Electronic Supplementary Information

Reconstructing the Intracellular pH Microenvironment for Enhancing Photodynamic Therapy

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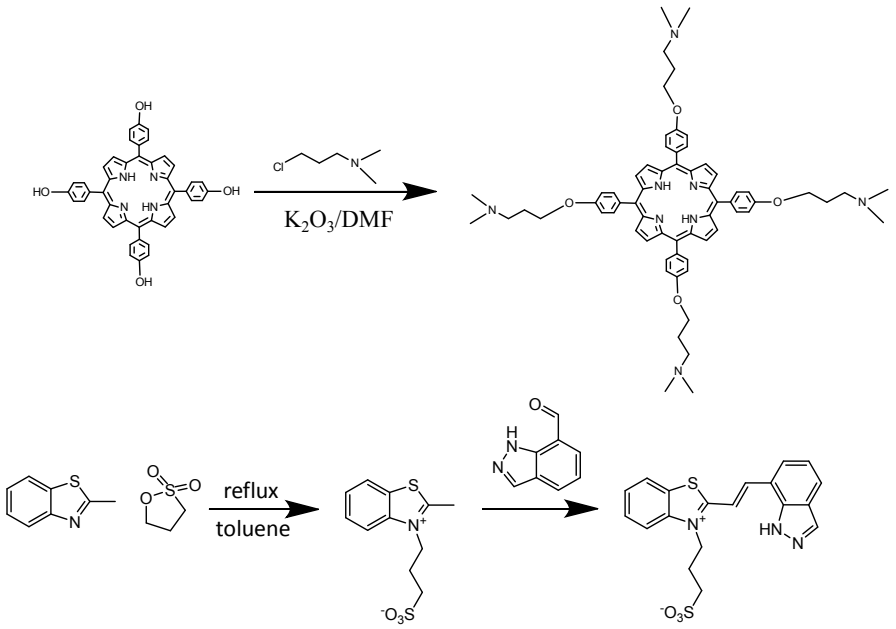
Materials

5,10,15,20-Tetrakis(4-hydroxyphenyl)porphyrin (THPP) was synthesized according to the literature.[1] Dimethyl formamide (DMF) was dried over calcium hydride before use. 2-methylbenzothiazole, propanesultone, 1-H-indazole-7-carbaldehyde, 2-methylimidazole, 3-chloro-N,N-dimethylpropan-1-amine 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and 4′, 6-diamidino-2-phenylindole (DAPI), 2′,7′-dichlorofluorescein diacetate(DCHF-DA), 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein,acetoxymethyl ester (BCECF-AM) were all purchased from Aladdin Reagents of China and used directly as received. 1-Octadecene (90%), Ammonium fluoride (NH_4F), PVP (Mw = 40000), $Zn(NO_3)_2 \cdot 6H_2O$, $TmCl_3 \cdot 6H_2O$, $YCl_3 \cdot 6H_2O$, $YbCl_3 \cdot 6H_2O$ were purchased from Sigma-Aldrich. All other reagents were purchased from commercial resources and used as received unless otherwise noted.

Characterization

^1H NMR spectra were recorded at 400 MHz, using BRUKER AV400 Spectrophotometer. The fluorescence spectra were recorded on a F-4500 fluorescence spectrophotometer at room temperature. The UV-Vis spectra of the samples were measured over different irradiation time intervals by using a Thermo Scientific Evolution 220 spectrophotometer. The hydrodynamic radius was collected with a Nanotrac Wave II Q Nanoparticle Size Analyzer, Microtrac. Fourier transform infrared (FT-IR) spectra were obtained with a Bruker TENSOR II FTIR Spectrometer. X-ray diffraction (XRD) patterns were acquired on a Rigaku D/MAX-2250V diffractometer and all the measurements were carried out at room temperature. Transmission electron microscopy (TEM) images were obtained with an electron microscope (JEM-2100F, 200 kV). MTT assays were conducted with a Spark™ Multimode Microplate Reader. Confocal laser scanning microscopy (CLSM) images were obtained using a Nikon $A1^+R$-980 confocal microscope.



Scheme of Synthesis 1

Synthesis of 5, 10, 15, 20-tetrakis(4-(3-(dimethylamino) phenyl)porphyrin (TPP)

3-chloro-N,N-dimethylpropan-1-amine(1.216 g, 10 mmol), THPP (0.678 g, 1.0 mmol) and anhydrous K_2O_3 (0.690 g, 5.0 mmol) were dissolved in 50 mL anhydrous DMF, and refluxed 24 h at 100 °C after purging with nitrogen for 20 min. The reaction mixture was evaporated in a vacuum, and the residual product dissolved in water at pH=4, and the insoluble part was removed. pH was then adjusted to weak alkalinity, and the sediments were collected. After that, the impurities in the sediments were removed by dialysis (MWCO = 500 Da) against deionized water at pH = 4, and dialysis products were freeze-dried. The final product was a purple-black solid with 30% yield. ^{1}H NMR (400 MHz, D_2O) δ 8.86 (m, 8H), 8.64 (m, 8H), 7.73 (m, 8H), 4.56 (t, 8H), 3.53 (t,8H), 3.21 (s, 24H), 2.42 (m, 8H).

Synthesis of the photoacid (PA)

The PA was synthesized according to the literature.[2] Firstly, the intermediate, 2-methyl-1-(3-sulfonatepropyl)-benzothiazolium, was synthesized. 2-

methylbenzothiazole (1.49 g, 0.01 mol) and propanesultone (1.22 g, 0.01 mol) were dissolved in 15 mL toluene. After purging with nitrogen for 20 min, the mixture was refluxed at 90 ℃ for 8 h. A white solid was obtained by filtration, washed with THF and dried in vacuo (1.28 g, 47 % yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.43 (m, 2H), 7.88 (t, 1H), 7.80 (t, 1H), 4.92 (t, 2H), 3.20 (S, 3H), 2.64 (t, 2H), 2.16 (m, 2H).

2-methyl-1-(3-sulfonatepropyl)-benzothiazolium (0.294 g, 1.00 mmol), 10 mg of ammonium acetate and 1-H-indazole-7-carbaldehyde (0.168 g, 1.16 mmol) were refluxed in absolute ethanol (2 ml) at 78 ℃ for 4h. An orange solid was obtained by filtration, washed with cold ethanol for three times, and dried in vacuo (0.208 g, 52% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 13.99 (S, 1H), 8.67 (d, 1H), 8.52 (d, 1H), 8.47 (d, 1H), 8.35 (m, 3H), 8.12 (d, 1H), 7.94 (t, 1H), 7.85 (t, 1H), 7.38 (t, 1H), 5.22 (t, 2H), 2.75 (t, 2H), 2.31 (m, 2H).

Synthesis of the UCNP ($NaYF_4$:Yb:Tm)

According to our previous research, the monodisperse UCNP (NaYF4:20%Yb,0.2%Tm) were prepared by thermal decomposition method. $YCl_3 \cdot 6H_2O$ (484.1 mg, 1.596 mmol), $TmCl_3 \cdot 6H_2O$ (1.53 mg, 0.004 mmol), and $YbCl_3 \cdot 6H_2O$ (155.0 mg, 0.4 mmol) dissolved in 1 mL deionized water were added to 100 mL flask containing 30 mL 1-Octadecene and 18 mL oleic acid, and the reaction solution was stirred at room temperature for 1 h. Then water was removed by heating the reaction solution to 120℃ under the protection of argon atmosphere and keeping the temperature for 0.5 h. In order to ensure that water was completely removed, the reaction solution was kept the temperature at 140℃ for 0.5 h. After the water was removed, the reaction solution was cooled down to room temperature. 10 mL methanol solution containing NH_4F (296.3 mg, 8 mmol) and NaOH (200 mg, 5 mmol) was then added to the system, and the reaction solution was stirred for about two hours at room temperature. After the methanol was completely removed by heating, the solution was fast heated to 280 ℃ and maintained the temperature at 280-290℃

for 1.5 h. After the reaction solution was cooled down to room temperature, the products were precipitated by adding 20 mL ethanol and collected by centrifugation at 13000 rpm for 10 min. After washing several times with cyclohexane and ethanol, the nanoparticles were dispersed in 20 mL cyclohexane (100 mM).

