Electronic supplementary information

Facile Synthesis of a Metal-Organic Framework Nanocarrier for NIR Imaging-Guided Photothermal Therapy

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Experimental Section

Materials and methods

All starting chemicals and solvents were purchased from commercial sources and used without further treatment, unless indicated otherwise. Carboxyl-functionalized heptamethine indocyanine (Cy) was synthesized as described in the literature.[1] Milli-Q water was collected from a Milli-Q system (Millipore, USA). FTIR was measured by Nicolet Impact 410 Fourier transform infrared spectrometer. UV and fluorescence were recorded on SHIMADZU UV-2450 and Edinburgh Instrument FLS-920 spectrometer, respectively. TEM and SEM images were recorded by JEOL JEM-1011 electron microscope (acceleration voltage of 100 kV) and JEOL JXA-840 (acceleration voltage of 15 kV). Size and zeta potential were measured by Malvern Zeta Sizer-Nano ZS90 instrument. PXRD was performed by a Riguku D/MAX2550 diffractometer using CuKα radiation, 40 kV, 200 mA with scanning rate of 0.4 ^{o} min^{-1}. TGA was performed using a NetzchSta 449c thermal analyzer system at a rate of 10 ^{o}C min^{-1} under air atmosphere. The temperature change in tumors under laser irradiation was monitored by infrared thermal camera (FLIR). Animal fluorescence images were obtained using a Maestro 500FL *in vivo* optical imaging system.

One-pot Synthesis of MOFs

The synthesis of Cy@ZIF-8 NPs was based on the previous procedure with some modifications.[2,3] In a typical experiment, 150 mg of zinc nitrate hexahydrate was dissolved in 5 mL of deionized water; on the other hand, 330 mg of 2-methyl imidazole and 10 mg of Cy were dissolved into 10 mL of methanol. Under stirring, the aqueous of zinc nitrate was added into the solution of 2-methyl imidazole and Cy at room temperature, the synthesis solution quickly turned turbid. After 3 min, Cy@ZIF-8 NPs were formed. The solution was centrifuged at 12000 rpm for 10 min to obtain the Cy@ZIF-8 NPs. Then Cy@ZIF-8 NPs was washed with methanol three times to completely remove the unreacted reagents. Finally, the products were freeze-dried and stored at -20 ^{o}C until further use. Yield was about 80% based on the amount of zinc. As a control, ZIF-8 NPs were synthesized with the same method.

In Vitro PTT Effects

Cy@ZIF-8 NPs in aqueous solution with different concentrations (5, 10, 20, 40, 80 μg mL^{-1}) were irradiated with NIR laser (808 nm, 0.8 W cm^{-2}, 5 min) and temperatures were recorded every 30 s. To a fixed Cy@ZIF-8 NPs concentration (20 μg mL^{-1}), the influence from different power density (0.6 W cm^{-2}, 0.8 W cm^{-2}, 1.0 W cm^{-2}) were recorded as well. The photothermal response of Cy@ZIF-8 NPs in water (20 μg mL^{-1}) was recorded with laser irradiation (808 nm, 0.8 W cm^{-2}, 5 min) and then shut off. The photostability was investigated by determining their absorbance changes upon continuous NIR laser irradiation (808 nm, 0.8 W cm^{-2}).

Cell Culture

Human epithelial carcinoma HeLa cells and the human lung adenocarcinoma A549 cells were obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China. HeLa cells and A549 cells were routinely grown in DMEM medium and cultured in medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 ^{o}C under 5% CO_2.

Cellular Uptake

Cellular uptakes by HeLa cells were examined using a confocal laser scanning microscope (CLSM) and flow cytometry analyses (FCS) *via* reported procedures.[4] Cells were seeded in 6-well culture plates (a sterile cover slip was put in each well) at a density of 5×10^4 cells per well and allowed to adhere for 24 h. After that, the cells were treated with Cy@ZIF-8 NPs (20 μg/mL) for 0.5 h at 37 ^{o}C. Thereafter cells were incubated for additional 0.5 h, 2 h, and 4 h at 37 ^{o}C. Subsequently, the supernatant was carefully removed and the cells were washed three times with PBS. Subsequently, the cells were fixed with 500 μL of 4% formaldehyde in each well for 20 min at room

temperature and washed twice with PBS again. Cells were visualized using blue channel for DAPI (λ_{ex} 405 nm) and red channel for Cy (λ_{ex} 633 nm) under a confocal laser scanning microscope (Carl Zeiss LSM 700). The cells for FCS were analyzed by flow cytometer (Beckman, California, USA).

