Supplementary Information

Fluorescent and "breathable" CO_2 responsive vesicles inspired from green fluorescent protein†

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1.1 Materials

$mPEG_{48}$-CH_2CH_2COOH (M_n=2.0 kDa) was purchased from Biomatrik Inc. and dried at 50 ℃ under vacuum for 2 days prior to use. 2-(Diethylamino)ethyl methacrylate (DEAEMA) was purified by passing through an activated basic Al_2O_3 column, and then stirred overnight over CaH_2 and distilled under reduced pressure. Dichloromethane (CH_2Cl_2), triethylamine (TEA), N,N-dimethylformamide (DMF) and toluene were refluxed with CaH_2, and ethanol (EtOH) was refluxed with CaO. Copper(I) bromide (CuBr, 98%, Aldrich) was treated by stirring in acetic acid and washed with ethanol and diethyl ether, and then dried at 50 ℃ for 2 days and stored under an argon atmosphere. Ethanolamine (98%, Alfa), 2-bromoisobutyryl bromide (97%, Alfa), Copper(I) iodide (98%, J&K), triphenylphosphine (Aladdin,99%), bis(triphenylphosphine)palladium(II) dichloride (Aladdin, Pd 15.2%), quinine sulfate (99%, J&K) were used as received and other reagents and solvents like potassium carbonate were purchased from Shanghai Sinopharm reagent Co. Ltd., Shanghai, and used without further purification.

1.2 Instruments and Measurements

^1H NMR and ^{13}C NMR spectra were recorded on a Varian MERCURY plus-400 spectrometer with dimethyl sulfoxide-d_6 (DMSO-d_6) or deuterated chloroform ($CDCl_3$) as the solvents at 298 K. The chemical shifts were referenced to residual peaks of TMS. Fourier transform infrared (FT-IR) spectra were measured as KBr pellets on a Perkin-Elmer Spectrum 100 FTIR spectrometer (U.K.) in the range of 4000-450 cm^{-1}. The

molecular weight and polydispersity index (PDI) of the samples were determined by gel permeation chromatography on a Malvern GPC with polystyrene as standard. Tetrahydrofuran (THF) was used as the eluent at a flow rate of 1.0 mL/min at 35 ^{o}C. Dynamic light scattering (DLS) was performed with a Malvern Zetasizer Nano ZS90 apparatus equipped with a 4.0 mW He-Ne laser operating at λ= 633 nm at 25 ^{o}C and a scattering angle of 90°. Transmission electron microscopy (TEM) measurements were performed with a JEOL JEM-100CX-II instrument at a voltage of 200 kV. Atomic force microscopy (AFM) measurements were performed on a Burker Dimension FastScan atomic force microscope. Confocal microscopy measurements were measured on a Leica TCS SP8 STED 3X Super-resolution multiphoton confocal microscope. High resolution mass spectrometer (HRMS) was performed on a Waters Premier Q-TOF Mass Spectrometer. HRMS data were acquired for each sample from 50 to 1000 Da with a 0.10 s scan time and a 0.01 s interscan delay over a three-min analysis time. UV-Vis absorption (UV-Vis) measurement was performed on a Perkin-Elmer Lambda 35 UV-Vis spectrophotometer (U.K.) in the range of 200-700 nm. The slit-width was set as 2 nm, and scan speed was set as 480 nm/min. The fluorescence emission measurements were carried out on a Perkin Elmer LS 50B fluorescence spectrometer. Excitation wavelength was λ_{ex}=405 nm in THF and 375 nm in H_2O. Scan speed was set at 480 nm/min, and the slit-width was 10/10. The conductivity of the copolymer aggregates was recorded by using a Mettler Toledo FE30 conductivity meter at 25 ℃ and the pH was tested by Mettler Toledo FE20 pH meter. CO_2 and N_2 gas was bubbled through the solution at 25 ℃ to record the conductivity and pH variation, and the CO_2

gas flow rate was modulated at 4 mL/min while the N_2 was 8 mL/min for all experiments.

1.3 General Procedure

Synthesis of the chromophore IE

The synthesis of the imidazole chromophore precursor is referenced to previous reports.[1] The alkyne terminated and iodine terminated chromophore precursors were coupled by Sonogashira coupling with the following procedure: To a three-neck round-bottle flask equipped with condenser and stir bar, 1.424 g (1 equiv.) of iodine terminated chromophore, 1.118 g (1.1 equiv.) of alkyne terminated chromophore, 71.18 mg (0.1 equiv.) of CuI, and 104.92 mg (0.1 equiv.) of PPh_3, and 5 mg of $Pd(PPh_3)_2Cl_2$ were dissolved in triethylamine. The reaction was refluxed under argon for 8 h and then allowed to cool down to room temperature. The solution was concentrated under reduced pressure to obtain raw product. The raw product was further purified by a silica column with $CHCl_3$/MeOH as eluent to obtain a pure product (0.6358 g, 43.35 %).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.24 (2H, Ar-H), 7.63 (2H, Ar-H), 6.97 (1H, CH), 4.98 (1H, OH), 3.63 (2H, CH_2CH$_2$OH), 3.52 (2H, CH$_2$CH_2OH), 2.39 (3H, CH_3).

^{13}C NMR, (DMSO-d_6) δ (ppm): 170.5 (-C=O), 166.2 (CH3-*C*), 135.4 (Ar-CH-*C*), 140.2 (Ar), 132.3 (Ar), 123.8 (Ar), 110.0 (Ar-CH2), 92.3 (-CH≡CH-), 59.4 (-CH$_2$*C*H$_2$OH), 43.7 (-*C*H$_2$CH$_2$OH), 16.5 (-CH_3). HRMS: m/z calculated for $[C_{28}H_{27}N_4O_4]^+$ 483.2027, found 483.2032.

