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Supporting Information

Biomimetic NIR-II Aggregation-Induced Emission Nanoparticles for Targeted Photothermal Therapy of Ovarian Cancer

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Experiment section

1. Materials

CH_2Cl_2 of analytical grade was obtained from Lingfeng Chemical Reagent Co. Ltd. (China). 1,2-Distearoyl-sn-Glycero-3-Phospho-ethanolamine-N-[Amino (Polyethylene Glycol) 2000] (DSPE-PEG-2000) was obtained from Ponsure Biological Co., Ltd. (China). CCK-8 kit and Calcein/PI Cell Viability/Cytotoxicity Assay Kit were purchased from Beyotime Biotech Co., Ltd. RPMI 1640, Dulbecco's Modified Eagle Medium (DMEM), penicillin-streptomycin and fetal bovine serum were obtained from Gibco.

2. Instruments

The absorption spectra and photoluminescence (PL) spectra were measured on a UV-8000 UV-vis absorption spectrophotometer and an Edinburgh F35 fluorescent spectrometer equipped with a xenon arc lamp, respectively. The surface charge and hydrodynamic size of nanoparticles were measured by Zeta-sizer Nano ZS (Malvern, UK) at 25°C. Transmission electron microscope (TEM) images were recorded with Tecnai G2 F20 S-TWIN (FEI). The samples for TEM were prepared by dripping the nanoparticle solution onto a 200-mesh-mesh copper grid and then drying the sample in the clean window at 25°C. The protein contents of ID8 membranes were detected by SDS-PAGE gel electrophoresis. The 808 nm laser (Lasever Inc., China) was used as a therapeutic light source in vitro and in vivo.

3. Cell culture

ID8, 4T1, CT26, and HUEVC cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin, and streptomycin in a humidified incubator containing 5% CO_2 at 37°C.

4. Preparation of 2TB NPs

Dissolve 9 mg of DSPE-PEG-2000 and 3 mg of 2TT-oC26B (referred to as 2TB) in 3 mL of CH_2Cl_2 (with a mass ratio of 3:1). Then, add the mixed solution dropwise to 9 mL of deionized water and sonicate the mixture in an ice bath using an ultrasonic processor at a frequency of 40 kHz and a power of 100 W for 5 minutes. Subsequently, remove the CH_2Cl_2 on a rotary evaporator set at a rotation speed of 130 rpm/min with the water bath temperature maintained at 40°C. After half an hour, remove the sample to obtain a clear blue solution.

5. Extraction of ID8 Cell Membrane

The mature ID8 cells were collected in centrifuge tubes and washed with PBS for twice, then cell lysis buffer was added with protease inhibitors, and crushed by sonication on ice for 15-20 min. The products were centrifuged at 12000 × g for 10 min at 4°C and the supernatant was collected for further centrifuging at 20000 × g for 20 min at 4°C. Then, the supernatant was collected for further centrifuging at 100000 × g for 45 min at 4°C, and the sediment containing mature ID8 membrane was acquired from the bottom of the centrifuged tube. Then, the ID8

membrane was stored at -80°C until used.

6. Preparation of 2TB-NPs@TM

To prepare cell membrane-coated nanoparticles, the obtained cell membrane proteins were dispersed thoroughly in PBS with the aid of ultrasonic disruption and mixed with P-TN-Dox nanogels through sonication for 3 min. The mixture was extruded by an Avanti mini extruder using a polycarbonate porous membrane (200 nm). 2TB-NPs@TM was obtained by centrifugation, and they were quantitatively measured using UV-vis spectroscopy.

7. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was used to characterize the cell membrane proteins. Briefly, protein samples extracted from ID8 cells were lysed using RIPA buffer containing protease inhibitors to maintain the integrity of the proteins. Their concentrations were determined using a BCA protein assay kit, and then the samples were denatured by heating at 90°C for 5 minutes. Fifteen microliters of each sample were loaded into the wells of a 10% sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel and subjected to electrophoresis. Subsequently, the gel was stained with Coomassie Brilliant Blue to visualize the proteins, including ID8 cell membrane fractions (TM), 2TB-NPs, and 2TB-NPs@TM. Finally, a photograph of the stained gel was taken to provide a visual representation of the protein separation.

