Electronic Supplementary Material (ESI) for RSC Advances.

Rhodamine B Derivatives Modified Upconversion Nanoparticles as a Fluorescent Turn-Off-On Sensor for the Highly Sensitive Detection of Cu^{2+} and Pyrophosphate

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Supporting Figures and Tables.

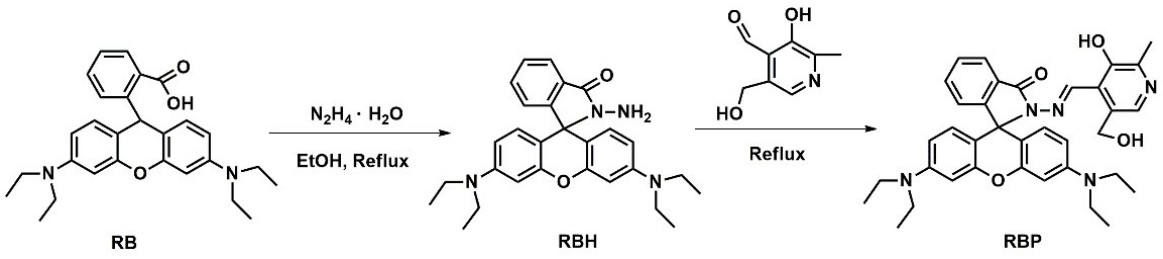

Fig. S1 Diagram of synthesis process of Rhodamine B derivative.

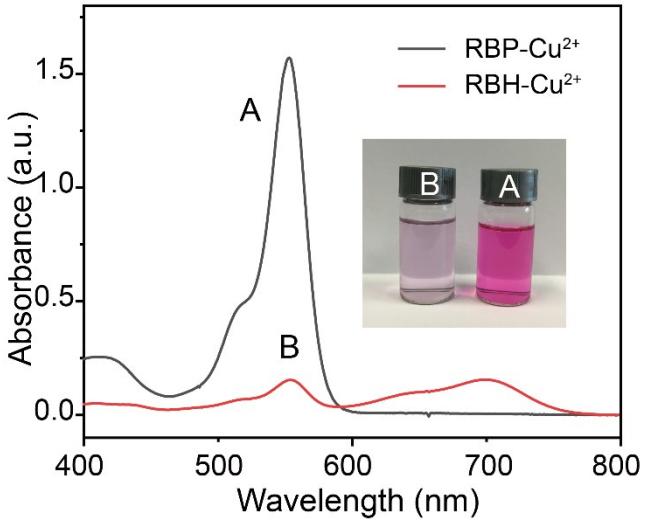

Fig. S2 The absorbance spectrum of RBP-Cu^{2+} and RBH-Cu^{2+}. The inset is the digital image of the RBP-Cu^{2+} and RBH-Cu^{2+} (The concentration of RBP, RBH and Cu^{2+} is 20 μM).

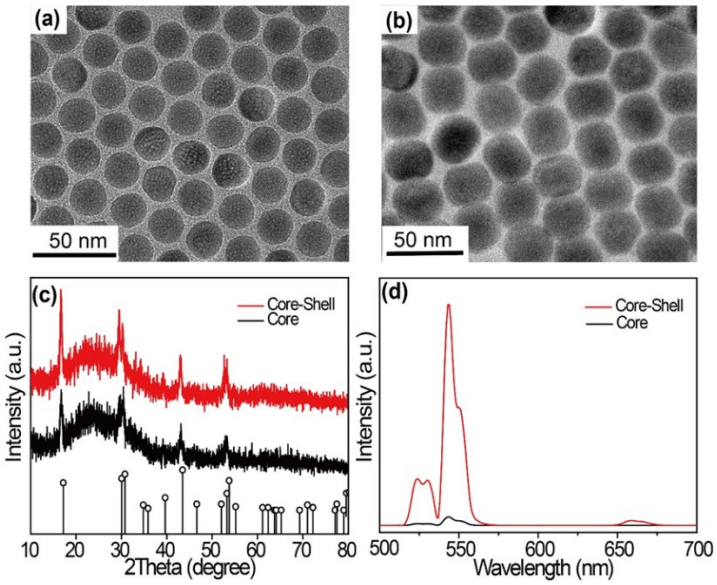

Fig. S3 (a)TEM images of core $NaYF_4$:Yb^{3+},Er^{3+} (b) $NaYF_4$:Yb^{3+},Er^{3+}@$NaYF_4$ core-shell UCNPs, (c) XRD patterns of $NaYF_4$:Yb^{3+},Er^{3+} core and $NaYF_4$:Yb^{3+},Er^{3+}@$NaYF_4$ core-shell UCNPs and (d) their luminescence spectra.

