Supporting Information

Self-assembly Nanoparticles by Human Serum Albumin and Photosensitizer for Targeted Near-infrared Emission Fluorescence Imaging and Effective Phototherapy of Cancer

Yan Huang,[†,‡] *Na He,*[‡] *Yunqing Wang,*[‡] *Dazhong Shen,*[†] *Qi Kang,* *[†] *Rongfang Zhao,*[‡] *Lingxin Chen* *[‡#]

[†] College of Chemistry, Chemical Engineering and Materials Science, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Shandong Normal University, Jinan 250014, China.

[‡] CAS Key Laboratory of Coastal Environmental Processes and Ecological Remediation, The Research Center for Coastal Environmental Engineering and Technology, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China.

[#] College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu 273165, China

Phone: +86-535-2109130 Fax: +86-535-2109130
*E-mails: kangqi@sdnu.edu.cn (Q. Kang); lxchen@yic.ac.cn (L. Chen)

List of Contents

1. General Experimental Section
2. Synthetic Procedures and Characterization Details
3. The Decomposition Products of Cy-HPT upon Laser Irradiation
4. pH Effects on Cy-HPT and HSA@Cy-HPT
5. The Bright Field of Cells in Figure 2
6. The Bright Field of Cells in Figure 3
7. The Bright Field of Cells in Figure 4
8. The Mean Fluorescence Intensity of Figure 4
9. Tumor-bearing Mice Imaging of HSA@Cy-HPT
10. The Mean Fluorescence Intensity in vivo Images of Figure 6
11. ^{1}H NMR, ^{13}C NMR and LC-MS of Cy-HPT
12. References

1. General Experimental Section

Instruments. Mice fluorescence imaging was performed on Perkinelmer IVIS Lumina XRMS Series III In Vivo Imaging System. Mice IR thermal imaging was performed by Infrared Thermal Camera (TESTO 865). Fluorescence spectra were obtained by a HORIBA Scientific Fluoromax-4 spectro fluorometer with a Xenon lamp and 1.0-cm quartz cells. Absorption spectra were measured on Thermo Scientific NanoDrop 2000/2000C spectrophotometer. Mass spectra were taken on LCQ Fleet LC-MS System (Thermo Fisher Scientific). The mean particle size was determined by DLS Zetasizer Nano ZS90 (Malvern Instruments Ltd, UK) and by TEM (JEOL, model JEM-1230, Japan). All pH measurements were performed with a basic pH-Meter PH-3C digital pH-meter (Lei Ci Device Works, Shanghai) with a combined glass-calomel electrode. ^{1}H NMR, ^{13}C NMR spectra were recorded on a Bruker AVANCE IIITM 500 spectrometer. MTT Assay was carried out by a microplate reader (Tecan, Austria). The fluorescence images of cells were taken using a confocal laser scanning microscope (Japan Olympus Co., Ltd) with an objective lens (×60). Intracellular fluorescence detection was carried out on flow cytometry (Aria, BD) with excitation at 633 nm and emission at 750-810 nm.

Materials. All solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. Other chemicals were purchased from Sigma-Aldrich unless otherwise stated and straightforward used without further purification, unless otherwise stated. The purity of **Cy-HPT** was separated on a Shimadzu LC-20AT HPLC system equipped with fluorescence and UV-vis absorption detectors. When it was used for imaging, the purity of **Cy-HPT** was greater than 95%. HEPES was obtained from Aladdin. All reactions were performed under argon protection and dark, monitored by TLC (Hailang, Yantai). Flash chromatography was carried out using silica gel (300-400 mesh). Human liver cancer cells (HepG2 cells) and human normal liver cells (L02 cells) were obtained from the Committee on Type Culture Collection of the Chinese Academy of Sciences. The antibody of Hsp70 and β-Actin were obtained from Cell Signaling Technology (Beverly, MA. USA).

Preparation of analytes. **Cy-HPT** (1 mM) and **HSA@Cy-HPT** (1 mg/mL) were prepared in DMSO and in ultrapure water, respectively, then stored at 4°C in darkness. All other reagents and chemicals were all from commercial sources and used without further purification. Toluene was distilled over sodium and benzophenone. Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, Bedford, MA, USA).

Loading Ratio. The encapsulation efficiency and release of **Cy-HPT** were determined by a HPLC

(Agilent 1260) with a UV-vis detector. Acetonitrile was used as the mobile phase. The **HSA@Cy-HPT** we obtained had the loading ratio of 6.7% for **Cy-HPT**.

Spectroscopic Methods. UV-visible spectra were obtained with 1.0-cm glass cells. **Cy-HPT** (DMSO, 20 μL, 2 μM) and **HSA@Cy-HPT** (ultrapure water, 20 μL, 40 μg/mL (containing equivalent concentrations of **Cy-HPT** 2 μM)) were added to a 10.0-mL color comparison tube, and diluted to 2 μM with HEPES buffer (10 mM, 1% DMSO, pH 7.4). Fluorescence spectra were obtained with a Xenon lamp and 1.0-cm quartz cells. **Cy-HPT** (DMSO, 10 μL, 2 μM) and **HSA@Cy-HPT** (ultrapure water, 20 μL, 40 μg/mL) were added to a 5.0-mL color comparison tube. After diluted to 2 μM with HEPES buffer (10 mM, 1% DMSO, pH 7.4), the mixture was measured. Then the fluorescence emission spectra of **Cy-HPT** were integrated from 830 to 900 nm with excitation at 810 nm, and **HSA@Cy-HPT** were integrated from 830 to 900 nm with excitation at 810 nm.