8. In Vitro Photothermal Conversion Properties

The photothermal effects were studied by monitoring temperature changes in real-time under 808 nm laser irradiation using an infrared thermal imaging camera. Aqueous solutions of water and 100 μg/mL concentrations of 2TB, 2TB-NPs, and 2TB-NPs@TM were continuously irradiated with an 808 nm laser at a power density of 1.0 W cm^{-2} for 5 minutes. The solution temperatures were periodically recorded every 30 seconds using the infrared thermal imager, and the corresponding thermal images of the samples were obtained through the accompanying software. Water irradiated with the laser served as a reference. Different concentrations (10-200 μg/mL) of 2TB-NPs@TM were irradiated with an 808 nm (1.0 W cm^{-2}) laser for 5 minutes to reach a maximum temperature steady state, then the laser was turned off and the sample was allowed to cool for 5 minutes. An 808 nm laser with different power densities (0.2-1.0 W cm^{-2}) was used to irradiate 100 μg/mL of 2TB-NPs@TM for 5 minutes followed by a 5-minute cooling period. Temperature changes during these processes were measured to investigate the effects of concentration and power density on photothermal conversion performance. Aqueous solutions of ICG (50 μg/mL) and 2TB-NPs@TM (50 μg/mL) were irradiated with an 808 nm (1.0 W cm^{-2}) laser for 5 minutes, then allowed to cool for 5 minutes, and this was repeated for six on/off cycles to assess the photothermal stability and repeatability of the 2TB-NPs@TM aqueous solution.

9. Cytotoxicity Assay

To evaluate the toxicity of 2TB-NPs and 2TB-NPs@TM, ID8 cells were seeded in a 96-well plate at a density of 1×10^4 cells per well in 100 μL of culture medium. After a 12-hour incubation, the cells were treated with varying concentrations of 2TB-NPs and 2TB-NPs@TM ranging from 5 μg/mL

to 200 μg/mL. Following an additional 24 hours of incubation, 10% CCK-8 solution was added, and the absorbance at 450 nm was measured using a microplate reader. Photothermal toxicity was also assessed using the CCK8 method. ID8 cells were seeded in a 96-well plate at a density of 1×10^4 cells per well in 100 μL of culture medium. After a 12-hour incubation, the cells were treated with varying concentrations of 2TB-NPs and 2TB-NPs@TM ranging from 5 μg/mL to 200 μg/mL, incubated for 4 hours at 37°C then irradiated with an 808 nm laser (1.0 W cm^{-2}) for 5 minutes, and further incubated in the incubator for 4 hours before detecting cell viability using the CCK8 method. To assess the targeted cytotoxicity of 2TB-NPs@TM, ID8, 4T1, CT26, and HUEVC cells were seeded in a 96-well plate at a density of 1×10^4 cells per well in 100 μL of culture medium. After a 12-hour incubation, the cells were treated with 75 μg/mL of 2TB-NPs@TM, incubated for 4 hours at 37°C, then irradiated with an 808 nm laser (1.0 W cm^{-2}) for 5 minutes, and further incubated in the incubator for 4 hours before detecting cell viability using the CCK8 method.

10. Live/Dead Cell Imaging

Live/dead cell staining experiments were conducted using a confocal laser scanning microscope (CLSM). ID8 cells were seeded into a confocal-specific 8-well plate and cultured at 37°C for 24 hours. Subsequently, 100 μg/mL of 2TB-NPs and 2TB-NPs@TM were added and the cells were incubated at 37°C. After 4 hours, the cells in the light group were irradiated with an 808 nm laser for 5 minutes and then returned to the incubator for another 4 hours. The supernatant was then removed, and the cells were washed twice with PBS. Afterward, the cells were irradiated with an 808 nm laser for 10 minutes, followed by a further 4-hour incubation. After removing the culture medium, the cells were incubated with fresh medium containing Calcein-AM (1 μM) and PI (1 μM) at 37°C for 30 minutes. The cells were then washed three times with PBS before acquiring CLSM images.

