Electronic Supplementary Information

CO_2-Acidolysis of Iminoboronate Ester Based Polymersomes

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1. Materials and Methods

Poly(ethylene glycol) diglycidyl ether (PEGDGE, Mn = 394), 2, 2'-Diamino-N-methyldiethylamine (DNMDA), cysteamine, formylphenylboronic acid (FPBA), caffeic acid phenethyl ester (CAPE), methoxypolyethylene glycol amine (mPEG-NH_2, Mn = 2000), Alizarin Red S (ARS), tributylphosphine, dimethylsulfoxide (DMSO) were purchased from Aladdin and used without further purification. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and Hoechst-33342 were obtained from Sigma Chemical Co. (USA). Ultrapure water was prepared utilizing a FDY-1002-UV-P purification system.

^{1}H-NMR (400 MHz) spectra were investigated by a Bruker spectrometer with $CDCl_3$, d_8-THF and D_2O as solvent, respectively. Absorption spectra were acquired on Evolution 220 spectrophotometer at 25 ^oC with a thermostatted cuvette holder. Fluorescence emission spectra were measured with a PTI fluorescence master system at 25 ^oC with a thermostatted cuvette holder. The slit width was maintained at 3 nm for excitation and 1 nm for emission, respectively. Dynamic light scattering (DLS) measurements were performed with a Malvern Nano-ZS90 instrument to determine the average diameter and size distribution of the micelles at 25 ^oC. Transmission electron microscopy (TEM) observations were carried out using a JEM-2100 microscope. The samples were prepared by dropping the micellar solution onto copper specimen grids (400 mesh, supported with formvar/carbon film), and dried at room temperature overnight. Field-emission scanning electron microscope (FE-SEM) observations were conducted on a high resolution HITACHI SU8220 field-emission scanning electron microscopy. The samples for SEM observations were prepared without gold coating. AFM was taken with a CSPM5500A scanning probe microscope system (Beijing Nano-Instruments Co., Ltd.) in the tapping mode. Samples were prepared by dropping the micellar solution onto mica sheets and drying the sheets at room temperature overnight. Confocal laser scanning microscopy (CLSM) images were acquired using a Leica TCS SP5 microscope.

2. Polymer Synthesis

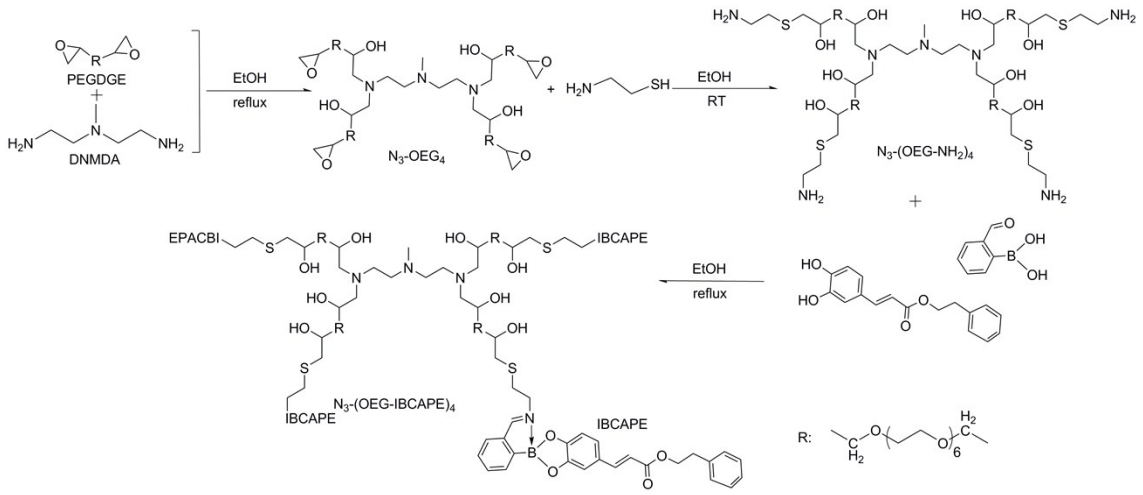

Figure S1. The synthetic route to the amphiphilic starlike prodrug of N_3-(OEG-IBCAPE)$_4$.

Figure S1 showed the synthetic route to the amphiphilic starlike prodrug of N_3-(OEG-IBCAPE)$_4$. The preparation for N_3-(OEG-IBCAPE)$_4$ is detailed below.