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[1] A. Baldridge, S. R. Samanta, N. Jayaraj, V. Ramamurthy, L. M. Tolbert, *J. Am. Chem. Soc.,* 2010, **132**, 1498-1499.

Synthesis of PEG-IE

mPEG-CH_2CH_2COOH (1 equiv. 1 g), EDC (2 equiv. 0.1917 g), DMAP (0.2 equiv. 0.0242 g), and trace amount of TEA was dissolved in 6 ml of DMF in a sealed 25 ml Shlenk flask. The solution was stirred under 0 °C for 30 min, and then added dropwise to a DCM (15 ml) solution of IE (1.8 equiv. 0.433 g) in a 50 ml Shlenk flask. The reaction was kept under 35 °C for 48 h and then concentrated to obtain the raw product. The raw product was first washed with brine and $CHCl_3$ 3 times each and then purified with a silica column with $CHCl_3$/MeOH as eluent (0.820 g, 66.56%).

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.31 (2H, Ar-H), 7.63 (2H, Ar-H), 7.00 (1H, CH), 4.97 (1H, OH), 4.20 (2H, NCH_2CH_2), 3.86 (2H, NCH_2CH_2), 3.49 (2mH, CH_2CH_2O), 3.32 (3H, OCH_3), 2.39 (3H, CH_3).

Synthesis of PEG-IE-Br

To a DCM solution (5 ml) of PEG-IE (0.460 g, 1 equiv.) and TEA (0.189 g, 10 equiv.), 2-bromoisobutyryl bromide (0.429 g, 10 equiv. in 2 ml DCM) was added dropwise under 0 °C. The reaction was kept at 30 °C for 24 h and then washed with brine for 3 times and concentrated. The raw polymer was obtained by precipitating with diethyl ether twice. The raw product was further purified by a silica column with $CHCl_3$/MeOH as eluent to obtain a pure product (0.1521 g, 31.17%).

^{1}H NMR (400 MHz, DMSO-d_6), ppm: 8.31 (2H, Ar-H), 7.63 (2H, Ar-H), 7.00 (1H, CH), 4.97 (1H, OH), 4.32-3.84, (NCH_2CH_2O), 3.49 (2mH, CH_2CH_2O), 3.32 (3H, OCH_3), 2.39 (3H, CH_3), 1.84 (3H, $BrCCH_3$).

Synthesis of PEG-IE-PDEAEMA

To a sealed Shlenk flask equipped rubber septum with PEG-IE-Br (0.1 g, 1 equiv.), CuBr (0.0108 g, 2 equiv.), and PMDETA (0.0270 g, 2 equiv.), DEAEMA (1.062 g, 150 equiv.) were injected. The mixture was immediately immersed into liquid nitrogen for three freeze-pump-thaw cycles to remove any trace amount of residual oxygen. The reaction was stirred under 80 ^{o}C for 12 h and then quenched by cooling down and exposure to the air. The solution was first passed through a short Al_2O_3 column to remove the copper catalyst and then concentrated and precipitated in n-hexane to obtain the product PEG-IE-PDEAEMA (Mn:16.2 kDa, PDI:1.46 by GPC).

^{1}H NMR (400 MHz, $CDCl_3$), ppm: 4.09-3.91 ($CH_3CH_2NCH_2CH_2O$), 3.65-3.60 (2mH, CH_2CH_2O), 2.74-2.50 (4nH, $CH_3CH_2NCH_2CH_2O$), 1.99-1.65 (2nH, CH_2CCH_3CO), 3.32 (3H, OCH_3), 1.07-0.98 (3nH, CH_3CH_2N), 0.92-0.79 (3nH, CH_2CCH_3CO).

Self-assembly of the polymer vesicles

PEG-IE-DEAEMA vesicles were prepared according to the method proposed by Yu et al[2], in which the THF solution of the copolymer was added dropwise water (equal volume), followed by an excessive of 9-fold water and dialysis in water.

Degree of protonation of the PDEAEMA segment

The CO_2 responsive behavior of the vesicles should be directly related to the degree of protonation of the PDEAEMA segment of the di-block copolymer. Since the pK_a of a

[2] S. Y. Yu, T. Azzam, I. Rouiller and A. Eisenberg, *J. Am. Chem. Soc.*, 2009, **131**, 10557-10566.

polymer is not only related to its certain functional groups, but also the environment of the nano-sized assembles, the pK_a of different polymers with similar PDEAEMA segments varies. According to reference reports, the pK_a of a typical PDEAEMA homopolymer should be around 7.5.[3] However, the pK_a for copolymer could range from 8.1 to 5.0 for different copolymer compositions, and the corresponding pH values were higher than 7, suggesting that the solution was basic.[4,5] In our case, however, the initial pH value was 6.8, quite closed to the water used for experiment, the pK_a for the polymer could be lower compared to those reported values in the literature. Since a pK_a value close to 5.0 would provide an estimation that about 2% of the initial protonated PDEAEMA segment, the actual degree of protonation could be even lower than that. This could also indicate that upon CO_2 bubbling, about 50% of the PDEAEMA segment was protonated. Such a condition that the pH value of the initial polymeric solution is close to 7 was also reported previously in other literatures with PDEAEMA as the CO_2 responsive segment.[5]

[3] A. Darabi, P. G. Jessop and M. F. Cunningham, *Chem. Soc. Rev.,* 2016, **45**, 4391.
[4] B. Yan, D. Han, O. Boissière, P. Ayotte and Y. Zhao, *Soft Matter*, 2013, **9**, 2011.
[5] H. Liu, Z. Guo, S. He, H. Shuai, H. Yin, C. Fei and Y. Feng, *Polym. Chem.*, 2014, **5**, 4756.

