# Supporting Information

## Novel Acceptor-Donor-Acceptor Structured Small Molecule-Based Nanoparticles for High Efficient Photothermal Therapy

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## Experimental Section

### Materials and Apparatus

3,9-Bis(2-methylene-(3-(1,1-dicyanomethylene)-indanone))-5,5,11,11-tetrakis(4-hexylphenyl)-dithieno[2,3-d:2',3'-d']-s-indaceno[1,2-b:5,6-b']dithiophene (ITIC) is purchased from Derthon Optoelectronic Materials Science Technology Co LTD. The absorption spectra are measured on an UV-3600 UV-Vis spectrophotometer (Shimadzu, Japan). The fluorescence emission spectrumis determined using NIR-II spectroscopy (Fluorolog3) equipped with an InGaAs detector. The size of nanoparticles is recorded by a 90 Plus particle size analyzer (Brookhaven Instruments, USA). The morphology of nanoparticles was carried out on a scanning electron microscope (SEM, Hitachi S-4800, Japan). The cell imaging was viewed by an Olympus IX 70 inverted microscope. In vivo fluorescence imaging was conducted using the PerkinElmer IVIS Lumina K. Thermal images are obtained by an E50 infrared camera (FLIR, Arlington, VA).

### Ethical Statement

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of the Medical School of Nanjing University and Experiments were approved by the Animal Ethics Committee of Nanjing Stomatological Hospital.

### Preparation of ITIC NPs

200 $\mu$L (2 mg/mL$^{-1}$) ITIC tetrahydrofuran (THF) solution is added into 5 mL water under vigorous stirring at room temperature. After stirring for 5 min, THF is removed by air blowing. ITIC NPs in the solution is obtained by centrifugation. The size and morphology of ITIC NPs are determined by SEM and DLS.

**Photostability**

ITIC NPs (80 $\mu$g/mL) PBS solution is irradiated by laser (660 nm, 1 W/cm$^2$) for 0, 5, 10 and 20 min, respectively. The absorbance of ITIC NPs is measured by UV-Vis spectrophotometer.

**Fluorescence Quantum Yields ($\Phi_F$).**

Rhodamine 101 in ethanol ($\Phi_{F(s)}$ = 1.00) was used as a standard to calculate $\Phi_F$ of ITIC and ITIC NPs. The $\Phi_{F(x)}$ value was calculated according to the following equation:

$$\Phi_{F(x)} = \Phi_{F(s)} \times \frac{A_s}{A_x} \times \frac{F_x}{F_s} \times (\frac{n_x}{n_s})$$

Where A is the absorbance, F is the area under the fluorescence emission curve, n is the refractiveindex of the solvent used in the measurement, and the subscripts s and x represent the standard andsample, respectively.

***In Vitro* Fluorescence Imaging**

ITIC NPs (0, 5, 10, 20, 40 μg/mL, respectively) in PBS solutions were subjected to fluorescence imaging. All solutions were put in eppendorf tubes and fluorescence image is by a fluorescence imaging system (PerkinElmer IVIS Lumina K) with excitation at 680 nm and emission at 820 nm.

***In Vitro*Photothermal Effect**

ITIC NPs (0, 10, 20, 40 $\mu$g/mL, respectively) in PBS are put into eppendorf tubes and irradiate by 660 nm laser (0.5, 0.75, 1.0 W/cm$^2$, respectively) for 10 min. The change of temperature is record by an infrared camera. The experiments were repeated three times.

**Photothermal conversion efficiency ($\eta$)**

The photothermal conversion efficiency (η) of ITIC NPs was calculated using the

following equation:

$$\eta\,(\%) = \frac{cm\Delta t}{wt} \times 100\%$$

Where c is specific heat capacity of ITIC NPs solution, m is mass of ITIC NPs solution, Δt is temperature increase of ITIC NPs solution, w is laser power, t is laserirradiation time.

**Cell Line**

SCC4 cell line is purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). They are cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) contained 10% fetal bovine serum (FBS) under 5% $CO_2$ at 37 $^oC$.

**Cellular Uptake Study**

SCC4 cells incubated with ITIC NPs PBS (40 $\mu$g/mL, 2 mL) in confocal dish in a 5% $CO_2$ incubator at 37$^oC$ for 24 h. The medium is removed and rinsed with PBS. The images are viewed with Olympus IX 70 inverted microscope and the samples are excited at 635 nm laser and collect from 660 to 800 nm. The cells are stained with DAPI (nuclei-specific dye).