Singlet Oxygen Generation and Quantum yield. In all the experiments, the irradiation intensity was fixed at 1.5 W/cm^2. Briefly, 10 μM of Cy-HPT or 200 μg/mL HSA@Cy-HPT were dissolved in ultrapure water and added with a singlet oxygen probe Singlet oxygen sensor green (SOSG, Invitrogen co., USA) (1 μM) in water containing 2% methanol, respectively. Then, the mixture solutions were irradiated for 5 min (808 nm, 1.5 W/cm^2). The fluorescence intensity of the irradiated solution was promptly determined by NIR fluorescence spectrometer. The emission from 500-600 nm was afforded by exciting with a light resource of 494 nm wavelength and quantified for the singlet oxygen generation. Singlet oxygen generation and singlet oxygen quantum yield (Φ_Δ) were measured using SOSG.[1] **HSA@Cy-HPT** in DMF at the concentration of 0.02 or 0.6 μg/mL were mixed with 30.0 μM DPBF, respectively, followed by 5 min irradiation (808 nm laser). Then, the absorbance of DPBF at 415 nm was monitored at various times. The value of Φ_Δ was calculated according to the equation of $\Phi_{\Delta(\text{sample})} = \Phi_{\Delta(\text{HSA@Cy-HPT})} W I_{\text{HSA@Cy-HPT}}/(W_{\text{HSA@Cy-HPT}} I_{\text{sample}})$,[2] where W_{sample} and $W_{\text{HSA@Cy-HPT}}$ are the DPBF photobleaching rates in the presence of sample and **HSA@Cy-HPT**, respectively, and I_{sample} and $I_{\text{HSA@Cy-HPT}}$ are the rates of light absorption by sample and HSA@Cy-HPT, respectively.

Photothermal Effect and Photothermal Conversion Efficiency. The solutions of free **Cy-HPT** (at the concentrations of 2, 4, 6, 8, and 10 μM) and **HSA@Cy-HPT** (at the concentrations of 40, 80, 120, 160, and 200 μg/mL) in 0.5 mL glass vials were irradiated by 808 nm laser for 5 min, respectively. Meanwhile, the temperatures of solutions were recorded using a thermometer at an interval of 30 s. To assess the photothermal conversion efficiency, the solutions of free **Cy-HPT** (10 μM) and **HSA@Cy-HPT** (200

μg/mL) in quartz cuvettes were irradiated at 808 nm, respectively. When the temperature reached a plateau, the irradiation was removed for cooling down to room temperature. The temperature of the solutions was recorded at an interval of 30 s, and then their photothermal conversion efficiencies were calculated.

Photostability. To evaluate the ability to maintain the temperature elevations under irradiation, 0.5 mL **Cy-HPT** (10 μM) and **HSA@Cy-HPT** (200 μg/mL)were respectively illuminated at 808 nm for 5 min (LASER ON), and then the irradiation was removed for cooling the samples to room temperature (LASER OFF). Subsequently, another four cycles of this LASER ON/OFF cycles were carried out. During this process, the temperature was recorded at an interval of 30 s.

Cell Staining Procedures and Colocalization-imaging Experiments. The cells were plated on 6-well plates and allowed to adhere at 37 ℃, 5% CO_2, 24 h before imaging. The culture medium was then removed, and the cells were washed once with 1 mL of Dulbecco's Modified Eagle Medium (DMEM). HepG2 cells were placed in 1 mL of DMEM and loaded with 2 μM Hoechst 33342 for 10 min before staining with 40 μg/mL **HSA@Cy-HPT**, washing the cells three times with DMEM to remove the excess **HSA@Cy-HPT**. Finally, 1 μg/mL LysoTracker Green DND-26 (Thermo Fisher Scientific Inc.) were added and the cells were incubated for another 10 min at 37°C, respectively. Finally, the cells were rinsed with DMEM three times and mounted on the microscope. Fluorescent images were acquired on an Olympus Fluo View FV1000 confocal laser-scanning microscope (Japan) with an objective lens (×60). The spectrally separated images acquired from the three dyes were estimated using Image-Pro Plus software.

Flow cytometry. FCM assay was carried out for the detection of the intracellular **Cy-HPT** and **HSA@Cy-HPT**. The cells were cultured at 2.0×10^5 cells/well in 6-well plates, and treated with 2 μM **Cy-HPT** and 40 μg/mL **HSA@Cy-HPT** at 37 ℃. After harvest, cells were washed, and resuspended in PBS and analyzed by flow cytometry. And then the cells were further exposed to 808 nm laser light irradiation (1.5 W/cm^2) for 5 min after incubated with singlet oxygen sensor green (SOSG, Invitrogen co., USA) for 20 min at 37 ℃. After harvest, cells were washed, and resuspended in PBS and analyzed by flow cytometry.

Cytotoxicity of Cy-HPT and HSA@Cy-HPT. The cytotoxicity of **Cy-HPT** was assessed by the MTT assay. HepG2 cells and L02 cells were seeded into 96-well cell culture plate at a final density of 8×10^3 cells/well. And then different concentrations of **Cy-HPT** (at the concentrations of 2, 4, 6, 8, and 10 μM) and **HSA@Cy-HPT** (40, 80, 120, 160, and 200 μg/mL, containing equivalent concentrations of **Cy-HPT**) were added to the wells. The cells were then incubated for 24 h at 37 ℃ under 5% CO_2.

