**Supporting Information**

**Fluorescence Lifetime Imaging-Guided Photodynamic Therapy over Two-Photon Responsive Metal-Organic Frameworks**

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**1. Experimental and methods**

**1.1 Materials and apparatus**

The general chemicals were obtained from Shanghai Macklin (China). Annexin V-FITC/PI Apoptosis Detection Kit were obtained from Shanghai Bestbio (China). The 1H-NMR spectra were recorded on at 25°C, using Bruker 400/600 Ultra shield spectrometer were reported as parts per million (ppm) from TMS (δ). UV-vis absorption spectra were recorded on a PerkinElmer Lambda 1050 + UV/vis/NIR spectrophotometer. SEM were detected by REGULUS8230\*. TEM were carried on a JEM-2100. XRD were recorded on SmartLab 9KW. Fluorescence measurements were carried out on a Hitachi F-7000 fluorescence spectrophotometer. IR spectra were recorded on a Nicolet FT-IR instrument. Quantum yield was determined by FLUORMAX-4P. Confocal laser scanning microscope imaging data acquisition and processing were performed using Lecia STELLARIS 8 equipped with one photon excitation source (405 nm), a white laser, and a femtosecond laser

**1.2 Two-photon excited fluorescence (2PEF) spectroscopy and two-photon action absorption (2PA) cross-section**

2PEF spectra were obtained by the two-photon excited fluorescence method with a femtosecond laser pulse and a Ti: sapphire system (680-1080 nm, 80 MHz, 140 fs) as the light source. The reference sample is Rhodamine B with a concentration of 1.0 × 10-3 M. The concentration of **ZrTc@HA** and **ZTBH** was 500 μg/mL. The calculations for the two-photon action absorption cross section are derived from our previous work.1-2

**1.3 Singlet oxygen (1O2) generation detection**

The singlet oxygen was measured by a singlet oxygen indicator (9,10-Anthracenediyl-bis(methylene)dimalonic, ABDA). Briefly, 2 mL of deionized water solution was mixed with **ZTBH** (50 μg/mL)) and DPBF (100 μM). Then the cuvette was exposed to NIR laser (850 nm, 1 W/cm2) irradiation or under dark condition for different time. The absorption spectra of ABDA showed a gradual decline with the irradiation.

**1.4 Superoxide anion radical (O2•-) detection.**

Dihydrorhodamine 123 (DHR123) was used as the superoxide anion radical indicator. Both **ZTBH** (50 μg/mL) and DHR123 (100 μM) were prepared in DI water,respectively. Then the cuvette was exposed to NIR laser (850 nm, 1 W/cm2) or under dark condition for different time, and the fluorescence spectra was observed to rise immediately after irradiation.3

**1.5 Electron spin resonance (ESR) assay.**

ESR was used to assess the generation of 1O2 and O2•- by **ZTBH**. The spin traps 2,2,6,6-tetramethylpiperidine (TEMP for trapping 1O2, 20 μL) and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO for trapping O2•-, 20 μL) were used to verify the species of reactive oxygen species (ROS) generated by **ZTBH** (50 μg/mL). The ESR signals of the **ZTBH** before and after NIR laser (850 nm, 1 W/cm2) irradiation were recorded.4-5

**1.6 Cell culture**

The HepG2 cells a were cultured in 25 cm2 culture flasks in DMEM, supplemented with fetal bovine serum (10%), penicillin (100 units/mL) and streptomycin (50 units/mL) at 37 °C in a CO2 incubator (95% relative humidity, 5% CO2). Cells were seeded in 35 mm glass bottom cell culture dishes, at a density of 1 × 105 cells and were allowed to grow when the cells reached more than 60% density. The three compounds were dissolved in DMSO with concentration of 1 mM as stock solution, and the commercial dyes were prepared as 1 mM PBS solution and diluted to working concentration as protocol required.

**1.7 Cell uptake analysis**

HEK 293T cells (CD44-negative cells) and HepG2 cells (CD44-positive cells) were seeded onto corresponding cell culture dishes and grown to about 70% density before used. HEK 293T cells were treated with **ZTBH** (50 μg/mL), HepG2 cells were treated with **ZrTc@BODIPY** (50 μg/mL) and **ZTBH** (50 μg/mL), respective. Furthermore, HepG2 cells were precultured with free HA to block the cancer-specific targeting of **ZTBH**. After 12 h incubation, the cellular uptake ability of **ZTBH** were analyzed using CLSM.