11. Measurement of Mitochondrial Membrane Potential

The mitochondrial membrane potential of ID8 cells treated with 2TB-NPs and 2TB-NPs@TM was assessed using a mitochondrial membrane potential assay kit. ID8 cells were seeded in a confocal-specific 8-well plate and cultured for 24 hours. Subsequently, 100 μg/mL of 2TB-NPs and 2TB-NPs@TM were added and the cells were incubated at 37°C. After 4 hours, the cells in the light group were irradiated with an 808 nm laser for 5 minutes and then returned to the incubator for another 4 hours. The supernatant was then removed, and the cells were washed twice with PBS. An equal volume of fresh medium containing JC-1 dye (5 mg/L) was added and co-incubated with the cells. After a 20-minute incubation, the cells were rinsed twice with a buffer solution to remove excess dye. Finally, the corresponding green and red channel fluorescence images were captured using a confocal microscope. The fluorescence signals of JC-1 monomers (green) were collected under 488 nm excitation and 530 nm emission, while the fluorescence signals of JC-1 aggregates (red) were collected under 543 nm excitation and 590 nm emission.

12. Animal Model

Female C57BL/6J mice, aged 4-5 weeks, were purchased from Guangdong Yuekang Biotechnology Co., Ltd. All experimental procedures were approved by the Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences (SIAT-IACUC-20230712-YYS-NMZX-CLT-1-02).

To establish a mouse model of ID8 ovarian cancer, 5×10^7 ID8 cells suspended in 100 μL of PBS were injected subcutaneously on the right flank of C57BL/6J mice (7-8 weeks old).

13. In Vivo Fluorescence Imaging and Photothermal Imaging

The in vivo fluorescence imaging was conducted on the orthotopic murine ovarian tumor-bearing mice. The tumor-bearing C57BL/6J mice were intravenously injected separately with 200 μL of a 2TB-NPs and 2TB-NPs@TM nanoparticle solution at a concentration of 0.1 mg/mL. At the given time points, the fluorescence images of mice were captured using a full spectrum in vivo fluorescence imaging system with an excitation wavelength of 808 nm. At 48 h post-injection, mice were sacrificed and their tumors and organs (heart, lung, kidney, spleen, and liver) were resected for vitro fluorescence signals acquiring, and the corresponding fluorescence signal intensity of tumors and major organs were quantified to evaluate the distribution of nanomaterials. For in vivo photothermal imaging evaluation, different groups of mice were intravenously injected with 200 μL of PBS, 2TB-NPs, and 2TB-NPs@TM nanoparticle solution, respectively. At 24 h after administration, the mice were gaseous anesthetized, and continuous 808 nm laser irradiation at a power density of 1.0 W cm^{-2} was performed locally on their tumors for 10 min. The real-time temperature variations of the tumor sites and infrared thermographs of mice were monitored and recorded with a visual FLIR system.

14. In Vivo Anti-Tumor Activity Study

The in vivo anti-tumor effects were assessed in the subcutaneous mouse ovarian cancer model established as described above. When the tumor volume reached approximately 60 mm^3, tumor-bearing C57BL/6J mice were randomly divided into 6 groups. The mice were treated via tail vein injection with 200 μL of PBS and 200 μL of 2TB-NPs and 2TB-NPs@TM nanoparticle solutions at a concentration of 0.1 mg/mL. Twenty-four hours after intravenous injection, the laser groups of mice were irradiated with an 808 nm laser for 10 minutes. Tumor length and width were recorded with calipers, and tumor volume was calculated using the formula: Volume = Length × $Width^2$ × 0.5. Mouse tumor volume and body weight were recorded every two days. On day 14, the mice were euthanized, and tumors and major organs (heart, liver, spleen, lungs, kidneys) were immediately excised from the mice. These organs were fixed in 10% neutral buffered formalin, then routinely processed into paraffin, sectioned at a thickness of 4 μm, and stained with hematoxylin and eosin (H&E). H&E stained sections were imaged using a light microscope. Additionally, blood samples were collected, and AST, ALP, ALT, ALB, TBA, GGT, DBIL, TBIL, UA, UREA, and CREA were measured by Servicebio Company (Wuhan, China).

