Supporting Information

A logic-gated modular nanovesicle enables programmable drug release for on-demand chemotherapy

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Materials and instruments. Methyl isonipecotate, thiophene-2-thiol, Fmoc-Lys-OH, piperidine, phenyl isothiocyanate, HATU, *N,N*-diisopropylethylamine (DIPEA), EDCI, N-hydroxysuccinimide (NHS), Thiazolyl blue tetrazolium bromide (MTT) and doxorubicin hydrochloride (DOX, >99%) were purchased from Sigma-Aldrich. Croconic acid, 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA), culture medium and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific. The water used in all experiments was obtained by filtering through a set of Millipore cartridges (Epure, Dubuque, IA). All solvents were purchased from Sigma without further purification unless specified. Waters 600 high-performance liquid chromatography (HPLC) system with a Waters 996 Photodiode Array Detector (PDA) and a semi-preparative C18 HPLC column (XTerra Prep RP18, 10 μm, 7.8 x 300 mm, Waters) was used for the purification of products. HPLC utilized a linear gradient starting from 5% A (acetonitrile) and 95% B (50 mM ammonium acetate buffer) for 5 min and increasing to 65% A at 35 min with a flow rate of 5 ml/min for semi-prep HPLC. A Perkin-Elmer 200 series HPLC pump with a Waters 2487 UV detector and an analytical C18 HPLC column (XTerra 5 μm, 150 x 4.6 mm, Waters) was used for analysis of compounds. The sample solution was filtered and loaded onto the analytical HPLC column eluting with the mobile phase (A: 50 mM ammonium acetate buffer; B: CH_3CN) at a flow rate of 1 mL/min according to the following gradient program: 0-2 min, 5% of B; 2-15 min, 5%-80% of B; 15-20 min, 80% of B; 20-25 min, 80%-5% of B. The ^{1}H NMR spectra were recorded on a Bruker spectrometer at 300 MHz. Mass spectra were obtained using a Waters Q-tof LCMS system (Waters, Milford, MA) that includes an Acquity UPLC. Transmission electron microscopy (TEM) images were acquired on a FEI Tecnai12 equipment with a voltage at 120 kV. UV-vis spectrum was recorded by a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA) using quartz

cuvettes with an optical path of 1 cm. Dynamic light scattering measurements were performed at a SZ-100 nano particle analyzer (HORIBA Scientific, USA). Cell viability was assessed by cell count kit 8 (CCK8) assay (Dojindo Laboratories, Japan). Athymic nude mice were purchased from Envigo (USA). Tissue and tumor samples for hematoxylin and eosin (H&E) staining were prepared by BBC Biochemical (Mount Vernon, WA) and observed using a BX41 bright field microscopy (Olympus). Fluorescence spectrophotometry was carried out on a Hitachi F-7000 fluorescence spectrophotometer. NIR-I imaging was performed on a CRi Maestro in vivo imaging system.

Synthesis of compound 2. Compound **1**(**CR780**) was prepared as previously described.[1] Compound **1** (0.528 g, 1 mmol), EDC·HCl (0.766 g, 4 mmol), and NHS (0.46 g, 4 mmol) were dissolved in 10 mL of DMF. Finally DIPEA (0.516 g, 4 mmol) was added into the solution. The mixture was stirred in an ice bath for 30 min, followed by the addition of Fmoc-lys-OH (1.104 g, 3 mmol). After stirring at room temperature for 24 h, CR780 was completely consumed, as confirmed by HPLC. The mixture was concentrated using rotatory evaporator and subsequently purified by reversed-phase HPLC. Yield: 1.019 g (83% yield), LC-MS (ESI) calcd for $[M + H]^+$: 1229.43, Found 1229.1527. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 8.518 (s, 2H), 7.921 (s, 2H), 7.88 (d, J = 7.5 Hz, 4H), 7.673 (d, J = 7.2 Hz, 4H), 7.401 (t, J = 7.2 Hz, 4H), 7.317 (t, J = 7.2 Hz, 4H), 6.996 (s, 2H), 6.493 (d, J = 3.9 Hz, 2H), 4.222 (s, 4H), 4.006 (s, 4H), 3.553 (d, J = 5.1 Hz, 2H), 3.419 (s, 4H), 2.998 (d, J = 6.3 Hz, 4H), 1.894 (s, 2H), 1.614-1.746 (m, 5H), 1.469-1.581 (m, 2H), 1.289-1.401 (m, 4H), 1.220 (s, 4H).

Synthesis of compound 3. Piperidine (0.689 g, 8.1 mmol) was slowly added into a solution of compound **2** (1.0 g, 0.81 mmol) in 2 mL anhydrous DMF, and the mixture was stirred at room temperature for 2 h. The DMF was removed under reduced pressure, the residue was purified by

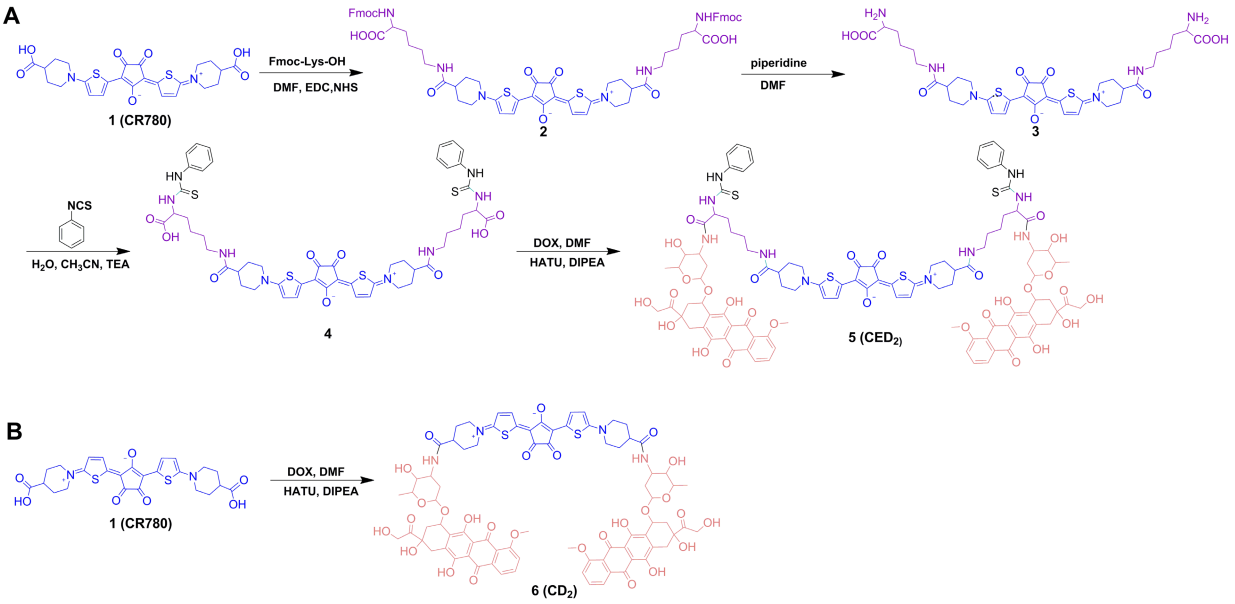
the reversed-phase HPLC to give the compound **3** (0.394 g, 62%). LC-MS (ESI) calcd for $[M + H]^+$: 785.29, Found 785.1205.

Synthesis of compound 4. To a solution of compound **3** (0.3 g, 0.38 mmol) in 10 mL of CH_3CN, a solution of phenyl isothiocyanate (0.154 g, 1.14 mmol) and triethylamine (0.04 g, 0.4 mmol) in 5 mL CH_3CN were added dropwise. The mixture was stirred at room temperature for 2 h and then purified by reversed-phase HPLC. Yield: 0.232 g (58% yield). LC-MS (ESI) calcd for $[M + H]^+$: 1055.32, Found 1055.1267. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 9.905 (s, 2H), 8.721 (s, 1H), 8.494 (s, 2H), 8.015 (s, 2H), 7.912 (s, 2H), 7.519 (d, *J* = 7.8 Hz, 4H), 7.308 (t, *J* = 8.1 Hz, 4H), 7.081 (t, *J* = 7.2 Hz, 2H), 6.979 (d, *J* = 4.8 Hz, 2H), 4.723 (s, 2H), 4.01 (d, *J* = 9.3 Hz, 4H), 3.041 (d, *J* = 5.4 Hz, 5H), 1.858 (s, 3H), 1.650-1.682 (m, 5H), 1.403 (d, *J* = 6.6 Hz, 4H), 1.306 (s, 4H), 1.228 (s, 2H), 1.106 (t, *J* = 7.2 Hz, 2H).

Synthesis of CED_2 (5). Compound **4** (0.211 g, 0.2 mmol), HATU (0.228 g, 0.6 mmol) and DIPEA (0.077 mg, 0.6 mmol) were added to a solution of DOX (0.232 g, 0.4 mmol) in dry DMF. The mixture was vigorously stirred at room temperature for 24 h, and the solvent was evaporated under reduced pressure. The residue was then purified by reversed-phase HPLC to afford compound CED_2 (0.219 g, 52% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 9.978 (s, 2H), 8.474 (s, 2H), 8.043 (s, 2H), 7.897 (s, 6H), 7.648 (t, *J* = 4.8 Hz, 3H), 7.537 (d, *J* = 7.8 Hz, 4H), 7.311(t, *J* = 7.8 Hz, 4H), 7.082 (t, *J* = 7.5 Hz, 2H), 5.457 (s, 2H), 5.287 (s, 3H), 4.936 (s, 4H), 4.692 (s, 2H), 4.585 (s, 4H), 4.189 (d, *J* = 6.6 Hz, 3H), 3.985 (s, 6H), 3.592 (s, 3H), 2.924-3.056 (m, 9H), 2.145 (s, 3H), 1.875 (d, *J* = 8.4 Hz, 4H), 1.687 (d, *J* = 9 Hz, 5H), 1.408 (d, *J* = 7.2 Hz, 5H), 1.312 (s, 3H), 1.233 (s, 6H), 1.158 (d, *J* = 6.6 Hz, 6 H), 1.057 (t, *J* = 7.2 Hz, 1H).

