Open-to-Air Preparation of Cross-linked CO_2-Responsive Polymer Vesicles by Enzyme-Assisted Photoinitiated Polymerization-Induced Self-Assembly

Liangliang Yu[a], Yuxuan Zhang[a], Xiaocong Dai[a], Qin Xu [a], Li Zhang[a,b], Jianbo Tan*[a,b]

a. *Department of Polymeric Materials and Engineering, School of Materials and Energy, Guangdong University of Technology, Guangzhou 510006, China.*

b. *Guangdong Provincial Key Laboratory of Functional Soft Condensed Matter, Guangzhou 510006, China.*

**Corresponding author: tanjianbo@gdut.edu.cn*

Liangliang Yu and Yuxuan Zhang contributed equally to this work.

EXPERIMENTAL SECTION

Material

2-Hydroxypropyl methacrylate (HPMA, Aladdin), glycidyl methacrylate (GlyMA, Aladdin), 2-(dimethylamino)ethyl methacrylate (DMAEMA, Aladdin), and allyl methacrylate (AMA, Aladdin) were purified by pass through a basic aluminum oxide column. 4,4′-Azobis(4-cyanovaleric acid) (ACVA, Aladdin), glucose, and glucose oxidase (GOx, Aladdin, lyophilized powder, >180 U/mg) were used as received. Sodium phenyl-2,4,6-trimethylbenzoylphosphinate (SPTP) was synthesized according to a literature [1]. 4-Cyano-4-(dodecylsulfanylthiocarbonyl)sulfanylpentanoic acid (CDPA) was synthesized according to a literature[2]. LUDOX®AM colloidal silica (30% w/w suspension in H_2O, Sigma-Aldrich) was dialyzed against water prior to use.

Characterization

^{1}H NMR Spectroscopy. Nuclear magnetic resonance (NMR) spectra were recorded in d_6-DMSO or $CDCl_3$ 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 phosphotungstic acid solution (1% w/w in PBS buffer, pH = 7) 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 100 kV.

Gel Permeation Chromatography (GPC). The molecular weight and polydispersity of the block copolymers were measured by gel permeation chromatography (GPC) using a Waters 1515 GPC instrument with dimethylformamide (DMF) as the mobile phase and Waters styragel HR1, HR4 columns. The eluent 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 the apparatus.

Dynamic light scattering (DLS). Intensity-average hydrodynamic diameters of the copolymer dispersions (0.1% v/v) were determined using a Brookhaven nanoparticle size-Zeta potential instrument. Dilute aqueous dispersions were analyzed using disposable cuvettes and all data were averaged over three consecutive runs.

Conductivity meter. The change of conductivity was recorded with a conductivity meter (DDS-11) at room temperature.

Thermogravimetric analysis (TGA). TGA was performed using a STA449F5 instrument under a stream of nitrogen. The samples were heated from 25 to 600 °C with a heating rate of 10 °C/min.

Synthesis of glycerol monomethacrylate (GMA)

Glycidyl methacrylate (80.0 g) was added to water (720.0 g, 10% w/w) in a round bottom flask connected with a condenser while stirred for 9 h at 80 °C. The resulting transparent solution was then saturated with NaCl and extracted with dichloromethane. The organic phase was dried with $MgSO_4$ overnight, filtered, and concentrated under reduced pressure. The obtained monomer was further purified by silica column chromatography (CH_2Cl_2: CH_3OH = 20:1), and then concentrated and dried at 45 °C under vacuum.

Synthesis of PGMA-CDPA macro-RAFT agent

GMA (45.60 g, 0.284 mol), ACVA (0.35 g, 1.25 mmol), and CDPA (2.01 g, 0.005 mol, GMA/CDPA molar ratio = 57, CTA/ACVA molar ratio = 4.0), and 1, 3, 5-trioxane (1.37 g, 0.0152 mol) were added into a 150 mL round-bottom flask, and then dissolved with anhydrous ethanol (68.3 g, 40% w/w) to form a homogeneous solution. The reaction mixture was purged with nitrogen for 45 min, sealed, and then immersed in a 70 °C pre-heated oil bath for 2.5 h. The polymerization was quenched by immersion in ice-water and exposure to air and analyzed by ^{1}H NMR spectroscopy. ^{1}H NMR indicated a mean degree of polymerization of 43 (75.5% conversion). The polymer was precipitated by adding excess *n*-hexane (300 mL) and washed several times. The obtained product was dried at 45 °C under vacuum overnight, and then analyzed by DMF GPC. DMF GPC measurement confirmed its M_n = 15.0 kg/mol and M_w/M_n = 1.26.