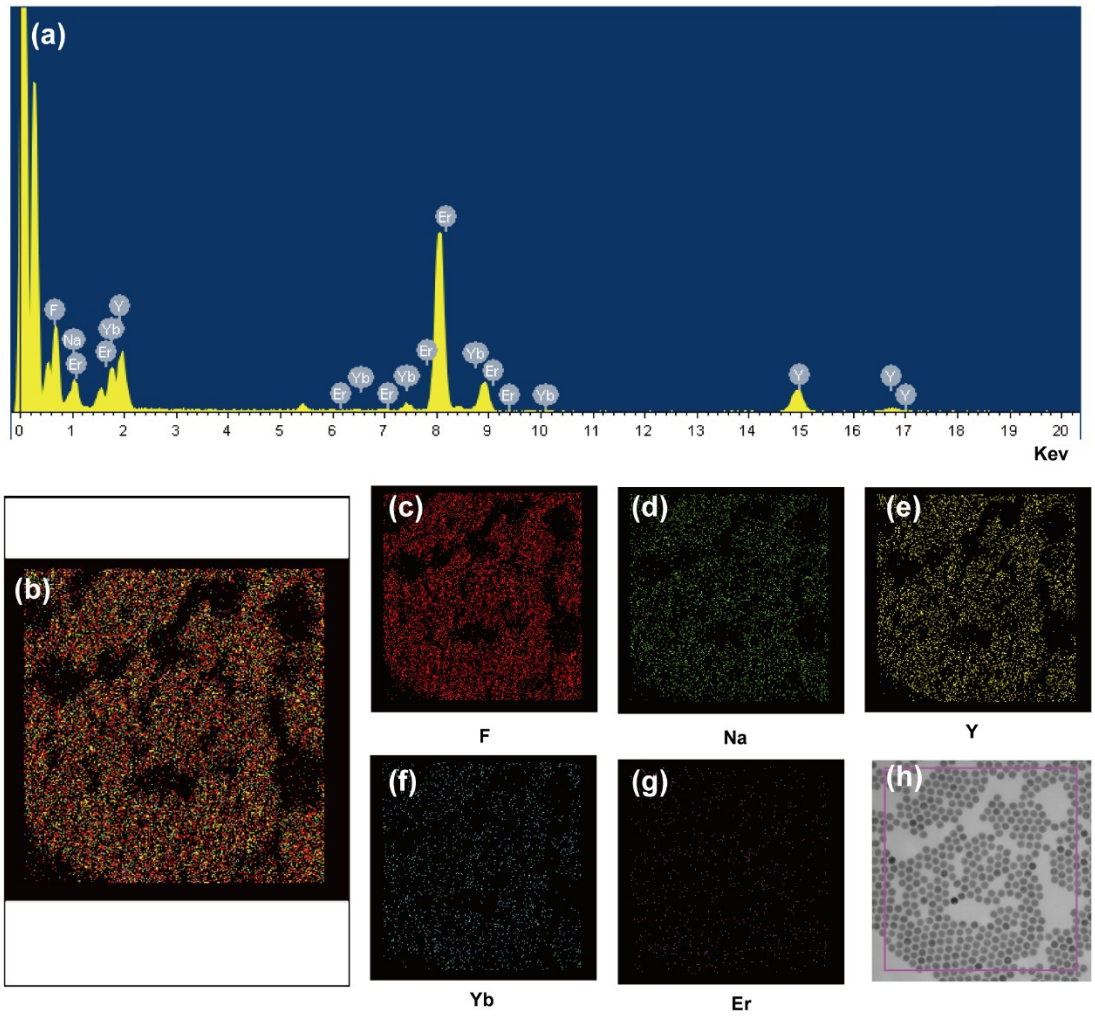

Fig. S4 (a) EDX spectrum and (b)-(g) elemental mapping of the $NaYF_4$:Yb^{3+},Er^{3+} nanoparticles.

