Supporting Information

Manipulation of Block Copolymer Vesicles Using CO_2: Dissociation or "Breathing"

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1. Materials

All chemicals were purchased from Aldrich. N, N'-dimethylacrylamide (DMA) and 2-(diethylamino)ethyl methacrylate (DEAEMA) were purified by passing through an activated basic Al_2O_3 short column to remove the inhibitor. Poly(ethylene oxide) (PEO) macroinitiator was prepared by reacting PEO monomethyl ether (M_n = 2000 g/mol) and 2-bromoisobutyryl bromide.[S1] 4-Methyl-[7-(methacryloyl)oxylethyoxyl]coumarin (coumarin methacrylate CMA) was synthesized using the method previous reported.[S2] The starting chain transfer agent, 2-(2-cyanopropyl)dithiobenzoate (CPDB), was synthesized using a literature method.[S3]

2. Characterization

^{1}H NMR spectra were recorded on a Bruker 300MHz spectrometer using deuterated chloroform as the solvent and tetramethylsilane as the internal standard. They were used to determine the block copolymer compositions. Size exclusion chromatograph (SEC) measurements were performed on a Waters system equipped with a photodiode array detector (PDA 996) and a refractive index detector (RI 410). THF was used as the eluent at an elution rate of 1 mL/min, while polystyrene standards were used for calibration. Change in the transmittance (at 550 nm) of a given polymer solution on heating was recorded using a Varian 50 Bio UV-vis spectrophotometer. The steady-state fluorescence emission spectra were obtained with a Varian Cary Eclipse fluorescence spectrometer. The morphologies of the polymer vesicles were examined using a Hitachi H-7500 transmittance electron microscope (TEM) operating at 60 kV. For the TEM measurements, a drop of a given vesicle solution was put on a TEM copper grid, kept still for 5 min, before the excess solution was removed by using a filter paper and the sample was let to dry at room temperature overnight. The pH measurements were carried out using a pH meter (AB15, Fisher Scientific).

3. Block Copolymer Synthesis

RAFT Synthesis of PDMA RAFT agent. RAFT polymerization of DMA was conducted at 78 ^{o}C, employing AIBN as the radical initiator and CPDB as the RAFT chain transfer agent. A typical reaction was as follows. In a 25 mL one-pot round flask. DMA (3 g, 0.03 mol), CPDB (0.382 g, 0.001 mol), AIBN (0.0324 g, 0.0002 mol) were added. Then 3 mL of anhydrous anisole was added to dissolve the mixture. After purging with argon of high purity for 30 min, the flask was immersed into a preheated oil bath for 4 h at 78 ^{o}C under continuous stirring. The crude product was purified by precipitation into excess ethyl ether twice, followed by vacuum-drying. The monomer conversion was almost 100% as estimated from the ^{1}H NMR spectrum. The sample obtained is denoted as $PDMA_{30}$ macroinitiator (PDI = 1.08, M_n = 4,000, from SEC).

RAFT Synthesis of PDMA-*b*-PDEAEMA Diblock Copolymer. $PDMA_{30}$ macroinitiator was used as the chain transfer agent to prepare the block copolymer. $PDMA_{30}$ macroinitiator (0.075 g,

0.025 mmol), DEAEMA (1.65 g, 0.01 mol), AIBN (0.0016 g, 0.001 mmol) were dissolved in 2 mL of 1, 4-dioxane. After purging the mixture with argon for 30 min, the reaction was allowed to last for 24 h at 78 ^{o}C under continuous stirring. The product was purified by precipitating the polymer solution into excess cold hexane three times, followed by vacuum-drying overnight. The sample obtained was characterized by ^{1}H NMR (Figure S1) yielding PDMA$_{30}$-*b*-PDEAEMA$_{400}$. From SEC: PDI = 1.27, M_n = 51,400.

