Supporting Information

Biocompatible Conjugated Polymer Nanoparticles for Highly Efficient Photoacoustic Imaging of Orthotopic Brain Tumor in Second Near-Infrared Window

Bing Guo, Zonghai Sheng, Kenry, Dehong Hu, Xiangwei Lin, Shidang Xu, Chengbo Liu, Hairong Zheng and Bin Liu**

Experimental Section

Materials. (4,8-bis((2-octyldodecyl)oxy)benzo[1,2-b:4,5-b']dithiophene-2,6-diyl)bis(trimethylstannane) (BDT) and 4,8-bis(5-bromo-4-(2-ethylhexyl)thiophen-2-yl)benzo[1,2-c:4,5-c'] bis[1,2,5]thiadiazole (BBT) were obtained from Derthon. 1, 2-distearoyl-*sn*-glycero- 3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000 (DSPE-PEG_{2000}) was purchased from Avanti Polar Lipids. Toluene, tetrahydrafuran (THF), tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$), tri(o-tolyl)phosphine ($P(o\text{-}tol)_3$), methanol, and acetone were purchased from Sigma Aldrich.

Characterization. NMR spectra were obtained using a Bruker 500NMR spectrometer. Polymer molecular weight and distribution was evaluated by gel

permeation chromatography (GPC) (Waters 996) equipped with an aphenogel GPC column, in which narrow distributed polystyrene was used as standard and THF was the eluent at 1.0 mL/min at 23 ^{o}C. UV-vis (Shimadzu Model UV-1700 spectrometer) was used to test the absorption properties of NP suspension. The NP size and distribution was characterized using dynamic light scattering (DLS) and field emission transmission electron microscopy (FE-TEM). PA characterization was conducted using a home-made acoustic-resolution PA microscopy system (supporting information). The PA spectra of the **P1** NPs (0.01 mg/mL) were obtained from 740 nm to1210 nm with a 20 nm-interval. The brain tumor growth was monitored using MRI (3T Magnetom Trio, Erlangen, Germany). Density functional theory (DFT) and time-dependent DFT (TDDFT) calculations were done using the Gaussian 09 program on a trimer model *via* the B3LYP functional with the 6-31G(d,p) basis set. To reduce the computational time, the alkyl side-chains were replaced with the methyl groups.

Synthesis of P1. A solution of (4,8-bis((2-octyldodecyl)oxy)benzo[1,2-b:4,5-b'] dithiophene-2,6-diyl)bis(trimethylstannane) (94.26 mg, 0.085 mmol), 4,8-bis(5-bromo-4-(2-ethylhexyl)thiophen-2-yl)benzo[1,2-c:4,5-c']bis[1,2,5]thiadiazole (62.9 mg, 0.085 mmol), $Pd_2(dba)_3$ (1.55 mg, 1.70 μmol) and $P(o\text{-}tol)_3$ (4.14 mg, 13.60 μmol) in toluene (10 mL) was vigorously stirred at 100 ^{o}C under argon for 24 h. After the reaction completed, the resultant viscous polymer solution was precipitated in

excess acetone. The precipitant was collected via filtration and dried in vacuum. The crude product was further dissolved in chloroform, precipitated in acetone again and collected by filtration. Subsequently, the precipitate was dissolved in chloroform and filtered through a short silica column. The polymer solution was concentrated by rotary evaporator, precipitated in excess acetone and washed with methanol. The precipitant was dried in vacuum at 40 °C to yield **P1** as a dark solid (98 mg, yield 85 %). ^{1}H NMR (500 MHz, $C_2D_2Cl_4$, 100 °C) δ 9.06 (b, 2H), 7.80 (br, 2H), 4.40 (br, 4H), 3.12 (b, 4H), 2.05 (br, 2H), 1.79 (br, 2H), 1.68-0.90 (m, 104H); GPC (THF, polystyrene standard), M_n: 2.17×10^4 g/mol; M_w: 5.91×10^4 g/mol, PDI: 2.73.