Subsequently, MTT was added to each well (final concentration 5 mg/mL) for an additional 4 h at 37 °C under 5% CO_2, then formazan crystals which were dissolved in 150 μL DMSO formed. The amount of MTT formazan was qualified by the absorbance (OD) at 570 nm using a microplate reader (Tecan, Austria). Calculation of IC_{50} values were done according to Huber and Koella. The results are the mean standard deviation of six separate measurements.

In Vitro Synergetic PDT, PTT Effect of Cy-HPT and HSA@Cy-HPT: To evaluatesynergetic PDT and PTT effectsof **Cy-HPT** and **HSA@Cy-HPT**, HepG2 cells were used. Firstly, 3×10^3 cells were seeded in 96-well plates and cultured overnight and then incubated with different concentrations of **Cy-HPT** and **HSA@Cy-HPT** for 24 h. Cells were exposed to 808 nm laser irradiation (1.5 W/cm^2) for 5 min. In the control group, cells were incubated with different concentrations of **Cy-HPT** and **HSA@Cy-HPT** straightforwardly for 48 h without laser irradiation. The dose-dependent synergetic anticancer effect of **Cy-HPT** and **HSA@Cy-HPT** were further intuitionally verified on HepG2 cell lines by using Calcein AM and PI co-staining. After 24 h incubation, the relative cell viabilities were determined by the MTT assay. In order to investigate the effect of PDT or PTT,[3] cells were maintained below 4 °C by ice treatment to scavenge photothermal conversion or treated with 10 mM N-acetylcysteine (NAC, ROS scavenger) to scavenge ROS, during laser irradiation. We used the dark group (no laser irradiation) to investigate the single effect of chemotherapy. Calcein AM and propidium iodide (PI) co-staining were carried out on HepG2 cells to further confirm the synergistic PDT/PTT effects of **Cy-HPT** and **HSA@Cy-HPT**. Briefly, 5×10^4 HepG2 cells per well were seeded in 6-well plates and cultured overnight. Then cells were incubated with **Cy-HPT** and **HSA@Cy-HPT** for 4 h. Afterward, the cells of experimental groups (single group of PDT, PTT; synergistic PDT/PTT group) were irradiated by an NIR (808 nm) laser with energy density of 1.5 W/cm^2 for 5 min. Incubation 4 h later, cells were stained with 2 μM Calcein AM and with 2 μM PI for visualization of live cells or visualization of dead/late apoptotic cells respectively. Cellular apoptosis and necrosis were also evaluated by Annexin V-FITC Apoptosis Detection Kit and observed by flow cytometry. Lastly, the fluorescent images of cells in all groups were acquired with a biological inverted microscope after being rinsed with PBS.

Animals and Tumor Xenografts: BALB/c mice (aged 5-6 weeks, weighed 20-25 g) and athymic nude mice (aged 5-6 weeks, weighted 20-25 g) were purchased from Binzhou Medical University. All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals, and protocols were approved by the Institutional Animal Care and Use Committee in

Binzhou Medical University, Yantai, China. For HepG2 tumor xenografts, 5 ×10^6 HepG2 cells suspended in 200 μL PBS were subcutaneously implanted into the right flank of each athymic nude mouse. All animals were maintained under aseptic conditions and were housed in a group of five in standard cages with free access to food and water and a 12 h light/dark cycle.

In vivo anticancer effect of HSA@Cy-HPT under light irradiation: To determine the in vivo tumor inhibitory effect of **HSA@Cy-HPT**, HepG2 tumor xenograft models were used. After tumor xenografts were established, they were randomly divided into four groups in each xenograft model: PBS non-irradiation group, PBS irradiation group, **HSA@Cy-HPT** non-irradiation group and **HSA@Cy-HPT** irradiation group. Animals in **HSA@Cy-HPT** non-irradiation group and **HSA@Cy-HPT** irradiation group for **HSA@Cy-HPT** (200 μg/mL, 200 μL in saline) every two days and repeated for 5 times. Animals in the PBS non-irradiation group and PBS irradiation group were given the same volume of blank PBS each time. Post-injection, tumors in the irradiation groups were exposed to an 808 nm laser at the power density of 1.5 W/cm^2 for 5 min. Tumor volumes and body weights were recorded during the treatments. Tumor volumes were calculated as length × $(width)^2/2$. 2-3 days post fifth drug injection, animals were sacrificed, and tumors were dissected and weighted. Meanwhile, tumors in each group were collected and fixed in 4% formaldehyde, made into paraffin sections for H&E staining. The major organs from each group were also collected for H&E staining to determine the toxicside effect of this treatment.

Single Oxygen Detection: Singlet oxygen sensor green (SOSG, Invitrogen co., USA) was employed to evaluate the singlet oxygen generation of **Cy-HPT** or **HSA@Cy-HPT** in living cells. Briefly, HepG2 cells were treated with **Cy-HPT** or **HSA@Cy-HPT**, and then added with SOSG (1 μM) in water containing 2% methanol. The cells were irradiated for 5 min (808 nm, 1.5 W/cm^2). The fluorescence imaging of the HepG2 cells were promptly determined by laser scanning confocal microscope. The emission from 500-600 nm was afforded by exciting with a light resource of 494 nm wavelength and quantified for the singlet oxygen generation.

