Supporting Information

$NaYbF_4$ nanoparticles as near infrared light excited inorganic photosensitizers for deep penetration photodynamic therapy

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1. Experimental Section

1.1 Synthesis of NPs

The method to synthesize $NaYbF_4$NPs has been previously descried.[1] A mixture comprising 1 mmol $YbCl_3$, 6 mL oleic acid and 15 mL 1-octadecene was poured into a 50-mL round bottom flask. N_2 was used as a shielding gas. The mixture was stirred vigorously for 1 h at 160 °C to produce a clear solution. The solution was then cooled down to 50 °C, and NaOH (0.1 g) and NH_4F (0.148 g) in 10 mL methanol were added, the new mixture was stirred for 30 min. The methanol and water were evaporated at 100 °C under a strong stream of N_2, and the solution was heated to 300 °C for 60 min with constant stirring. Finally, the mixture was cooled down to room temperature and precipitated by ethanol. The synthesized NPs were washed three times with a mixture of ethanol and cyclohexane to remove impurities. The NPs were then collected by centrifugation. After purification, the NPs were dispersed in cyclohexane.

1.2 Hydrophilic modification of $NaYbF_4$ NPs

We use ligand attraction to get hydrophilic $NaYbF_4$ NPs.[2] TWEEN 80 (100 μL) was added to a 25-mL flask containing 5 mg of $NaYbF_4$ NPs and 2 mL of $CHCl_3$, and the solution was stirred for 1 h at room temperature. Deionized water (10 mL) was then poured into the flask. The mixed solution was stirred and kept at 70 °C for 3 h. During this period, the $CHCl_3$ was evaporated and the NPs were gradually transferred to the hydrophilic system. The TWEEN-coated $NaYbF_4$ NPs were obtained by centrifugation.

1.3 Determination of ROS generation

The time-dependent concentration of DPBF during ROS generation can be described as follows:[3]

$$\frac{\mathrm{d}[\mathrm{DPBF}]}{\mathrm{d}t} = -\sum_{i=1}^{n} k_i[\mathrm{ROS}_i][\mathrm{DPBF}] = -k[\mathrm{DPBF}], \quad (1)$$

where [DPBF] and [ROS_i] are the concentrations of DPBF and ROS, respectively; k_i is the rate constant of chemical reaction between O_2 and DPBF; and k is the consumption rate of DPBF or the reciprocal of the time constant in the decrease of DPBF.

The concentration of DPBF can be determined by:

$$[\mathrm{DPBF}] = [\mathrm{DPBF}_0]e^{-kt}. \tag{2}$$

According to the Beer–Lambert law, the transmission intensity can be described as:

$$I_t = I_0 \exp(\sigma[\mathrm{DPBF}]L), \tag{3}$$

Where σ is the absorption cross section of PS, and L is the optical path. Here, $A = \sigma[\mathrm{DPBF}]L \propto [\mathrm{DPBF}]$. A can be used to estimate the change of DPBF concentration, which is given by

$$A = \ln(I_0 / I_t) \tag{4}$$

1.4 Absorption spectra of DPBF

DPBF solution (0.02 μmol/mL) without and with $NaYbF_4$ (20 μmol/mL) were exposed to a 980 nm laser with the power of 1.3 W. The transmission spectra of DPBF in the solutions were measured every ten minutes. A deuterium lamp served as the light source of transmission spectra, and a miniature QE65000 fiber optic spectrometer (Ocean Optics, USA) was used to detect the transmission from UV to visible light. The absorption spectrum of DPBF can be obtained according to the Beer–Lambert law.

1.5 Luminescence spectrum of Yb^{3+}

A focused 980 nm laser with a power of 1.3 W was used to irradiate the solution containing $NaYbF_4$ NPs. The fluorescence of Yb^{3+} was collected by a lens-coupled monochromator (Zolix Instruments Co. Ltd, Beijing). The fluorescence signals from Yb^{3+} were detected using an NIR-sensitive PbS detector with a lock-in preamplifier (Stanford Research System Model SR830 DSP) at the chopping rate of 3000 rps.