Figures and tables

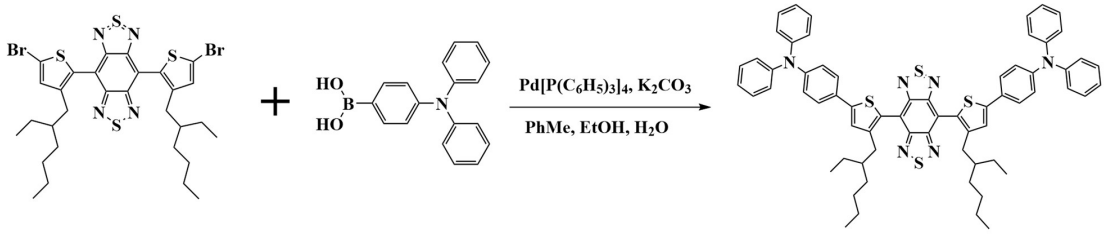

Figure S1. Synthetic route of 2TT-oC26B（2TB）.

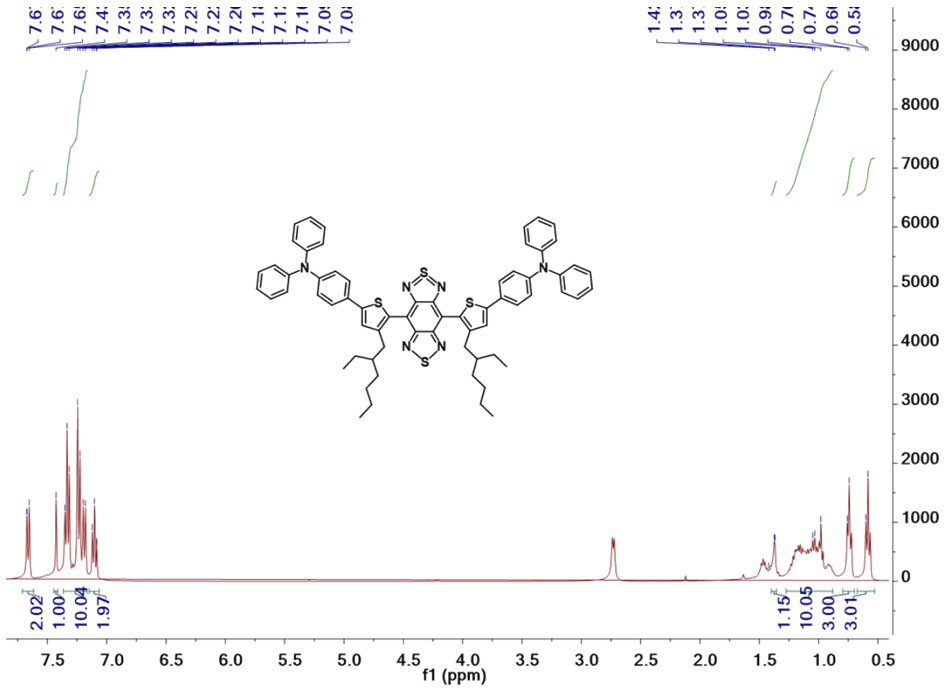

Figure S2. ^{1}H NMR spectrum of 2TB in $CDCl_3$.