Poly(ethylene glycol) diglycidyl ether (PEGDGE, Mn = 394, 12.04 g, 30.54 mmol) and 10 mL of anhydrous ethanol were placed into a 50 mL round bottom flask. After 30 min of stirring at room temperature, the round-bottom flask was placed in a preheated oil bath (80 °C). Then, 2, 2'-Diamino-N-methyldiethylamine (DNMDA, 0.37 g, 3.15 mmol) in anhydrous ethanol solution (2 mL) dropped wisely added while stirring and the reaction was maintained at 80 °C for 12 h. Thereafter, the reaction mixture was concentrated and precipitated with anhydrous ether. The obtained product was dried under vacuum to yield a white sticky semi-solid substance of N_3-OEG$_4$ 4.88 g (yielding, 93.38%).

^{1}H-NMR (400 MHz, D_2O, δ): 4.04-3.49 (-OCH_2CH_2O- and -CH(OH)-), 3.49-3.33 (-CH_2-O-C-), 3.33-3.08 (-(HO)CCH_2N<), 3.02-2.96 (-C-O-CH-), 2.74-2.65 (>NCH_2CH_2N< and >NCH_3).

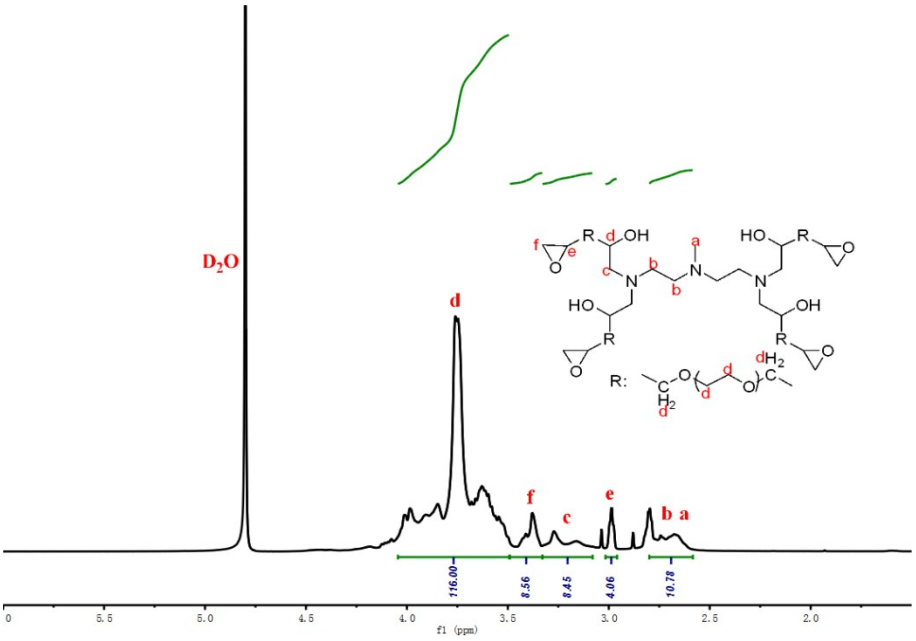

Figure S2. The ^{1}H-NMR spectrum of N_3-OEG$_4$ in D_2O.

The obtained prepolymer of N_3-OEG$_4$ (4.60 g, 2.71 mmol) in ethanol (3 mL) was added drop wisely in the ethanol solution (5 mL) of cysteamine (1.26 g, 16.33 mmol) at room temperature and the system was maintained for 4 h under N_2. After this, the system was concentrated and precipitated with anhydrous ether. The obtained product was dried under vacuum to yield a white sticky semi-solid substance of N_3-(OEG-NH_2)$_4$ 5.18 g (yielding, 95.26%).

^{1}H-NMR (400 MHz, D_2O, δ): 4.11-3.44 (-OCH_2CH_2O-, -$CH(OH)CH_2SCH_2CH_2NH_2$), 3.35-3.11 (-(HO)CCH_2N<), 2.70-2.65 (>NCH_2CH_2N<, >NCH_3).

