Supporting information

Intra-arterial infusion of PEGylated upconversion nanophosphors to improve the initial uptake by tumors *in vivo*†

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Experimental Section

Materials

Rare earth oxides Y_2O_3 (99.999%), Yb_2O_3 (99.999%) and Tm_2O_3 (99.98%) were purchased from Shanghai Yuelong New Materials Co. Ltd. Rare earth chlorides ($LnCl_3$, Ln: Y, Yb, Tm) were prepared by dissolving the corresponding metal oxide in 10% hydrochloric solution at elevated temperature and then evaporating the water completely. Tetraethyl orthosilicate (TEOS), absolute ethanol, aqueous ammonia (28 %wt), cyclohexane and hydrochloric solution were obtained from Sinopharm Chemical Reagent Co., China. Igepal CO-520, oleic acid (>90 %) and 1-octadecene were purchased from Sigma-Aldrich Co. Ltd. The mPEG-silane (2K Da) is bought from Creative PEGWorks Co. Ltd., USA. All the other chemical reagents were of analytical grade and were used directly without further purification. Deionized water (DI) was used throughout.

Characterization

The size and morphologies of nanoparticles were determined at 200 kV using a JEOL JEM-2010F low to high resolution transmission electron microscope (TEM). Samples were prepared by

placing a drop of dilute dispersion in cyclohexane or water on the surface of a copper grid. Powder X-ray diffraction (XRD) measurements were measured with a Bruker D4 X-ray diffractometer (Cu Kα radiation, λ = 0.15406 nm). Dynamic light scattering was carried out on an ALV-5000 spectrometer-goniometer equipped with an ALV/LSE-5004 light scattering electronic and multiple tau digital correlator and a JDS Uniphase He-Ne laser (632.8 nm) with an output power of 22 mW. The size distribution was measured at 25 ˚C with a detection angle of 90°. Fourier-transform infrared (FTIR) spectroscopy was measured using an IR Prestige-21 spectrometer (Shimadzu) from samples in KBr pellets. Upconversion luminescence (UCL) spectrum was measured with an Edinburgh LFS-920 fluorescence spectrometer, using an external 0~800 mW adjustable CW laser at 980 nm (Connet Fiber Optics, China), as the excitation source, instead of the Xenon source in the spectrophotometer.

Synthetic procedure

Synthesis of $NaYF_4$:Yb,Tm nanoparticles: Typically, YCl_3 (0.79 mM), $YbCl_3$ (0.20 mM), and $TmCl_3$ (0.01 mM) were added to a 100 mL 3-necked round bottom flask equipped with a magnetic stir bar. Oleic acid (7.4 g, 0.026 mol) and 1-octadecene (11.4 g, 0.045 mol) was added and the mixture was stirred and heated at 140 ˚C for 30 minutes to form a transparent solution. Ammonium fluoride (148 mg), sodium hydroxide (100 mg) were dissolved in methanol (5 mL) in a separate vial and then added dropwisely to the round bottom at room temperature. Methanol was evaporated off at 50 ˚C (2 h). Air and moisture were then removed from the reaction mixture by attaching a vacuum and then injecting N_2 gas into the round bottom for 3 times. The mixture was then heated to 300 ˚C (1 h). After cooling to room temperature, the mixture was centrifuged and washed by cyclohexane (10 mL) for 3 times. The as-prepared nanoparticles were dispersed in cyclohexane (20 mL) for

further silica coating.

Synthesis of $NaYF_4$:Yb,Tm@SiO_2-PEG nanocomposites: Igepal CO-520 (9 g), cyclohexane (100 mL) and $NaYF_4$:Yb,Tm-solution (8 mL) were added to a flask and sonificated (30 mins). Ammonia-solution (1000 μL) was added and the mixture was shaken at 25° C (160 rpm, 2 hr). Tetraethyl orthosilicate (200 μL) was then added and the mixture was put back in the shaker (160 RPM, 2 days). The mPEG-silane (26 mg) was dissolved in water (2 mL), and 2 μL of solution was then pipetted into the flask and shaken for another 2 h. To precipitate $NaYF_4$:Yb,Tm@SiO_2-PEG nanoparticles, acetone (50 mL) was added. The mixture was then centrifuged and washed by ethanol:water (1:1) for 1 time, and water for 2 times. The as prepared $NaYF_4$:Yb,Tm@SiO_2-PEG nanoparticles can be easily dispersed in ethanol or water.

