

Supporting Information

Enzyme Catalysis-induced RAFT Polymerization in Water for the preparation of Epoxy-Functionalized Triblock Copolymer Vesicles

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Experimental section

Material

2-Hydroxypropyl methacrylate (HPMA, Aladdin), glycidyl methacrylate (GlyMA, Aladdin), horseradish peroxidase (HRP, Aladdin, ≥ 300 units/mg dry weight), acetylacetone (ACAC, Aladdin, $\geq 99\%$), sodium dodecylsulfate (SDS, Aladdin), silver nitrate (AgNO_3 , Aladdin), sodium borohydride (NaBH_4 , Aladdin), methylene blue (MB, 0.05 wt.%, Aladdin), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, Aladdin), hydrogen peroxide (Aladdin, 30 wt.% stabilized aqueous solution) were used directly. 4-Cyano-4-(ethylthiocarbonothioylthio) pentanoic acid (CEPA) was synthesized according to the literature.^[1]

Characterization

¹H NMR Spectroscopy. Nuclear magnetic resonance (NMR) spectra were recorded in d_6 -DMSO using a Bruker Avance III 400 MHz NMR spectrometer at a temperature of 25 °C.

Transmission Electron Microscopy (TEM). The obtained samples were diluted 100-fold with water. A drop of the solution was placed on a copper grid for 1 min and then blotted with filter paper to remove excess solution. A drop of uranyl acetate solution (0.5 wt%) was soaked on the same copper grid for 1.5 min, and then blotted with filter paper. TEM observations were carried out on a Hitachi 7700 instrument operated at 120 kV.

Gel Permeation Chromatography (GPC). The molecular weight and polydispersity of the samples were measured by gel permeation chromatography (GPC) using a Waters 1515 GPC instrument with dimethylformamide (DMF) as the mobile phase and Waters styragel HR1 and HR4 columns. The eluent used was HPLC grade DMF containing 10 mM LiBr and was filtered prior to use. The flow rate of DMF was 1.0 mL/min. Linear poly(methyl methacrylate) polymers with narrow molecular weight distributions were used as the standards to calibrate apparatus.

Dynamic light scattering (DLS). Intensity-average hydrodynamic diameters of the copolymer dispersions were determined using a Malvern Zetasizer Nano ZS instrument. Dilute aqueous dispersions were analyzed using disposable cuvettes and all data were averaged over three consecutive runs.

Aqueous electrophoresis. Zeta potentials were determined as a function of solution pH using a

Malvern Zetasizer Nano ZS instrument equipped with an auto-titrator for triblock nanoparticles diluted to approximately 0.3% w/w. The solution pH was adjusted using NaOH or HCl.

Optical microscopy (OM). The emulsion images were recorded using an optical microscope (BK-POL, Chongqing, China) with a built-in-camera.

UV-visible absorption spectroscopy. All UV-visible spectra were recorded with a 1.0 cm quartz cuvette using a UV2450 spectrometer.

Synthesis of glycerol monomethacrylate (GMA)

Glycidyl methacrylate (40.0 g) was added to water (360.0 g, 10% w/w) in a round bottom flask connected with a condenser while stirred for 9 h at 80 °C. The resulting solution was then saturated with NaCl and extracted with dichloromethane. The organic phase was dried with MgSO₄ overnight, filtered, and concentrated under reduced pressure. The obtained monomer was further purified by silica column chromatography (CH₂Cl₂: CH₃OH = 20: 1), and then concentrated and dried at 45 °C under vacuum.