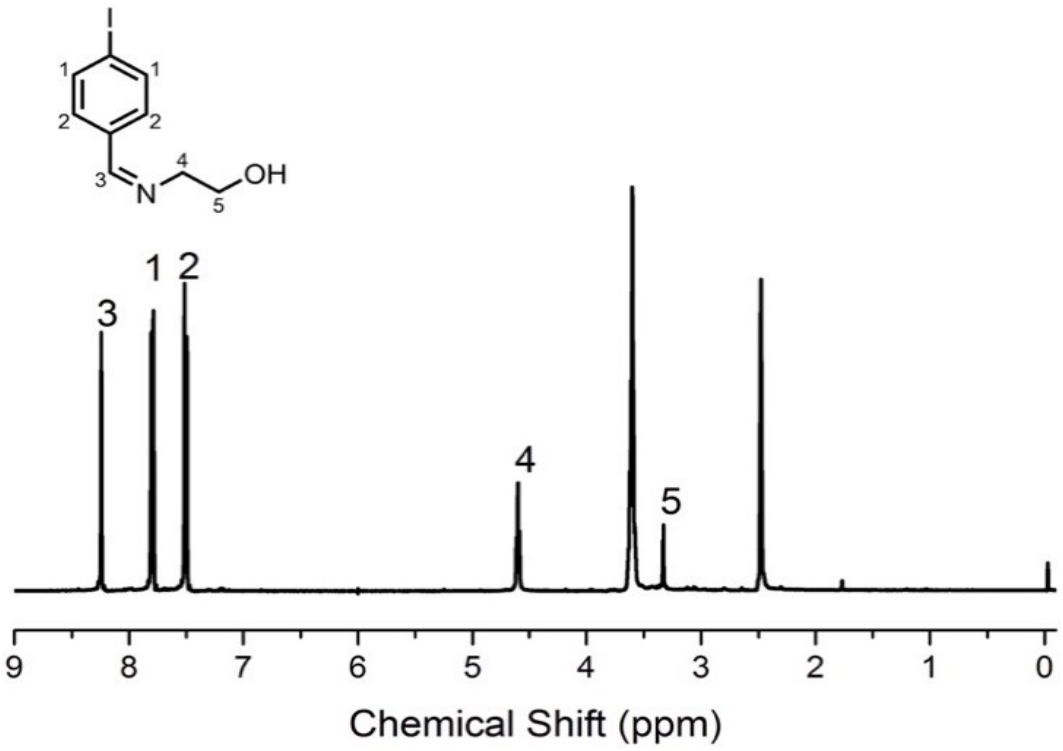

Figure S1. ^{1}H NMR spectrum of compound C1 in DMSO-d_6.

HRMS of compound C1: m/z calculated for $[C_9H_{11}INO]^+$ 275.9880, found 275.9884.

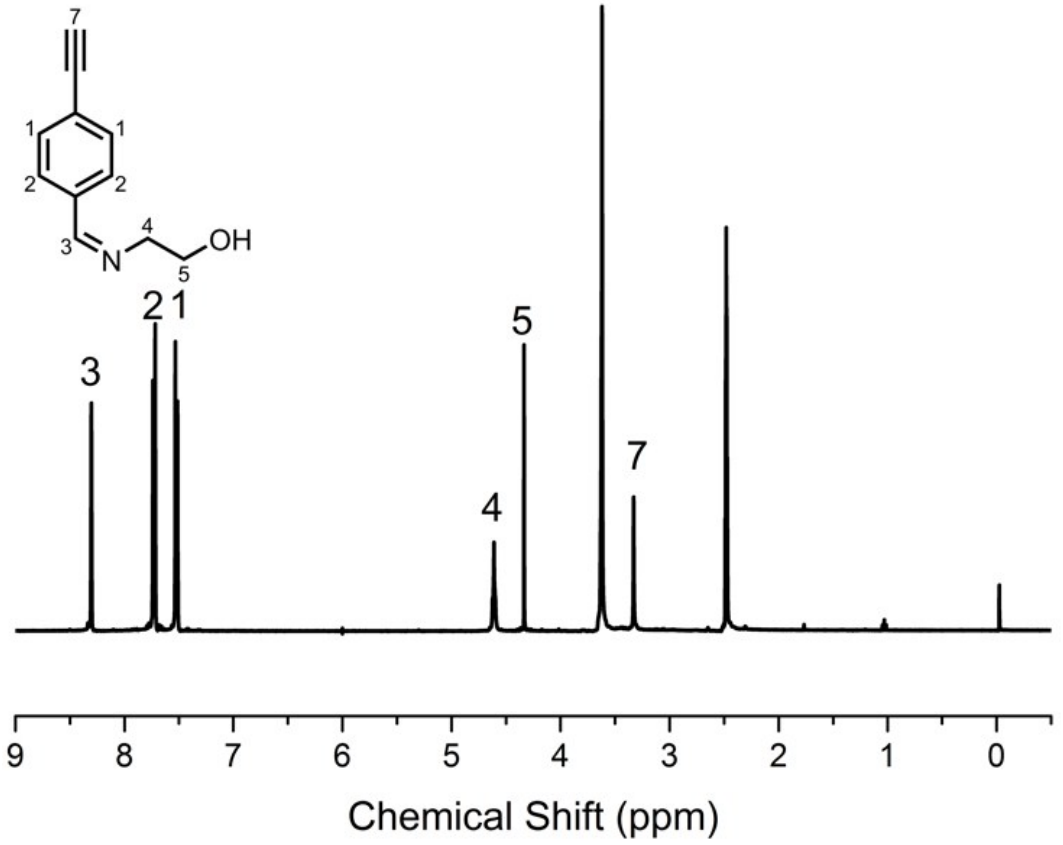

Figure S2. ^{1}H NMR spectrum of compound C2 in DMSO-d_6.