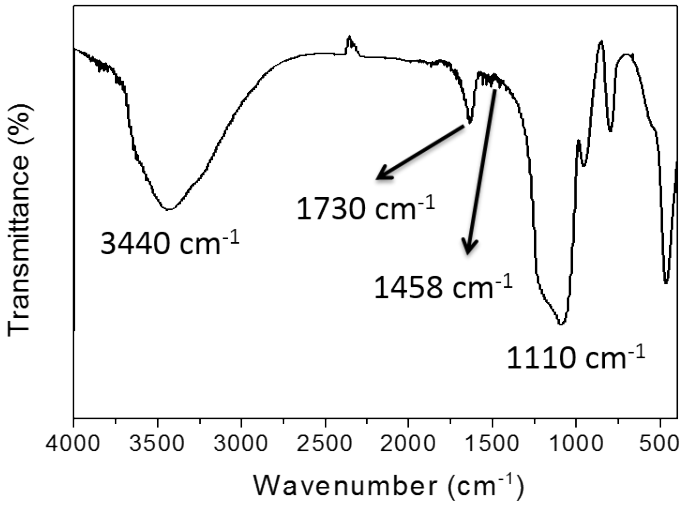

Figure S1. FTIR spectrum of PEG-UCNPs

Cell culture and cytotoxicity

A breast cancer cell line MCF-7 cell was provided by Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS, China). The MCF-7 cells were grown in RPMI 1640 (Roswell Park Memorial Institute's medium) supplemented with 10% FBS (fetal bovine

serum) at 37 °C and 5% CO_2. *In vitro* cytotoxicity was measured by performing methyl thiazolyl tetrazolium (MTT) assays on the MCF-7 cells. Cells were seeded into a 96-well cell culture plate at 5 × 10^4/well, under 100% humidity, and were cultured at 37 °C and 5% CO_2 for 24 h; different concentrations of PEG-UCNPs (0, 100, 200, 300, 400 and 500 mg/mL, diluted in RPMI 1640) were then added to the wells. The cells were subsequently incubated for 4 h or 24 h at 37 °C under 5% CO_2. Thereafter, MTT (10 mL; 5 mg/mL) was added to each well and the plate was incubated for an additional 4 h at 37 °C under 5% CO_2. After the addition of 10% sodium dodecyl sulfate (SDS, 100 mL/well), the assay plate was allowed to stand at room temperature for 12 h. The optical density OD570 value (Abs.) of each well, with background subtraction at 690 nm, was measured by means of a Tecan Infinite M200 monochromator-based multifunction microplate reader. The following formula was used to calculate the inhibition of cell growth: Cell viability(%) = (mean of Abs. value of treatment group/mean Abs. value of control) × 100%.

Upconversion luminescence (UCL) imaging

Laser scanning upconversion luminescence microscopy (LSUCLM) imaging *in vitro*

UCL bioimaging of living cells MCF-7 cells (5 × 10^8/L) were plated on 14 mm glass coverslips and allowed to adhere for 24 h. Subsequently, cells were washed with phosphate buffer solution (PBS). The MCF-7 cells were incubated in a serum-free medium containing 200 mg/mL PEG-UCNPs for 1 h at 37 °C under 5% CO_2, and then washed with PBS sufficiently to remove excess nanoparticles. Laser scanning upconversion luminescence (LSUCL) imaging was performed with our previously reported laser scanning luminescence microscope with an Olympus FV1000 scanning unit (60 × oil-immersion objective lens; NA: 1.35; pinhole: 785 μm). Cells were excited by a CW

infrared laser at 980 nm (Connet Fiber Optics, China) with output power of 300 mW (corresponding to ~16 mW in the focal plane). Upconversion luminescence signals were detected in the blue channel (420–480 nm).

Upconversion luminescence (UCL) imaging *in vivo*

Animal procedures were in agreement with the guidelines of the regional ethic committee for animal experiments. *In vivo* and *ex vivo* UCL imaging was performed with a modified Kodak in vivo imaging system F using an external 0–5 Wadjustable CW infrared laser (980 nm, Shanghai Connet Fiber Optics Co., China) as the excited source and an Andor DU897 EMCCD as the signal collector. Images of UCL signals were analyzed with Kodak Molecular Imaging Software. Excitation was provided by the CW infrared laser at 980 nm and UCL signals were collected at 800 ± 12 nm. Tumor-bearing nude mice (weight = 20 g) were anesthetized and were injected intravenously and intra-arterially with PBS solution with and without of PEG-UCNPs (200 mL, 5.0 mg/mL). The injection speed of PEG-UCNPs solution is kept at 20 μL/s, which is much lower than the flow rates (36.4±7.5 μL blood per cardiac cycle) of mice, to exclude the factors affecting the delivery of PEG-UCNPs. The imaging experiments were carried out 20 min post-injection.