Synthesis of P1 Nanoparticles (P1 NPs). To prepare NPs, **P1** (0.5 mg) and DSPE-PEG_{2000} (1 mg) were dissolved in 1 mL of THF and sonicated at room temperature. The mixture was quickly poured into 10 mL of water. Subsequently, a microtip probe sonicator (12 W) was used to sonicate the suspension and disperse organic components vigorously in water for 2 minutes. Then, the mixture was transferred to fume hood and was stirred with a magnetic stirring bar for two days to remove THF. Finally, the NP suspension was filtered using membrane filter (diameter = 200 nm) and further concentrated to 1 mg/mL with corning concentrators (Mw = 30K). The NPs were stored at 4 °C in fridge.

Cell Culture. U87 cells and bEnd.3 cells were supplied by American Type Culture Collection. Once received, they were cultured using Dulbecco's Modified Eagle Medium (DMEM) media in an incubator (5% CO_2 at 37 ^oC). DMEM was mixed with fetal bovine serum (10%) and penicillin streptomycin (1%). The cells were pre-cultured to reach confluency before all *in vitro* tests.

Orthotopic Transplantation Brain Tumor Model. All animal experiments were carried out under the animal usage and care regulations at Shenzhen Institutes of Advanced Technology. 5-8 weeks old Balb/c nude mice were supplied by Guangdong Medical Experimental Animal Center. To prepare orthotopic U87 tumor-bearing mice, 1×10^6 of U87 cells labeled by luciferase in PBS (6 μL) were injected into the mice striatum (bregma + 1.0 mm, left lateral 2.0 mm and depth 3.0 mm). MRI was used to monitor the growth of the mice brain tumor.

Cell uptake via PA Signal Detection

U87 cells were treated with 0.1 mg/mL of P1 NPs and were cultured for 2 h. Subsequently, the cells were washed using PBS for three times to remove free NPs. The cells were then collected and diluted in PBS into different concentrations from 500 to 10^5 cells in 100 μL of PBS. Meanwhile, the untreated cells with a concentration of 10^5 cells in 100 μL of PBS were used as control. All the cell samples were tested using the AR-PAM system to collect the PA signals.

***In Vitro* Biocompatibility.** To study the cytotoxicity of the NPs, U87 and bEnd.3 cells were seeded in 96-well plates (concentration: 5×10^4 cells in 100 μL medium per well) and treated with **P1** NPs of different concentrations. The cells were further cultured for 24 h and the relative cell viability was characterized *via* CCK-8 assay. The *in vitro* hemolysis assay was as well carried out to evaluate the biocompatibility. After the Balb/c nude mice blood was totally collected, centrifugation was conducted at 1500 rpm for 3 min to separate red blood cells (RBC). The RBC were further washed by PBS for three times. Then the RBC at 10% (v/v) concentration in PBS were treated with different concentrations of CP NPs and then were incubated for 3 h at 37 °C. Finally, the supernatant of the suspensions were collected by centrifugation (10 000 rpm, 1 min), and subsequently analyzed by UV-vis spectroscopy at wavelength of 541 nm. According to equation 1, the hemolytic percentage (HP) was obtained.

$$HP\ (\%) = \frac{D_t - D_{nc}}{D_{pc} - D_{nc}} \times 100\% \qquad (1)$$

Where D_t, D_{pc}, and D_{nc} are the extinction coefficient value of the tested samples, DI water as positive control and PBS as negative control, respectively.

***In Vivo* PA Imaging Under Skin.** Before starting the PA imaging test, the mice were anesthetized with 2% isoflurane in oxygen and then put in prone position. To evaluate the signal/background ratio under pulsed laser excitation of different wavelengths, **P1**

NPs were mixed with the matrigel matrix at volume ratio of 1/1. The NP concentration was 0.01 mg/mL. Subsequently, 10 μL of the mixture was injected into the mice flank for *in vivo* PA imaging at different excitation wavelengths *via* the AR-PAM system.

***In Vitro* PA Imaging under Mouse Brain Skull.** To evaluate the penetration of **P1** NPs under pulse laser excitation at different wavelengths, NPs were mixed with the matrigel matrix at volume ratio of 1/1. The NP concentration was 0.01 mg/mL. Next, the mixture (10 μL) was applied onto the mice brain skull. The PA signals were collected using the AR-PAM system.