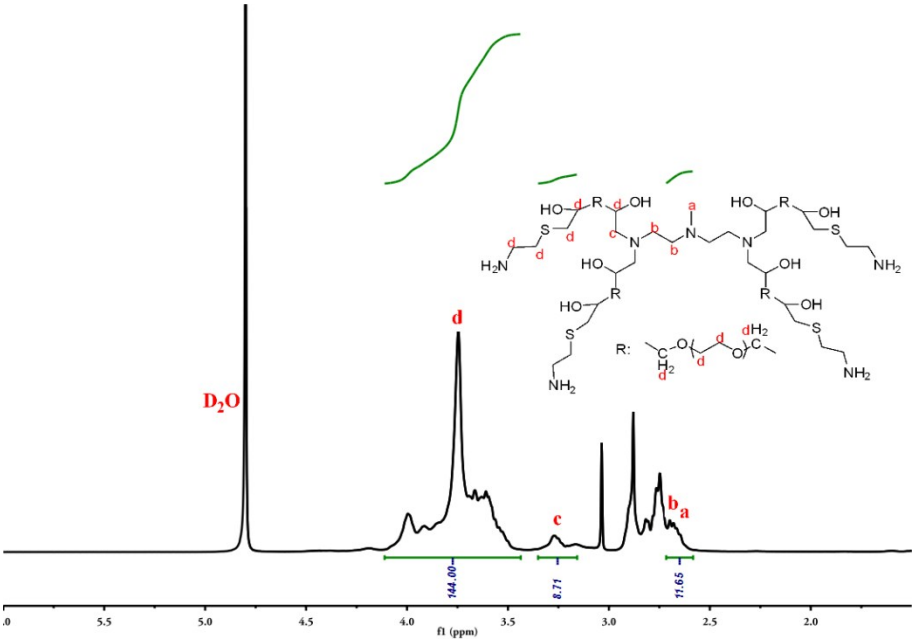

Figure S3. The ^{1}H-NMR spectrum of N_3-(OEG-NH_2)$_4$ in D_2O.

N_3-(OEG-NH_2)$_4$ (2.78 g, 1.39 mmol), formylphenylboronic acid (FPBA, 0.87 g, 5.84 mmol), caffeic acid phenethyl ester (CAPE, 1.66 g, 5.84 mmol) and anhydrous ethanol (5 mL) were quickly added into a 50 mL round-bottom flask. After 30 min of stirring at room temperature, the round-bottom flask was placed in a preheated oil bath (80 °C) for 12 h. The crude product was collected by precipitation twice in the anhydrous ether. Finally, the polymer was dried to constant weight under vacuum oven to give a light yellow solid substance of N_3-(OEG-IBCAPE)$_4$ 4.98 g (yielding, 95.78%). Anal. Calcd for $C_{181}H_{247}B_4N_7O_{52}S_4$: C, 61.68; H, 7.06; N, 2.78. Found: C, 61.65; H, 7.11; N, 2.70.

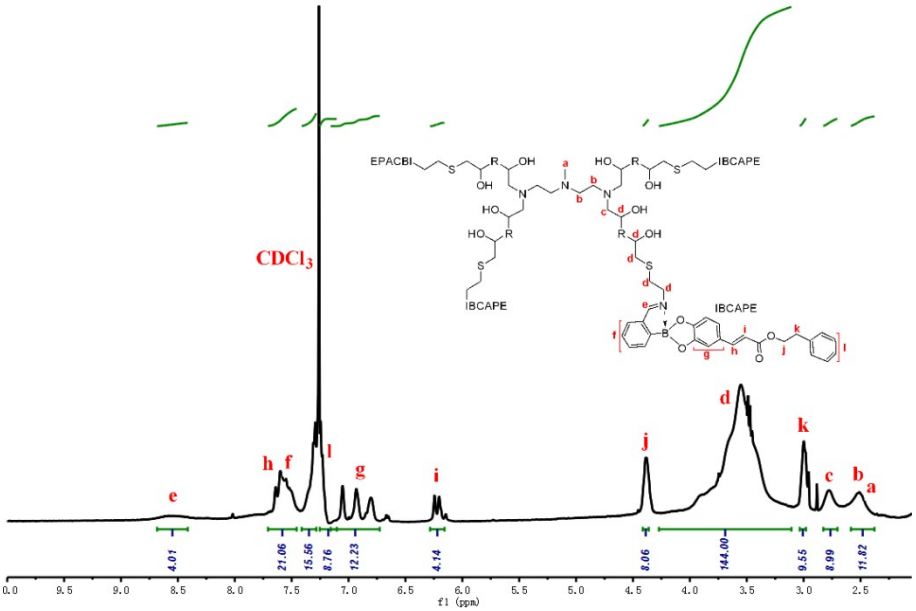

Figure S4. The ^{1}H-NMR spectrum of N_3-(OEG-IBCAPE)$_4$ in $CDCl_3$.

^{1}H-NMR (400 MHz, $CDCl_3$, δ): 8.74-8.38 (-C=N-), 7.72-6.60 (Ar-H, Ar-CH_2=C-), 6.34-6.04 (Ar-C=CH-), 4.42-4.35 (-C(O)OCH_2-), 4.29-3.07 (-OCH_2CH_2O-, -CH(OH)CH_2S$CH_2CH_2NH_2$), 3.04-2.98 (-C(O)OC-CH_2-Ar), 2.82-2.71 (-(HO)CHCH_2N<), 2.58-2.38(>NCH_2CH_2N<, >NCH_3).

3. Polymersomes Preparation

In the polymersomes preparation experiment, N_3-(OEG-IBCAPE)$_4$ (9.0 mg), H_2O (6.0 mL) were added together and stirred for 12 h. Finally, the polymersomes solution was diluted to 1.5 mg mL^{-1} (with respect to polymer in water).

