Supplementary Information

Polydiacetylene Liposomes with Phenylboronic Acid Tags: A Fluorescence Turn-On Sensor for Sialic Acid Detection and Cell Surface Glycan Imaging

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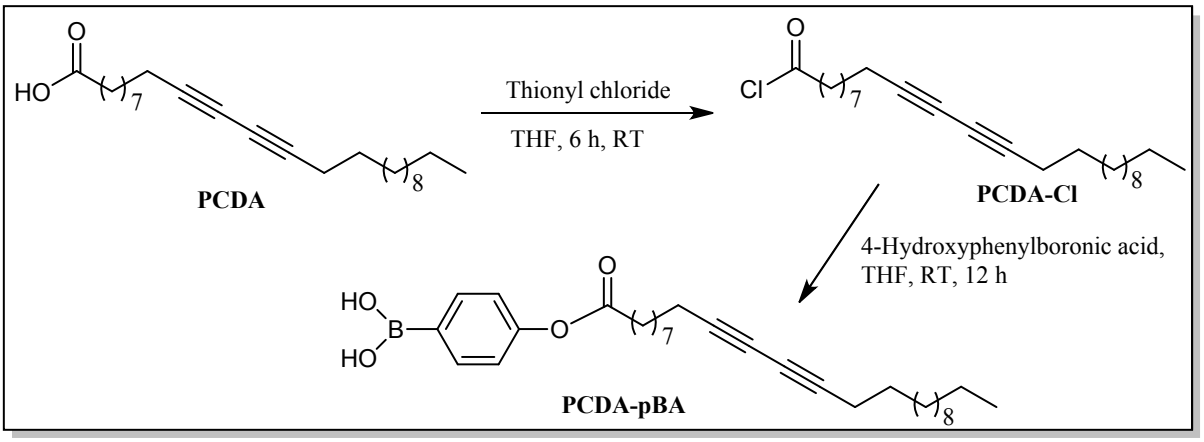
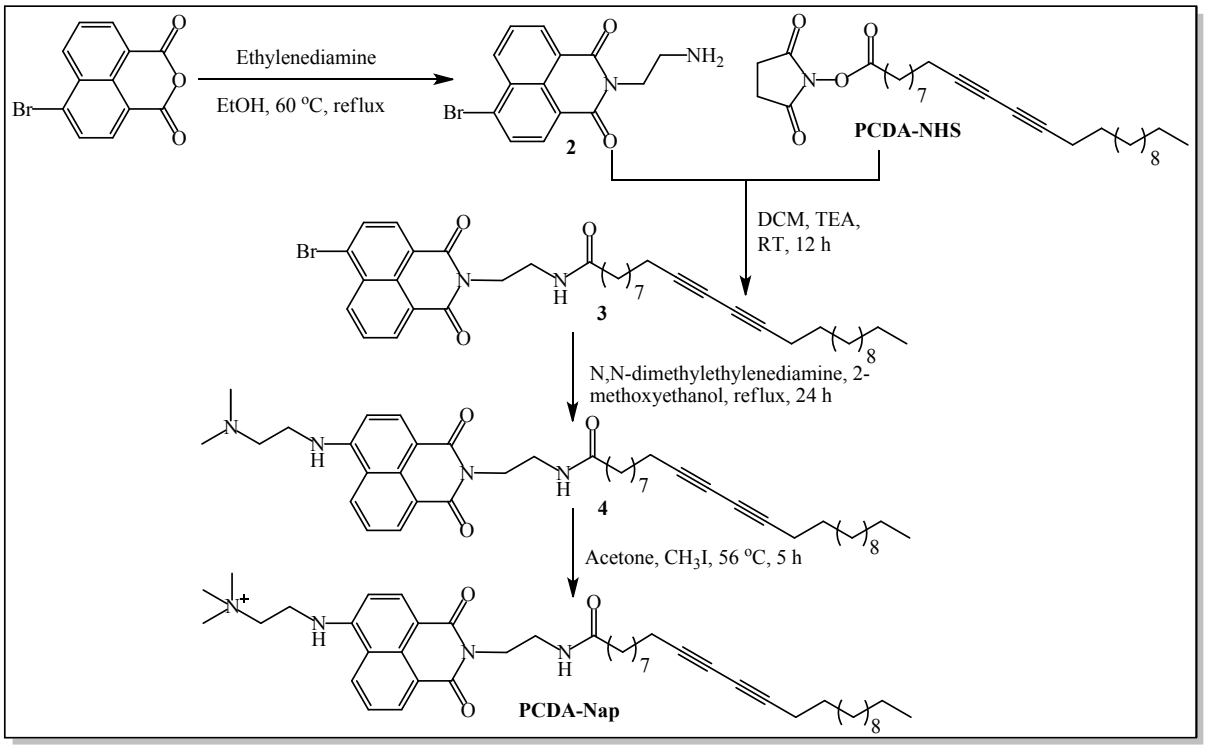
Characterization and general methods. ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR spectra were recorded using a Bruker Avance DMX 500 MHz/125 MHz spectrometer. Peaks were based on a tetramethylsilane (TMS) internal standard. Electrospray ionization mass spectroscopy (ESI-MS) data were obtained using a Thermo Scientific LCQ FLEET mass spectrometer equipped with an electrospray ion source and controlled by Xcalibur software (Thermo Fisher Scientific, Waltham, MA, USA). Steady-state fluorescence spectra and decay curves were obtained using an Hamamatsu C11367-11 fluorescence spectrometer equipped with a 450 W Xe lamp and a time-correlated single photo counting card.