Synthesis of PGMA-CEPA macro-RAFT agent

GMA (31.48 g, 0.197 mol), ACVA (0.2 g, 0.714 mmol), and CEPA (0.94 g, 3.57 mmol, CTA/ACVA molar ratio = 5.0), and 1, 3, 5-trioxane (1.7 g, 0.0189 mol) were added into a 150 mL round-bottom flask, and then dissolved with anhydrous ethanol (47.22 g, 40% w/w) to form a homogeneous solution. The reaction mixture was purged with nitrogen for 30 min, sealed, and then immersed in a 70 °C pre-heated oil bath for 2 h. The polymerization was quenched by immersion in ice-water and exposure to air and analyzed by ¹H NMR spectroscopy, which indicated a mean degree of polymerization of 46 (83.3 % monomer conversion). The polymer was precipitated by adding excess hexane (500 mL) and washed several times. The obtained product was dried at 45 °C under vacuum overnight, and then analyzed by DMF GPC. The DMF GPC measurement confirmed its $M_n = 14800$ g/mol and $M_w/M_n = 1.12$.

Synthesis of PGMA₄₆-PHPMA₃₀₀ diblock copolymer precursor vesicles via HRP-initiated aqueous RAFT dispersion polymerization

PGMA₄₆-CEPA (0.76g, 0.1mmol), HPMA (4.33g, 0.03 mol), deionized water(15.18 g, to make

the HPMa concentration of 20% w/w), ACAC stock solution (940 μL , 0.425 mol/L) and HRP stock solution (1.08 mL, 8.8 mg/ml) were added to a 25 mL round bottom flask. Then the reaction mixture was degassed with nitrogen for 30 min and maintained at 32°C in a water bath. Finally, H_2O_2 (100 μL , 0.3 mol/L) was injected into the solution to initiate the polymerization for 3 h. The molar ratio of [CTA]: [HRP]: [H_2O_2]: [ACAC] was maintained at 1: 0.00216: 0.3: 4.

Synthesis of PGMA₄₆-PHPMA₃₀₀-PGlyMA_n (n = 100, 200, 300, 400) triblock copolymer vesicles via HRP-initiated RAFT seeded emulsion polymerization

PGMA₄₆-PHPMA₃₀₀ diblock precursor vesicles (5.18 g, 22.7 % w/w), deionized water (4.508 g, to make the HPMa concentration of 10% w/w), GlyMA (0.66 g, 4.6 mmol, target DP = 200), ACAC stock solution (220 μL , 0.425 mol/L) and HRP stock solution (250 μL , 8.8 mg/ml) were added into a flask. Then, it was put into a water bath set at 32°C. The mixture solution was degassed with nitrogen for 30 min and the H_2O_2 (22 μL , 0.3 mol/L) was injected into the solution to initiate the polymerization for 3 h.

Pickering emulsion formation

Hexane (2.0 mL) was homogenized with 5.0 mL of a 0.5-3 % w/w aqueous vesicle dispersion for 2 min using a FSH-2A homogenizer at 12000 rpm. The droplets were imaged by optical microscopy.

Surfactant Challenge Tests

Vesicle dispersions (4.0 g) were mixed with SDS solutions (1.0 g) to form the test solutions (final concentration of 1% w/w for both vesicles and SDS).

Cross-linking of PGMA₄₆-PHPMA₃₀₀-PGlyMA₃₀₀ nanoparticles

In a typical experiment, an aqueous dispersion of PGMA₄₆-PHPMA₃₀₀-PGlyMA₃₀₀ nanoparticles (2.58 g, 7.5% w/w, 0.62 mmol epoxy groups) were weighed into a vial, following the addition of diamine (0.0187 g, 0.62 mmol amine group). The sample vial was placed on a roller mixer for 24 h. Then, the free unreacted diamine was removed by centrifugation for several times.

Synthesis of Ag NPs Supported by cross-linked PGMA₄₆-PHPMA₃₀₀-PGlyMA₃₀₀ vesicles

0.025 g AgNO₃ was introduced into 2.5 g PGMA₄₆-PHPMA₃₀₀-PGlyMA₃₀₀ vesicles dispersion (7.5% w/w) and stirred for 30 min. Subsequently, 0.025 g PVP was added to the reaction mixture with gentle stirring and the reaction was conducted at 50 °C for 12 h. The product was then separated by centrifugation and rinsed with water.

Catalytic Reduction Experiments.

