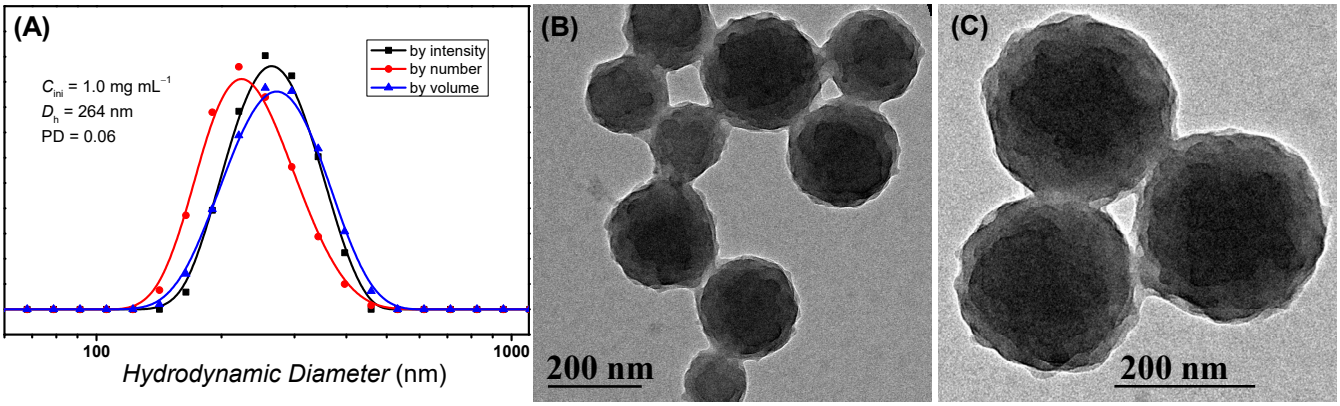

Fig. S5 (A) DIS size distribution of nanostructures self-assembled from PEO_{43}-*b*-$PACEMA_{10}$ at C_{ini} = 1.0 mg mL^{-1} in DMF/water (1/4, v/v); (B) and (C) TEM images of large compound micelles (LCMs) after dialyzing against deionized water without staining.

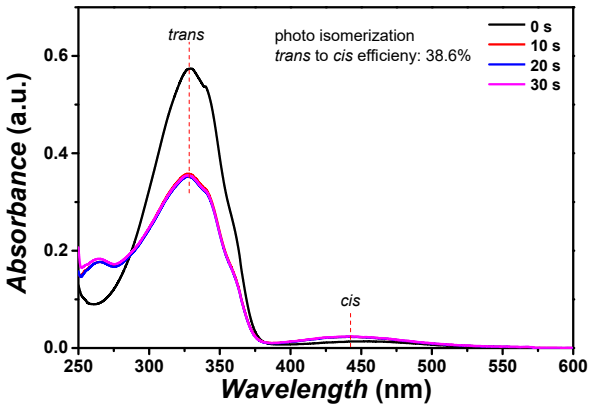

Fig. S6 UV-Vis spectra of the ACEMA monomer solution in THF irradiated at 450 nm (the photo-isomerization efficiency was 38.6% when UV-irradiated for 10 ~ 30 s).

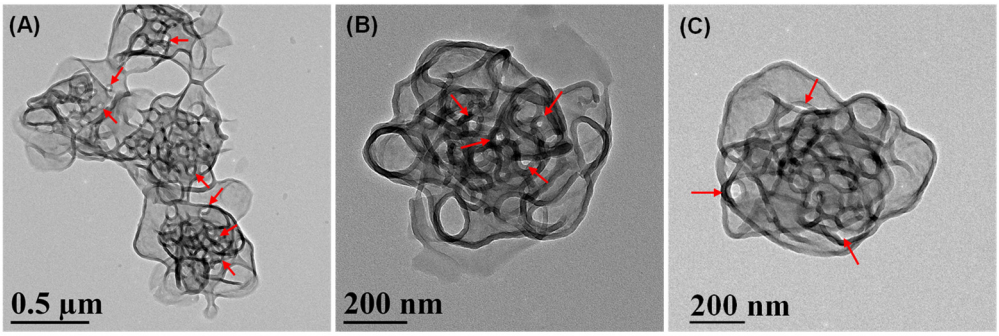

Fig. S7 TEM images of high-genus multicompartment vesicles (HGMVs) when UV-irradiated at 450 nm (the red arrows point to the internal cavities and perforated holes).

5 References

1. F. J. Lu, Y. W. Luo, B. G. Li, Q. Zhao and F. J. Schork, *Macromolecules*, 2010, **43**, 568-571.