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Supporting Information

Enhanced photothermal therapy performance for D-A conjugated polymers based on [1,2,3]triazolo[4,5-*g*]quinoxaline by manipulating molecular motion

Liwen Hu,[a,b] Linrui Shi,[a] Tianze Hu[a], Peiling Chen,[a] Ting Guo,[a] Chunxiao Wang,[c] Renqiang Yang[b] and Lei Ying*[a,d]*

[a]L. Hu, L. Shi, T. Hu, P. Chen, T. Guo, Prof L. Ying
Institute of Polymer Optoelectronic Materials and Devices, State Key Laboratory of Luminescent Materials and Devices, South China University of Technology, Guangzhou 510640, China
E-mail: mstguo@scut.edu.cn, msleiying@scut.edu.cn

[b]L. Hu, Prof R.Yang
Key Laboratory of Optoelectronic Chemical Materials and Devices (Ministry of Education), Flexible Display Materials and Technology Co-Innovation Centre of Hubei Province, School of Optoelectronic Materials & Technology, Jianghan University, Wuhan 430056, China

[c]C. Wang
State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510060, China

[d]Prof L. Ying
South China Institute of Collaborative Innovation, Dongguan 523808, China

Animals

Famale Balb/c mice (about 4-week-old 16-18 g) were purchased from animal's center of Southern Medical University. Tumor-bearing mice were prepared through subcutaneous injection of one million human cervical carcinoma cells into the mice' right leg. It spent about seven days for the tumor to be used for PTT. Then, twenty mice were separated into four groups according to the size of tumors. The four groups were (i) PBS group; (ii) PBS+Laser group; (iii) PBDT-QTz NPs; (iv) PBDT-QTz NPs+Laser group. The plan was approved by the local committee of animal experimental ethics of State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University. All animal procedures were operated strictly in accordance with the guidelines of the Regional Ethics Committee for Animal Experiments and all operations were to cut down the suffering of mice.

Cell Culture

In vitro experiments, a human cervical carcinoma cells and B16 melanoma cancer cell lines cultures were utilized. Two cells lines were cultured in DMEM supplemented with 100 U/mL penicillin, 100 U/mL streptomycin, and 5% (v/v) FBS in a humidified atmosphere containing 5% CO_2 at 37 ℃.

Cytotoxicity test

The cytotoxicity of PBDT-QTz NPs and PCDT-QTz NPs with or without laser irradiation was investigated by the classical CCK-8 assays. HeLa cells were seeded into 96-well plates and cultured for 24 h. Then PBDT-QTz NPs and PCDT-QTz NPs at a series of concentrations (prepared in fresh cell medium) were added, and the cells were incubated for 6 h followed by irradiation with 808 nm laser (1.0 W cm^{-2}) for 5 min for PTT cytotoxicity study. In parallel, PBDT-QTz NPs or PCDT-QTz NPs-incubated

HeLa cells without laser irradiation were conducted for dark cytotoxicity study under the same experimental conditions. After that, we added 10 μL of CCK-8 solution into each well and further incubated at 37 °C for 2 h. Finally, the absorbance of each well at 450 nm was measured by a microplate reader.

The cytotoxicity of PBDT-QTz NPs and PCDT-QTz NPs against B16 cells were measured using the aforementioned method.

Live-Dead Cell Staining

HeLa cells or B16 cells were divided into 2 groups and treated with PBDT-QTz NPs (50 μg mL^{-1}) with or without laser irradiation respectively. After 6 h of incubation at 37 °C, one group was irradiated by 808 nm laser (1.0 W cm^{-2}) for 5 min and incubated for 2 h. Then, all cells were stained with calcein-AM/propidium iodide (PI) solution for 30 min at room temperature. Finally, the samples were imaged by an inverted fluorescence microscope (Olympus IX71, Japan).

Flow cytometry

HeLa cells or B16 cells were seeded in 6-well plates at 37 °C for 24 h and treated by PBS, PBS+Laser, PBDT-QTz NPs (10 μg mL^{-1}), PBDT-QTz NPs (10 μg mL^{-1})+Laser. Laser irradiation at 808 nm with a power of 1.0 W cm^{-2} was used for 5 min. After treatments, all cells were incubated for 4 h and detached by 1.0 mL of trypsin solution without EDTA at 37 °C, centrifuged (3 min at 800 rpm). The cell suspension in binding buffer was stained by annexin v-alexa fluor 647 and propidium iodide (PI) for 5 min in the dark and analyzed by flow cytometer according to the product manual.