Scheme S1. The synthesis route of PCDA-pBA.

Synthesis of PCDA-pBA: To a sol ution of PCDA (200.0 mg, 0.53 mmol) in anhydrous tetrahydrofuran (THF, 10 mL) was dropwise added thionyl chloride (0.64 g, 5.3 mmol) under argon atmosphere. Afterwards, one drop of triethylamine was added and the resulting mixture was stirred at room temperature for 6 h. The solvent and excess thionyl chloride were thoroughly removed under reduced pressure. The residue was dissolved in 10 mL of anhydrous THF. This solution was then slowly added to a mixture of 4-hydrophenylboronic acid (73.7 mg, 0.53 mmol) and triethylamine (80.0 mg, 0.79 mmol) in 20 mL of THF under argon atmosphere. The resulting solution was stirred at room temperature overnight. After the reaction finished, the solvent was removed under vacuum. The crude product was then purified by column chromatography (silica, methylene chloride: methanol, 10:1, *v/v*)

to obtain the desired compound PCDA-pBA as a white solid (170.0 mg, 64.4%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.28 (d, *J* = 8.4 Hz, 2H), 7.28 (d, *J* = 8.5 Hz, 2H), 2.64 (t, *J* = 7.5 Hz, 2H), 2.27–2.32 (m, 4H), 1.80–1.86 (m, 2H), 1.59–1.54 (m, 4H), 1.30–1.38 (m, 26H), 0.93 (t, *J* = 7.0 Hz 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 171.94, 154.59, 137.14, 121.32, 77.65, 77.43, 65.41, 65.28, 34.49, 31.94, 29.67, 29.65, 29.63, 29.50, 29.36, 29.12, 29.08, 28.93, 28.89, 28.78, 28.39, 28.33, 24.89, 22.71, 19.24, 14.13. ESI-MS m/z: calculated for $C_{31}H_{47}BO_4$, 494.36, found $[M+Na]^+$, 517.34.



Scheme S2. The synthesis route of PCDA-Nap.

***Synthesis of N-(2-Aminoethyl)-4-bromo-1,8-naphthalimide (2)*:** To a solution of 4-bromo-1,8-naphthalic anhydride (1.1 g, 4.0 mmol) in absolute ethanol (85 mL) was slowly added ethylenediamine (270 mg, 4.5 mmol) at 60 ˚C. The resulting mixture was then stirred and refluxed before the reaction solution was getting turbid (~20 min). The resulting precipitate was filtered and the filtrate was concentrated under vacuum. The residue was purified by column chromatography (silica, methylene chloride: methanol, 10:1, *v*/*v*) to obtain compound **2** as a white solid (770.5 mg, 60.3 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.48 (d, *J* = 7.3 Hz, 1H), 8.43 (d, *J* = 8.5 Hz, 1H), 8.24 (d, *J* = 7.8 Hz, 1H),

8.13 (d, J = 7.8 Hz, 1H), 7.93 (t, J = 7.5 Hz, 1H), 4.06 (t, J = 6.8 Hz, 2H), 2.84 (t, J = 6.8 Hz, 2H).

***Synthesis of compound 3*:** PCDA-NHS (260 mg, 0.55 mmol) and compound **2** (180 mg, 0.56 mmol) were dissolved in 10 mL anhydrous dichloromethane (DCM). Then, triethylamine (66.4 mg, 0.58 mmol) was added into the solution. The reaction was carried out in the dark at room temperature overnight. The solvent was evaporated in *vacuo* and the residue was purified by column chromatography (silica, methylene chloride: methanol, 50:1, *v*/*v*) to obtain compound **3** as a white solid (110 mg, 29.7 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.70 (dd, J = 7.3, 1.1 Hz, 1H), 8.64 (dd, J = 8.5, 1.1 Hz, 1H), 8.46 (d, J = 7.9 Hz, 1H), 8.10 (d, J = 7.9 Hz, 1H), 7.91 (dd, J = 8.5, 7.3 Hz, 1H), 4.47–4.39 (m, 2H), 3.72 (q, J = 5.4 Hz, 2H), 2.31–2.23 (m, 4H), 2.14–2.09 (m, 2H), 1.52 (m, 6H), 1.44–1.15 (m, 26H), 0.92 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 173.49, 164.26, 133.66, 132.35, 131.52, 131.23, 130.75, 129.11, 128.19, 122.81, 121.93, 77.66, 77.48, 65.37, 65.28, 39.66, 39.18, 36.74, 31.94, 29.67, 29.65, 29.63, 29.50, 29.36, 29.15, 29.13, 28.89, 28.83, 28.77, 28.39, 28.31, 25.53, 22.71, 19.24, 19.21, 14.13.