**1.8 Cytotoxicity assays in cells**

The study of the effect of **ZTBH** on viability of cells was carried out using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. **ZTBH** stock solutions were diluted by fresh medium in to desired concentration (10, 30, 50, 70, 90, 100, 150, 200 μg/mL). HepG2 cells were cultured in a 96-well plate for 24 h before experiments. The cell medium was then exchanged by different concentrations of **ZTBH** medium solutions. They were incubated at 37 °C in 5% CO2 for 12 h before cell viability was measured by the MTT assay. The cell medium solutions were exchanged by 100 μL of fresh medium, followed by the addition of 20 μL (5 mg/mL) MTT solution to each well. The cell plates were then incubated at 37 °C in 5% CO2 for 4 h. After MTT medium removal, the formazan crystals were dissolved in DMSO (100 μL/well) and the absorbance was measured at 490 nm using a microplate reader.

**1.9 ROS generation under two-photon laser in vitro**

The ROS production in living cells was also assessed. HepG2 cells were seeded in Petri dishes and incubated for 24 h. **ZTBH** (50 μg/mL) was added and incubated with the cells for 12 h. Then, the cells were washed with PBS solution and incubated with SOSG (1 μM) and DHE (1 μM) for 30 min, after which the cells were incubated shielded from laser or irradiated for 5 min (850 nm laser). And then the cells were observed by CLSM.

**1.10 Annexin V-FITC and PI assays.**

HepG2 cells were incubated with **ZTBH** (0.5 mg/mL, 12 h) and then further stained with cell apoptosis detection kit. The cells were irradiated for 15 min (850 nm, 0.1 W/cm2), and the fluorescent signals were collected via CLSM.

**1.11 Live/dead assay with calcein AM/PI.**

HepG2 cells were incubated with **ZTBH** (0.5 mg/mL, 12 h). Calcein AM and PI were then used to confirm the viability of the HepG2 cells. The signals were collected via CLSM after laser irradiation (λex = 850 nm laser irradiation, 0.1 W/cm2).

**1.12 In Vivo TP-FLIM on Liver Tumor of Zebrafish.**

Zebrafish embryos xenotransplanted with HepG2 cells treated with **ZTBH** were purchased from Hunter Biotechnology, Inc. Zebrafish were immobilized in a mixture of agarose. The TP-FLIM images of the zebrafish were collected every 5 min under 850 nm laser (0.1 W/cm2) irradiation.

**1.13 DFT calculations**

Geometry optimization of **ZrTc** and **ZrTc@BODIPY** were perfromed via the B3LYP hybrid functional with 6-31G(d) basis sets in the Gaussian 16 package. Multiwfn 3.88 was used to analyze the molecular orbitals (MOs) and dipole moments, and VMD 193 software8 was used for molecular visualization.6-10

**1.14 Statistical analysis**

Statistical analyses were carried out via unpaired t test analysis via GraphPad Prism software. All experimental data are expressed as the means ± SDs. The differences in all the data were regarded as significant for the p value: ns, not significant, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

**1.15 Synthesis route of BODIPY**

**Synthesis of BODIPY-Br:** To a solution of 2,4-dimethyl-1H-pyrrole (1.19 g, 12.5 mmol) and 4-bromobenzaldehyde (0.925 g, 5 mmol) in dry CH2Cl2 (100 mL) is added trifluoroacetic acid (50 μL, 0.65 mmol) at room temperature. After 3 h of stirring under ice bath, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (1.128 g, 5 mmol) is added and stirred for additional 1 h at room temperature. Then, triethylamine (10 mL, 72 mmol) is added, followed by slow addition of BF3·Et2O (10 mL, 81 mmol). The reaction mixture is washed after 2 h of stirring at room temperature with saturated aqueous Na2CO3 solution (350 mL) and further purified by column chromatography on silica with petroleum ether/ethyl acetate = 100:1. The product fraction is dried to yield a red solid.