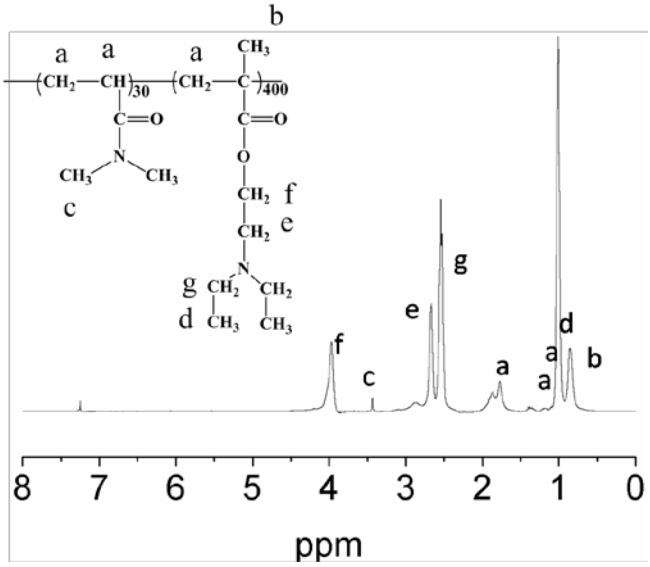

Figure S1. ^{1}H NMR spectrum of PDMA-*b*-PDEAEMA in $DCCl_3$.

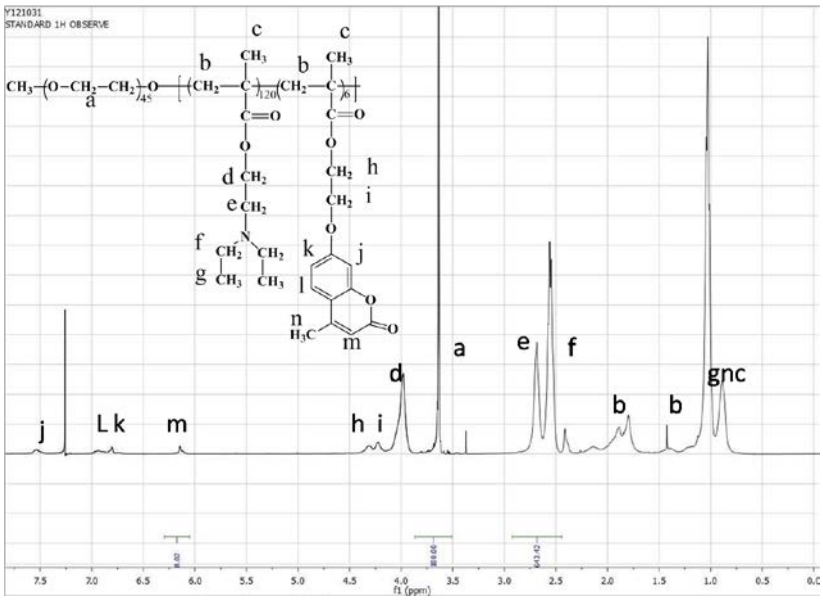

Figure S2. ^{1}H NMR spectrum of PEO-*b*-P(DEAEMA-*co*-CMA) in $DCCl_3$.

ATRP Synthesis of PEO-*b*-P(DEAEMA-*co*-CMA). The diblock copolymer was synthesized by means of ATRP using PEO_{45}-Br as the macroinitiator. The reaction was as follows. CuBr (0.0143g, 0.1 mmol), PEO_{45}-Br (0.2 g, 0.1 mmol), PMDETA (0.0173 g, 0.1 mmol), CMA (0.29 g, 1 mmol), DEAEMA (1.49 g, 9 mmol) were dissolved in 4 mL of anisole in a 10 mL flask under an argon atmosphere. The reaction mixture was degassed by three freeze-pump-thaw cycles and then filled with argon. The flask was then placed in a preheated oil bath at 70 ^{o}C for 2 h. After

polymerization, the solution was cooled to room temperature and diluted with THF. The mixture was passed through a neutral Al_2O_3 column to get rid of copper salt. The purification of the polymer was completed by two THF solution precipitations into cold hexane twice. The sample was finally dried in a vacuum overnight. Knowing the molecular weight of the PEO block, ^{1}H NMR analysis (Figure S2) yielded a block copolymer composition of PEO_{45}-*b*-P($DEAEMA_{100}$-*co*-CMA_6). From SEC: PDI = 1.21, M_n = 16,700.

4. Preparation of Block Copolymer Vesicles

Either PEO_{45}-*b*-P($DEAEMA_{100}$-*co*-CMA_6) or $PDMA_{30}$-*b*-$PDEAEMA_{400}$ was dissolved in THF at a concentration of 5%. Under sonication, 2 mL of the THF solution was added into 10 mL of deionized water. Afterwards, THF was evaporated under vacuum at room temperature. The final polymer concentration in the aqueous solution was adjusted to 1% (10 mg/mL) for further experiments.