***Synthesis of compound 4*:** Compound **3** (200 mg, 0.3 mmol) and *N,N*-dimethylethylenediamine (523.4 mg, 5.9 mmol) were dissolved in 10 mL of 2-methoxyethanol. The resulting mixture was stirred and refluxed for 24 h. After cooling to room temperature, the reaction solution was diluted with 30 mL of chloroform and poured into 50 mL of water. The organic layer was separated and the aqueous layer was extracted with chloroform (3 × 10 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by column chromatography with methylene chloride/methanol (25:1, *v*/*v*) as the eluent to afford compound **4** as a yellow solid (130 mg, 64.1 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.63 (d, J = 7.3 Hz, 1H), 8.50 (d, J = 8.4 Hz, 1H), 8.24 (d, J = 8.3 Hz, 1H), 7.68 (t, J = 7.5 Hz, 1H), 6.72 (d, J = 8.5 Hz, 1H), 6.45 (m, 2H), 4.50–4.37 (m, 2H), 3.73–3.63 (m, 2H), 3.53–3.41 (m, 2H), 2.82 (t, J = 5.8 Hz, 2H), 2.41 (s, 6H), 2.26 (m, 4H), 2.15 (t, J = 7.6 Hz, 2H), 1.59–1.51 (m, 4H), 1.50–1.44 (m, 2H), 1.44–1.20 (m, 26H), 0.92 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 173.48, 165.38, 164.90, 150.05, 135.00, 131.53, 129.99, 127.00, 124.76, 122.62, 120.47, 109.60, 104.48, 77.64, 77.55, 65.32, 56.89, 44.98, 40.13, 40.08, 38.99, 36.83,

31.93, 29.66, 29.64, 29.63, 29.50, 29.36, 29.16, 29.12, 28.89, 28.83, 28.40, 28.35, 25.60, 22.70, 19.24, 19.21, 14.13.

***Synthesis of PCDA-Nap*:** To a solution of compound **4** (130 mg, 0.19 mmol) in 10 mL of acetone was added methyl iodide (114 mg, 0.8 mmol). The reaction solution was stirred and refluxed at 56 ˚C for 5 h. After cooling to room temperature, the resulting yellow precipitate was filtered and wash with cold acetone to obtain the desired PCDA-Nap as a yellow solid (129 mg, 97.3 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.65 (d, J = 8.1 Hz, 1H), 8.48 (d, J = 6.8 Hz, 1H), 8.34 (d, J = 8.4 Hz, 1H), 7.85 (t, J = 6.0 Hz, 1H), 6.97 (d, J = 8.6 Hz, 1H), 4.13 (t, J = 6.1 Hz, 2H), 3.91–3.95 (m, 2H), 3.71 (t, J = 6.5 Hz, 2H), 3.44–3.36 (m, 2H), 3.25 (s, 9H), 2.29 (t, J = 6.9 Hz, 4H), 1.96 (t, J = 7.4 Hz, 2H), 1.29–1.47 (m, 6H), 1.35–1.16 (m, 26H), 0.87 (t, J = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 172.67, 164.36, 163.58, 149.82, 134.25, 131.17, 129.85, 128.57, 125.26, 122.77, 120.89, 109.88, 104.84, 78.49, 78.45, 65.84, 63.20, 53.31, 40.48, 37.43, 37.00, 35.94, 31.77, 29.48, 29.42, 29.34, 29.18, 29.13, 29.03, 28.87, 28.76, 28.70, 28.65, 28.23, 28.18, 25.56, 22.57, 18.80, 18.77, 14.42. ESI-MS m/z: calculated for $C_{44}H_{65}N_4O_3^+$, 697.51, found $[M]^+$, 697.41.

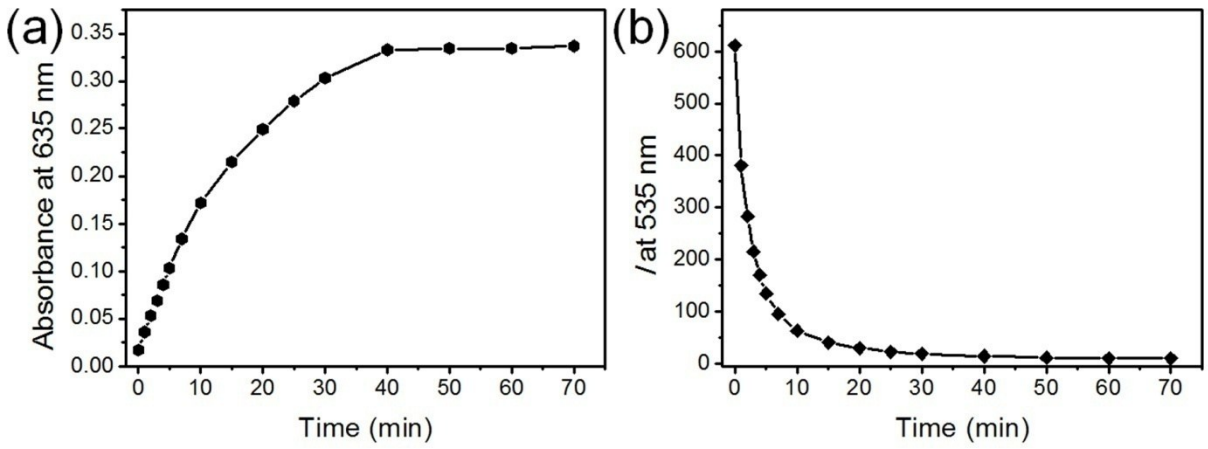


Fig. S1. (a) Absorbance at 635 nm and (b) fluorescence intensity at 535 nm of the composite liposome solution as a function of UV irradiation time from 0 to 70 min.