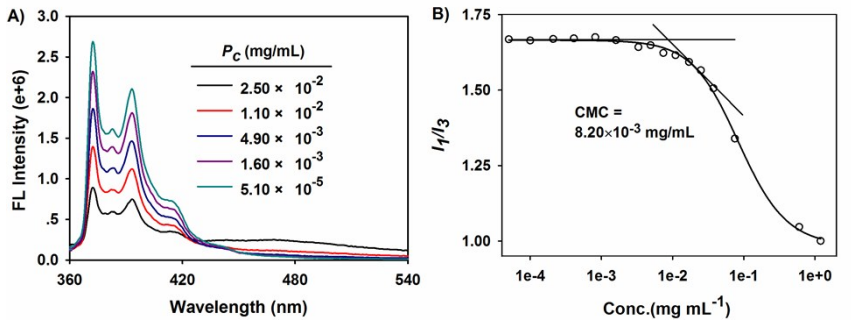

Figure S5. A) Fluorescence emission spectra excited at 340 nm as a function of polymer concentration in previously pyrene saturated aqueous solution. B) Changes in the ratio of I_1/I_3 of pyrene emission as a function of polymer concentration.

In order to determine the critical self-assembly concentration (CSAC) of N_3-(OEG-IBCAPE)$_4$ in aqueous solution, the fluorescence pyrene probe technique was applied. As shown in Figure S5A, the fluorescence emission spectra of N_3-(OEG-IBCAPE)$_4$ in pyrene saturated aqueous solution exhibited a characterized vibrational five peaks emission of pyrene. The ratio of the first (I_1 at 372 nm) and third peaks (I_3 at 382 nm) of pyrene emission remained constant up to a certain polymer concentration and decreases exponentially above it (see Figure S5B). This change reflected the onset of vesicle formation and the partitioning of the pyrene between the aqueous and vesicle phases. Therefore, the CSAC value for N_3-(OEG-IBCAPE)$_4$ in aqueous solution is 8.20 × 10^{-3} mg mL^{-1}.

4. pH Changes vs. amount of CO_2

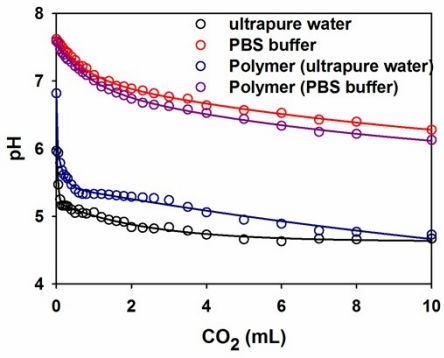

Figure S6. The pH changes of ultrapure water, PBS buffer and N_3-(OEG-IBCAPE)$_4$ in ultrapure water and PBS buffer

solutions (0.25 mg mL^{-1} × 1.5 mL) upon CO_2, respectively.

The pH changes of ultrapure water, PBS buffer and N_3-(OEG-IBCAPE)$_4$ in ultrapure water and PBS buffer solutions (0.25 mg mL^{-1} × 1.5 mL) along with the adding amount of CO_2 bubbling were automatically recorded by a pH sensor (Mettler-Toledo Instruments Co., Ltd) at 25 °C, respectively.

5. ^{1}H NMR Changes of N_3-(OEG-IBCAPE)$_4$ before and after CO_2 Bubbling

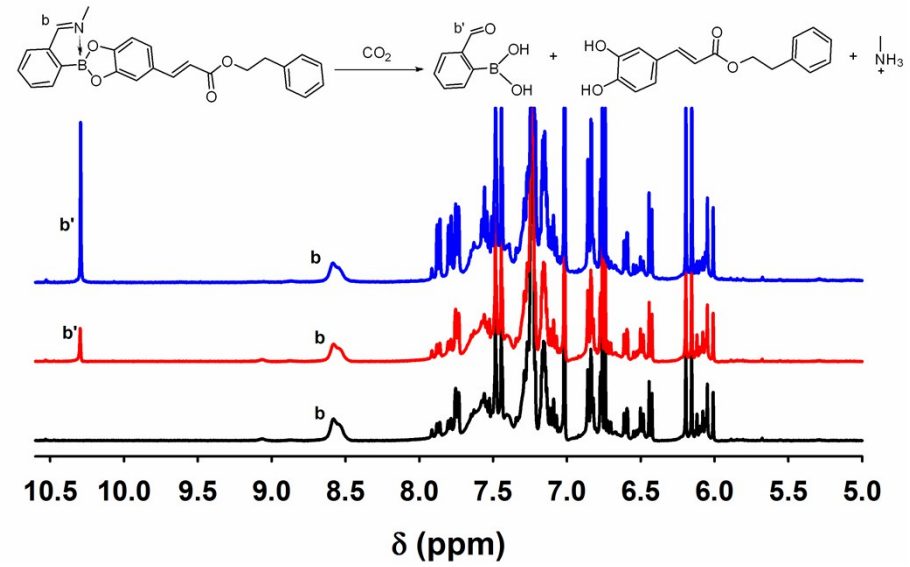

Figure S7. ^{1}H NMR spectra of N_3-(OEG-IBCAPE)$_4$ (25.0 mg mL^{-1} × 0.5 mL) in d_8-THF (black) and the mixture of d_8-THF/D_2O (v/v, 4/1) before (red) and after 20 mL CO_2 bubbling (blue).