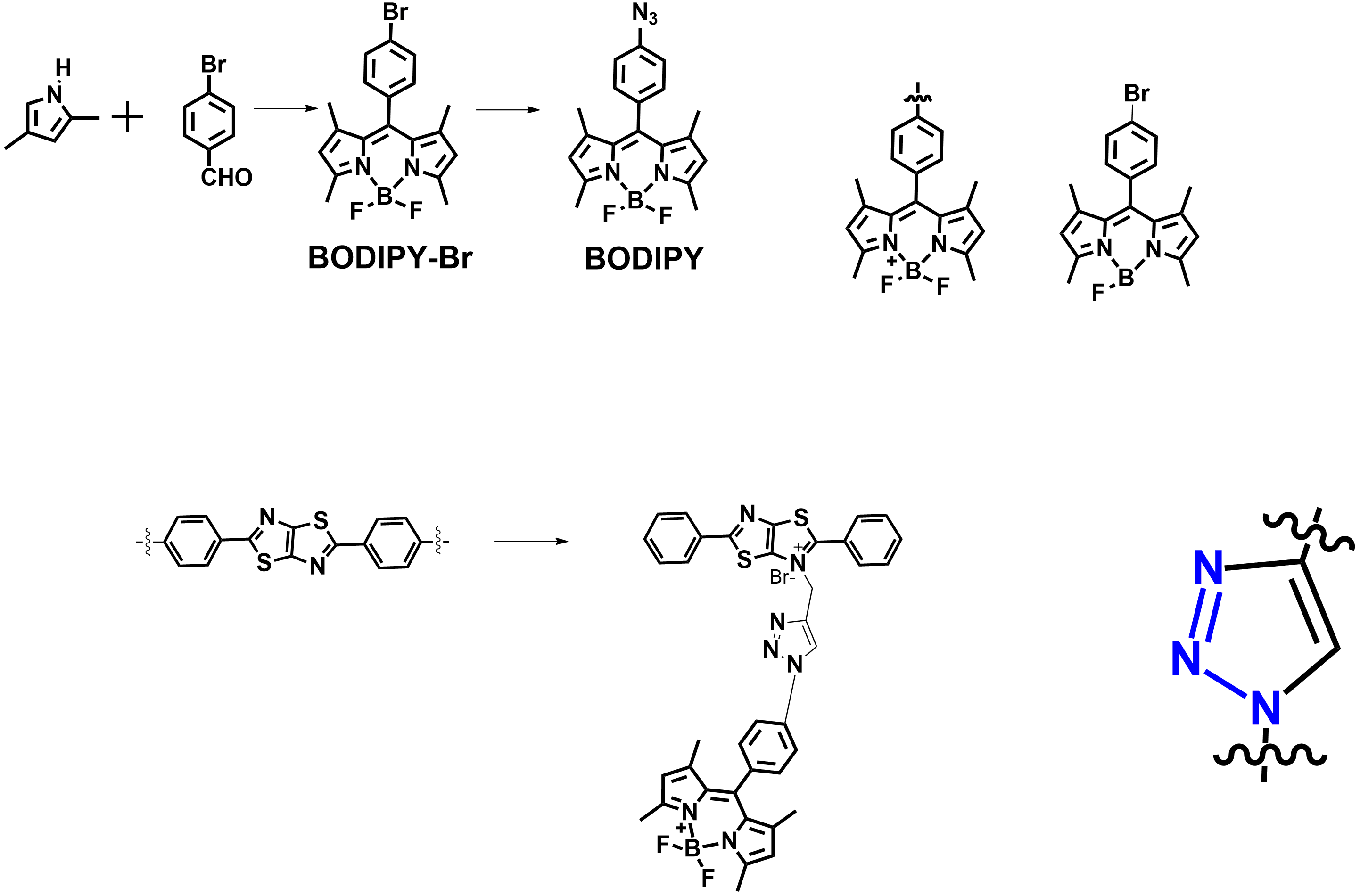
**Synthesis of BODIPY: BODIPY-Br** (0.785 g, 1.95 mmol), TMSN3 (0.674 g, 5.85 mmol) and TBAF (1.53 g, 5.85 mmol) were added to EtOH (10 mL), and then the mixture was heated to reflux under stirring for 24 h. After cooling down, the product **BODIPY** was purified by column chromatography.

**Synthesis of ZrTc:** 3 mL DMF solution containing **Tc** (0.0014 mol/L), ZrCl4 (0.001 mol/L) and benzoic acid (0.014 g) was added to 5 mL glass bottle and reacted in the oven for 72 h at 120°C. After dropping down to room temperature, the obtained solid was collected by centrifuging. The solid was washed with DMF and ethanol for three times, respectively, and then dried in a vacuum at 60°C before use.

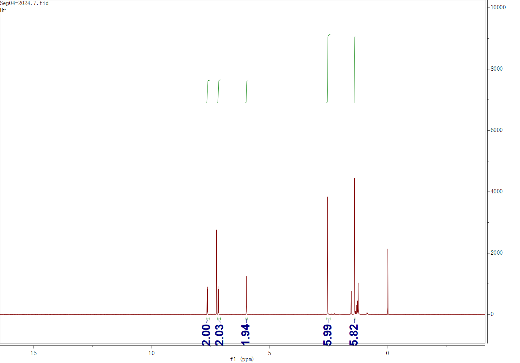
**ZrTc alkylation**: 50 mg **ZrTc** was dissolved in 8 mL ethanol. Then 300 μL propargyl bromide was added. The reaction mixture was heated at 60°C and continued stirring for 3 days. The obtained solid was collected by centrifuging. Then, the solid was washed with ethanol for three times. The product was dried in a vacuum at 60°C before use.