Synthesis of CD_2 (6). The compound **1**(0.105 g, 0.2 mmol), HATU (0.228 g, 0.6 mmol) and DIPEA (0.077 mg, 0.6 mmol) were added to a solution of DOX (0.232 g, 0.4 mmol) in dry DMF. After stirring at room temperature for 24 h, the DMF was evaporated under reduced pressure, and the residue was purified by the reversed-phase HPLC to give the compound CD_2 (0.205 g , 65% yield). HRMS (ESI) calcd for $[M + H]^+$: 1579.43, Found 1579.2802. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 14.011 (s, 2H), 13.245 (s, 2H), 8.451 (s, 2H), 7.869 (d, *J* = 4.2 Hz, 3H), 7.661-7.596 (m, 3H), 6.975 (d, *J* = 4.2 Hz, 1H), 5.438 (s, 2H), 5.222 (s, 1H), 4.850 (t, *J* = 21 Hz, 4H), 4.567 (s, 3H), 4.163 (d, *J* = 6.6 Hz, 2H), 3.959 (s, 9H), 3.394 (d, *J* = 4.8 Hz, 3H), 3.160 (s, 2H), 3.081-2.992 (m, 3H), 2.938 (s, 3H), 2.883 (s, 1H), 2.715 (d, *J* = 4.5 Hz, 3H), 2.265-2.067 (m, 5H), 1.830 (s, 5H), 1.723-1.611 (m, 4H), 1.424 (d, *J* = 8.4 Hz, 2H), 1.224 (s, 4H), 1.122 (d, *J* = 6.3 Hz, 6H), 0.976 (t, *J* = 7.2 Hz, 2H)

Synthesis of PEG-PPS diblock polymer. This polymer was synthesized according to our previous reports.[2] In brief, PEG thioacetate was dissolved in THF under a nitrogen atmosphere and was injected in sodium methoxide in a solution of MeOH. The reaction was kept at room temperature for 30 min, and then propylene sulfide was injected into the solution. After 45 min, the end capping agent iodoacetamide was added and the mixture stirred for overnight. The final product was obtained by precipitation with ethyl ether and repeatedly purified for three times.



Scheme S1. Chemical structures and synthetic routes of CED_2 (**A**) and CD_2 (**B**).

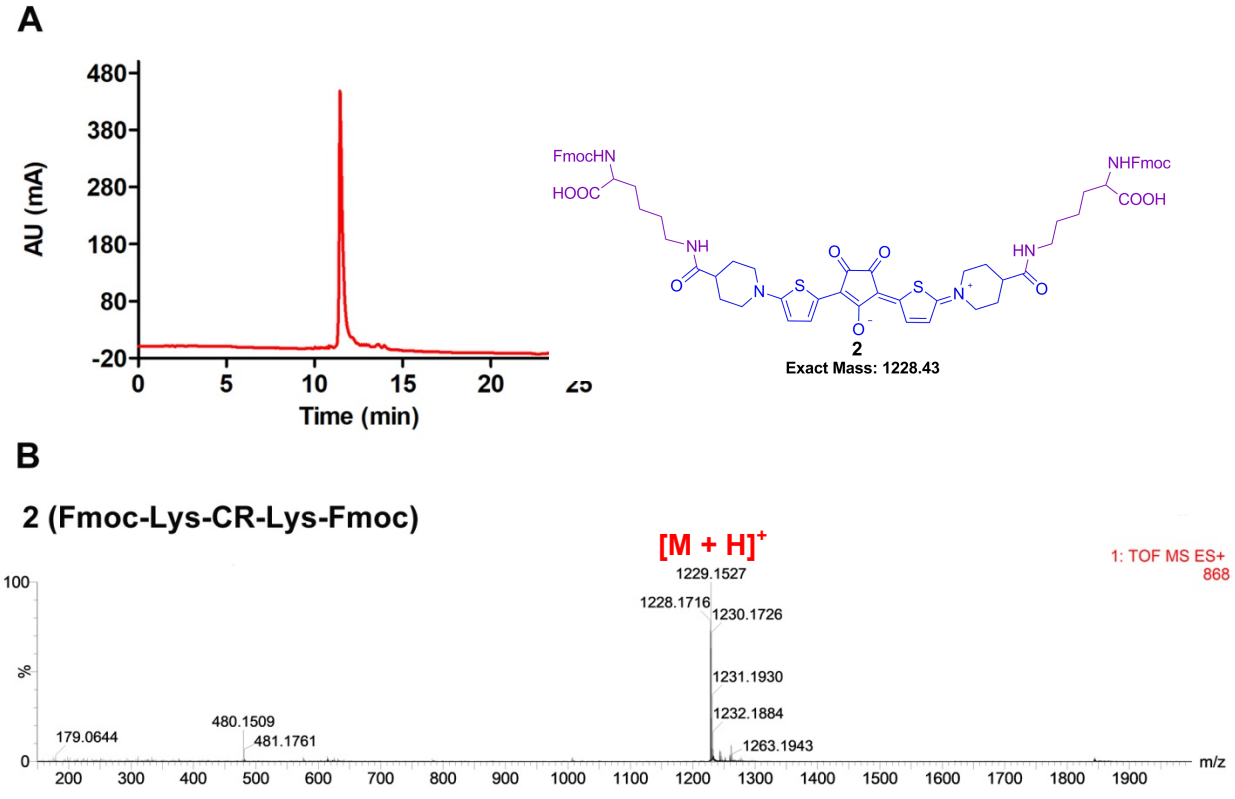
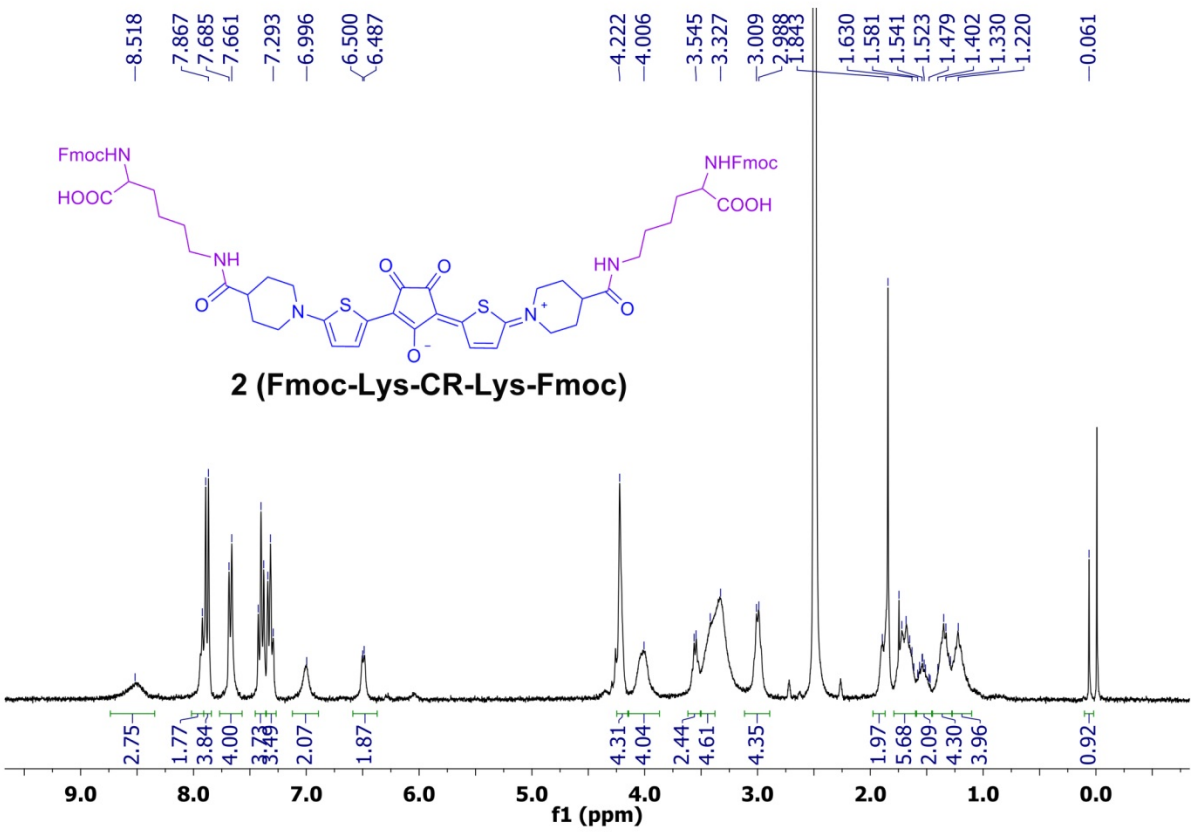


Figure S1. HPLC (**A**) and HRMS spectrum (**B**) of compound **2**.



