Supporting Information

Engineering Living Cells with Cucurbit[7]uril-based Supramolecular Polymer Chemistry: From Cell Surface Engineering to Manipulation of Subcellular Organelles

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I. Chemistry Section

II. Biological Section

III. References.

I. Chemistry Section

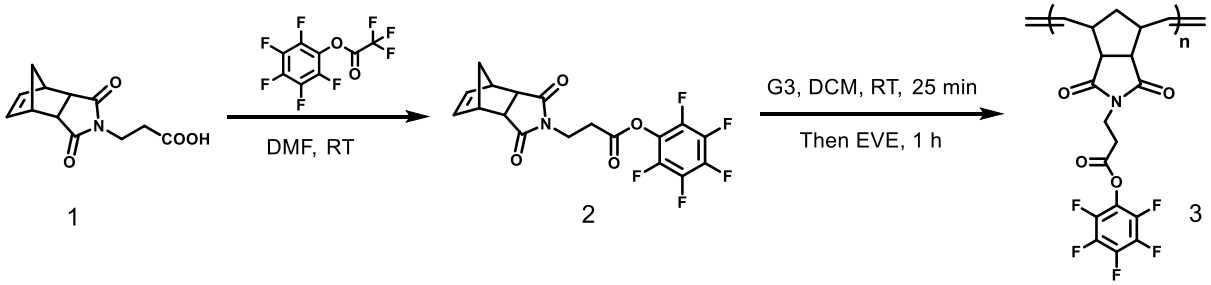
1. Materials and methods

Chemicals: DSPE-PEG_{2000}-NH_2, NH_2-PEG_{1000}-BOC, and NH_2-PEG_{2000}-BOC, NH_2-PEG_{1000}-N_3 were purchased from Tanshtech Co., Ltd. (China). Cy5-NH_2 was purchased from Xi'an Ruixi Biological Technology Co., Ltd. (China). Cucurbit[7]uril (CB[7]) [1], monohydroxyl cucurbit[7]uril (CB[7]OH) [2] and NTI fluorescence [3] were synthesized in our lab according to literatures. All other chemical reagents were purchased from commercial suppliers (Sigma-Aldrich, Energy Chemical). All reagents and solvents were used as supplied without further purification.

Instruments: Nuclear magnetic resonance (NMR) spectra were recorded on a JNM-ECZ500R (500 MHz) or Bruker Avance 400 MHz NMR spectrometer. Chemical shifts are reported in delta (δ) units, expressed in parts per million (ppm) downfield from tetramethylsilane using the residual protio-solvent as an internal standard ($CDCl_3$, 1H: 7.26 ppm; D_2O, 1H: 4.79 ppm). High resolution mass spectra (HRMS) was recorded on an ESI focus spectrometer (Agilent1290 / Bruker maXis impact). Gel permeation chromatography (GPC) analyses were performed on a Waters ACQUITY UPLC sample manager and a Waters ACQUITY differential refractive index detector.

2. Synthetic procedures and characterization of polymers

2.1 The synthesis of precursor polymer NB-PF_5



Scheme S1: Synthesis route of precursor polymer NB-PF_5 with the degree of polymerization of 50 (NB_{50}-PF_5) and 30 (NB_{30}-PF_5).

Synthesis of monomer 1: Monomer 1 was prepared according to the literature [4]. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.29 (s, 2H), 3.78 (t, *J* = 7.3 Hz, 2H), 3.27 (s, 2H), 2.72 – 2.63 (m, 4H), 1.51 (d, *J* = 9.9 Hz, 1H), 1.24 (d, *J* = 9.5 Hz, 1H).

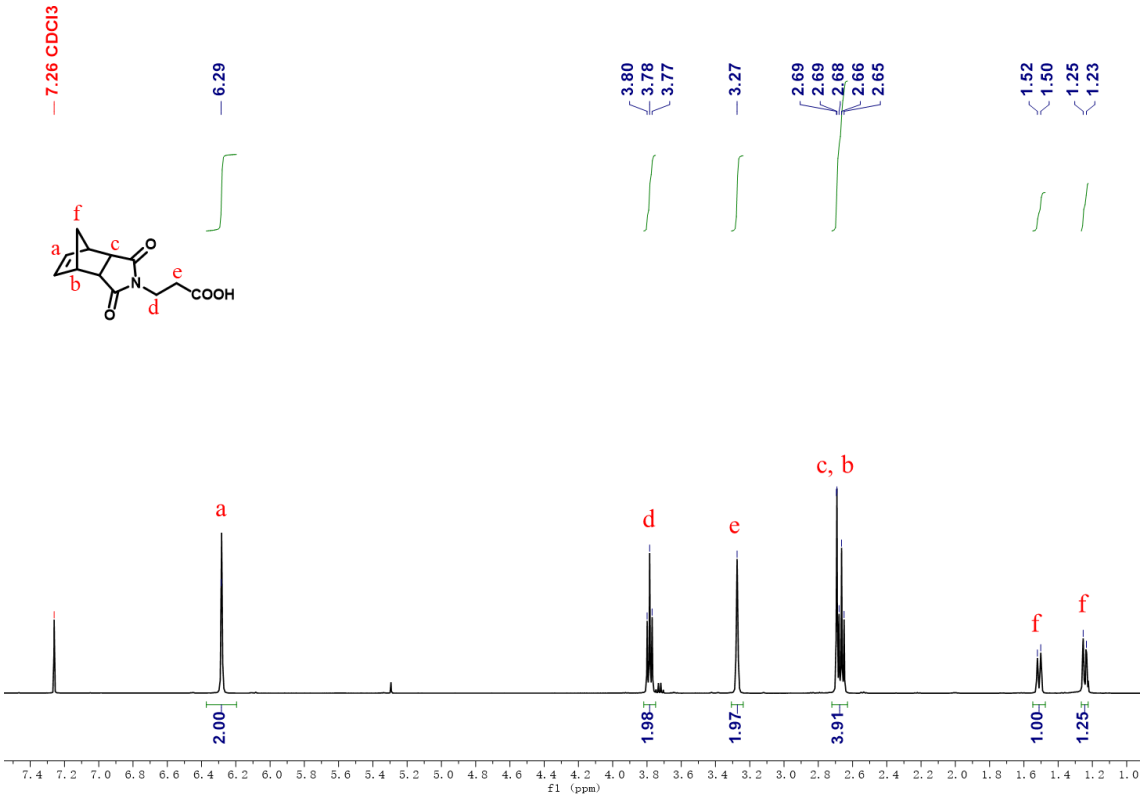


Figure S1. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of monomer 1.

Synthesis of NB-PF$_5$: The mixture of monomer 1 (1.75 g, 7.4 mmol), pentafluorophenyl trifluoroacetate (2.49 g, 1.53 mmol) and *N,N*-diisopropylethylamine (DIPEA, 2.87 g, 22.2 mmol) were dissolved into anhydrous dimethylformamide (DMF, 20 mL) and stirred overnight at room temperature. Then the reaction mixture was dissolved in ethyl acetate (EA, 100 mL) and washed by water (2 x 100 mL). The organic layer was dried over with anhydrous Na_2SO_4, then the solvent was removed under reduced pressure, the crude product was purified by a silica gel column with PE/EA (3/1, v/v) as the eluent to afford **NB-PF$_5$** (2.72 g, 91.2% yield) as a white powder. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.30 (s, 2H), 3.92 (t, *J* = 7.1 Hz, 2H), 3.31 (s, 2H), 3.03 (t, *J* = 7.1 Hz, 2H), 2.73 (s, 2H), 1.54 (d, *J* = 10.0 Hz, 1H), 1.23 (s, 1H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 177.88, 166.77, 137.94, 77.36, 77.30, 77.10, 76.85, 47.95, 45.33, 42.83, 33.98, 31.06. ^{19}F NMR (471 MHz, Chloroform-*d*) δ -152.22 (d, J = 19.0 Hz, 2F), -157.35 (m,1F), -161.90 (m, 2F). MS (ESI: m/z): calcd for $C_{18}H_{12}F_5NO_4$, $[M+Na]^+$: 424.28; found,424.06.

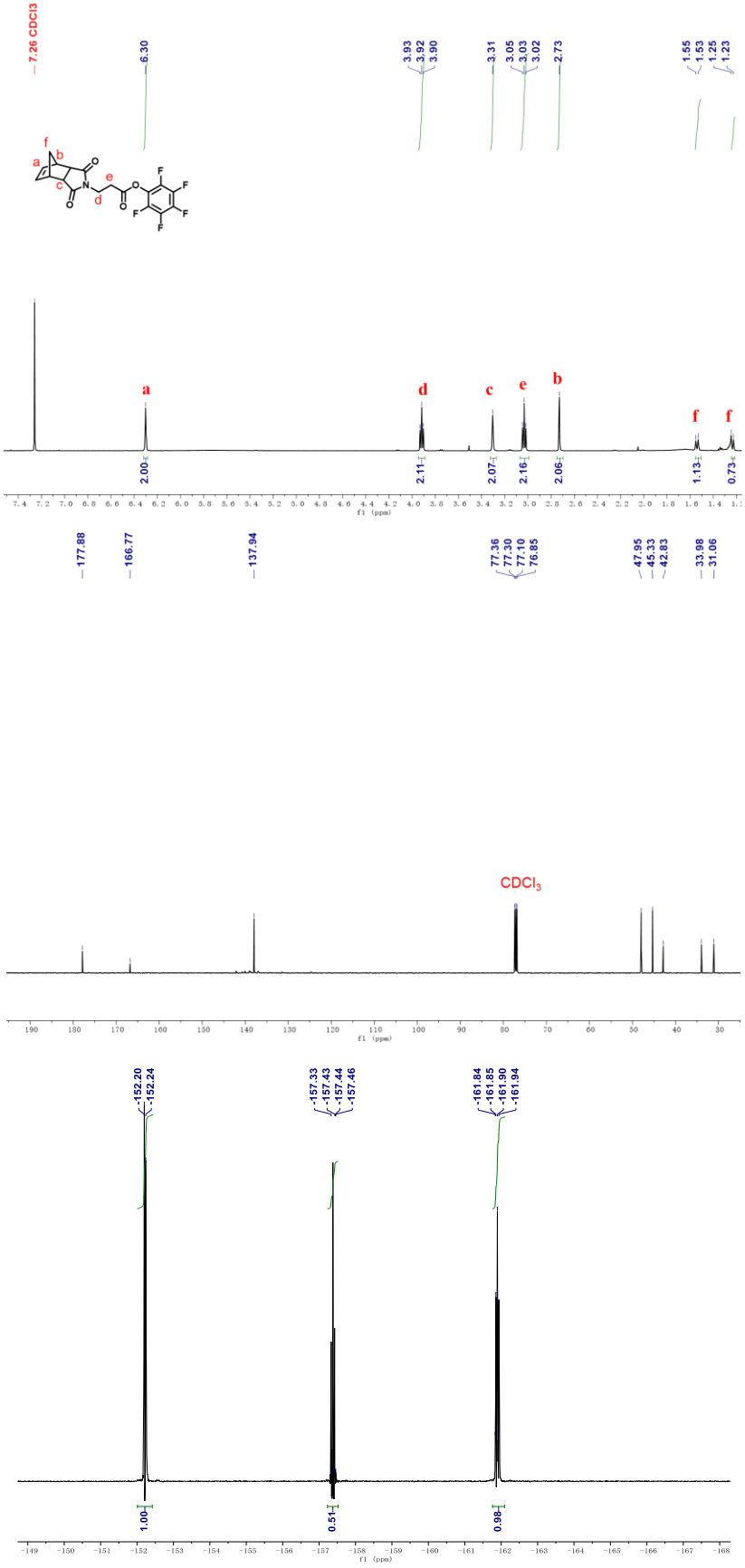


Figure S2. ^{1}H NMR (500 MHz, $CDCl_3$), ^{13}C NMR (126 MHz, $CDCl_3$) and ^{19}F NMR (471 MHz, $CDCl_3$) spectra of NB-PF_5.

