Supporting Information

Light-Responsive Prodrug-Based Supramolecular Nanosystems for Site-Specific Combination Therapy of Cancer

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Materials and methods

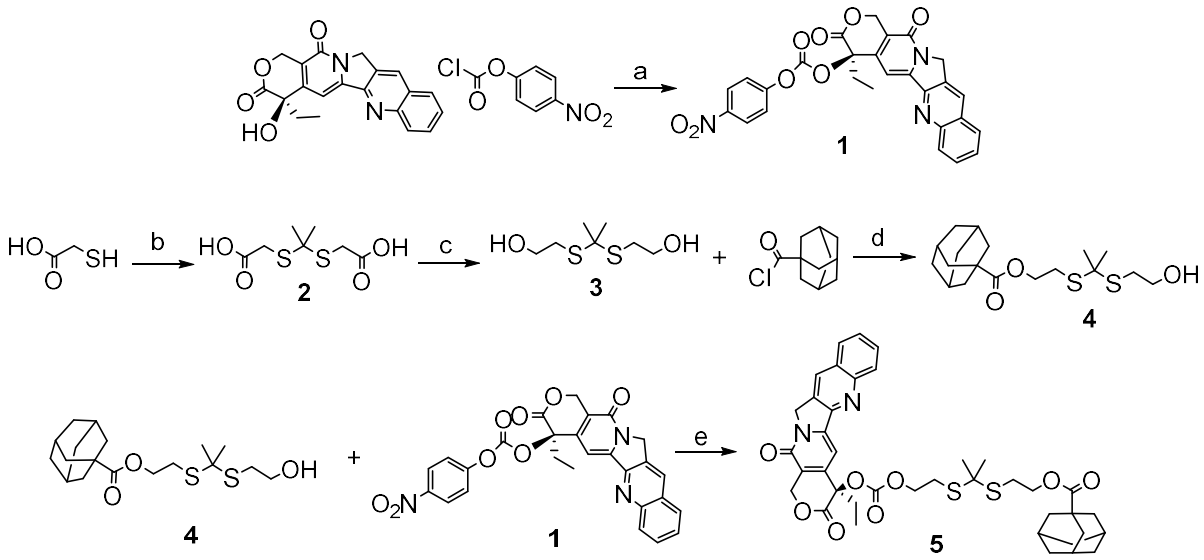
1.1 Materials

1-adamantanecarbonyl chloride, camptothecin (CPT), 4-nitrophenylchloroformate, and sodium hyaluronate (Mw = 8 to 12 kDa) were purchased from Aaron Pharmatech Ltd (Shanghai, China). 5,10,15,20-Tetrakis(4-hydroxyphenyl)-21H,23H-porphine (THPP), Amberlite® IR120 hydrogen form, anhydrous thioglycolic acid, dimethylaminopyridine (DMAP), 4-hydroxybenzaldehyde, 1-hydroxybenzotriazole hydrate (HOBt), pyrrole, and triethylamine (TEA) were purchased from Sigma Aldrich. β-Cyclodextrin (β-CD), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC•HCl), lithium aluminium hydride ($LiAlH_4$), propionic acid, and thiazolyl blue tetrazolium hydrate (MTT) were purchased from Tokyo Chemical Industry. Dry HCl gas was produced in situ by adding concentrated sulfuric acid to sodium chloride and then drying *via* concentrated sulfuric acid. Anhydrous THF and acetone were obtained by distilling over CaH_2 overnight. MDA-MB-231

and MCF-7 cells were obtained from American Type Culture Collection (ATCC). Dialysis membrane Spectra/Por 6 (MWCO: 2 kDa) was purchased from Thermo Fisher Scientific Pte Ltd. All chemicals were of analytical grade and used without further purification if not indicated otherwise.

1.2 Instruments

^{1}H NMR spectra were probed on a Bruker BBFO400 spectrometer, and D_2O, DMSO-d_6, and $CDCl_3$ were used as the deuterated solvents. The UV-vis-NIR absorption and fluorescence emission spectra were measured using Shimadzu UV-3600 and Shimadzu RF5301PC spectrophotometer, respectively. Transmission electron microscopy (TEM) images were taken at an acceleration voltage of 100 kV on JEOL JEM-1400. Hydrodynamic diameters and zeta potential values were measured at 25°C by a Malvern Zetasizer Nano-S system. HPLC analysis was performed with Shimadzu LC20AP with the Waters XSelect™ HSS C18 5 μm (4.6 x 250 mm) column at detection wavelength of 254 nm. Acetonitrile/water binary system containing 0.5% trifluoroacetic acid was used as the mobile phase with a flow rate at 1 mL/min. Confocal laser scanning microscopy (CLSM) images were acquired by ZEISS LSM 800 Confocal Laser Scanning Microscope.



Scheme S1. Synthesis procedure of aCPT prodrug. Conditions: (a) DMAP, DCM, 0°C, yield: 88%, (b) anhydrous acetone, HCl (g), yield: 56%, (c) $LiAlH_4$ in dry THF, yield: 59%, (d) pyridine in anhydrous diethyl ether, yield: 34%, (e) DMAP reflux in dry DCM, yield: 24%.

1.3 Synthesis of aCPT

*1.3.1 Synthesis of **1***

The synthesis was reported in previous literature.[1] Briefly, camptothecin (CPT, 300 mg, 861 μmol) and 4-nitrophenylchloroformate (608 mg, 3.01 mmol) were dissolved in dry DCM (45 mL) at 0°C. Dimethylaminopyridine (DMAP, 631 mg, 5.17 mmol) was added to result in a yellow solution. The solution was then stirred at 0°C for 6 h. The mixture was washed with water (60 mL). The organic layer was dried over sodium sulfate followed by the filtration, and excess solvents were removed in vacuo. Purification was conducted by silica column chromatography. DCM (100 mL) was first used, followed by 50% DCM/EtOAc (300 mL) and then EtOAc (200 mL) to give the product as a pale yellow solid (265 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) 8.42 (1H, s), 8.22 (3H, m), 7.96 (1H, m), 7.86 (1H, m), 7.69 (1H, m), 7.38-7.41 (3H, m), 5.71 (1H, d, $J_{1,2}$ 17.3), 5.42 (1H,d, $J_{1,2}$ 17.3), 5.31 (2H, d) 2.21-2.41 (2H, m), 1.07 (3H, t, J 7.5 Hz).

*1.3.2 Synthesis of **2***

The synthesis was reported in previous literature.[2] Anhydrous thioglycolic acid (9.21g, 100 mmol) and anhydrous acetone (11.6g, 200 mmol) were purged with dry HCl gas under stirring at room temperature for 6 h. The mixture was then cooled in an ice bath. The mixture was filtered, and washed with ice cold hexane and ice cold water to give white solid (6.24 g, 56%). ^{1}H NMR (400 MHz, d-DMSO) 12.6 (2H, s, br), 3.37 (4H, s), 1.54 (6H, s).

*1.3.3 Synthesis of **3***

The synthesis was reported in previous literature with some minor modifications.[2] Compound **2** (1.5 g, 6.69 mmol) was dissolved in anhydrous THF (150 mL) and cooled in an ice bath. $LiAlH_4$ (1.52 g, 40.1 mmol) was slowly added over 30 min, purging with N_2 to prevent the buildup of hydrogen gas. After the addition was done, the mixture was allowed to stir at room temperature for 4 h. The reaction was quenched by the addition of water (1 mL). After which, the mixture was filtered. The filtrate was evaporated to dryness. DCM was added followed by Na_2SO_4 to remove water. The mixture was filtered and evaporated to obtain an orange liquid. Purification was conducted by silica column chromatography at 5% MeOH/DCM to give product as a yellow liquid (0.774 g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) 3.73 (4H, t, J 6.4 Hz), 3.33 (2H, s), 2.81 (4H, t, J 6 Hz), 1.574 (6H, s).

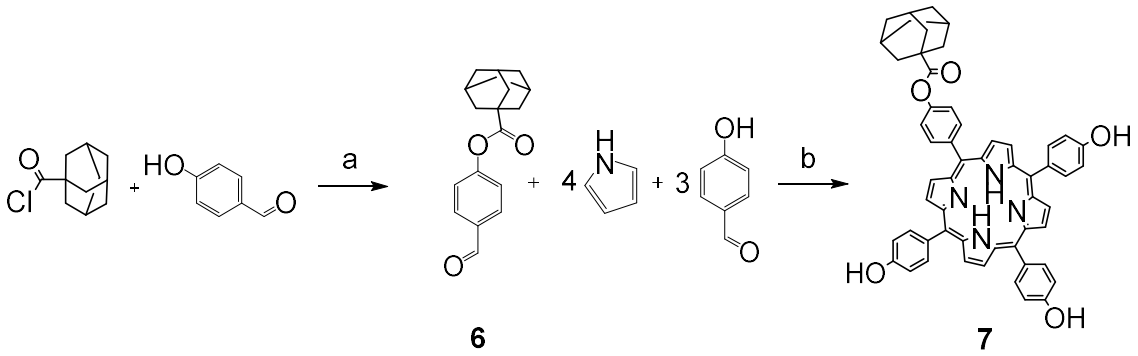
*1.3.4 Synthesis of **4** (TL)*

1-adamantanecarbonyl chloride (1.04 g, 5.24 mmol) and compound **3** (0.858 g, 4.37 mmol) were added to anhydrous diethyl ether (100 mL) and anhydrous pyridine (0.415 g, 5.24 mmol). The reaction was stirred at 23-25°C for 3 days. The organic layer was extracted with

DCM and brine. Purification was carried out by silica column chromatography using 10% EA/hexane to give compound **4** as an orange liquid (0.53 g, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) 4.21 (2H, t, 6.8 Hz), 3.79 (2H, t, 5.4 Hz), 2.86 (4H, m), 2.01 (3H, s), 1.89 (6H, s), 1.71 (6H, s), 1.62 (6H, s). HR-MS (ESI+): m/z $C_{18}H_{30}O_3S_2Na$, $[M+Na]^+$ calcd. 381.1534, found 381.1541.

*1.3.5 Synthesis of **5** (aCPT)*

Compounds **1** (265 mg, 516 μmol) and **4** (278 mg, 775 μmol) were dissolved in dry DCM (60 mL). DMAP (95 mg, 775 μmol) was then added and the mixture was left to reflux at 75 °C overnight. The mixture was then allowed to cool to room temperature. Purification was carried out by flash chromatography with DCM (100 mL), 5% EtOAc/DCM (100 mL), 10% EtOAc/DCM (100 mL), and 15% EtOAc/DCM (300 mL) to give aCPT as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) 8.39 (1H, s), 8.21 (1H, d, J 8.4 Hz), 7.93 (1H, d, J 8.4 Hz), 7.83 (1H, t, J 7.6), 7.67 (1H, t, J 7.6), 7.33(1H, s), 5.71 (1H, d, J 17.2), 5.42 (1H,d, J 17.2), 5.29 (2H, s) 4.30-4.19 (2H, m), 4.15-4.08 (2H, m), HR-MS (ESI+): m/z $C_{39}H_{45}N_2O_8S_2$, $[M+H]^+$ calcd. 733.2617, found 733.2631.