Synthesis of the UCNP@ZIF-8

PVP-stabilized UCNP: 5 mL ethanol was added to 5 mL the above of solution (UCNP), the UCNP were collected centrifugation at 13000 rpm for 10 min and dispersed in 20 mL of chloroform. A solution of PVP (250 mg, M_w = 40000) in 10 mL chloroform was added to the UCNP suspension and the mixture was stirred at room temperature for 24 h. The PVP-stabilized UCNP was collected by centrifugation at 13000 rpm for 10 min, washed three times with methanol, and dispersed in 5 mL methanol.

UCNP@ZIF-8: methanolic solution of 2-methylimidazole (25 mM, 25 mL), zinc nitrate (25 mM, 25 mL) and PVP-stabilized UCNP (5 mL) were mixed fast and kept at room temperature for 12 h. The product was collected by centrifugation at 13000 rpm for 10 min, washed three times with methanol, and dispersed in 5 mL methanol.

Synthesis of UCNP@ZIF+PA, UCNP@ZIF+TPP and UCNP@ZIF+TPP+PA

PA (10 mg) and UCNP@ZIF-8 (40 mg) were dissolved in 20 mL DMSO and stirred at room temperature under the condition of avoiding light. After 24 h, PEG (100 mg) was added to the mixture. UCNP@ZIF+PA was collected by centrifugation at 13000 rpm for 15 min, washed three times with deionized water and redispersed in PBS (pH = 7.2). The supernatant after centrifugation was also collected and was measured by UV-Vis spectroscopy at 450 nm wavelength. The absorbance of different concentration of PA was measured to make a concentration-absorbency-related standard curve. According the standard curve, the amount of unloaded PA was calculated, and the amount of loaded PA can also be calculated. UCNP@ZIF+TPP

and UCNP@ZIF+PA+TPP were synthesized as the same method. The amount of loaded of PA and TPP were 5.7% and 8.5%, respectively.

Study of the producing H^+ of PA

Bromothymol blue (BB) was an acid-base indicator with a trial range of pH = 7.2-6.0. It could be used to show the change of low H^+ concentration. PA was added into 2 mL BB, and the absorbance of the mixture was measured at different light time by UV-Vis spectroscopy at 300-800 nm wavelength. In the same time, A series of PA solutions with pH 7.4 were prepared (40, 80, 120, 160, 240, 320 μg mL^{-1}), and their pH were detected with light irradiation by pH detector.

Study of the UV-Vis absorptance and fluorescence of TPP in different pH

0.2 M NaH_2PO_4 (A) and 0.2 M Na_2HPO_4 (B) solution were prepared, and other buffer solutions with different pH were prepared according to a certain proportion (pH = 7.4, 7.2, 7.0, 6.8, 6.6, 6.4, 6.2, 6.0). The same concentration of TPP was added to the above buffer solutions with different pH, and then their UV-Vis absorptance and fluorescence spectra were detected.

Light-triggered UV-Vis absorptance and fluorescence spectra of UCNP@ZIF+TPP+PA

2 mL of UCNP@ZIF+TPP+PA, UCNP@ZIF+PA and UCNP@ZIF+TPP (2 mg mL^{-1}) were illuminated for different time by 980 nm laser (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 min) at 1.4 W/cm^2. After light irradiation, their UV-Vis absorptance and fluorescence spectra were detected, respectively.

Study of the producing $^1O_2$ of UCNP@ZIF+TPP

The $^1O_2$ could degrade specifically 1,3-diphenylisobenzofuran (DPBF), so the degradation of DPBF could be used to confirm the generation of $^1O_2$. UCNP@ZIF+TPP was dispersed in different PBS (pH = 7.4, pH = 6.5, pH = 6.0). 20

μL DPBF (2 mg mL^{-1}) was added to 2 mL UCNP@ZIF+TPP, and then the absorbance of the solution at 300-500 nm wavelength was measured at different light time (the control group without UCNP@ZIF+TPP). The singlet oxygen quantum yields (Φ_Δ) of TPP in nanomaterials were measured in according to the previous literatures.[3,4] 5,10,15,20-tetrakis(*p*-sulfonato)porphyrinate (TPPS) was used as a standard. The value of Φ_Δ was calculated according to *Eqn (1)*:

$$\Phi_\Delta = \Phi_\Delta^{\mathrm{Std}} \frac{R I_{\mathrm{abs}}^{\mathrm{Std}}}{R^{\mathrm{Std}} I_{\mathrm{abs}}} \qquad (1)$$

where $\Phi_\Delta^{\mathrm{Std}}$ is the singlet oxygen quantum yield of the standard TPPS ($\Phi_\Delta^{\mathrm{Std}}$,0.71);[5,6] R and R^{Std} are the degradation rate of DPBF in the presence of the samples and the standard TPPS, respectively; I_{abs} and $I_{\mathrm{abs}}^{\mathrm{Std}}$ are the absorbance of the samples and the standard TPPS, respectively.

Cell culture

The 4T1 cells were cultivated in high-glucose 1640 (Gico, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS), streptomycin (100 mg mL^{-1}) and penicillin (100 units mL^{-1}). The cell line was purchased from Shanghai Institute of cells, Chinese Academy of Sciences, and cultured at 37 °C in humid atmosphere with 5% CO_2.

The cellular uptake and localization in 4T1 cells

The cellular uptake and localization were carried out using confocal laser scanning. In brief, 4T1 cells were seeded in 2 mL culture medium at a density of 2*10^5 cells and grown for 24 h at 37 °C. The culture medium was removed, and the cells were treated with UCNP@ZIF+TPP+PA at another 1 h and 4 h at 37 °C respectively. The culture medium was removed, and the cells were washed with PBS for three times and were treated with 4', 6-diamidino-2-phenylindole (DAPI) for 10 min. After that, the cells

were washed with PBS for three times again. The cellular uptake and localization were evaluated by detecting the fluorescence of DAPI and TPP (λ_{ex} = 405 nm, λ_{em} = 435 nm and λ_{ex} = 640 nm, λ_{em} = 720 nm) with confocal laser scanning microscopy (CLSM).

Intracellular detection of the change of pH in 4T1 cells

The intracellular pH change was detected by BCECF-AM, which was a commonly used fluorescent probe for detecting intracellular pH. 4T1 cells were seeded in 2 mL culture medium at a density of 2*10^5 cells and grown for 24 h at 37 °C. The culture medium was removed, and the cells were treated with UCNP@ZIF+PA or UCNP@ZIF+TPP+PA. After 12 h, the cells were cultured with BCECF-AM for 30 min at 37 °C. And then the cells were washed with PBS for three times, and were illuminated by 980 nm laser for 5 min at a power of 1.4 W/cm^2. After that, the level of intracellular ROS and localization were evaluated by confocal laser scanning microscopic (CLSM) (λ_{ex} = 488 nm, λ_{em} = 535 nm).

Intracellular detection of ROS generation in 4T1 cells

The level of intracellular ROS and localization were evaluated by detecting the fluorescence of DCFH-DA (λ_{ex} = 488 nm, λ_{em} = 525 nm). In brief, 4T1 cells were seeded in 2 mL culture medium at a density of 2*10^5 cells and grown for 24 h at 37 °C. The culture medium was removed, and the cells were treated with UCNP@ZIF+PA, UCNP@ZIF+TPP or UCNP@ZIF+TPP+PA. After 12 h, the cells were cultured with DCFH-DA for 30 min at 37 °C. And then the cells were washed with PBS for three times, and were illuminated by 980 nm laser for 5 min at a power of 1.4 W/cm^2. After that, the level of intracellular ROS and localization were evaluated by CLSM.