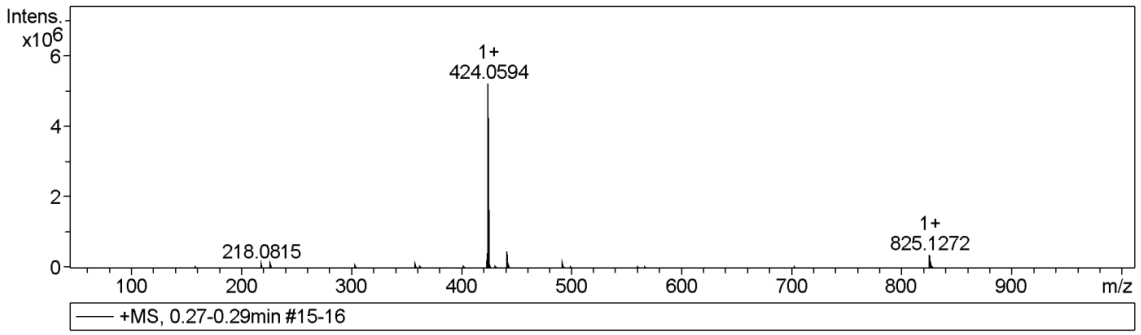


Figure S3. ESI-MS spectrum of NB-PF_5 in CH_3CN.

Synthesis of NB_n-PF_5: According to the literature [4], the third generation Grubbs' catalyst (G3) (8.82 mg, 9.97×10^{-3} mmol) and compound 2 (200 mg, 0.50 mmol) were respectively dissolved in 2 mL DCM. The catalyst G3 solution was added all at once to the compound 2 solution at room temperature while stirring vigorously. After reacting for 15 mins, the reaction was quenched by addition of 1 mL EVE for another 1 h. All the procedures were completed under N_2. After completion, the mixture was concentrated and then precipitated in methanol to obtain polymer NB_{50}-PF_5. Polymer NB_{30}-PF_5 was synthesized using the same method.

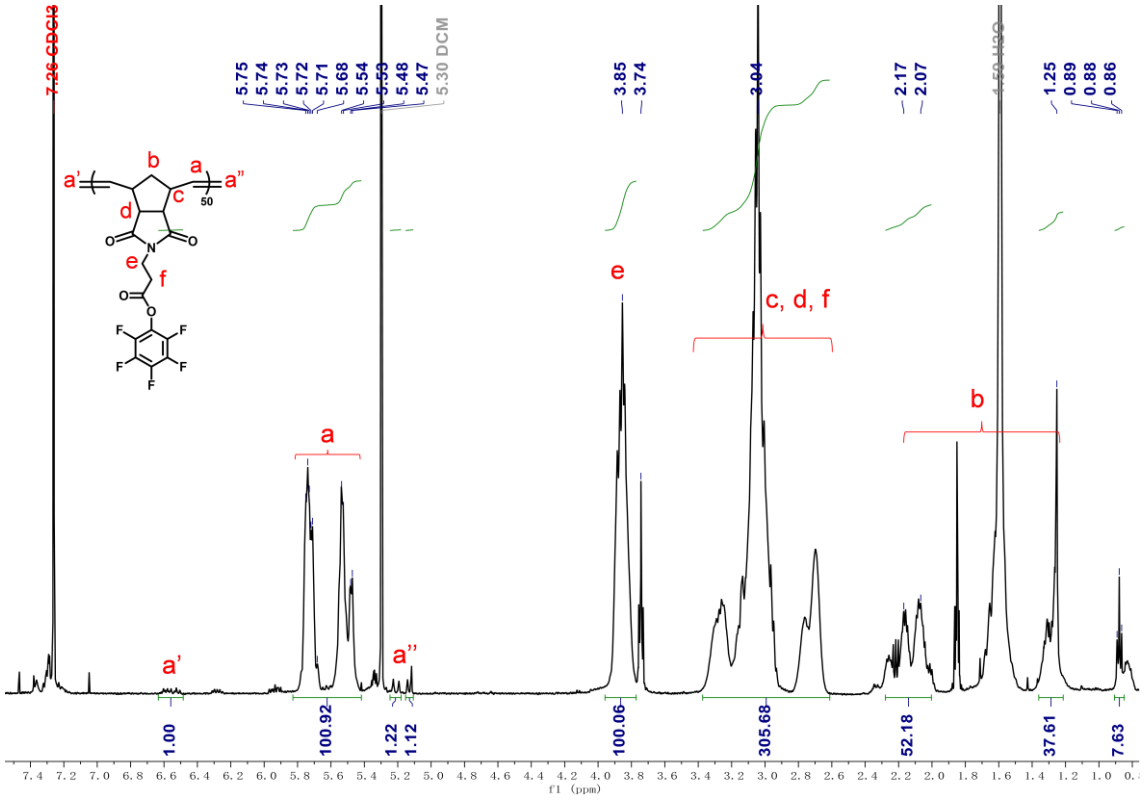


Figure S4. ^1H NMR spectrum (500 MHz, $CDCl_3$) of NB_{50}-PF_5.

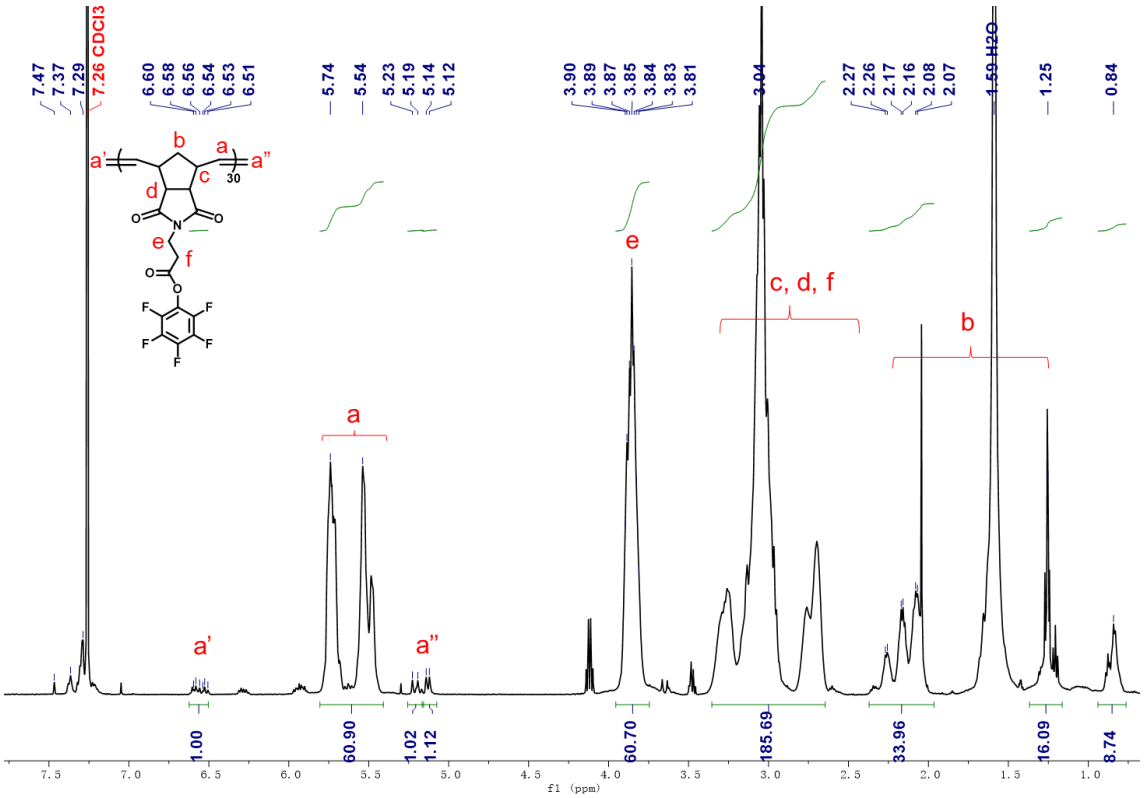


Figure S5. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of NB_{30}-PF_5.

2.2 The synthesis of functional motifs with amine for the post-modification of precursor NBn-PF_5

Preparation of monopropargyloxy CB[7] (CB[7]OP): CB[7]OP was prepared according to the literature [5]. CB[7]-OH (200 mg, 0.17 mmol) was dispersed in 5 mL of anhydrous DMSO using an ultrasonator. The solution was degassed under vacuum and re-filled with nitrogen for three times. NaH (300 mg, 60% in kerosene) was added and reacted at room temperature for 4 h. Then the solution was cooled down to 0 °C, and propargyl bromide (0.5 mL, 5.8 mmol) was added and stirred for 12 h. After that, 100 mL methanol was added into the solution and grey precipitate was isolated through centrifugation. The precipitate was washed with methanol for 3 times and dried under vacuum. Then 15 mL deionized water containing 69 mg p-xylylenediamine dihydrochloride (PXDA) was used to digest the precipitate. The mixture was centrifuged and the obtained supernatant was loaded onto column filled with CHP-20P macroporous resin. The column was eluted with deionized water and the separation process was monitored using ESI-MS. The combined fractions were concentrated using rotary evaporation and precipitated with methanol to obtain pure CB[7]OP in the form of CB[7]OP·PXDA host-guest complex.

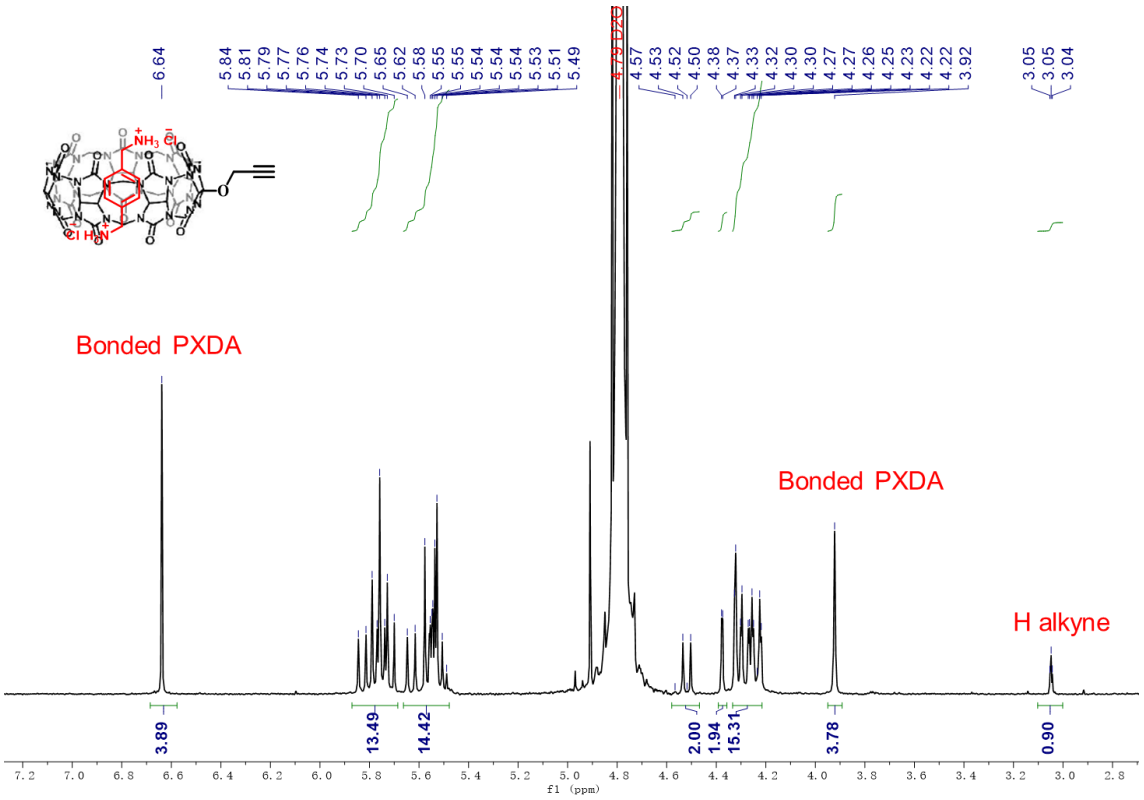


Figure S6. ^{1}H NMR spectrum of CB[7]OP·PXDA in D_2O with excess PXDA

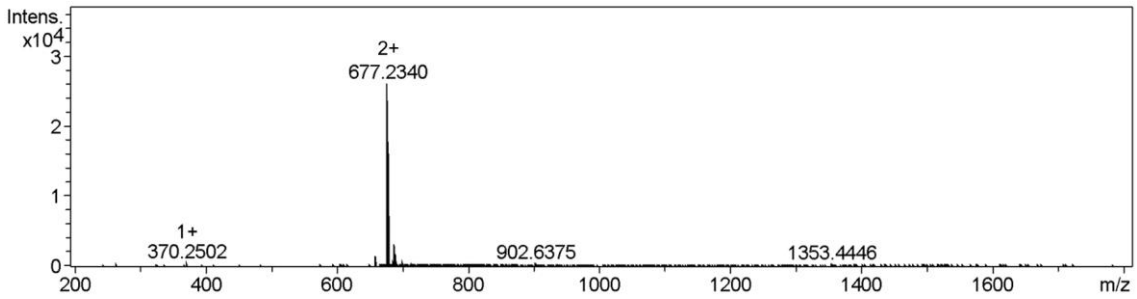


Figure S7. ESI-MS spectrum of CB[7]OP·PXDA in D_2O with excess PXDA.