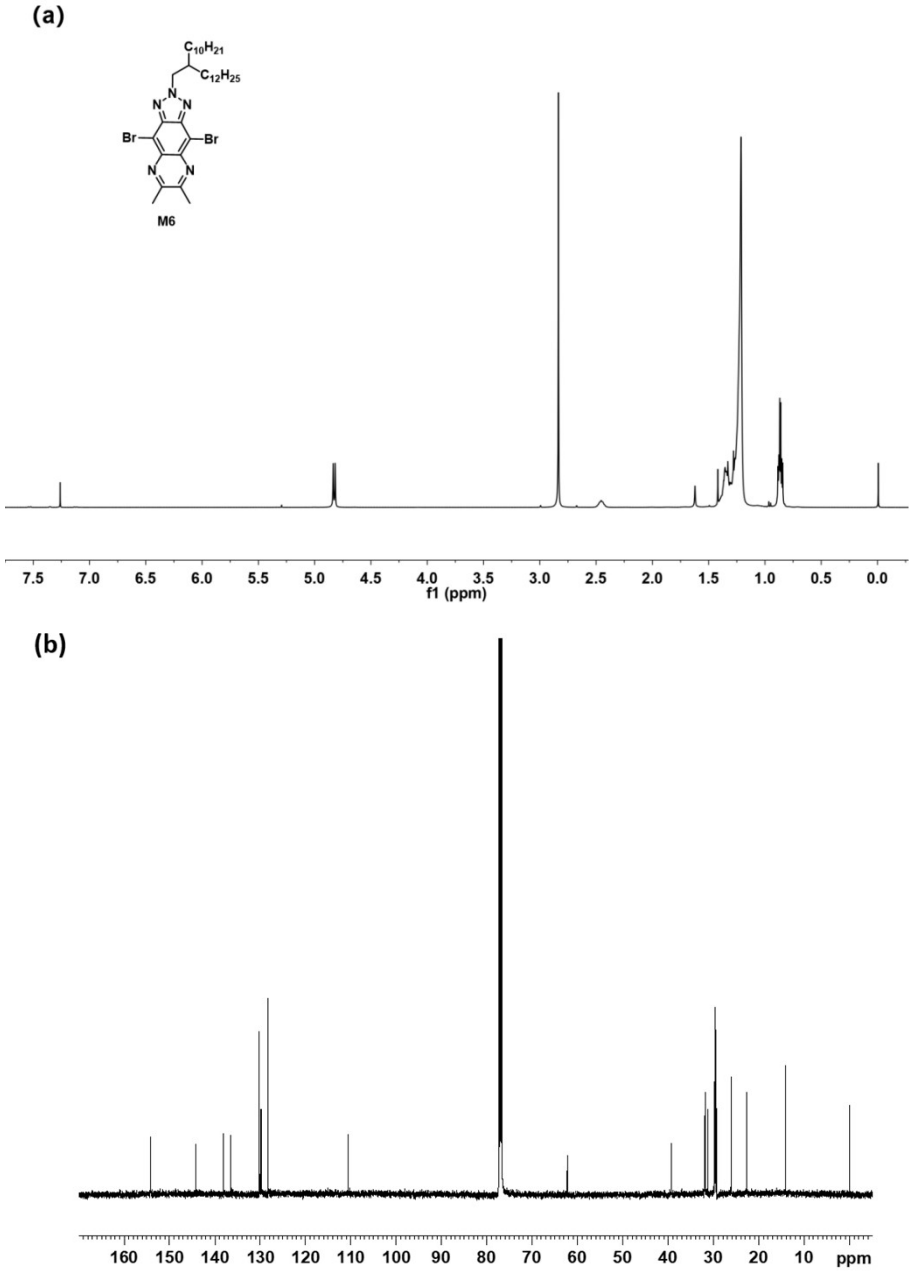

Fig. S1 The ^{1}H NMR (a) and ^{13}C NMR (b) spectra of Br_2QTz.

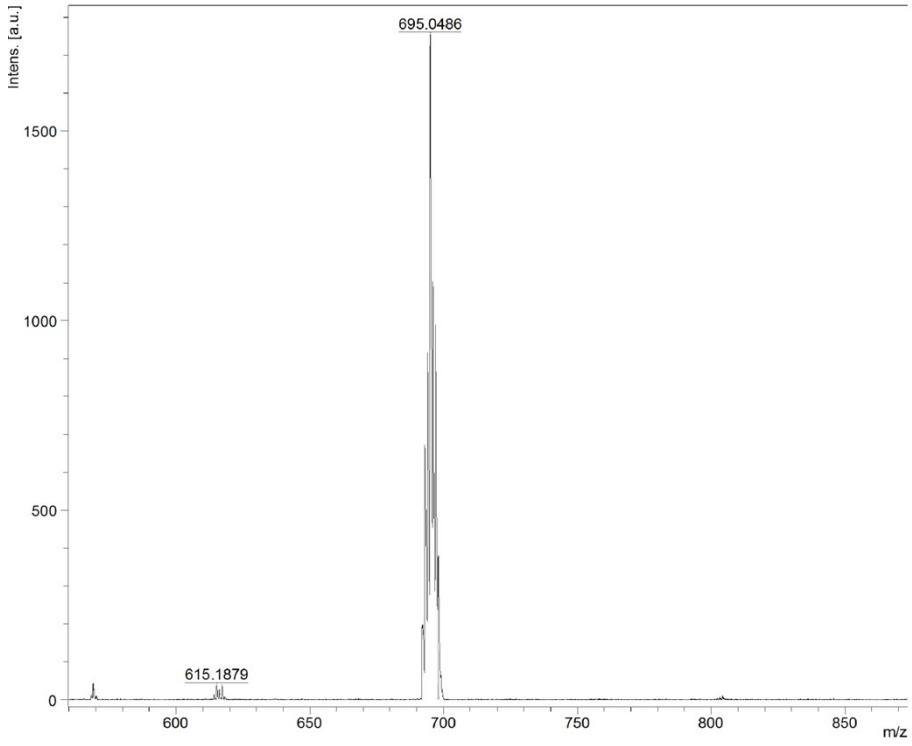

Fig. S2 The MALDI-TOF mass spectrum of Br_2QTz.