In vitro toxicity assay against 4T1 cells

4T1 cells were seeded into 96-well microplates at the density of 5*10^3 per well and incubated for 24 h at 37 °C. Then the culture medium was replaced by fresh medium containing different concentrations of UCNP@ZIF+TPP, UCNP@ZIF+PA or UCNP@ZIF+TPP+PA (0, 0.1.9, 3.9, 7.8, 15.6 31.2 and 62.5 μg mL^{-1}). After further incubation for 24 h, the culture media were replaced by FBS-free medium containing 0.6 mg mL^{-1} 3-[4,5-dimethylthiazol-2-yl-]-2,5-diphenyltetrazolium bromide (MTT) and incubated for 4 h. Finally, the MTT solution was replaced by 100 μL of dimethyl sulfoxide (DMSO). By using a microplate reader 5 (Bio-TekELx800, USA), cell proliferation was measured by comparing the absorbance at λ = 490 nm to the control. To verify the phototoxicity of UCNP@ZIF+TPP, UCNP@ZIF+PA or UCNP@ZIF+TPP+PA, 4T1 cells were irradiated with a 980 nm laser (1.4 W/cm^2) for 5 min and incubated for another 24 h before the MTT assay.

***In vivo* toxicity assay.**

All animal experiments operations were performed in accordance with the protocols approved by Institutional Animal Care and Use Committee (IACUC) and the care regulations approved by the administrative committee of laboratory animals of East China Normal University. 10 week female Kun mice (~40 g) were obtained from Shanghai Laboratory Animal Center, Chinese Academy of Sciences. Fifteen mice were randomly divided into three groups: control, 3 days treated and 30 days treated mice(n = 5 for each group). The experimental mice were intravenously injected with UCNP@ZIF+TPP+PA (in 150 μL PBS) with same volume of saline as the control. At the time points of 3 days and 30 days after the administration, mice were sacrificed to collect their major visceral organs (heart, liver, spleen, lung and kidney) in a 10% formalin solution for histopathology analysis using a typical hematoxylin and eosin (H&E) staining assay. Their body weight was measured every three days (n = 5 for each group).

***In vivo* evaluation of PDT treatment**

4T1 xenografted tumors were generated in female Balb/c nude mice at 7 weeks of age (~18 g, Shanghai Laboratory Animal Center, Chinese Academy of Sciences) by subcutaneously (s.c.) injecting 1×10^6 4T1 cells suspended in 120 μL of PBS into the right leg. Once the tumors reached a volume of 70-100 mm^3 (7 days after tumor inoculation), mice were randomly divided into 5 groups (n = 5 for each group) and were intratumorally injected with 20 μL of the following solutions (1: PBS, 2:UCNP@ZIF+TPP+PA, 3: UCNP@ZIF+PA +light, 4: UCNP@ZIF+TPP +light, 5: UCNP@ZIF+TPP+PA +light). Tumors were measured every two days with a digital caliper, and their volumes (V) were calculated from the following equation: $V = L \times W^2/2$, then normalized to their initial volume (V_0) to obtain the relative tumor volume (V/V_0). The pathological tissue sections of tumors were collected in 48 h post-treatment for hematoxylin and eosin (H&E) staining assay. Mice were euthanized once tumor volume reached 1000 mm^3.