***In Vivo* PA Imaging of Orthotopic Brain Tumor.** Before starting the PA imaging test, the mice were anesthetized with 2% isoflurane in oxygen and then put in prone position. To study the PA imaging ability of **P1** NPs in orthotopic brain tumor before and after the intravenous injection of **P1** NPs (0.5 mg/Kg), the PA and ultrasound images of the tumor area were acquired *via* the AR-PAM system.

Toxicology Evaluation. Healthy Balb/c mice were separated into three groups with five mice in each group: untreated control group 1, group 2 and 3, in which mice were intravenously injected with 0, 0.5 and 5 mg/Kg of **P1** NPs, respectively. After 7 days of **P1** NPs injection, the mice blood was collected through ocular vein and was

characterized by blood biochemistry assay and complete blood panel test. The **P1** NP-treated mice (5 mg/Kg) were sacrificed to collect major organs, such as heart, liver, spleen, lung and kidney. The major organs were characterized *via* H& E staining following the BBC Biochemical protocol. The images were taken by a digital microscope.

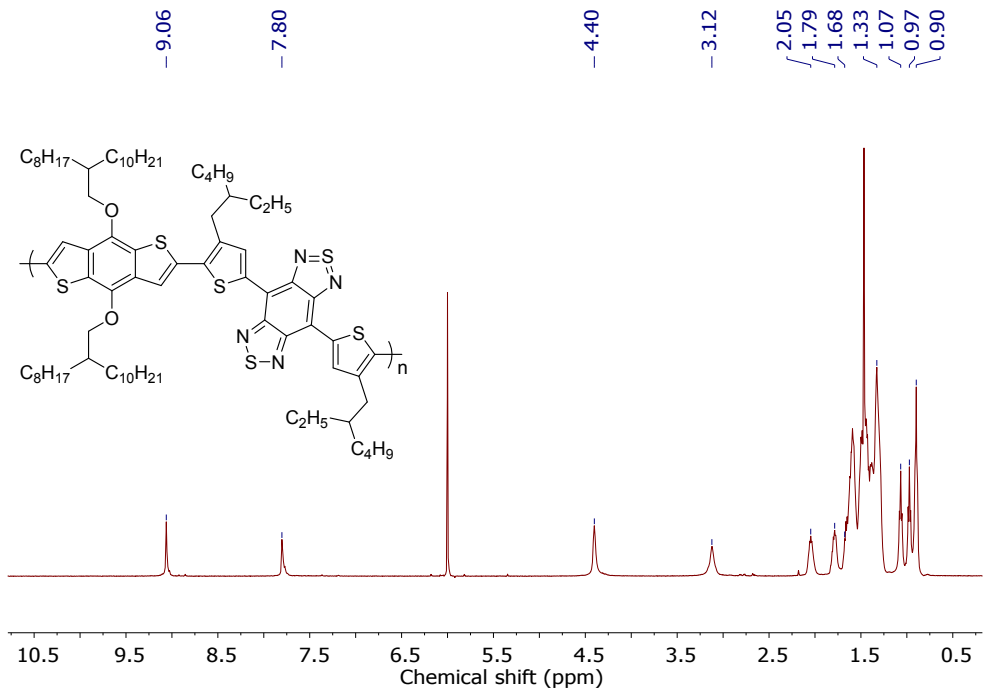

Fig. S1 ^{1}H NMR spectrum of **P1** in $C_2D_2Cl_4$ at 373K.