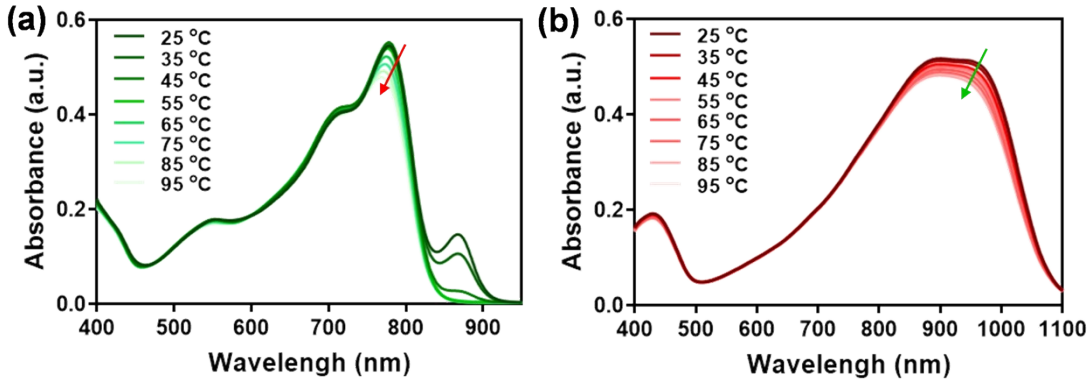

Fig. S3 Temperature-dependent absorption spectra of PBDT-QTz and PCDT-QTz.

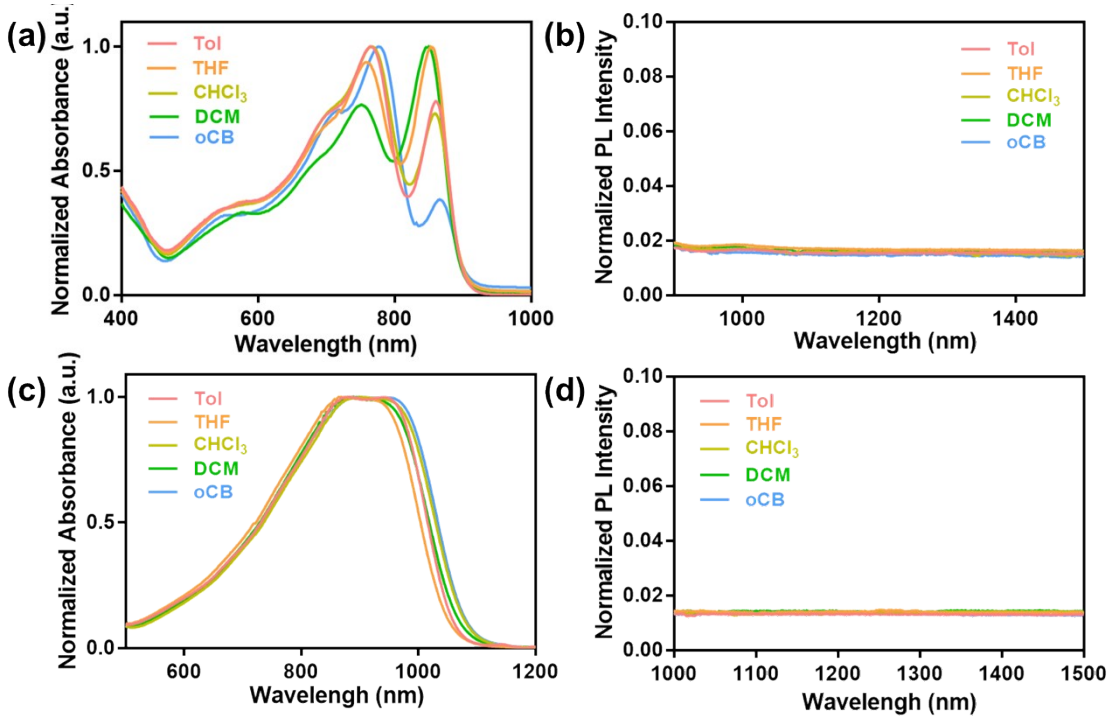

Fig. S4 UV-vis absorption (a, c) and PL (b, d) spectra of PBDT-QTz and PCDT-QTz in different solvents.

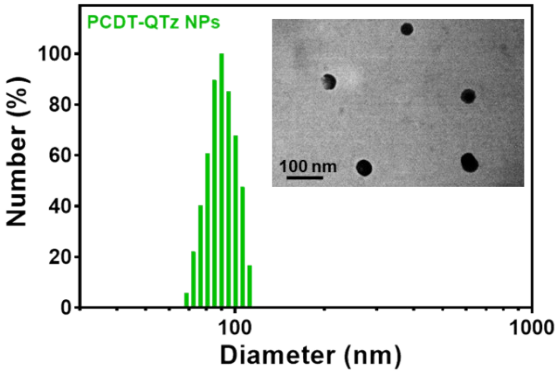

Fig. S5 Size distribution of PCDT-QTz NPs (DLS) characteristics. The inset shows its TEM image.