1.6 Oxygen sensitivity of the luminescence lifetimes of Yb^{3+}

The time-resolved spectra of $^2F_{5/2} \rightarrow {}^2F_{7/2}$ transition of Yb^{3+} in low and high oxygen concentration solutions were measured under the excitation of a 980 nm light in the pulse mode. Low and high oxygen concentrations were acquired by loading Ar and O_2 into solution separately and measured by an oxygen detector (AMT07, USA). For the detection, NIR sensitive detector (Zolix Instrument D InGaAs 2600-TE) was employed for the

measurements of temporal behavior of Yb^{3+} emissions. The decay profiles were recorded by a Tektronix TDS 5052 digital oscilloscope.

1.7 Cell line and cellular uptake

PC9 cells were purchased from the American Type Culture Collection (ATCC) and grown in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% antibiotics. The cells were maintained in 5% CO_2 at 37 °C in an incubator. The breast cancer cell line MDA-MB-231-luciferase cells were generously provided by Prof. Luo of East China Normal University, and were grown in Dulbecco's modified Eagle's medium (Gibco, USA) supplemented with 10% FBS (Gibco) and 1% antibiotics.

To investigate the intracellular accumulation of NPs in human lung adenocarcinoma cell line PC9, $NaYbF_4$ NPs were replaced by $NaYF_4$:Yb,Er NPs for the ease of detection. The intracellular accumulation of $NaYF_4$:Yb,Er NPs was investigated by confocal laser scanning microscopy (CLSM). $NaYF_4$:Yb,Er NPs were added for metabolism analyses to investigate cellular uptake by PC9 cells. Cells were observed following incubation with 200 μg/mL $NaYF_4$:Yb,Er in RPMI-1640 medium for 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 h at 37 °C. The PC9 cells were washed with cold phosphate-buffered saline (PBS) three times and fixed on a glass-bottom dish. The cellular uptake of $NaYF_4$:Yb,Er NPs was investigated using Er^{3+} emission at 540 nm and 654 nm, which was detected by CLSM (Leica TCS SP5; Leica Microsystems Co. Ltd., Solms, Germany). Different dosages of $NaYF_4$:Yb,Er NPs were used to quantify uptake by the PC9 cells. The cells were treated with $NaYF_4$:Yb,Er NPs at concentrations of 10, 40, 100, 200, 400, 800, and 1600 μg/mL, and CLSM was used to observe the emission of Er^{3+}.

1.8 Cytotoxicity and NIR exposure effect on the cells

Cell viability was assessed by a CCK-8 assay. Cells were seeded in 96-well plates at 2.5×10^3 cells per well and incubated in RPMI-1640 supplemented with 10% FBS for 24 h. The cells were then treated with NaYbF4 (0, 10, 20, 40, 80, 100, 200, 400, 800, 1600 μg/mL) for 24 h. Next, the drug (NPs)-containing medium was replaced with 100 μL FBS-free

RPMI-1640 medium and 10 μL CCK-8 per well, the cells were incubated in a CO_2 incubator at 37 °C for 0.5 h, then the absorbance (450 nm) was measured and analyzed.

To investigate the effect of the laser power on cell viability, a 980 nm laser was used to irradiate the cells in 96-well plates with different power densities (0, 133, 347, 575, 800, 1026, 1261 mW/cm^2) for different irradiation time (10 min, 30 min, 60 min). After the cells were incubated in a CO_2 incubator for 24 h, cell viability was measured by the CCK-8 assay.