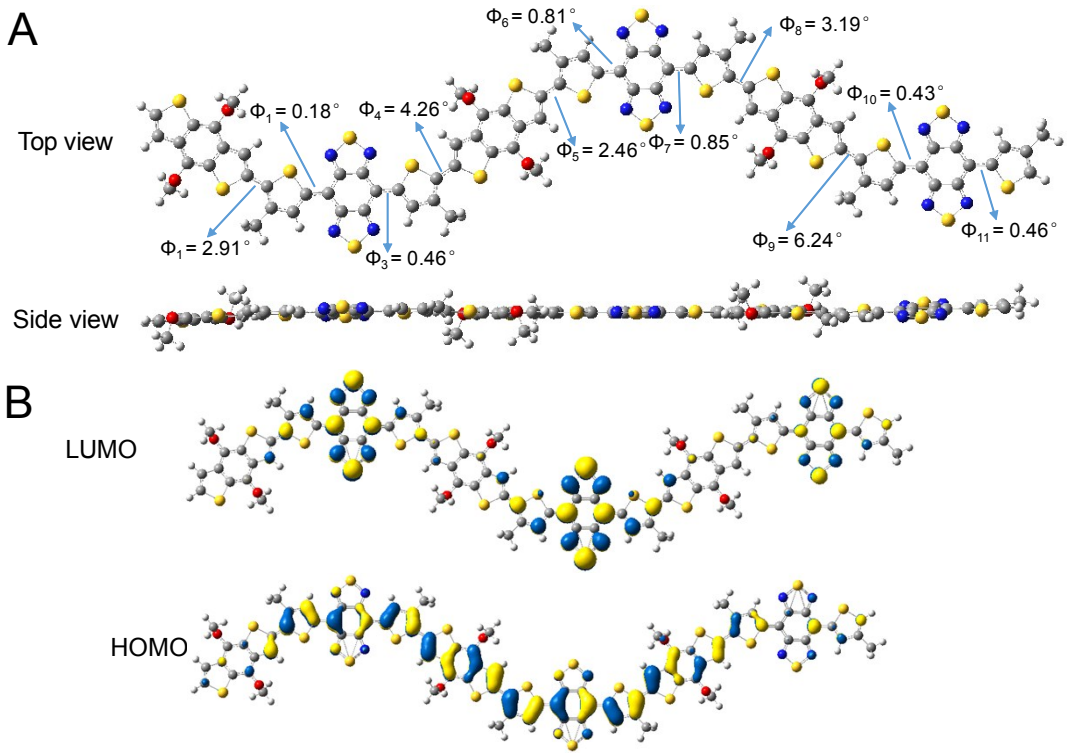

Fig. S2 (A) Optimized confirmation of **P1** trimer structures with the torsion angles along the conjugated backbones. (B) HOMO and LUMO wave functions of **P1** trimer in the geometrically optimized structures (B3LYP/6-31G (d,p))

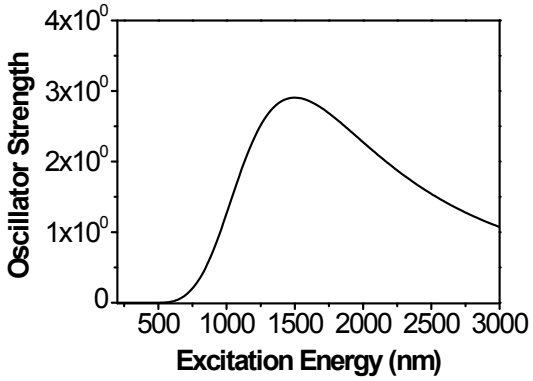

Fig. S3 TDDFT-calculated oscillator strength of the trimer model of **P1**.