Synthesis of Ada-PEG$_{1000}$-NH$_2$: Ada-PEG-NH$_2$ was prepared according to the study [6]with a little change. At first, to a solution of 1-adamantanecarbonylchloride (198.69 mg, mmol) in anhydrous DCM (5 mL), the complex solution of BOC-PEG$_{1000}$-NH$_2$ (100 mg, 0.2 mmol) and triethylamine (0.22 g, 2.2 mmol) in DCM (10 mL) was added dropwise at an ice bath. The reaction mixture was slowly warmed to room temperature and stirred for 48 h, then the solvent was removed under vacuum. The obtained product was dissolved in 3 mL deionized water and filtered out unreacted 1-adamantanecarbonylchloride and the Ada-PEG-Boc was obtained by lyophilization, then the structure of Ada-PEG-Boc was characterized by ^{1}H NMR (Figure S). After that, the given product was dissolved in 5 mL DCM, and trifluoroacetic acid (TFA, 1 mL) was added to remove the protection of Boc. The solution was stirred for 6 h at room temperature, and

the solvent was evaporated. The residue was dissolved in deionized water, the pH was adjusted to 10 with dilute NaOH solution, followed by extracted with DCM for 2 times. The organic phases were dried with anhydrous $MgSO_4$, evaporated and dried under vacuum to get the product Ada-PEG_{1000}-NH_2.

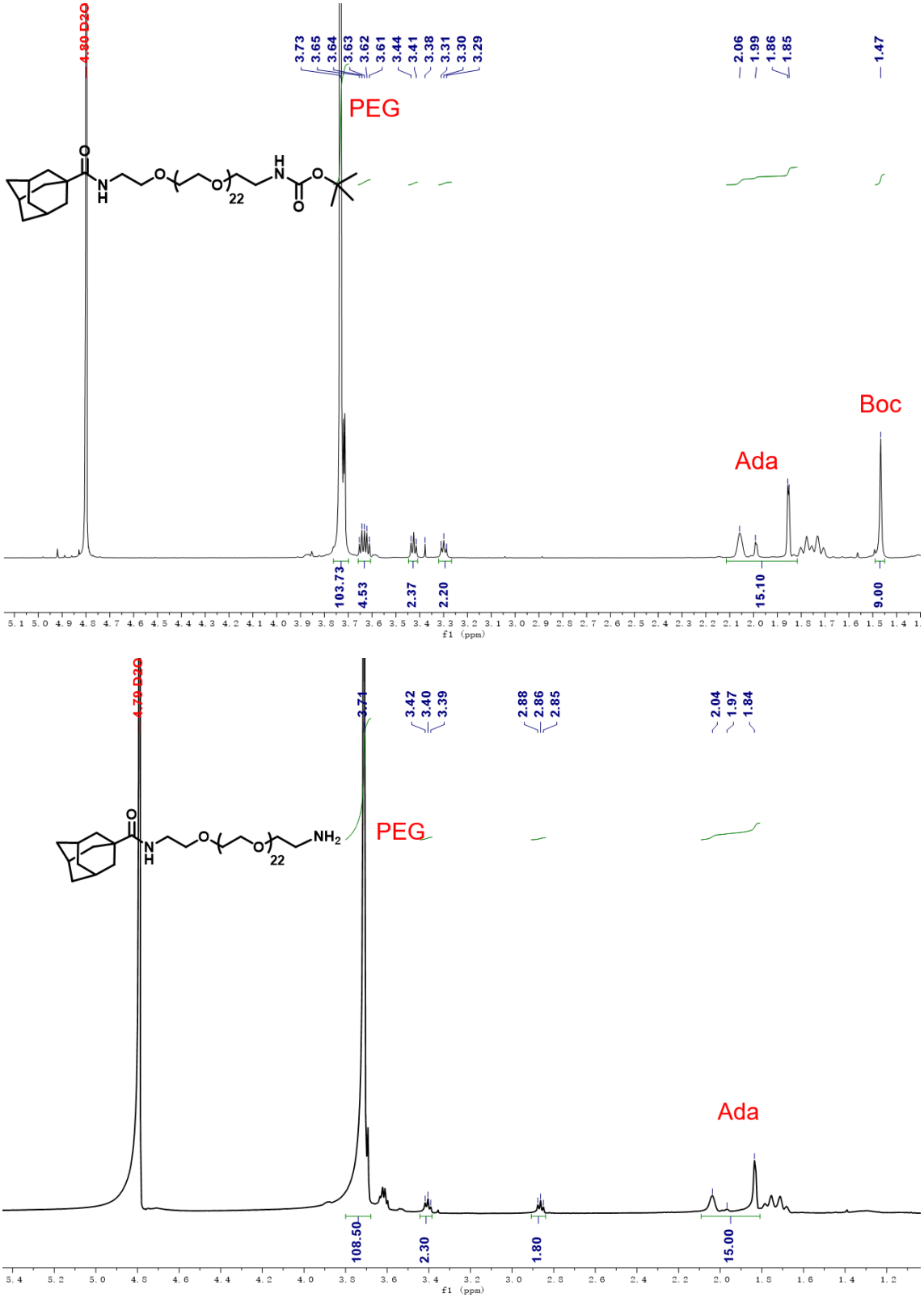
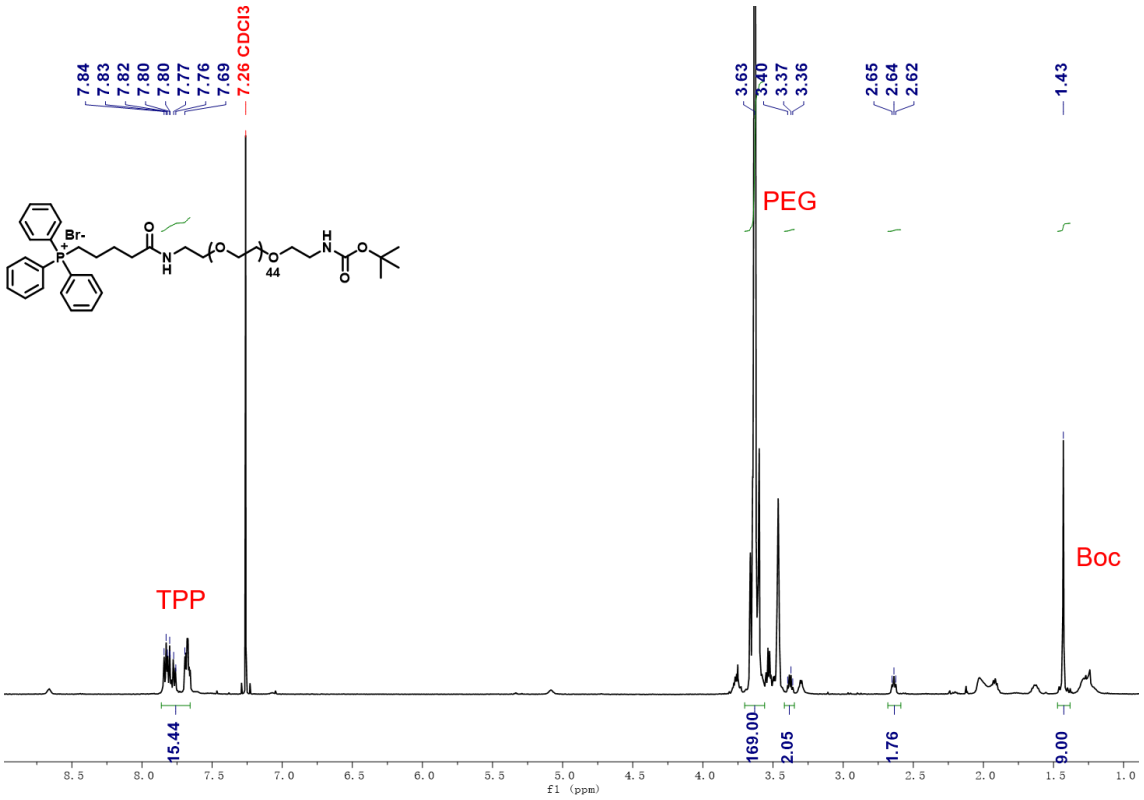


Figure S8. ^{1}H NMR spectrums of Ada-PEG-Boc (500 MHz, D_2O) and Ada-PEG_{1000}-NH_2 (400 MHz, D_2O).

Synthesis of TPP-PEG$_{2000}$-NH$_2$: 4-Carboxbutyltriphenylphosphonium bromide (TPP-C$_4$-COOH) was synthesized according to the study [7]. The structure of TPP-C$_4$ -COOH was confirmed by ^{1}H NMR. TPP-C$_4$ -COOH (221.7 mg, 0.5 mmol), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCl, 95.85 mg, 0.5 mmol) and 1-hydroxybenzotriazole (HOBT, 76.5 mg, 0.5 mmol) were dissolved in anhydrous $CHCl_3$ (6 mL) and stirred at room temperature for 6 h. Then the solution of NH$_2$-PEG$_{2000}$-Boc (200 mg, 0.1 mmol) in 1 mL $CHCl_3$ was added dropwise, and the reaction mixture was stirred at room temperature for 48 h. After the reaction, the solvent was subsequently removed *in vacuo* and the product TPP-PEG$_{2000}$-Boc was purified by dialysis against distilled water for 3 days (MWCO 1000 Da) and collected by lyophilization, then the structure of TPP-PEG$_{2000}$-Boc was characterized by ^{1}H NMR. At last, the deprotection procedure of Boc refers to **Synthesis of Ada-PEG$_{1000}$-NH$_2$** to give the final product TPP-PEG$_{2000}$-NH$_2$.



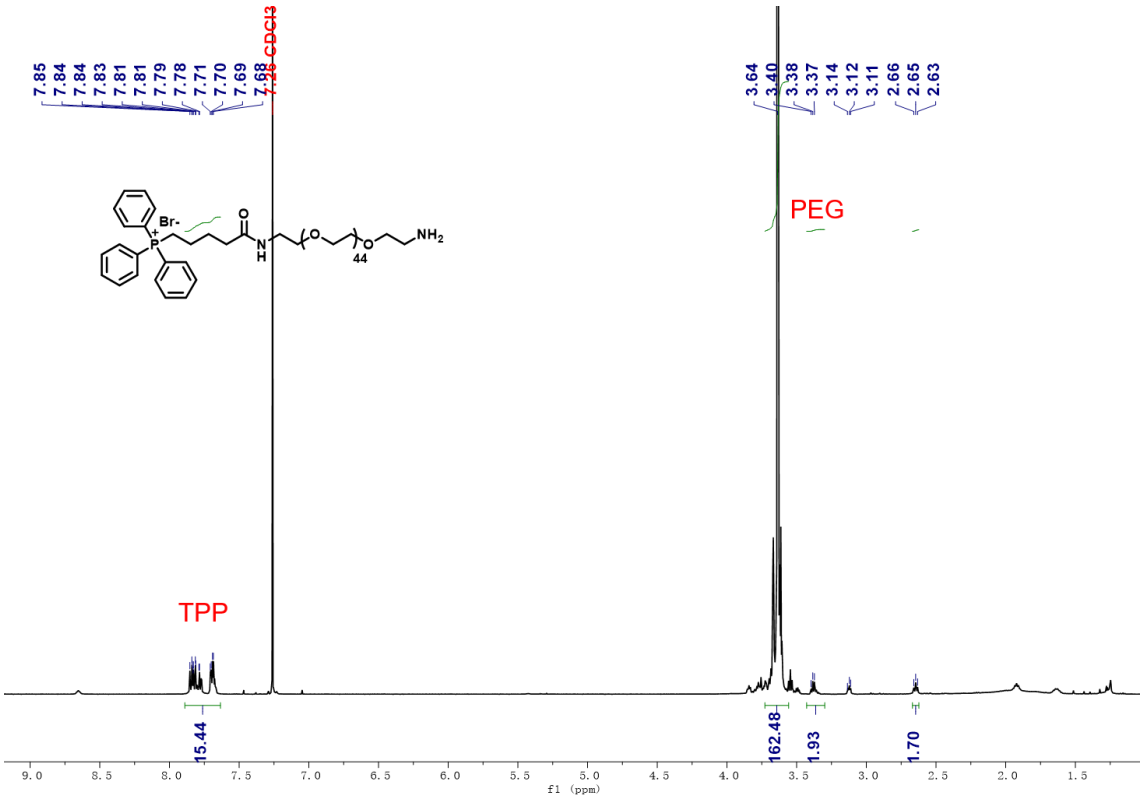


Figure S9. ^{1}H NMR spectrums of TPP-PEG$_{2000}$-Boc and TPP-PEG$_{2000}$-NH$_2$ (500 MHz, CDCl$_3$).

