Supporting Information

Water-Soluble Conjugated Polymer with Near-Infrared (NIR)-Absorbing for Synergistic Tumor Therapy by Photothermal and Photodynamic Activity

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Experimental Procedures

Materials and Measurements. All chemical reagents were purchased from Sigma-Aldrich and Alfa-Aesar commercial company and used as recieved. UV-Vis-NIR absorption spectra were recorded on a UH5300 spectrophotometer and fluorescence emission were measured on a Hitachi F-4600 fluorescence spectrophotometer with a Xenon lamp as the excitation source, respectively. The morphology of PTDBD was determined by using scanning electron microscopy (SEM, JSM-6700F). Size and zeta potential were measured by the Malvern ZetaSizer Nano ZS90. The NIR light was 808 nm laser and purchased from Changchun New Industries Optoelectronics Tech. Co., Ltd. Infrared (IR) thermal images and temperature were measured by a thermal imaging camera Ti480 (Fluke, USA). A microplate reader (Bio-Rad) was employed to record cell viability. Fluorescence images were captured by a confocal laser scanning microscopy (Zeiss LSM 880).

Synthesis of conjugated polymer PTDBD. Monomer 2 was synthesized according to the previously reported literatures.[39] The synthesis of PTDBD was completed by Suzuki cross-coupling reaction,[40] specific process was as follows: A solution of monomer 1 (13.0 mg, 0.038 mmol) and monomer 2 (300.0 mg, 0.38 mmol), and monomer 3 (130.0 mg, 0.34 mmol) in 1,4-dioxane (15 mL) and K_2CO_3 aqueous solution (2.0 M, 5 mL) was firstly degassed and then $Pd(PPh_3)_4$ was added. The resulting solution was stirred at 90 ^oC for 48 h under nitrogen. After the reaction was finished, the solvent was removed under vacuum. Then the residue was extracted with dichloromethane and then washed

by brine. The blue organic layer was dried by anhydrous Na_2SO_4. After concentrating the solvent to 1~2 mL, the solution was then added into 300 mL of methanol. The precipitate was collected by centrifugation. The process was repeated twice to offer a black solid (230 mg, 43%). Then, it was treated with trimethylamine aqueous solution at room temperature for 48 h. After removing the solvent under vacuum, PTDBD was obtained. ^{1}H-NMR (600 MHz, ppm): δ7.69 (s, 14H), 3.54 (s, 52H), 1.44 (s, 32H).

Reactive oxygen species (ROS) measurement. Activated 2,7-dichlorofluorescin (DCFH, 40.0 μM) was prepared to add to PTDBD solutions forming a mixture solution of DCFH (10.0 μM) and PTDBD (5.0 μM). DCFH solution (10.0 μM) alone with the same volume was performed as control group. Then the specimens were irradiated with 808 nm laser light (1.0 W/cm^2) and the fluorescence intensities changes were measured at intervals of 1 min. The recorded fluorescence intensity was measured at wavelength of 524 nm, and the excitation wavelength was 488 nm. As comparison, a reported conjugated polymer was measured by irradiation of white light with other condition same with PTDBD.

Photothermal performance measurement. (1) PTDBD solutions with different concentrations were irradiated by an 808 nm laser at a series of densities for 8 min to get the photothermal profiles of PTDBD. Pure water under the same condition was used as the control group. Thermal imaging camera Ti480 was used to monitor the temperature changes and record the thermal images. (2) The PCE of PTDBD was measured by reported method.[37] Briefly, PTDBD solution was irradiated with an 808 nm laser for 8 min to get a plateau. Then the laser was shut off and the temperature of

solution was monitored until it returned to original value.

Synergetic phototherapy in *vitro*. MTT assay was employed to evaluate the cell viability. HeLa cells were seeded into 96-well plates at a concentration of 1×10^4 cells/well. After incubation overnight, all cells were divided into four groups: dark, PDT, PTT, and PDT&PTT. Cells in each group further incubated in fresh culture medium containing different concentrations of PTDBD for 4 h. cells in PDT&PTT groups were irradiated with 808 nm NIR laser at a series of densities (0.5, 0.75, 1 W/cm^2) for different time (2, 4, 6, 8 min). For PDT group, the heat was eliminated by ice, and ROS was eliminated by sodium azide (NaN_3) for PTT group. After incubated for another 4 h, the cells were further incubated with MTT (500 μg/mL) solution for another 3 h. Then DMSO (100 μL) was added per well to replace the previous medium. Absorbance at 490 nm was then recorded by microplate reader. All results were performed in triplicate and presented as mean ± SD compared to untreated cells.