Synthesis of $PGMA_{43}$-*b*-P(HPMA-*co*-DMAEMA-*co*-AMA) vesicles via enzyme-assisted photo-PISA

A series of vesicles with different DPs of PDMAEMA were synthesized via enzyme-assisted photo-PISA. A typical protocol for the synthesis of $PGMA_{43}$-*b*-($PHPMA_{350}$-*co*-$PDMAEMA_{50}$-*co*-AMA_{20}) is as follow: $PGMA_{43}$-CDPA (0.145 g, 0.0198 mmol), HPMA (1.00 g, 6.94 mmol), DMAEMA (0.156 g, 0.992 mmol), AMA (0.0500 g, 0.396 mmol), and glucose (0.087 g, to ensure the final concentration of glucose is 0.1 M) were added to a 10 mL round bottom flask and dissolved with water (4.63 g to ensure the monomer concentration of 20% w/w). Then a GOx solution (193 μL, 2 mg/mL, to ensure the final concentration of GOx is 0.5 μM) was added. The reaction mixture was incubated for 20 min to remove dissolved oxygen, adding SPTP (0.0020 g, 0.0065 mmol), and irradiated by a

LED lamp (405 nm, 0.5 mW/cm^2) at room temperature for 1.5 h.

Loading methylene blue into P(HPMA$_{350}$-*co*-DMAEMA$_{50}$-*co*-AMA$_{20}$) vesicles

PGMA$_{43}$-CDPA (0.217 g, 0.0297 mmol), HPMA (1.50 g, 10.40 mmol), DMAEMA (0.234 g, 1.486 mmol), AMA (0.0750 g, 0.594 mmol), DSS (0.02 g), methylene blue (1.36 mL, 2.5×10^{-3} wt.%), and glucose (0.130 g, to ensure the final concentration of glucose is 0.1 M) were added to a 10 mL round bottom flask and dissolved with water (4.98 g to ensure the monomer concentration is 20% w/w). Then a GOx solution (289 μL, 2.0 mg/mL, to ensure the final concentration of GOx is 0.5 μM) was added. The reaction mixture was incubated for 15 min to remove dissolved oxygen and irradiated by a LED lamp (405 nm, 0.5 mW/cm^2) at room temperature for 2 h after the addition of SPTP (3.1 mg, 0.010 mmol). The product was purified by multiple cycles of centrifugation-redispersion in water and finally dispersed in water. UV-Vis spectrometer was used to monitor the variation in the optical properties of the MB in the supernatant solution after centrifugation.

Preparation of hybrid vesicles

In a typical experiment, PGMA$_{43}$-*b*-(PHPMA$_{350}$-*co*-DMAEMA$_{25}$-*co*-AMA$_{20}$) vesicle (1.72 g, 5% w/w), SiO_2 nanoparticles solution (2.42 g, 82.5% w/w) and water (0.71 g, to ensure the final concentration of vesicle at 3% w/w) were added into a 10 mL round-bottom flask. Then the reaction mixture was stirred for 24 h at room temperature. The sample was purified by five cycles of centrifugation-redispersion in water.

As a control, PGMA$_{43}$-*b*-(PHPMA$_{350}$-*co*-DMAEMA$_{25}$-*co*-AMA$_{20}$) vesicles (1.72 g, 5% w/w) and water (0.708 g) were added into a 10 mL round-bottom flask. Then the reaction mixture was purged with CO_2 for 30 min, added SiO_2 nanoparticles solution (2.42 g, 82.5% w/w), and stirred for 24 h at room temperature. The solution was further purged with N_2 for 30 min and purified by five cycles of centrifugation-redispersion in water.