**Cytotoxicity Assay**

The cell cytotoxicity of ITIC NPsis measured using cell counting kit-8 (CCK-8, Biotool) in SCC4 cells. The experiment was divided into two groups (Group A: ITIC NPs, Group B: ITIC NPs + 660 nm laser). Cells were seeded into a 96-well plate at $5\times10^3$ cells/well and cultured overnight. Medium containing drugs were then added to each well, with ITIC NPs concentrations ranging from 0 to 20 μg/mL and incubated for an additional 24 h. Besides, Group B cells were treated with 660 nm (500 mW/cm$^2$) laser for 5min after the incubation. The cell viabilities in each group were determined using CCK8. The 50% of

the cell growth inhibition ($IC_{50}$) was calculated by nonlinear regression analysis using GraphPad Prism 6 software (San Diego, CA).

**Animal Models**

All animal experiments are performed in compliance with the relevant laws and institutional guidelines, and the institutional ethics committee has approved the experiments. SCC4 cells are injected into the left flank subcutaneously athymic nude mice of ~ 6 weeks old. These mice are used for fluorescence imaging, thermal imaging and PTT at the volumes of the tumors were ~ 100 $mm^3$.

***In Vivo*Fluorescence Imaging andThermal Imaging**

100 $\mu$L of ITIC NPs (40 $\mu$g/mL) PBS is injected through tail vein into tumor-bearing mice. After that, fluorescence imaging of the tumors was monitored at different time and observed by a fluorescence imaging system (PerkinElmer IVIS Lumina K) with excitation at 680 nm and emission at820 nm. Thermal imaging is monitored by an E50 infrared camera when the tumor sites are administrated with 660 nm laser (1 $W/cm^2$) for different time after 2 hours post-injection of ITIC NPs (40 $\mu$g/mL).

***In Vivo* PTT**

SCC4 tumor-bearing nude mice are randomly divided into 3 groups with the same number of male and female (PBS with laser treatment, ITIC NPs only, ITIC NPs with 1 $W/cm^2$ laser). For each group, mice are injected 100 $\mu$LITIC NPs (40 $\mu$g/mL) PBS or PBS through tail vein, respectively. 2 hours later, mice are irradiated with laser (660 nm, 1 $W/cm^2$) for 8 min. The treatments are conducted every other day. Tumor volumes and body weights of mice are record every other day as well. Tumor volumes are calculated

by the commonly used equation: V= width$^2$*length/2.

***Ex Vivo* Histology Examination**

The mice were sacrificed after the treatments for 12 days, after that, the histology analyses were conducted. Tumors and the major organs including livers, hearts, lungs, kidneys, spleens were taken from all the mice in different groups, and respectively fixed with 4% formaldehyde solution. The tissues were embedded in the paraffin cassettes after dehydration and stained with hematoxylin and eosin (H&E) or Ki-67. After that, the histology imaging was observed with a microscope.

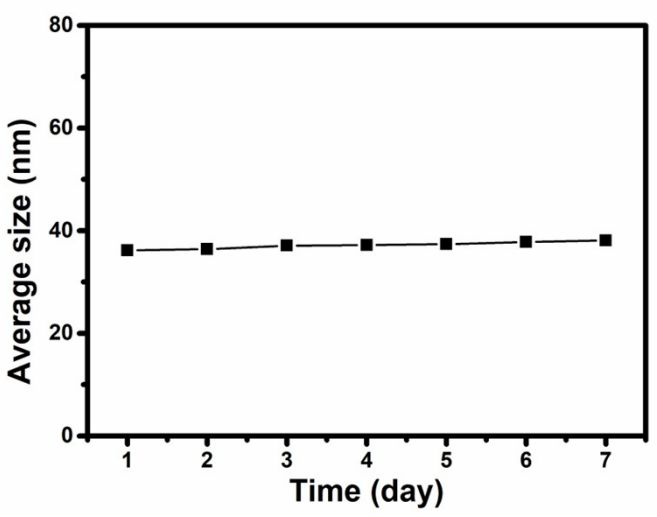


Figure S1. The average diameter change of ITIC NPs within 7 days.