Synthesis of ERT-PEG$_{2000}$-NH$_2$: The endoplasmic reticulum targeting motif ERT-PEG$_{2000}$-NH$_2$ was synthesized. Firstly, a mixture of 2-(4-methylphenylsulfonamido) acetic acid (1 g, 4.36 mmol) and thionyl chloride (1 mL) in 10 mL of dry THF was heated at reflux for 6 h. The excess thionyl chloride was removed in vacuo and the resulting solid was dissolved in 10 mL THF, and the complex solution of BOC-PEG$_{2000}$-NH$_2$ (200 mg, 0.095 mmol) and triethylamine (0.5 mL) in THF (1 mL) was added dropwise at an ice bath. The reaction mixture was slowly warmed to room temperature and stirred for 48 h, then the solvent were removed under vacuum. Post-processing step follows the similar procedure as **Synthesis of Ada-PEG$_{1000}$-NH$_2$**.

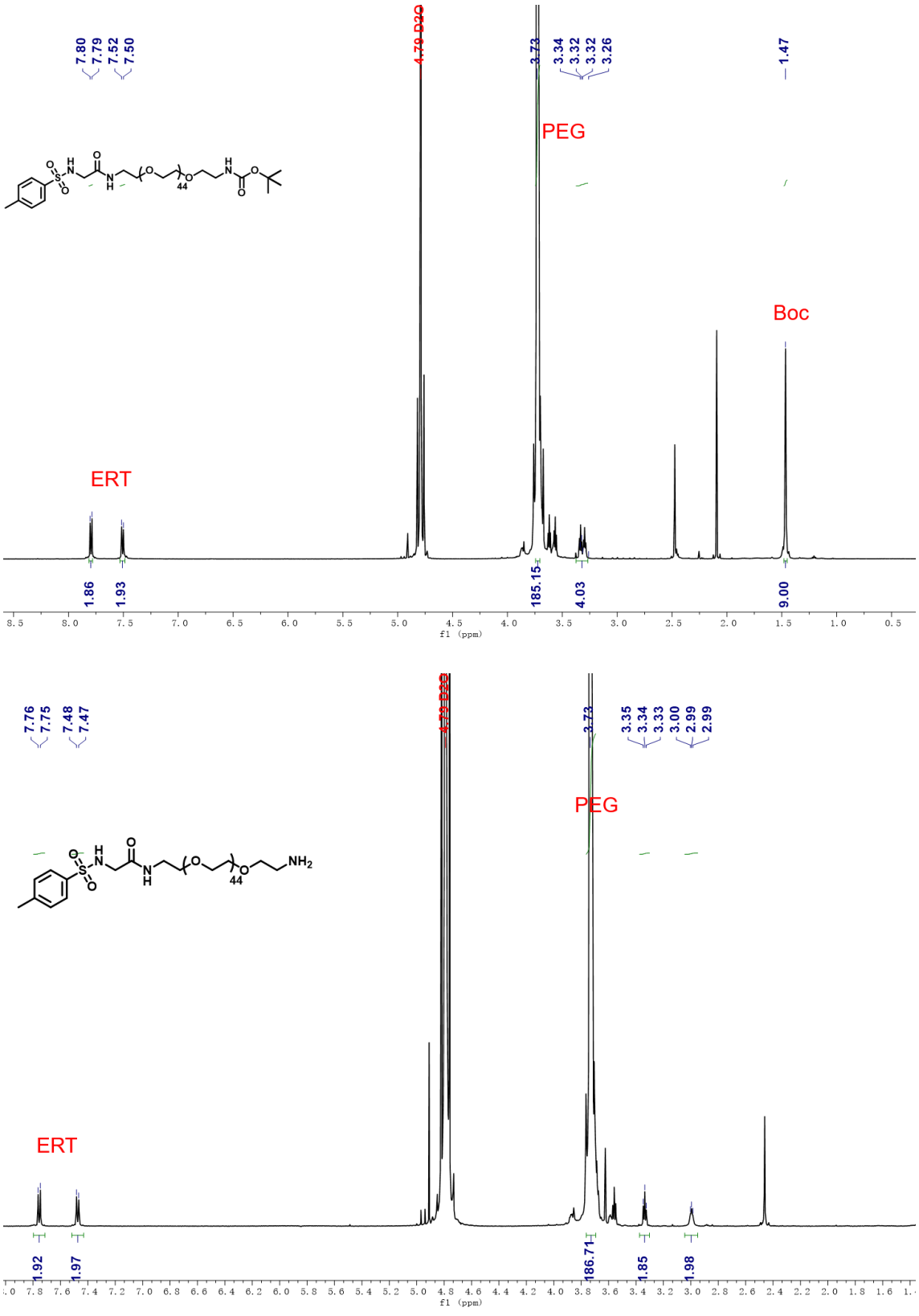
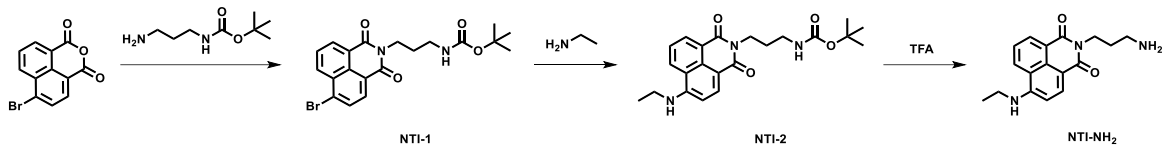


Figure S10. ^{1}H NMR spectrums of ERT-PEG$_{2000}$-Boc and ERT-PEG$_{2000}$-NH$_2$ (500 MHz, D$_2$O).

The Synthesis of NTI-NH_2:



Scheme S2: Synthesis route of NTI-NH_2 fluorescence.

NTI-1: 4-Bromo-1,8-naphthalic anhydride (277 mg, 1.0 mmol, 1 equiv.) and N-Boc-1,3-propanediamine (209 mg, 1.2 mmol, 1.2 equiv.) were dissolved into a mixture of DMF (5 mL), followed by the addition of TFA (0.175 mL). Then the mixture was stirring at 100 ℃ for 24 h under N_2. The reaction was quenched by H_2O (30 mL). The resulted solution was extracted with ethyl acetate (10 mL×3) and washed with brine (30 mL×3). The organic phase was dried by Na_2SO_4 and concentrated on a rotary evaporator. The residue was purified by column chromatography with DCM / ethyl acetate (v/v = 3/1) as the eluent to afford NTI-1 as a light-yellow solid (352 mg, 81.0 %). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.57 (d, J = 7.3 Hz, 1H), 8.46 (dd, J = 11.6, 8.3 Hz, 2H), 7.67 (t, J = 7.9 Hz, 1H), 7.12 (d, J = 8.2 Hz, 1H), 5.36 (s, 1H), 4.25 (t, J = 6.5 Hz, 2H), 3.14 (t, J = 8.2 Hz, 2H), 1.92 (m, J = 6.3 Hz, 2H), 1.45 (s, 9H).

NTI-2: NTI-1 (346 mg, 0.8 mmol, 1 equiv.) was dissolved in 15 mL DMSO, followed by 2 M ethylamine THF solution (4 mL, 10 equiv.). The reaction was heated at 100 °C for 12 h under N_2. Then the reaction mixture was cooled down and added into 50 mL 1M HCl solution. The resulted solution was extracted by ethyl acetate (100 mL×3) and washed with 1M HCl (50 mL×3). The organic phase was dried by Na_2SO_4 and concentrated on a rotary evaporator to afford NTI-2 as a orange solid (260 mg, 81.7 %). It was directly used in the next step without characterization.

NTI-NH_2: NTI-2 (250 mg, 0.63 mmol) was dissolved in 5 mL DCM, followed by the addition of TFA (1 mL). The mixture was stirred at room temperature for 4 h when TLC show full conversion. The resulted solution was concentrated on a rotary evaporator and adding 1 M NaOH solution to adjust the pH ≥10. Then 20 ml ethyl acetate was added and extracted by ethyl acetate (20 mL ×2). Then the organic phase washed by H_20 (50 mL×2)and dried over by Na_2SO_4. The resulted solution was concentrated on a rotary evaporator to afford the NTI-NH_2 as sticky orange compound (172 mg, 93.8 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 8.50 (dd, J = 8.4, 1.2 Hz, 1H), 8.45 (dd, J = 7.3, 1.2 Hz, 1H), 8.33 (d, J = 8.3 Hz, 1H), 7.74 (dd, J = 8.5, 7.3 Hz, 1H), 7.20 (d, J = 8.3 Hz, 1H), 4.07 (t, J = 6.9 Hz, 2H), 3.51 (q, 2H), 2.68 (t, J = 7.3 Hz, 2H), 1.81 (d, J = 10.8 Hz, 2H), 1.21 (t, 3H). ^{13}C NMR (126 MHz, DMSO-d6) δ 164.39, 163.73, 157.18, 132.90, 132.18, 131.16, 130.21, 125.56, 124.71, 122.82, 113.73, 113.51, 44.92, 38.66, 37.56, 29.56, 22.63. MS (ESI:m/z) : calcd for $C_{17}H_{19}N_3O_2$, $[M+Na]^+$: 298.36; found,298.50.