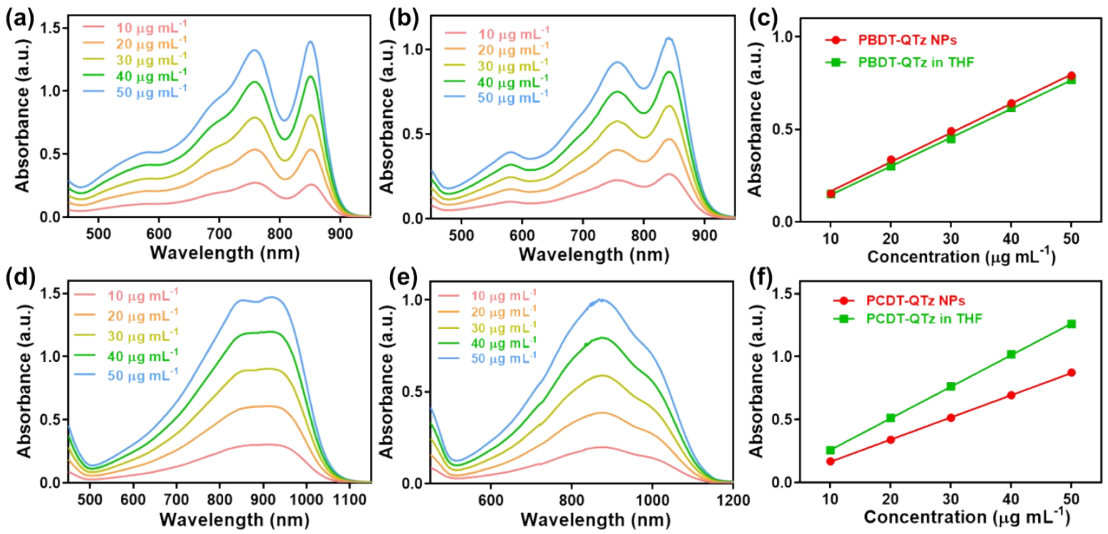

Fig. S6 The UV-vis absorption spectra of PBDT-QTz, PCDT-QTz in THF solution (a, d) and PBDT-QTz NPs, PCDT-QTz NPs aqueous solution (b, e) with different concentrations. The plot of absorbance at 808 nm versus their concentrations of PBDT-QTz in THF solution, PBDT-QTz NPs aqueous solution (c), PCDT-QTz in THF solution, PCDT-QTz NPs aqueous solution (f).

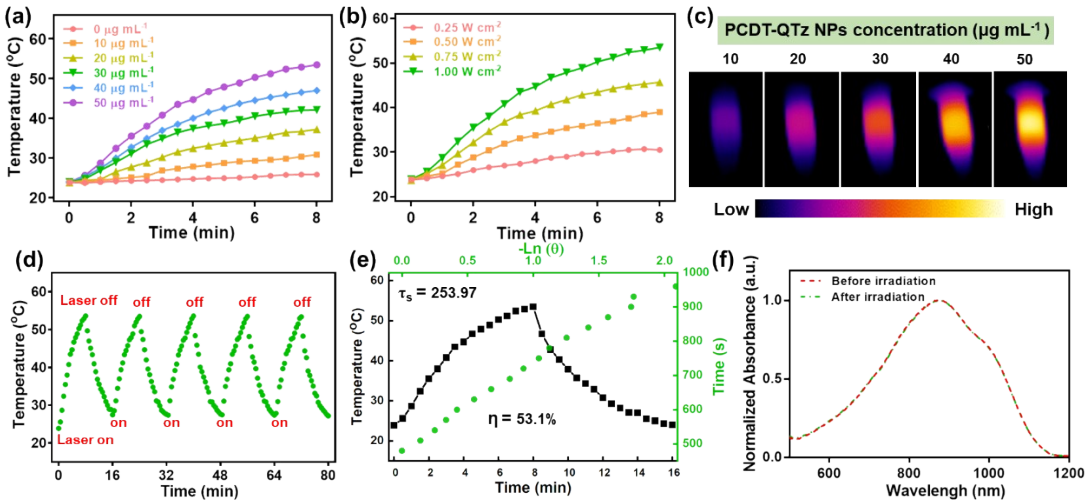

Fig. S7 (a) The photothermal heating curves of PCDT-QTz NPs at various concentrations under 808 nm laser irradiation at a power density of 1.0 W cm^{-2}. (b) PCDT-QTz NPs with concentration of 50 μg mL^{-1} under 808 nm laser irradiation at diversity power densities. (c) Infrared thermographs of PCDT-QTz NPs with different concentrations after 8 min of laser irradiation. (d) Photothermal curves of PCDT-QTz

NPs aqueous solution for five heating/cooling cycles. (e) Photothermal performance of PCDT-QTz NPs and the corresponding linear relationship between -Ln (θ) and time (s). (f) Normalized UV-vis spectra of PCDT-QTz NPs aqueous solution before and after 8 min irradiation with 808 nm laser (1.0 W cm^{-2}).