ADDITIONAL RESULTS

Table S1. Hydrodynamic diameter of cross-linked CO_2-responsive polymer vesicles ($PGMA_{43}$-P(HPMA-*co*-DMAEMA-*co*-AMA)) prepared by enzyme-assisted photo-PISA before and after CO_2 treatment.

Sample number	DP of PGMA	DP of PHPMA	DP of PDMAEMA	DP of PAMA	D_h (nm)	D_h (nm), treated with CO_2
1	43	400	0	20	217.6	216.2
2	43	375	25	20	230.4	235
3	43	350	50	20	355.2	531.6
4	43	325	75	20	333.0	629.4
5	43	300	100	20	281.7	691.9

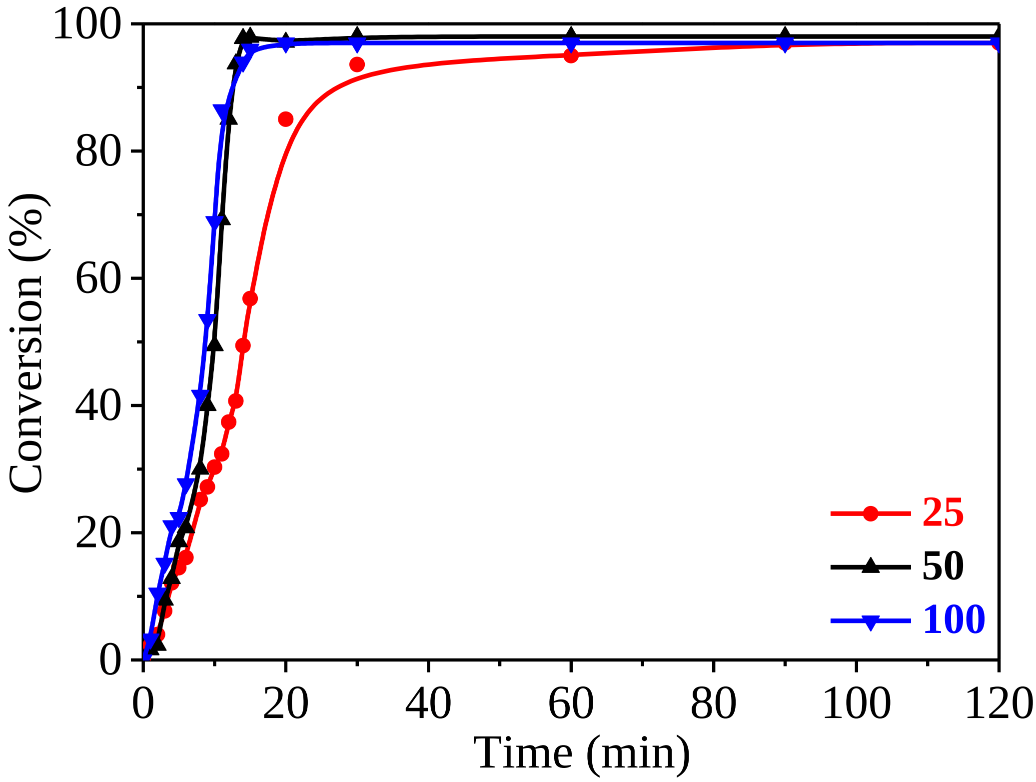

Figure S1. Polymerization kinetics of enzyme-assisted photoinitiated polymerization-induced self-assembly using $PGMA_{43}$-CDPA with different DPs of PDMAEMA (25, 50, 100). Target composition of $PGMA_{43}$-P($HPMA_m$-*co*-$DMAEMA_n$-*co*-AMA_{20}) (m+n = 400).