A dispersion of Ag/cross-linked PGMA₄₆-PHPMA₃₀₀-PGlyMA₃₀₀ vesicles (20 μL, 7.5% w/w) was mixed with an aqueous solution of MB (0.05 × 10⁻³ wt.%, 5 mL). Then, an aqueous solution of NaBH₄ (0.6 M, 0.5 mL) was added to the mixture. UV-Vis spectrometer was used to monitor the variation in the optical properties of the dye.

Additional results

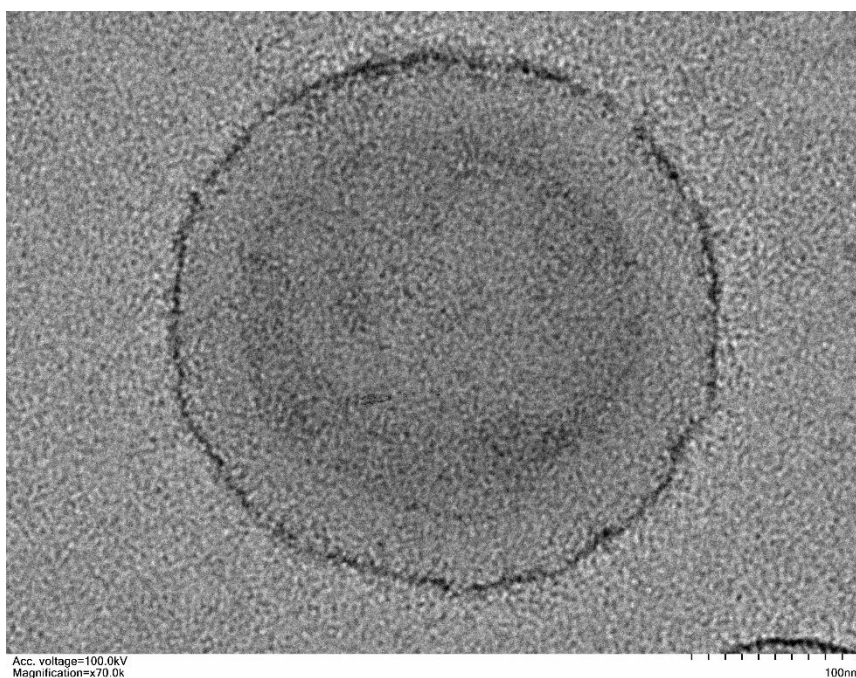


Figure S1. PGMA₄₆-PHPMA₆₀₀ vesicles prepared via enzyme-initiated seeded RAFT dispersion polymerization using PGMA₄₆-PHPMA₃₀₀ vesicles as seeds.