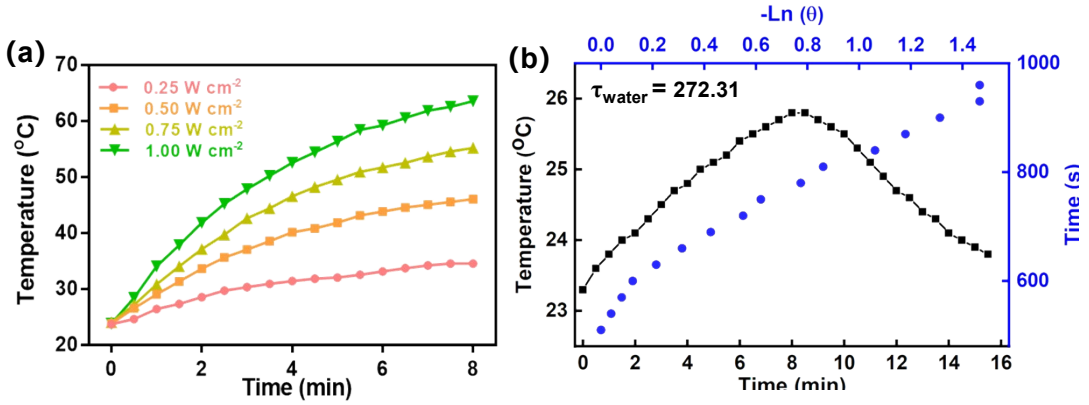

Fig. S8 (a)The photothermal heating curves of PBDT-QTz NPs with concentration of 50 μg mL^{-1} under 808 nm laser irradiation at diversity power densities, (b, c) photothermal performance of H_2O and the corresponding linear relationship between -Ln (θ) and time (s) at a power density of 1.0 W cm^{-2}.

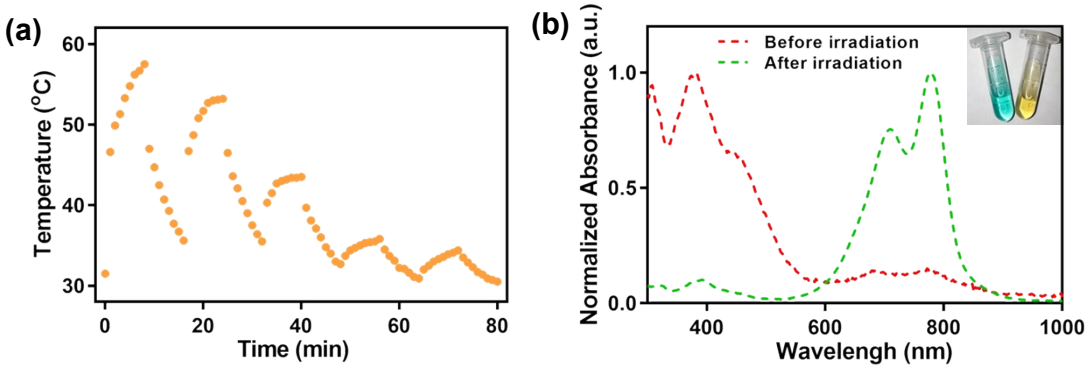

Fig. S9 (a) Photothermal curves of ICG aqueous solution for five heating/cooling cycles. (b) Normalized UV-vis spectra of ICG aqueous solution before and after 8 min irradiation with 808 nm laser (1.0 W cm^{-2}).

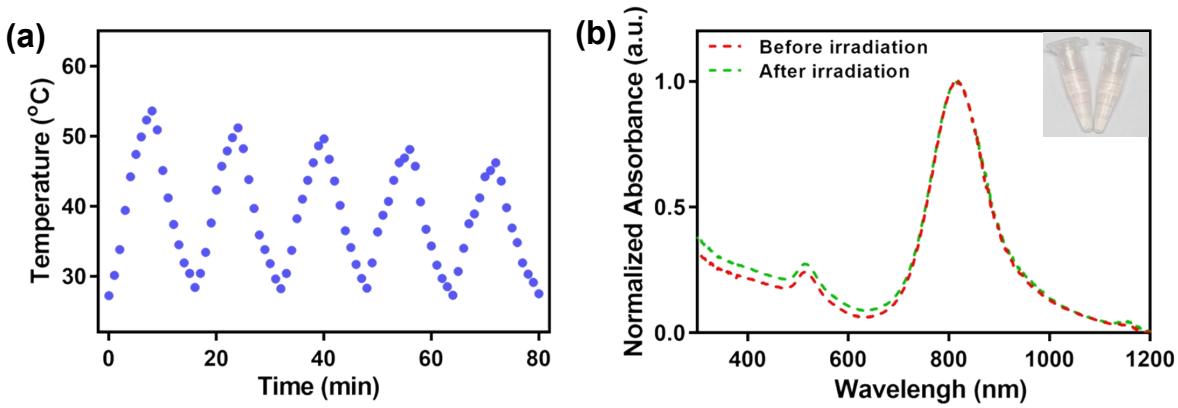

Fig. S10 (a) Photothermal curves of Au NPs aqueous solution for five heating/cooling cycles. (b) Normalized UV-vis spectra of Au NPs aqueous solution before and after 8 min irradiation with 808 nm laser (1.0 W cm^{-2}).

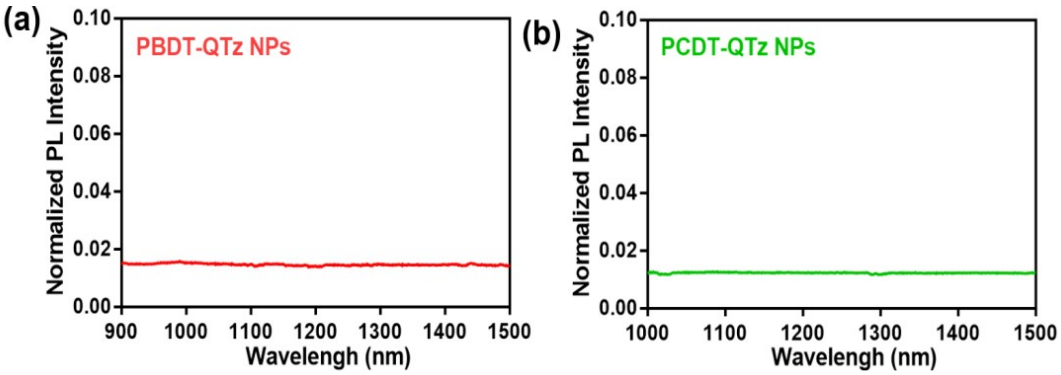

Fig. S11 The PL spectra of (a) PBDT-QTz NPs and (b) PCDT-QTz NPs.