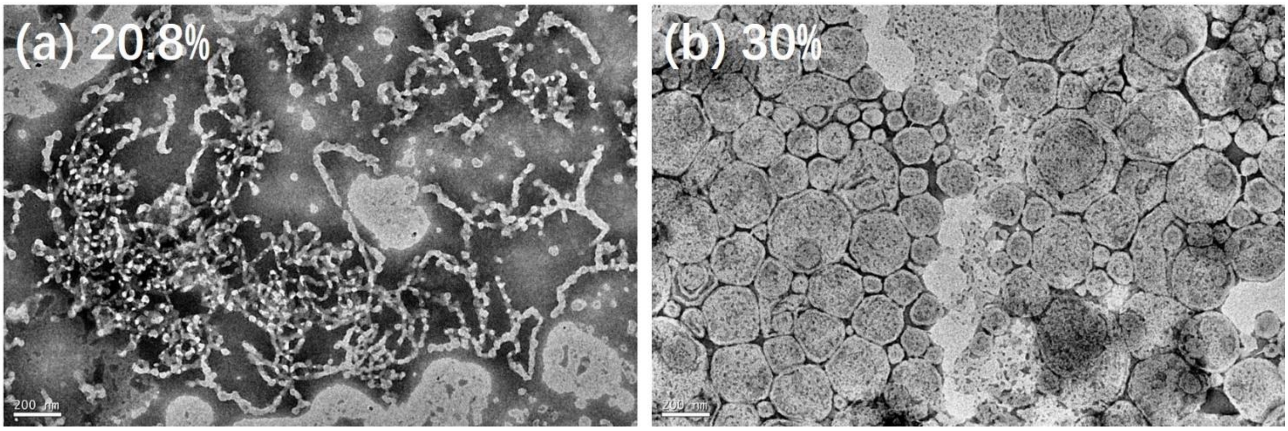

Figure S2. TEM images of polymer nanoparticles obtained by withdrawing samples during enzyme-assisted photoinitiated polymerization-induced self-assembly using PGMA$_{43}$-CDPA (target composition of PGMA$_{43}$-P(HPMA$_{350}$-*co*-DMAEMA$_{50}$-*co*-AMA$_{20}$)): (a) monomer conversion = 20.8%, (b) monomer conversion = 30%.

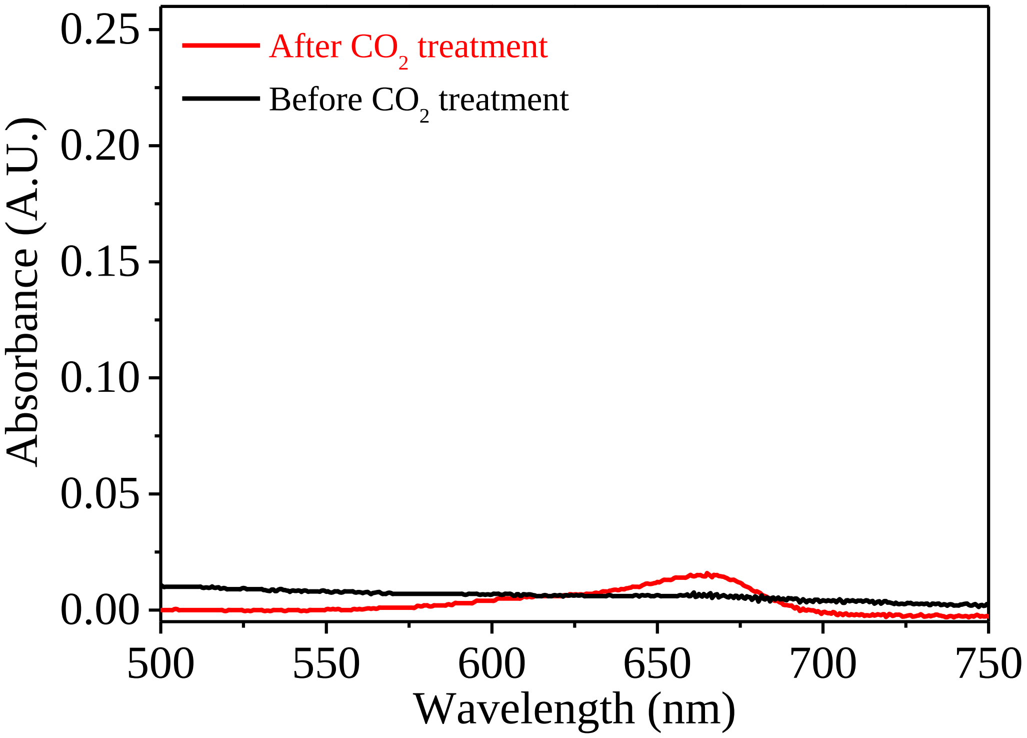

Figure S3. UV-vis absorbance spectra of supernatants obtained from aqueous dispersions of MB-loaded PGMA$_{43}$-PHPMA$_{400}$ vesicles before and after 20 min CO$_2$ treatment.