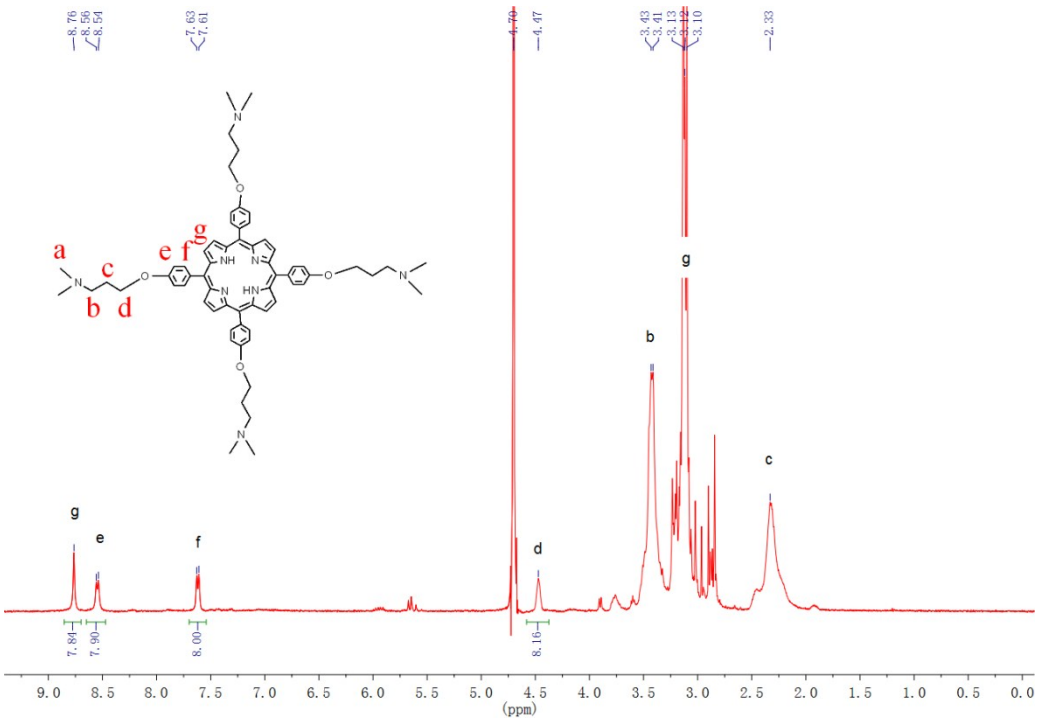


Figure S1. ^{1}H NMR spectrum of TPP in D_2O

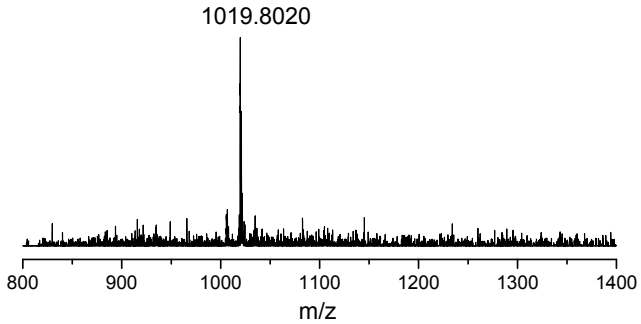


Figure S2. Mass spectrum of TPP

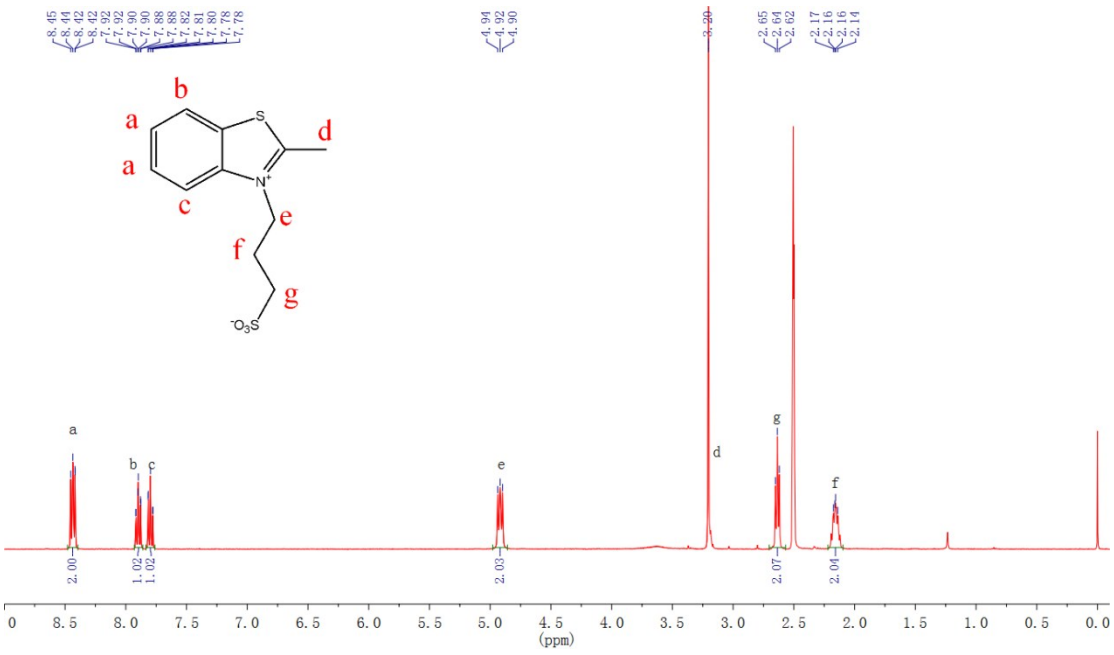


Figure S3. [1]H NMR spectrum of the intermediate in DMSO-d_6

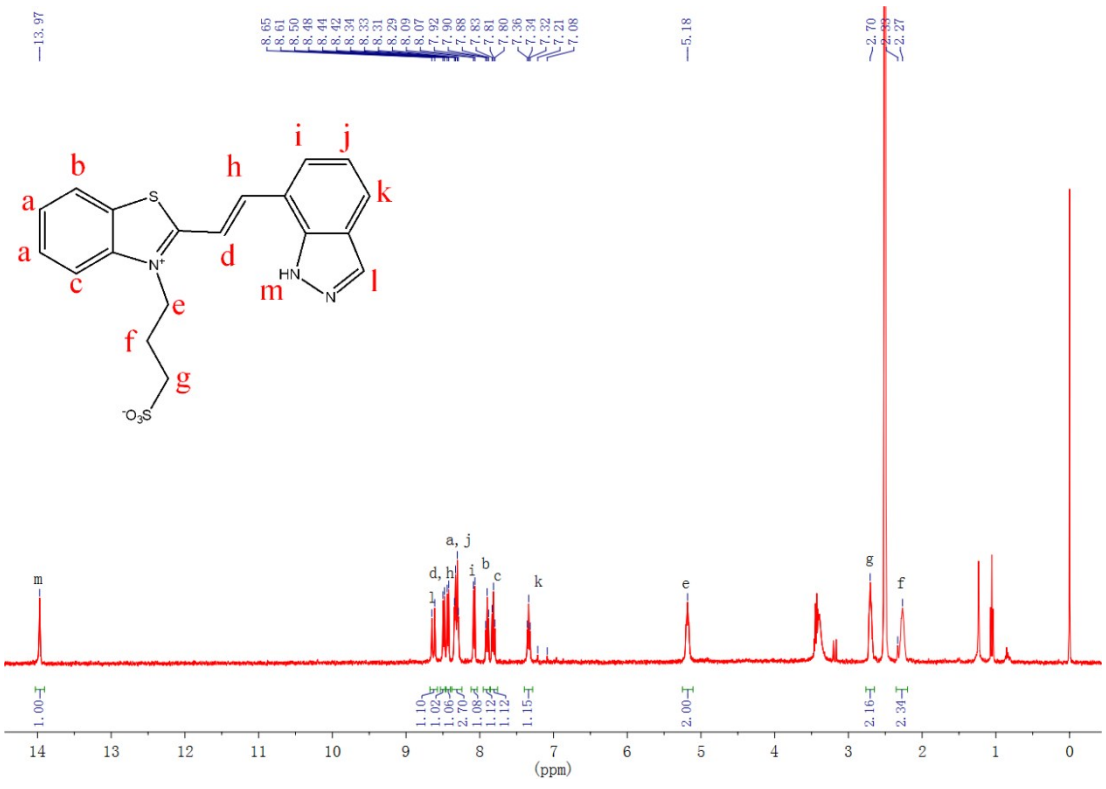


Figure S4. [1]H NMR spectrum of PA in DMSO-d_6

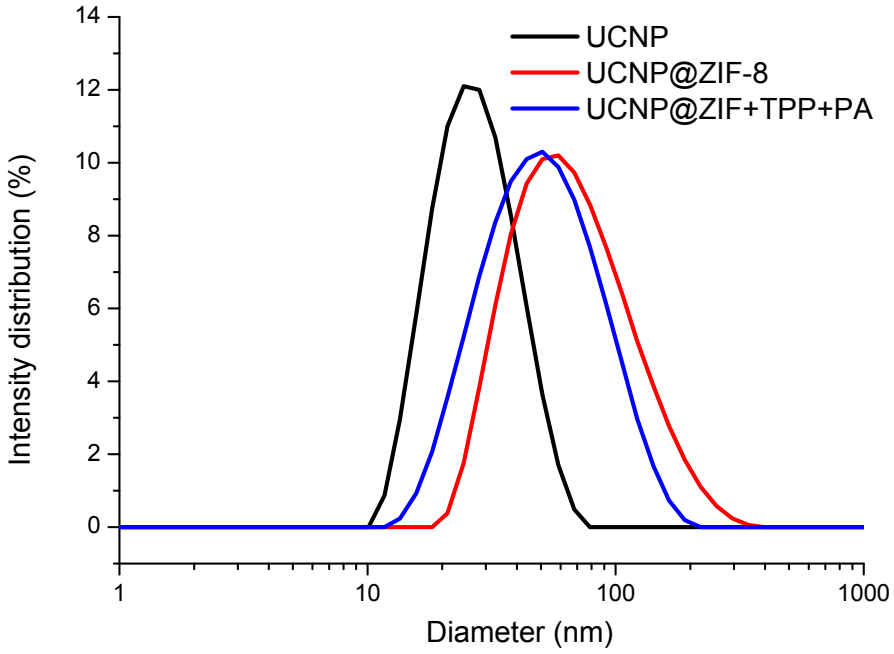


Figure S5. Size distributions of UCNP@ZIF+TPP+PA

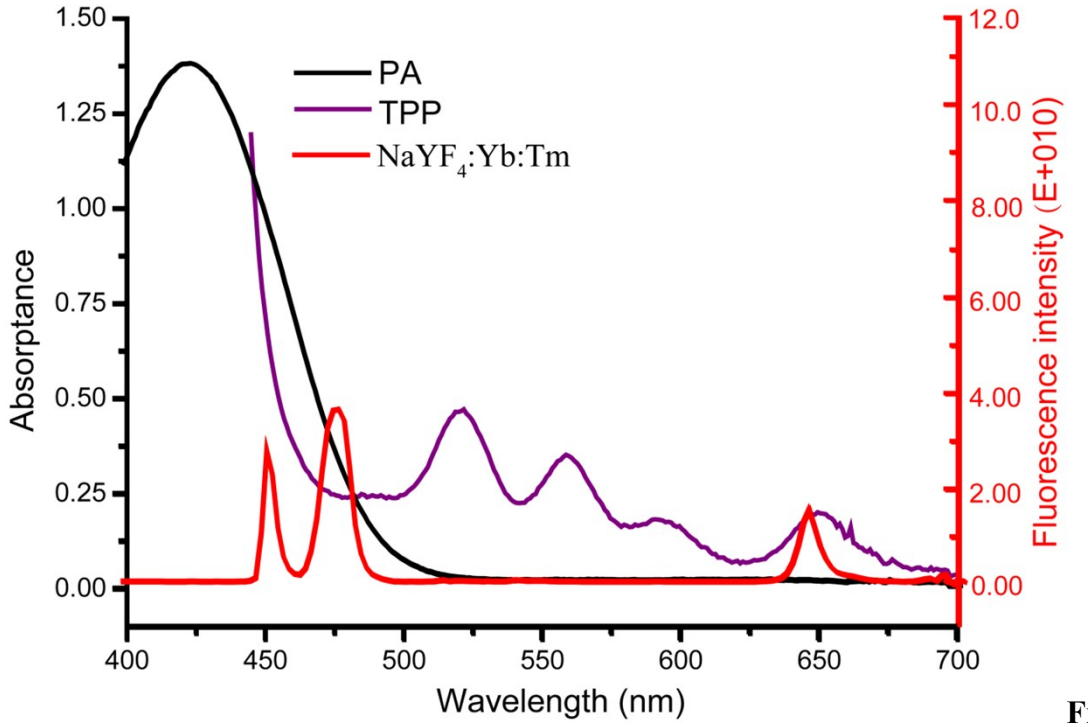


Figure S6. UV-Vis absorptance spectra of TPP and PA and the emission spectrum of UCNP