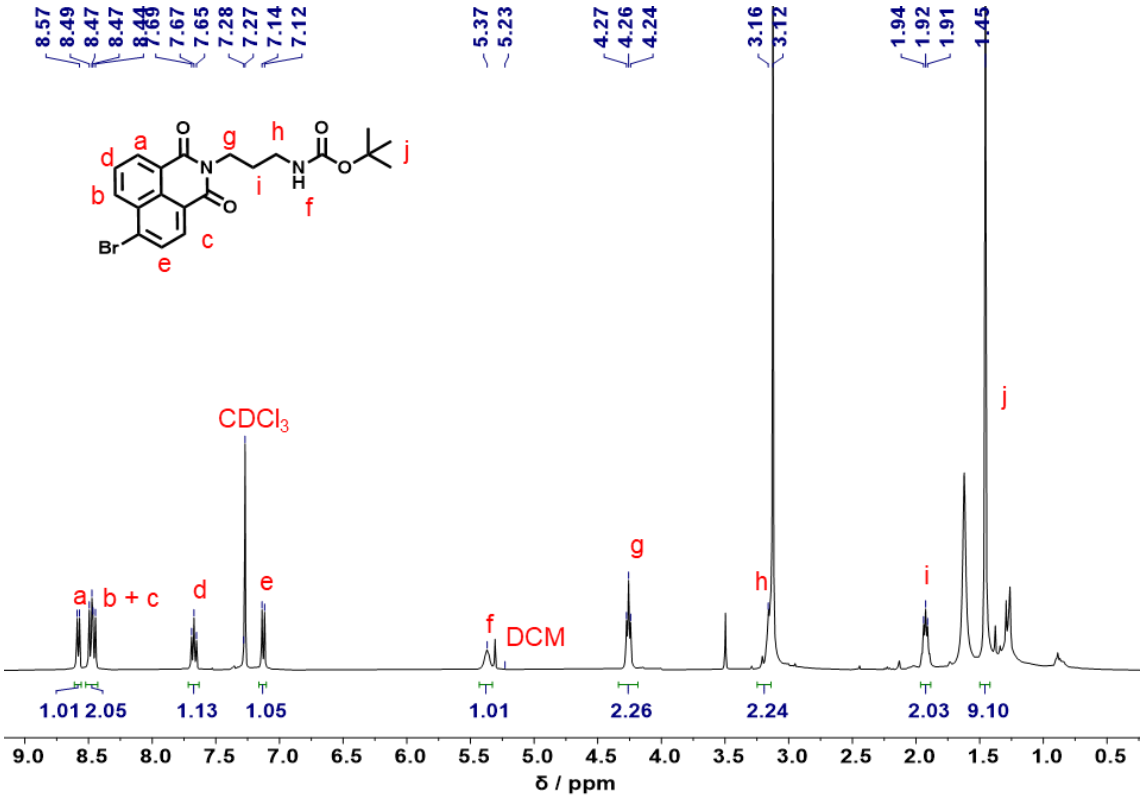
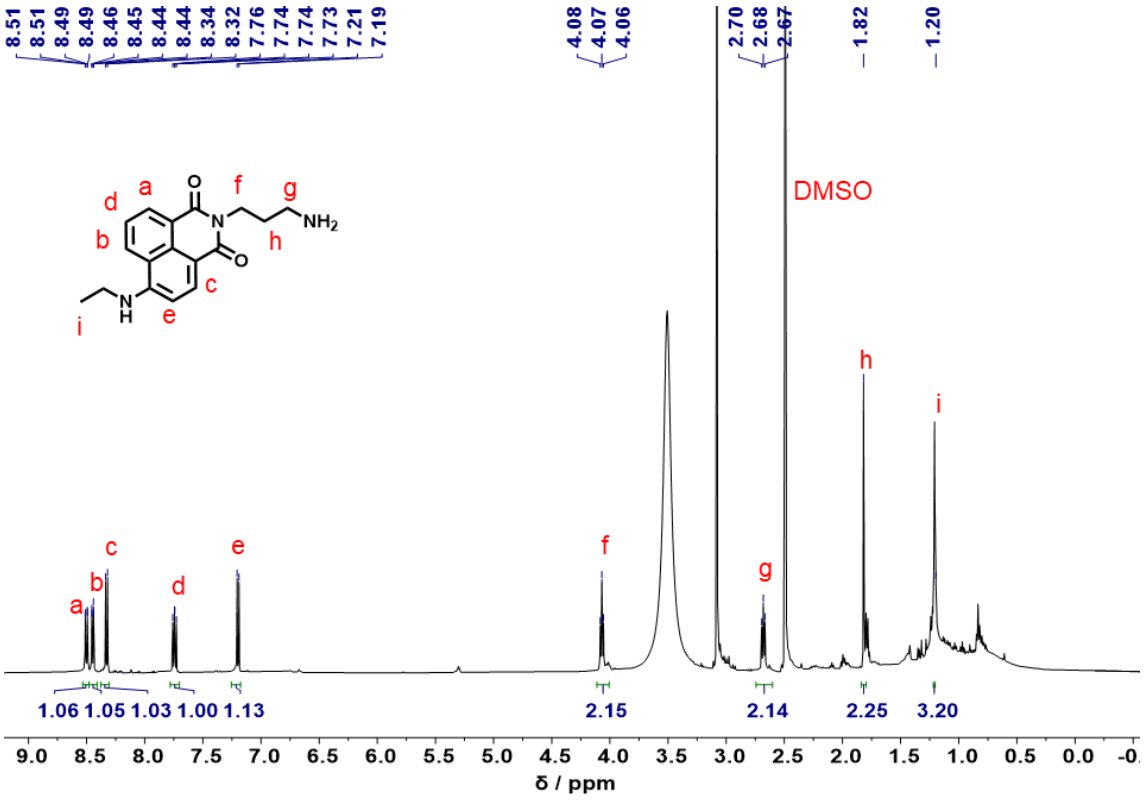


Figure S11. ^{1}H NMR spectrum of NTI-1 (400 MHz, $CDCl_3$).



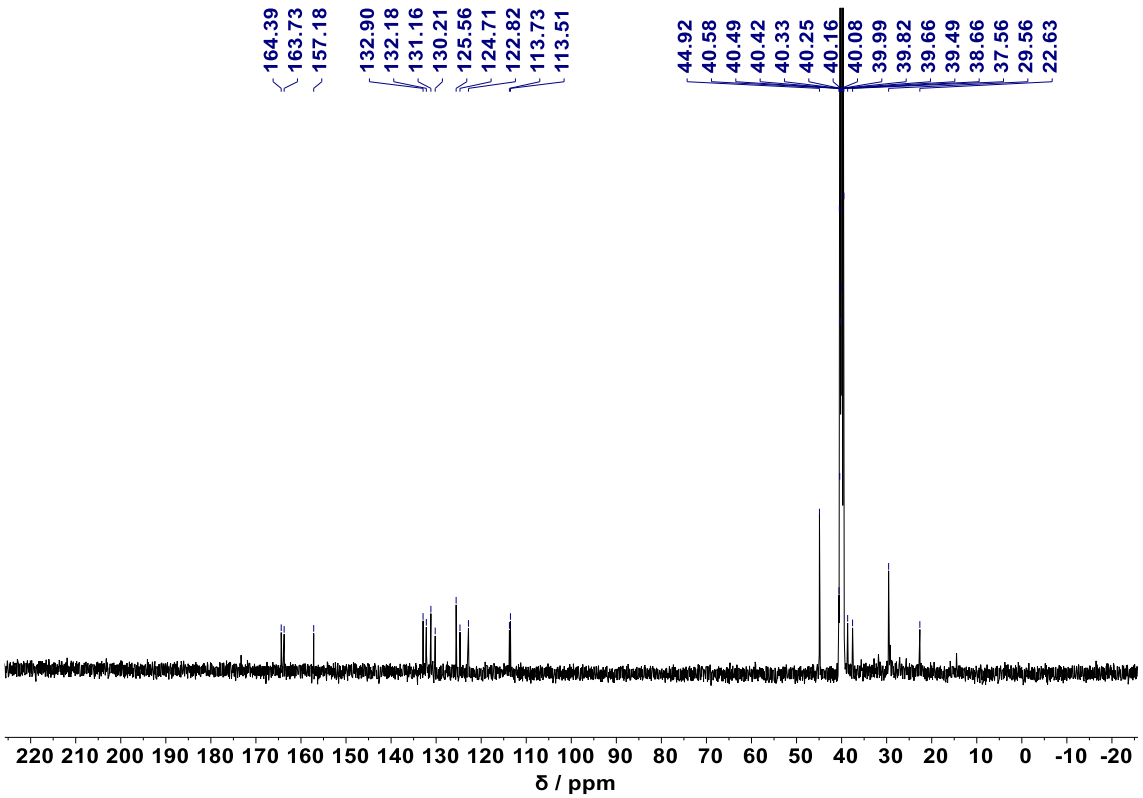


Figure S12. The spectra of ^{1}H NMR (500 MHz, DMSO) and ^{13}C NMR (126 MHz, DMSO) of NTI-NH_2.

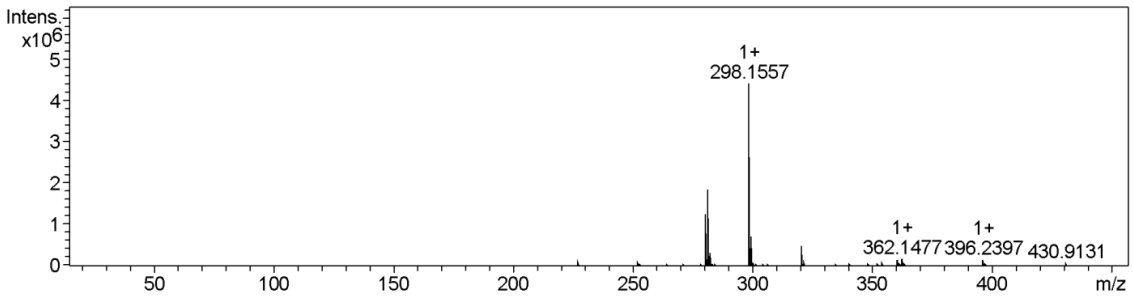


Figure S13. ESI-MS spectrum of NTI-NH_2 in MeOH.

The procedure for post-modification with amines:

Synthesis of NB_{50}-DSPE-CB[7]: DSPE-PEG_{2000}-NH_2 (24 mg, 0.01 mmol) and 8 μL of DIPEA were dissolved in 1 mL dry THF, then mixed with 1 mL of NB_{50}-PF_5 (40 mg, 1.99 μmol) in THF solution and the mixture was stirred at room temperature for 4 h. Next, N_3-PEG_{1000}-NH_2 (10 mg, 0.01 mmol) in 1 mL THF was added and reacted for 4 h. Then the solution of NTI-NH_2 (1.78 mg, 1 μmol) in 1 mL THF was added into the reaction mixture and reacted for another 4 h. Finally, 60 μL PEG_{350}-NH_2 was added and stirred overnight. The reaction solution was dialyzed against THF (MWCO:7000 Da) for 3 days and the polymer NB_{50}-DSPE-N_3 was then obtained under reduced pressure. NB_{50}-DSPE-CB[7] was prepared using CuAAC reaction. Briefly, the complex of CB[7]OP and PXDA (14 mg, 9.86 μmol) were

dissolved into 3 mL deionized water, then mixed with 2 mL of NB_{50}-DSPE-N_3 (30 mg, 0.66 μmol)in deionized water under N_2. $CuSO_4$ (7.38 mg, 29.58 μmol) and THPTA (38.53 mg, 88.74 μmol) were added afterward. After the reaction temperature reach to 65 °C, 500 mg Sodium L-Ascorbate was added and the mixture was stirring for 48 h. After centrifugation, the supernatant was dialyzed against deionized water (MWCO:7000 Da) for 3 days and methanol for 2 days, a certain amount of NaOH was added during dialysis to remove PXDA. Then, the mixture was dialyzed against deionized water again for 1 day and the final product was obtained by lyophilization.

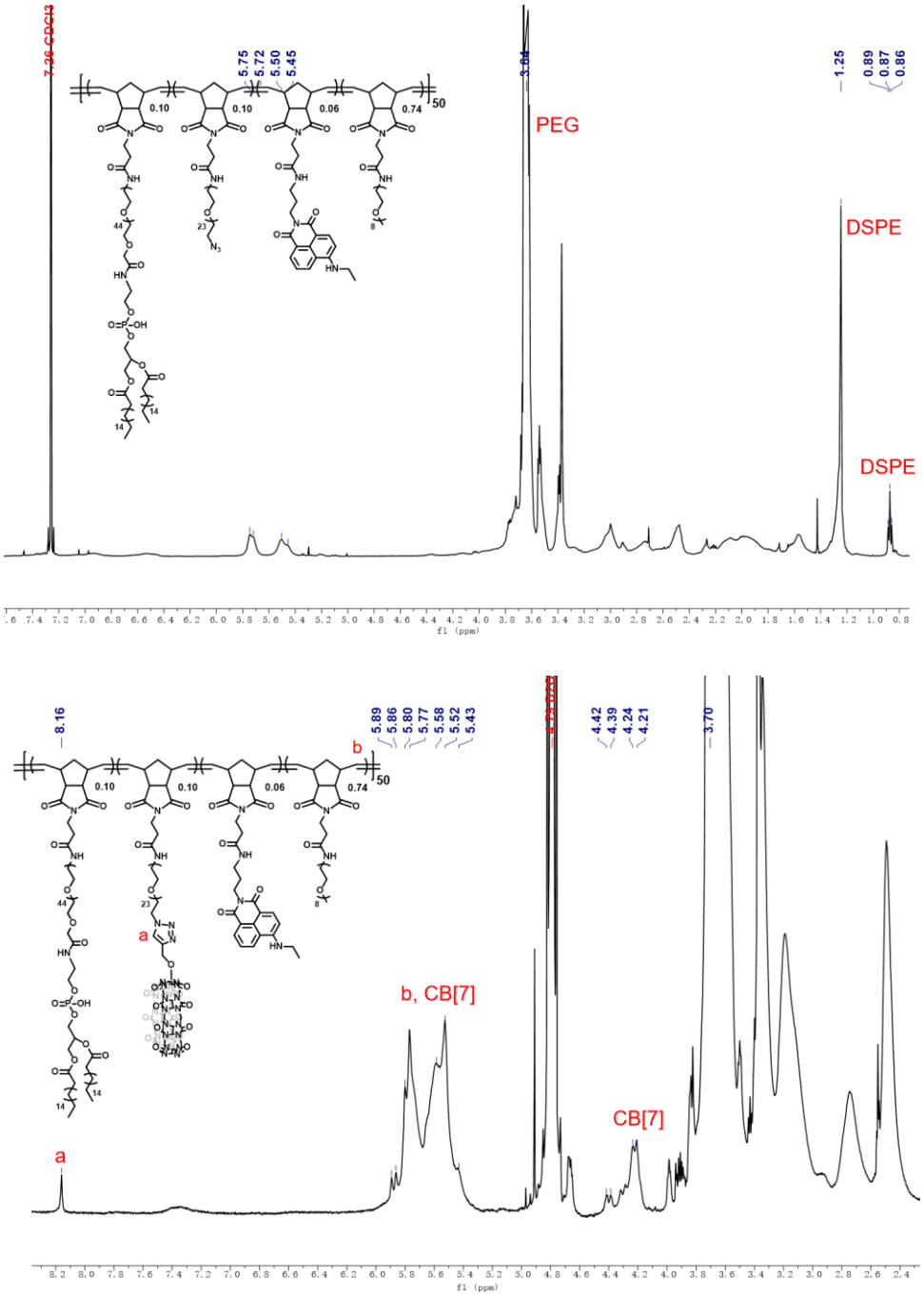


Figure S14. ^{1}H NMR spectrums of NB_{50}-DSPE-N_3 (500 MHz, $CDCl_3$) and NB_{50}-DSPE-CB[7] (500 MHz, D_2O).