Figrue S2. ^{1}H NMR spectrum (in d_6-DMSO) of compound **2.**

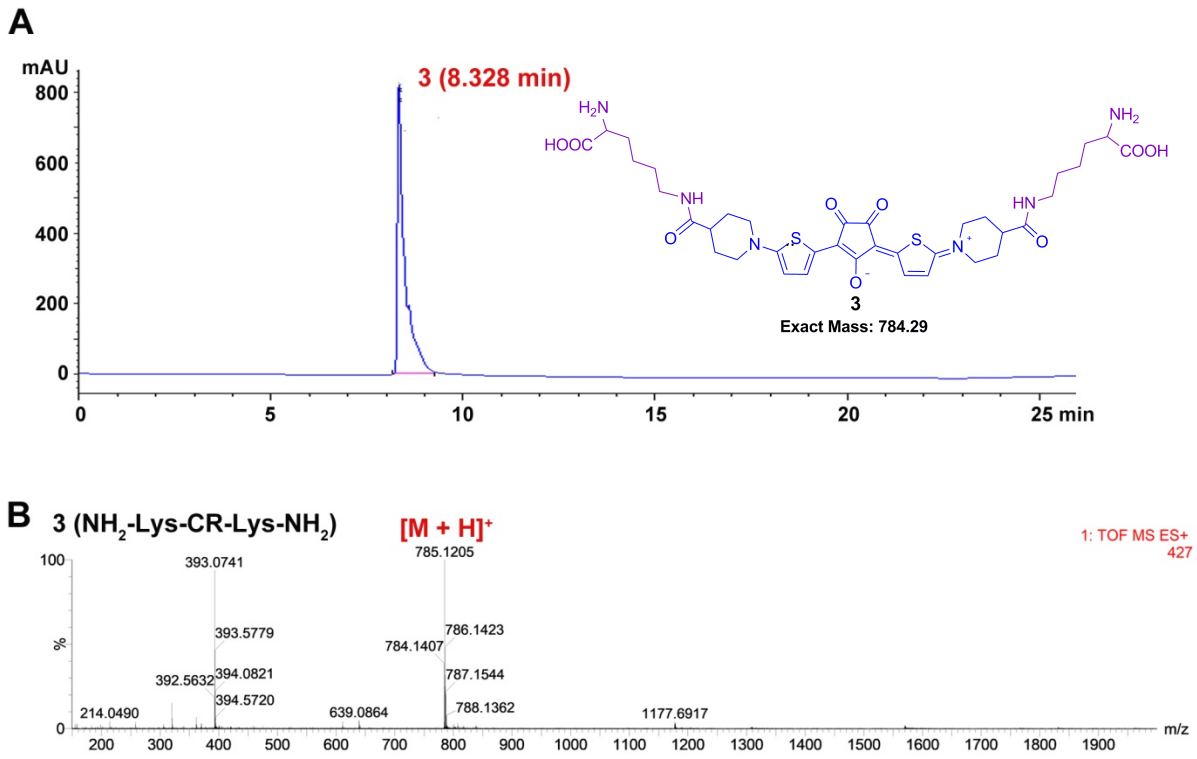


Figure S3. HPLC (**A**) and HRMS (**B**) spectrum of compound **3**.

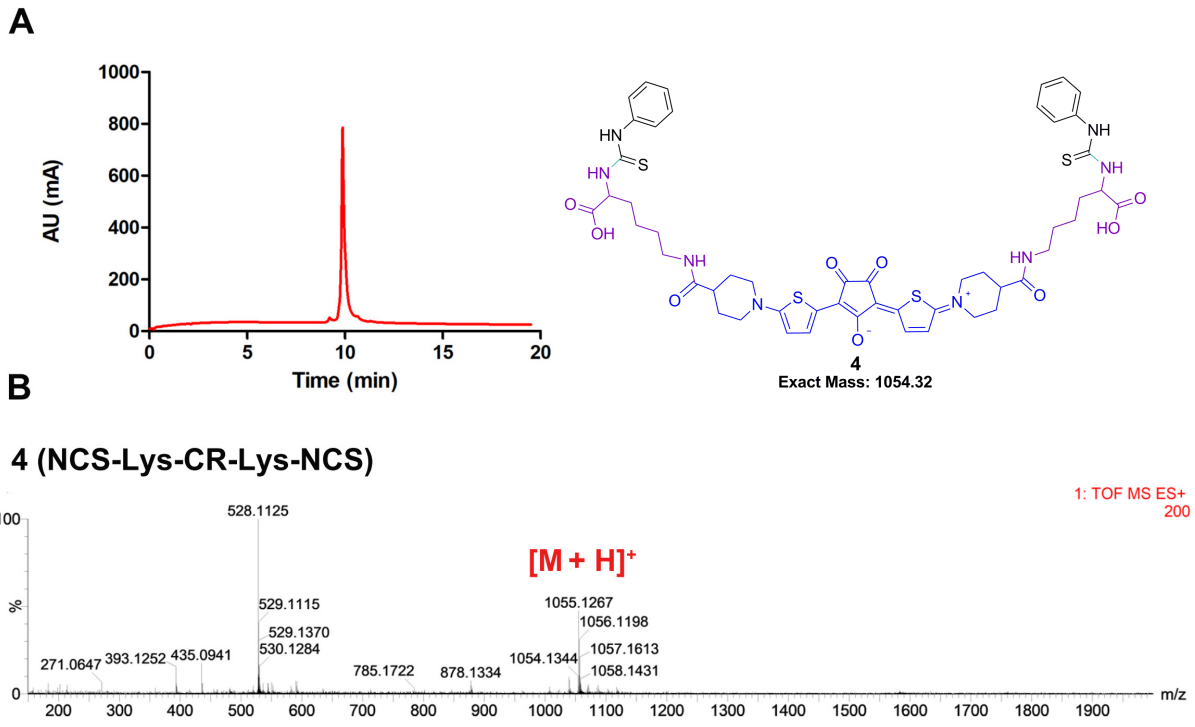
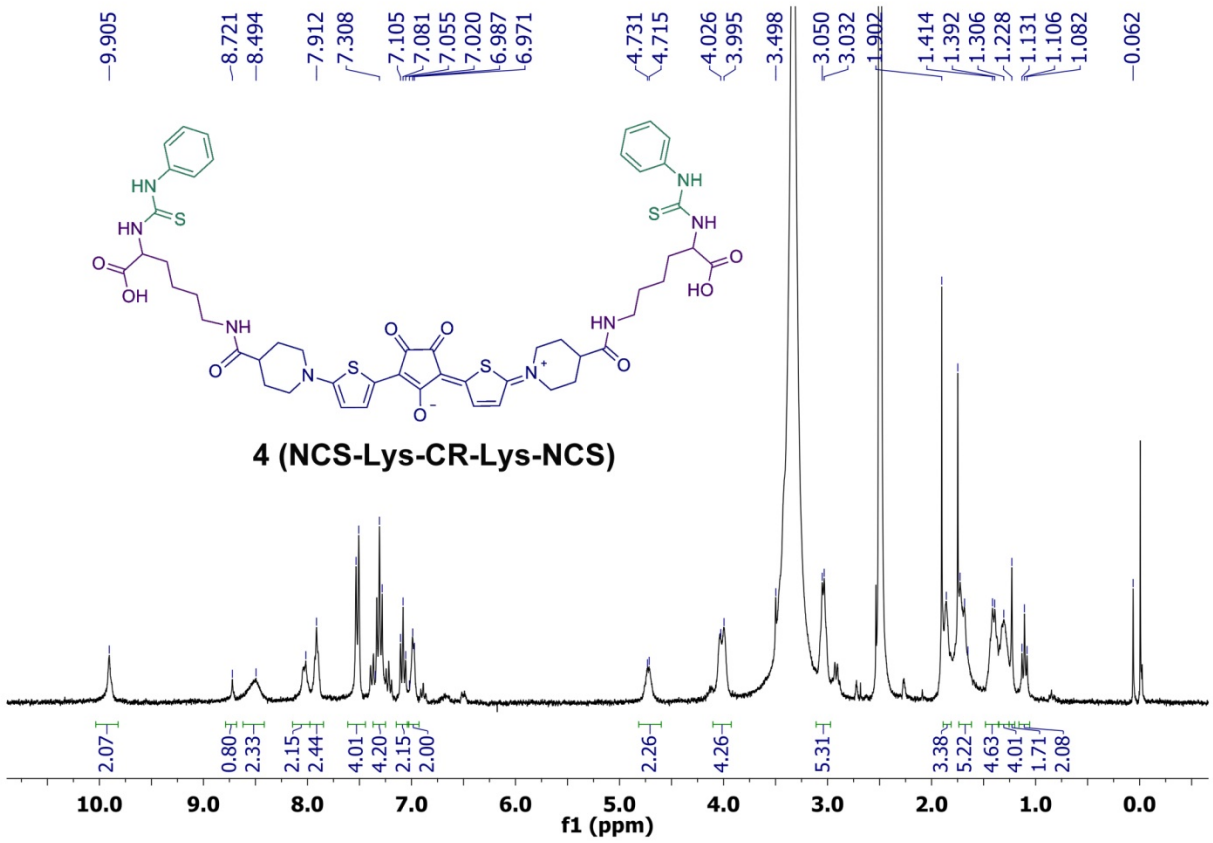


Figure S4. HPLC (**A**) and HRMS spectrum (**B**) of compound **4**.



Figrue S5. ^{1}H NMR spectrum (in d_6-DMSO) of compound **4.**

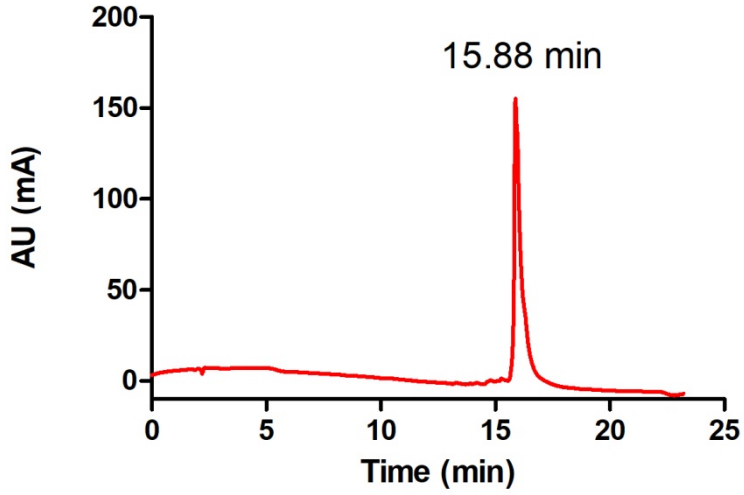


Figure S6. HPLC spectrum of compound **5 (CED_2)**.