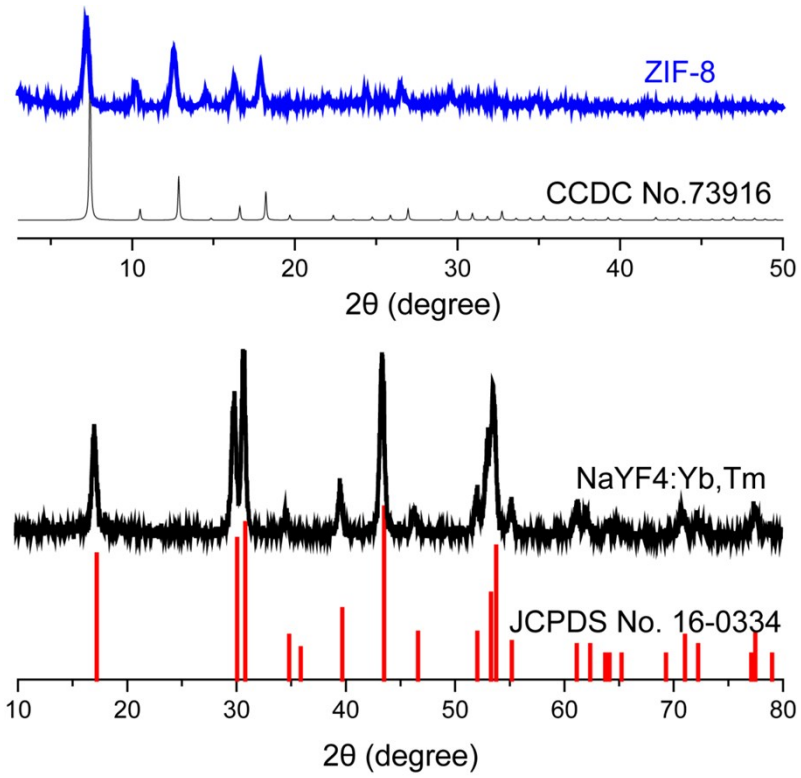


Figure S7. XRD of $NaYF_4$:Yb/Tm and ZIF-8

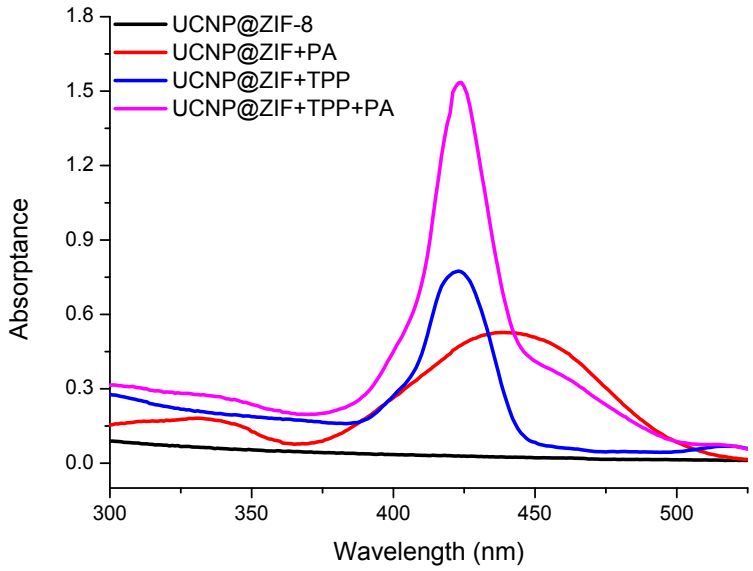


Figure S8. UV-Vis absorptance spectra of UCNP@ZIF-8 loading TPP or PA

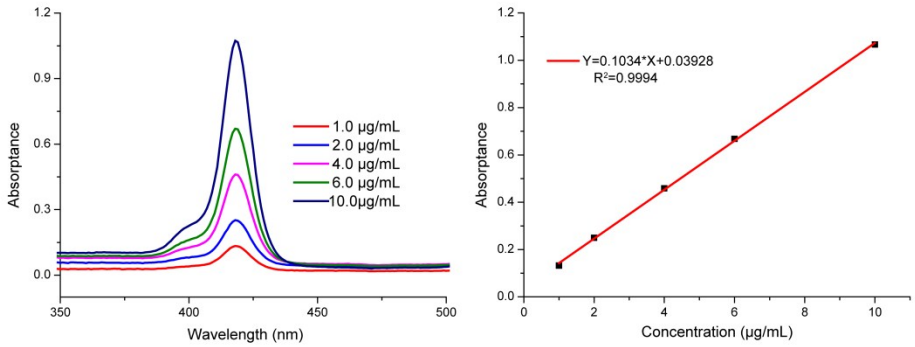


Figure S9. UV-Vis absorptance spectra of TPP

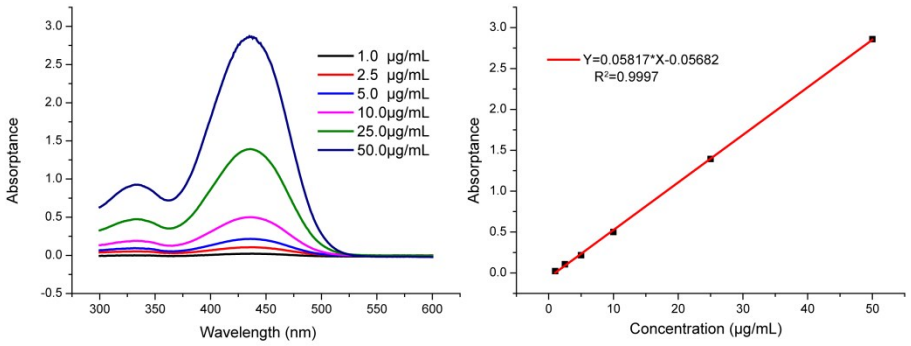


Figure S10. UV-Vis absorptance spectra of PA

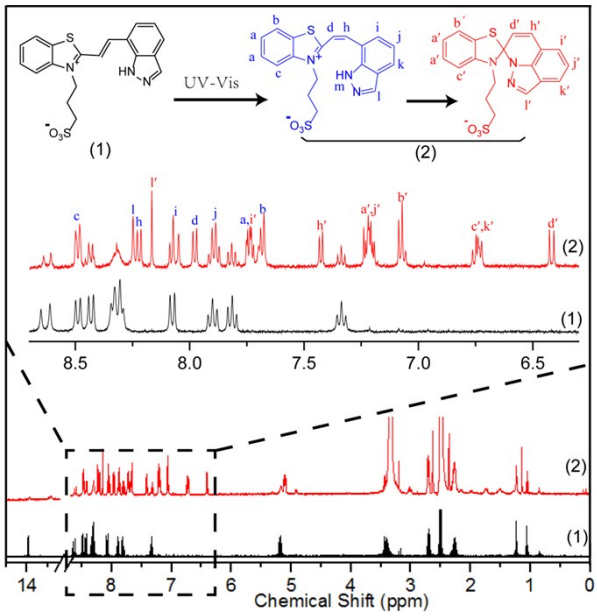


Figure S11. ^{1}H NMR of PA after irradiation (1:before light irradiation; 2: after light irradiation).

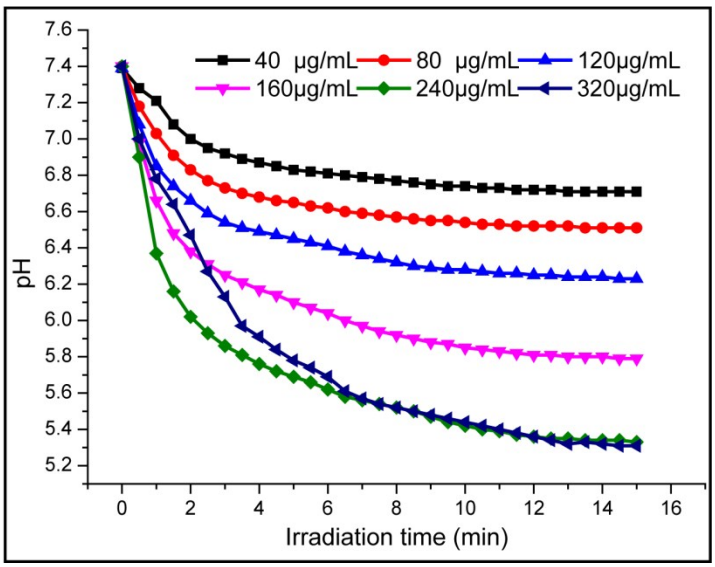


Figure S12. The pH changes of different concentrations of PA solution with light irradiation time.