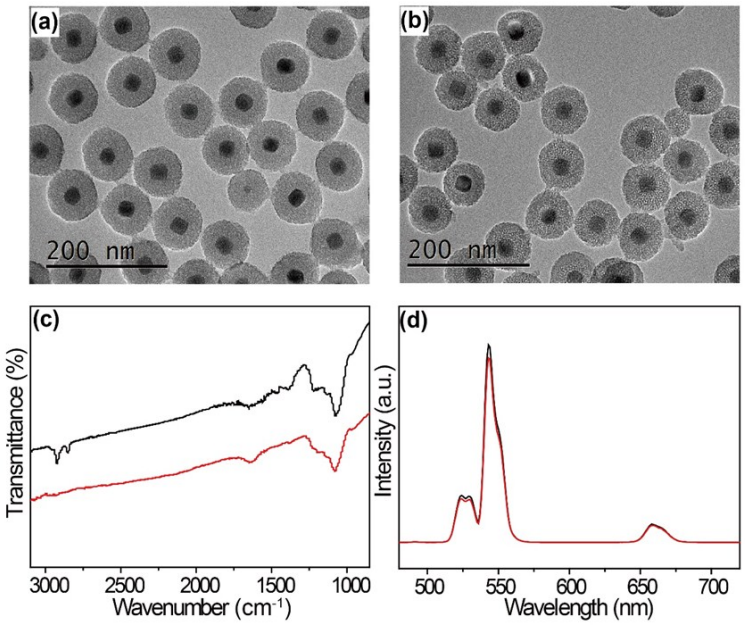

Fig. S5 (a) TEM images of UCNPs@SiO_2 and (b) UCNPs@mSiO_2, (c) IR spectra of UCNPs@SiO_2 before and after the removal of CTAB molecules, (d) The emission spectra of UCNPs@SiO_2 before and after the removal of CTAB molecules. (The blank and red line of (c) and (d) represent UCNPs@SiO_2 before and after the remove of CTAB molecules respectively.)

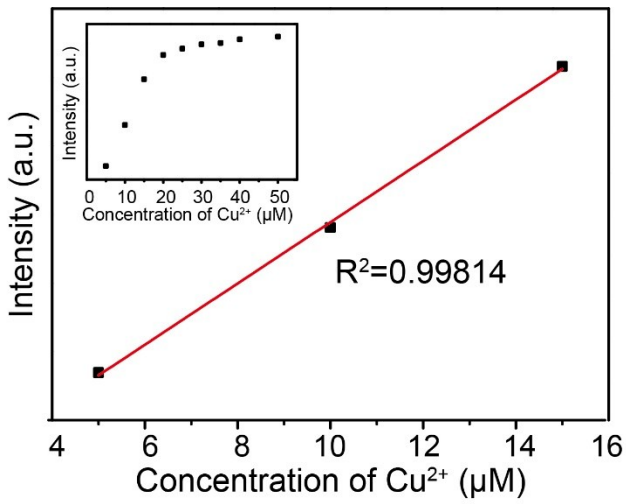

Fig. S6 The linear plot of detection (absorbance) of Cu^{2+}, the inset is scatter figure of intensity of absorbance with different concention of Cu^{2+} (0-45 μM)

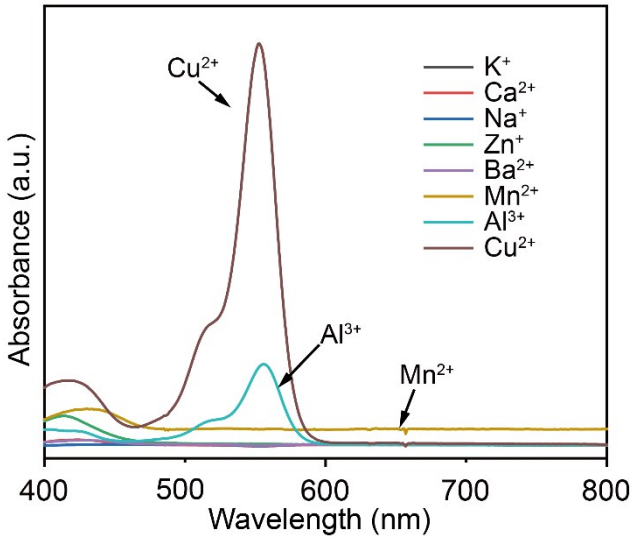

Fig. S7 Selectivity for Cu^{2+} detection. (The concentration of Cu^{2+} is 20 μM, and the other ions is 100 μM