Cytotoxicity Test

The cytotoxicity test was measured *via* MTT assay and live-dead cell staining assay according to the previous work.[2] Cells were incubated with Cy@ZIF-8 NPs (0-40 μg/mL) for 4 h, then irradiated by the NIR laser (808 nm, 0.8 W cm^{-2}, 5min), then cells were incubated for another 24 h for MTT assays. Finally, the absorbance at 490 nm was recorded by microplate reader (BioTek, ELX808). Live-dead cell staining assay was performed according to Live-Dead Cell Staining Kit. After 4 h of incubation with Cy@ZIF-8 NPs (40 μg/mL) and irradiated with NIR laser (808 nm, 0.8 W cm^{-2}, 5 min). Then, cells were stained with Calcein-AM/PI for 30 min at room temperature, and the result was detected by a fluorescence microscope (Nikon Eclipse Ti, Optical Apparatus Co., Ardmore, PA, USA).

Apoptosis

The apoptosis and necrosis induced by PTT of Cy@ZIF-8 NPs were evaluated by flow cytometry. HeLa cells were seeded at 1×10^6 cells/well in 6-well plates and further cultured for another 48 h. Then, the culture media were replaced by 1 mL of fresh culture media containing 10% FBS. Cy@ZIF-8 NPs (40 μg/mL) was added to the cells, and cells incubated with PBS served as a blank control. After 6 h of incubation, the cells were irradiated with NIR laser (808 nm, 0.8 W cm^{-2}) for 5 min. Following incubation of 48 h, the floating dead cells and adherent alive cells were all collected and stained with propidium iodide (PI) (dead cells) and an Annexin V-FITC (apoptosis cells) apoptosis detection kit (Beyotime Biotechnology) according to the manufacturer's instructions. The apoptosis and necrosis results were analyzed using a flow cytometry.

Western Blot and Antibodies

Western blotting was performed using standard methods. After being treated with Cy@ZIF-8 NPs at a concentration of 40 μg/mL with or without NIR laser (808 nm, 0.8 W cm^{-2}, 5 min), the cell pellets were suspended in radio immunoprecipitation assay buffer (150 mM sodium chloride, 50 mM Tris pH 8.0, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with 10 mM NaF, 1mM Na_3VO_4, 5 mM EDTA, 1 mM EGTA, 5 mg/ml leupeptin, 1 mg/ml pepstatin A, 1 mM phenyl methylsulfonyl fluoride, and protease and phosphatase inhibitor for 15 min at 4 °C and centrifuged at 12000 rpm 20 min at 4 °C. Protein concentration of the supernatants was determined using a BCA protein Assay Kit (Beyotime Biotechnology, China). Equal amounts (50 μg) of the proteins were resolved by 12% SDS–polyacrylamide gel electrophoresis gels and transferred to PVDF membranes (Millipore) using a Bio-Rad Trans-blot instrument. Membranes were blocked in 5% milk for 1 h at room temperature following incubation with the indicated primary antibody overnight at 4 °C, washed 3 times with TBS-T buffer, incubated with secondary antibody IgG-HRP at 1:20000 dilutionsin TBS-T buffer (Cat. #A 11034 for anti-rabbit; #A 32723 for anti-mouse by Invitrogen). After washing 3 times with TBS-T buffer, the membrane was developed with ECL substrate (Thermo Scientific) and the signal was detected by a BIO-RAD Fluorescent Imager, following quantification by Image Lab software.

The antibodies used in this study were diluted in TBS-T including 3% milk. Antibodies directed against HSP70 (sc-66048) was obtained from Santa Cruz (1:1 000 dilution), β-actin were obtained from Invitrogen (1:5 000 dilution).

Animal Model

All experiments related to live subjects were performed in compliance with the relevant laws and guidelines of China-Japan Union Hospital of Jilin University and approved by the ethical committee of China-Japan Union Hospital of Jilin University. Kunming (KM) female mice were obtained from Jilin University, China (56-84 d, 20-25 g) and maintained under required conditions. The xenograft tumor models of U14 cervical cancer were established by injecting U14 cervical carcinoma cells into the right leg region of the mice. After about 7 days, mice with tumor volumes of about 150 mm^3 were selected for the NIR fluorescence imaging and PTT treatment.