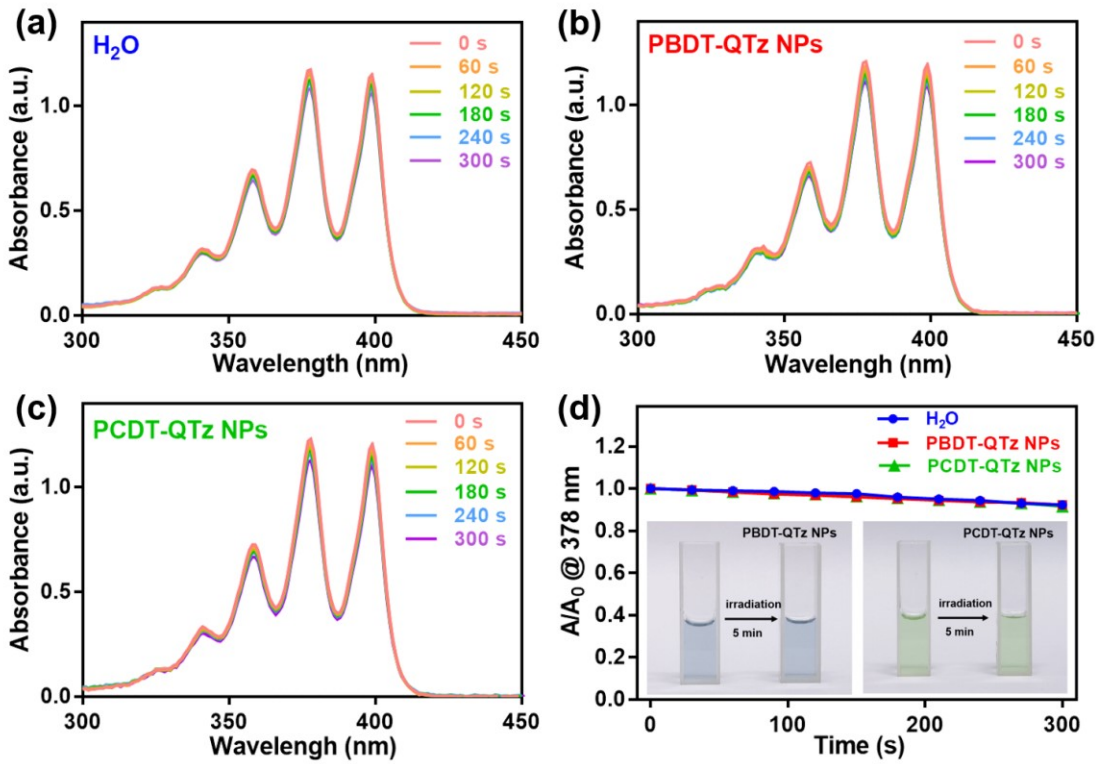

Fig. S12 The photogradation of ABDA in pure H_2O (a), PBDT-QTz NPs aqueous solution (b) and PCDT-QTz NPs aqueous solution (c) with the same concentration of 50 μg mL^{-1} under white light exposure for continuous time (0-300 s). (d) The relative absorbance of ABDA at 378 nm at various exposure time point. The insets are PBDT-QTz NPs aqueous solution and PCDT-QTz NPs aqueous solution before and after white light irradiation for 300 s.

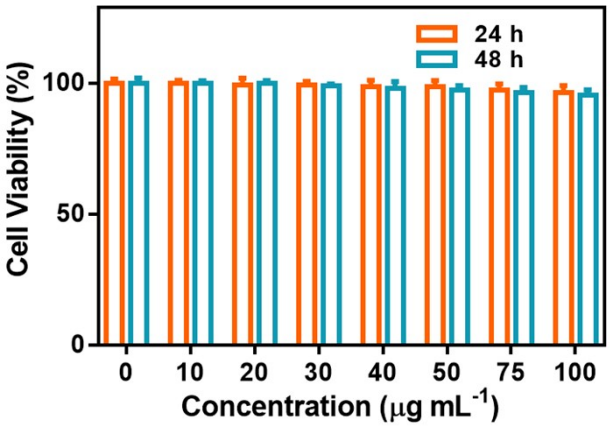

Fig. S13 Cell viability of B16 cells after co-incubation with various concentrations of PBDT-QTz NPs for 24 h and 48 h.