Scheme S2. Synthesis of aPS. Conditions: (a) TEA at 0^{o}C in THF, (b) propionic acid, reflux for 1 h.

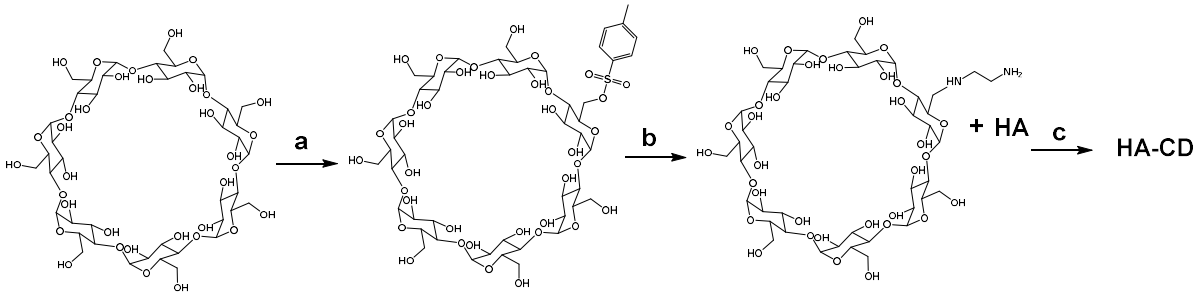
1.4 Synthesis of aPS

*1.4.1 Synthesis of **6***

Triethylamine (1.53 mL) and 4-hydroxybenzaldehyde (1.24 g, 10.2 mmol) in THF (10 mL) were first cooled in ice bath before dropwise addition of 1-adamantanecarbonyl chloride (3.03 g, 15.2 mmol) in THF (10 mL) into the solution. The mixture was left to stir at room temperature for 12 h before THF was removed under vacuo. The residue was dissolved in ether (50 mL), followed by washing with 1M Na_2CO_3 (3x 50 mL) and brine (50 mL). The resultant solution was dried over Na_2SO_4. After the filtration, excess solvents were removed in vacuo to yield **6** as a white solid (1.42 g, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) 9.99 (1 H, s), 7.91 (2 H, m), 7.24 (2 H, m), 2.10 (3 H, s), 2.60 (6 H, s), 1.75 (6 H, m).

1.4.2 Synthesis of 7 (aPS)

Compound **6** (1.42 g, 5.00 mmol) and 4-hydroxybenzaldehyde (1.83 g, 15.0 mmol) first underwent reflux in propionic acid (150 mL). Upon the addition of pyrrole (1.34 g, 20.0 mmol), the reaction mixture was refluxed for another 1 h. Reaction mixture was cooled to room temperature and then cooled further to 0°C for 15 min. The cooled mixture was filtered, and purple solid residue was obtained. The solid was dissolved in DCM (250 mL) and filtered to remove impurities. The organic solution was washed with 1 M $NaHCO_3$ (50 mL), follow by brine (50 mL) and then dried over Na_2SO_4. Purification was carried out by flash chromatography with 2 % MeOH/DCM to give aPS as a purple solid. ^{1}H NMR (400 MHz, $CDCl_3$) 8.86 (8 H, s), 8.20 (2 H, m), 8.05 (6 H, m), 7.45 (2 H, m), 7.19 (6 H, m), 2.38 (3 H, s), 2.24 (6 H, s), 2.18 (6 H, s). HR-MS (ESI+): m/z $C_{55}H_{45}N_4O_5$, [M+H]+ calcd. 841.3390, found 841.3378.



Scheme S3. Synthesis procedure of HA-CD polymer. Condition: (a) NaOH, TsCl, (b) ethylenediamine, reflux in DMF under N_2, (c) EDC.HCl, HOBt for 2 days.

1.5 Synthesis of β-cyclodextrin modified hyaluronic acid (HA-CD)

Sodium hyaluronate was pretreated with cationic exchange resin (Amberlite® IR120 hydrogen form) to give hyaluronic acid in the following procedure. Sodium hyaluronate (1 g) was first dissolved in deionized water (300 mL), passed through the cationic exchange resin slowly, and then lyophilized to give hyaluronic acid. *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC•HCl) (0.23 g, 1.21 mmol) and 1-hydroxybenzotriazole hydrate (HOBT, 0.16 g, 1.05 mmol) were added into a solution of hyaluronic acid (0.14 g, 14.6 μmol) in DMSO (10 mL). The mixture was then stirred at 25°C for 30 min. Mono-6-deoxy-6-ethylenediamino-β-CD (0.81 g, 0.69 mmol) in DMSO (10 mL) was added into the mixture, and the obtained solution was stirred at room temperature for 24 h. The resulting mixture was dialyzed against DMSO (4 x 100 mL) and then an excess amount of deionized water for 3 days (2 L x 10). The sample was freeze-dried to give HA-CD as white solid.

The degree of β-CD substitution onto HA is defined as the number of β-CD molecule per 100 sugar residues of HA estimated using ^{1}H NMR spectra in D_2O. The chemical structure of the HA-DA conjugate was confirmed using ^{1}H NMR spectra, as shown in **Figure S4.** The characteristic resonances of HA appeared at 2.03 ppm and 3.34–4.54 ppm, whereas those of β-CD were found at the range of 3.39 – 3.92 and 5.07 ppm. Successful introduction of β-CD onto HA polymers was confirmed by the proton peaks of CD. The amount of β-CD in the conjugate was quantitatively characterized from the integration ratio between the characteristic peak of the *N*-acetyl group in HA (δ =∼ 2.03 ppm [3H, -COC*H*3-]) and that of the proton on the anomeric carbon in β-CD (δ = ∼ 5.07 ppm [7H, -C*H*-]). The degree of substitution (defined as the number of β-CD per 100 sugar residues of HA polymer) was around 6.56% for the HA-CD conjugate. The concentration of β-CD in 1 mg/mL of HA-CD was calculated to be 135 μM.

1.6 Drug release using HPLC

A reverse phase HPLC was used. Buffer A was 0.5% trifluoroacetic acid (TFA) in water, while buffer B was 0.5% TFA in acetonitrile (ACN). The analysis was performed at 25ºC with elution gradient. Briefly, the procedures are: 5% buffer B for 5 min, ramping of 5% buffer B to 95% buffer B in 30 min, followed by a decrease of 95% buffer B to 5% in 2 min. Retention times of CPT, aPS and aCPT were first determined to be 12.0, 15.3 and 16.1 min. aCPT (45 μM) with different concentrations of aPS (0, 45, 90, and 135 μM) were prepared and illuminated with light of 660 nm. The amount of free CPT released was then calculated from the standard calibration curve obtained from different CPT concentrations (0, 6.25, 12.5, 25, 50 and 100 μM) in 10% DMSO/H_2O using the external standard method, whereby the area under the curve of peak corresponding to CPT was directly proportionate to the concentrations of CPT prepared.

1.7 Stability constants determined by Hildebrand-Benesi plot

For the determination of the stability constants, the Hildebrand-Benesi method was employed. UV-vis spectrum of aPS aqueous solution (2 x 10^{-5} M) was measured in the absence of light. After which, β-cyclodextrin aqueous solution (20.0 mg mL^{-1}, 5 μL) was added to the aPS solution, and the UV-vis spectrum of the resulting solution was measured. The process was repeated systematically by the addition of β-cyclodextrin aqueous solution (20.0 mg/mL) into the same solution. The absorbance at 435 nm was recorded. Bensi-Hildbrand plot was then illustrated taking $\frac{1}{\Delta A}$ against $\frac{1}{[H]_0}$ by following equation (1):

$$\frac{1}{\Delta A} = \frac{1}{b\Delta\varepsilon[G]_0[H]_0K_a} + \frac{1}{\Delta\varepsilon[G]_0} \quad (1)$$

where ΔA is the change in the absorbance of aPS at respective concentration of β-cyclodextrin, b is the path length, $\Delta\varepsilon$ is the change in molar absorptivity of the guest (in this case aPS), $[G]_0$ is the concentration of the guest (in this case aPS), $[H]_0$ is the concentration of the host (in this case β-cyclodextrin), and K_a is the association or stability constant.

Similar experiment was conducted for aCPT with an initial concentration of 4×10^{-5} M and β-cyclodextrin with 10 µL of 20.0 mg mL^{-1} added. The absorbance was taken at 370 nm.

The association or stability constant can be calculated by the following equation (2):

$$K_a = \frac{y\ intercept}{slope} \quad (2)$$

1.8 Determination of critical aggregation concentration (CAC) by pyrene probe

Pyrene (1 mg) was first dissolved in acetone (4 mL) to yield a concentration of 1.24 mM. This pyrene solution (1.6 µL) was added to 4 mL glass vial, and it was left to evaporate in the dark overnight. After which, HA-aPs-aCPT solutions with different concentrations (0.02 mg/mL to 1 mg/mL) were prepared and added to the vials under stirring overnight to give final concentration of 1 µM for pyrene. The solutions were stirred in the dark overnight. Fluorescence of pyrene was then measured from 350 nm to 450 nm by the excitation at 335 nm. The pyrene emission wavelengths at 373 nm and 383 nm were recorded for different concentrations. Pyrene 1:3 ratio was calculated to determine the critical aggregation concentration.

1.9 Preparation of HA-aPS-aCPT NPs

HA-CD (1 mg) was first dissolved in deionized water (0.948 mL). A separate aliquot of aPS (0.0755 mg, 90 µM) and aCPT (0.0324 mg, 45 µM) in DMSO (52.6 µL, 5 %) was prepared and mixed well. Under sonication, the aliquot of aPS and aCPT in DMSO was added dropwise into the HA-CD suspension. The mixture was then dialyzed against phosphate buffer saline (PBS, 500 mL) to give HA-aPS-aCPT NPs. The loading capacity was computed by using the following equations (3) and (4):

$$Loading\ capacity\ of\ aCPT(\%) = \frac{Mass\ of\ aCPT}{Mass\ of\ nanoparticles} \times 100\% \quad (3)$$

$$Loading\ capacity\ of\ aPS(\%) = \frac{Mass\ of\ aPS}{Mass\ of\ nanoparticles} \times 100\% \quad (4)$$

1.10 Singlet oxygen detection using singlet oxygen sensor green (SOSG)

Singlet oxygen detection was determined by following previously reported protocol with slight modifications.[3] Briefly, SOSG dissolved in methanol was incubated with different samples ([SOSG] = 2.5 µM, [aPS] = 4.5, 9 and 13.5 µM) to measure $^1O_2$ generation after irradiation of 660 nm light. The solution was diluted 5 times with deionized water and then the

diluted solution (150 µL) was pipetted into 96 well plate for measurements. The generated $^1O_2$ was determined by measuring SOSG fluorescence maximum at 530 nm under 494 nm excitation.