HRMS of compound C2: m/z calculated for $[C_{11}H_{12}NO]^+$ 174.0919，found 174.0897.

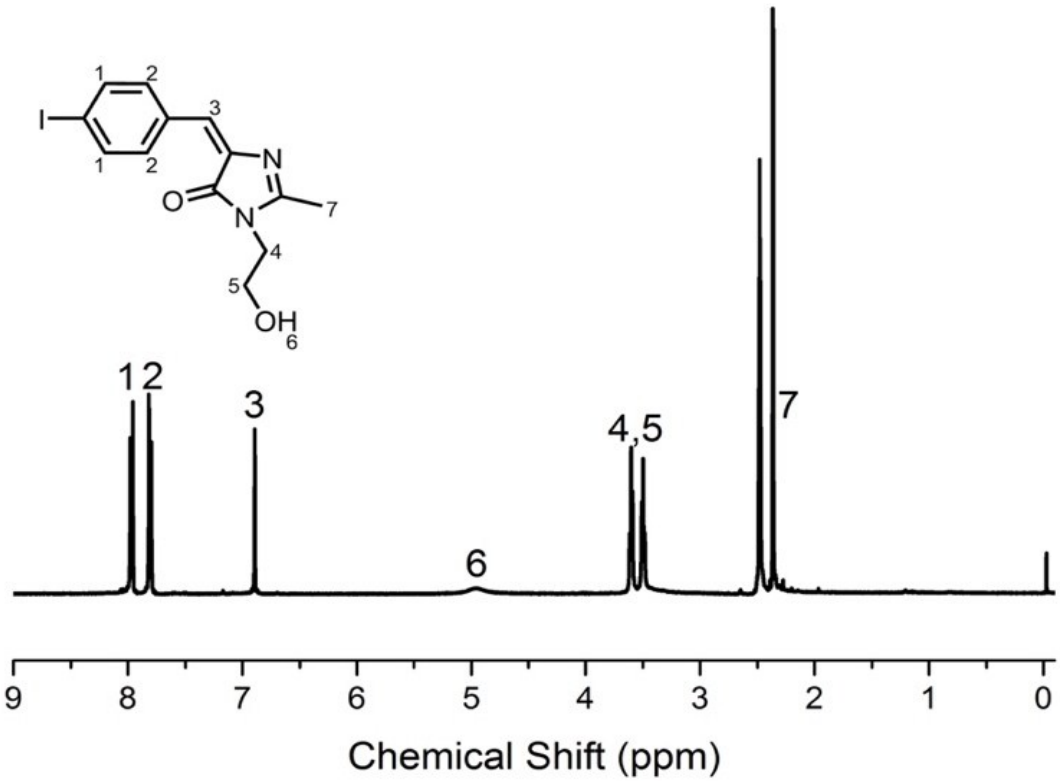

Figure S3. ^{1}H NMR spectrum of compound C3 in DMSO-d_6.

HRMS of compound C3: m/z calculated for $[C_{13}H_{14}IN_2O_2]^+$ 357.0095, found 357.0098.

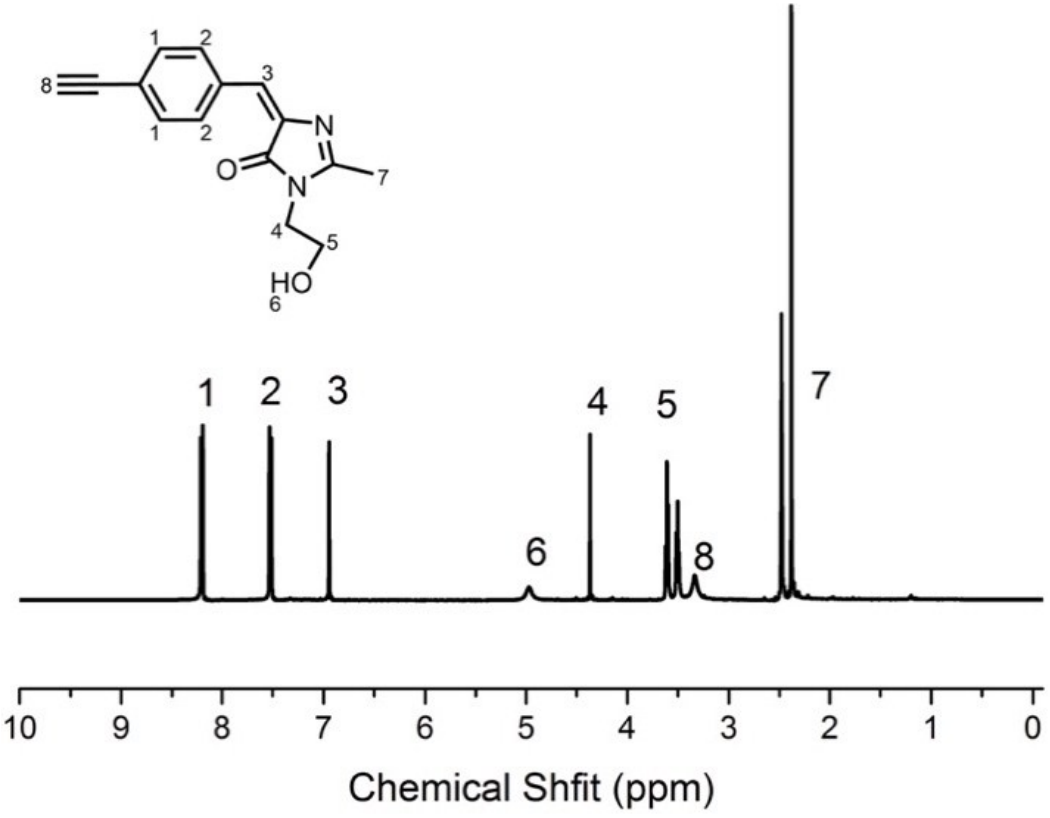

Figure S4. ^{1}H NMR spectrum of compound C4 in DMSO-d_6.