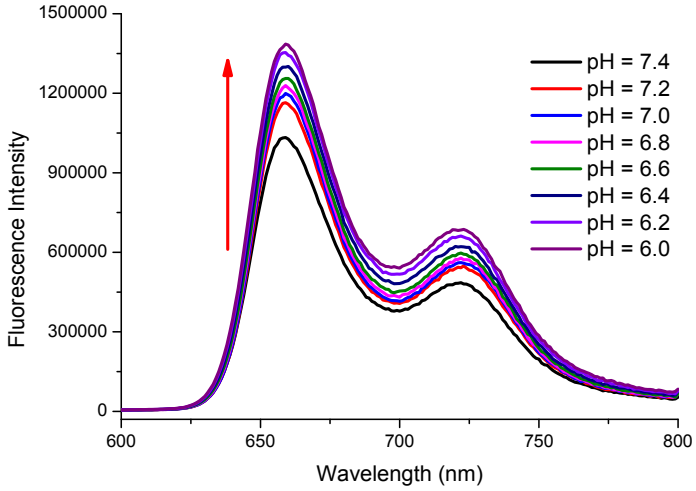


Figure S13. Fluorescence spectra of TPP at different pH

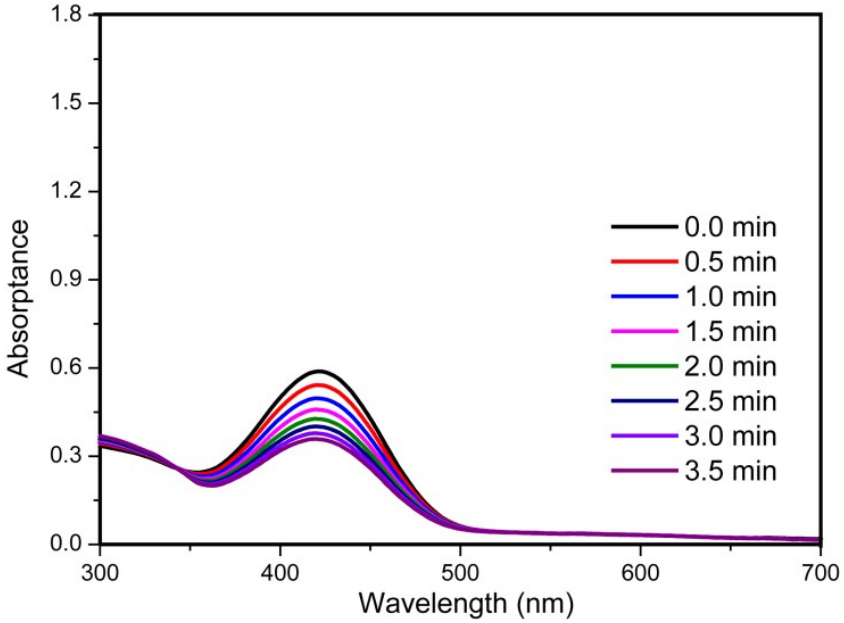


Figure S14. UV-Vis absorptance of UCNP@ZIF+PA with light irradiation.

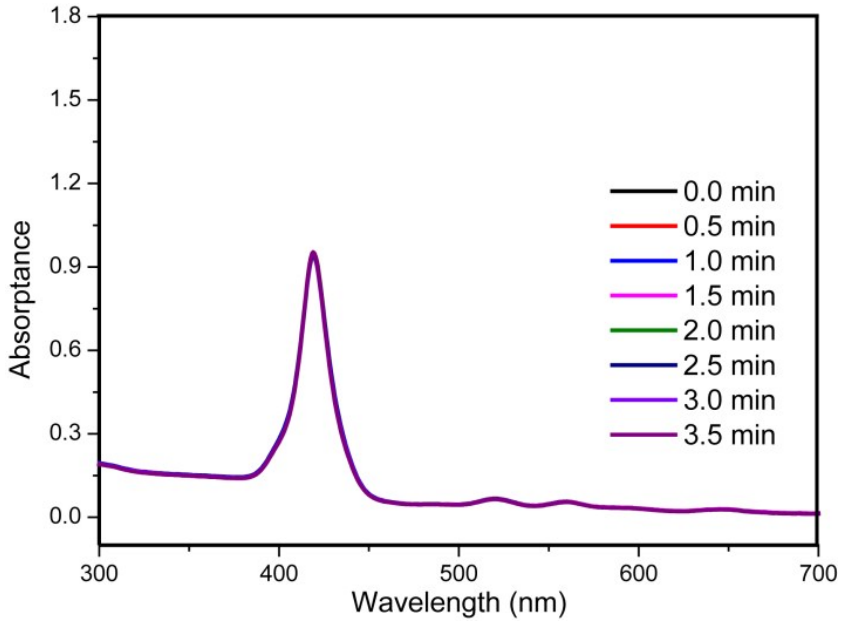


Figure S15. UV-Vis absorptance of UCNP@ZIF+TPP with light irradiation.

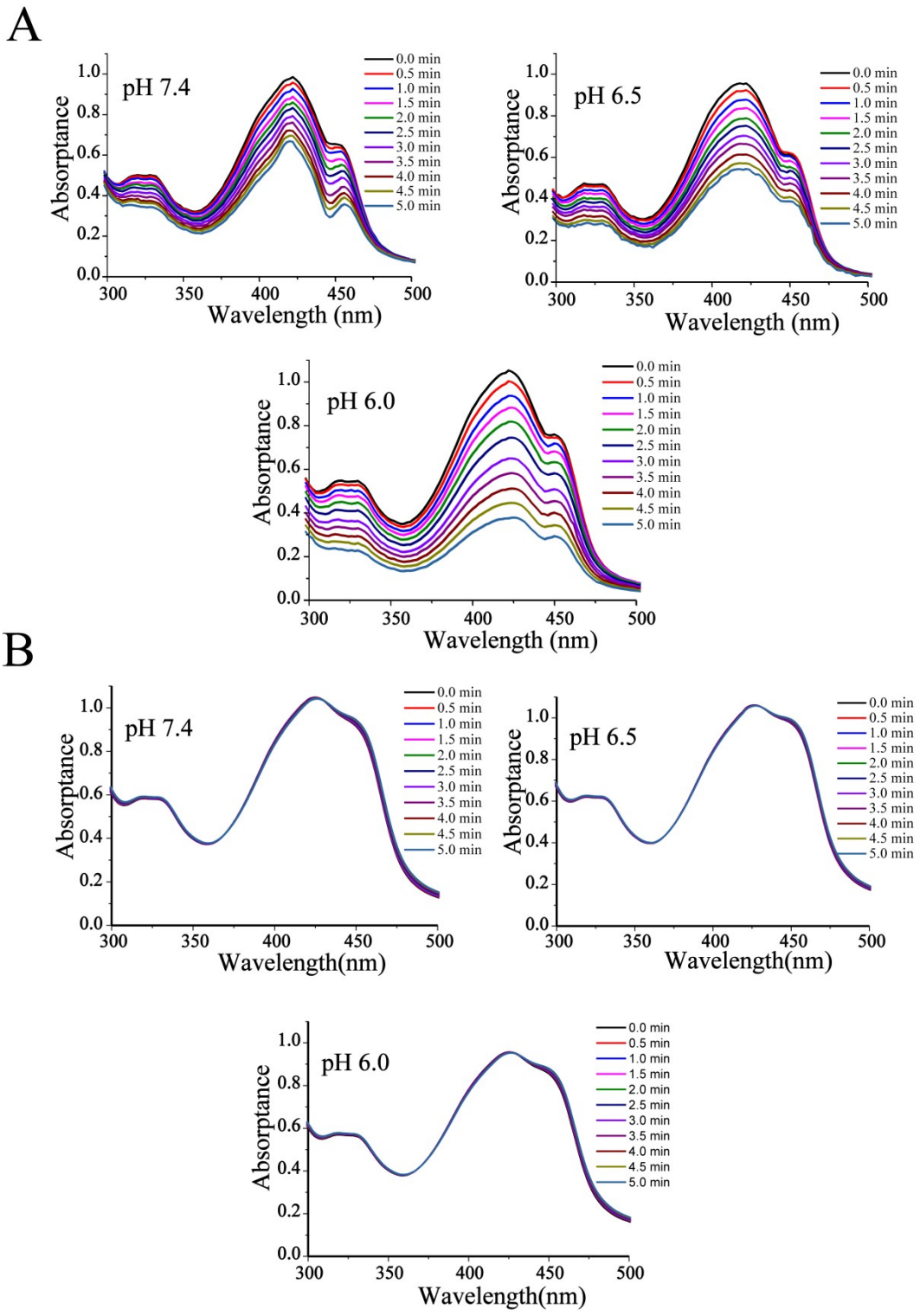


Figure S16. UV-Vis absorptance spectra of DPBF with light irradiation (A: containing nanomaterials, B:not containing nanomaterials)