1.11 Cell culture

MDA-MB-231 ($CD44^+$) and MCF-7 ($CD44^-$) human breast carcinoma cells were cultured in cell incubator with DMEM high glucose containing 10% fetal bovine serum and 1% penicillin-streptomycin under 5% CO_2 at 37ºC.

1.12 *In vitro* ROS detection

The generation of $^1O_2$ from aPS was qualitatively determined using 2',7'-dichlorofluorescin diacetate (carboxy-H_2DCFDA). HA-aPS-aCPT NPs (50 µg/mL) in PBS were first incubated with MDA-MB-231 cells for 4 h. Following which, the sample was irradiated with light of 660 nm for 30 min. The cells were then dyed with carboxy-H_2DCFDA (25 µM) in PBS at 37ºC for 30 min, protected from light. At the 25th min, Hoechst 33342 dye was added to stain the nucleus. The dyes were then removed, and excess dyes were washed away with PBS and cells were visualized under the confocal microscope immediately. The excitation/emission of carboxy-H_2DCFDA was taken as 495/592 nm. The excitation/emission of Hoechst 33342 was taken as 350/461 nm.

1.13 Cellular uptake and *in vitro* targeting ability

MDA-MB-231 cells were seeded onto 6-well plate with coverslips at the bottom at the density of 2 x 10^5 cells/well and left to adhere for 12 h. To determine cellular uptake by CLSM, HA-aPS-aCPT NPs (100 µg/mL) in PBS were incubated at different durations of 2 h and 4 h. The cells were then washed thrice with PBS and fixed with 4% formaldehyde for visualization under the CLSM. To validate targeting ability of the HA-aPS-aCPT NPs, MDA-MB-231 ($CD44^+$) and MCF-7 ($CD44^-$) cells were cultured and seeded onto 6-well plates at a density of 5 x 10^5 cells/well and left to adhere for 12 h. After which, equivalent concentrations of HA-aPS-aCPT NPs (100 µg/mL) in PBS were added to both MDA-MB-231 and MCF-7 cells for two different durations of 2 h and 4 h. The cells were then washed thrice with PBS and trypsinized for flow cytometry analysis using excitation/emission of 633/730 nm. Both flow cytometric analysis and CLSM imaging were conducted for HA competitive assays to test the targeting ability of the HA-aPS-aCPT NPs to CD44 receptors in MDA-MB-231 cells. In both experiments, MDA-MB-231 cells (2 x 10^5 cells/well) were pretreated with free HA (5 mg/mL) for 2 h. HA-aPS-aCPT NPs (100 µg/mL) were then added and incubated for 2 h. The treated cells were washed thrice with PBS. To carry out flow cytometry analysis, the cells were

trypsinized and collected. For CLSM imaging, cells were fixed with 4% formaldehyde, mounted and visualized under excitation/emission of 640/730 nm.

1.14 *In vitro* cytotoxicity test of HA-PS-CPT NPs and apoptosis study

The cytotoxicity of various materials was investigated with MDA-MB-231 cells by MTT assay. MDA-MB-231 cells (1 x 10^4 cells per well) were seeded onto 96-well plates and incubated for 12 h. After cell confluency reached 60 - 70%, culture medium was replaced with a medium (100 µL) containing CPT drug, aCPT prodrug, HA-aPS-TL NPs (6.25, 12.5, 25, 50, 100 and 200 µg/mL), and HA-aPS-aCPT NPs (6.25, 12.5, 25, 50, 100 and 200 µg/mL) followed by incubation at 37°C in the dark for 48 h. The cell viability was evaluated by MTT assay. The MTT dye dissolved in DMEM high glucose was incubated for 4 h to form the formazan crystals. DMSO (100 µL) was then added to dissolve the crystals. The plate was then read at 570 nm with reference to 630 nm. The percentage cell viabilities of each sample at different concentrations were calculated using the following formula:

$$\frac{Abs_{570\,nm}\,(sample) - Abs_{630\,nm}(sample)}{Abs_{570\,nm}\,(control) - Abs_{630\,nm}(control)} \times 100$$

To test for the cytotoxicity of HA-aPS-aCPT NPs under light irradiation, the above procedure was repeated with the same concentrations of NPs added. However, instead of incubation in the dark for 48 h, light of 660 nm at 70 mW/cm^2 was irradiated on the cells for 30 min. After incubation in the dark for another 44 h, the cell viability was measured in a similar procedure.

To visualize the phototoxicity using CLSM, MDA-MB-231 cells were seeded on to petri dish (2 x 10^5) and incubated for 48 h. The medium was replaced with fresh medium containing HA-aPS-aCPT (50 µg/mL) and incubated in dark for 6 h. After which, light of 660 nm was irradiated. After 18 h of incubation in the dark, the cells were washed with PBS and stained with calcein-AM (3 µM, ex/em: 488/515 nm) and propidium iodide (PI, 8 µM, ex/em: 488/610 nm) for 2 h before visualizing under the microscope.

1.15 Animal model

Female BALB/c nude mice (6 weeks old) were obtained from the Animal laboratory of Xinqiao Hospital (Chongqing). Any operations done on the mice strictly followed the Animal Management Rules of the Ministry of Health of People's Republic of China and under the protocols of the Care and Use of Laboratory Animals of the Third Military Medical University. To develop the MDA-MB-231 tumor xenograft model, 5 × 10^5 MDA-MB-231 cells suspended in 100 µL of 3:2 matrigel:PBS were subcutaneously injected into the mice. The tumors were

left to grow to about 60 to 80 mm^3. The tumor volumes were computed according to the following formula: ($width^2$ × length) / 2

1.16 *In vivo* optical imaging

Female BALB/c nude mice were intravenously injected with 200 μL of THPP as well as HA-aPS-aCPT NPs ([THPP] = [aPS] = 4.0 mg/kg, [aCPT] = 2.0 mg/kg) *via* their tail vein. At 2, 6 and 24 h intervals, the mice were anesthetized and visualized using *in vivo* imaging system under the excitation wavelength of 670 nm and emission wavelength of 740 nm. To visualize the bioaccumulation, after 24 h, the mice were sacrificed and major organs such as heart, liver, spleen, lung, kidney, brain as well as tumor were collected and imaged using the same excitation/emission wavelength.

1.17 *In vivo* combination therapy

BALB/c nude mice bearing subcutaneous MDA-MB-231 tumors (~80 mm^3) were randomly divided into five groups (n = 6 per group). The respective groups were: (I) Saline, (II) HA-aPS-aCPT, (III) CPT, (IV) THPP, and (V) HA-aPS-aCPT + light irradiation. Intravenous injections were used on all mice with volume of 100 μL administrated each time. Mice in group I were treated with saline, group II were treated with HA-aPS-aCPT NPs in the dark, group III were treated with camptothecin, group IV were treated with THPP, and group V were treated with HA-aPS-aCPT NPs and irradiated by the 660 nm light (100 mW/cm^2) for 30 min after the administration for 24 h. Concentrations of different species administrated each time are as follows: [HA-CD] = 0.5 mg/kg, [aPS] = [THPP] = 0.375 mg/kg, and [CPT] = [aCPT] = 0.16 mg/kg of mouse. The mice were administrated with respective treatment once every three days for a total of six times in 21 days period. The tumor sizes were recorded every 2-3 days for 21 days, with their lengths and widths measured by a digital vernier caliper. The weight of the mice was also recorded using a digital weighing balance every 2-3 days for 21 days. The tumor volumes were computed according to the following formula: ($width^2$ × length) / 2. On the 21^{st} day, all the mice were euthanized and major organs such as heart, liver, spleen, lung, and kidney were harvested and fixed by 10% formalin at 4^oC for 2 days. Subsequently, these fixed tissues were embedded with paraffin and then sectioned for histological studies.

1.18 TUNEL and histological assay

To investigate the tumor apoptosis in BALB/c nude mice, tumor biopsies were performed using a TUNEL apoptosis detection kit (Beyotime biotechnology, China). The samples were stained using Hoechst 33258 to mark the nuclei for CLSM observation. Standard protocol was followed for hematoxylin and eosin (H&E) staining and observed under an optical microscope.

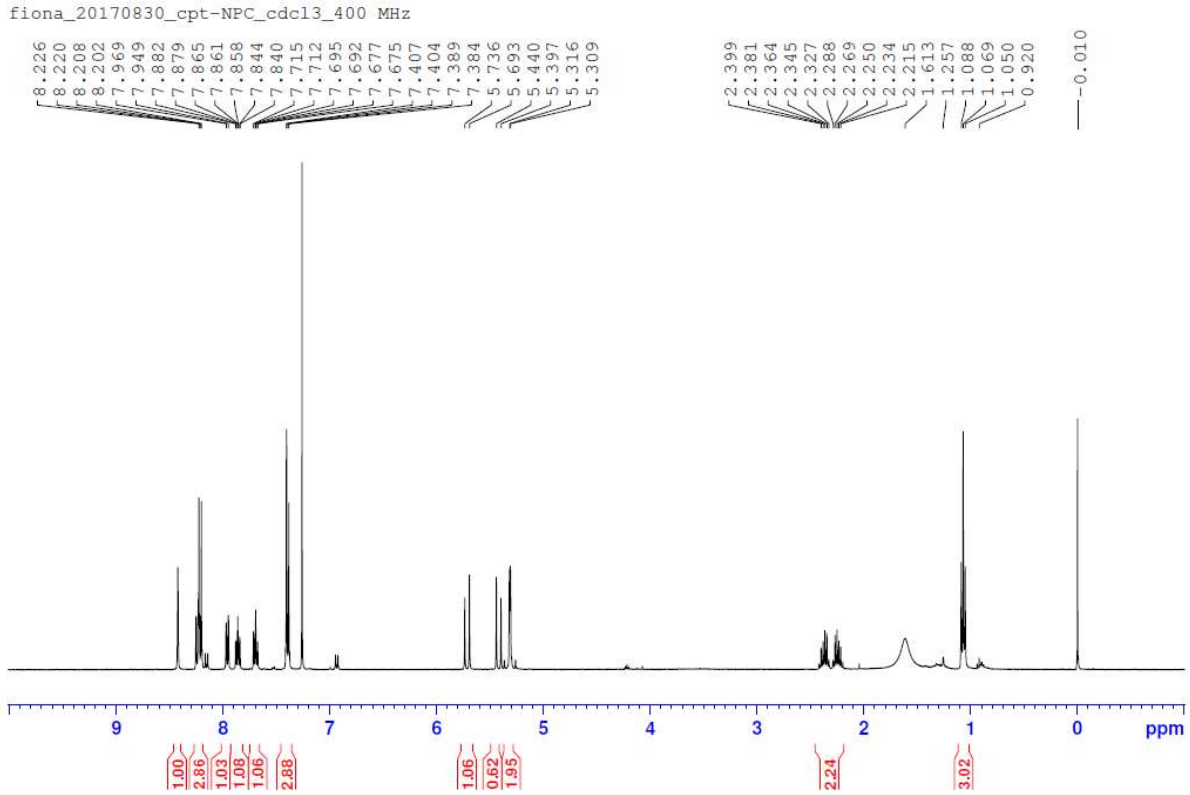


Figure S1. [1]H NMR spectrum of compound **1**.