HRMS of compound C4: m/z calculated for $[C_{11}H_{12}NO]^+$ 255.1134, found 255.1129

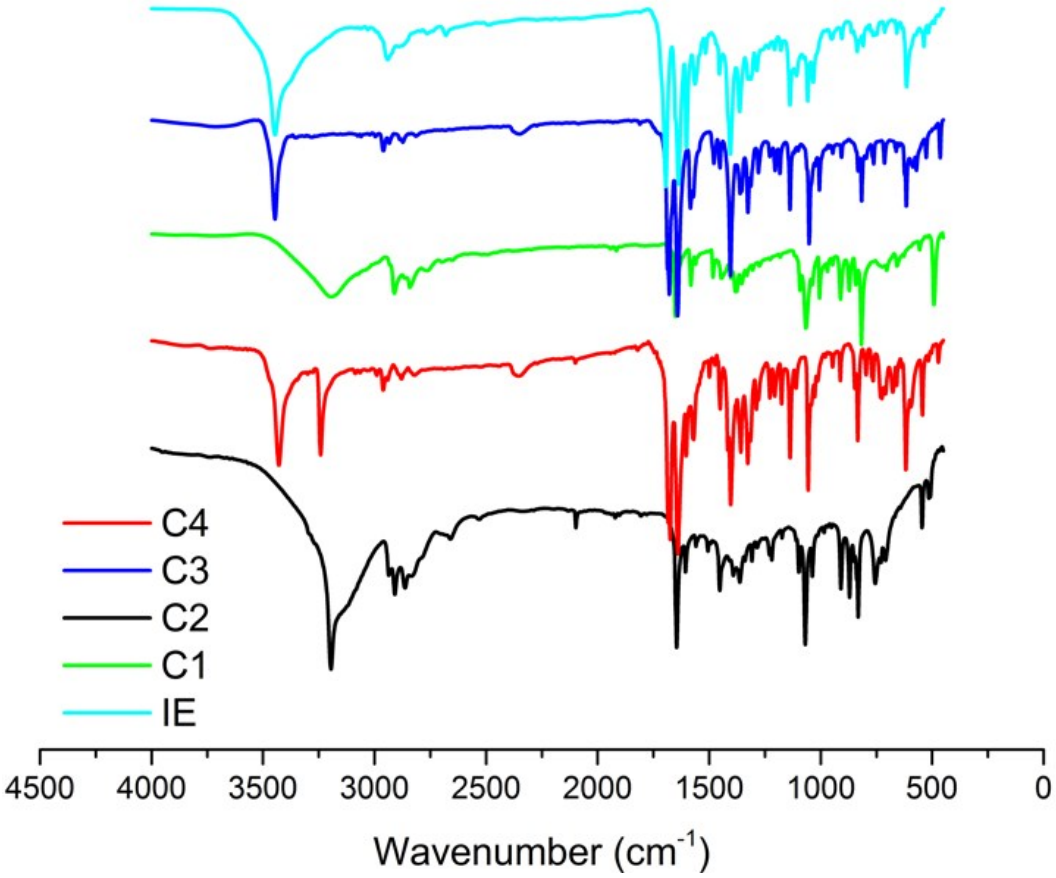

Figure S5. FTIR spectra of the compound C1-C4 and the IE chromophore. The sharp peaks appeared at 1679 cm^{-1} ($\nu_{C=O}$) in compound C2 and C4 demonstrate the formation of the imidazole ring, while the disappearance of the sharp peak at 3192 cm^{-1} ($\nu_{\equiv C-H}$) in compound IE illustrates the consumption of the proton on the terminal alkyne in compound C4, thus demonstrating the coupling of the two chromophore precursors to the final chromophore.