5. Transmittance Measurements of Vesicle Solutions on Heating in the Presence of CO_2

An aqueous vesicle solution of $PDMA_{30}$-*b*-$PDEAEMA_{400}$ (2 mL, 10 mg/mL, concentration of DEAEMA groups ~ 0.05 mol/L) was placed in a quartz cuvette and sealed by a rubber stopper. Using a pressure-lock precision analytical syringe, different amounts of CO_2 (1 atm) was injected into the vesicle solution. Before CO_2 injection, absorption spectra of the solution were recorded for measuring the transmittance (at 550 nm). After each CO_2 injection, the transmittance measurement was made after every 2 min with the solution under stirring. All the measurements were carried out at room temperature.

6. Loading and Release of Pyrene-1, 3, 6, 8-tetrasulfonic acid tetrasodium

For loading the model dye into the vesicles, pyrene-1, 3, 6, 8- tetrasulfonic acid tetrasodium salt was dissolved in 4 mL of either block copolymer vesicle solution (25 mg/mL) to obtain a dye concentration of 100 μM. The solution was sealed with parafilm and kept under stirring in the dark. After 72 h, dye-loaded vesicles were separated by centrifugation at 20,000 rpm. These vesicles were further subjected to dialysis against deionized water (2 L, pH 8.6) for 12 h; fresh water was used for every 3 h. The used pH=8.6 for the dialysis was important to keep the PDEAEMA block insoluble and thus the integrity of vesicles during the dialysis process. Finally, 10 mL of the vesicle solution was obtained at a concentration of 10 mg/mL.

To observe the release behavior of the dye in the presence of CO_2 in the vesicle solution (2 mL, 10 mg/mL), the typical experiment conducted was as follows. 2 mL of dye-loaded vesicle solution was sealed with a rubber stopper; then a certain amount of CO_2 was injected into the solution through a pressure-lock precision analytical syringe. The transmittance of the solution was monitored by recording the absorption spectra. When the transmittance became constant, the vesicle solution was transferred into a dialysis bag (MW cutoff: 3500) sitting on top of a cuvette and immersed in 10 mL deionized water (pH=8.6). The increase in the amount of dye released from the vesicles and diffusing into the solution underneath the dialysis bag was monitored from its fluorescence emission intensity at the 384 nm peak (λ_{ex} = 354 nm).

The accumulative amount of released dye was determined by using a calibration curve ploting the fluorescence intensity at 384 nm (*I*) as a function of dye concentration. To obtain the calibration

curve, a series of aqueous solutions of different dye concentrations (*C:* mg/mL) were prepared and their fluorescence emission spectra were recorded to measure the intensity of the 384 nm peak (Figure S3). Linear curve fitting yields: $I = 3.53192 \times 10^6 C + 125.09636$, with $R^2 = 0.99627$. Afterwards, to obtain the amount of dye loaded in the vesicle, a dye-loaded vesicle solution was completely dissociated by purging an excessive amount CO_2 in the solution for long time, from the recorded fluorescence spectrum corresponding to 100% release of the dye, the concentration of dye in the vesicle solution was calculated based on the calibration curve, This concentration was set as the reference to obtain the dye release percentage as shown in Figure 4. For the crosslinked vesicles, the above experiment was carried out before the crosslinking reaction. Moreover, knowing the amount of dye in the vesicle solution and the amount of dye used for preparing dye-loaded vesicles, the loading efficiency of the dye was found to be about 1 wt% for uncrosslinked PDMA-*b*-PDEAEMA vesicles and 0.6 wt% for crosslinked PEO-*b*-P(DEAEMA-co-CMA) vesicles. These low loading efficiencies are due to the used diffusion-based loading method, with which most of dye molecules remain in the exterior solution outside the vesicles.