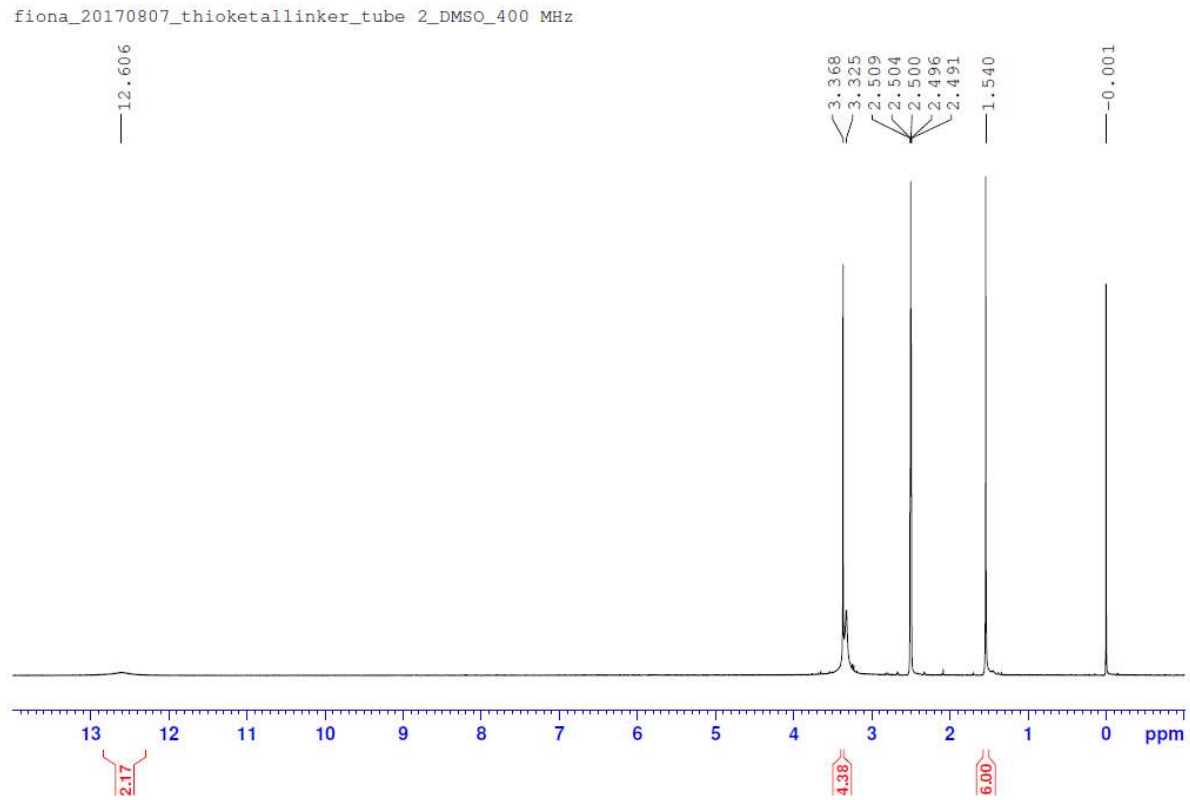


Figure S2. [1]H NMR spectrum of compound **2**.

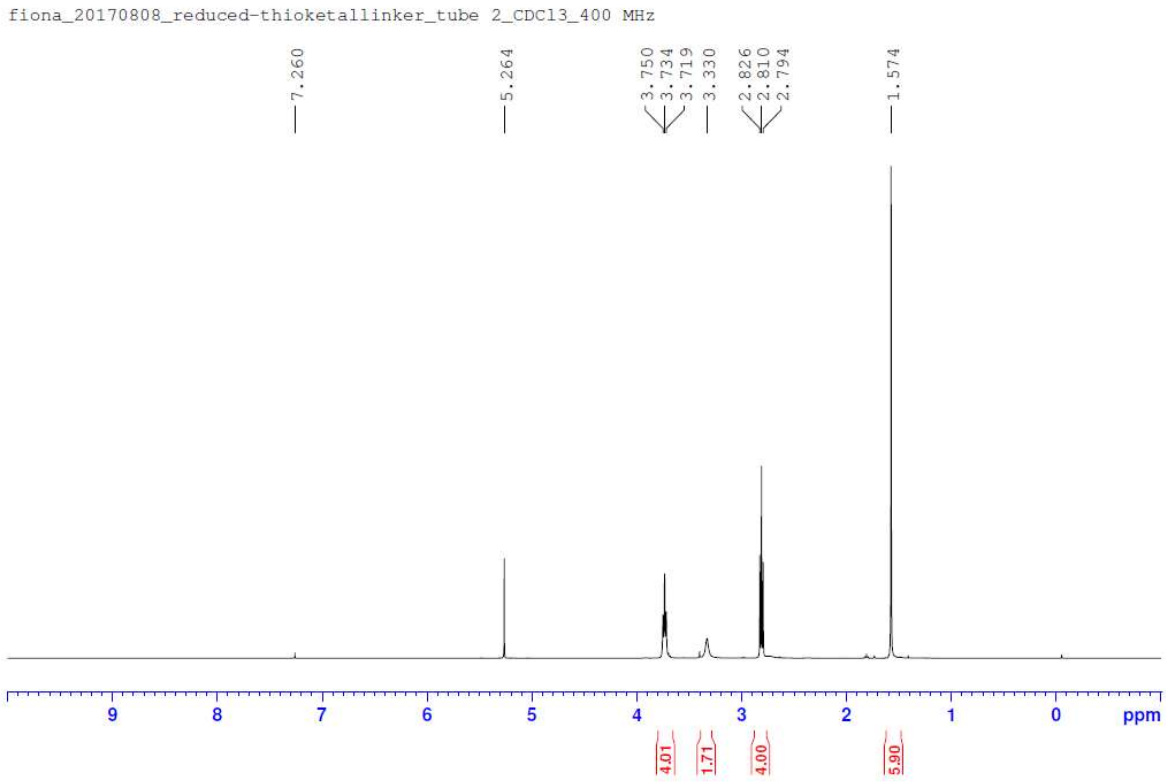
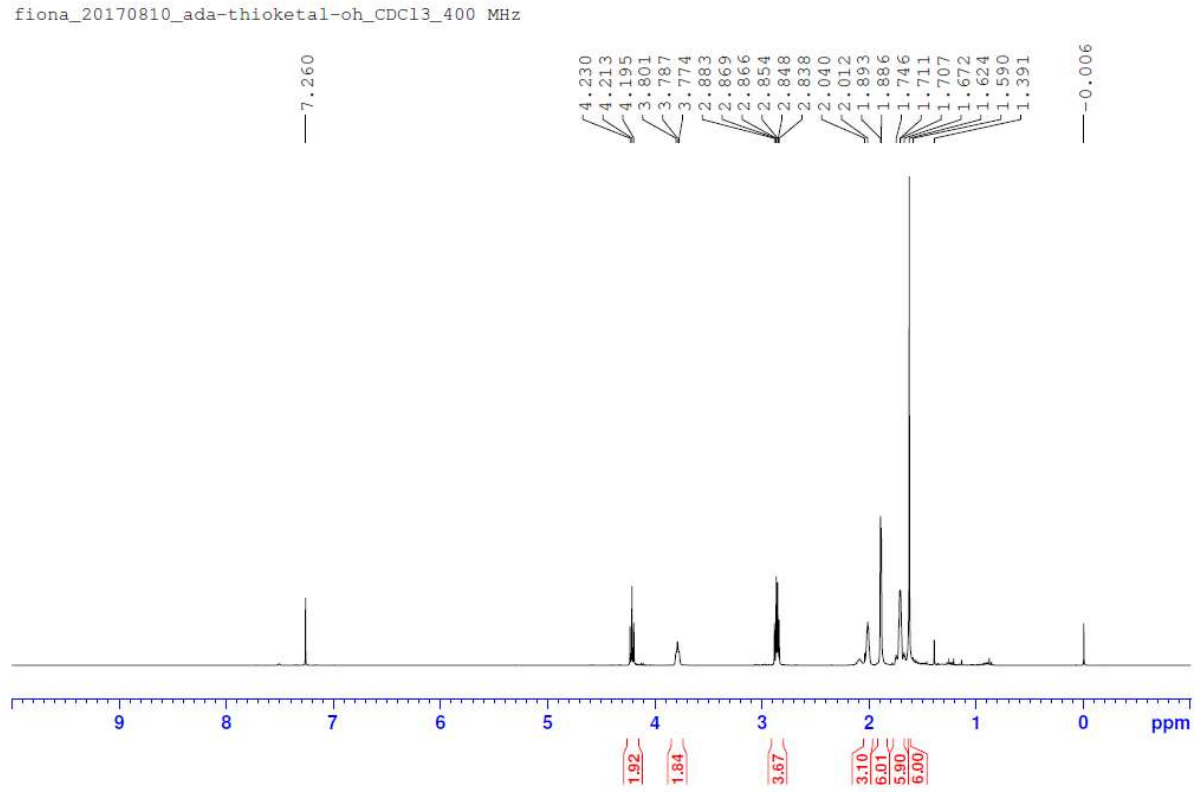


Figure S3. ^{1}H NMR spectrum of compound **3**.