1.9 *In vitro* PDT

For the in vitro PDT tests, PC9 cells seeded in a 96-well plate were incubated with $NaYbF_4$ NPs at concentrations of 0, 10, 20, 40, 100, 200 μg/mL for 2.5 h, then irradiated with a 980 nm NIR laser with an output power density of 575 mW/cm^2 for 30 min and 1 h. After the experiments, cells were maintained in 5% CO_2 atmosphere at 37 °C in an incubator for 24 h. Then FBS-free RPMI-1640 medium and 10 μL CCK-8 were added to each well and the cells were incubated at 37 °C for 0.5 h. Absorbance was measured at 450 nm.

1.10 *In vivo* PDT

Six-week-old male mice weighing 18.8±1.6 g were used in our study. PC9 cells (2×10^7) suspended in 100 μL PBS were implanted subcutaneously into the right axilla of the mice. One week later, tumors had developed and the subcutaneous tumor mouse model was established and the subcutaneous tumor growth in each mouse was carefully monitored. The diameters of tumors were measured using a Vernier caliper.

When the tumors of the mice reached a diameter of ~5–6 mm and a whole body weight of ~20.3±2.1 g, the mice were randomly divided into four groups with three mice per group: the BC (blank control, PBS only) group; the NO ($NaYbF_4$ only) group; the LO (laser only) group; and the PDT ($NaYbF_4$ and laser) group. The hydrated $NaYbF_4$ NPs in PBS was sonicated to thoroughly disperse the NPs. The mice in NO and PDT groups were administered a single dose of 20 mg/cm^3 via local tumor injection. After 30 min, the tumors were exposed to a 980 nm laser at a power density of 366 mW/cm^2 for 2 h. The following detection indices were introduced to analyze the therapeutic effects in vivo: the

shortest diameter (d_a) and the longest diameter (d_b) of tumors were measured, and the volume (V) of the tumor was estimated using the formula $V = d_a^2 \times d_b/2$. Ten days after treatments, the tumors were measured again. The normalized tumor volume was calculated as the ratio of the differential volume over the volume before the treatment.

In order to see the effect of PDT in deep tissues, we developed the bone metastasis model by injecting MDA-MB-231-Luc cells into the tibias from the tibial plateau of nude mice. Seven days after the injection, d-luciferin potassium salt was administrated via intraperitoneal injection to monitor the tumor growth. The overexpressed luciferase in MDA-MB-231-Luc tumors can catalyze the oxidation of luciferin to emit luminescence, which can be monitored by an *in vivo* imaging system. The treatments were performed on luminescence-positive mice. A single dose of $NaYbF_4$ NPs in PBS (0.15 mg/g) was injected into the bone matrix of mice in the NO and PDT groups. After 4 h, the legs bearing tumors were exposed to 980-nm laser radiation at a power density of 255 mW/cm^2 for 2 h. Seven days after the treatment, the luminescence images of the animals were taken again. Luminescence intensity was normalized and the fold change in luminescence intensity before and after the treatment was obtained.[4]

2. Characterization

We used simple methods to observe the dispersion stability of hydrophilic nanoparticles. One method is by water solution photograph of hydrophilic nanoparticles. The photograph of $NaYbF_4$ water solution shows that there is no precipitate and aggregation (Fig. S1a), which indicates the hydrophilic $NaYbF_4$ nanoparticles can steadily exist in water. The other method is based on fluorescence image of hydrophilic nanoparticles. When nanoparticles aggregate, size of nanoparticles would increase, leading to a severe fluorescence scattering. Conversely, if there is no aggregation, fluorescence has little scattering and spread along a straight line. To observe fluorescence images, $NaYF_4$:Yb,Er nanoparticles were employed. Hydrophilic modification of $NaYF_4$:Yb,Er nanoparticles uses the same method as those for $NaYbF_4$ nanoparticles. The photograph of $NaYF_4$:Yb,Er water solution and corresponding fluorescence images in dark and bright field are

displayed in Fig. S1b, c, d, respectively. The fluorescence imaging did not show any significant amount of scattering, which further confirmed the dispersion stability of the nanoparticles in water. In fact, $NaYbF_4$ nanoparticles can still show dispersion stability in water after 3days.