As shown in Figure S7, the starting ^{1}H NMR spectra of N_3-(OEG-IBCAPE)$_4$ (25.0 mg mL^{-1} × 0.5 mL) in d_8-THF (black) displayed a newly formed signal of -C*H*=N- at ≈8.58 ppm, indicating the efficient occurrence of the aldehyde-amine condensation reaction of the iminoboronate ester reaction system. In order to better observe the spectral changes, the prepared prodrug was dissolved in d_8-THF/D_2O mixture (v/v, 4/1) (red). It can be seen a newly forming peak of -CHO group appeared at ~10.30 ppm, indicating the hydrolysis-induced disintegration of the segmental iminoboronate moieties. However, even after the polymer solution was placed for 24 h, the proton signal intensity of -C*H*=N- at ≈8.58 ppm almost completely remain. Further, in the presence of 20 mL CO_2, a newly formed formyl proton (-C*H*=O, ~10.30 ppm) greatly increased while the imine proton (-C*H*=N-, ~8.58 ppm) declined slightly, ascribing to the weak acidity of CO_2 in aqueous solution and also the extremely high concentration of iminoboronates in the system. After hydrolysis, iminoboronate moities were cleaved into three components of primary amine, CAPE and 2-formylphenylboronic acid (FPBA).

6. The Morphology Analysis of Polymer Self-Assemblies

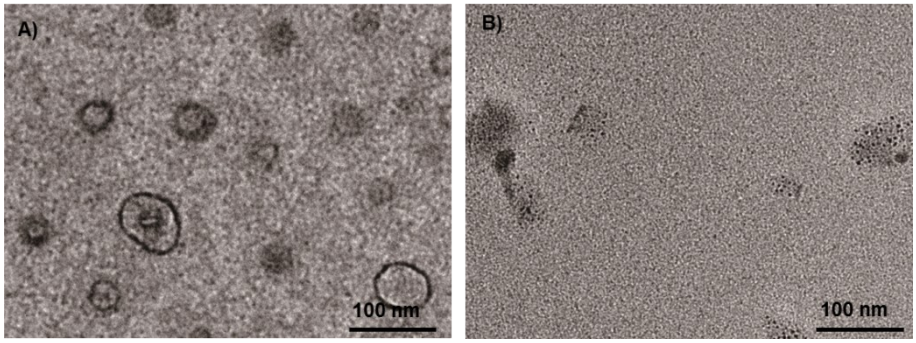

Figure S8. TEM images of the N_3-(OEG-IBCAPE)$_4$ polymersomes before (A) and after (B) CO_2 bubbling.

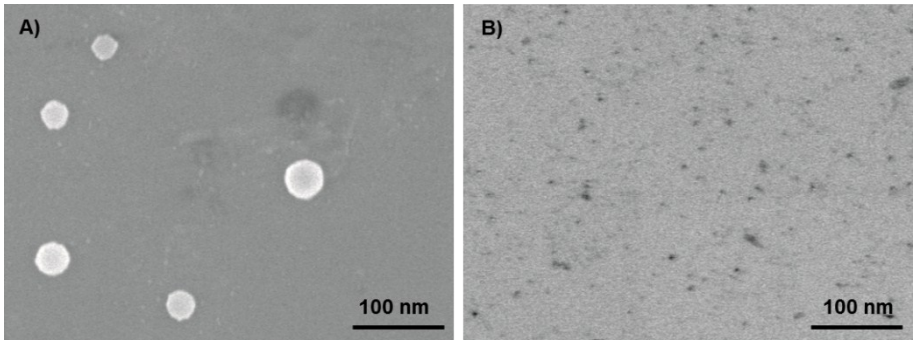

Figure S9. SEM images of the N_3-(OEG-IBCAPE)$_4$ polymersomes before (A) and after (B) CO_2 bubbling.