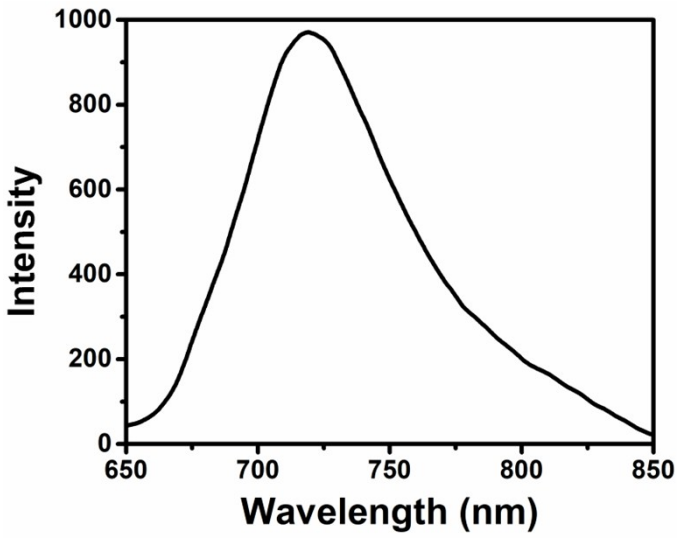


Figure S2. ITIC molecule fluorescence emission spectrum.

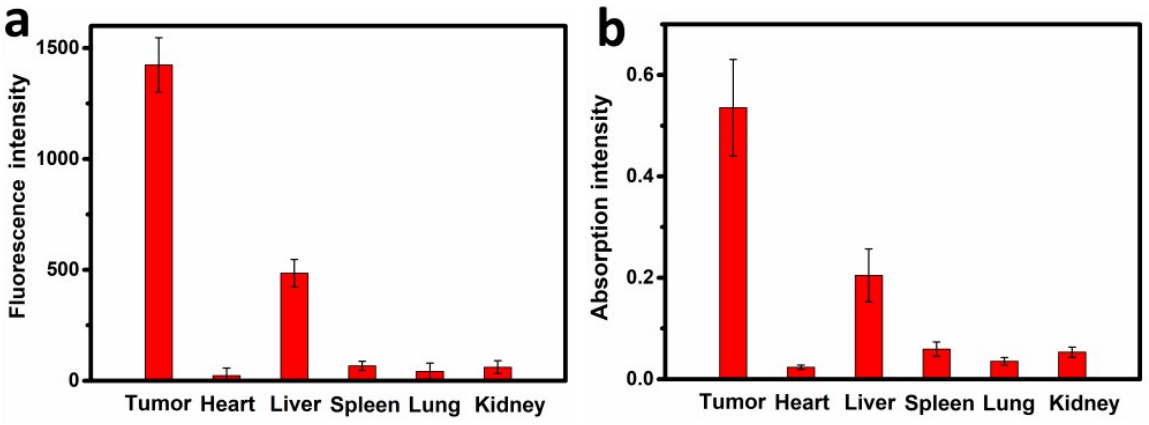


Figure S3. The distribution quantification ofITIC NPs in tumorsand major organs by fluorescence intensity (a) and UV-visabsorption spectra intensity (b), respectively.

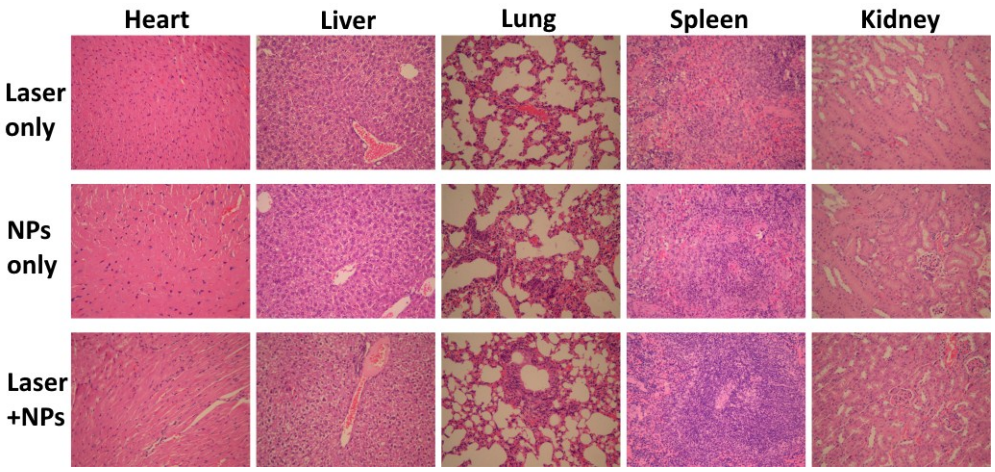


Figure S4. H&E staining images of major organs, including heart, liver, lung, spleenand kidney, collected from tumor bearing mice after different treatment. Magnification: 200×.