Synthesis of NB_{50}-DSPE-Ada: The synthesis of NB_{50}-DSPE-Ada follows the similar procedure as NB_{50}-DSPE-N_3. Briefly, NB_{50}-PF_5 (20 mg, 1 μmol) was dissolved in 2 mL DMF, the complex solution of DSPE-PEG_{2000}-NH_2 (12 mg, 0.005 mmol) and DIPEA (8 μL) was then added and the mixture was stirred at room temperature for 4 h. Next, Ada-PEG_{1000}-NH_2 (6 mg, 0.005 mmol) in 1 mL DMF was added and reacted for 4 h. Then the solution of Cy5-NH_2 (1.31 mg, 0.5 μmol) in 1 mL DMF was added into the reaction mixture and reacted for another 4 h. Finally, 30 μL PEG_{350}-NH_2 was added and stirred overnight. The reaction solution was dialyzed against methanol (MWCO:7000 Da) for 3 days and the product NB_{50}-DSPE-Ada was obtained under reduced pressure.

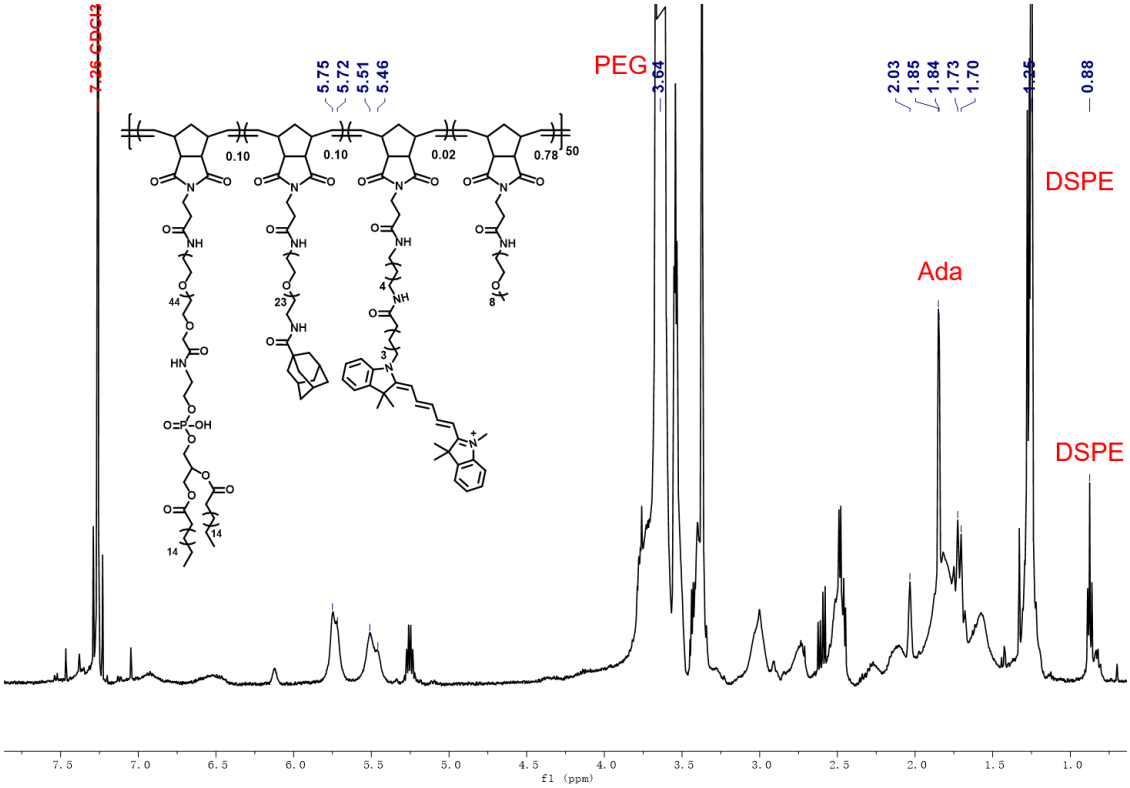


Figure S15. ^1H NMR spectrums of NB_{50}-DSPE-Ada (500 MHz, D_2O).

Synthesis of NB_{30}-ERT-CB[7]: NB_{30}-PF_5 (40 mg, 3.3 μmol) and ERT-PEG_{2000}-NH_2 (23 mg, 0.01 mmol) were dissolved in 2 ml THF and stirred at room temperature for 4 h. Next, N_3-PEG_{1000}-NH_2 (10 mg, 0.01 mmol) in 1 mL THF was added and reacted for 4 h. Then the solution of NTI-NH_2 (0.89 mg, 1 μmol) in 1 mL THF was added into the reaction mixture and reacted for another 4 h. Finally, 60 μL PEG_{350}-NH_2 was added and stirred overnight. The reaction solution was dialyzed against THF (MWCO:7000 Da) for 3 days and the solution was then concentrated under reduced pressure to give the product of NB_{30}-ERT-N_3. NB_{50}-DSPE-CB[7] was also synthesized via click chemistry and the preparation process is the same as **Synthesis of NB_{50}-DSPE-CB[7]**. At the same time, the NB_{30}-ERT-CB[7]-2 which without adding NTI fluorophore was prepared using the similar procedure.

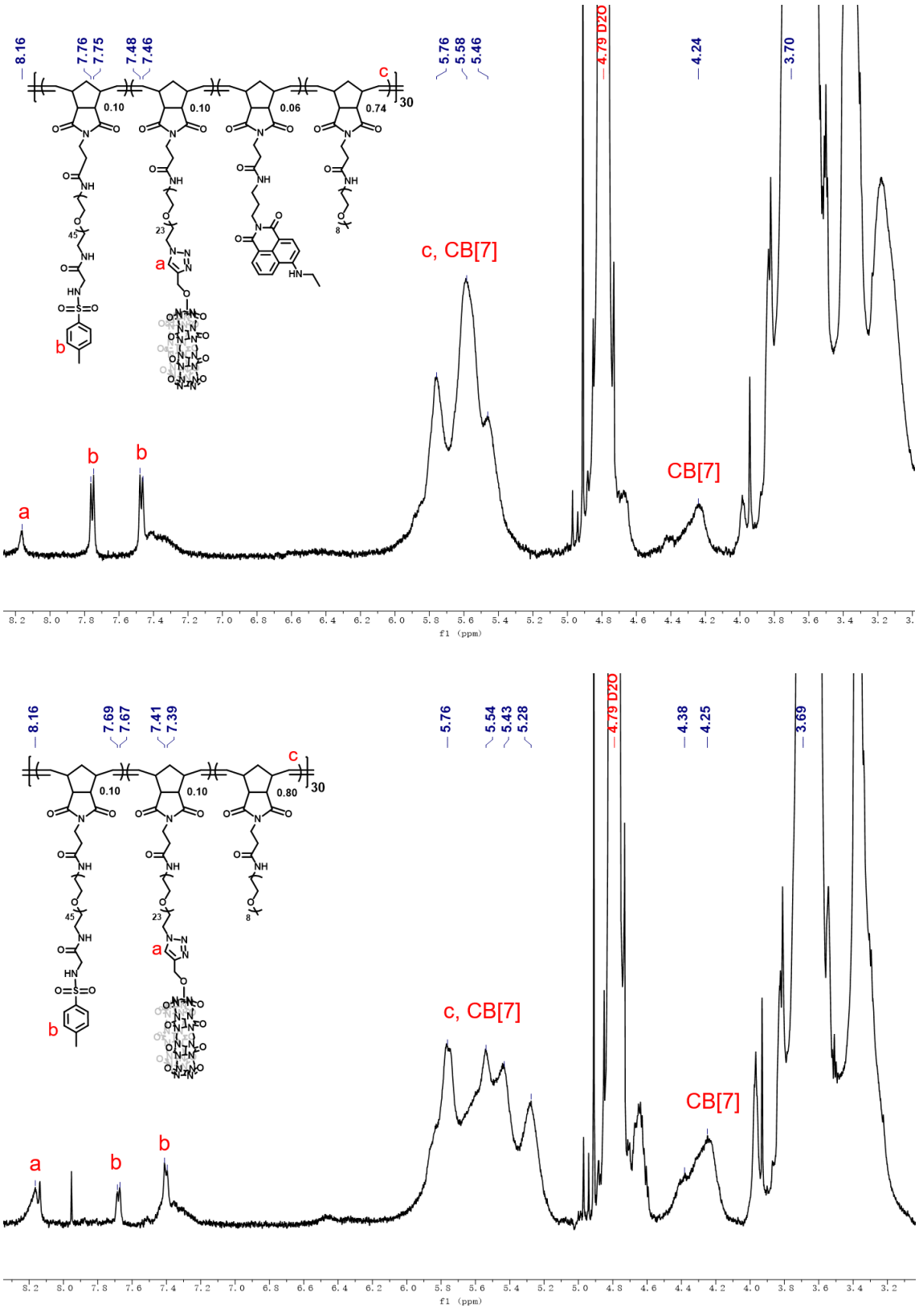


Figure S16. ^{1}H NMR spectrums of NB_{30}-ERT-CB[7] and NB_{30}-ERT-CB[7]-2 (500 MHz, D_2O).

Synthesis of NB_{30}-TPP-Ada: NB_{30}-PF_5 (20 mg, 1.65 μmol) and TPP-PEG_{2000}-NH_2 (11.5 mg, 0.005 mmol) were dissolved in 2 ml dry DMF and the mixture was stirred at room temperature for 4 h. Next, TPP-PEG_{1000}-NH_2 (6 mg, 0.005 mmol) in 1 mL DMF was added and reacted for 4 h. Then the solution of Cy5-NH_2 (0.65 mg, 0.5 μmol) in 1 mL DMF was added into the reaction mixture and reacted for another 4 h. Finally, 30 μL PEG_{350}-NH_2 was added and stirred overnight. The reaction solution was dialyzed against methanol (MWCO:7000 Da) for 3 days and the product NB_{30}-TPP-Ada was obtained under reduced pressure. The polymer NB_{30}-TPP-Ada-2 which without adding Cy5 fluorescence was also prepared.