**Synthesis of ZrTc@BODIPY:** **BODIPY** (0.46 g, 1.25 mmol) were added to a mixture of **ZrTc alkylation** (50 mg) and CuI (3.0 mg) in DMF (10 mL) in 50mL round-bottom flask. The reaction mixture was stirred at 60 °C for 72 h. The resultant precipitate was collected by centrifugation, washing with DMF and methanol, and dried under freeze drying.

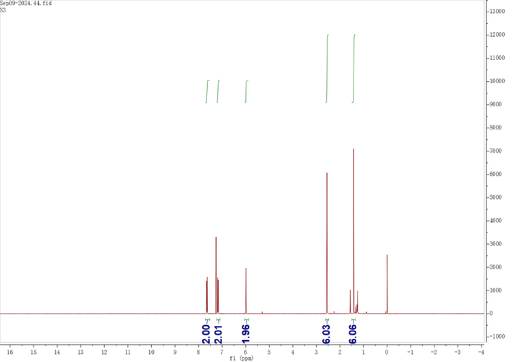
**Synthesis of ZTBH:** 1 mg of **ZrTc@BODIPY** were dispersed in 20 mL DI water (pH = 7) containing 2 mg of hyaluronic acid (HA), and the mixture was stirred for 24 h at room temperature. Then, **ZTBH** was washed with DI water three times by repeated centrifugation and redispersion, before being dried under freeze drying.



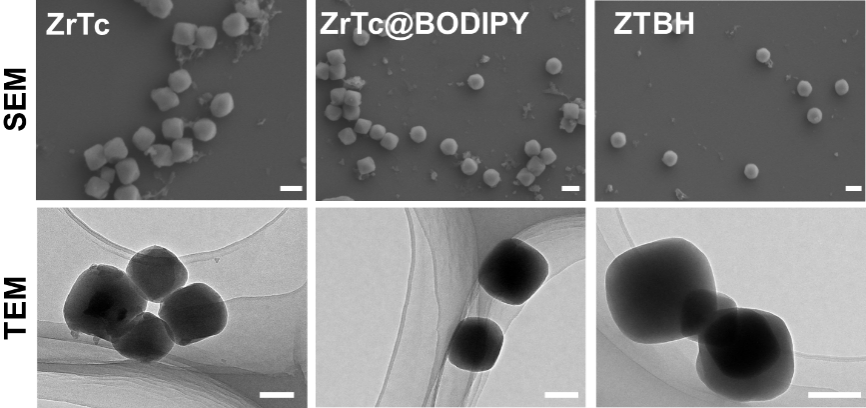
**Fig. S1.** Synthesis route of **BODIPY**.



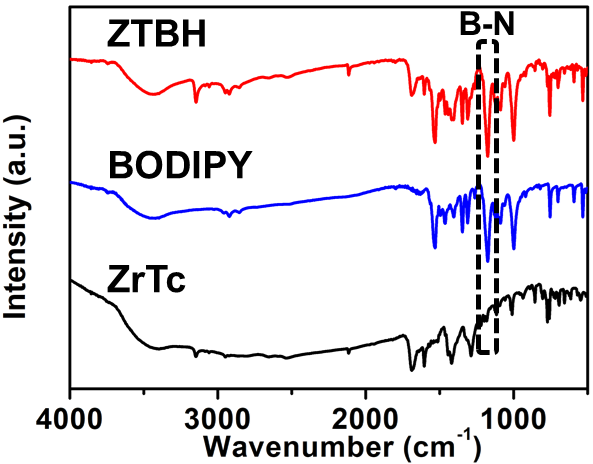
**Fig. S2.** A) 1H NMR (400 MHz, CDCl3, r.t.) spectrum **BODIPY-Br**.



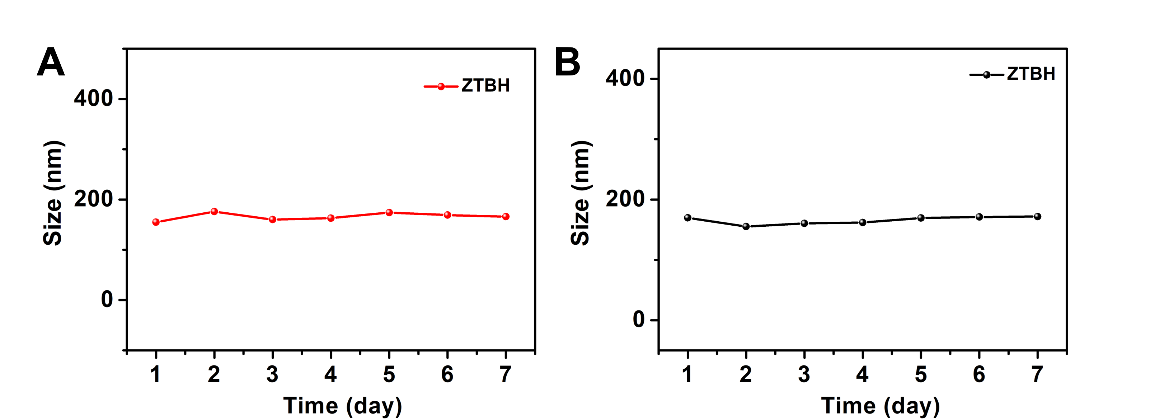
**Fig. S3.** 1H NMR (400 MHz, CDCl3, r.t.) spectrum of **BODIPY**.