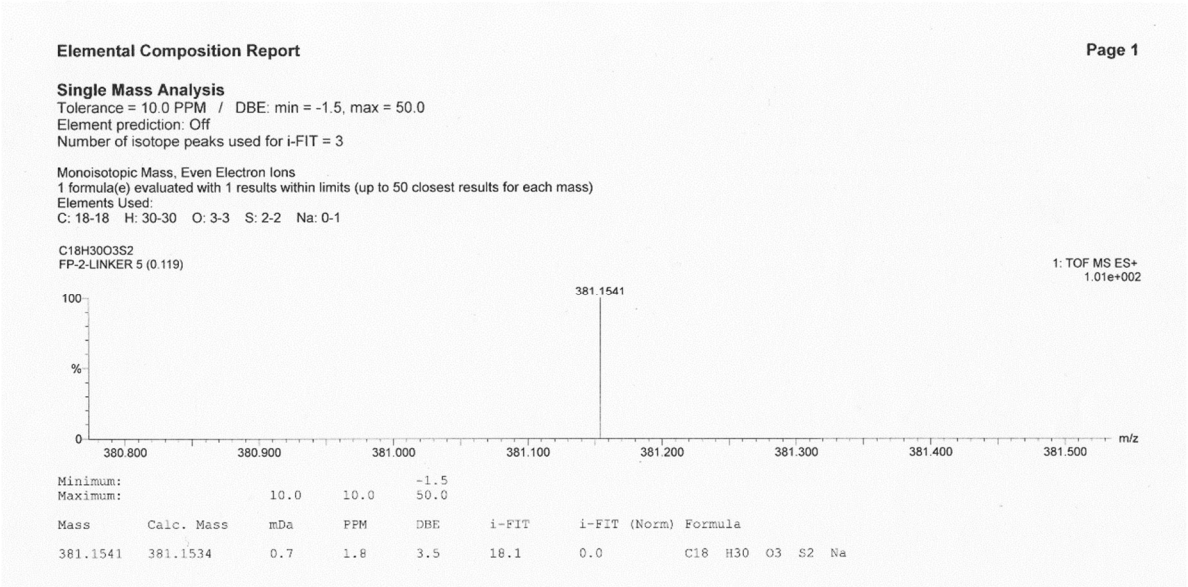
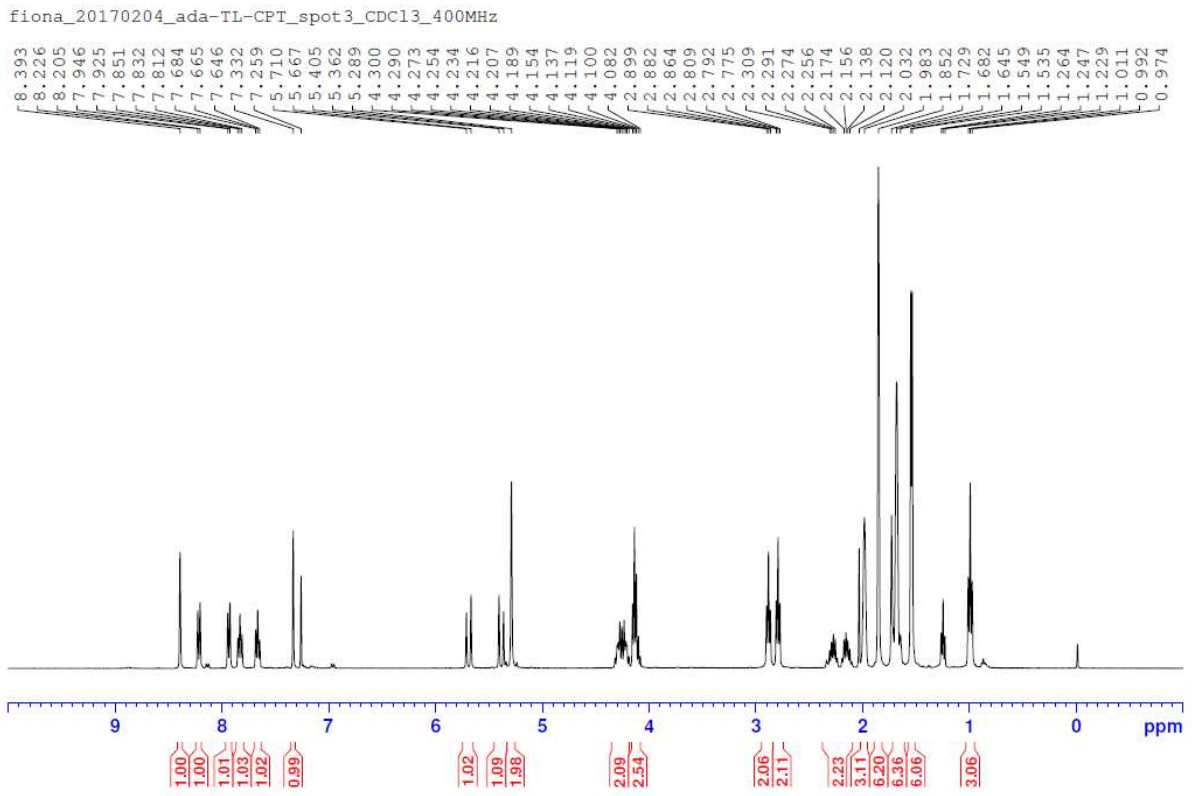


Figure S4. ^{1}H NMR (top) and ESI-MS (bottom) spectra of compound **4**.



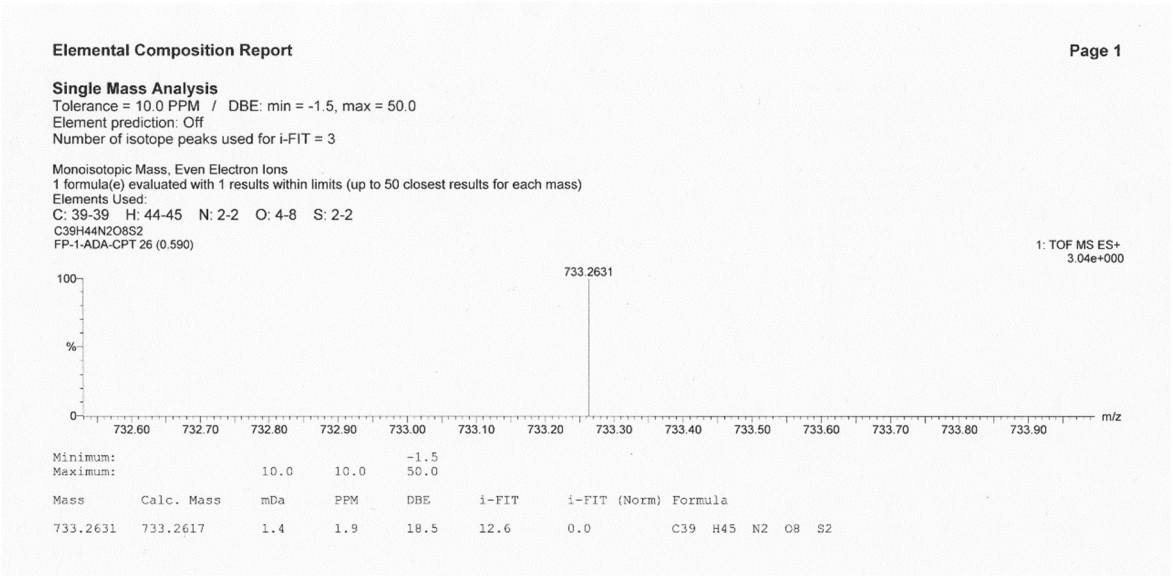


Figure S5. ^{1}H NMR (top) and ESI-MS (bottom) spectra of compound **5**.

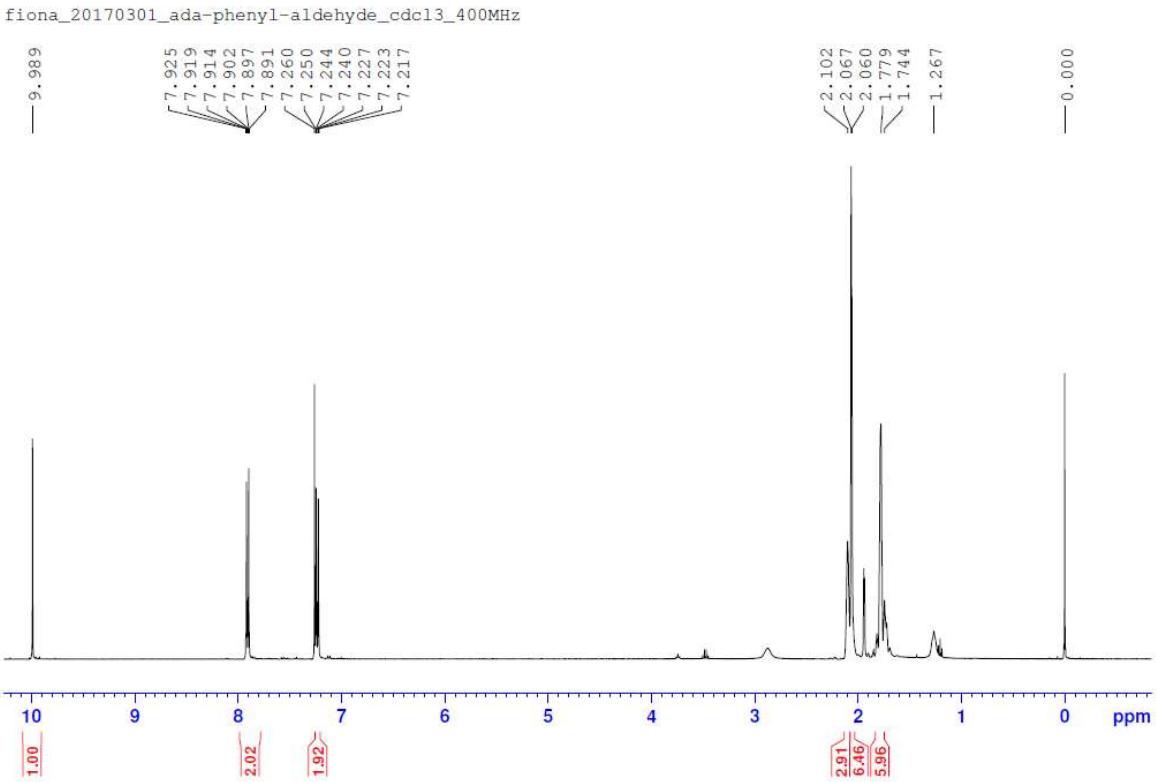
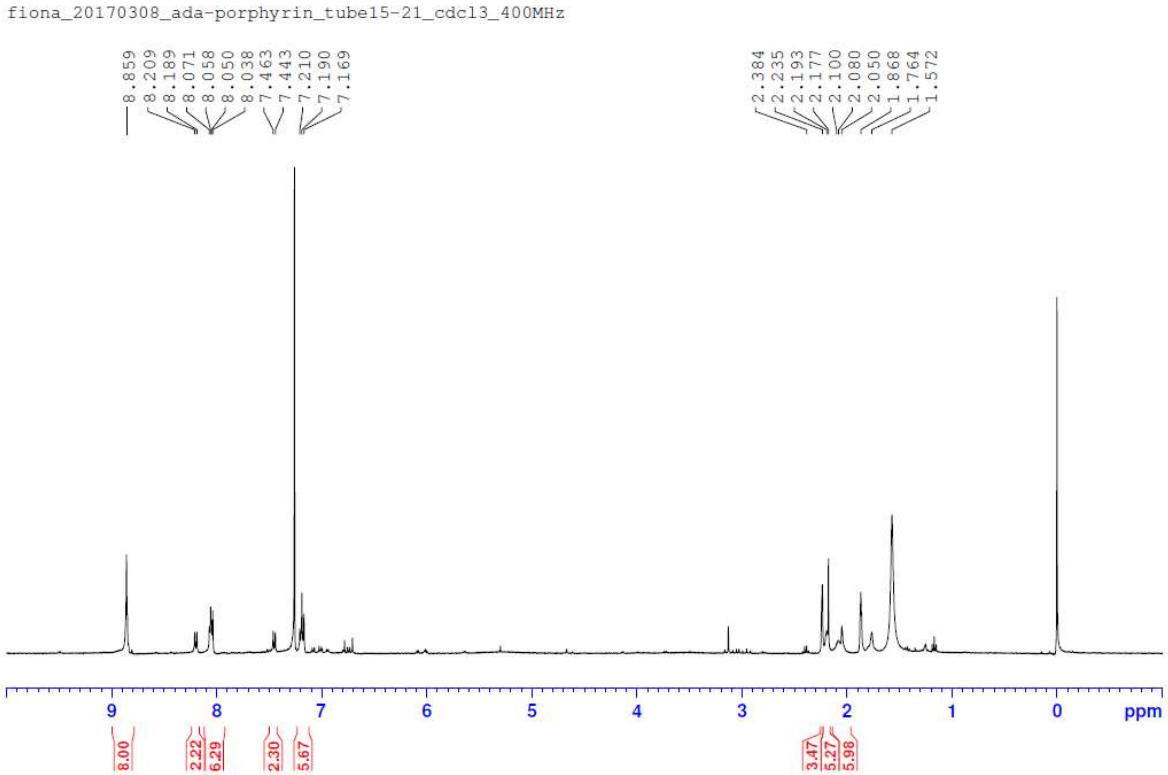