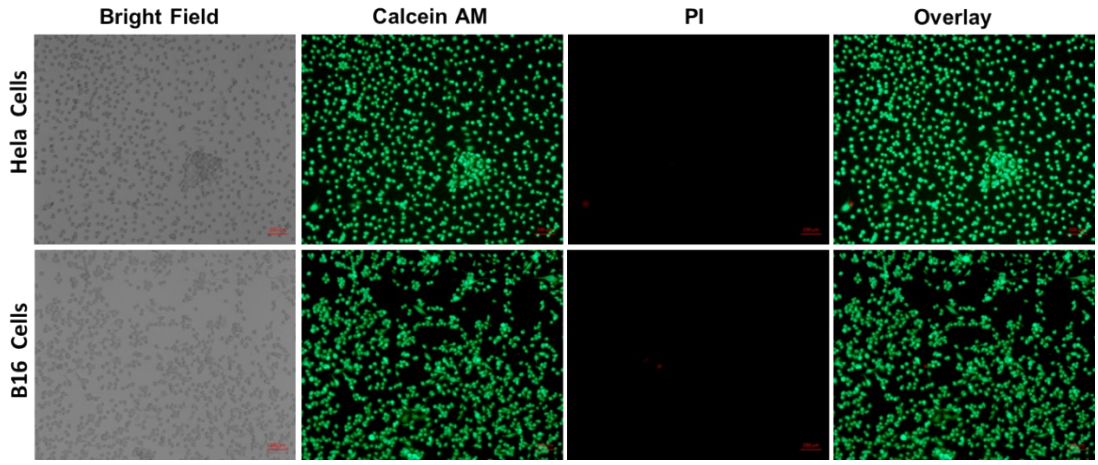

Fig. S14 Fluorescence imaging of live/dead Hela cells and B16 cells co-incubation PBDT-QTz NPs with the concentrations of 50 μg mL^{-1} without 808 nm laser irradiation (1.0 W cm^{-2}) treatment.

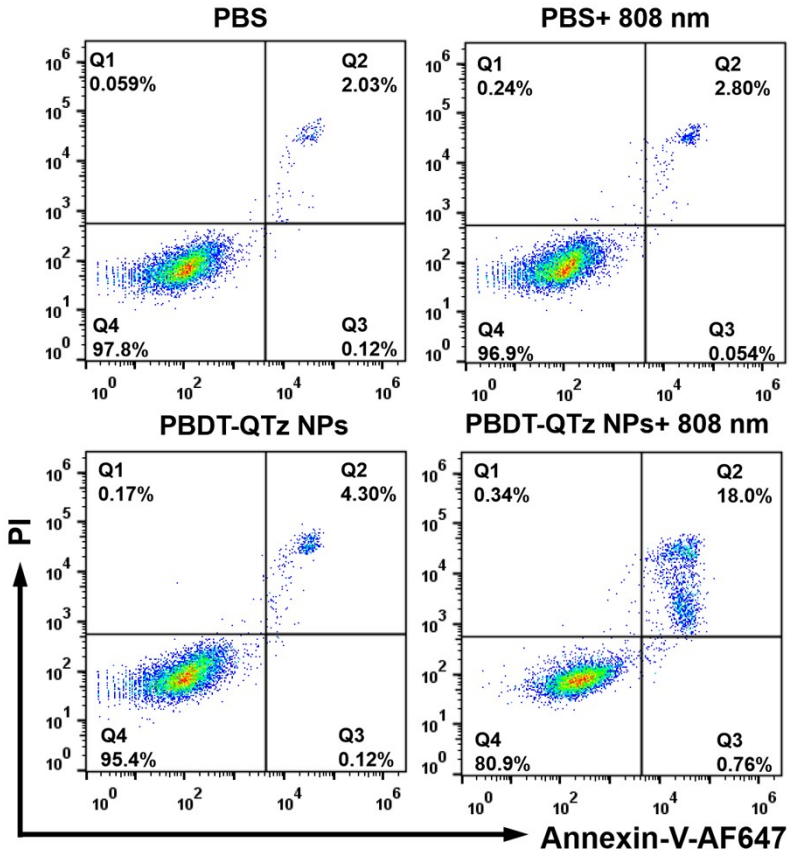

Fig. S15 The flow cytometric results on B16 cell apoptosis after different treatments with PBS, PBS+Laser, PBDT-QTz NPs, PBDT-QTz NPs+Laser, successively.