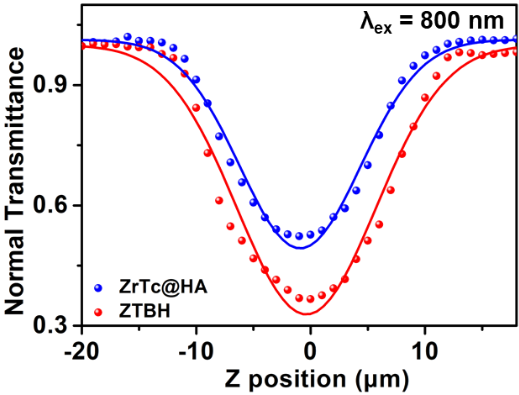
**Fig. S4.** SEM image of **ZrTc**, **ZrTc@BODIPY**, and **ZTBH** (scale bar: 200 nm). TEM image of **ZrTc**, **ZrTc@BODIPY**, and **ZTBH** (scale bar: 100 nm).



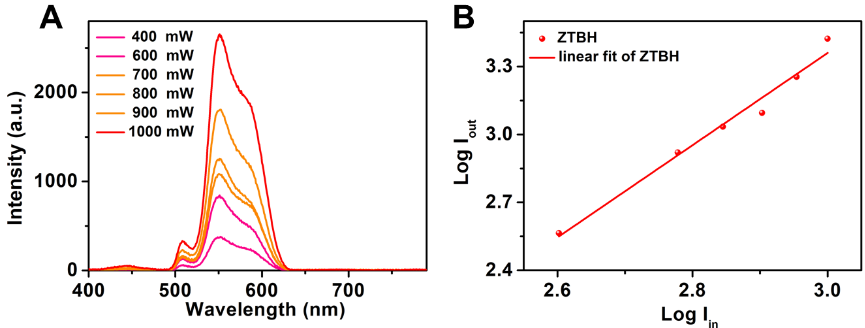
**Fig.S5.** FT-IR spectra of **ZrTc**, **BODIPY** and **ZTBH**.



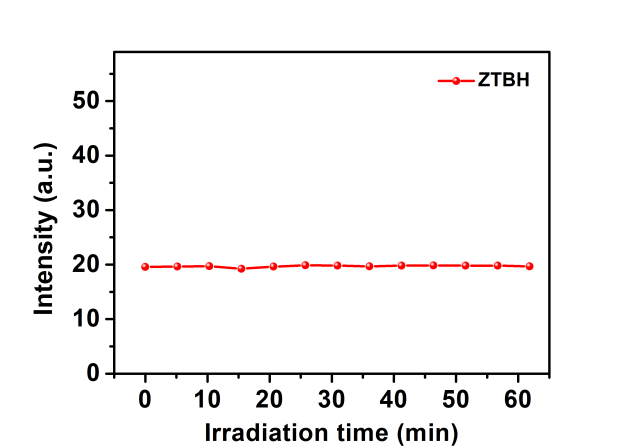
**Fig. S6.** A) DLS measurement of **ZTBH** in PBS solution during 7 days. B) DLS measurement of ZTBH in serum-containing medium during 7 days.