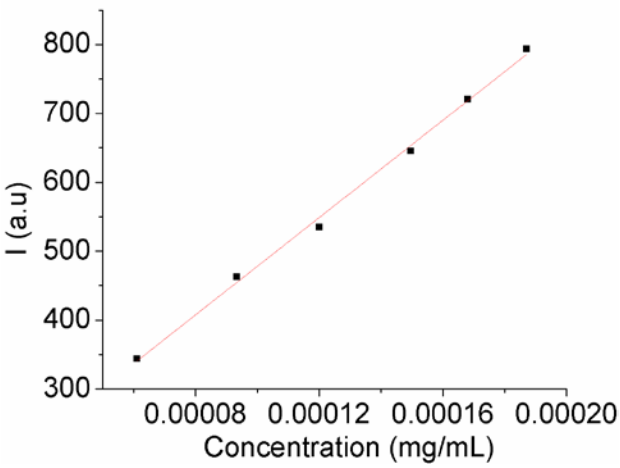

Figure S3. The calibration curve of fluorescence emission intensity at 384 nm vs. concentration of pyrene-1, 3, 6, 8-tetrasulfonic acid tetrasodium in aqueous solution.

7. pKa, pH and PDEAEMA Protonation of Vesicle Solutions in the Presence of CO_2

For CO_2-dissociable vesicles of $PDMA_{30}$-*b*-$PDEAEMA_{400}$, pH measurements were carried out in order to determine the pKa of the PDEAEMA block inside the vesicles (using an ORION 8115BNUWP pH electrode with an Accumet AB15 pH-meter). Basically, pH of the vesicle solution was measured by titration with a hydrochloric solution at the concentration 0.05mol/L. After determining the equivalence, pH corresponding to the half of the equivalence was taken as the average pKa (assuming the solutions were ideal), yielding pKa ~ 8.1. The protonation degree of PDEAEMA δ in the aqueous solution in response to CO_2 was calculated based on the following equations:

$$Ka = \frac{[PDEAEMA][H^+]}{[PDEAEMAH^+]} \quad \text{.....(1)}$$

$$\delta = \frac{[PDEAEMAH^+]}{[PDEAEMA]+[PDEAEMAH^+]} \quad \text{.....(2)}$$

$$pH = -\log[H^+] \quad \text{.....(3)}$$

$$\delta = \frac{1}{1+10^{pH-pKa}} \quad \text{.....(4)}$$

According to the pH change of the vesicle solution upon injection of different amounts of CO_2, the protonation degree of PDEAEMA changed from 24% (before CO_2), to 33% (7 mol% CO_2 injection), 39% (9 mol%), 44% (13 mol%) and finally 50% (18 mol%) at which total dissolution of the vesicles was observed.

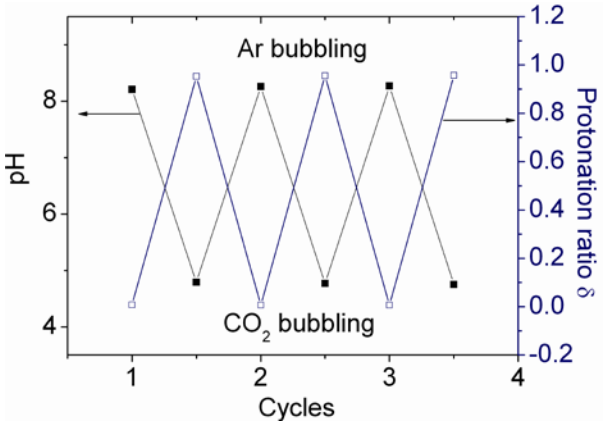

Figure S4. Reversible changes in pH and the protonation degree of DEAEMA groups of the cross-linked vesicle solution of PEO_{45}-*b*-P($DEAEMA_{100}$-*co*-CMA_6) with 90% coumarin dimerization (2 mL, 1 mg/mL) following alternating bubbling of CO_2 and Ar in the solution.

For the "breathing" vesicles of PEO_{45}-*b*-P($DEAEMA_{100}$-*co*-CMA_6), the reversible volume expansion and contraction was accompanied by a reversible change in pH and the protonantion degree of the amine groups. Figure S1 shows the result obtained with the vesicle solution at 90% dimerization of coumarin. After CO_2 bubbling, pH of the vesicle solution decreased from about 8.3 to 4.8; while after Ar bubbling, the initial higher pH was recovered. Using the same titration method, pKa of the random copolymer block of P($DEAEMA_{100}$-*co*-CMA_6) in the crosslinked vesicle solution was estimated to be about 6.1. As compared to the pKa in $PDMA_{30}$-*b*-$PDEAEMA_{400}$, this lower value is quite surprising and implies that the pKa is much affected by the actual microenvironment of the tertiary amine groups. Based on this pKa, the reversible change in the protonation degree in response to alternating CO_2 and Ar bubbling is also shown in Figure S4.