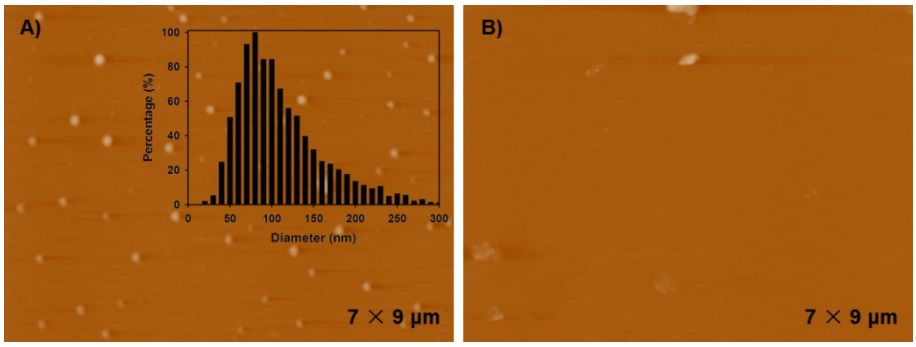

Figure S10. AFM observations of the N_3-(OEG-IBCAPE)$_4$ polymersomes before (A) and after (B) CO_2 bubbling, the size distribution of nanoparticles (the inset in A).

As shown in Figure S10A, the polymeric self-assemblies of N_3-(OEG-IBCAPE)$_4$ were visualized with nano-spheres with the average diameter (*D*AFM) of 100 nm. By contrast, no vesicles remained in the solution after CO_2 bubbling because of the cleavage of iminoboronate ester of N_3-(OEG-IBCAPE)$_4$ upon bubbling of CO_2.

7. Absorption, Emission Spectra and DLS Analysis of Polymer Aqueous Solutions

During the experiment, the vesicular dispersion of N_3-(OEG-IBCAPE)$_4$ (0.25 mg mL^{-1} × 1.5 mL) was sealed in a quartz cuvette at 25 °C with mild stirring, and different amounts of CO_2 were injected into the solution.

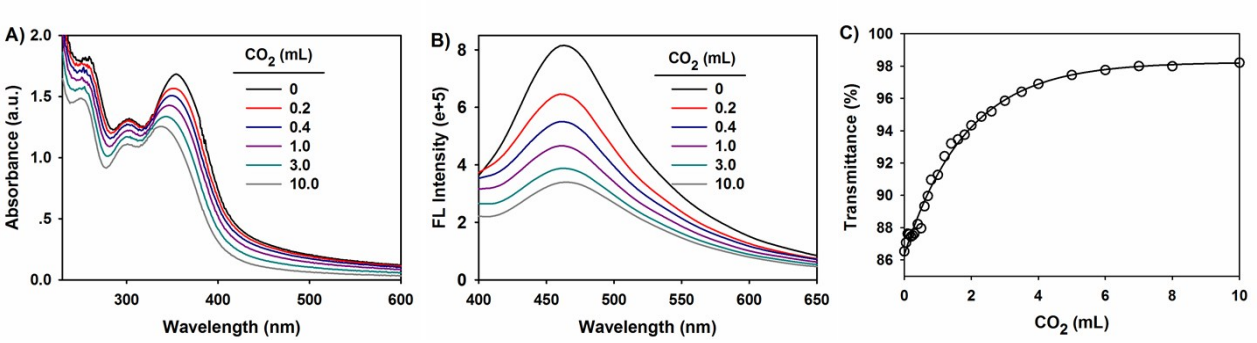

Figure S11. Absorption (A), fluorescence emission (B, λ_{ex} = 360 nm) spectra and plot of optical transmittance (C) of vesicular dispersion of N_3-(OEG-IBCAPE)$_4$ in ultrapure water (0.25 mg mL^{-1} × 1.5 mL) upon CO_2 bubbling.