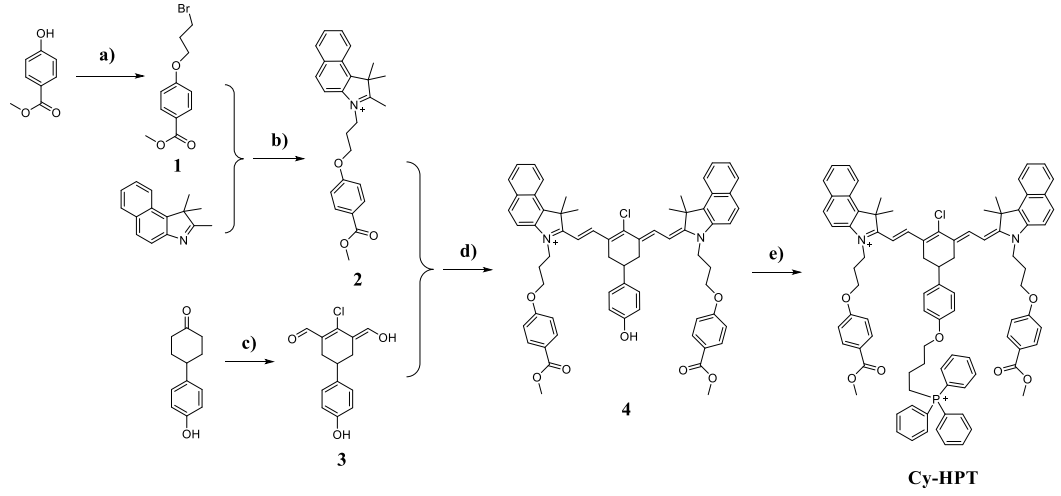
Western Blot. 1×10^6 HepG2 cells were seeded in 6-well plate and incubated overnight. They were treated with HSA@Cy-HPT (200 μg/mL) for another 24 h incubation. Then, cells were irradiated with NIR laser light (808 nm, 1.5 W/cm^2) for 5 min. For comparison, cells only incubation of HSA@Cy-HPT were used for control, while cells with incubation of HSA@Cy-HPT for single PDT or single PTT as the positive control. After 4 h laser irradiation, all cells were washed with PBS, protein extracts were prepared by suspending the cells in 200 μL RIPA lysis buffer containing 1% PMSF (Solarbio, China) and 20%

PhosSTOP (Roche, Germany). Then the extracts were quantified with BCA protein assay kit (Biogot, China). After denatured, the equal amounts of protein were electrophoresed on 10% SDS-polyacrylamide gels (Bio-Rad, USA) and transferred to PVDF membranes. The membrane was incubated with 5% BSA (Sigma-Aldrich, USA) and incubated with primary antibodies overnight at 4 °C with gentle shake. A horseradish peroxidase (HRP)-conjugated secondary antibody (Cell Signaling Technology, USA) was used to mirror the quantity of proteins and signals were detected with an enhanced chemiluminescence (ECL) detection system. The results were analyzed by ImageJ to acquire the grey value of every bond. The primary antibodies (dilution) were incubated, as follows: Hsp70 (abcam, 1/1000), β-actin (Mouse, Sigma) (1/1000), followed by secondary antibody incubation.

H&E staining: Heart, liver, spleen, lung and kidney of tumor-bearing mice and normal mice in each group, tumor tissue of tumor-bearing mice in each group were excised and fixed in 10% formaldehyde and embedded in paraffin. Then the treated synovial membrane tissue of ankle joint were prepared to frozen sections and stained with hematoxylin and eosin (H&E) to confirm histology.

Statistics Analysis: A comparison between experimental and control groups was analyzed by the unpaired Student's t test. The statistical analysis was determined using Kaplan-Meier survival plot (SPSS), and the curves were compared using the log-rank (Mantel-Cox) test. $p < 0.05$, 0.01, and 0.001 were considered a statistically significant difference and remarked with *, **, ***, respectively.

2. Synthetic Procedures and Characterization Details



Scheme S1. Synthetic Approaches of **Cy-HPT**. a) 1,3-dibromopropane, K_2CO_3, acetone, refluxed for 12 h, 92%; b) acetonitrile, refluxed for 12 h, 90%; c) DMF, CH_2Cl_2, $POCl_3$, 45 °C, 3 h, 85%; d) n-Butyl alcohol:benzene = 7:3 (v/v), refluxed, 3 h, 70%; e) (4-Bromobutyl) triphenylphosphonium bromide, K_2CO_3, acetone, refluxed for 24 h, 53%.

Synthesis of Compound 1: Methyl 4-hydroxybenzoate (152 mg, 1 mmol) and 1,3-dibromopropane (200 mg, 1 mmol) were dissolved in dry acetone (250 mL), then anhydrous potassium carbonate (275 mg, 2 mmol) was added. The mixture was stirred under a dry argon atmosphere for 12 h and monitored by TLC. After the reaction was finished, the mixture was cooled to room temperature, evaporated under reduced pressure and partitioned with CH_2Cl_2 and saturated KBr solution. Finally, the organic layer was separated. Purification by column chromatography on silica eluting with CH_2Cl_2/EtOAc (8:1) gave the product compound 1 as white powder (248.1 mg, yield: 92%).