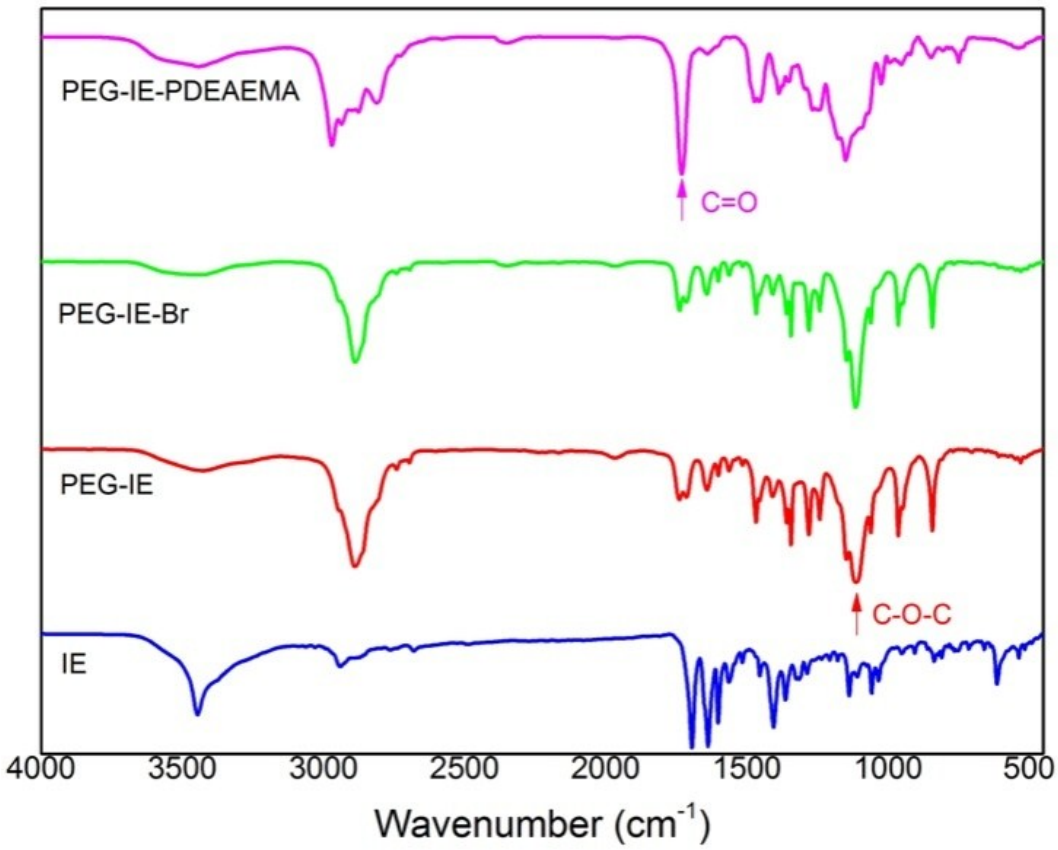

Figure S6. FTIR spectra of the synthesized polymers. The appearance of peak at around 2900 cm^{-1} (ν_{C-H} of ether) and the 1120 cm^{-1} (ν_{C-O-C}) in the spectrum of PEG-IE demonstrates the successful addition of PEG segment to the chromophore, while the strong peak at 1740 cm^{-1} ($\nu_{C=O}$) in the spectrum of PEG-IE-PDEAEMA shows the successful growth of PDEAEMA chain on the other side of the chromophore.

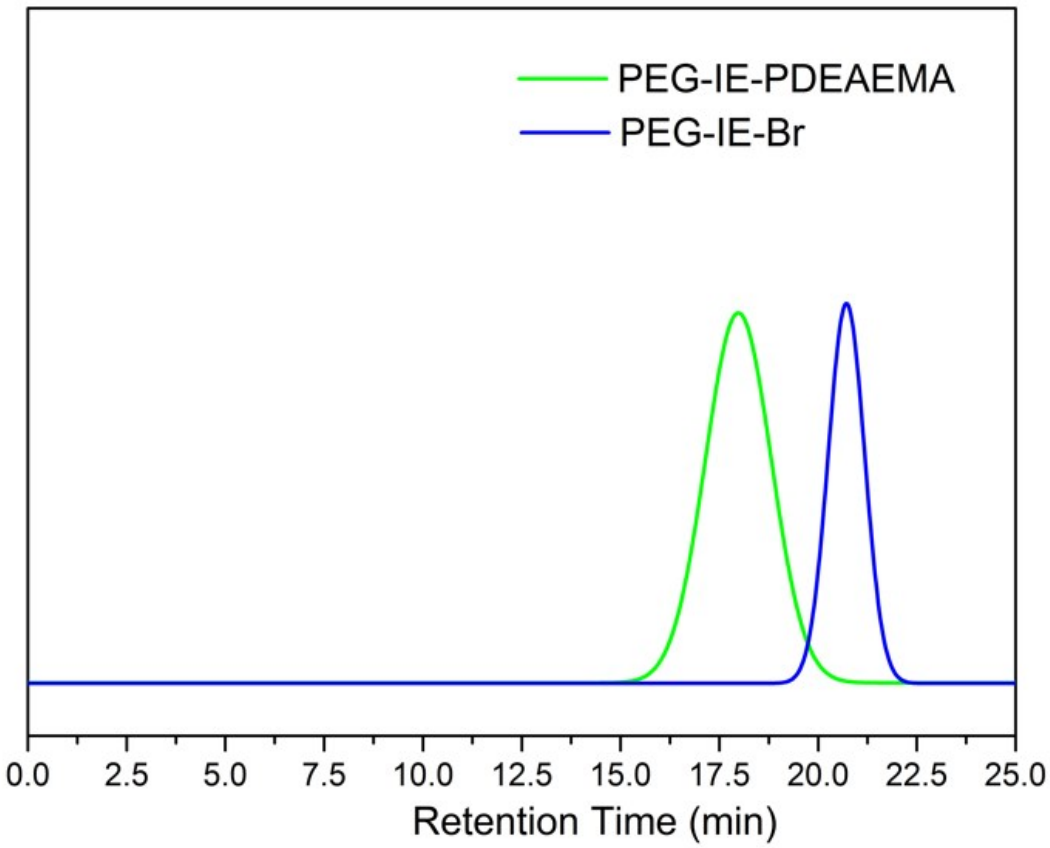

Figure S7. GPC measurements of the macro-initiator and the final polymer with THF as eluent.