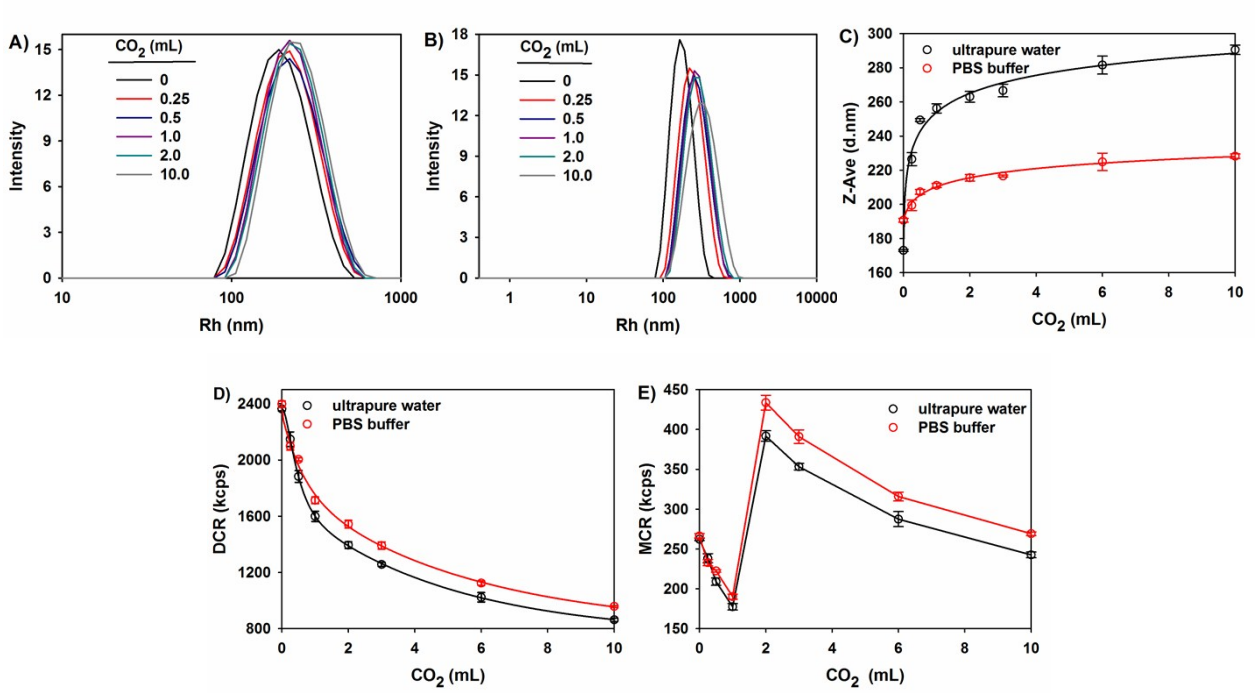

Figure S12. The size distribution of N_3-(OEG-IBCAPE)$_4$ in ultrapure water (A), PBS buffer solutions (B) (0.25 mg mL^{-1} × 1.5 mL) upon CO_2**,** respectively. C) Plots of diameter of N_3-(OEG-IBCAPE)$_4$ (0.25 mg mL^{-1} × 1.5 mL) vs injected amount of CO_2 in ultrapure water and PBS buffer solutions**,** respectively. D), E) DLS analysis of N_3-(OEG-IBCAPE)$_4$ in ultrapure water and PBS buffer solutions (0.25 mg mL^{-1} × 1.5 mL) upon CO_2.