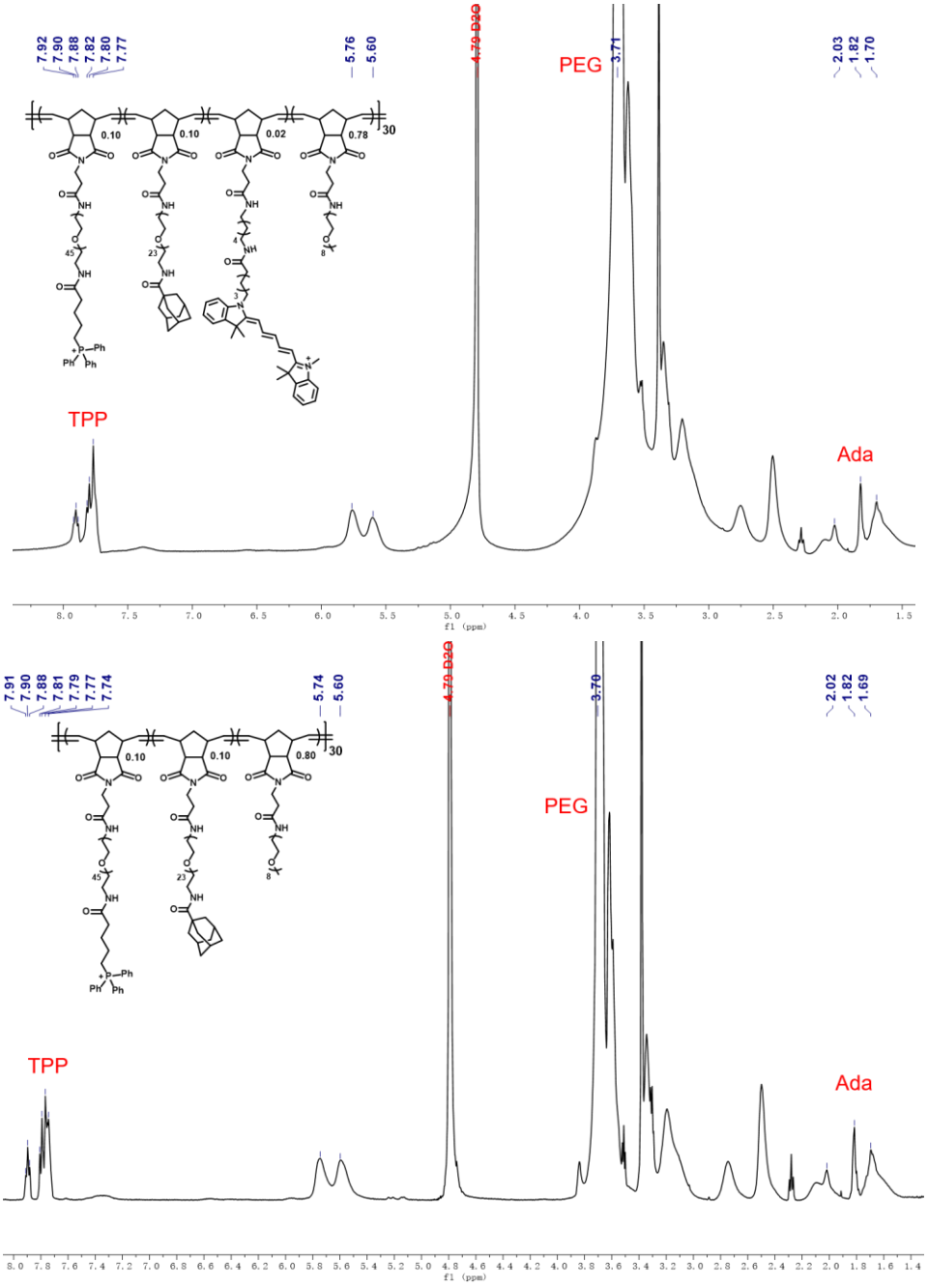


Figure S17. ^1H NMR spectrums of NB_{30}-TPP-Ada and NB_{30}-TPP-Ada-2 (500 MHz, D_2O).

Table S1. GPC results of ROMP random copolymers in this study

Polymers	Amines	GPC[a] M_n / kDa	PDI[a]	DP[b]	Calculated[c] M_n / kDa
NB_{50}-PF_5	—	8.91	1.08	50	20.1
NB_{50}-DSPE-N_3	DSPE-PEG_{2000}-NH_2 N_3-PEG_{1000}-NH_2 NTI-NH_2 PEG_{350}-NH_2	19.5	1.03	50	45.4
NB_{50}-DSPE-Ada	DSPE-PEG_{2000}-NH_2 Ada-PEG_{1000}-NH_2 Cy5-NH_2 PEG_{350}-NH_2	17.7	1.07	50	43.5
NB_{50}-Ada	Ada-PEG_{1000}-NH_2 Cy5-NH_2 PEG_{350}-NH_2	17.2	1.04	50	33.8
NB_{30}-PF_5	—	8.38	1.31	30	12.1
NB_{30}-ERT-N_3	ERT-PEG_{2000}-NH_2 N_3-PEG_{1000}-NH_2 NTI-NH_2 PEG_{350}-NH_2	15.5	1.17	30	28.9
NB_{30}-TPP-Ada	TPP-PEG_{2000}-NH_2 Ada-PEG_{1000}-NH_2 Cy5-NH_2 PEG_{350}-NH_2	14.4	1.12	30	26.5

[a] The molecular weights and PDI were measured by GPC in THF

[b] Degree of polymerization (DP) was calculated from the monomer conversion by ^{1}H NMR

[c] The calculated molecular weights derived from the DP

II. Biological Section

1. Materials and apparatus:

Materials: DC 2.4 (mouse dendritic cells) and PC-3 (human prostate cancer cells) were purchased from Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). RPMI-1640, fetal bovine serum (FBS), phosphate-buffered saline (PBS) and penicillin-streptomycin were purchased from Gibco Life Technologies (USA). The cell proliferation assay kit (Cell Counting Kit-8, CCK-8), Mito-Tracker Red CMXRos, Mito-Tracker Green, Lyso-Tracker Red, Lyso-Tracker Green, ER-Tracker Red, ER-Tracker Green, Calcein-AM and Did were purchased from Shanghai Beyotime Biotech Co. Ltd. (Shanghai, China).

Apparatus: Laser confocal microscopy (LSM 880 NLO, Zeiss, Germany), Flow cytometry (FACS Verse, BD Bioscience, USA), Microplate reader (SpectraMax iD3, Molecular Devices, USA).

2. Cell culture

DC 2.4 and PC-3 cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum and 100 u/mL Penicillin/Streptomycin in a 5% CO_2 incubator at 37 °C. Upon reaching 80%-90% confluence, the cells were washed with PBS, blown gently after trypsin digestion to form a cell suspension for the following cell experiments.

3. Retention of polymers on cell surface

DC 2.4 and PC-3 cells were seeded in 35 mm confocal laser culture dishes, respectively, at a density of 5.0×10^4 cells/mL for 24 h. Subsequently, the cells were washed with PBS for 2 times. Then PC-3 and DC 2.4 cells were treated with 500 μg/mL NB_{50}-DSPE-CB[7] and NB_{50}-DSPE-Ada. After a 25 mins incubation, the cells were washed with PBS for 3 times to remove unbound polymers and fresh serum-free media was added. Retention of polymers on cell surface was observed under a confocal microscope (CLSM) at different time intervals (0 h, 3 h, 8 h and 24 h).

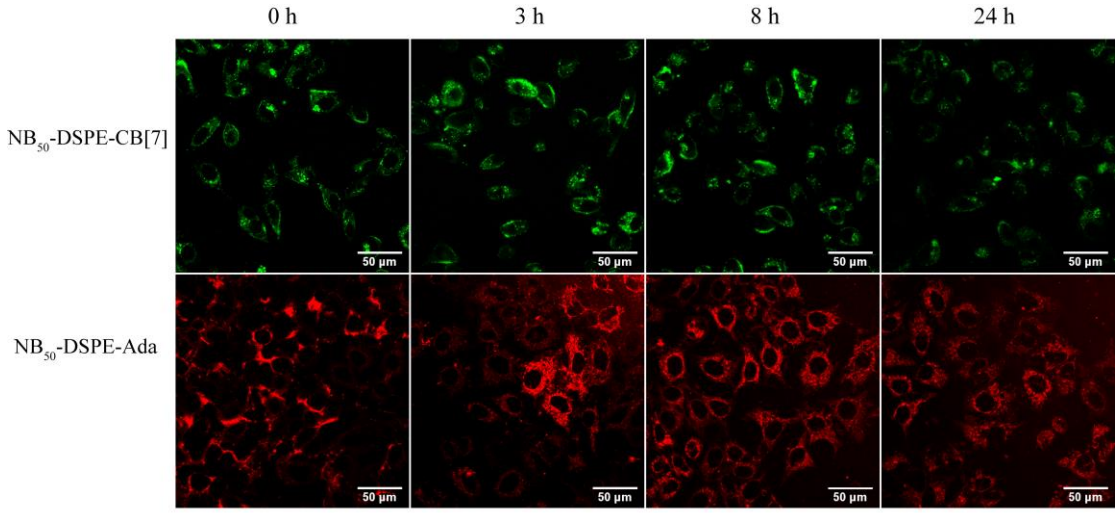


Figure S18. The retention of NB_{50}-DSPE-CB[7] (green) and NB_{50}-DSPE-Ada (red) on PC-3 and DC 2.4 cells surface. Scale bar, 50 μm.

4. Manipulation of cell-cell interactions

The interaction between DC 2.4 and PC-3 cells was measured qualitatively and quantitatively by CLSM and flow cytometry. Firstly, DC 2.4 and PC-3 cells were labeled with NB_{50}-DSPE-Ada (500 μg/mL) and NB_{50}-DSPE-CB[7] (500 μg/mL), respectively. After washing with PBS for 3 times by centrifugation, DC 2.4 cells (1.0×10^6 cells) were mixed with the same number of PC-3 cells and shaken at 300 rpm for 30 mins. The cells were then observed under a CLSM. To calculate the aggregation ratio of cells, DC 2.4 and PC-3 cells were firstly labeled with Did and Calcein-AM according to the manufacturer's instructions, this step is conducive to reduce the impact of fluorescence quenching during flow analysis process. After centrifugation to remove the staining solution, DC 2.4 and PC-3 cells were re-suspended in the serum-free media containing NB_{50}-DSPE-Ada (500 μg/mL) and NB_{50}-DSPE-CB[7] (500 μg/mL), respectively. After a 25 mins incubation, the cells were washed with PBS for 3 times to remove free polymers and the same number of DC 2.4 cells and PC-3 cells were mixed, then shaken at 300 rpm for 30 mins. The aggregation ratio of DC 2.4 and PC-3 cells were analyzed by flow cytometry.

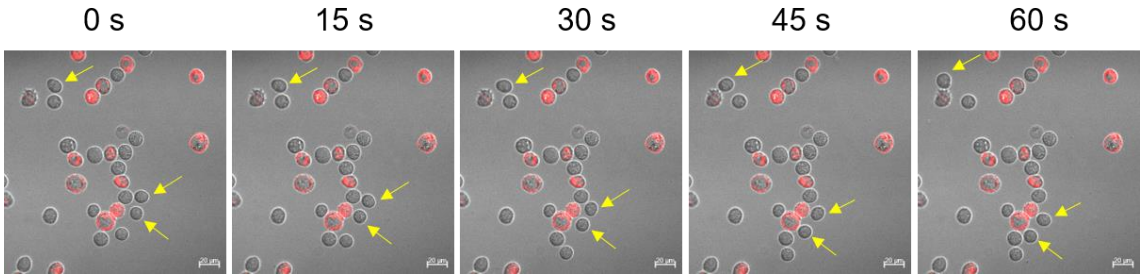


Figure S19. The time scanning images of cellular assembly induced by the host-guest interaction, DC 2.4 cells modified with NB_{50}-DSPE-Ada (red fluorescent cells) were mixed with PC-3 cells modified with NB_{50}-DSPE-CB[7] (non-fluorescent cells) in a ratio of 1:1. Scale bar, 20 μm.

As can be seen from the time scanning images (Figure S19), most of the cells were firmly bound together and not moving. Most likely, these cells have already bound with the counterpart cells through host-guest recognition during the 30 mins shaking process. However, there were still a few stray suspension cells which have not completed the binding, were still capable of moving around. These stray cells were monitored and imaged time-dependently. As shown in Figure S19, some of these stray cells gradually found the other type of cells and bound together. These purposeful binding events vividly showed the dynamic process of cell-cell attachment, provided further evidence to support our protocol of cell surface engineering.