Figure S6. ^{1}H NMR spectrum of compound **6**.



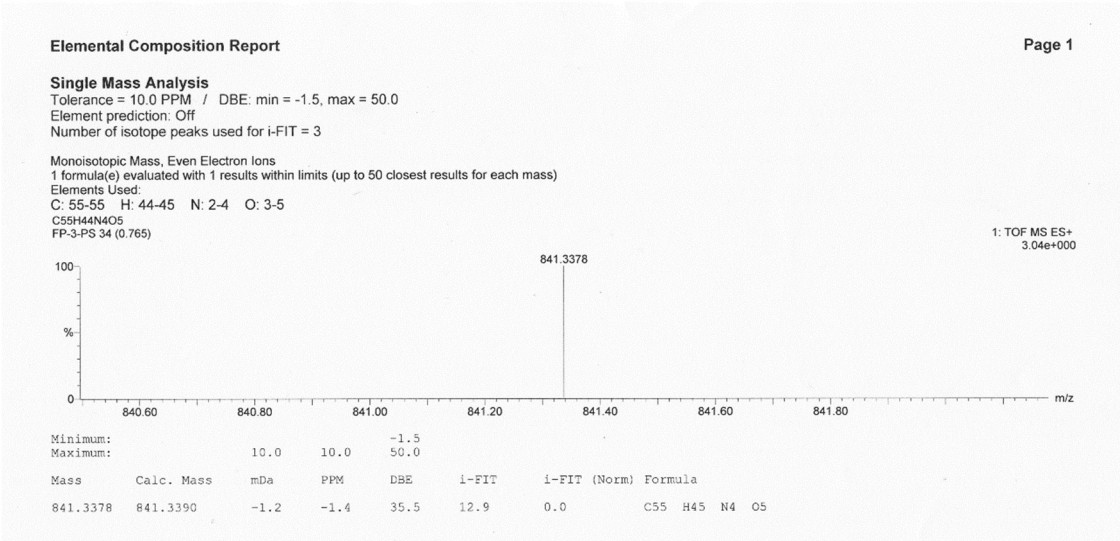


Figure S7. ^{1}H NMR (top) and ESI-MS (bottom) spectra of compound **7**.

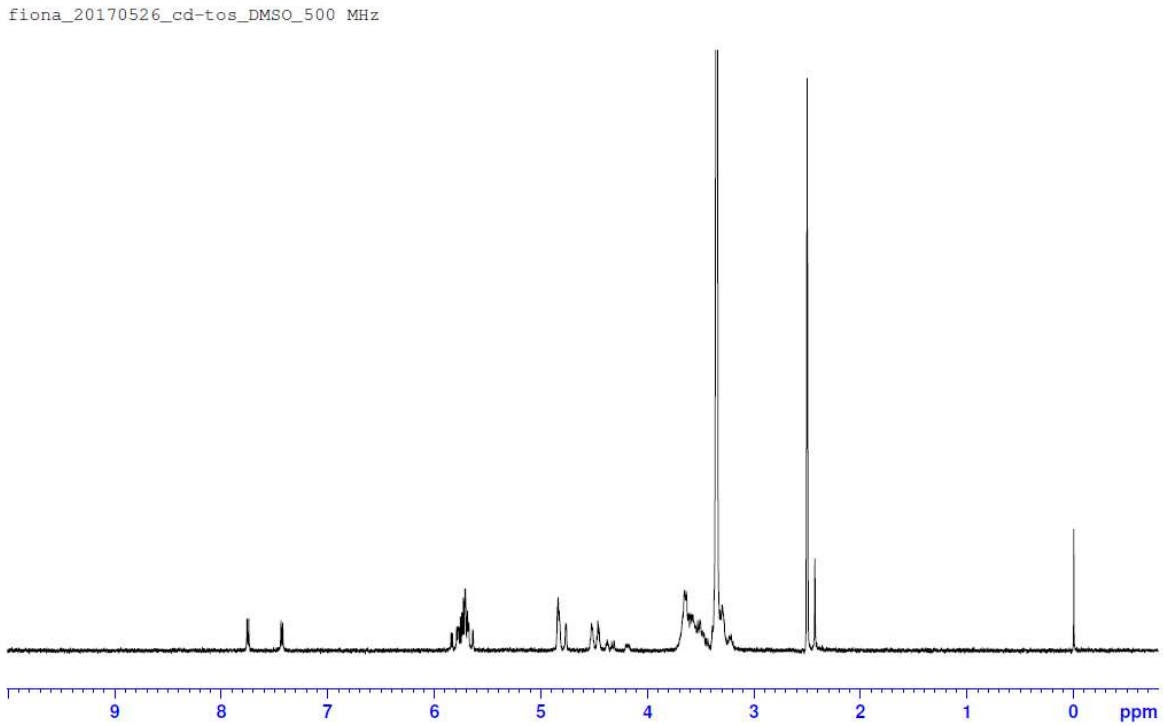


Figure S8. [1]H NMR spectrum of Tosylated-HA.

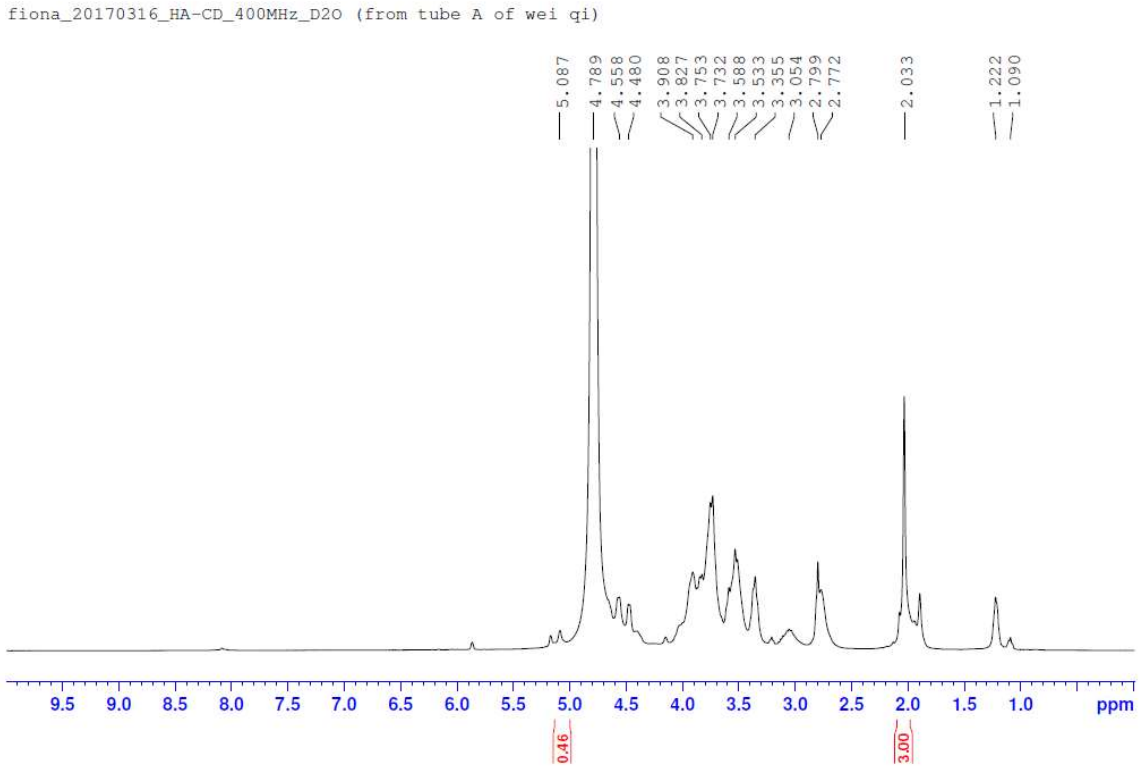


Figure S9. [1]H NMR spectrum of HA-CD.

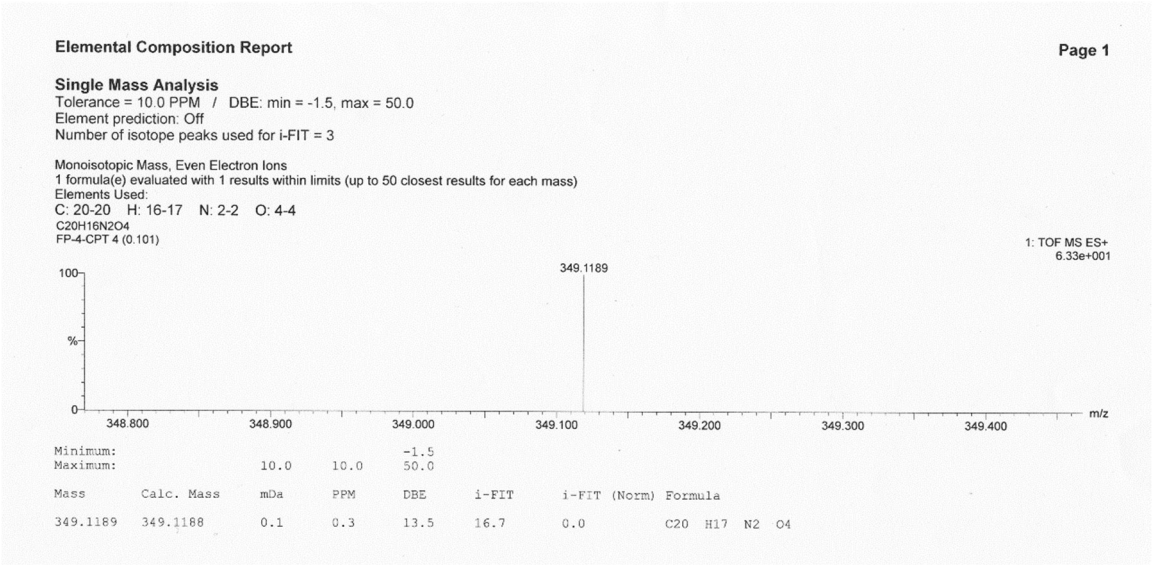


Figure S10. ESI-MS spectrum of CPT found after light irradiation when aCPT and aPS were incubated.