Fluorescence imaging characterization. (1) After incubated overnight, HeLa cells were divided into four groups: blank, laser, PTDBD, PTDBD + laser. Expect blank group, the other three groups were further incubated with PTDBD (100 μg/mL)-contained fresh culture medium for another 4 h. Then the cells in laser and PTDBD + laser group were irradiated with 808 nm NIR laser (1.0 W/cm^2, 8 min). After irradiation, the cells both with and without irradiation were further incubated for another 4 h. Then the cells were washed with PBS (pH 7.4), and then stained with AO/EB for 4 min. After washed by PBS, the samples were characterized by fluorescence microscopy using a 488 nm laser for AO, and 550 nm laser for EB. (2) For imaging of ROS in cellular level,

HeLa cells were divided into three groups: blank, laser (+) and laser (-). The cells after incubation with PTDBD (40 μg/mL, 4 h) were further incubated with DCFH-DA (10 μM) for 50 min in the dark. Then the cells in laser (+) group were irradiated with 808 nm NIR laser (1.0 W/cm^2, 8 min), the other groups were in the dark for the same time. Then the cells were stained by DAPI (nuclei dye). After washed by PBS, the samples were characterized by fluorescence microscope using a 488nm laser for DCF, and a 405 nm for DAPI.

Synergetic phototherapy in *vivo*. All animal procedures were performed in accordance with the relevant laws and guidelines approved by the Animal Care and Use Committee of Shanxi University**.** Female BALB/c Nude mice (5 weeks old, Vital River Company) were used to build the HeLa tumor models. When the tumor size reached ~7 mm, the mice were divided into 4 groups (3/group): PBS, PBS + laser, PTDBD, PTDBD + laser. The intratumorally injected volume of solution in each group was 100 μL. The concentration of injected PTDBD was 192.8 μg/mL. Then the laser groups were irradiated with 808 nm NIR laser (1 W/cm^2) for 10 min. The temperature changes and IR thermal images were recorded by thermal imaging camera Ti480. The tumor size and body weight were monitored every three days. After 21 days treatment, all mice were executed and tumors were collected to weight. The major organs (heart, liver, spleen, lung, and kidney) of mice were then dissected from the mice to be processed by freezing microtome. Then Hematoxylin and Eosin (H&E) was used to stain the organs for further observation of pathologies by an optical microscope.

Results and Discussion

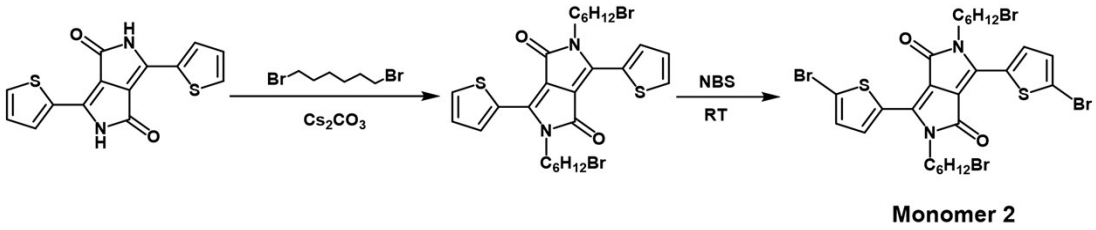


Figure S1. Synthetic routes of Monomer 2.