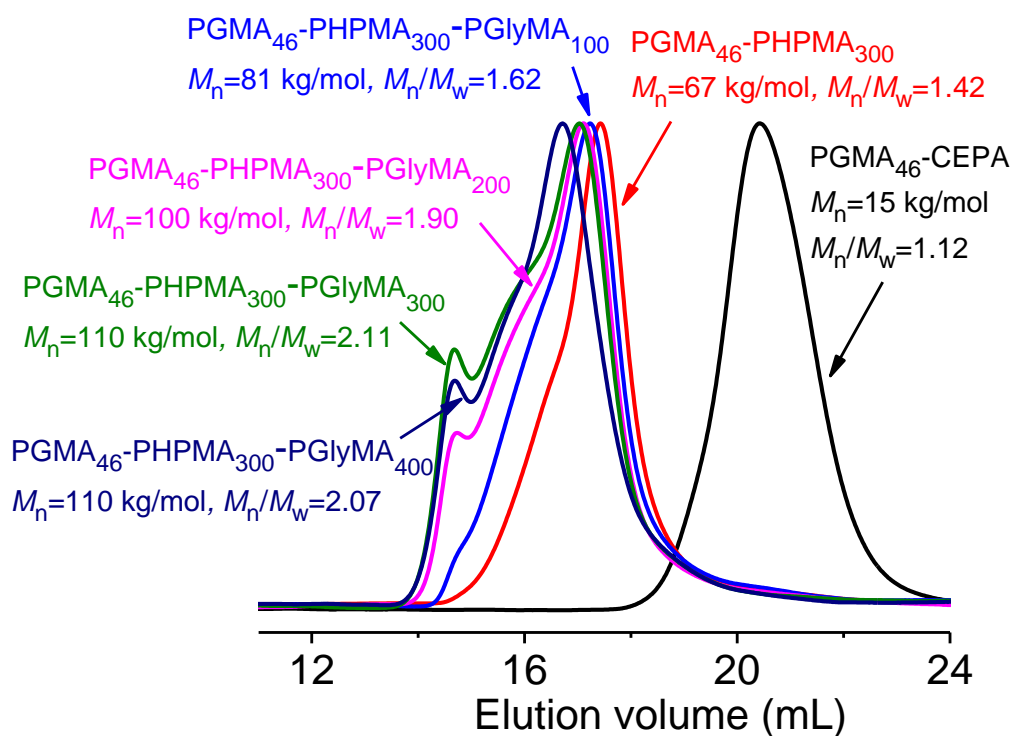


Figure S2. DMF GPC traces of PGMA₄₆-CEPA and PGMA₄₆-PHPMA₃₀₀-PGlyMA_n ($n = 0, 100, 200, 300, 400$) block copolymer vesicles prepared by enzyme-initiated aqueous RAFT polymerization.

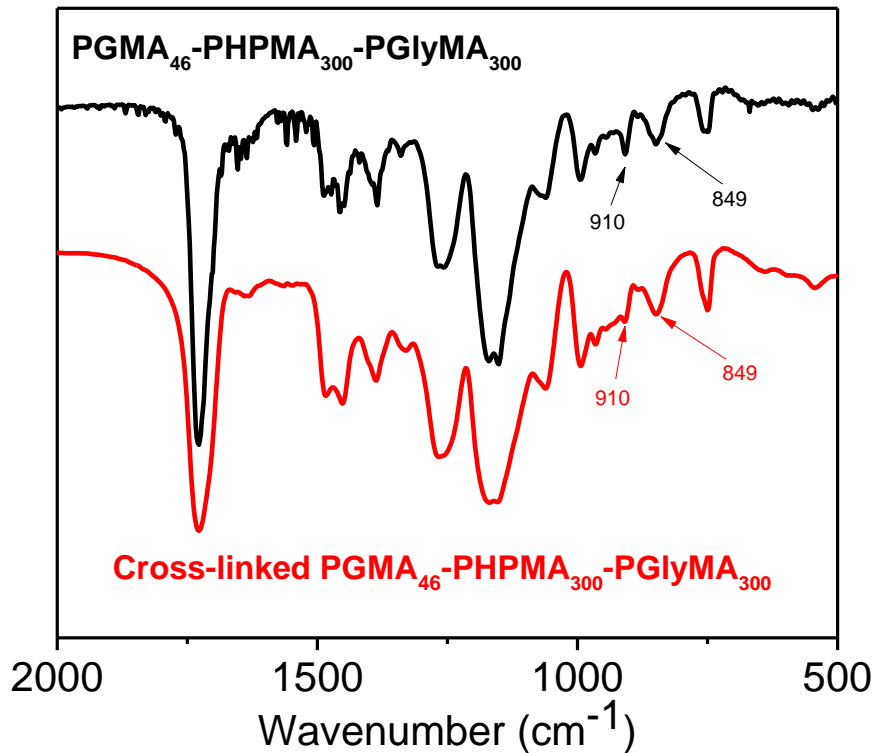


Figure S3. FT-IR spectra of PGMA₄₆-PHPMA₃₀₀-PGlyMA₃₀₀ vesicles and cross-linked PGMA₄₆-PHPMA₃₀₀-PGlyMA₃₀₀ vesicles (amine/epoxy ratio of 7.0)

Reference

- [1] W. Shen, Y. Chang, G. Liu, H. Wang, A. Cao, Z. An, *Macromolecules* **2011**, *44*, 2524–2530.