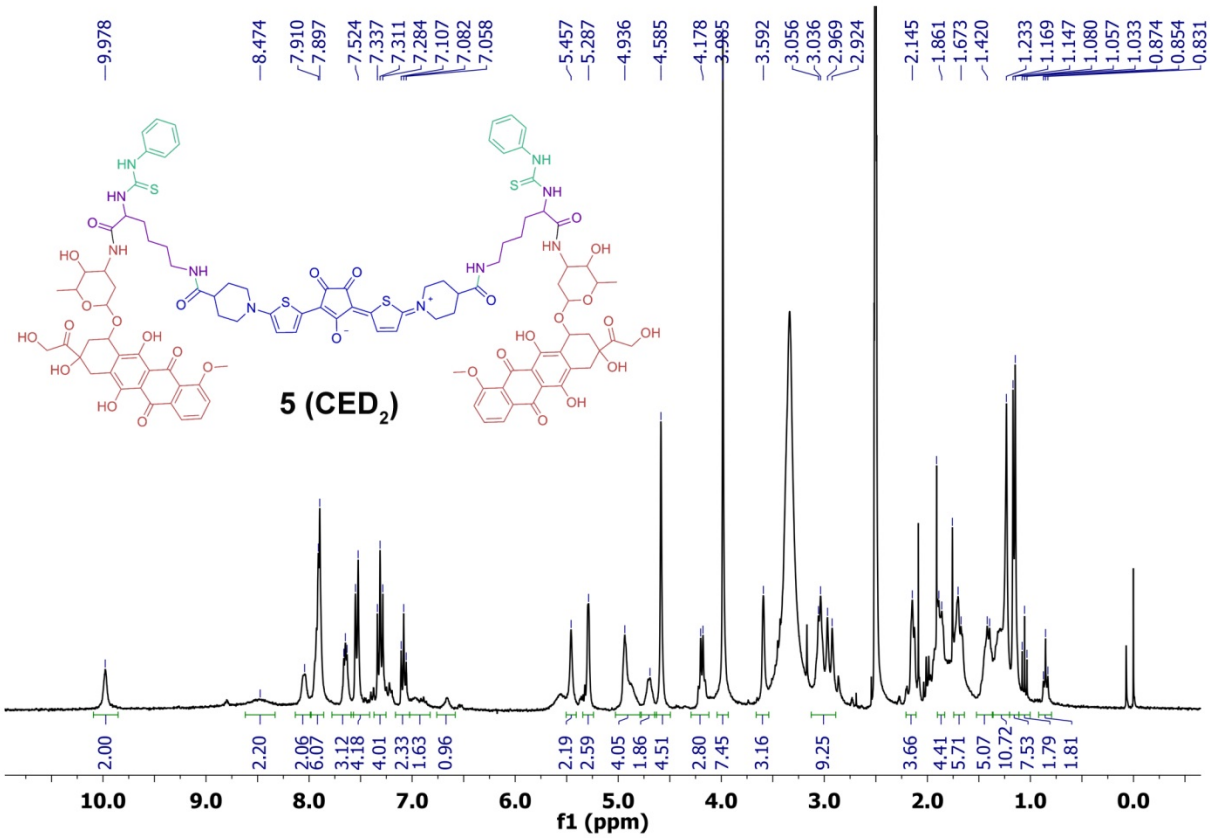


Figure S7. ^{1}H NMR spectrum (in d_6-DMSO) of compound **5** (**CED$_2$**).

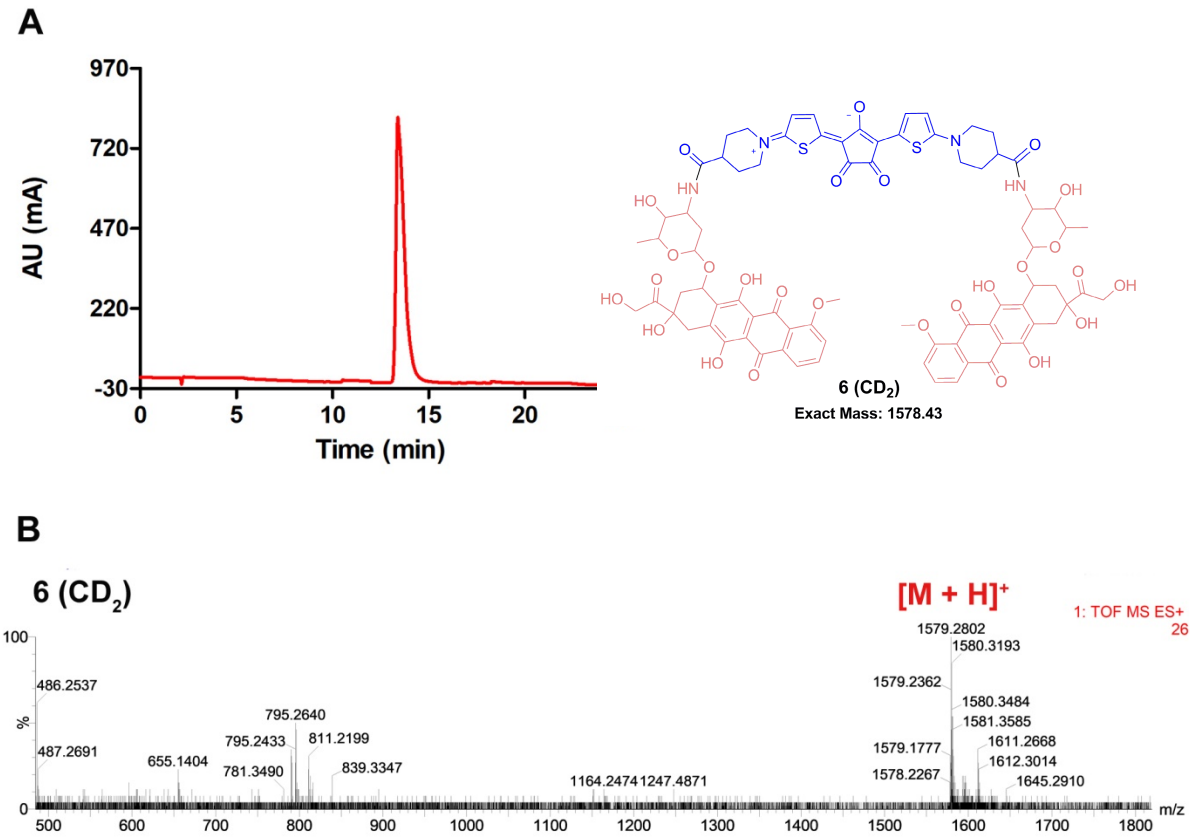


Figure S8. HPLC (**A**) and HRMS spectrum (**B**) of compound **6 (CD_2)**.

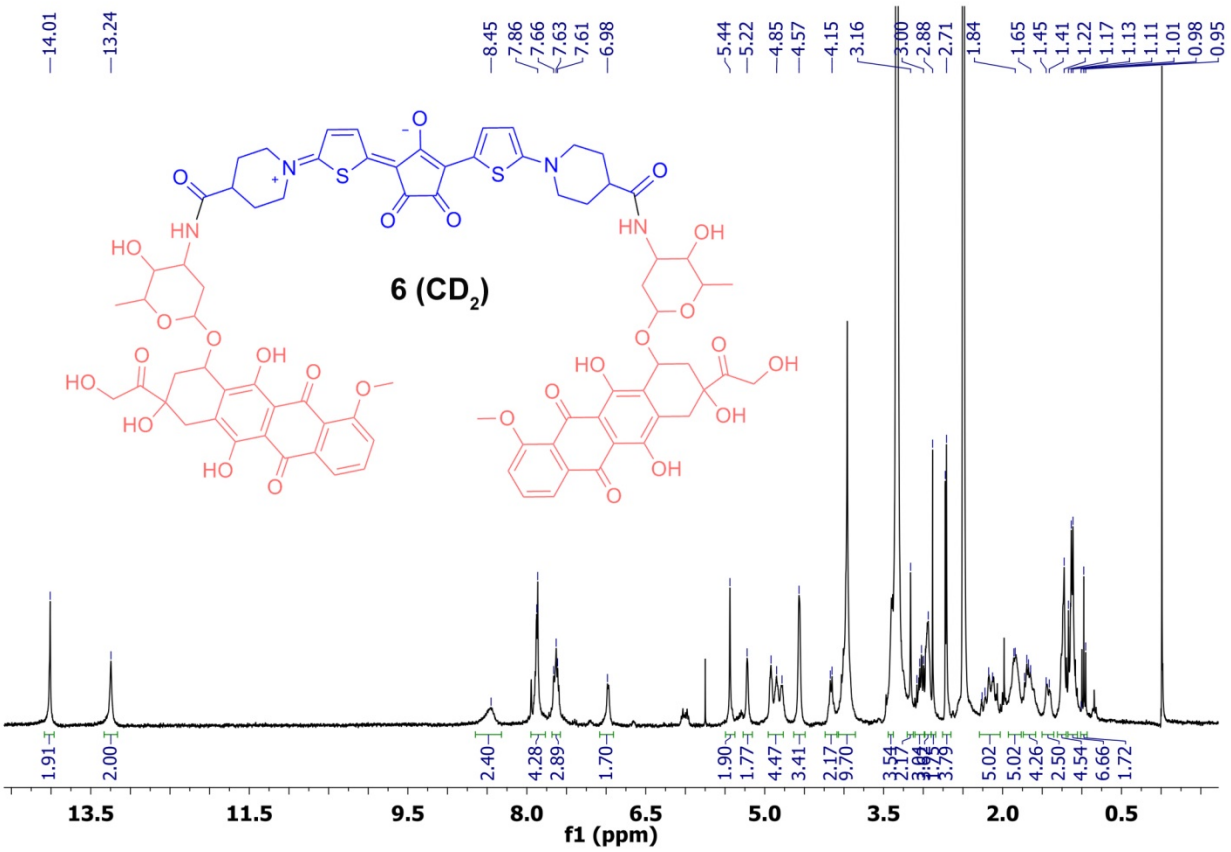


Figure S9. ^{1}H NMR spectrum (in d_6-DMSO) of compound **6** (**CD_2**).