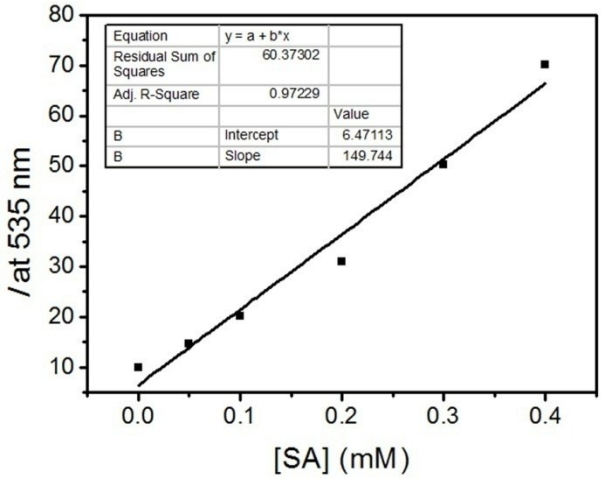


Fig. S2. Linear relationship between fluorescence intensity of the PBDA liposomes and SA concentration.

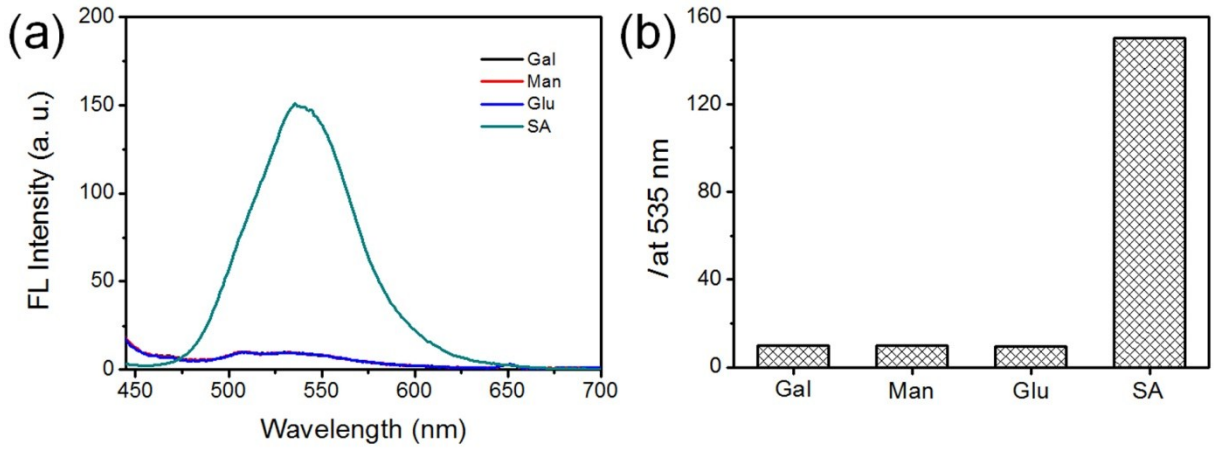


Fig. S3. (a) Fluorescence emission spectra and (b) related emission intensity at 535 of the PBDA liposome solution (100 μM) after adding galactose, mannose, glucose and SA (1 mM) in PBS (10 mM, pH 7.4), respectively.