8. Absorption and Emission Spectra Analysis of mPEG-IBARS Aqueous Solutions

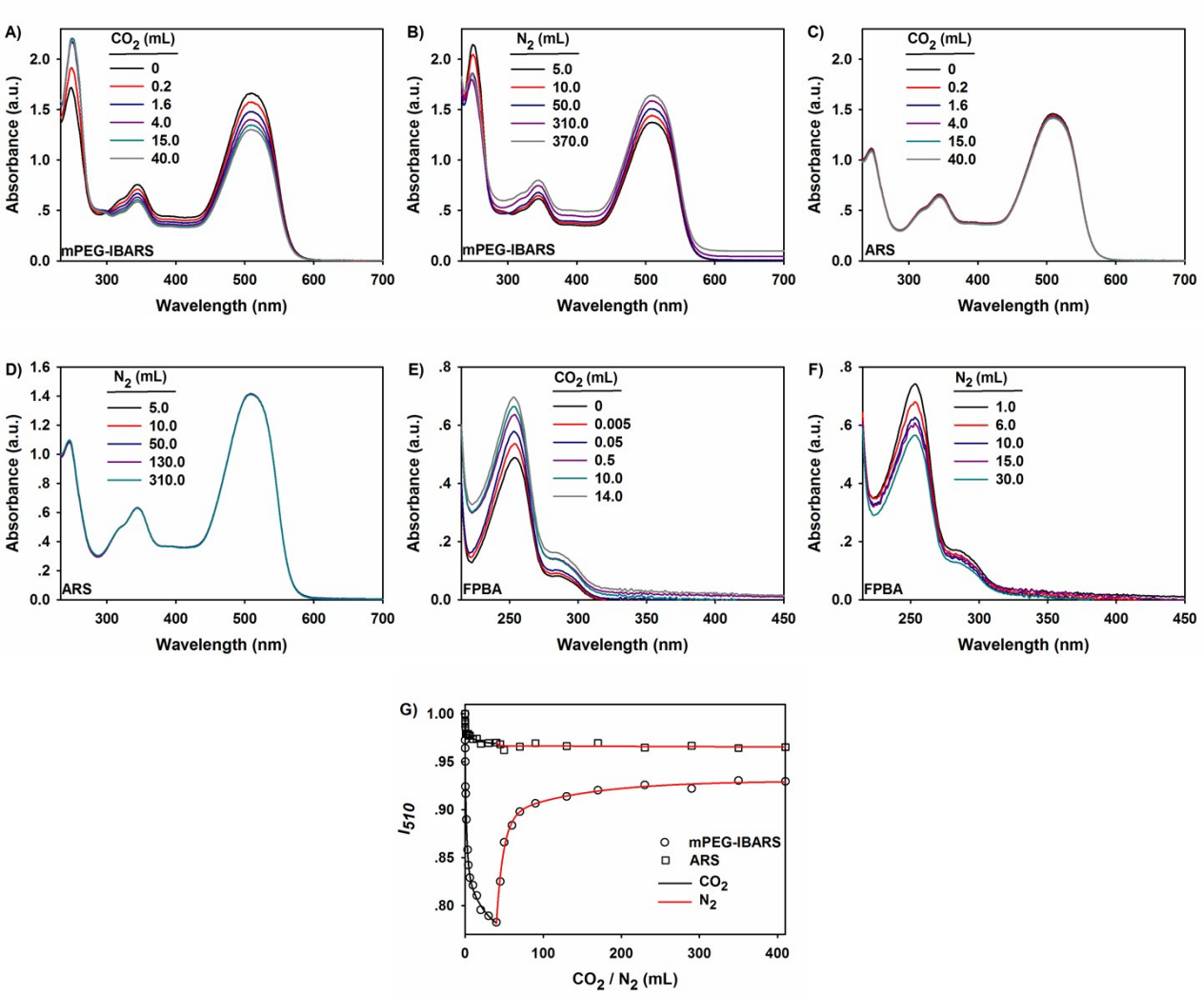

Figure S13. The absorption spectra change upon the addition and removal of CO_2. A, B) mPEG-IBARS. C, D) ARS. E, F) FPBA. G) The reversible change of the normalized intensity at 510 nm (I_{510}) of mPEG-IBARS and ARS aqueous solution upon addition and removal of CO_2.

The primary CO_2-induced hydrolysis process of mPEG-IBARS aqueous solution (0.25 mg mL^{-1} × 1.5 mL, pH 7.4) was also verified using absorption spectroscopy. As shown in Figure S13A, mPEG-IBARS aqueous solution before CO_2 bubbling showed a characteristic absorption band of IBARS moieties at 250 nm, 342 nm and 510 nm. In the presence of CO_2, the absorption band at 250 nm increased with a gradually decreased at 342 and 510 nm, respectively, ascribing to the cleavage of iminoboronic acid ester of dynamic B−O and C=N bonds and the generation of FPBA and ARS as byproduct. As shown in Figure S13B, these absorption band can be mostly regained to their initial intensity after removing CO_2 with N_2, intensely indicating the reversible hydrolysis of iminoboronate binding upon alternative CO_2 and N_2. Figure S13C and S13D showed a very weak change of characteristic absorption bands at at 245 nm, 345 nm and 510 nm of ARS upon the

bubbling of CO_2 and N_2. While Figure S13E and S13F showed that the characteristic absorption band of FPBA at 205-350 nm intervals changed much and reversibily upon the bubbling of CO_2 and N_2. Figure S13G gave the reversible changes of the normalized intensity at 510 nm (I_{510}) of mPEG-IBARS and ARS aqueous solution. These results confirmed the iminoboronate ester can be reversibly hydrolyzed in aqueous solution through the alternative bubbling of CO_2 and N_2.

9. The Cellular Uptake of Polymersomes Observed with Confocal Laser Scanning Microscopy

N_3-(OEG-IBCAPE)$_4$ or free CAPE was dispersed in the medium by slightly ultrasonication right before their introduction to the cells. For confocal microscopy study, cells were seeded at initial densities of 5×10^4 cells/mL in dishes (35 mm confocal imaging dish, ibidi, Cat. No. 81156) and incubated for 24 hours before introducing N_3-(OEG-IBCAPE)$_4$ or free CAPE. Live cell confocal microscopy was used to assess the cellular uptake of the N_3-(OEG-IBCAPE)$_4$ or free CAPE. HL-60 cells were incubated with N_3-(OEG-IBCAPE)$_4$ and free CAPE at the same drug concentration (50 μg/mL). After incubation for 24 hours, cells were carefully washed with PBS, and stained by Hoechst-33342 ThermoFisher Scientific H1399, with excitation/emission ~350⁄461 nm) for 5 min. After that, the stain solution was removed and the cells were washed 2-3 times in PBS. Images were taken using a Leica TCS SP5 Confocal Microscope.

10. In Vitro Cytotoxicity Assay

MTT assay was conducted to evaluate the cell viability. Briefly, after incubation with a drug free medium for 24 hours, cells were transferred to a series concentration of N_3-(OEG-NH_2)$_4$, N_3-(OEG-IBCAPE)$_4$ or CAPE. Then all the cell samples were further incubated for 24 hours. After that, cell culture medium was replaced with MTT assay solution (0.5 mg/mL) and the cells were further incubated for 4 hours at 37 ^oC. Then MTT solution was removed and DMSO was added. The absorbance was measured at 570 nm with a reference of 690 nm using a microplate reader (#680, Bio-Rad). The relative cell viability was calculated as a percentage compared to the control samples (treated with fresh medium).