Synthesis of Compound 2: 1,1,2-trimethyl-1H-benz[e]indole (2 g, 10 mmol) and compound 1 (2.7 g, 10 mmol) were mixed in 10 mL anhydrous acetonitrile in 50 mL round flask, then the mixture was refluxed for 12 h, then stopped heating and cooled down. The precipitate was filtered through a Buchner funnel, and the solid product was washed by diethyl ether and dried in vacuum to afford dark violet product (4.32 g, yield: 90%).

Synthesis of Compound 3: A solution of 40 mL of anhydrous N, N-dimethylformamide (DMF) and 40 mL of anhydrous CH_2Cl_2 was placed in a 250 mL round-bottom flask, chilling the solution to -10 °C and then stirring for 20 min. Phosphorus oxychloride (37 mL), with 35 mL of anhydrous CH_2Cl_2 was dropwise added into above solution through a constant pressure drop of liquid funnel. 4-(4-Hydroxyphenyl)cyclohexanone (10 g, 52.6 mmol) was added into the mixture in batches, the solution changed from colorless into yellow immediately. Then the solution was slowly heated to 45 °C for 3 h, then cooled down, poured into a lot of ice, and allowed to stand overnight. The yellow solid was collected through a buchner funnel and dried in vacuum (12.9 g, yield: 85%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 9.22 (s, 1H), 8.12 (s, 1H), 8.08 (s, 1H), 7.65-7.34 (m, 2H), 7.14 (s, 1H), 6.69-6.64 (m, 2H), 2.38-2.71 (m, 1H), 2.53-2.45 (m, 2H), 2.28-2.26 (m, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 191.8, 162.9, 155.7, 148.5, 145.7, 142.4, 131.2, 127.6, 127.5, 117.8, 115.1, 37.2, 31.7, 30.6. LC-MS (ESI$^-$): m/z $C_{14}H_{13}ClO_3$ calcd. 264.0553, found $[M-H]^-$ 263.0481.

Synthesis of Compound 4: Compound 2 (0.96 g, 2 mmol) and 3（0.26 g, 1 mmol） were resolved in 100 mL mixed solution of n-butyl alcohol and benzene (7:3, v/v) in 250 mL round flask, refluxed for 3 h, dried in vacuum, to obtain green solid. The crude product was purified by silica gel chromatography using EtOAc/CH_3OH (8:1, v/v) as eluent to afford compound 4 as green solid (0.651 g, 70% yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 9.37 (s, 1H), 8.41-8.04 (m, 8H), 7.83-7.77 (m, 5H), 7.69-7.51 (m, 2H), 7.25-7.23 (m, 2H), 6.85-6.83 (m, 5H), 6.39-6.36 (m, 2H), 4.51-4.50 (m, 3H), 4.14 (s, 4H), 3.80 (s, 7H),

3.04-3.01 (m, 2H), 2.55-2.51 (m, 6H), 2.24 (m, 2H), 1.97-1.92 (m, 11H), 1.56-1.22 (m, 4H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm): 174.2, 166.2, 162.3, 156.7, 147.6, 142.7, 140.1, 135.3, 134.1, 131.9, 131.6, 130.9, 130.4, 128.6, 128.2, 127.8, 126.5, 125.5, 122.7, 122.4, 115.7, 114.9, 114.7, 112.1, 101.6, 65.2, 52.2, 51.2, 41.6, 40.5, 40.4, 40.3, 40.2, 40.1, 39.9, 39.8, 39.6, 39.4, 37.8, 33.9, 27.5, 27.2. LC-MS (ESI$^+$): m/z $C_{66}H_{64}ClN_2O_7^+$ calcd. 1031.45, found [M^+] 1031.69.

Synthesis of Compound Cy-HPT: Compound 4 (46.5 mg, 0.05 mmol) and (4-Bromobutyl) triphenylphosphonium bromide（47.8 mg，0.1 mmol）were dissolved in dry acetone (25 mL), then anhydrous potassium carbonate (27.5 mg，0.2 mmol) was added. The mixture was stirred under a dry argon atmosphere for 24 h and monitored by TLC. After the reaction was finished, the mixture was cooled to room temperature, evaporated under reduced pressure and partitioned with CH_2Cl_2 and saturated KBr solution. Finally, the organic layer was separated. Purification by column chromatography on silica eluting with EtOAc/CH_3OH (3:1) gave the product Cy-HPT as dark green crystals (35.74 mg, 53%). ^{1}H NMR (CH_3DO, 500 MHz) δ (ppm): 8.47-8.45 (m, 2H), 7.87-7.76 (m, 6H), 7.63-7.50 (m, 5H), 7.36-7.28 (m, 19H), 7.05-6.91 (m, 5H), 6.37-6.10 (m, 1H), 5.33 (m, 1H), 4.57- 4.27 (m, 2H), 4.13-4.03 (m, 2H), 3.87-3.44 (m, 8H), 3.30 (m, 8H), 2.96-2.93 (m, 1H), 2.63-2.62 (m, 2H), 2.18-2.09 (m, 4H), 1.94-1.88 (m, 8H), 1.76-1.62 (m, 4H), 1.28 (m, 6H), 0.89 (m, 2H). ^{13}C NMR (CH_3DO, 125 MHz) δ (ppm): 175.7, 171.5, 165.4, 165.1, 160.6, 137.5, 133.4, 132.0, 131.9, 131.8, 129.8, 129.0, 128.7, 128.6, 128.5, 126.3, 117.3, 113.7, 113.6, 112.4, 112.3, 49.7, 49.6, 49.5, 49.2, 46.7, 46.5, 46.4, 46.2, 46.0, 45.9, 45.7, 45.5, 42.6, 30.1, 27.8, 27.5, 27.4, 25.3, 25.1, 25.0, 24.8, 18.4. LC-MS (ESI$^+$): m/z $C_{88}H_{86}ClN_4O_7P^{2+}$ calcd. 674.29, found [M^+] 674.69.