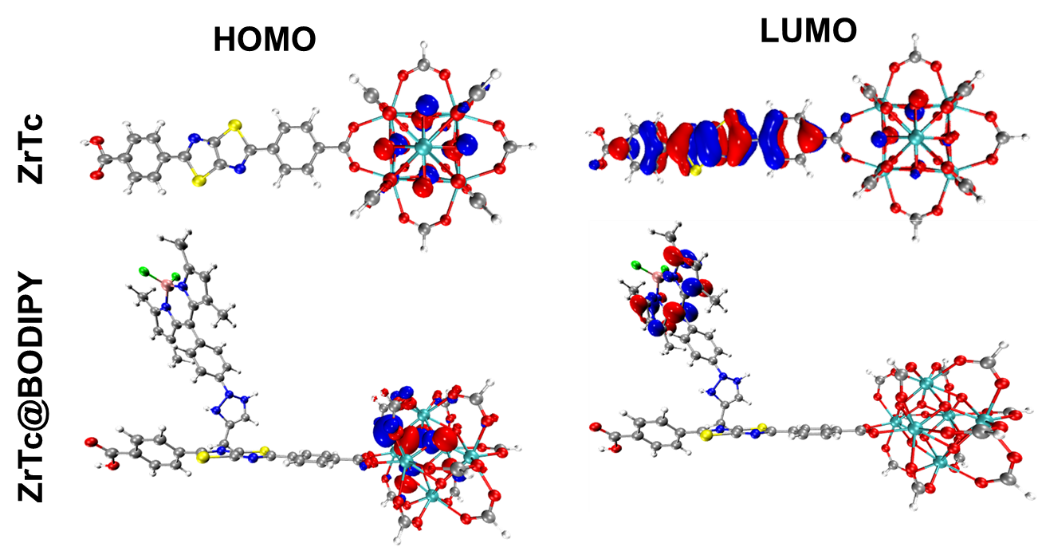
**Fig. S7.** Open aperture Z-scan plots of **ZrTc@HA** and **ZTBH** (0.5 mg/mL, λex = 800 nm, fixed power:1 W/cm2).



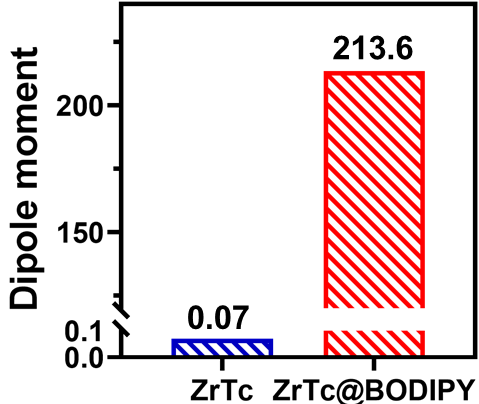
**Fig. S8.** A) Two-photon excited fluorescence sprctra of **ZTBH** with femtosecond laser excitation at 850 nm with different input power. B) Two-photon absorption verification of **ZTBH**.



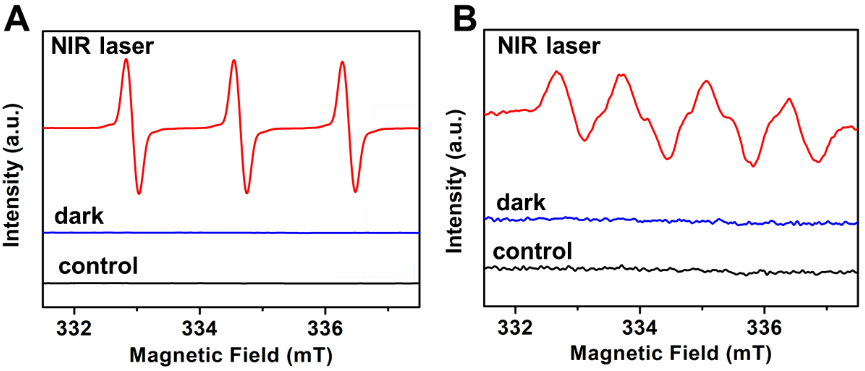
**Fig. S9.** The photostability of **ZTBH** excited by 850 nm (1 W/cm2).



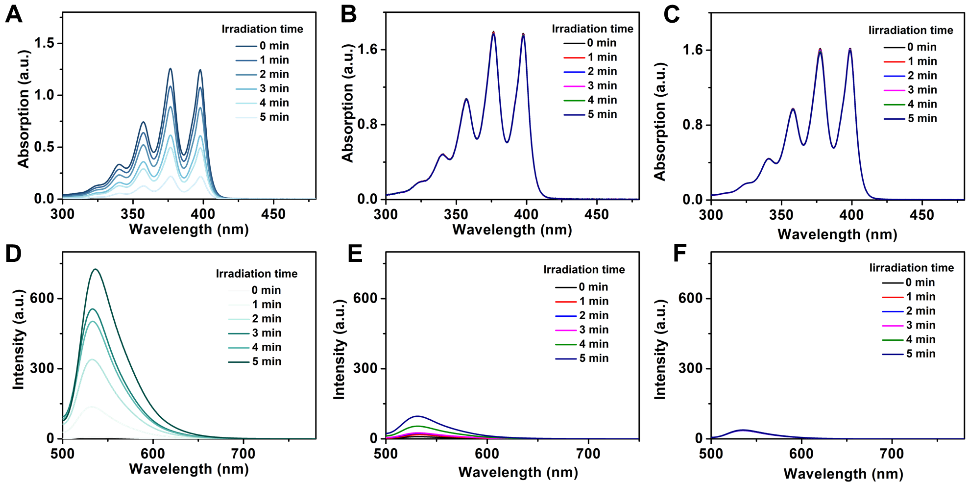
**Fig. S10.** Simulated molecular orbital spin density distribution of **ZrTc** and **ZrTc@BODIPY** (Gaussian 16).