***In vivo* NIR Imaging and Photothermal Treatments**

NIR fluorescence signals were acquired on a commercial Maestro *in vivo* fluorescence imaging system (CRI Maestro 500FL). U14 bearing Kunming mice were injected with Cy@ZIF-8 NPs through intratumor (0.5 mg kg^{-1}) or tail vein (1 mg kg^{-1}), *in vivo* spectral imaging from 650 to 800 nm was carried out with an exposure time of 3000 ms. These mice were monitored with *in vivo* fluorescent imaging system at different time points. For photothermal imaging and therapeutic assays, the tumors of mice were exposed to 808 nm laser for 10 min at 0.8 W cm^{-2}. Photothermal imaging was captured by a FLIR Ax5 camera. The tumor volume and body weight were measured every other day in 12 days. After 12 days of observation and measurement, the mice were sacrificed and the tumors were excised to intuitionally evaluate the tumor inhibition.

Statistical Analysis

All experiments were performed at least three times and all results were expressed as mean ± standard deviation

(SD). Student's t-test was used to determine the statistical difference between various experimental and control groups. Significant differences between the groups are indicated by * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$, respectively.

Results

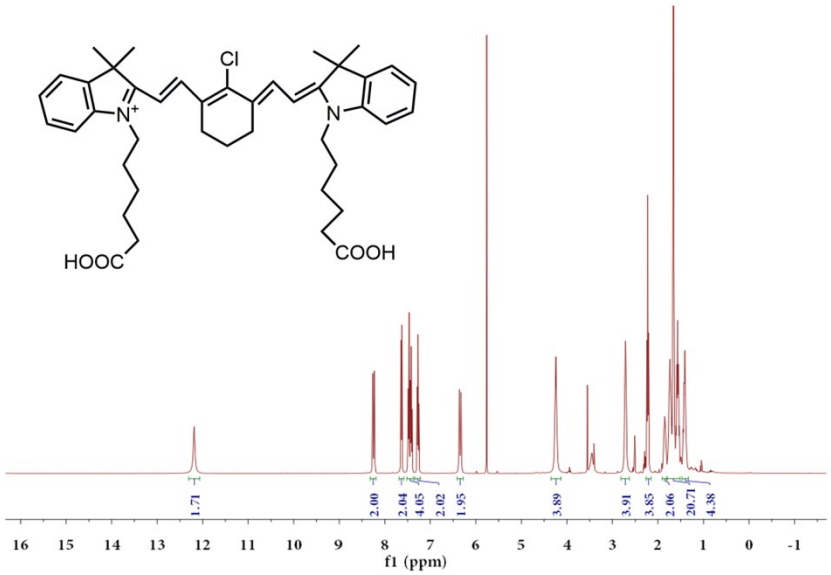

Fig. S1 The ^{1}H NMR of Cy in DMSO-d_6.

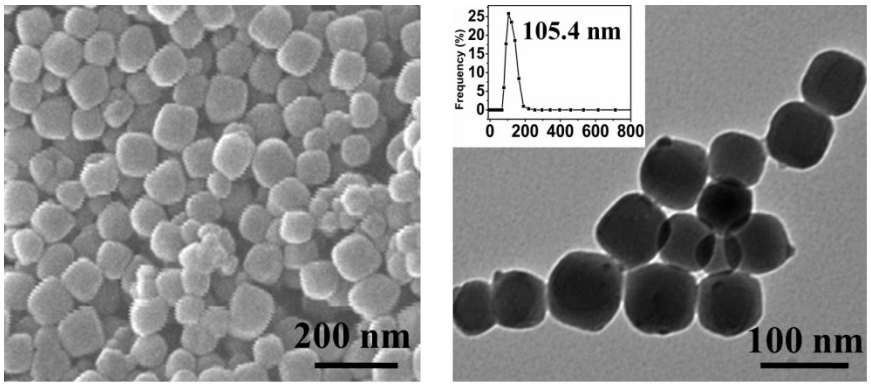

Fig. S2 (A) SEM image and (B) TEM image of ZIF-8 NPs. Insert: Particle size distribution of ZIF-8 NPs dispersed in water.