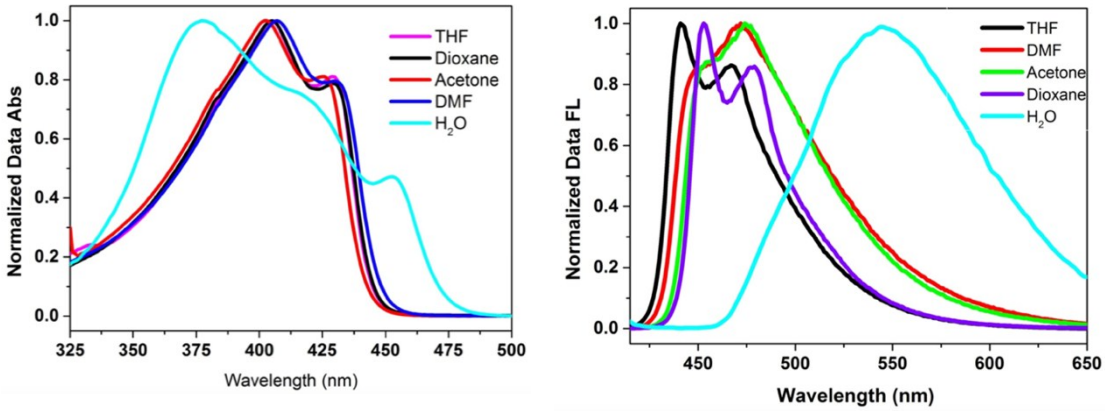

Figure S8. UV Absorption spectra of the chromophore IE in various solvents (left) and fluorescence emission spectra of the chromophore IE in various solvents (right, λ_{ex}=405 nm).

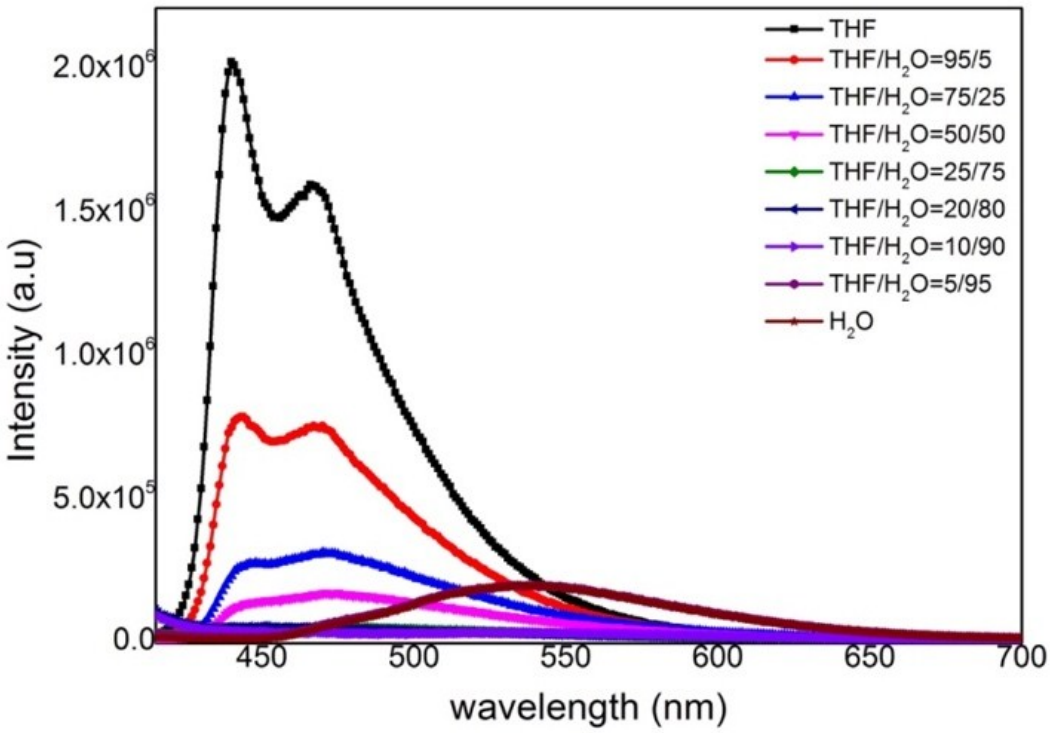

Figure S9. Fluorescence emission spectra of the chromophore IE in THF with changing water content (λ_{ex}=405 nm).