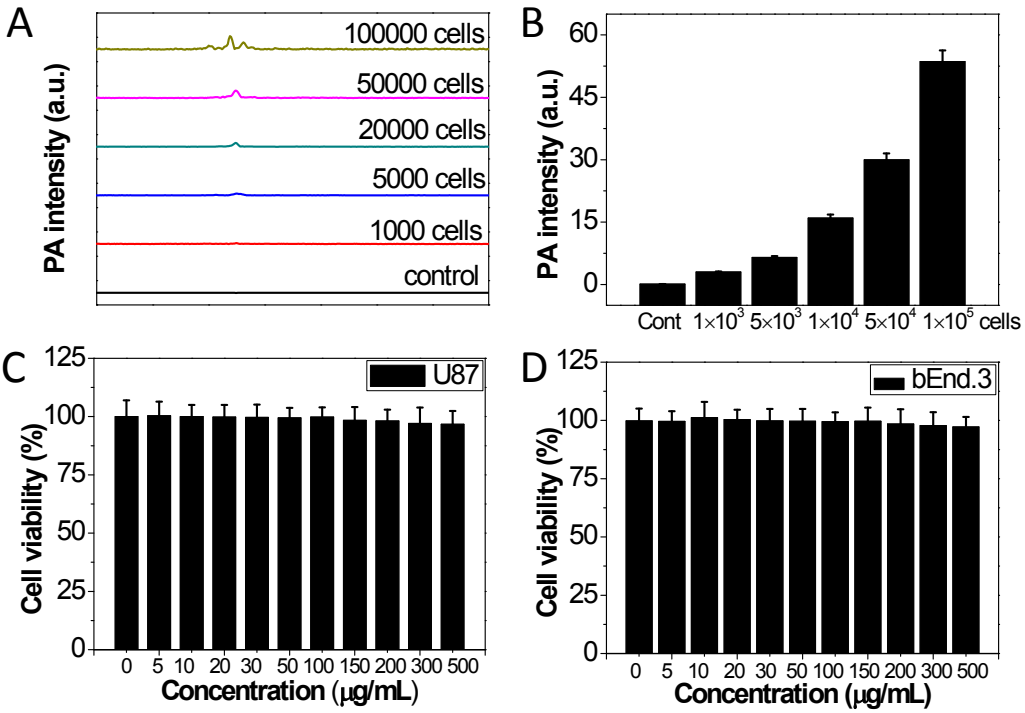

Fig. S4 (A) 1064 nm PA signals (with pulse laser of 5.6 mJ/cm^2) of U87 glioma cells incubated with **P1** NPs (0.1 mg/mL) for 3 h. (B) PA signal intensity of U87 glioma cells treated with **P1** NPs, as a function of the cell quantity. (C-D) Cytotoxicity evaluation of **P1** NPs. (C) Viabilities of U87 glioma cells and (D) bEnd.3 endothelial cells treated with different concentration of **P1** NPs.

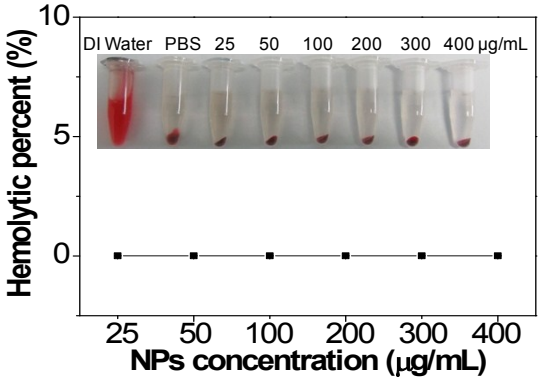

Fig. S5 Hemolytic analysis of red blood cells after 3 h of P1 NPs treatment. DI water and PBS were used as the positive and negative control, respectively. The representative hemolysis photos are inserted in the figure.

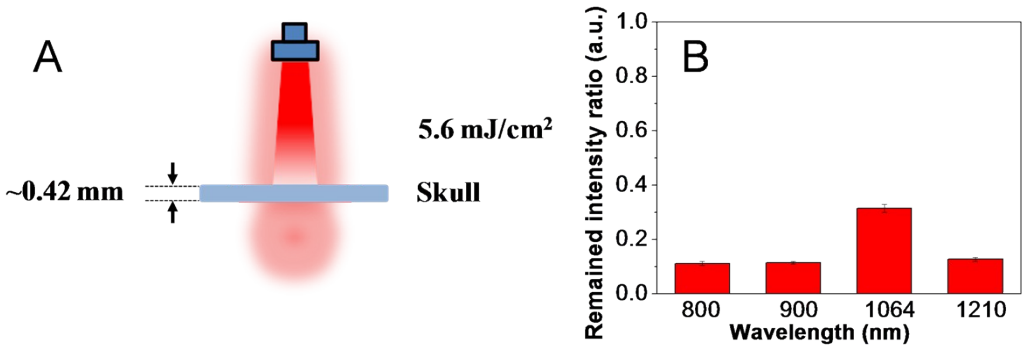

Fig. S6 Measurement of the attenuation of laser excitation of different wavelengths (*e.g.,* 800, 900, 1064 and 1210 nm) after penetration into the mice skull. The initial power of the pulse laser was 5.6 mJ/cm^2. (A) Schematic illustrating the attenuation measurement. (B) Ratio of the remained laser intensity to the initial laser power at different excitation wavelengths.