3. The Decomposition Products of Cy-HPT upon Laser Irradiation

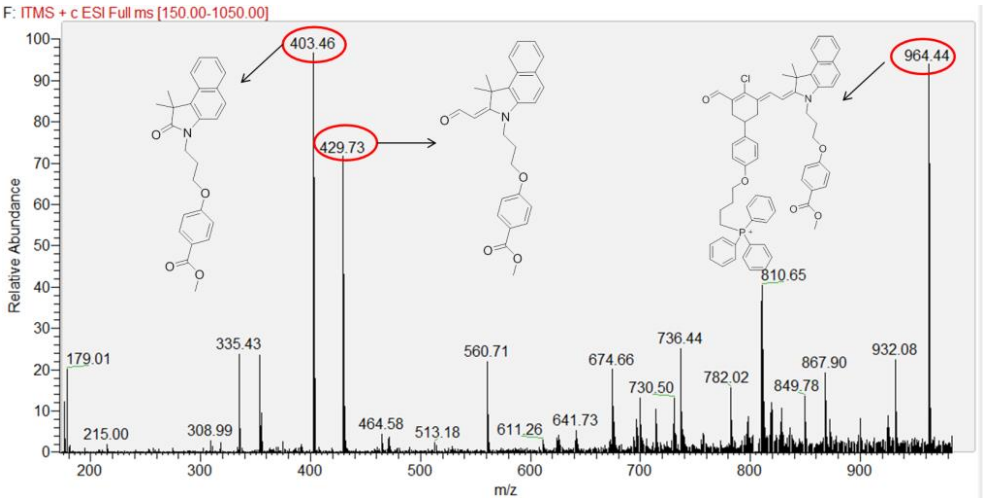


Figure S1. Mass spectra of **Cy-HPT** solution (a) before and (b) after laser irradiation (808 nm, 1.5 W/cm^2, 15 min).

4. pH Effects on Cy-HPT and HSA@Cy-HPT

Standard fluorescence pH titrations were performed in 10 mM HEPES solution at a concentration of Cy-HPT and HSA@Cy-HPT. As shown in Figure S1, the pH of the mediums hardly had effect on fluorescence intensity within the range from 3.0 to 8.4, which meant that the Cy-HPT and HSA@Cy-HPT would work well under physiological conditions (pH = 7.40).

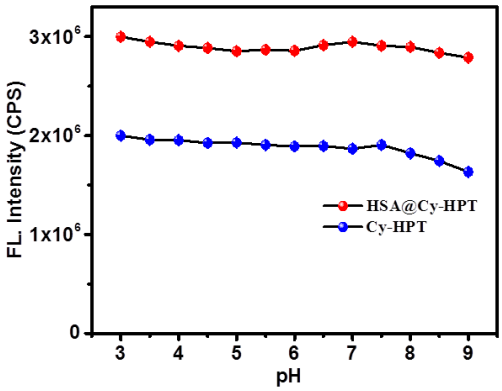


Figure S2. The pH Effects on Cy-HPT and HSA@Cy-HPT.

5. The Bright Field of Cells in Figure 2

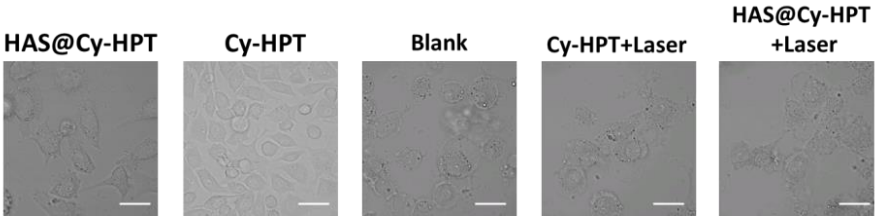


Figure S3. The bright field of cells in Figure 2. Scale bar = 20 μm.

6. The Bright Field of Cells in Figure 3

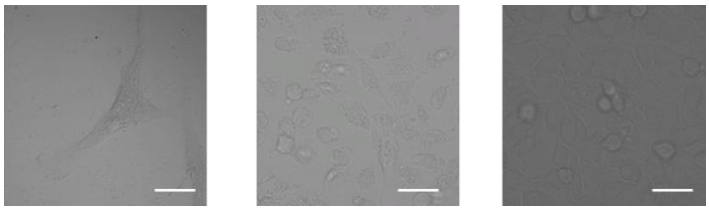


Figure S4. The bright field of cells in Figure 3. Scale bar = 20 μm.

7. The Bright Field of Cells in Figure 4

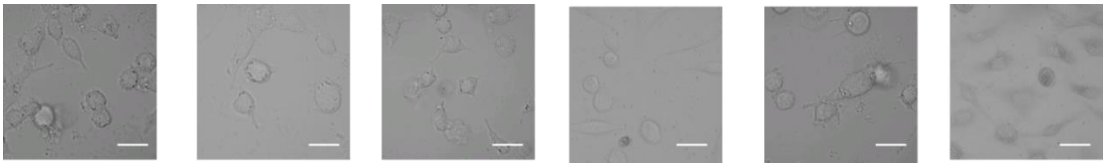


Figure S5. The bright field of cells in Figure 4. Scale bar = 20 μm.