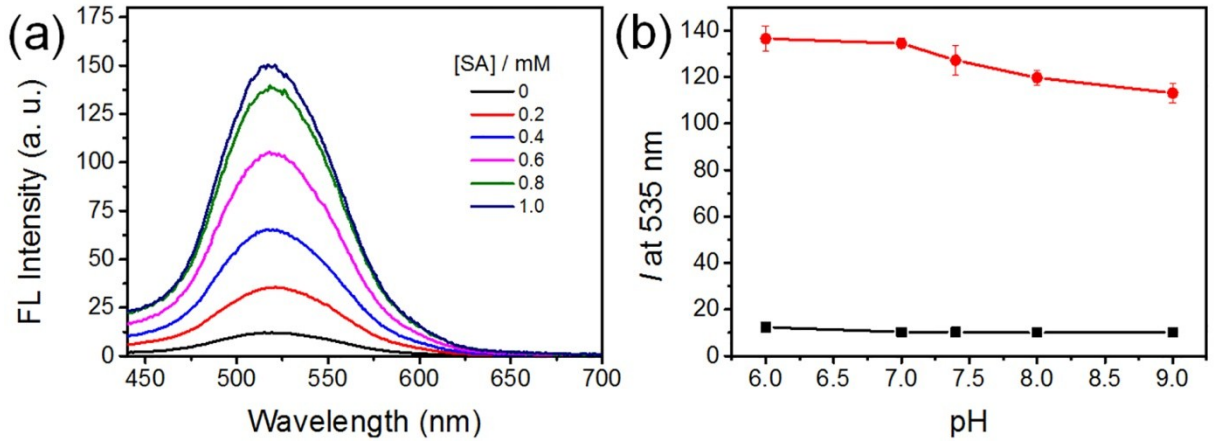


Fig. S4. (a) Fluorescence intensity at 535 nm of the PBDA liposomes (100 μM) in PBS (10 mM) with different pH (6.0, 7.0, 7.4, 8.0, 9.0) before (black spot) and after (red spot) adding free SA (0.7 mM), and (b) fluorescence emission spectra of the PBDA liposomes (100 μM) after the addition of different concentrations of SA in PBS (10 mM, pH 7.4) containing 10 % FBS.

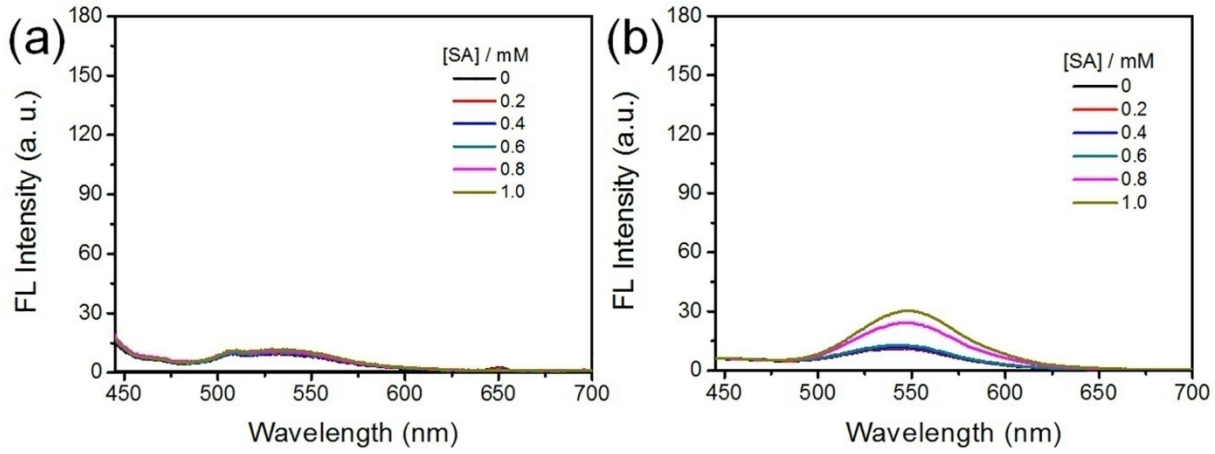


Fig. S5. (a) Fluorescence emission spectra of the PDA liposomes (100 μM) prepared from PCDA-pBA and PCDA-Nap (molar ratio of 9:1) after adding different concentrations of free SA in PBS (10 mM, pH 7.4), and (b) fluorescence emission spectra of the PDA liposomes (100 μM) prepared from PCDA-EA and PCDA-Nap (molar ratio of 9:1) after adding different concentrations of free SA in PBS (10 mM, pH 7.4).

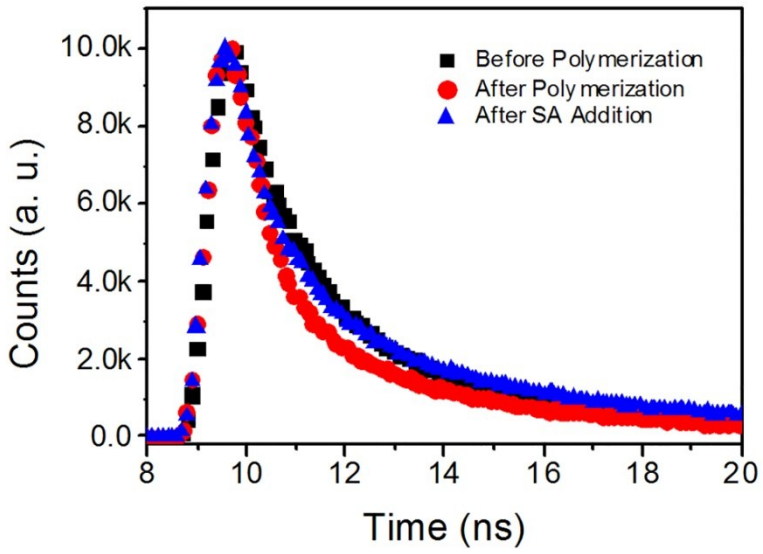


Fig. S6. Time-resolved fluorescence decays of the PBDA liposomes before polymerization, after polymerization and then after SA addition.