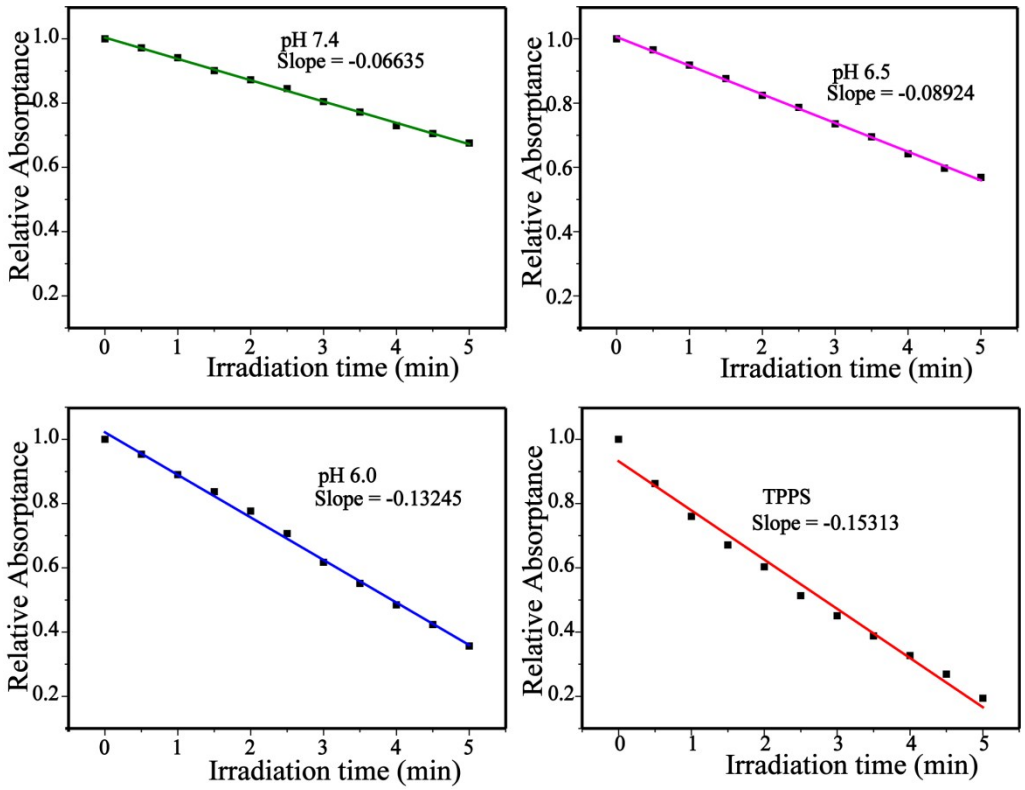


Figure S17. The degradation rate of DPBF with nanomaterials and standard TPPS.

Table S1. Singlet oxygen quantum yield of TPP in nanomaterials at different pH.

Samples	Φ_{Δ}^{a}
pH = 7.4	0.308
pH = 6.5	0.414
pH = 6.0	0.613

[a] Singlet oxygen quantum yield of TPP in nanomaterials was carried out according to Eqn (1)

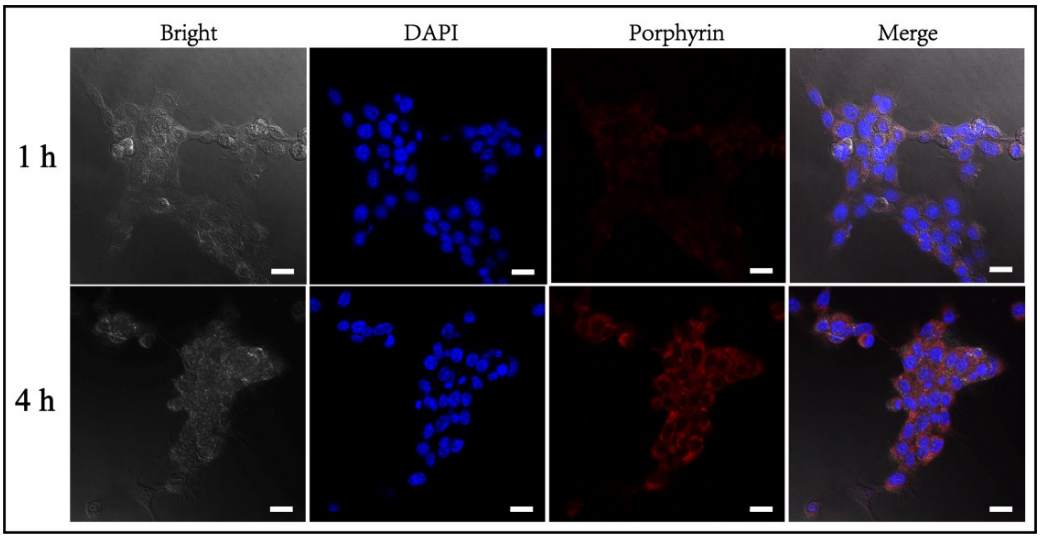


Figure S18. Cellular uptake of UCNP@ZIF+TPP+PA for 1 h and 4 h (scale bars, 20 μm)

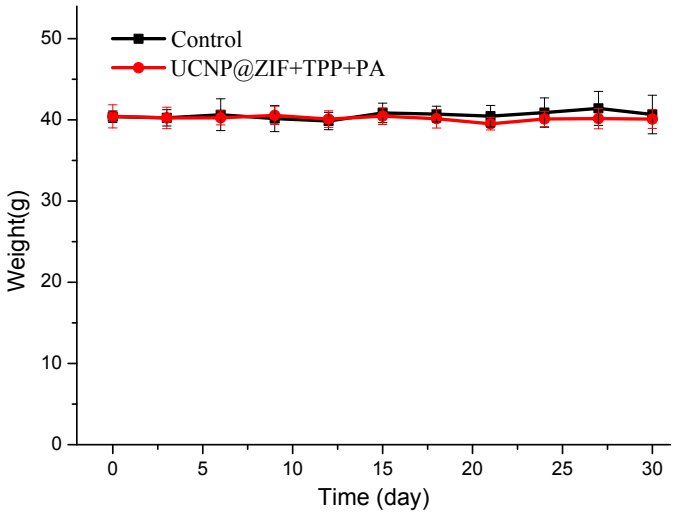


Figure S19 Body weights of K.M. mice in different groups.