**Fig. S11.** The calculated dipole moment of **ZrTc** and **ZrTc@BODIPY**.



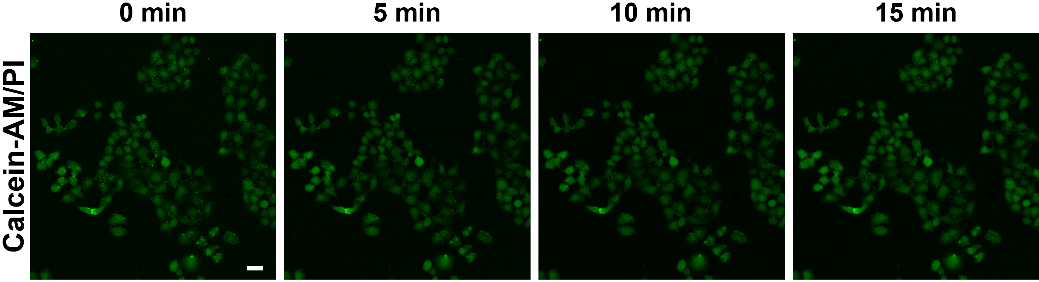
**Fig. S12.** A) ESR signals of **ZTBH** trapped by TEMP (λex = 850 nm, 1 W/cm2). B) ESR signals of **ZTBH** trapped by DMPO (λex = 850 nm, 1 W/cm2).



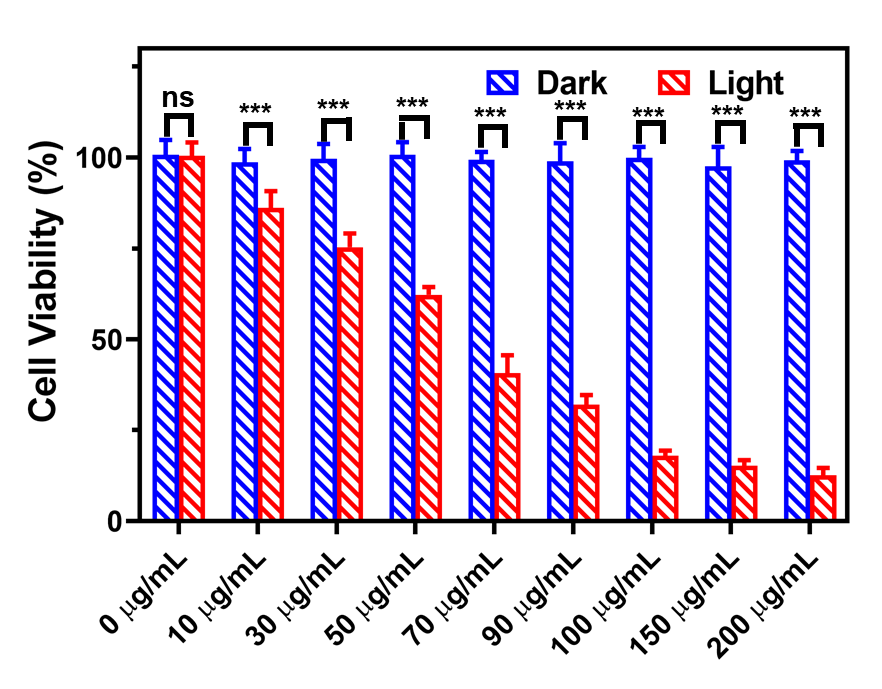
**Fig. S13.** A) Absorption spectra of ABDA treated with **ZTBH** (50 μg/mL) under NIR light irradiation. B) Absorption spectra of ABDA under NIR light irradiation. C) Absorption spectra of ABDA treated with **ZTBH** (50 μg/mL) under dark condition. D) Emission spectra of DHR123 treated with **ZTBH** (50 μg/mL) in the solution under NIR light irradiation. E) Emission spectra of DHR123 under NIR light irradiation. F) Emission spectra of DHR123 treated with **ZTBH** (50 μg/mL) in the solution under dark condition.