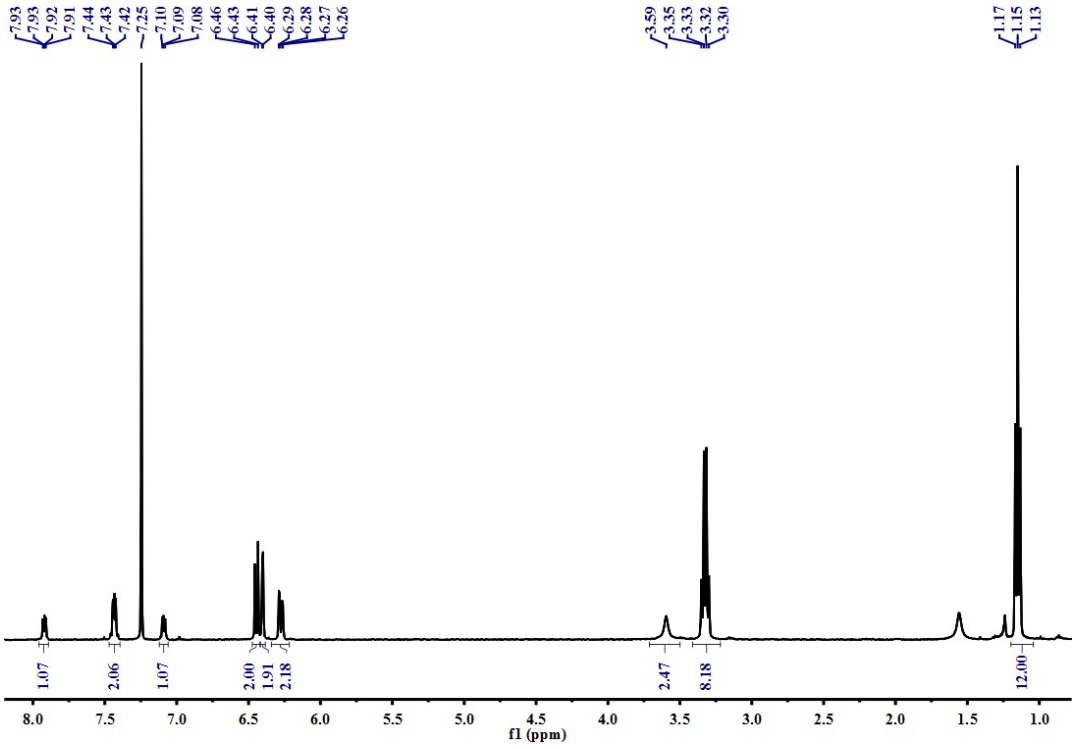

Fig. S8 ^{1}H NMR spectrum of Rhodamine B hydrazide.