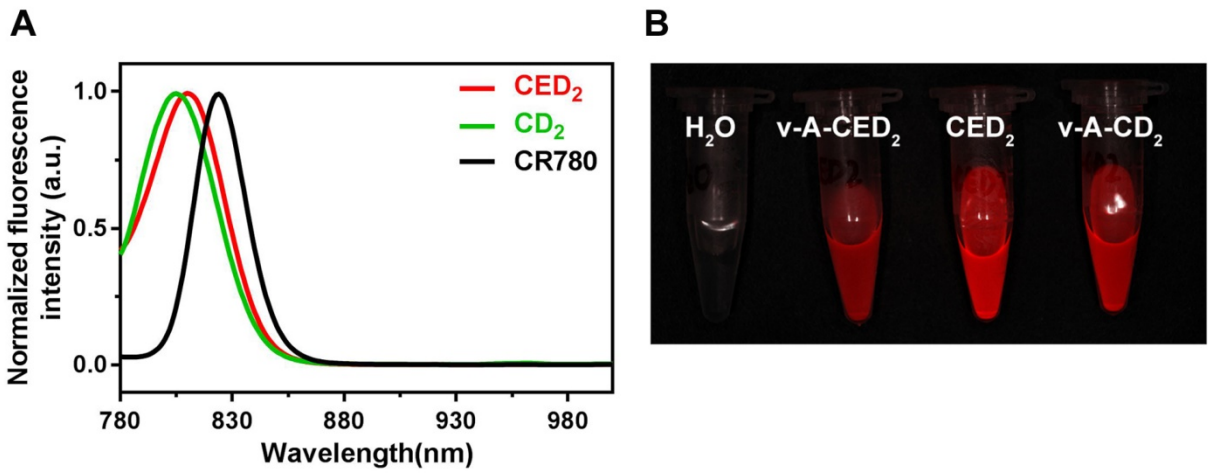


Figure S10. (A) Normalized fluorescence intensity of CED_2, CD_2 and CR780 recorded at room temperature using the same excitation wavelength (763 nm). (**B**) The NIR-I fluorescence images of different samples at the same concentration (10 μM).

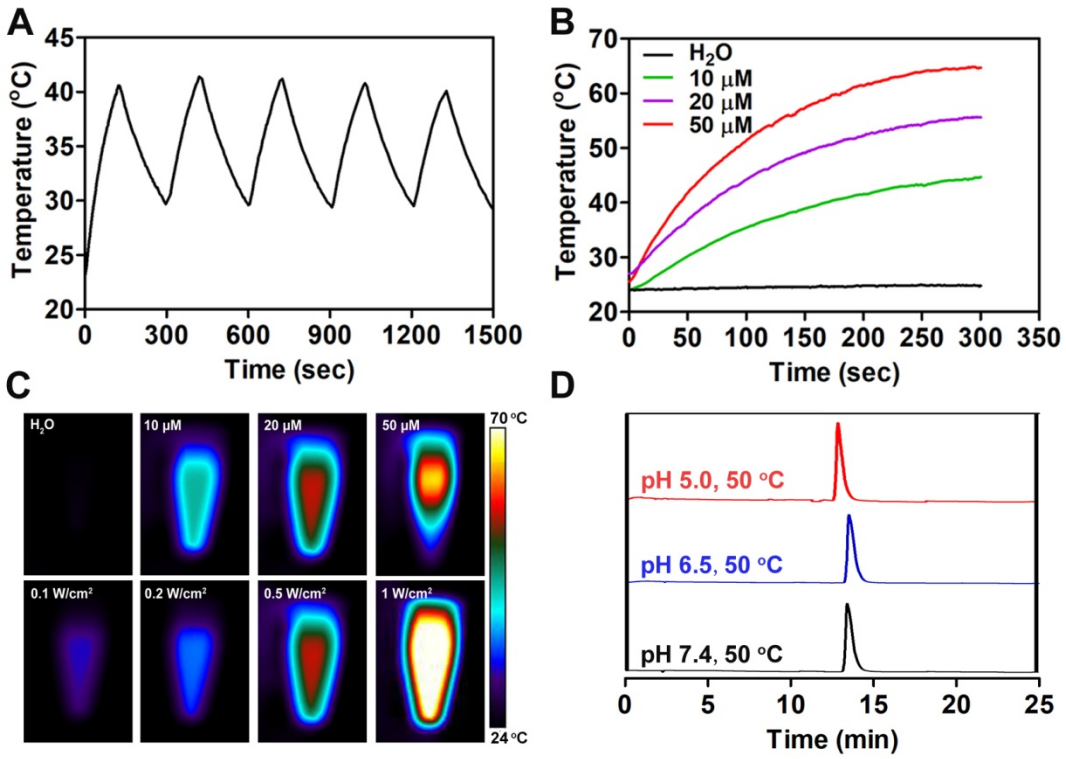


Figure S11. (A) Photothermal stability of CED_2 within five cycles of NIR laser irradiation. (B) Temperature increase profiles of CED_2 aqueous solution (10% DMSO) with different concentrations upon NIR laser irradiation (power density: 0.5 W/cm^2) for 5 min. (C) Photothermal images of CED_2 aqueous solution (10% DMSO) with different concentrations upon NIR laser irradiation (laser power density: 0.5 W/cm^2, upper row) for 5 min and 20 μM of CED_2 aqueous solution (10% DMSO) upon NIR laser irradiation with various power densities (bottom row) for 5 min. (D) The stability of CD_2 under different conditions; no degradation was found.

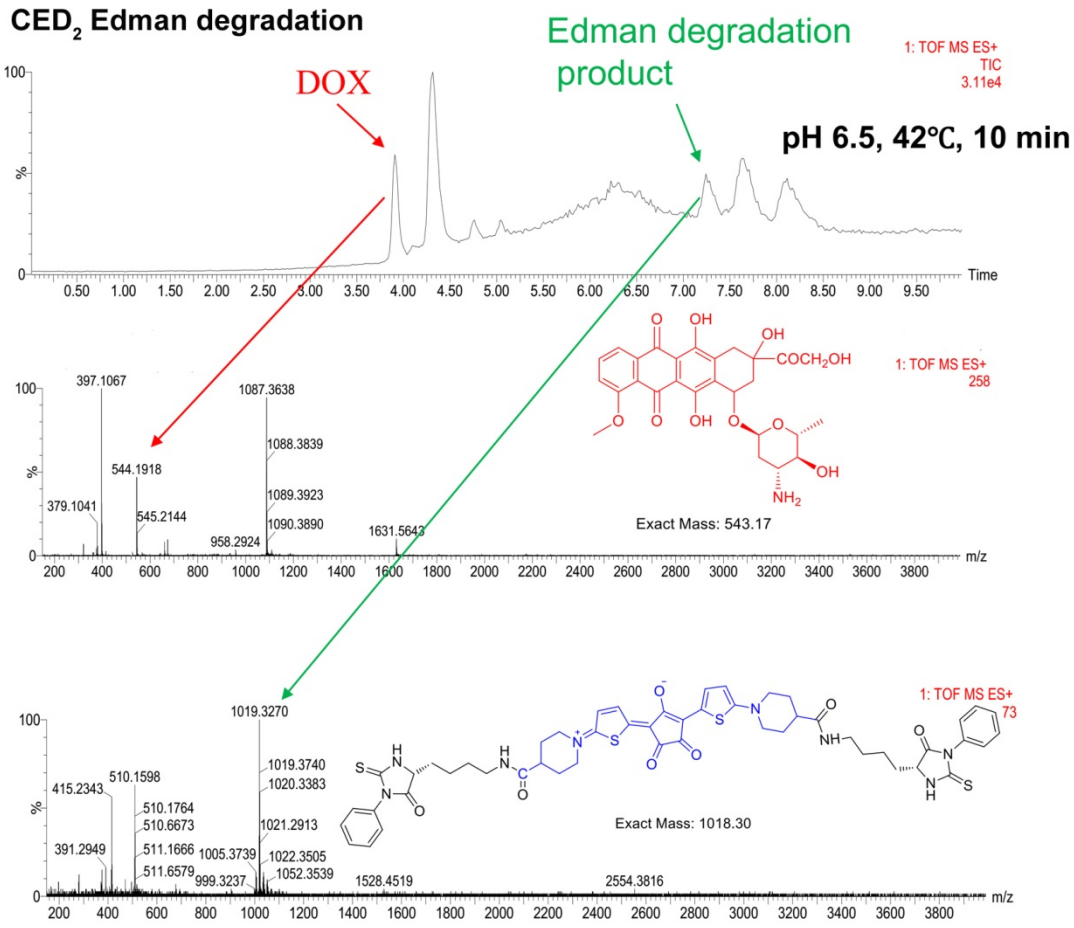


Figure S12. LC-MS analysis of the Edman degradation products of CED_2 after heating at 42 ^{o}C for 10 min in the phosphate buffer, pH 6.5.

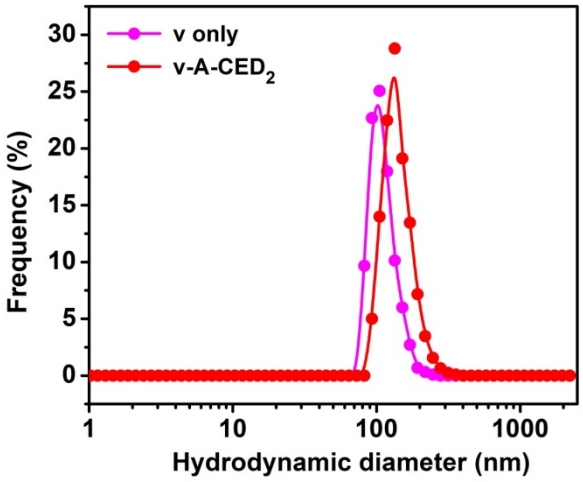


Figure S13. DLS measurements of the PPS-PEG (v only) and v-A-CED_2 vesicles, respectively.