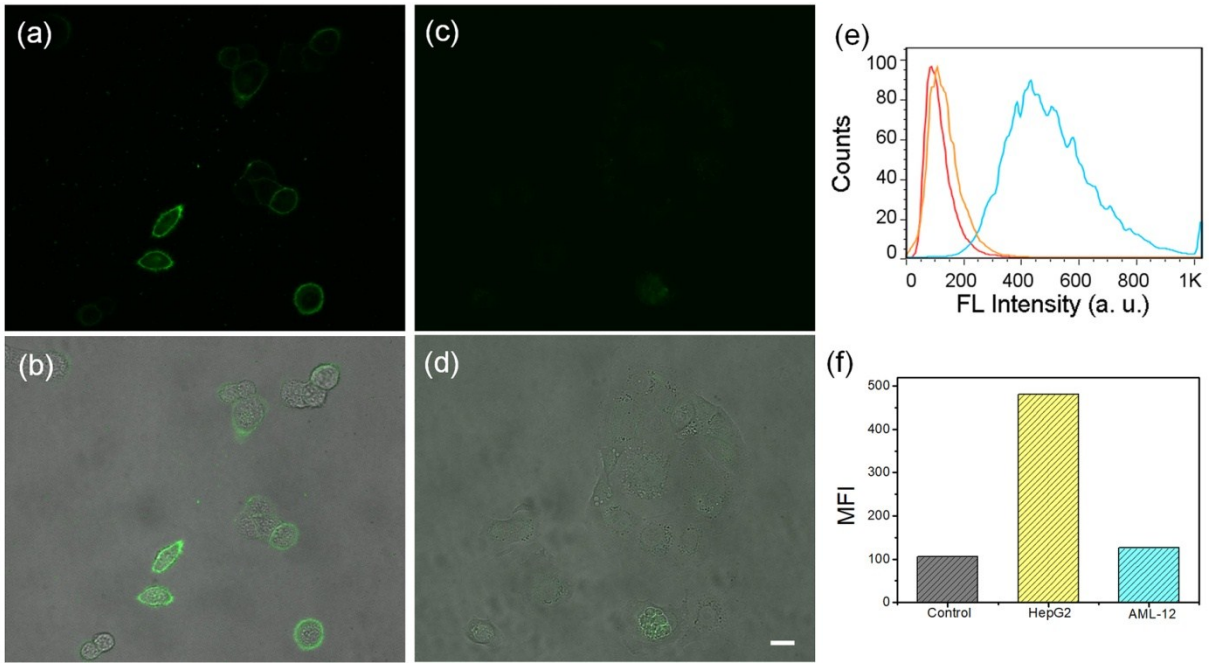


Fig. S7. Confocal fluorescence images of HepG2 cells (a) and AML-12 cells (b) after being treated with the PBDA liposomes, and corresponding overlapped fluorescence and bright-field images of HepG2 cells (b) and AML-12 cells (d) after being treated with the PBDA liposomes, scale bar: 20 μm. (e) and (f) Flow cytometry profile (e) and MFI (f) of HepG2 and AML-12 cells incubated with the PBDA liposomes, red line: control, orange line: AML-12 cells and blue line: HepG2 cells.