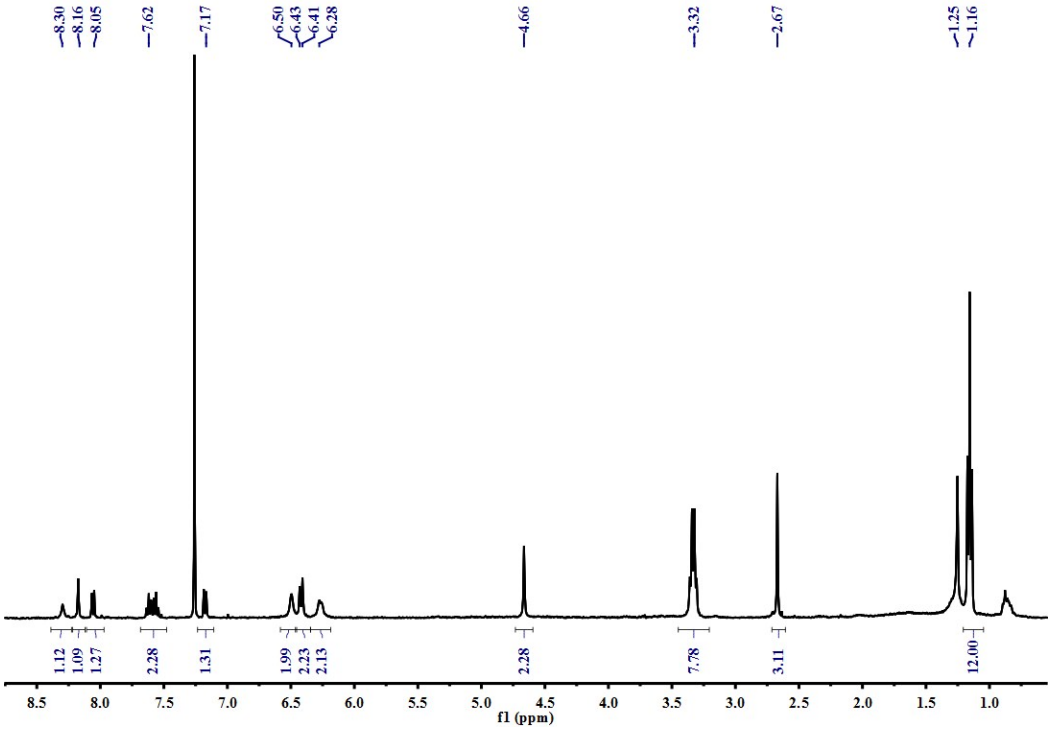

Fig. S9 ^{1}H NMR spectrum of RBP

Table S1 Summary of the probe for detection of Cu^{2+}

Probe for Cu^{2+}	Linear range	Detection limit
TSPP on UCNPs@SiO_2-NH_2 [1]	0-0.16 mM	2.16 μM
$NaYF_4$:Yb^{3+}/Er^{3+}:Rd-NH_2 [2]	2-14 μM	----
UCNPs@mSiO_2–RBH [3]	0-20 μM	4.6 ppb
UCNPs@RBH [4]	0-14 μM	1 μM
UCNPs@ mSiO_2-P [5]	0-14 μM	0.17 μM
PEI-capped UCNPs [6]	0.1-2 μM	57.8 nM
AuNR@SiO_2@TCPP [7]	0.15-2.5 μM	75 nM

Table S2 Summary of the probe for detection of PPi

Probe for Cu^{2+}	Linear range	Detection limit
Cu^{2+}-PEI-UCNPs [6]	0.5-8 μM	184 nM
CQD/Cu^{2+} [8]	3.3-85.8 μM	0.3 μM
AuNR@SiO_2@TCPP-Cu^{2+} [7]	5-75 μM	0.82 μM

Experimental

Preparation of hexagonal phase (β-phase) $NaYF_4$: Yb, Er core nanoparticles

3.2 mL of 0.2 M $Y(CH_3COO)_3$, 0.72 mL of 0.2 M $Yb(CH_3COO)_3$ and 0.08 mL of 0.2 M $Er(CH_3COO)_3$ were mixed with 6 mL of oleic acid (OA) and 14 mL of 1-octadecene (1-ODE). The mixture was heated to 150 °C and keep heating for at least 40 min to form a clear and transparent solution, and then cooled down to room temperature slowly with the stirring. Then, 4 mL of 0.5 M NaOH (methanol solution) and 8 mL of 0.4 M NH_4F (methanol solution) were mixed together and injected to the solution and heated to 50 °C and maintained at least for 30 min to evaporation solvent. Subsequently, the solution was heated up to 100 °C slowly and keep for this temperature 30 min under vacuum to remove water and oxygen left in the solution. Under a nitrogen atmosphere, the solution was heated up to 275 °C and kept for 1.5 h. Then the solution was cooled down to room temperature with stirring slowly. NaYF4:Yb^{3+}, Er^{3+} nanoparticles were collected by the addition of ethanol and centrifugation at 6000 rpm for 5 min. and dispersed in10 mL of cyclohexane.