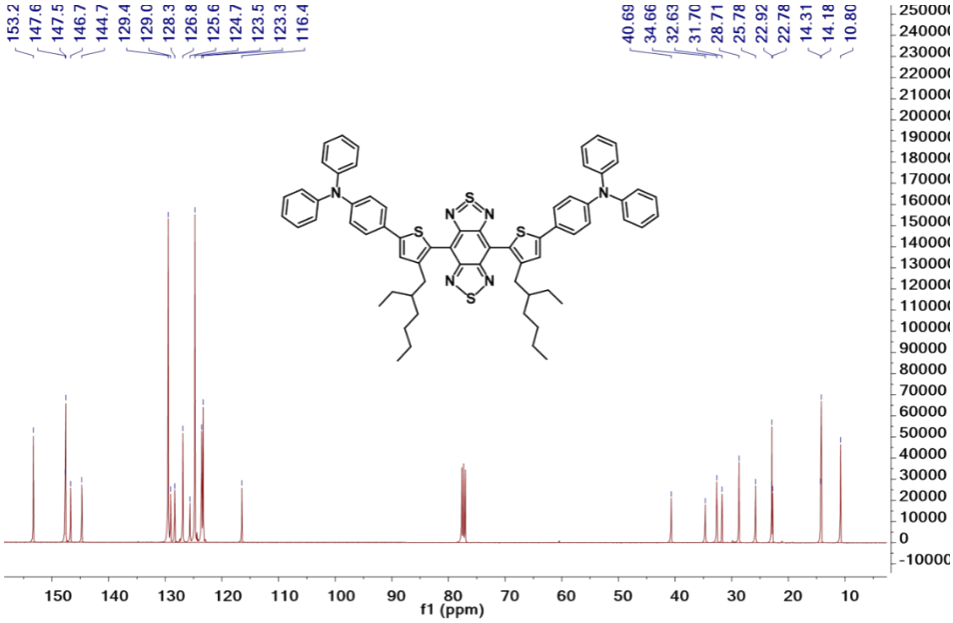

Figure S3. ^{13}C NMR spectrum of 2TB in $CDCl_3$.

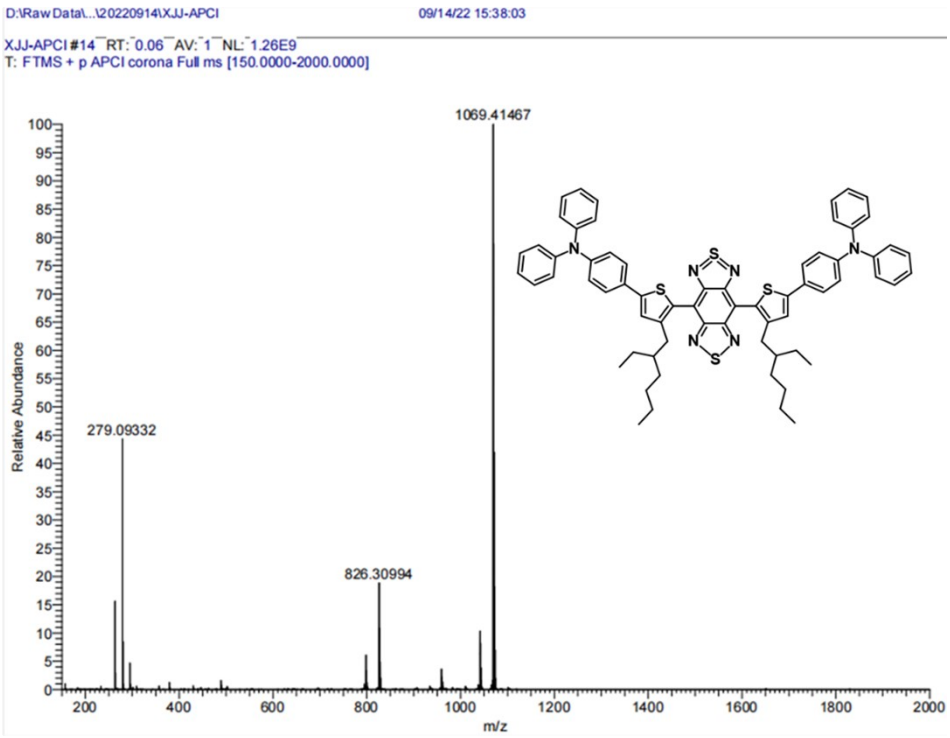

Figure S4. HR-MS of 2TB.