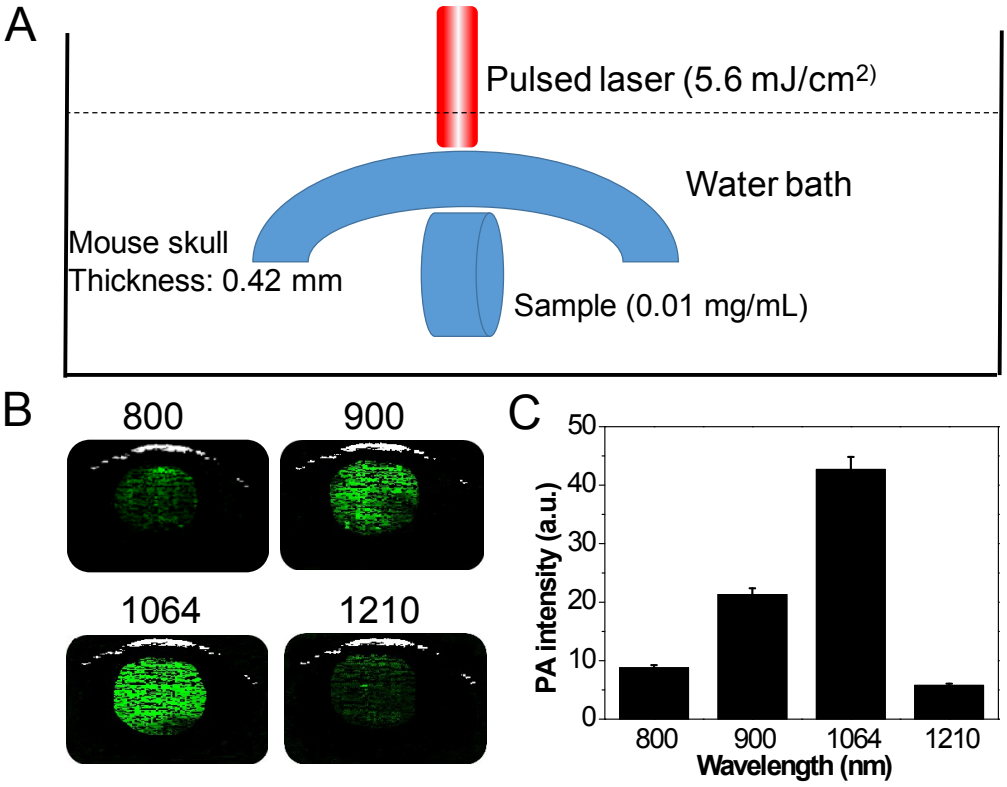

Fig. S7 PA imaging of **P1** NPs under mice brain skull. (A) Schematic illustrating the PA imaging under mice brain skull with a pulsed laser (5.6 mJ/cm^2). (B) PA images and (C) intensity of **P1** NPs under mice brain skull with different excitation wavelengths (NPs concentration = 0.01 mg/mL, matrigel matrix-v/v = 1:1).