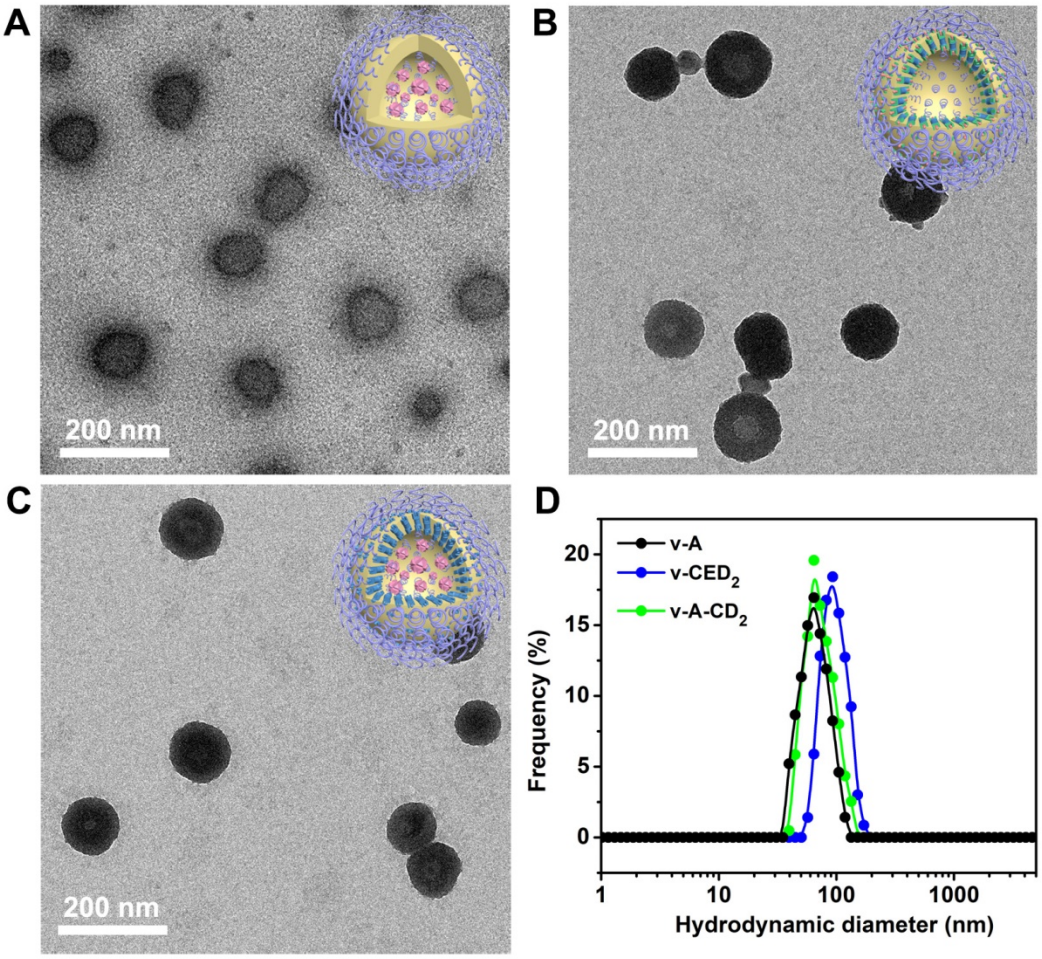


Figure S14. TEM measurements of different vesicle formulations, including v-A (A), v-CED_2 (B) and v-A-CD_2 (C), as well as DLS analysis of the above vesicles (D). Insets are cartoons for each vesicle formulation.

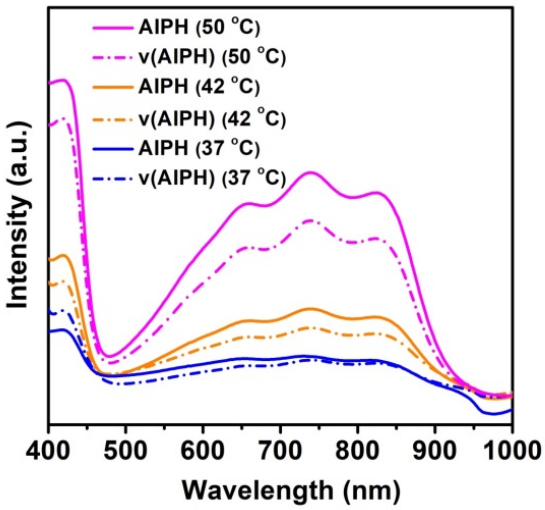


Figure S15. Generation of $ABTS^{+\bullet}$ as induced by the free radicals released from free AIPH and v(AIPH) (v-A) vesicles at different temperatures.

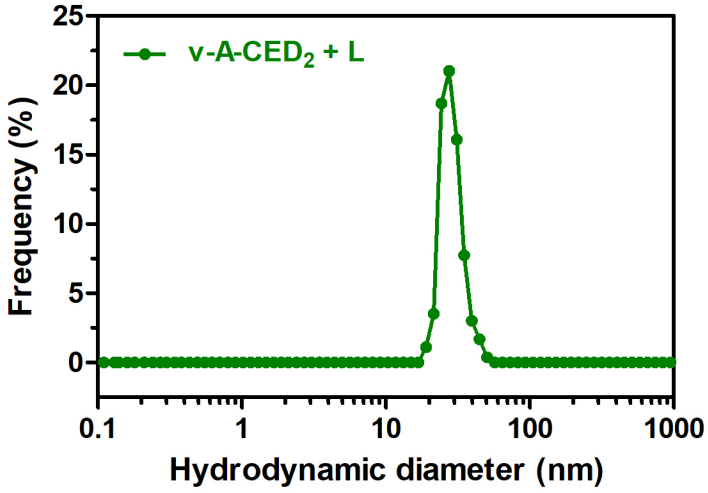


Figure S16. DLS-measured size of the v-A-CED_2 nanovesicles after NIR laser irradiation (0.5 W/cm^2, 5 min)

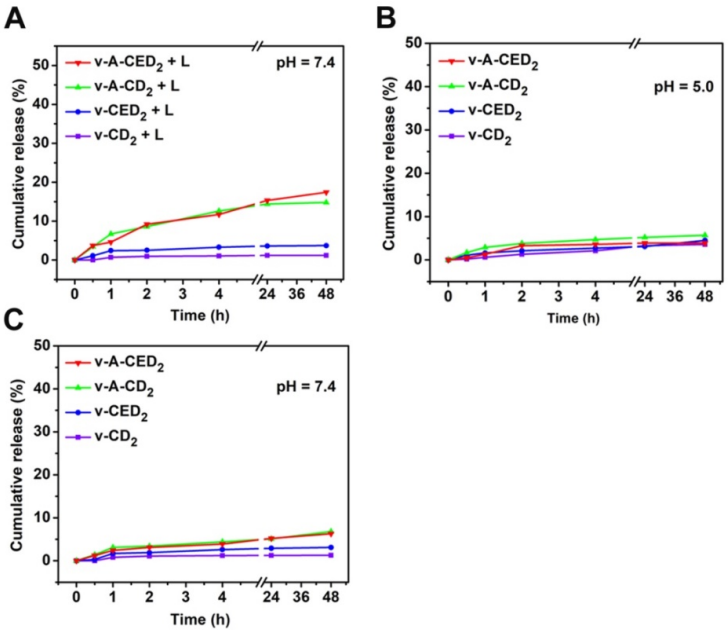


Figure S17. Additional drug release profiles of vesicle samples after treated with NIR laser (0.5 W/cm^2, 5 min) incubated with PBS (A) or without laser in phosphate buffer with pH 5.0 (B) or 7.4 (C).

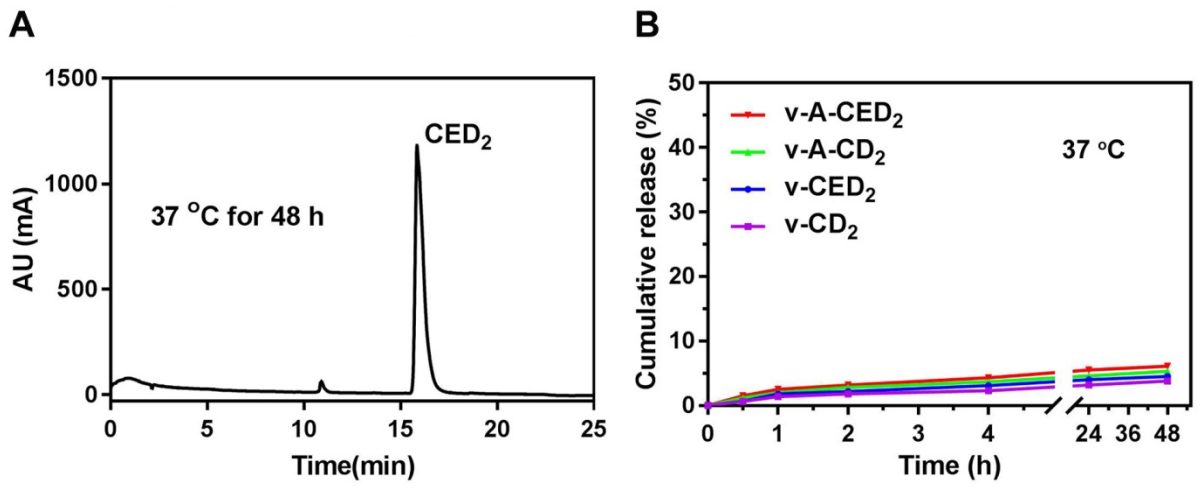


Figure S18. (A) The stability of CED_2 in mouse serum incubated at 37 °C for 48 h analyzed by HPLC. (B) The stability of different vesicles in mouse serum at 37 °C by testing the fluorescence intensity of DOX at different time points.