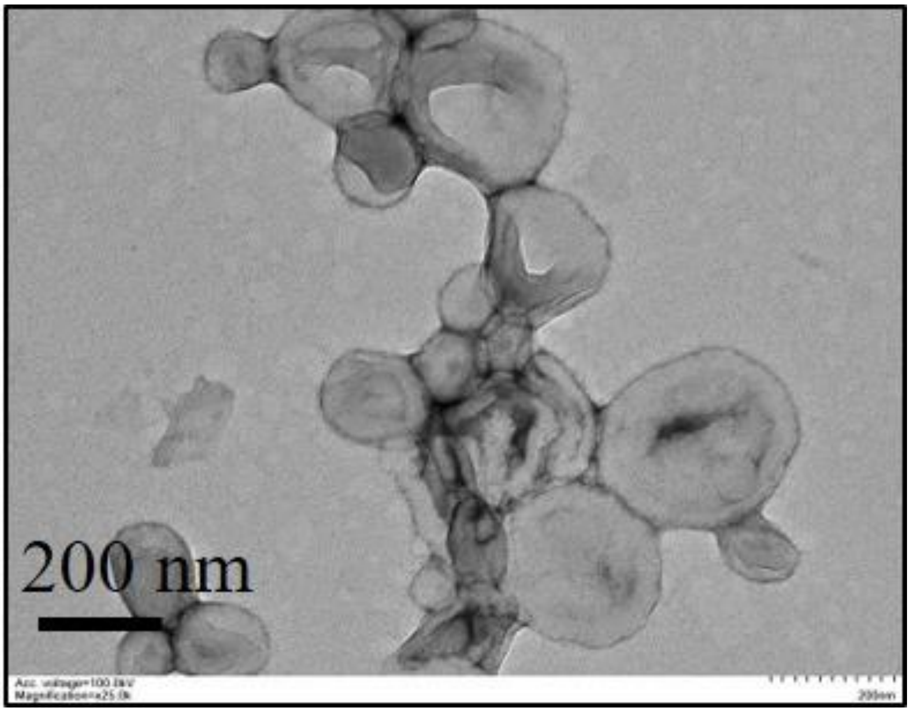

Figure S4. TEM image of PGMA$_{43}$-P(HPMA$_{400}$-*co*-AMA$_{20}$) vesicles prepared by enzyme-assisted photo-PISA.