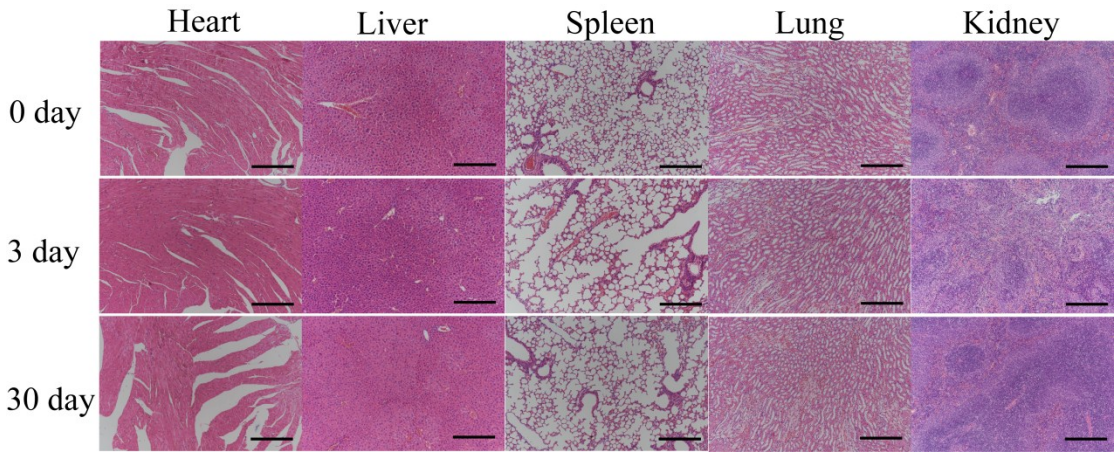


Figure S20. Pathological H&E stained images of tissue sections from heart, liver, spleen, lung and kidney of the mice treated with UCNP@ZIF+TPP+PA. The tissue sections were harvested in 3 and 30 days after the intravenous injection of a 10 mg kg-1 dosage, showing no significant change of H&E tissue sections. Scale bars, 200 μm.

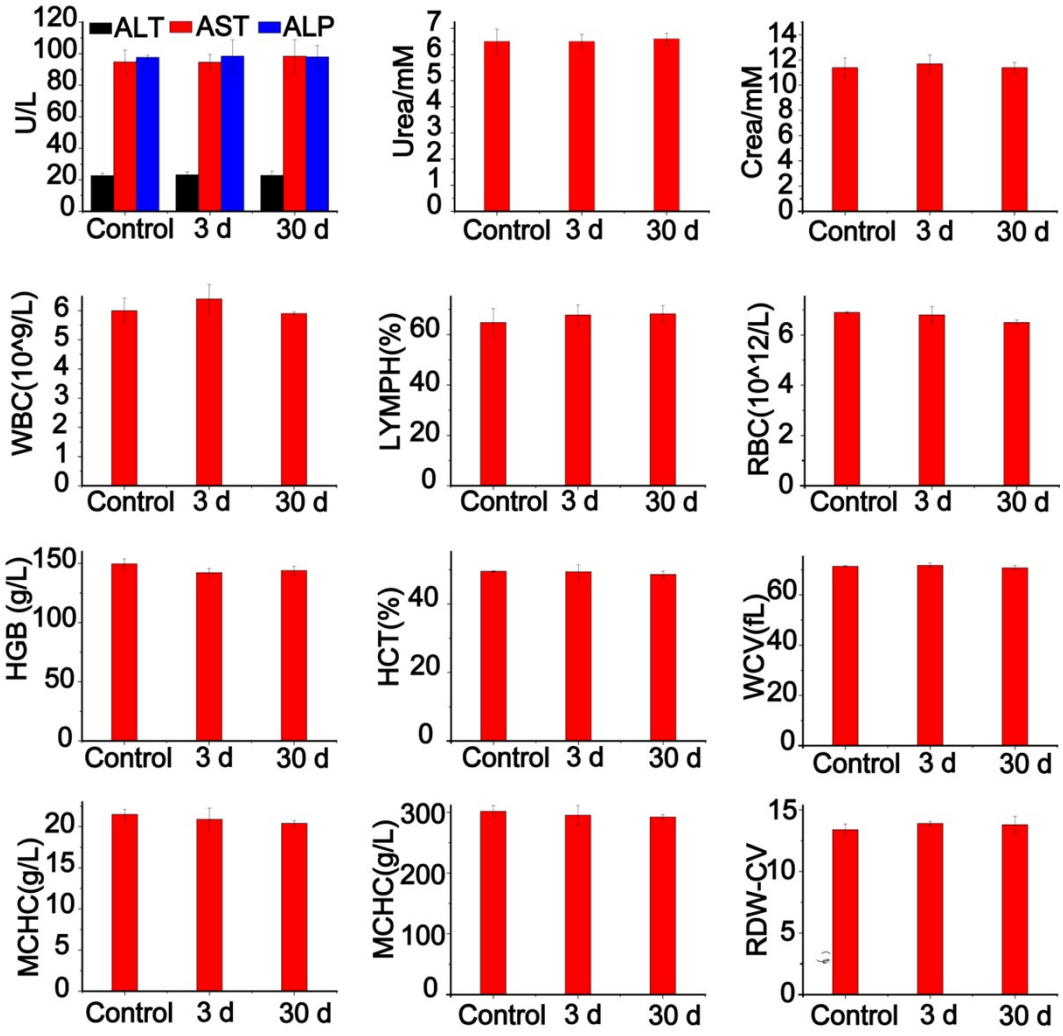


Figure S21. Toxicity studies of UCNP@ZIF+TPP+PA. a) Blood biochemical parameters and b) hematology data obtained from the mice after the intravenous injection of UCNP@ZIF+TPP+PA (10 mg kg-1, 150 μL, n = 3, mean ± s.d.) in 3 and 30 days with 150 μL saline injection as control.

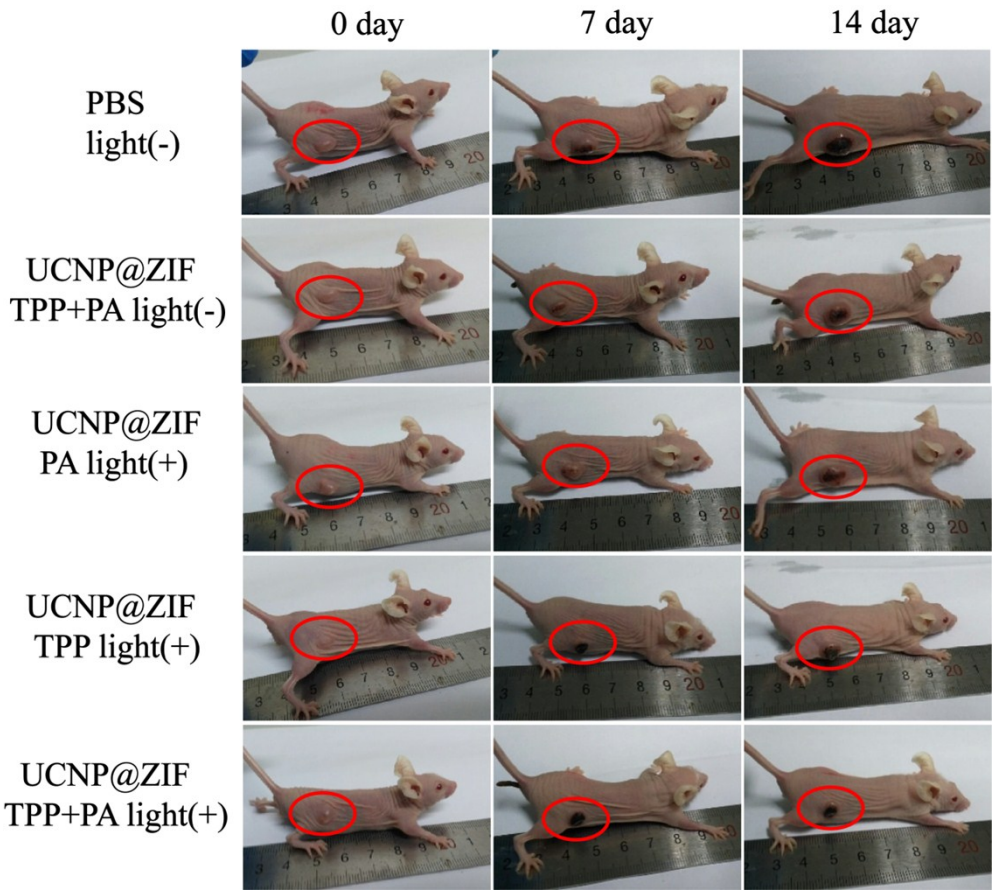


Figure S22. Representative photographs of 4T1 tumor-xenografted Balb/c mice in different times after treatment.

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