Preparation of $NaYF_4$: Yb, Er@$NaYF_4$ core-shell (named UCNPs) nanoparticles

4 mL of 0.2 M $Y(CH_3COO)_3$ (0.8 mmol) was added into 6 mL of oleic acid (OA) and 14 mL of 1-octadecene (1-ODE) The mixture was heated to 150 °C and keep heating for at least 40 min, and then cooled down to room temperature slowly under stirring, then, prepared $NaYF_4$:Yb, Er core solution in first step was added to the mixture, and 4 mL of 0.5 M NaOH (methanol solution) and 8 mL of 0.4 M NH_4F (methanol solution) were mixed together within 10 seconds and injected to the solutions, then heated to 50°C and maintained for 30 min. the solution was heated up to 100 °C under vacuum and repeated the same operation in the core steps, the solution was cooled down to room temperature slowly with stirring and the product were collected by the addition of ethanol and centrifugation at 6000 rpm for 5 min, finally, the collected products were dispersed in 10 mL of cyclohexane.

Synthesis of UCNPs@mSiO_2 nanoparticles

0.1 g of CTAB was added in 20 mL of water, and then 10 mg UCNPs was added into the mixture under vigorously stirring to evaporate cyclohexane solvent resulting in a transparent solution, then transfer the mixture to a 250 mL three-necked flask, 40 mL of water, 6mL of ethanol and 300 μL of 2 M NaOH, were added to the mixture and heated to 70°C slowly under vigorously stirring. 300 μL of tetraethylorthosilicate (TEOS) was then added dropwise and the reaction for 1 h. The products were collected by centrifugation at 10000 rpm for 20 min and washed with ethanol 3 times. The surfactants were removed by the process of ion exchange to form the mesoporous layer of SiO_2, the nanoparticles were transferred into 100 mL of ethanol containing 0.6 g of NH_4NO_3 in a 250 mL three-necked flask and kept at 60 °C for 4 h.

Synthesis of RBP

Rhodamine B hydrazide (RBH) was prepared by the reported method.[17] As shown in Fig. S1, the RBP was synthesized by RBH and pyridoxal hydrochloride, 0.92 g of RBH (2 mmol) was firstly dissolved in 30 ml of ethanol in a 100 mL flask, and then 0.4 g pyridoxal hydrochloride (4 mmol) was added to the mixture. The mixture was refluxed for 10 h under sirring. The reaction system were cooled to room temperature, then vacuumed concentrating and purified by recrystallization with ethanol, finally get compound RBP (0.35 g) as brownish-red solid.

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.30 (1H, s), 8.16 (1H, m), 8.05 (1H, s), 7.62 (2H, m), 7.17 (1H, d, *J* = 6.9 Hz), 6.50 (2H, d, *J* = 8.8 Hz), 6.43 (2H, d, *J* = 2.4 Hz), 6.28 (2H, dd, *J* = 8.9 Hz, 2.6 Hz), 4.66 (2H, s), 3.32 (8H, q), 2.67 (3H, s), 1.16 (12H, t, *J* = 7.1 Hz).

Preparation of UCNP@mSiO$_2$-RBP

A certain amount of UCNPs@mSiO$_2$ was added to the 20 μM rhodamine B derivative RBP and stirred overnight to give a fluorescent probe.

Fluorescence Detection of Cu^{2+} and PPi

Different concentrations of Cu^{2+} were added into the as-prepared UCNP@mSiO$_2$-RBP dispersion and the fluorescence intensities of system were recorded from 450 to 600 nm with excitation wavelength of 980 nm after the incubation time for 20 min. For the determination of PPi, 20 μM Cu^{2+} was first added into as-prepared UCNP@mSiO$_2$-RBP dispersion. Different concentration of PPi solution (0-45 μM) was added into the above mixture. After 20 min incubation, the fluorescence spectrum was recorded with excitation wavelength of 980 nm.