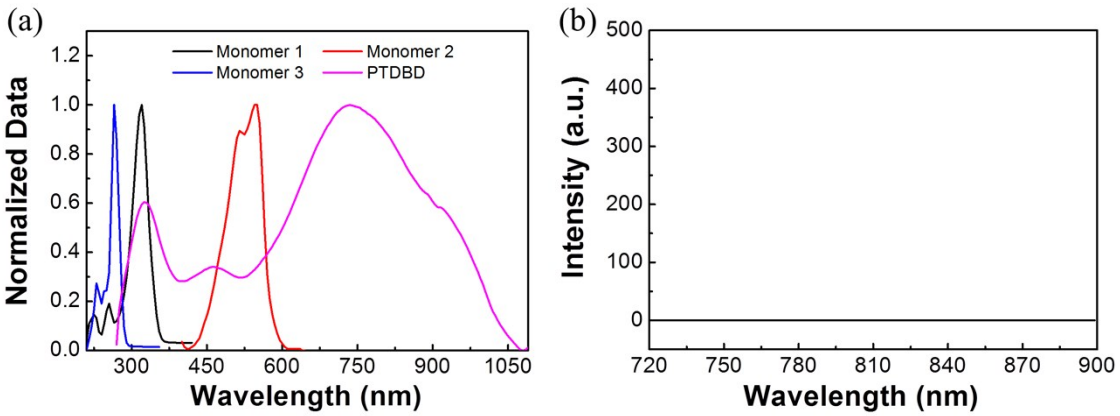
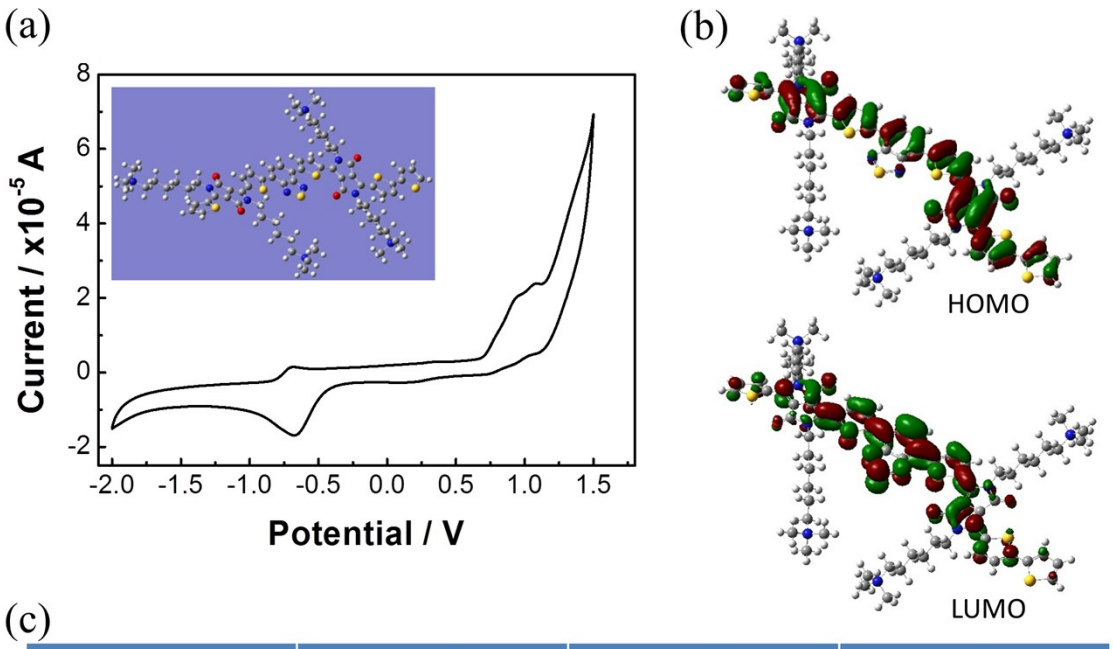


Figure S2. (a) Normalized UV-vis-NIR absorption of monomers and polymer PTDBD in DMSO. (b) Fluorescence emission spectra of PTDBD in water.



(c)

	HOMO / eV	LUMO / eV	Eg / eV
Theory	- 5.01	- 3.30	1.71
Actual	- 5.94	- 3.81	1.68

Figure S3. (a) Cyclic voltammograms of PTDBD. The supporting electrolyte was 0.1 M Bu_4NClO_4 and the scan rate was 0.05 V/s. [PTDBD] = 38 μM in RUs. The inset is the stereostructure of PTDBD. (b) HOMO and LUMO electron distributions of PTDBD. (c) Comparison of energy levels between theory and actual for PTDBD.