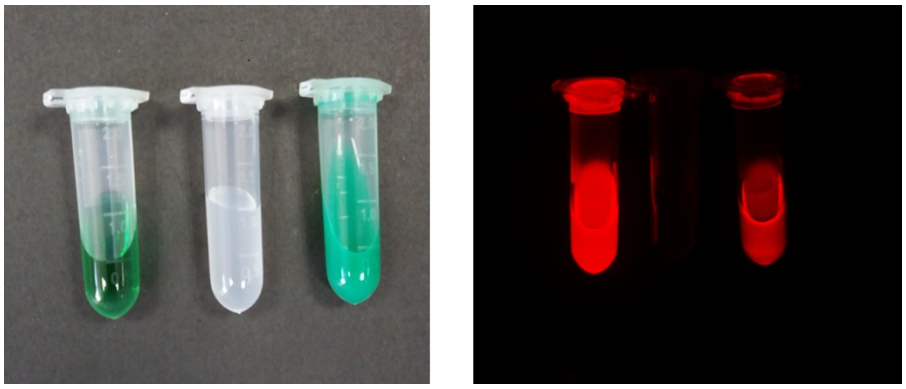

Fig. S3 Photographs of free Cy, ZIF-8 NPs, and Cy@ZIF-8 NPs in methanol solution under white light (left photo), and NIR light (right photo) at 750 nm.

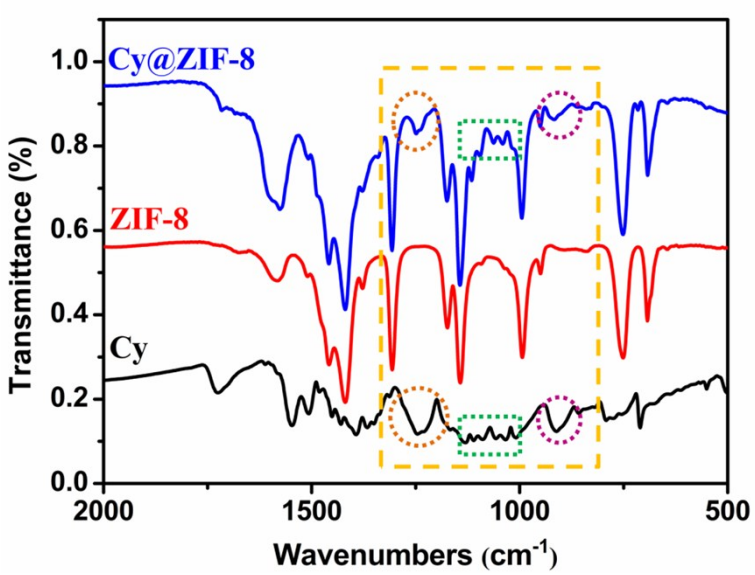

Fig. S4 FTIR spectra of Cy, ZIF-8 NPs and Cy@ZIF-8 NPs.

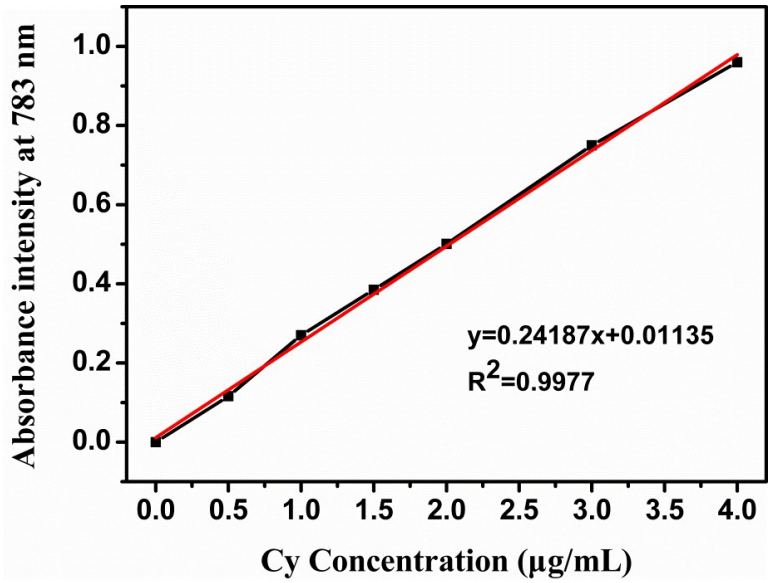

Fig. S5 The standard curve of Cy in methanol solution.