5. Cell fusion by the PEG-induced method

The homogeneous cell fusion induced by PEG was performed according to the literature [8]. At first, DC 2.4 and PC-3 cells were labeled with NB_{50}-DSPE-Ada (500 μg/mL) and NB_{50}-DSPE-CB[7] (500 μg/mL), respectively. After washing with PBS for 3 times by centrifugation, DC 2.4 cells (1.0×10^6 cells) were mixed with the same number of PC-3 cells and shaken at 300 rpm for 30 mins. Then, the cell pellet was carefully added to 1 mL of pre-warmed PEG solution (50 wt% in PBS) at 37 °C warm bath, followed by gentle mixing for 1 min. Next, 9 mL of culture medium was added to the cell suspension. After PEG was removed by centrifugation, the cells were re-suspended in culture medium and transferred to a glass-bottom culture dish. The cells were observed under a CLSM at 0 h and 24 h.

6. Subcellular localization of NB_{30}-ERT-CB[7] and NB_{30}-TPP-Ada

The subcellular localization of NB_{30}-ERT-CB[7] and NB-TPP-Ada were investigated by CLSM. Briefly, PC-3 cells were incubated with serum-free media containing NB_{30}-ERT-CB[7] and NB-TPP-Ada, respectively, with the concentration of 200 μg/mL. After 8 h of incubation, the cells were washed with PBS for 3 times and stained with ER-Tracker, Mito-Tracker or Lyso-Tracker at 37 °C for 30 mins. Next, cells were washed with PBS for 3 times and the location of NB_{30}-ERT-CB[7] and NB_{30}-TPP-Ada in the cells were observed by CLSM. The excitation was 488 nm for NB_{30}-ERT-CB[7]; 633 nm for NB_{30}-TPP-Ada; 561 nm for ER Tracker Red, Mito-Tracker Red CMXRos and Lyso-Tracker Red; 514 nm for ER-Tracker Green, Lyso-Tracker Green and Mito-Tracker Green. All the images were processed using Fiji plugin.

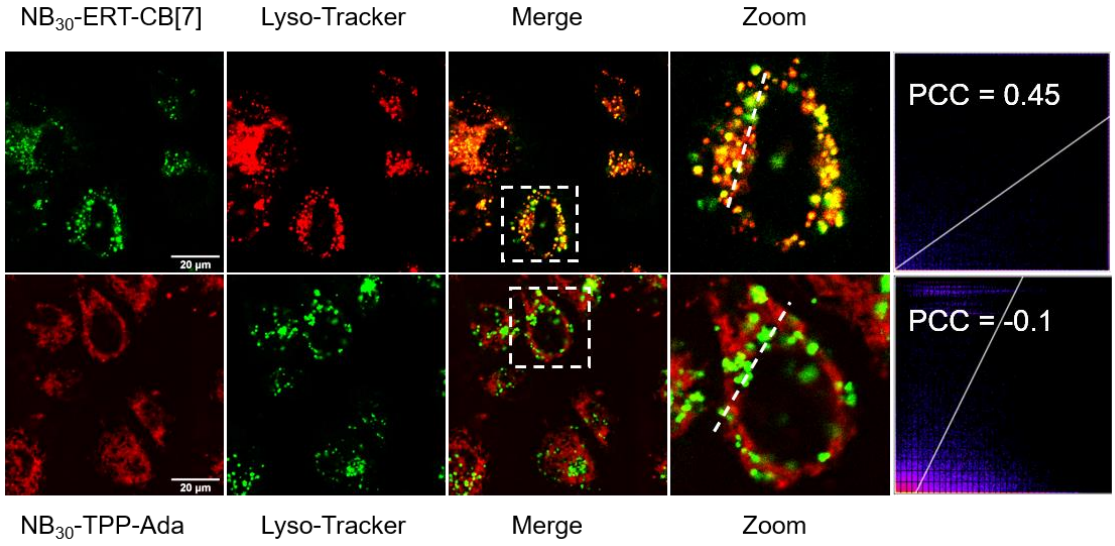


Figure S20. Colocalization images of NB_{30}-ERT-CB[7] and NB_{30}-TPP-Ada with lysosome. Scale bar, 20 μm.

7. Conjugation of ER and mitochondrial

In order to investigate the conjugation between ER and mitochondrial, PC-3 cells were treated with serum-free media containing 200 μg/mL NB_{30}-ERT-CB[7] (without NTI labelling) for 12 h. After washed with PBS for 3 times, the cells subsequently incubated with same concentration of NB_{30}-TPP-Ada (without Cy5 labelling) for another 12 h. Then, the cells were stained by ER Tracker Red and Mito-Tracker Green sequentially, and observed under CLSM. The control group was directly stained with ER Tracker Red and Mito-Tracker Green. The images were processed using Fiji plugin. For transmission electron microscopy (TEM) observation, cells were fixed in 2.5% glutaraldehyde at 4 °C for 24 h and then transferred to centrifuge tubes. The fixative was pipetted off by centrifugating for 10 min, then fresh fixative was added. After 2 h, cells were stained by 1% OsO_4 for 1h and dehydrated continuously in 50%, 70%, 95%, and 100% ethanol for 15 min. Next, the cells were embedded in epoxy resin and placed in a 60 °C oven. Ultrathin sections were obtained with an ultramicrotome, stained with 5% aqueous uranyl acetate and 2% lead citrate, and finally imaged using TEM.

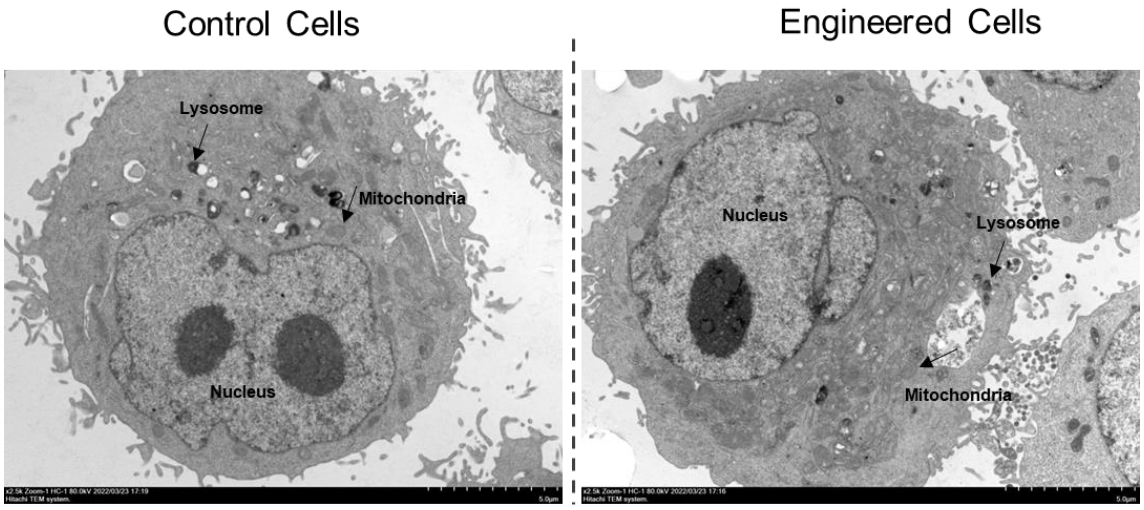


Figure S21 . TEM images of entire cell section. PC-3 cells without (left) or with (right) the treatment of NB30-ERT-CB[7]-2 and NB30-TPP-Ada-2. Scale bar, 5 μm.

8. Biocompatibility studies

The cytotoxicity of NB_{50}-DSPE-CB[7]/NB_{50}-DSPE-Ada to DC 2.4 or PC-3 cells, and the cytotoxicity of NB_{30}-ERT-CB[7]/NB_{30}-ERT-Ada to PC-3 cells were evaluated. The general method was described. Briefly, DC 2.4 and PC-3 cells were respectively seeded in a 96-well plate at a density of 1×10^4 cells per well. After incubation for 24 h. The cell culture medium was replaced with 100 μL of a fresh medium containing NB-ERT-CB[7] or NB-TPP-Ada and the concentration of polymer ranged from 0-1000 μg/mL. After 24 h of incubation, 10 μL of CCK-8 reagent was added to each well and incubated for 1.5 h at 37 °C. Absorbance was measured using a microplate reader at 450 nm. Each trial was performed

with five parallel wells. Cell viability was determined using the equation below.

$$\text{Cell viability}\,(\%) = \frac{\text{A(treatment group)}}{\text{A(control group)}} \times 100$$

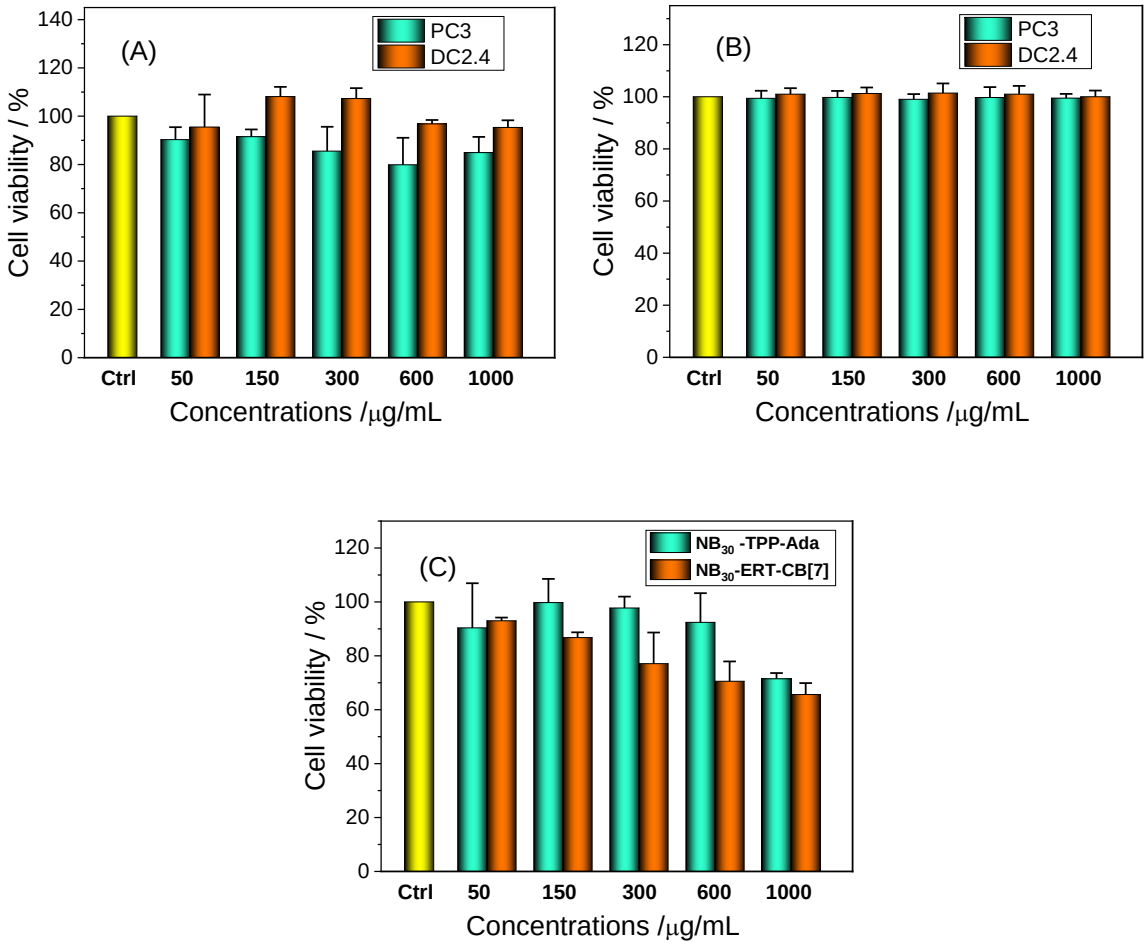


Figure S22. Cell toxicity tests. (A, B) PC-3 and DC 2.4 cells were treated with NB_{50}-DSPE-CB[7] or NB_{50}-DSPE-Ada for 24 h before cell viability measurement; (C) PC-3 cells were incubated with NB_{30}-TPP-Ada or NB_{30}-ERT-CB[7] for 24 h before cell viability measurement.

9. Statistics and reproducibility

All experiments are repeated at least three times, and each data has more than three parallel samples. For flow cytometry analysis, about 10000 events were counted for each sample group. Bar are represented as mean±s.d.

III. References

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