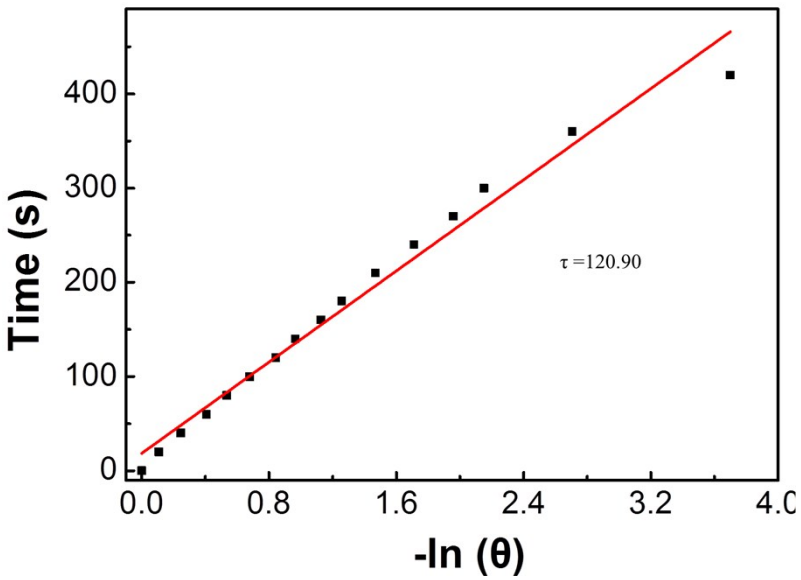


Figure S4. Linear time data versus -lnθ obtained from the cooling period of Figure 2d.

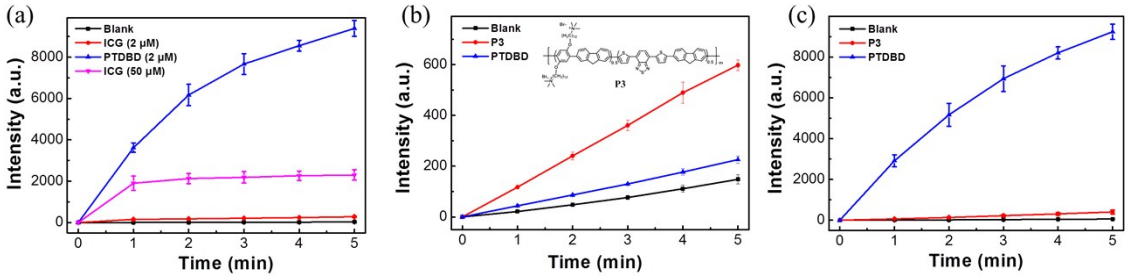


Figure S5. (a) Comparison of the ROS generation ability of PTDBD (2 μM in RUs) and ICG (2 μM, 50 μM). ROS generation of PTDBD and reported polymer P3 (2 μM in RUs) under irradiation of (b) white light and (c) 808 nm laser at the same condition (1 W/cm^2).

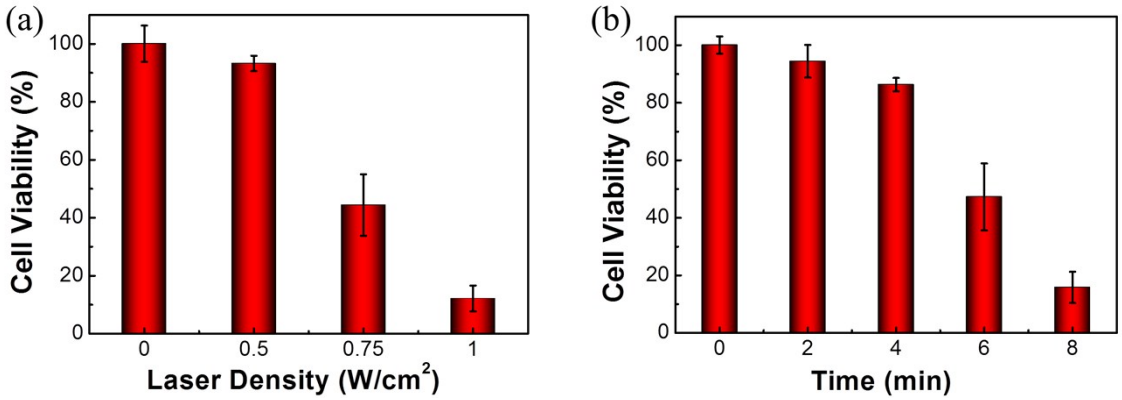


Figure S6. Cell viability of HeLa cells treated with PTDBD under irradiation (a) at different laser density and (b) for different irradiation time.