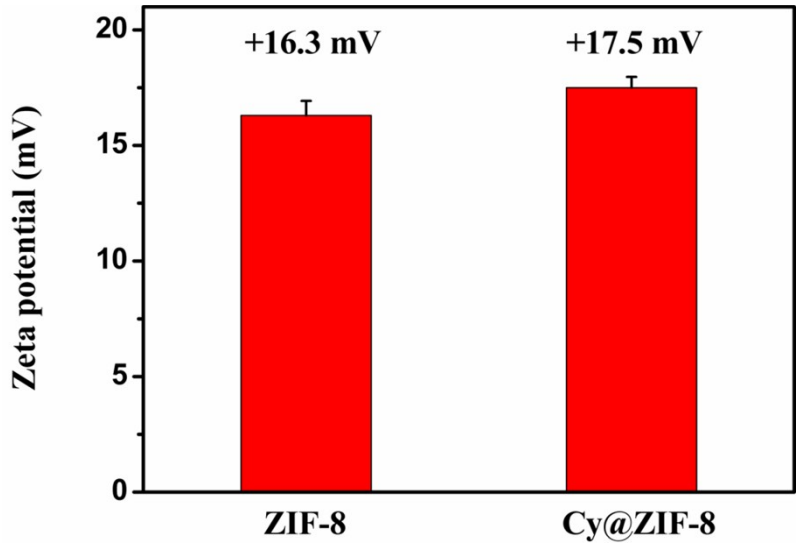

Fig. S6 Zeta potentials of ZIF-8 NPs and Cy@ZIF-8 NPs.

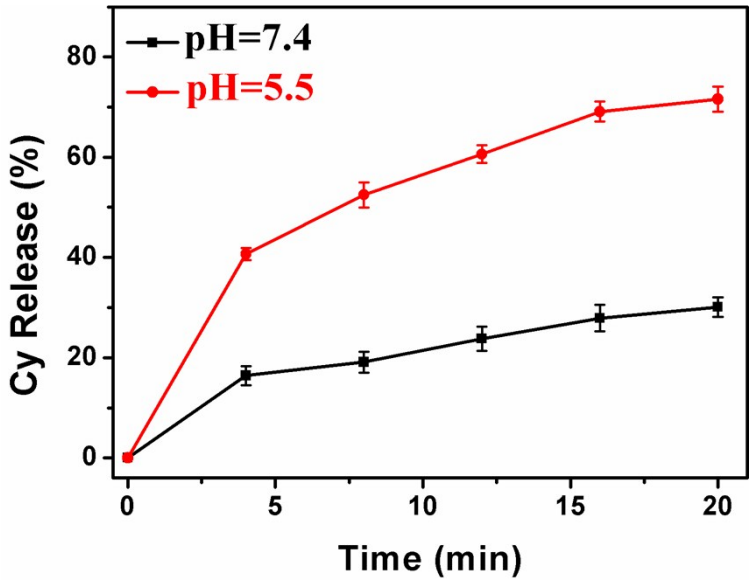

Fig. S7 Cy release profiles in different PBS solutions (pH 5.5 and 7.4) under 808 nm laser irradiation.

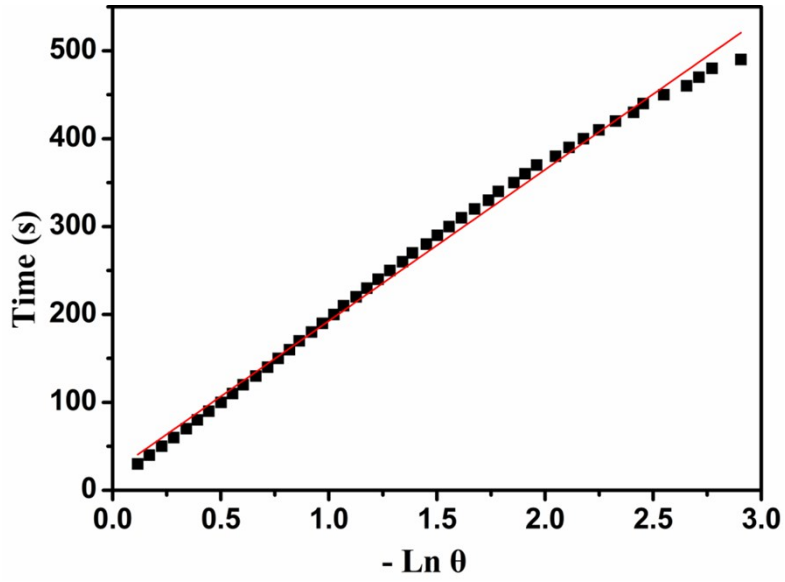

Fig. S8 Linear time data versus −Ln θ obtained from the cooling period.