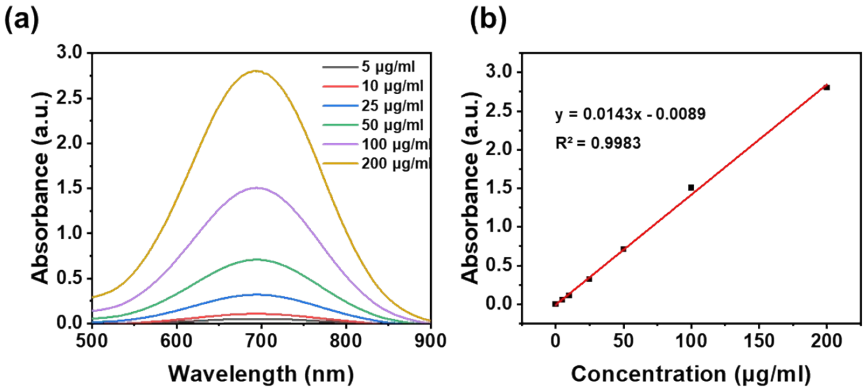

Figure S5. The absorption spectra of 2TB solution at different concentrations (a) and the concentration fitting curve (b).

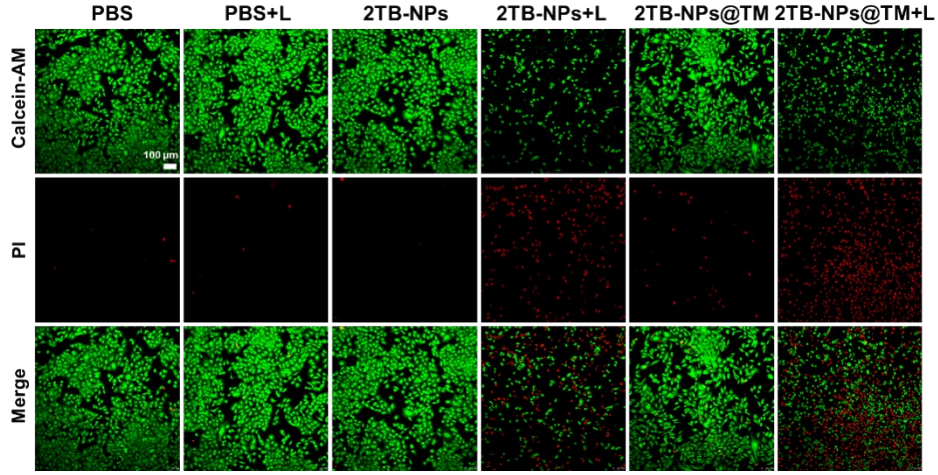

Figure S6. Live/dead cell staining of ID8 cells after different treatments, including PBS, PBS with laser (PBS + L), 2TB-NPs, 2TB-NPs with laser (2TB-NPs + L), 2TB-NPs@TM, and 2TB-NPs@TM with laser (2TB-NPs@TM + L). Laser: 808 nm, 1.0 W cm^{-2}, 10 minutes. Concentration: 2TB concentration of 50 μg/mL. Scale bar: 100 μm.