8. The Mean Fluorescence Intensity of Figure 4.

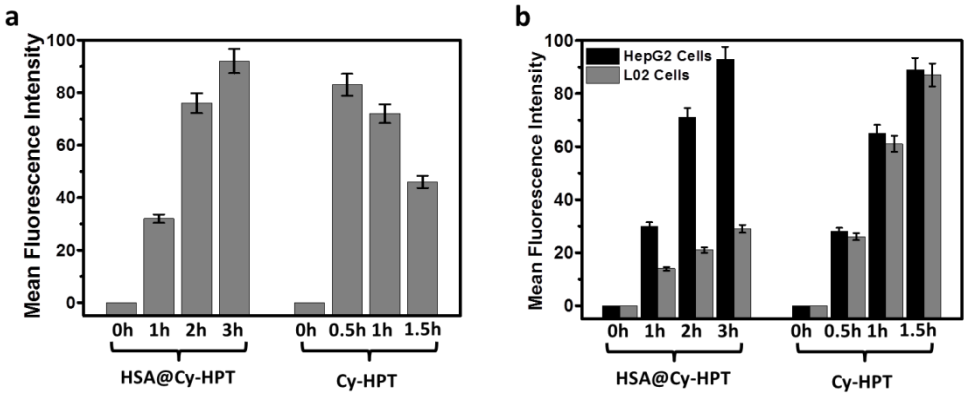


Figure S6. (a) The mean fluorescence intensity of Figure 4a. (b) The mean fluorescence intensity of Figure 4b. Data are presented as mean ± SD (n = 5).

9. Tumor-bearing Mice Imaging of HSA@Cy-HPT.

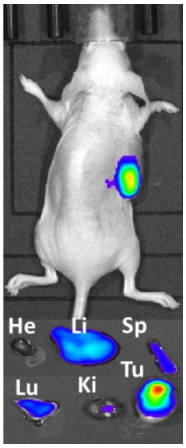


Figure S7. Tumor-bearing mice in vivo and ex vivo fluorescent imaging of dissected main organs of HSA@Cy-HPT at time point of 12 h. He: heart; Li: liver; Sp: spleen; Lu: lung; Ki: kidney; Tu: tumor. Images displayed represent emission intensities collected window: 830 - 880 nm, λ_{ex}= 808 nm.

10. The Mean Fluorescence Intensity in vivo Images of Figure 6.

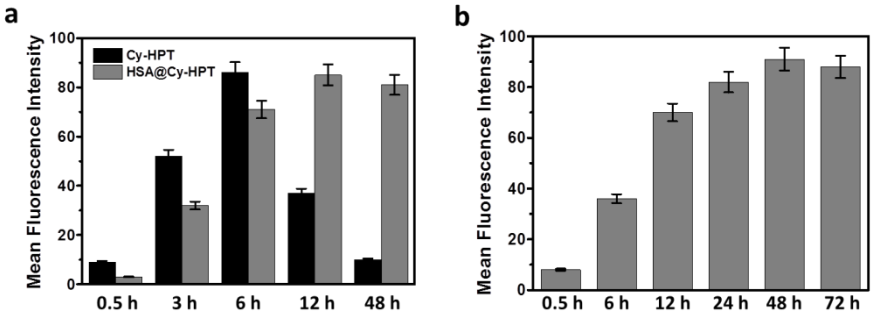
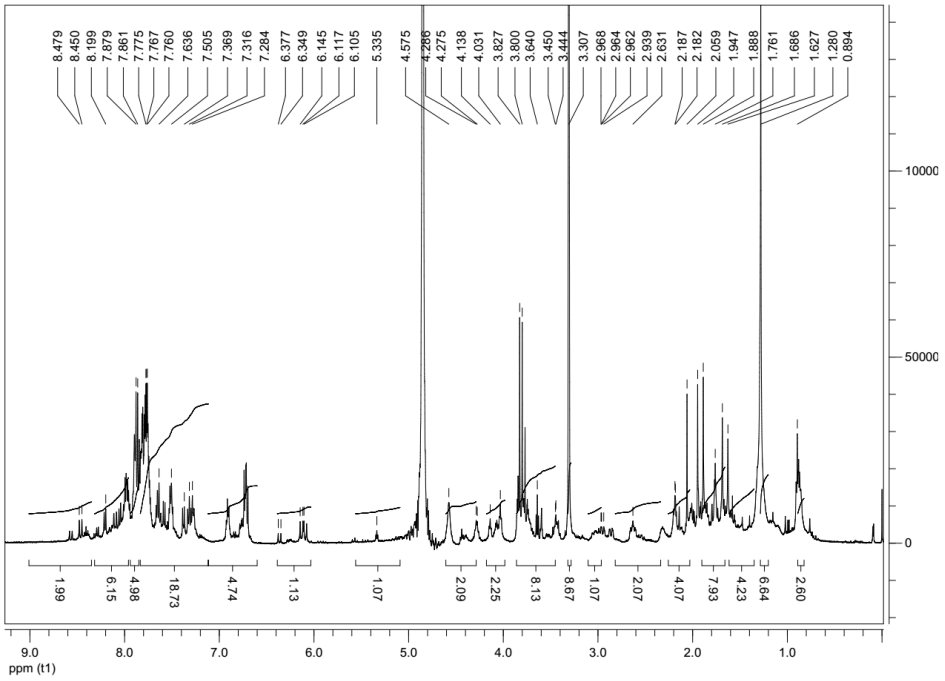