Effects on cell growth and cytotoxicity experiment

Hela cells were obtained from xiao'sgroup. All cell lines were maintained under standard culture conditions (atmosphere of 5% CO_2 and 95% air at 37 °C) in DMEM with 10% FBS (fetal calf serum). The cytotoxic effects of probe UCNPs@mSiO$_2$-RBP were assessed using the MTT assay. Briefly, in the experimentation, the cells in the exponential phase of growth were used. 3×10^4 cells/well were seeded onto 96-well plates and allowed to grow for 24 h. Then treated cells with UCNPs@mSiO$_2$-RBP. (The concentration of probe is 0 100 200 300 400 500 600 μg/mL respectively) The incubation time of probe were kept for another 24 hours. At the end of this time, the UCNPs@mSiO$_2$-RBP –containing medium was replaced with PBS, and 20 μL 0.5% MTT (5 mg/mL) was then added to each well for 4h at 37°C. The culture medium were removed, and the cells were dissolved in 200 μL DMSO. The optical density (O.D.) of the plate wells was recorded by an AC100-120 Automated Microplate Reader (TECAN, Switzerland) at a test wavelength of 570 nm and reference wavelength of 630 nm. The inhibition rate of each compound was calculated based on the following formula: Cell survival rate =average A_{570} nm of treated group/average A_{570} nm of control group×100%.Each treatment was replicated six wells.

Fluorescence imaging in nude mice

All procedures and operations were performed with the Guide for the Care and Use of Laboratory Animal Resources and the National Research Council, and were approved by the Institutional Animal Care and Use Committee of the NIH. The modified NightOWL II LB983 small animal in vivo imaging system with the excitation of 980 nm laser and equipped with a sensitive Charge Coupled Device (CCD) camera were used to bioimaging on living mice. Nude mice were purchased from the Dalian Medical University (Dalian, China) and were used in imaging studies. For subcutaneous injection and UCL imaging, the UCNP@mSiO$_2$-RBP aqueous solutions (100 μL,10 mg/mL) in 0.9% NaCl saline solution were subcutaneously injected into the back area of nude mice (~10 g) anesthetized with chloral hydrate. And the other mice was performed with the same operations and sequence inject 100 μL of Cu^{2+} solution (50 μM) to quench the fluorescence of probe.

Characterization

Distribution and morphology of the prepared nanoparticles were obtained with Transmission electron microscopy (TEM, Tecnai G220 S-TWin) at an acceleration voltage of 200 kV, X-ray Powder diffraction (XRD) measurements were carried out on a Rigaku D/MAX-2400 diffractometer with Cu-Kα radiation (40 kV, 40 mA, λ=1.54184 Å). Flourescence spectra were measured with a modified Edinburgh FS5 fluorescence spectrometerwith the excitation 980 nm CW laser (Changchun New Industries Optoelectronics Technology Co., Ltd, China). UV-Vis absorption spectra were measured by a UV-Vis Spectrophotometer (Agilent, HP8453), MTT experiments were carried out by using a microplate reader (Thermo Multiskan FC). The bioimaging experiments on living mice utilized the modified

NightOWL II LB983 small animal in vivo imaging system equipped with a sensitive Charge Coupled Device (CCD) camera, with the excitation of 980 nm laser.

1. X. Huang, L. Wang, X. Zhang, X. Yin, N. Bin, F. Zhong, Y. Liu and Q. Cai, *Sens. Actuators, B*, 2017, **248**, 1-8.
2. X. Jiang and G. Meng, *J. Lumin.*, 2013, **135**, 227-231.
3. C. Li, J. Liu, S. Alonso, F. Li and Y. Zhang, *Nanoscale*, 2012, **4**, 6065-6071.
4. J. Zhang, B. Li, L. Zhang and H. Jiang, *Chem. Commun.*, 2012, **48**, 4860-4862.
5. X. Mou, J. Wang, X. Meng, J. Liu, L. Shi and L. Sun, *J. Lumin.*, 2017, **190**, 16-22.
6. F. Wang, C. Zhang, Q. Xue, H. Li and Y. Xian, *Biosens. Bioelectron.*, 2017, **95**, 21-26.
7. L. Wang, Q. Song, Q. Liu, D. He and J. Ouyang, *Adv. Funct. Mater.*, 2015, **25**, 7017-7027.
8. Z. S. Qian, L. J. Chai, Y. Y. Huang, C. Tang, J. Jia Shen, J. R. Chen and H. Feng, *Biosens. Bioelectron.*, 2015, **68**, 675-680.