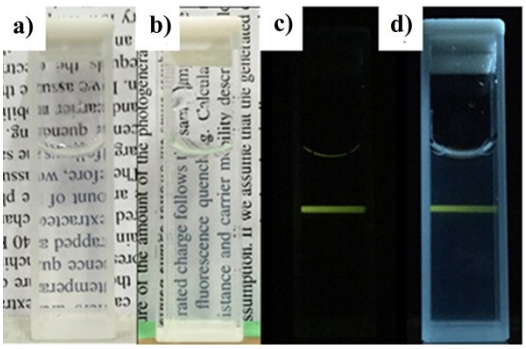

Fig. S1 a) The photograph of $NaYbF_4$ nanocrystals in water; b) the photograph of $NaYF_4$:Yb^{3+},Er^{3+} nanoparticles in water; c) and d) the images of upconversion fluorescence of $NaYF_4$:Yb^{3+},Er^{3+} nanoparticles in dark and bright field.

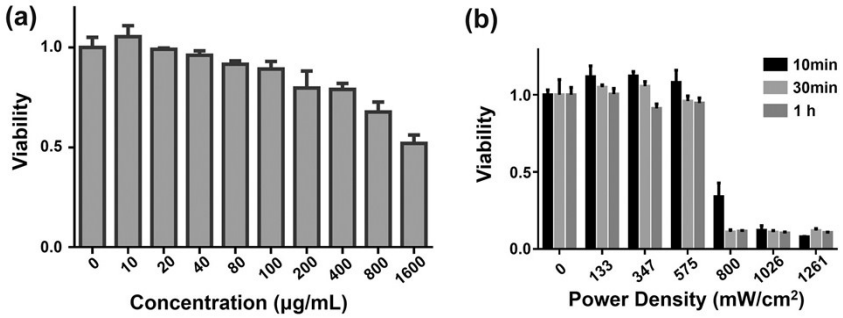

Fig. S2 Viability of PC-9 cells (a) incubated with various doses of nano-particles for 24 h. (b) treated with different power density of 980 nm irradiation without nanoparticle.