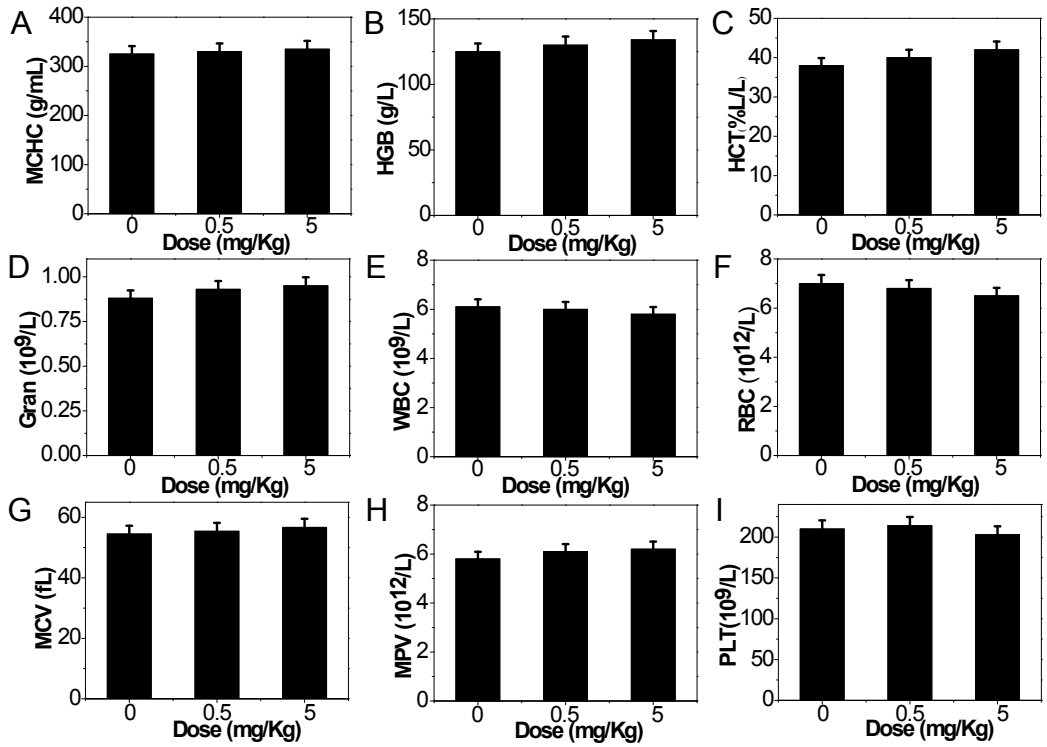

Fig. S8 Hematology and blood biochemical assay for healthy mice after intravenous injection of **P1** NPs for 7 days. (A) Mean corpuscular hemoglobin concentration (MCHC); (B) Concentration of hemoglobin (HGB); (C) Hematocrit (HCT); (D) Neutrophilic granulocytes number (Gran); (E) white blood cells number (WBC); (F) Red blood cells number (RBC); (G) Mean corpuscular volume (MCV); (H) Mean platelet volume (MPV); and (I) Platelets (PLT).

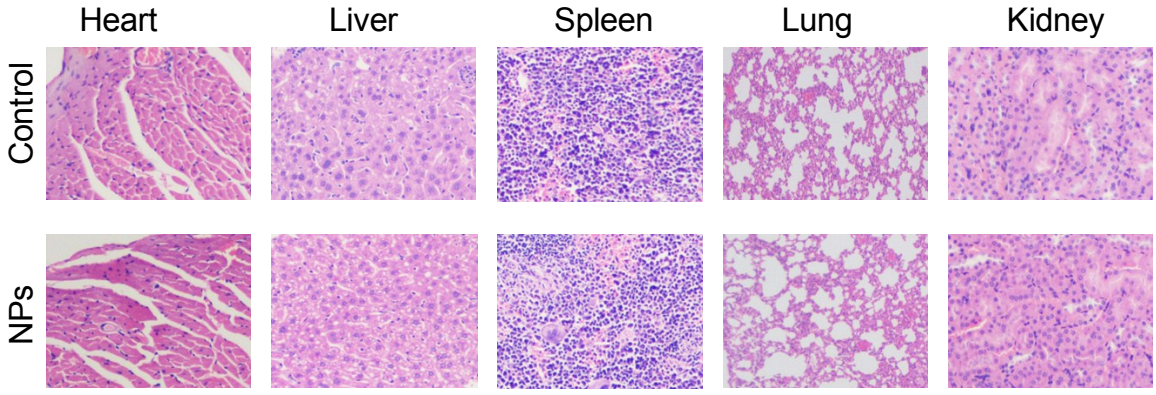

Fig. S9 Representative hematoxylin and eosin (H&E) stained images of major organs composed of heart, liver, spleen, lung and kidney, which were collected from the mice sacrificed after 7 days of **P1** NPs injection (magnification: 400 ×).