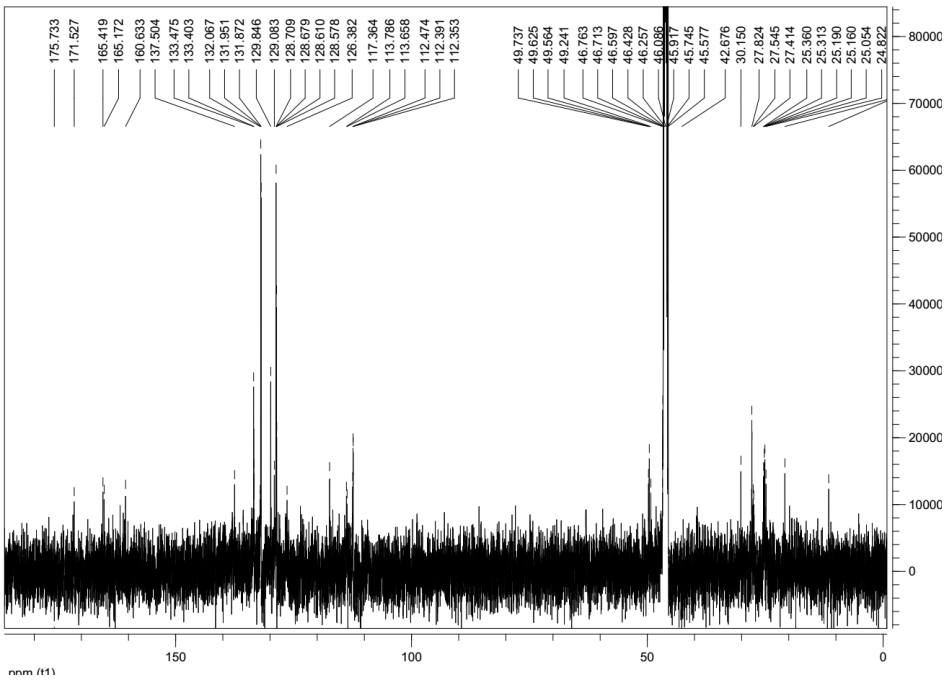
Figure S8. (a) The mean fluorescence intensity of Figure 6a. (b) The mean fluorescence intensity of Figure 6b. Data are presented as mean ± SD (n = 5).

11. ^{1}H NMR, ^{13}C NMR and LC-MS of Cy-HPT

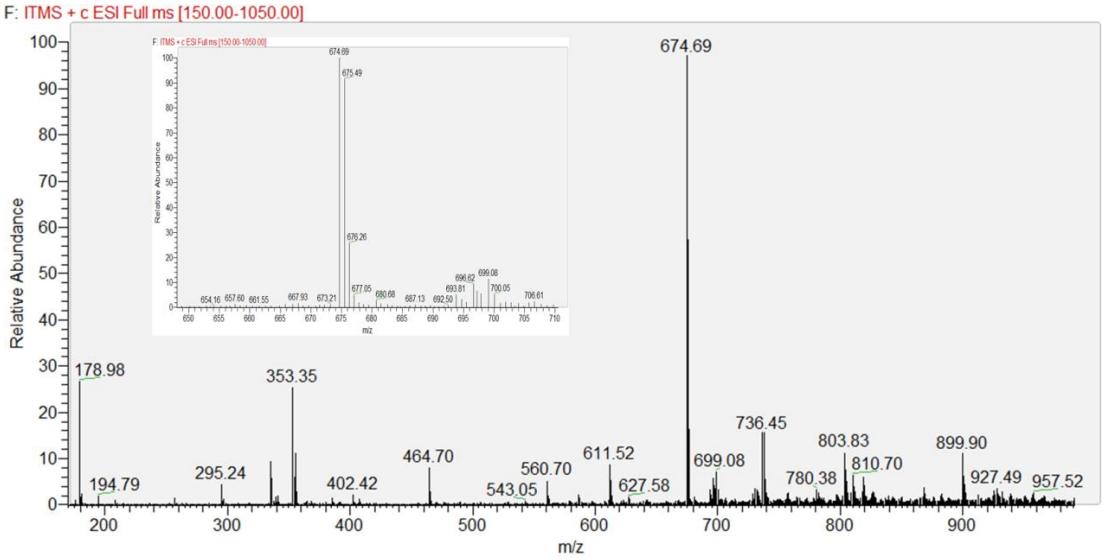
^{1}H NMR



^{13}C NMR



LC-MS



12. References

[1] D. F. Eaton, Pure Appl. Chem. 1988, 60, 1107.

[2] I. Scalise, E. N. Durantini, Bioorg. Med. Chem. 2005, 13, 3037.

[3] S. Luo, X. Tan, S. T. Fang, Y. Wang, T. Liu, X. Wang, Y. Yuan, H. Q. Sun, Q. R. Qi, C. M. Shi, Adv. Funct. Mater. 2016, 26, 2826.