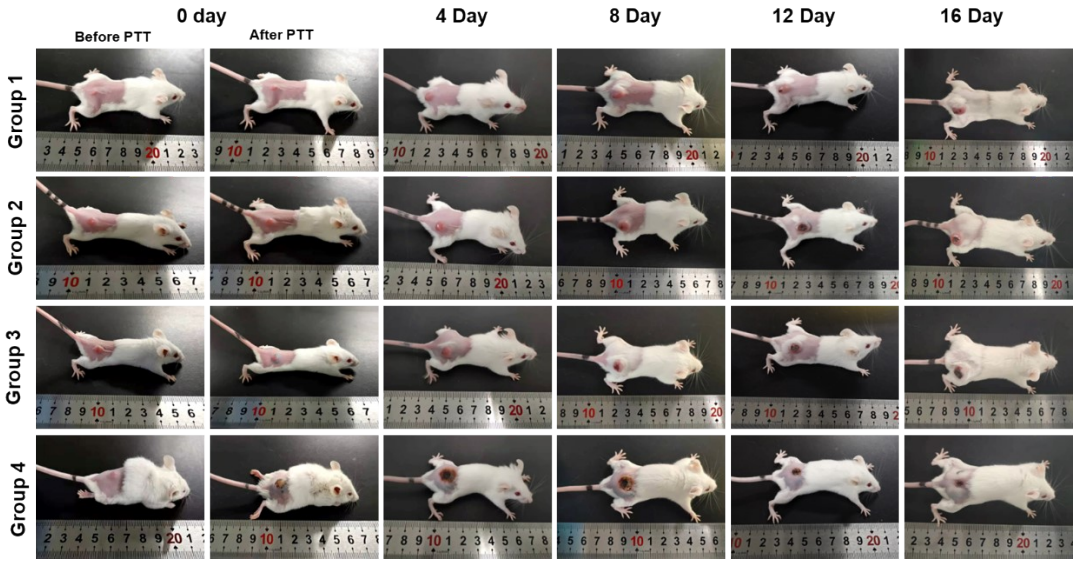

Fig. S16 Representative pictures of Hela tumor-bearing BALB/c mice after various treatments. [Group 1: PBS, Group 2: PBS+Laser, Group 3: PBDT-QTz NPs and Group 4: PBDT-QTz NPs+Laser].

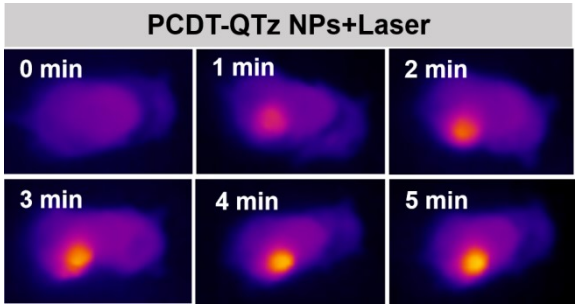

Fig. S17 Infrared thermographs of temperature change of PCDT-QTz NPs treated mice under 5 min 808 nm laser irradiation (1.0 W cm^{-2}).

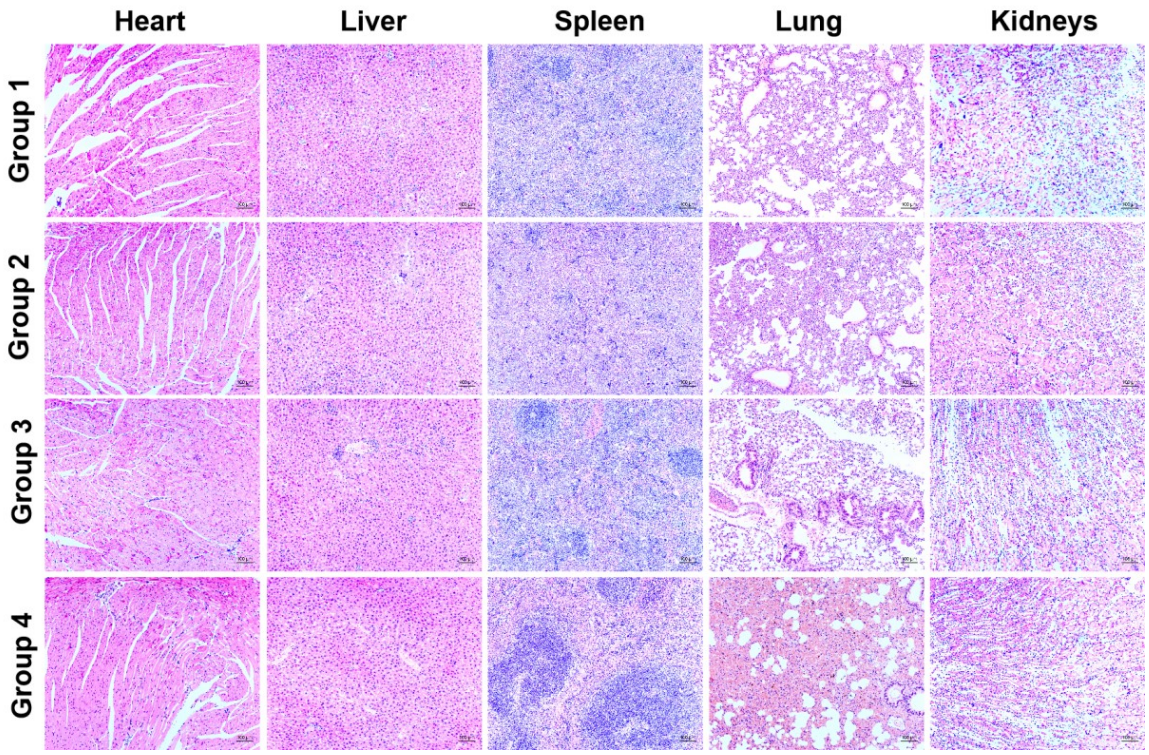

Fig. S18 H&E staining images of major organs (heart, liver, spleen, lung, and kidneys) in four treatment groups. Scale bars are 100 μm.

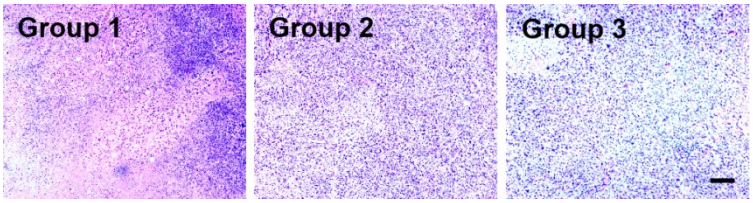

Fig. S19 Histological H&E staining for tumors after 16 days photothermal treatment with Group 1, Group 2, or Group 3. Scale bars are 100 μm.