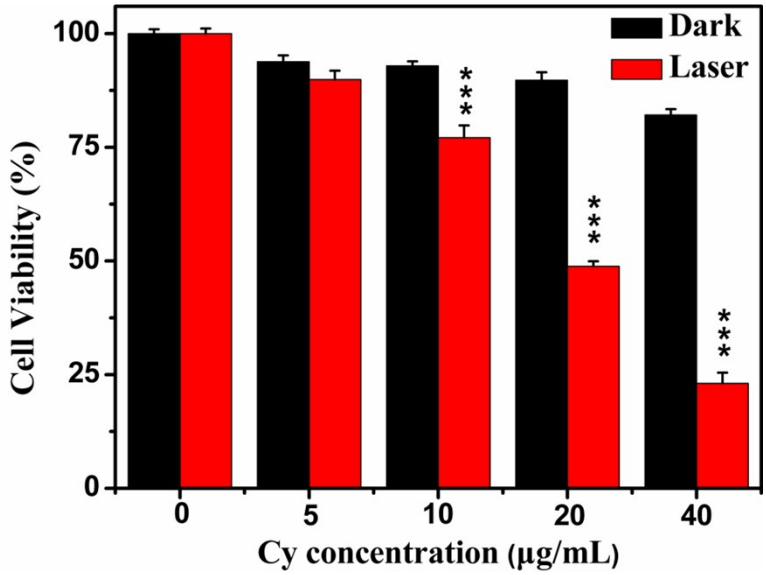

Fig. S9 Relative cell viabilities of A549 cells after incubated with different concentrations of Cy@ZIF-8 NPs without or with NIR laser irradiation. The level of significance was set at probabilities of $^{**}p < 0.01$, and $^{***}p < 0.001$.

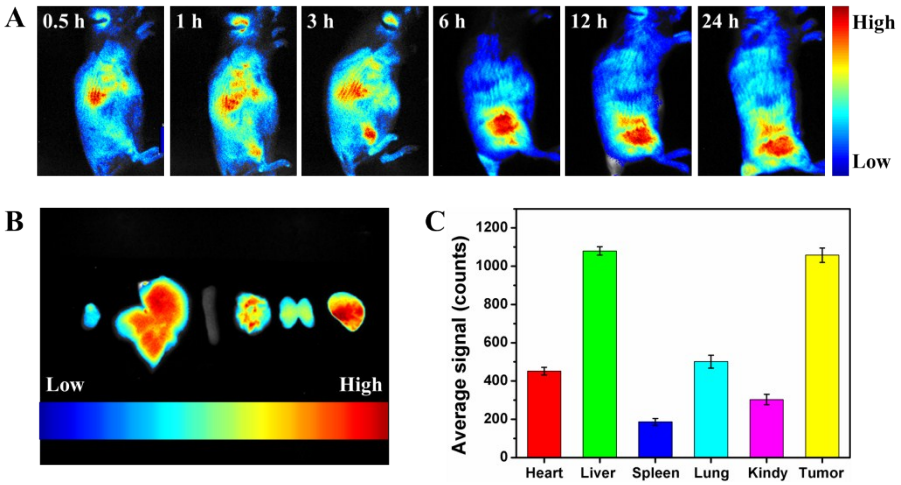

Fig. S10 (A) Time based *in vivo* NIR fluorescence images of mice after intravenous injection of Cy@ZIF-8 NPs (1 mg kg^{-1}). (B) *Ex vivo* imaging of the tissues 6 h post-injection (from left to right: heart, liver, spleen, lung, kidney, and tumor). (C) Mean fluorescence intensity of tissues along with extension of time.

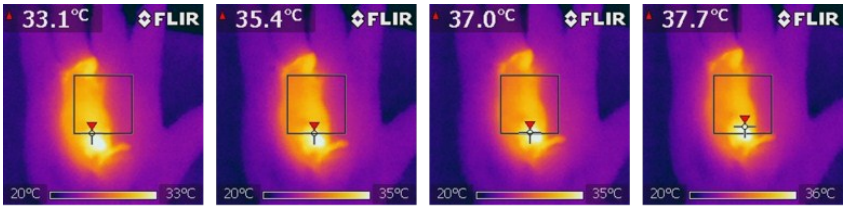

Fig. S11 The whole-body IR images of mice in the control group under 808 nm laser (0.8 W cm^{-2}) at different time points.