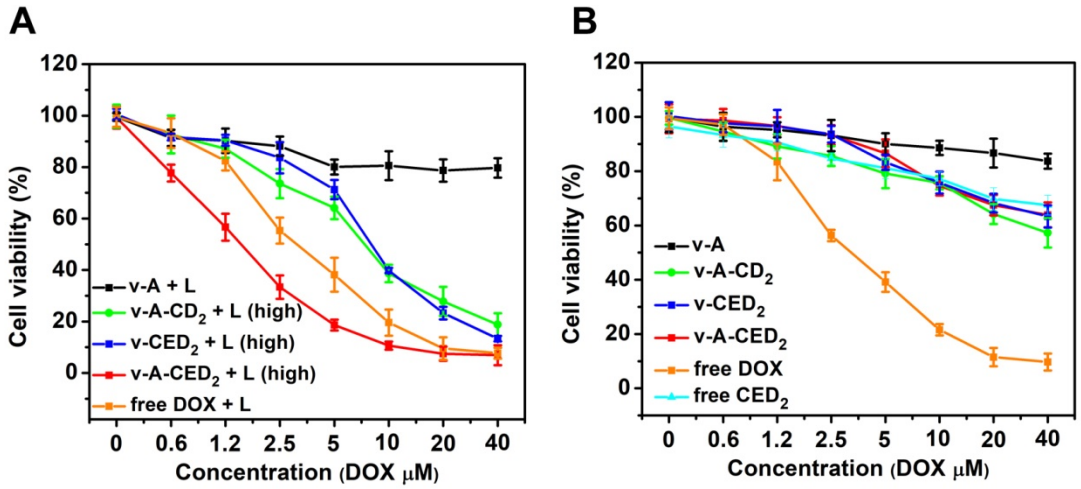


Figure S19. Cell viability assay on U87 MG cells at 24 h after different vesicles treatments with low laser intensity (L, 0.5 W/cm^2) or with high laser intensity (L (high), 1 W/cm^2) (A), or without laser (B). The concentrations were normalized to those of DOX included.

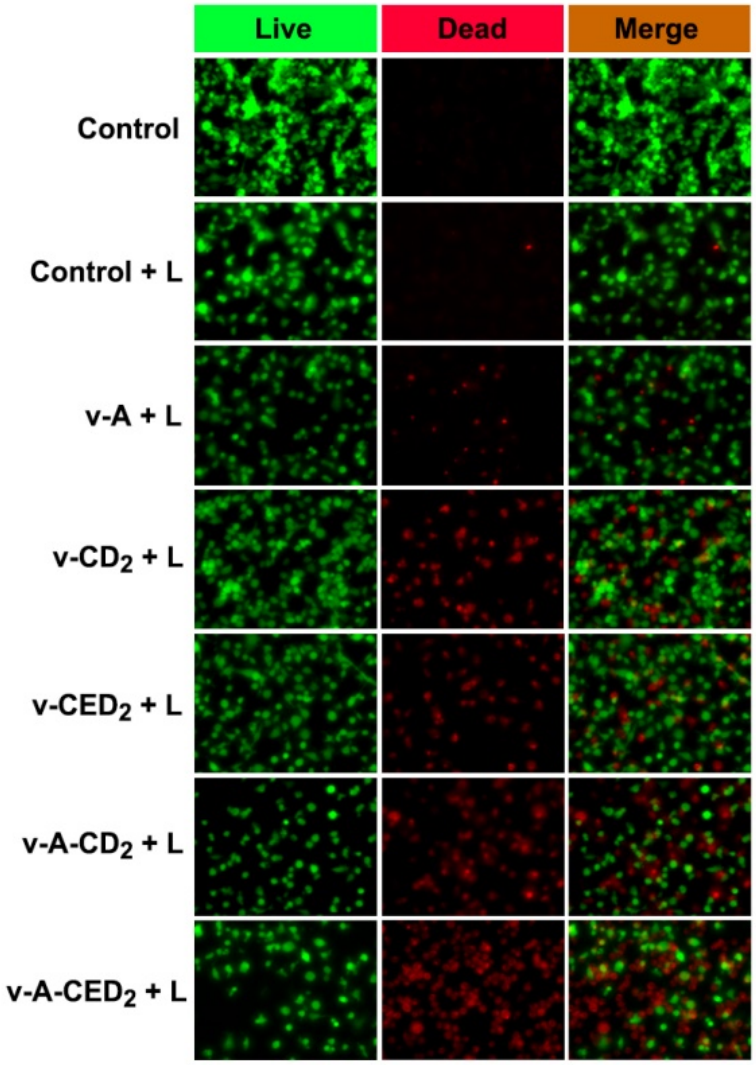


Figure S20. Fluorescent images of calcein AM/PI stained U87MG cells incubated with fresh medium or different vesicle formulations for 24 h after exposed to NIR laser irradiation ($0.5W/cm^2$, 5 min).

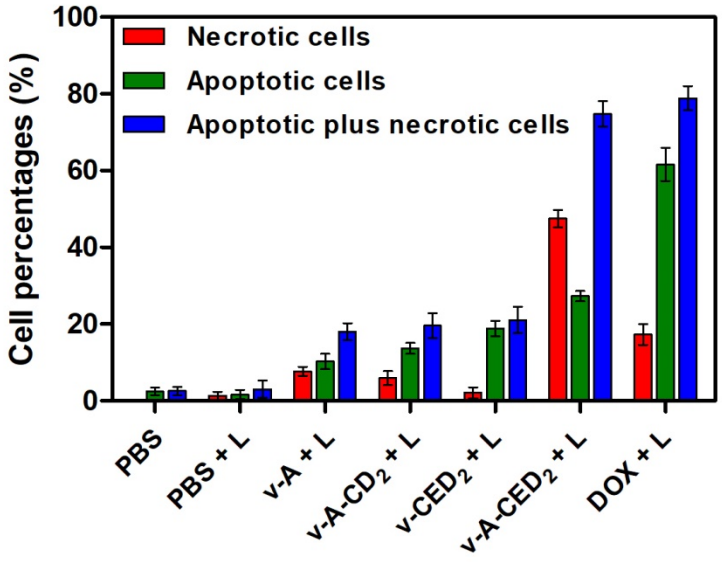


Figure S21. Analysis of the apoptotic and necrotic cell populations from the Annex V/PI co-staining test on U87MG cells after different treatments. L represents to NIR laser irradiation (0.5 W/cm^2, 5 min). Values are presented as mean ± s.d.

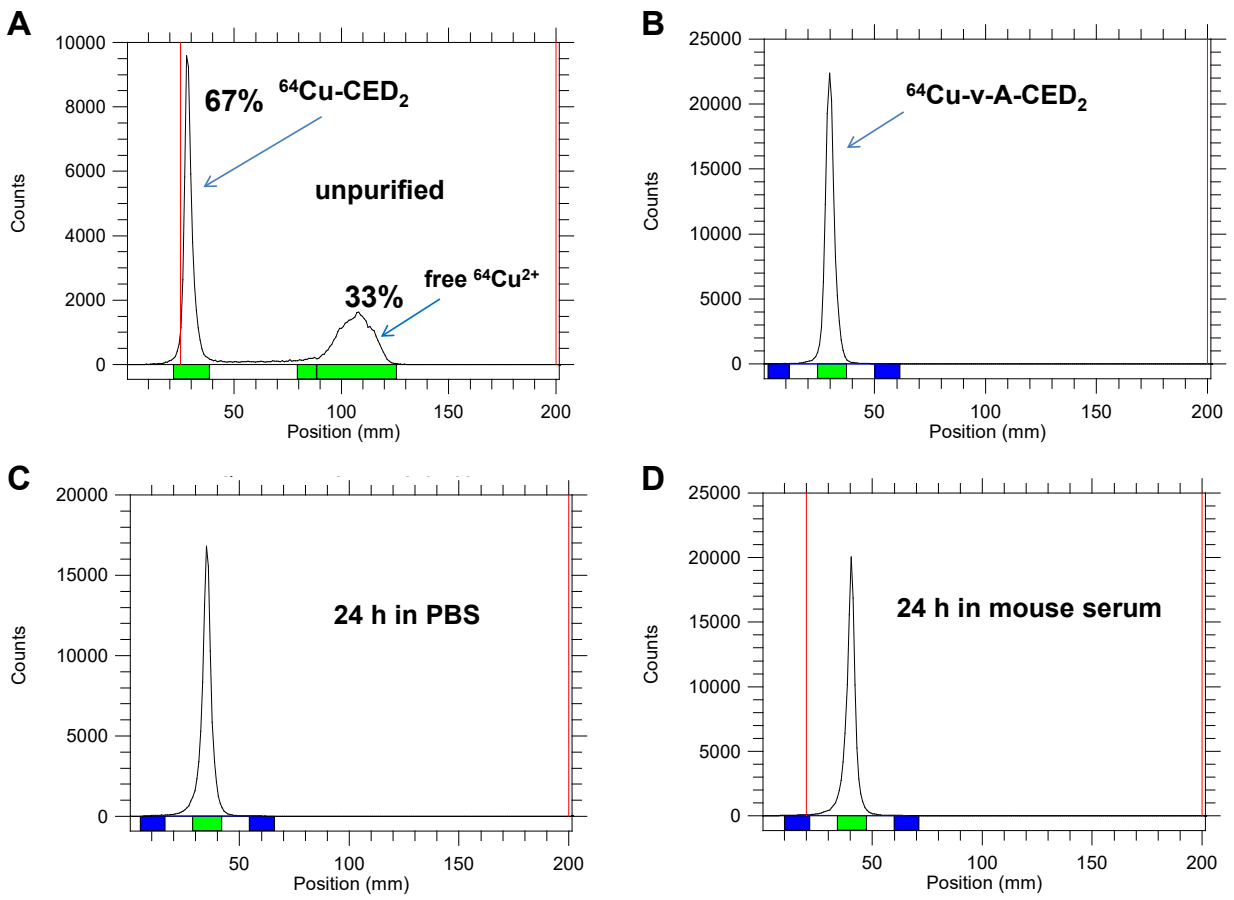


Figure S22. (A) Thin-layer chromatography (TLC) plate analyses of ^{64}Cu-CED$_2$ after labeling. (B) TLC plate analyses of ^{64}Cu-v-A-CED$_2$ after purification. (C) (D) The stability test of ^{64}Cu-v-A-CED$_2$ after incubated in PBS or mouse serum at 37 ^{o}C for 24 h.