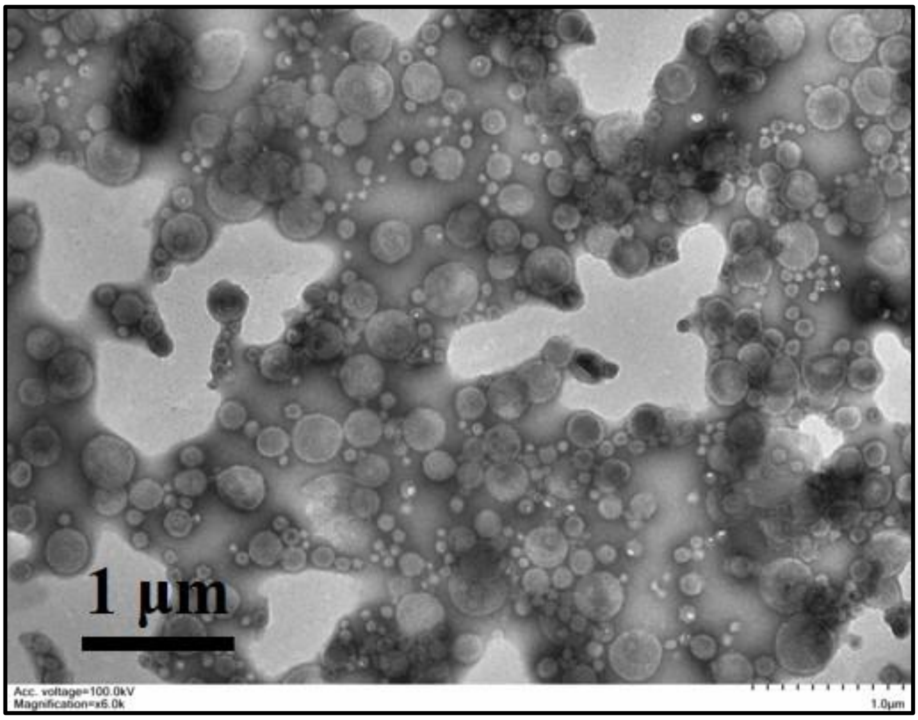

Figure S5. TEM image of PGMA$_{43}$-P(HPMA$_{325}$-*co*-DMAEMA$_{75}$-*co*-AMA$_{30}$) vesicles prepared by enzyme-assisted photo-PISA.

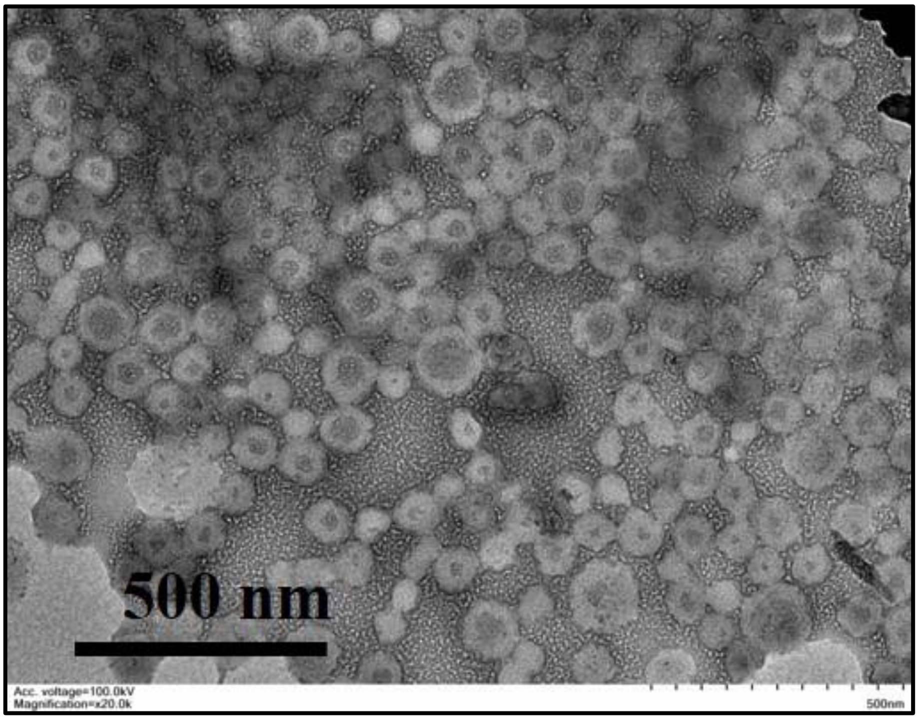

Figure S6. TEM image of PGMA$_{43}$-P(HPMA$_{325}$-*co*-DMAEMA$_{75}$-*co*-AMA$_{40}$) vesicles prepared by enzyme-assisted photo-PISA.

REFERENCES

[1] Jiang, Y.; Xu, N.; Han, J.; Yu, Q.; Guo, L.; Gao, P.; Lu, X.; Cai, Y. *Polym. Chem*. 2015, *6*(27), 4955.

[2] Shen, W., et al., Biocompatible, Antifouling, and Thermosensitive Core-Shell Nanogels Synthesized by RAFT Aqueous Dispersion Polymerization. *Macromolecules*. 2011. *44*(8): 2524-2530.