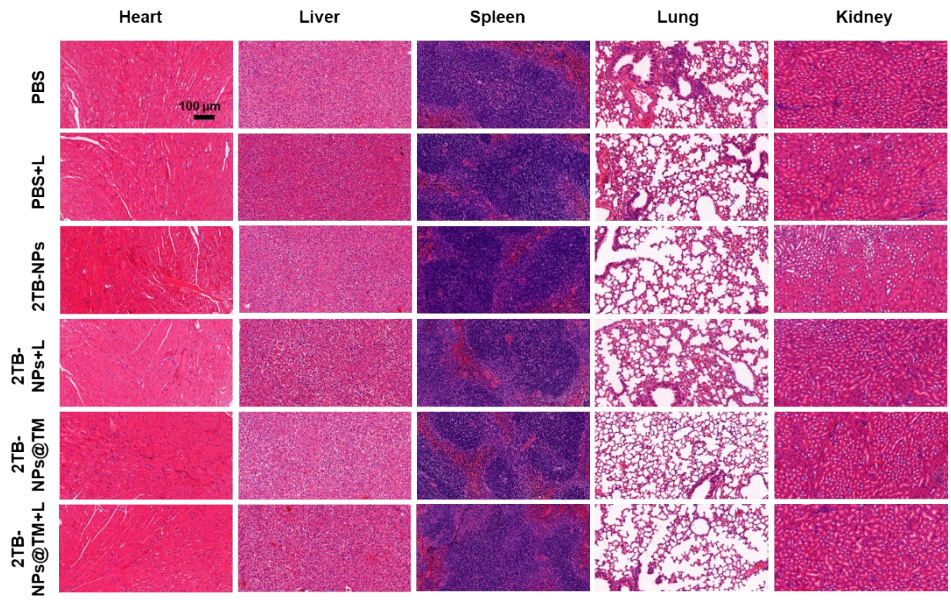

Figure S7. Hematoxylin-eosin (H&E) staining of major organs of different groups post treatments.

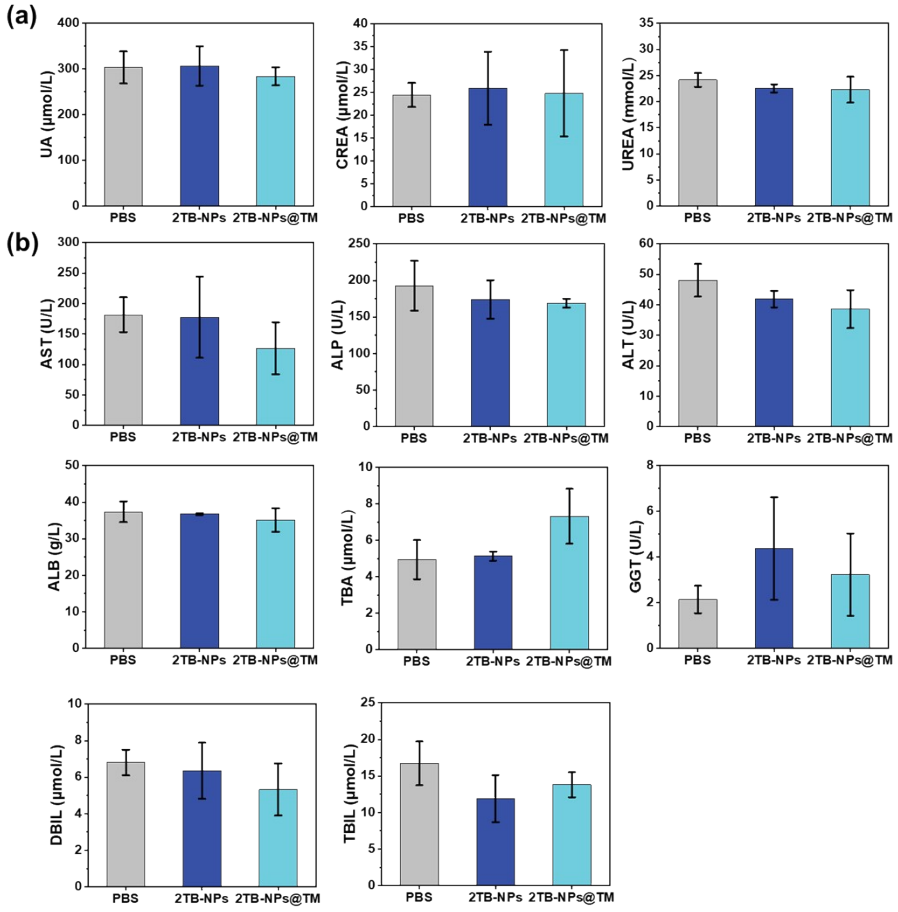

Figure S8. Blood biochemical analysis. (a) Renal function indicators include uric acid (UA), urea nitrogen (UREA), and creatinine (CREA); (b) Liver function indicators include aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), albumin

(ALB), total bile acid (TBA), gamma-glutamyl transpeptidase (GGT), direct bilirubin (DBIL), and total bilirubin (TBIL).