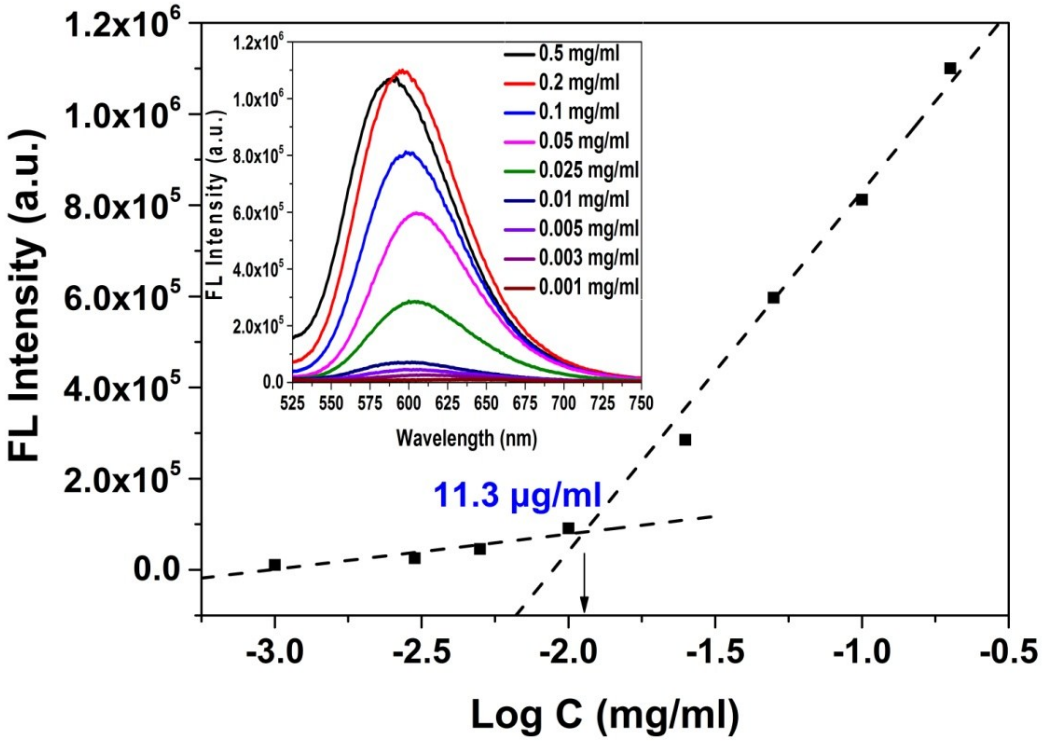

Figure S10. Critical aggregation concentration (CAC) of the PEG-IE-PDEAEMA (determined by the fluorescence intensity of Nile red versus the concentration of the polymer). Nile red was used to evaluate the aggregation of PEG-IE-PDEAEMA. As shown in Fig. S10, the CAC value of PEG-IE-PDEAEMA assembly is 11.3 μg/mL, indicating the high stability of PEG-IE-PDEAEMA nanoparticles.

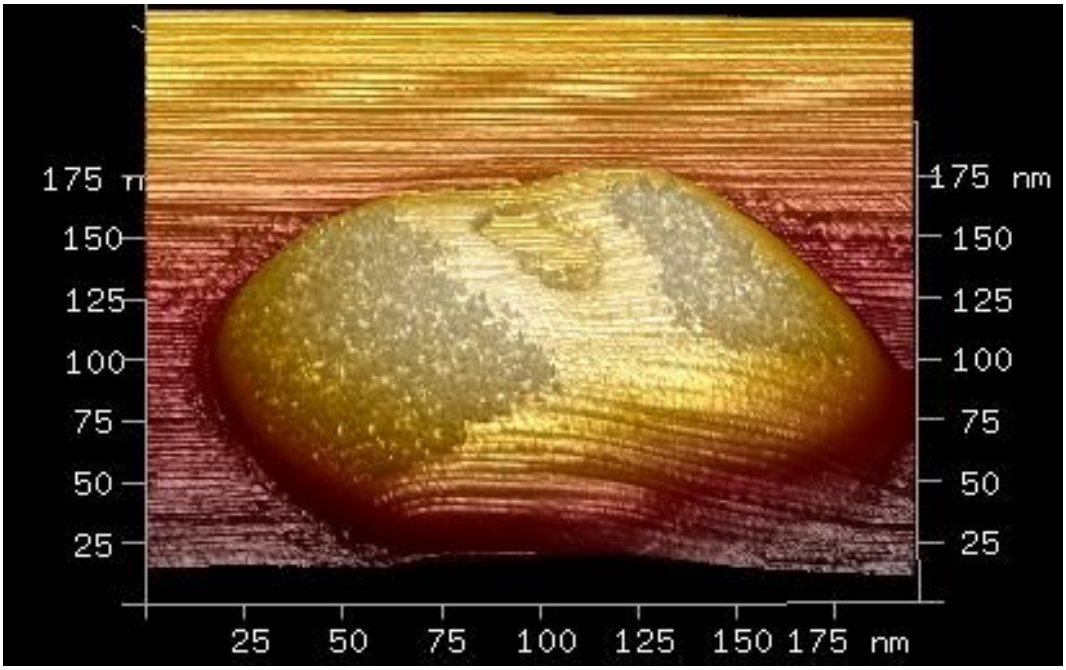

Figure S11. Characteristic AFM image of a single vesicle (before CO_2 purging).

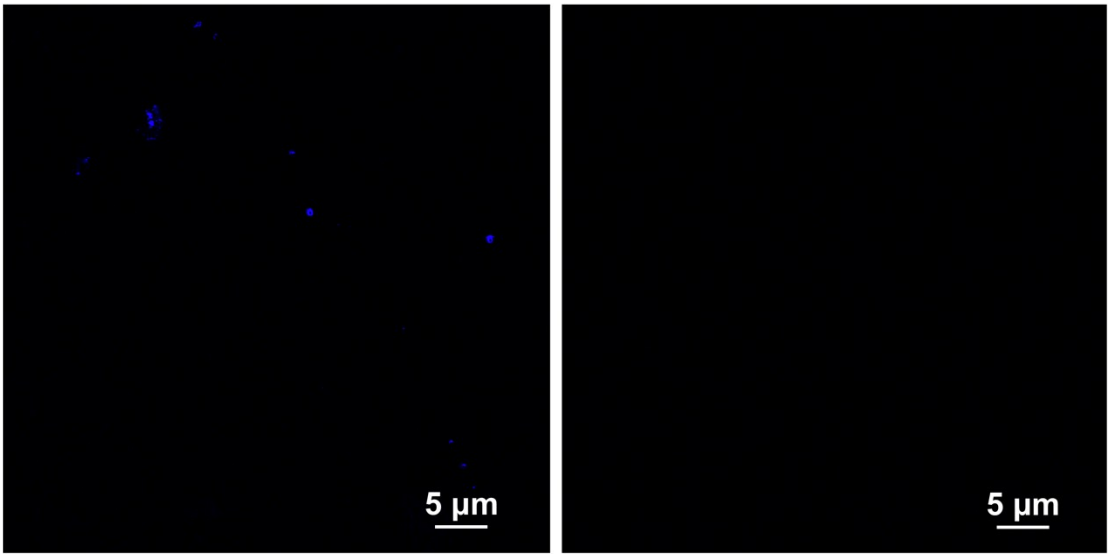

Figure S12. Confocal microscope images of the vesicles before (left) and after CO_2 purging (right). Although the fluorescence spectra (Figure 6) suggest that the fluorescent behavior was indeed weakened instead of disappeared, the fluorescent vesicles under confocal microscope could be hardly recorded within the required time for imaging, so the blue circle of the fluorescent vesicle didn't appear in the confocal image after CO_2 purging.