9. Photo-Crosslinking of Block Copolymer Vesicles

A vesicle solution of PEO_{45}-*b*-P($DEAEMA_{100}$-*co*-CMA_6) (1 mg/mL, 2 mL) was exposed to UV light for vesicle wall cross-linking. Figure S5 shows the absorption spectra of the solution as a function of UV irradiation time. The continuous decrease of the absorption peak at around 320 nm indicates a growing degree of coumarin dimerization.[S5,S6] After 7 min UV irradiation, the

dimerization reaction was almost completed. In this study, three different dimerization degrees, corresponding to different vesicle wall cross-linking densities, were obtained by adjusting the UV irradiation time.

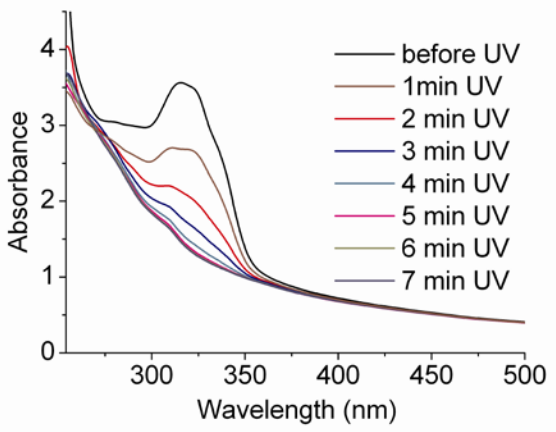

Figure S5. UV-vis spectra of a PEO_{45}-*b*-P($DEAEMA_{100}$-*co*-CMA_6) vesicle solution (1 mg/mL, 2 mL) upon UV irradiation (320-500 nm, intensity: 500 mW/cm^2).

10. Reversible Size Changes of Cross-linked Vesicles Triggered by CO_2 and Ar

By controlling the dimerization degree of coumarin groups in the vesicle wall, vesicles of PEO_{45}-*b*-P($DEAEMA_{100}$-*co*-CMA_6) with three cross-linking densities were obtained. The extent of their gas-controllable expansion and contraction (breathing) is determined by the cross-linking density. Lower cross-linking density resulted in larger volume change. This is shown by the DLS results in Figure S6.

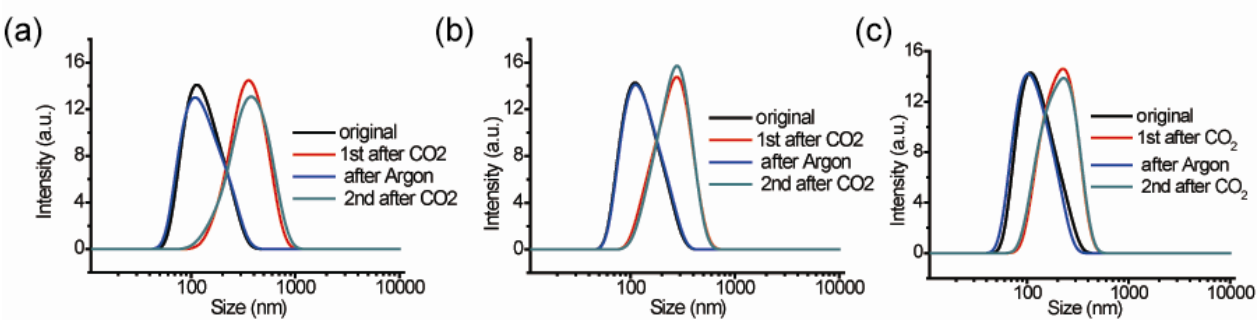

Figure S6. DLS data showing the reversible size change of PEO_{45}-*b*-P($DEAEMA_{100}$-*co*-CMA_6) vesicles (2 mL, 1 mg/mL) with different dimerization degrees of coumarin groups upon CO_2 and Ar bubbling in solution: (a) 30 %, (b) 60 % and (c) 90 %.

References

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