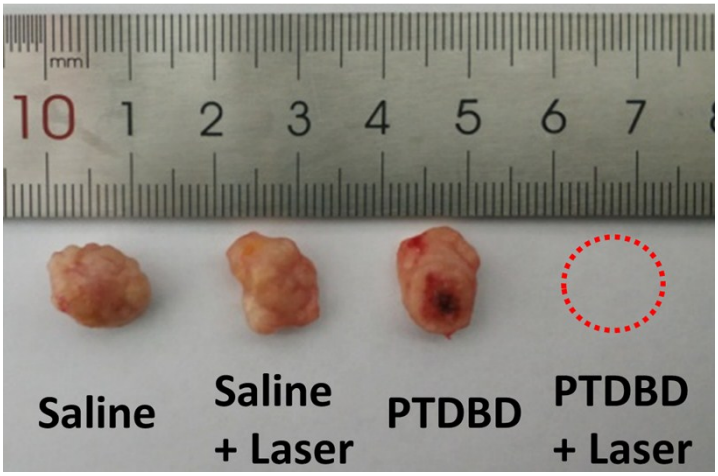


Figure S7. Photograph of tumors extracted from mice with different treatments.

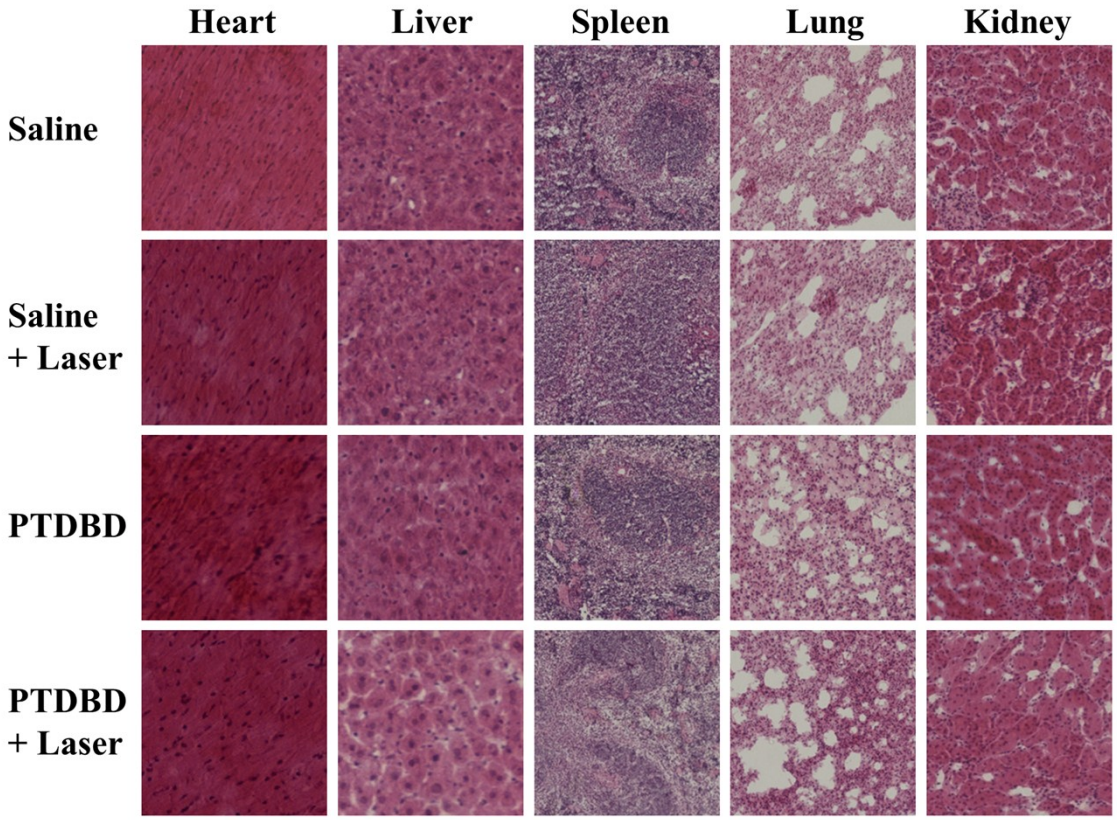


Figure S8. Images of H&E stained tissues of mice with different treatments.