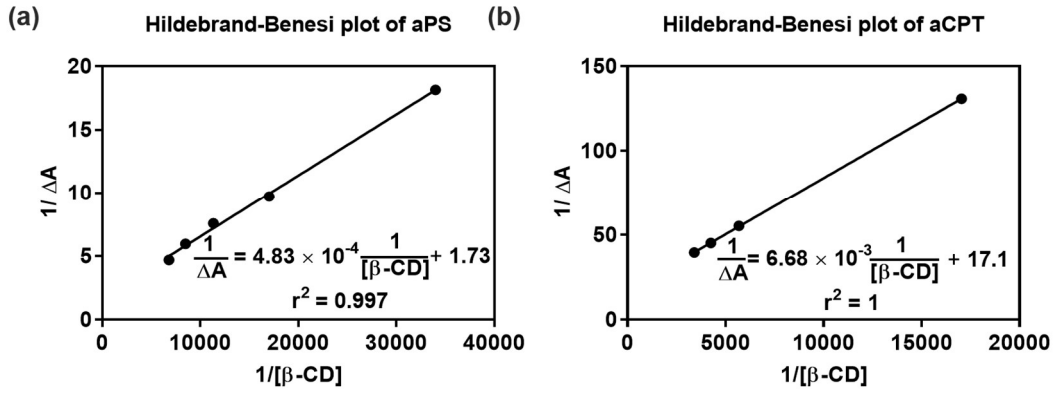


Figure S11. Hildebrand-Benesi plots for (a) aPS and (b) aCPT.

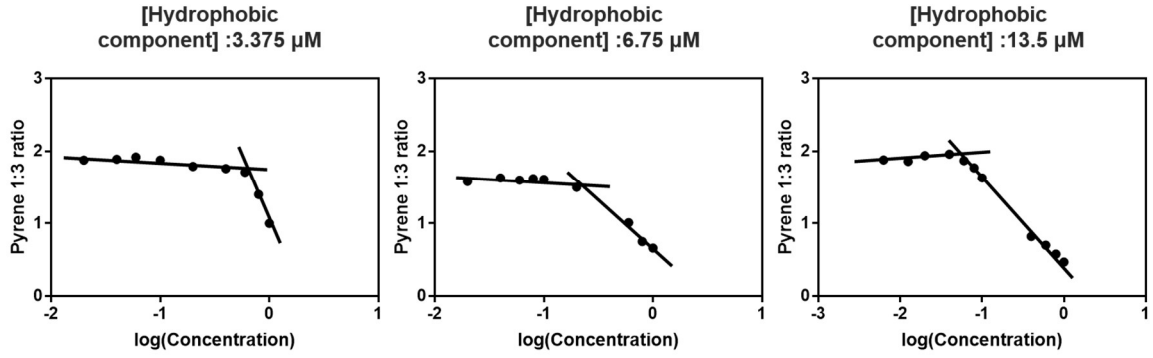


Figure S12. CAC determination using pyrene probe by taking the ratio of fluorescence between 373 nm and 383 nm at different concentrations of hydrophobic component and keeping [HA-CD] polymer constant.

Table S1. Polymer concentration, hydrophobic component, and respective CAC as determined by pyrene probe from Figure S12, hydrodynamic diameter and PDI value.

HA-CD (mg)	[Hydrophobic component] (μM)	CAC (mg/mL)	Hydrodynamic diameter (nm)	Polydispersity index (PDI)
1	3.375	0.49	135.1	0.342
1	6.75	0.16	102.7	0.360
1	13.50	0.05	76.3	0.060

Table S2. Compilation of the NP labelled, ratio of photosensitizer and camptothecin prodrug, and corresponding hydrodynamic size and polydispersity index.

Name	Ratio of aPS : aCPT	Hydrodynamic size (nm)	PDI
NP1	1:0	81.82	0.074
NP2	3:1	78.09	0.083
NP3	2:1	76.32	0.056
NP4	1:2	100.9	0.088
NP5	1:3	95.13	0.062
NP6	0:1	302.4	0.567

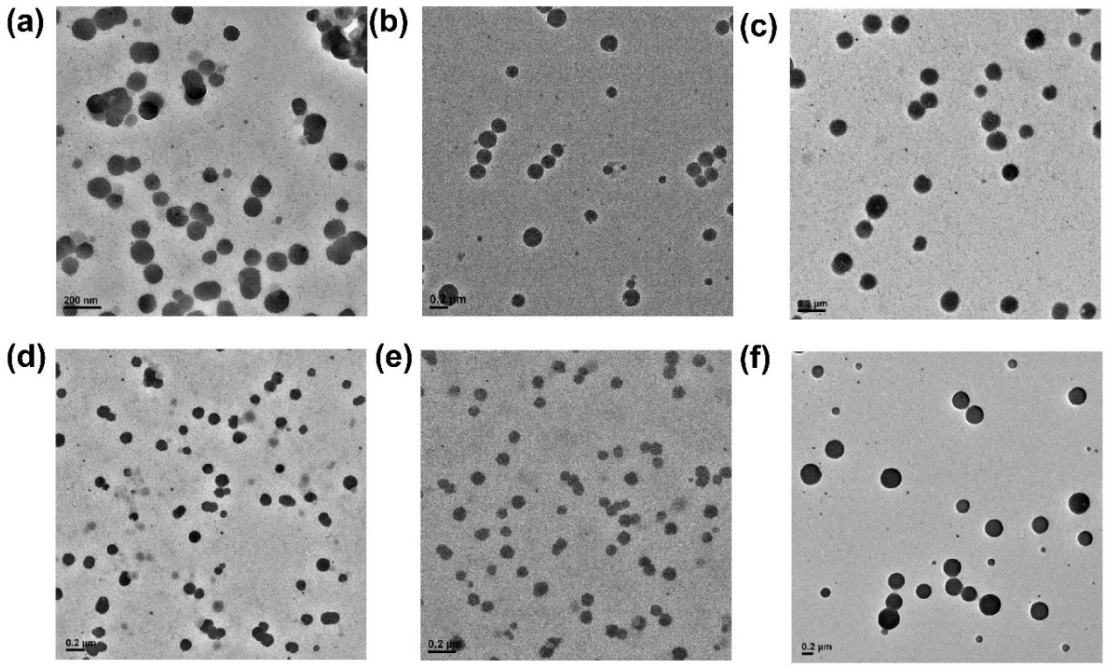


Figure S13. TEM images of (a) NP1, (b) NP2, (c) NP3, (d) NP4, (e) NP5, and (f) NP6. Scale bar: 200 nm.

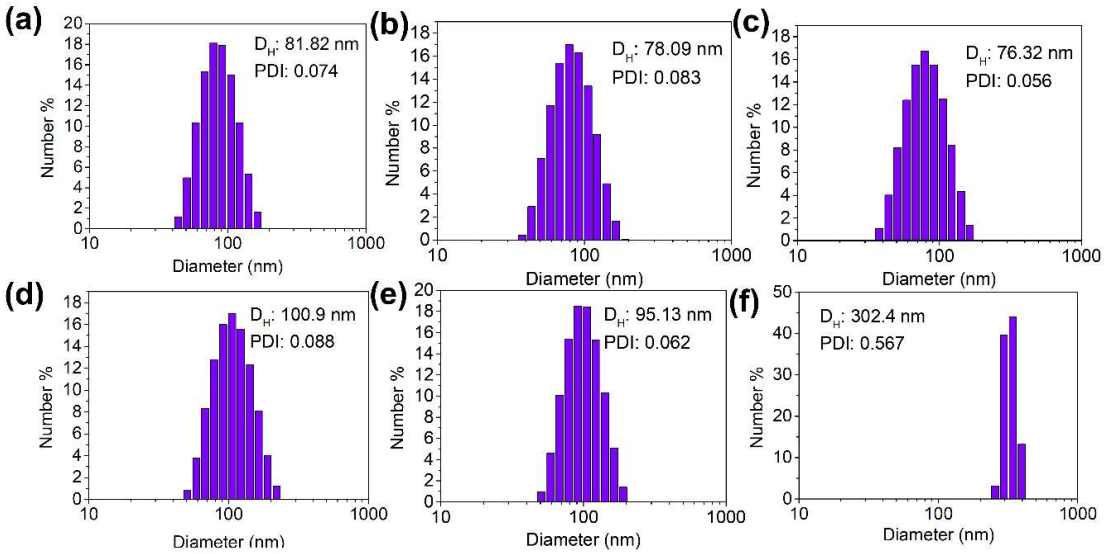


Figure S14. Hydrodynamic diameters of (a) NP1, (b) NP2, (c) NP3, (d) NP4, (e) NP5, and (f) NP6 measured using DLS.

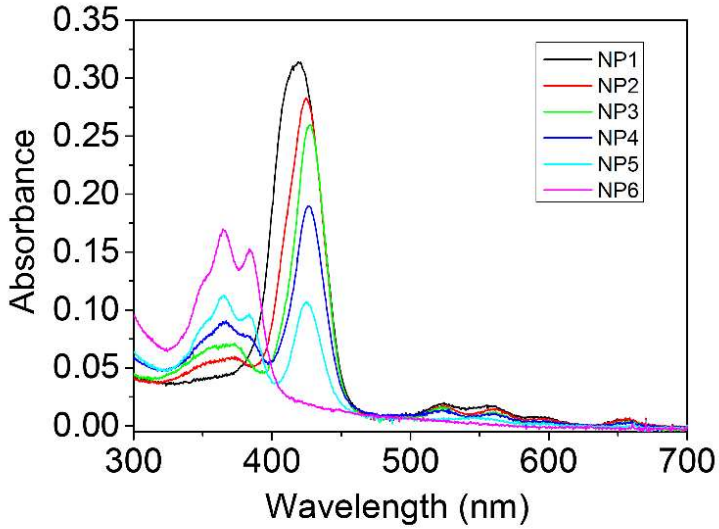


Figure S15. Absorption curves of NP1 (black), NP2 (red), NP3 (green), NP4 (blue), NP5 (cyan), and NP6 (pink).