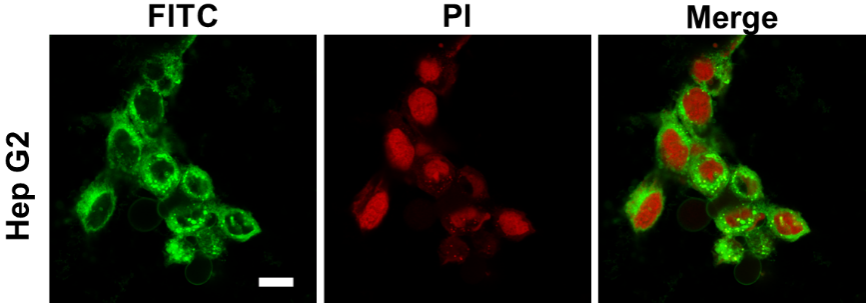
**Fig. S14.** Confocal images to check the cell uptake of **ZTBH** (scale bar: 20 μm). G1: HepG2 cells treated with **ZTBH**. G2: HEK 293T cells treated with **ZTBH**. G3: HepG2 cells treated with **ZrTc@BODIPY**. G4: HepG2 cells treated with free HA and **ZTBH**.



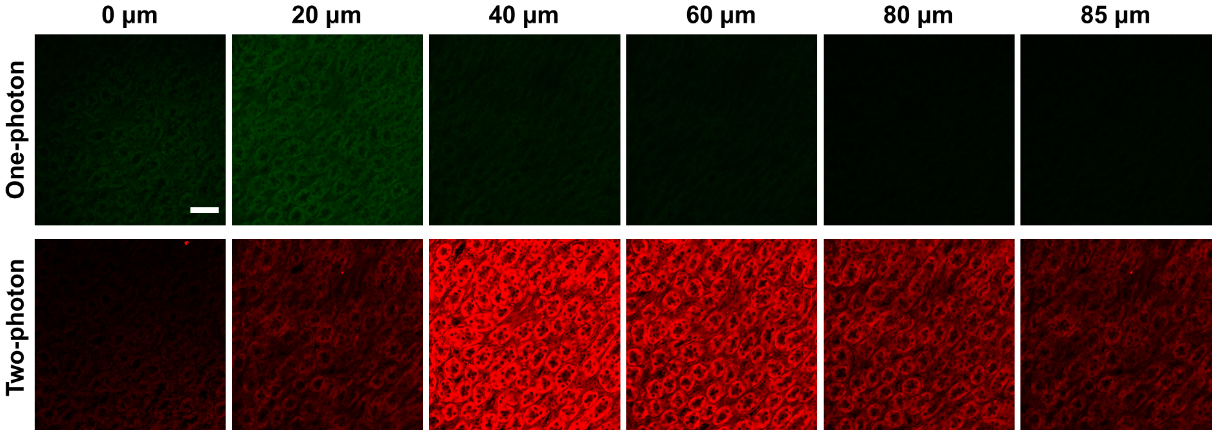
**Fig S15.** CLSM images of calcein AM/PI stained Hep G2 cells under dark and 850 nm laser irradiation (laser power: 0.1 W/cm2; irradiation time: 15 min; scale bar: 50 μm).



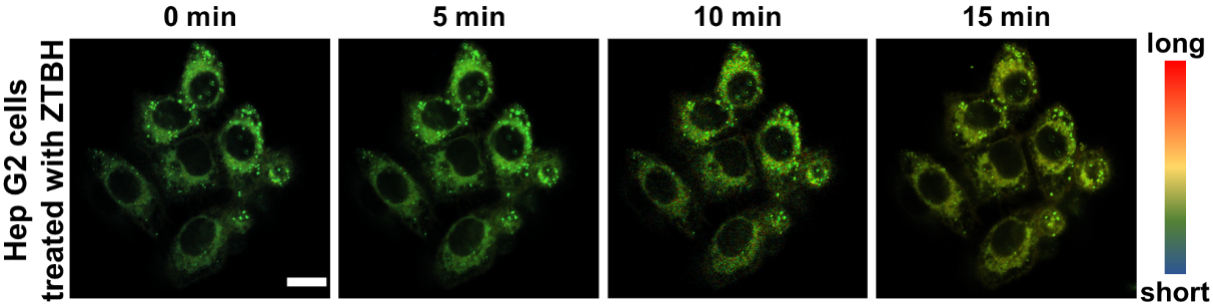
**Fig. S16.** Cell viability of HepG2 cells treated with **ZTBH** at different concentration with (red) and without (blue) NIR irradiation.



**Fig. S17.** HepG2 cells treated with **ZTBH** and Annexin V-FITC / PI under 850 nm irradiation (scale bar: 20 μm).



**Fig. S18.** One-photon (λex = 500 nm, 0.1 W/cm2) and B) two-photon (λex = 850 nm, 0.1 W/cm2) fluorescence images of mouse kidney tissue incubated with **ZTBH** at different penetration depth along the z axis (scale bar:200 μm).



**Fig. S19.** TP-FLIM images of HepG2 cells treated with **ZTBH** under different irradiation time (λex = 850 nm, 0.1 W/cm2; scale bar:20 μm).

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