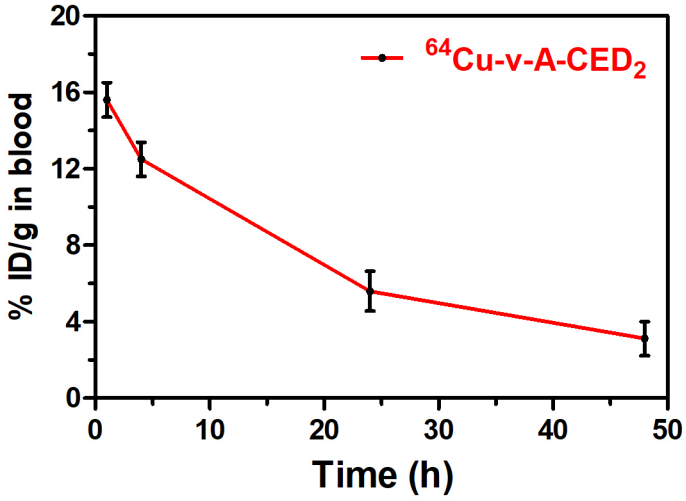


Figure S23. Quantification of %ID/g of ^{64}Cu-v-A-CED$_2$ in blood calculated from PET images.

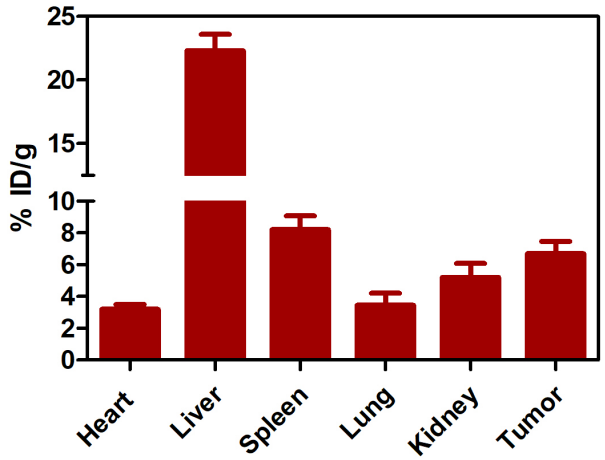


Figure S24. Biodistribution of the ^{64}Cu-labeled v-A-CED$_2$ determined by gamma counting at 48 h post injection (n = 3).

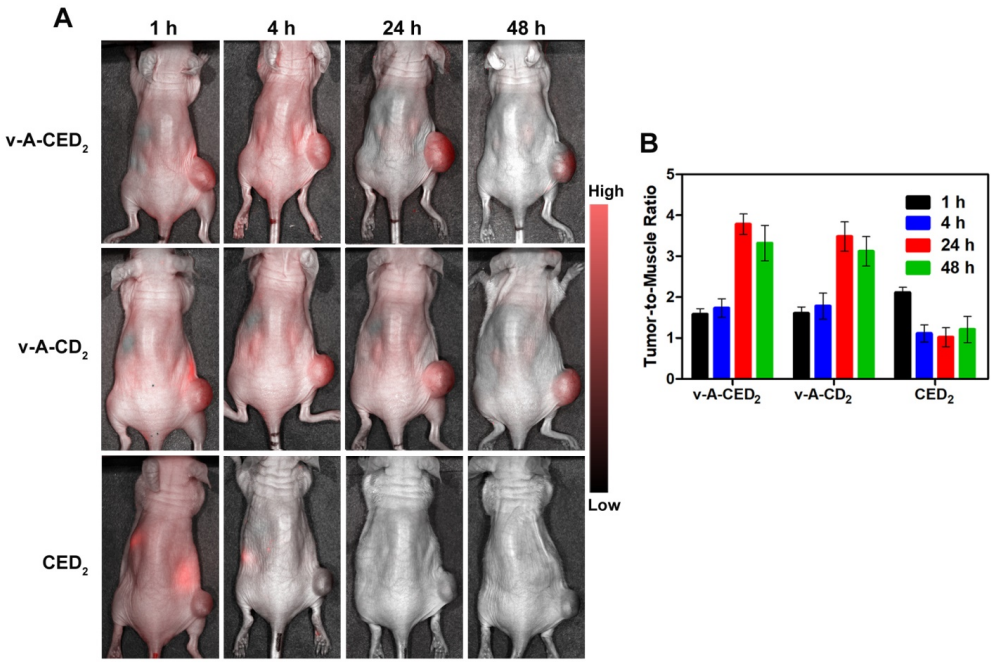


Figure S25. Representative whole-body NIR-I fluorescence imaging (A) and the corresponding tumor-to muscle ratios (B) of U87MG tumor-bearing mice after intravenous injection of different agents.

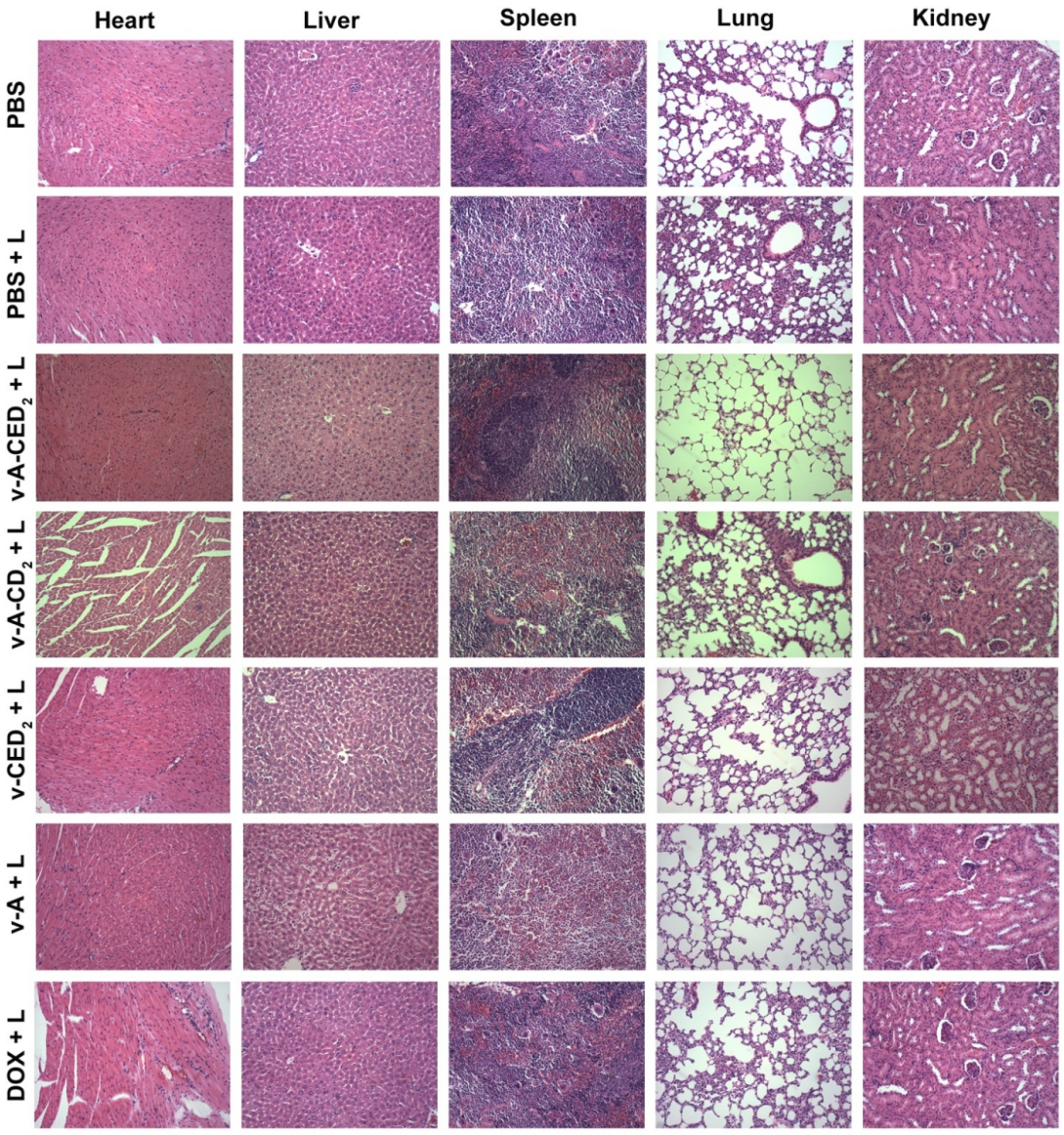


Figure S26. H&E staining results of major organs from mice with different treatments. The treatments include PBS, PBS + L, v-A-CED_2 + L, v-A-CD_2 + L, v-CED_2 + L, v-A + L, and DOX + L. L represents that mice were applied with NIR laser irradiation (0.5W/cm^2, 4 min). Mice were sacrificed at 16 days post-irradiation and the major organs for each mouse were collected, including heart, liver, spleen, lung, and kidney.

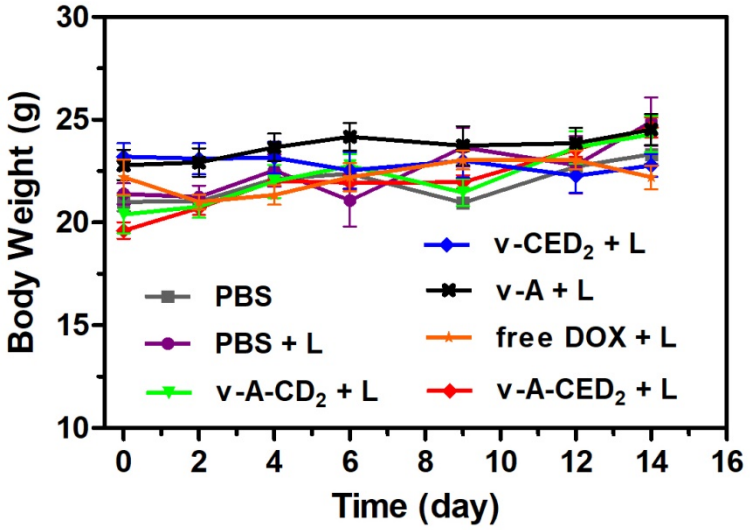


Figure S27. Body weight changes of mouse groups (5 mice for each group) with different treatments, including PBS, PBS + L, v-A-CD_2 + L, v-CED_2 + L, v-A + L, free DOX + L and v-A-CED_2 + L. L represents that mice were applied with NIR Laser irradiation (0.5 W/cm^2, 4 min) after 24 h administration of different formulations by intravenous injection.

References

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