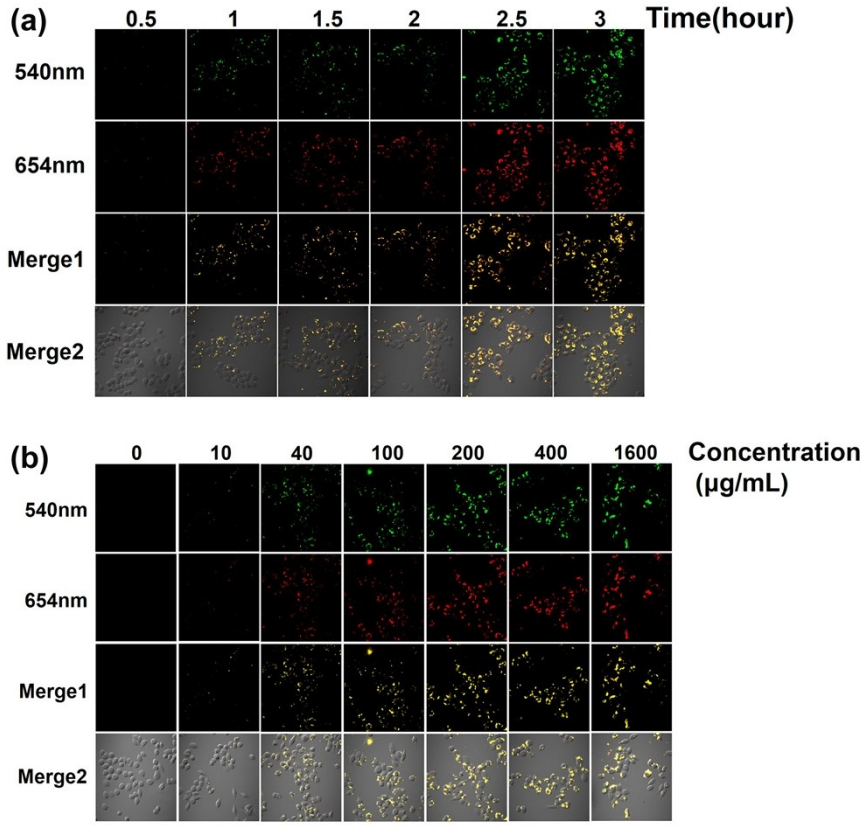

Fig. S3 Confocal images of PC-9 cells (a) incubated with fixed concentration of 400μg/mL $NaYF_4$:Yb,Er for various incubating time. (b) for various nanoparticle concentrations with the same incubating time of 2.5 h.

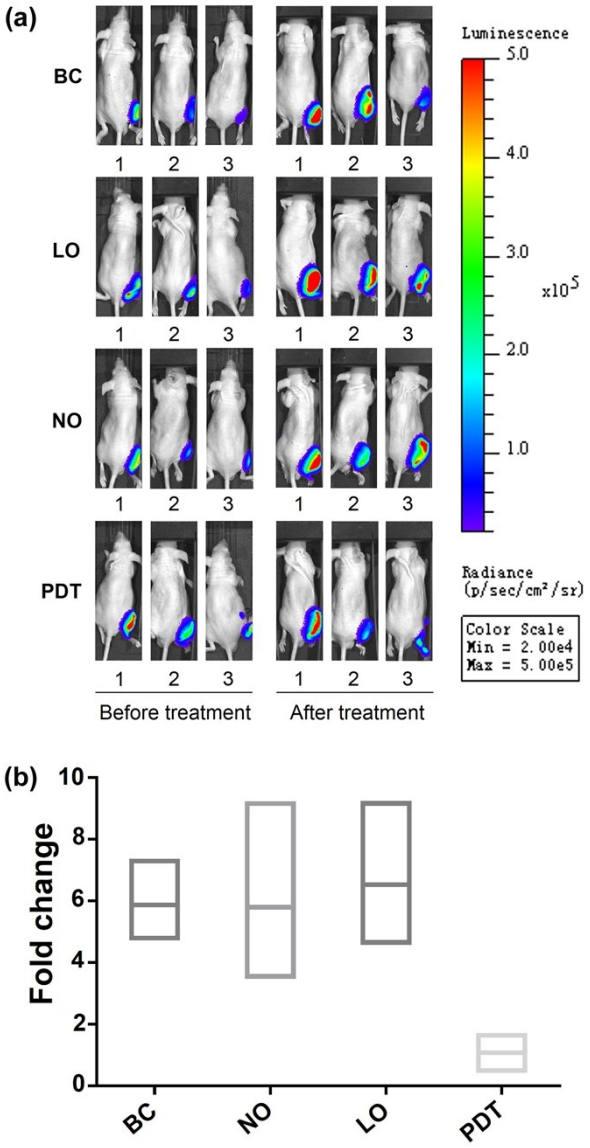

Fig. S4 (a) Luminescence photos of mice before and after different treatments. (b) Fold change of luminescence intensity values before and after the treatment.

Additional references:

1 Z. Q. Li and Y. Zhang, *Nanotechnology*2008, **19**, 345606.

2 W. L. Ren, G. Tian, S. Jian, Z. J. Gu, L. J. Zhou, L. Yan, S .Jin, W. Y. Yin and Y. L. Zhao, *RSC Adv.* 2012,**2**, 7037-7041.

3 F. Wilkinson, W. P. Helman and A. B. Ross, *J. Phys. Chem. Ref. Data.*1993, **22**, 113-262.

4 C. P. Wang, X. P. Cai, J. S. Zhang, X. Y. Wang, Y. Wang, H. F. Ge, W. J. Yan, Q. Huang, J. R. Xiao and Q. Zhang, *Small* 2015, **17**, 2080-2086.