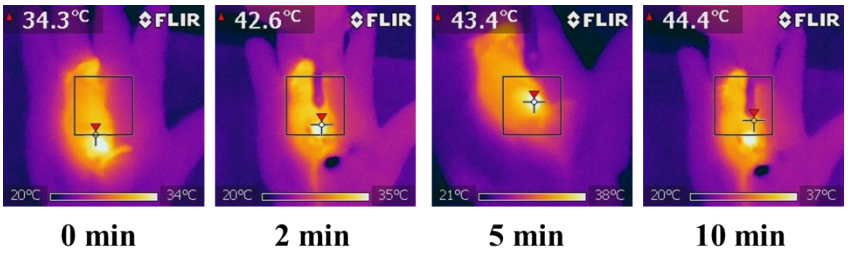

Fig. S12 The whole-body IR images of mice after intravenous injection of Cy@ZIF-8 NPs under irradiation (808 nm, 0.8 W cm^{-2}) at various time points.

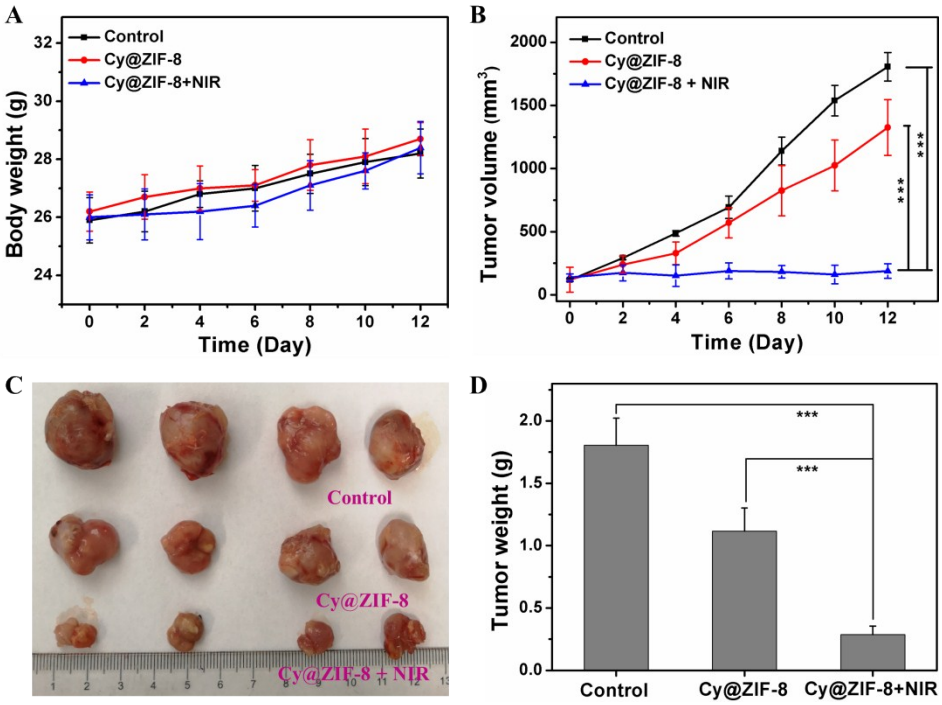

Fig. S13 *In vivo* PTT study of U14 tumor-bearing Kunming mice *via* intravenous injection of Cy@ZIF-8 NPs. (A) Changes in body weight and (B) Tumor volume of mice after different treatments as a function of time. (C) Representative photo of the excised tumors and (D) tumor weight of every group. Statistical significance: $^{**}p < 0.01$, and $^{***}p < 0.001$.

Notes and references

1. X. Tan, S. Luo, D. Wang, Y. Su, T. Cheng and C. Shi, *Biomaterials*, 2012, **33**, 2230-2239.
2. M. Zheng, S. Liu, X. Guan and Z. Xie, *ACS Appl. Mater. Interfaces*, 2015, **7**, 22181-22187.
3. Y. Pan, Y. Liu, G. Zeng, L. Zhao and Z. Lai, *Chem. Commun.*, 2011, **47**, 2071-2073.