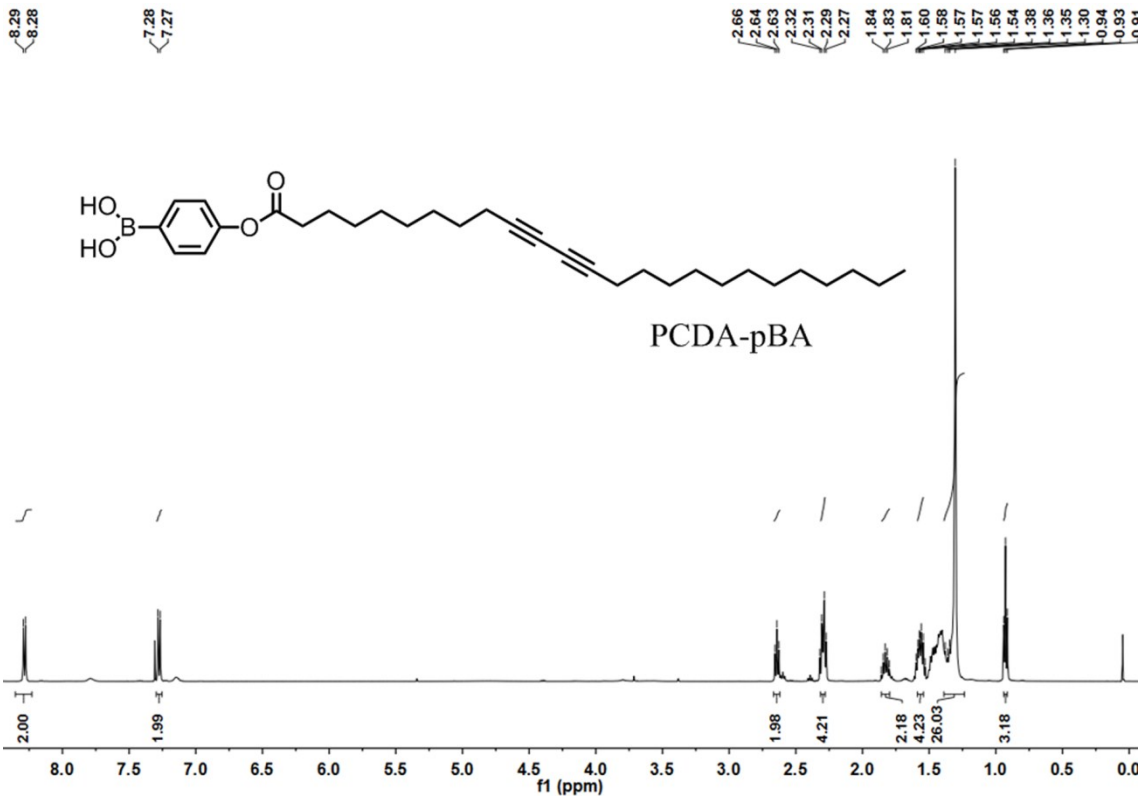


Fig. S8. ^{1}H NMR spectrum of PCDA-pBA in $CDCl_3$.

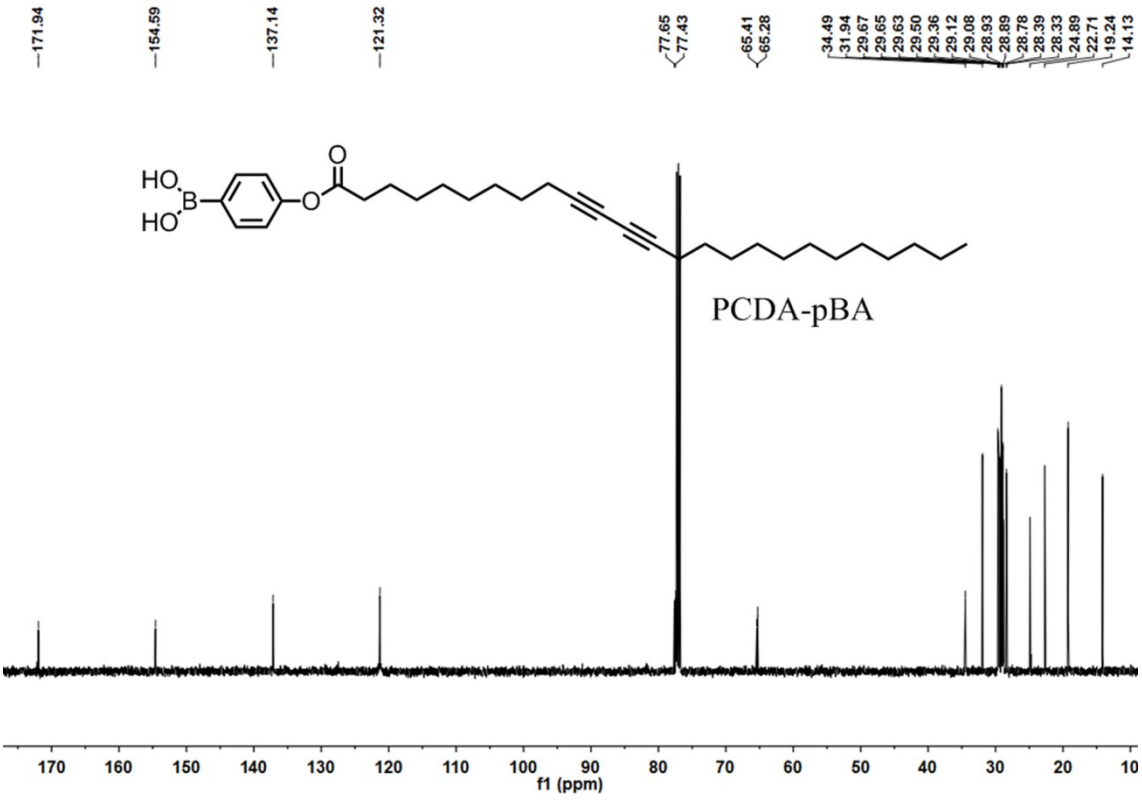


Fig. S9. ^{13}C NMR spectrum of PCDA-pBA in $CDCl_3$.

