Supporting Information

Dye-conjugated upconversion nanoparticles for ratiometric imaging of intracellular pH values

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Experimental Section

Materials: Rare earth oxides, such as Y_2O_3 (99.99%), Yb_2O_3 (99.99%), and Tm_2O_3 (99.99%), were purchased from J&K Chemical Limited. Oleic acid, 1-octadecene, ethanol, ammonia, and tetraethylorthosilicate (TEOS) were obtained from Sinopharm Chemical Reagent. Xylenol orange (XO), 3-aminopropyltrimethoxysilane (APTS, >99%), carbodiimide (EDC), and *N*-hydroxysuccinimide (NHS) were obtained from Aladdin Chemistry. Deionized water (Millipore Milli-Q grade) was used throughout the experiments.

Instrumentation: Luminescent spectral measurements were carried out by using an Edinburgh LFS-920 spectrometer with high stability of 980 nm pump laser source. Electronic absorption spectra were recorded with Shimadzu UV-3600 UV-VIS-NIR spectrophotometers. Powder small-angle X-ray diffraction measurements were carried out on a Bruker Smart APEX CCD diffractometer at 40 kV and 20 mA using Cu-Kα radiation (λ = 1.54 Å). Transmission electron microscopy observations were carried out on a JEOL JEM-2100 transmission electron microscope at an acceleration voltage of 200 kV. Particle size was measured with a nanoparticle size analyzer Brookhaven 90Plus. Fourier transform infrared spectra were performed using an IRPRESTIGE-21 spectroscope (Shimadzu) with KBr pellets.

Synthesis of Oleic Acid Capped UCNPs [abbreviated as UCNPs]:

UCNPs were prepared by a solvothermal process. YCl_3(0.798 mmol), $YbCl_3$(0.2 mmol) and $TmCl_3$(0.002 mmol) were mixed with 6 mL oleic acid and 15 mL octadecene in a 100 mL flask. Next, the reaction solution was directly heated to 150 ^oC to remove water and oxygen, with vigorous magnetic stirring in the current of nitrogen for 2 h. After the reaction was completed, 10 mL of methanol solution (2.5 mmol NaOH + 0.4 mmol NH_4F) was added dropwise into the solution at room temperature. At this point, the reaction mixture was a turbid solution. Then the solution was heated to 50 ^oC for half an hour. Next, the reaction solution was directly heated to 100 ^oC to remove the remaining water and some low boiling substance. Then the solution was heated to 298 ^oC under nitrogen and maintained at this temperature for 1 h. After the reaction was complete, 3 mL of cyclohexane was poured into the solution at room temperature. The resultant mixture was centrifugally separated (10000 rpm, 5 min every time in 20 ^oC). The nanoparticles were then washed with ethanol/cyclohexane (9:1, v/v) for three times and redispersed in 5 mL cyclohexane finally.

Synthesis of UCNPs@SiO_2-NH_2 nanoparticles: The as prepared UCNPs (5 mg) were dispersed in cyclohexane (5 mL), and then CO-520, ammonium hydroxide (0.1 mL, 28 wt %) was added, followed by ethanol (2 mL) containing TEOS (8 μL) under vigorous stirring. The entire system was vigorously stirred for 36 h at RT. UCNPs@SiO_2 were collected by centrifugation at 8500 rpm for 15 min and were washed three times with water and twice with ethanol. The obtained purified UCNPs@SiO_2 samples were redispersed in 20 mL of ethanol, and then 2 mL of APTS was added to form a mixture and allowed to react under refluxing at 80 °C for 3 h. The resultant was washed with deionized water for several times, and dried at 60 °C for 3 h in a vacuum oven to obtain the UCNPs@SiO_2-NH_2.

Synthesis of UCNPs@SiO_2-XO nanoparticles:

Covalent binding of XO to the UCNPs@SiO_2-NH_2 was conducted using a modification of the standard EDC-NHS reaction. Carboxyl groups of XO (5.7 mg, 1.1 × 10−5 moL) were activated by an EDC/NHS solution (molar ratio of FA/EDC/NHS = 1:1:2.5) for 30 min. Afterward, 5 mg of UCNPs@SiO_2-NH_2 were added to form a mixed solution and allowed to react at RT for 12 h. The resultants were washed with deionized water and ethanol alternatively for removing unreacted chemicals by centrifugation. The obtained purified UCNPs@SiO_2-XO samples were redispersed into 10 mL of PBS for further characterization and applications.

Cell Culture: The HeLa lines were provided by the Institute of Biochemistry and Cell Biology, SIBS, CAS (China). The HeLa cells were grown in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum), and culture was at 37 ^oC under 5% CO_2.

Cytotoxicity Assay: The *in vitro* cytotoxicity was measured using a standard methyl thiazolyl tetrazolium (MTT, Sigma Aldrich) assay in HeLa cell lines. Briefly, cells growing in log phase were seeded into 96-well cell culture plate at 1 × 10^4/well. Nanoprobe was added to the wells of the treatment group at concentrations of 100, 200, 400, and 600 μg/mL. For the negative control group, 1 μL/well solvent was diluted in RPMI 1640 with the final concentration of 1 %. The cells were incubated for 24 h at 37 $^{\circ}C$ under 5 % CO_2. The combined MTT/PBS solution was added to each well of the

96-well assay plate and incubated for an additional 4 h. An enzyme-linked immunosorbent assay (ELISA) reader was used to measure the OD570 (absorbance value) of each well referenced at 490 nm. The following formula was used to calculate the viability of cell growth:

Viability (%) = (mean of absorbance value of treatment group / mean of absorbance value of control) × 100

Luminescence Bioimaging: Cells (5×10^8/L) were plated on 14 mm glass coverslips and allowed to adhere for 24 h. The cells were washed with PBS and then incubated solely with 200 μg/mL UCNPs@SiO_2-XO in DMDEM for 1 h at 37 ^oC. Cell imaging was then carried out after washing the cells with PBS. Luminescence imaging was performed with an Olympus FluoView FV1000 confocal fluorescence microscope and a 60 × oil immersion objective lens. Cells incubated with UCNPs@SiO_2-XO were excited at 980 nm with a CW laser, and the emission was collected at 540 ± 20 nm. Quantization by line plots was accomplished using the software package provided by Olympus instruments.

The titration curve:

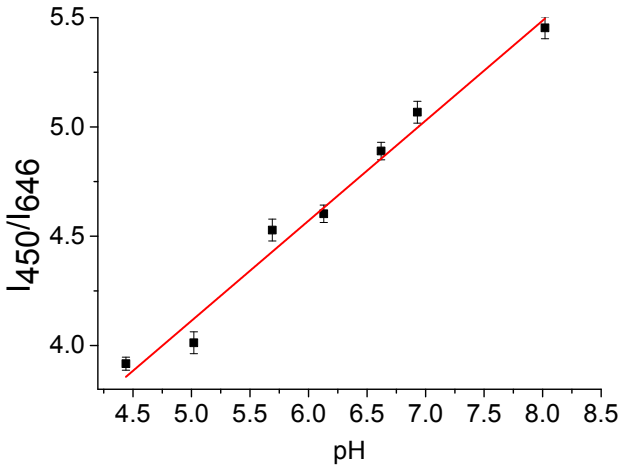

Fig. S1 The luminescence intensity ratio (I_{450nm}/I_{646nm}) of UCNPs@SiO_2-XO as a function of pH value. Solid line represents fit with a correlation coefficient of 0.99.