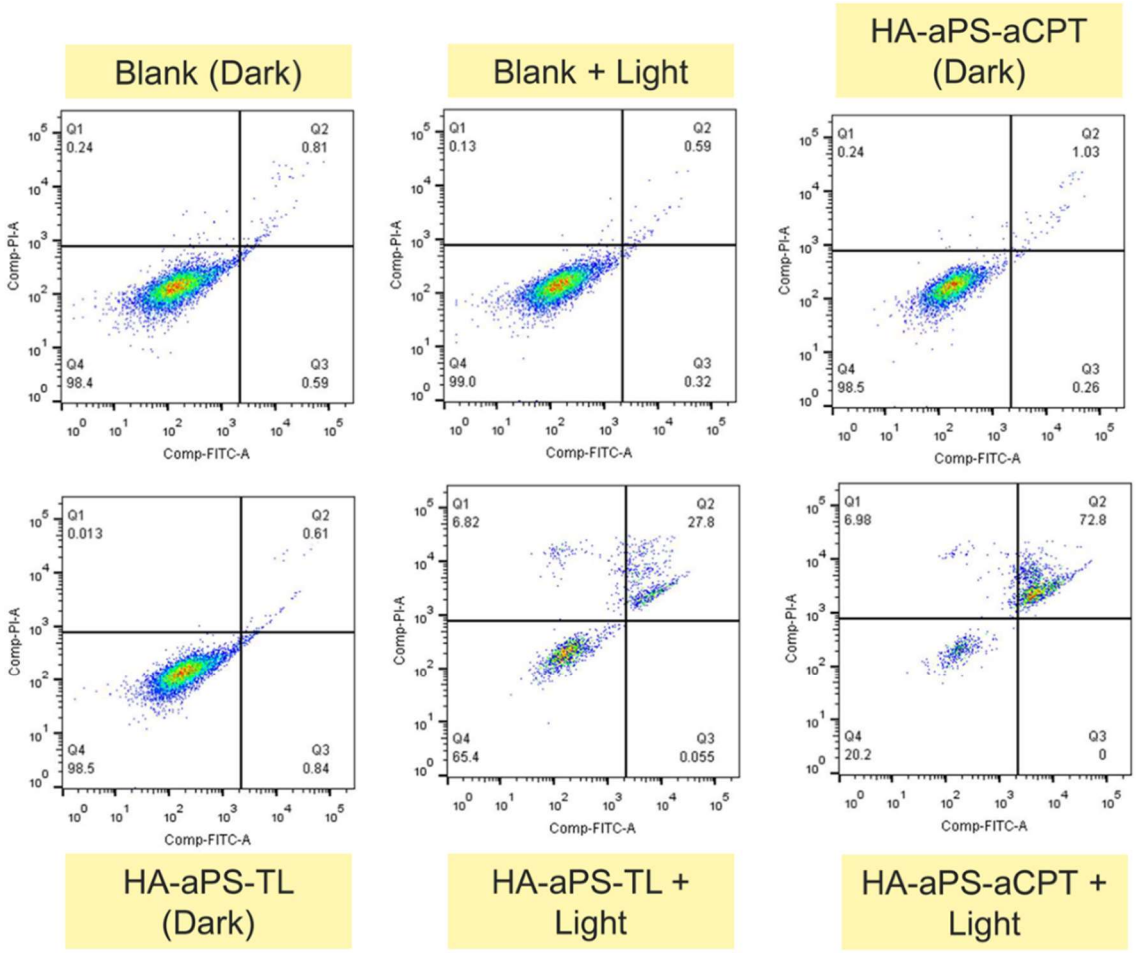


Figure S16. Apoptosis assay by flow cytometry on different samples, *i.e.*, blank (dark), blank with light, HA-aPS-TL in the dark and irradiated with light, and HA-aPS-aCPT NPs (50 μg/mL) in the dark and irradiated with light.

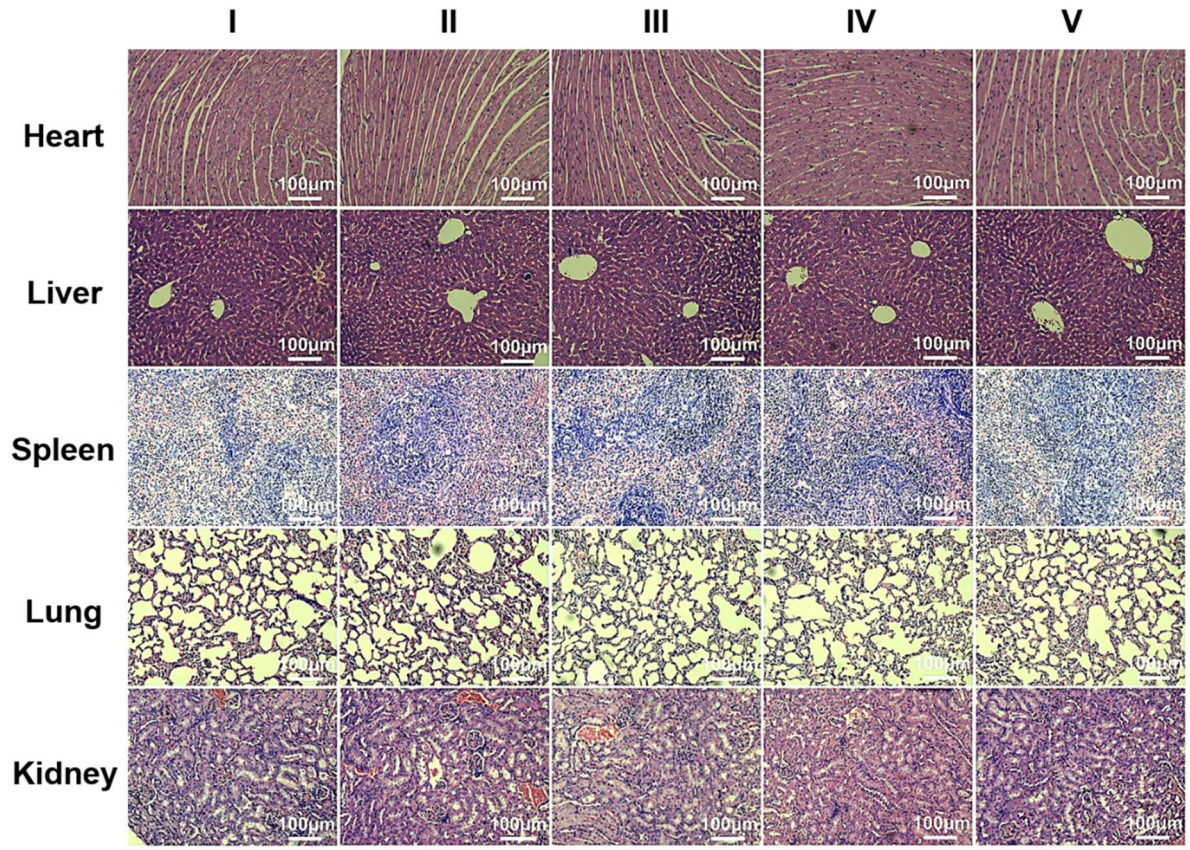


Figure S17. H&E staining on major organs (Heart, Liver, Spleen, Lung and Kidney) for different groups of mice (Group I: Saline, II: HA-aPS-aCPT NPs, III: CPT, IV: THPP, V: HA-aPS-aCPT NPs + light irradiation).

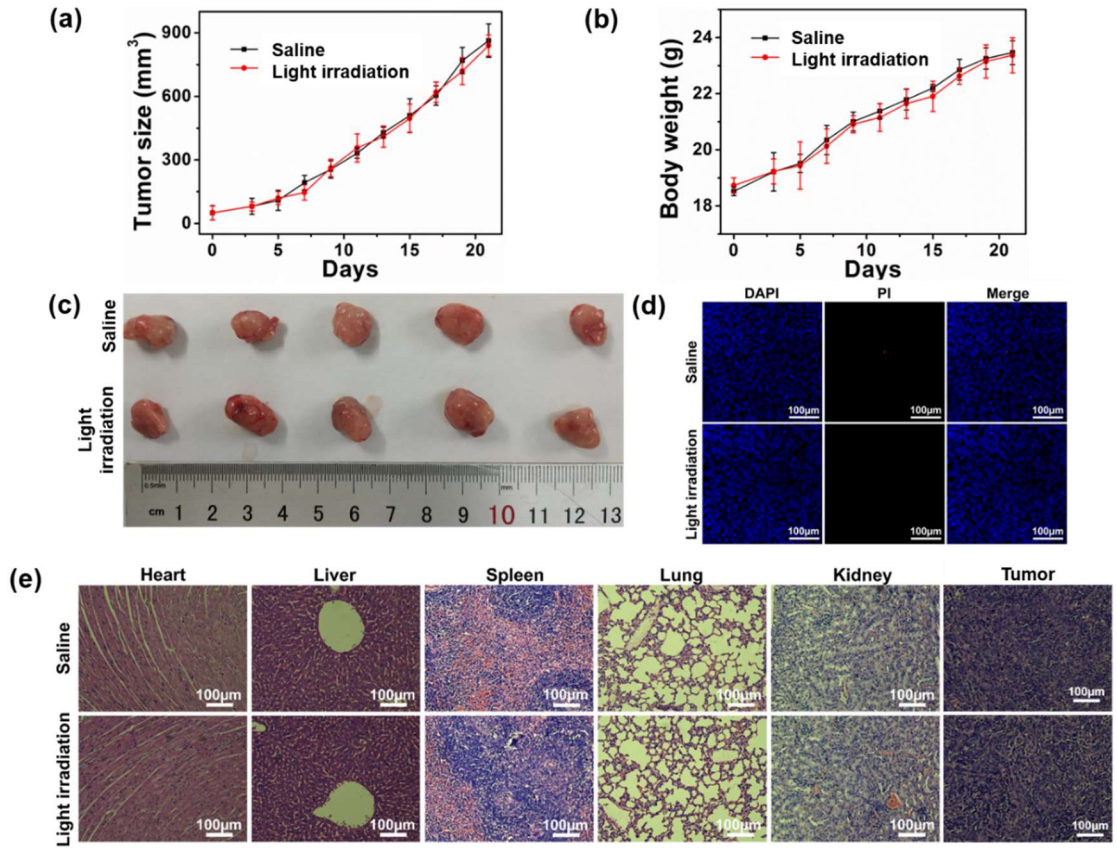


Figure S18. (a) Time dependent tumor growth for different groups of mice with different treatments over a period of 21 days. (b) Body weights of the mice during different treatments for a period of 21 days. (c) Five excised tumors from euthanized mice after various treatments. (d) TUNEL assay on the tumor tissues after various treatments. Red fluorescence shows dead cells. (e) H&E staining of the major organs in mice after various treatments.

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