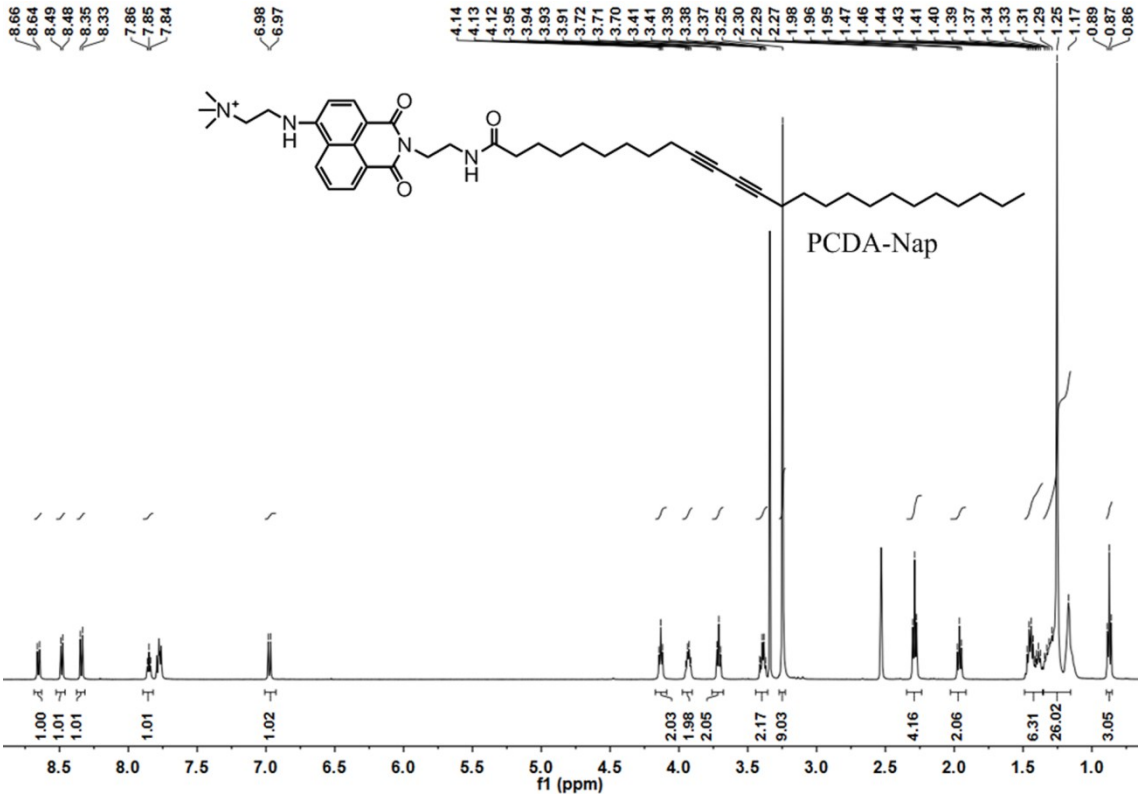


Fig. S10. ^{1}H NMR spectrum of PCDA-Nap in DMSO-d_6.

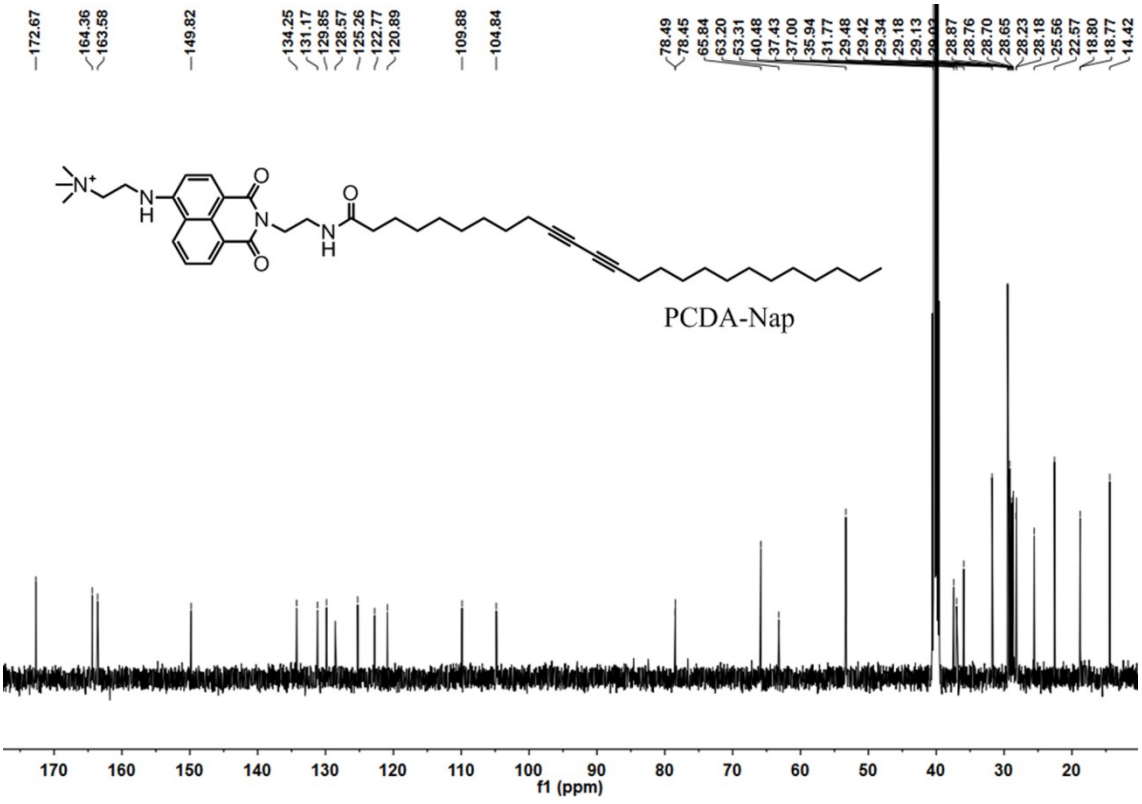


Fig. S11. ^{13}C NMR spectrum of PCDA-Nap in DMSO-d_6.