**Development of upconversion luminescent probe for ratiometric sensing and bioimaging of hydrogen sulfide**

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## Experimental Section

**Materials:** All reagents and chemicals were procured from commercial sources and used without further purification. 2,2,3-Trimethyl-3H-indole, iodomethane, 4-(dimethylamino)benzaldehyde, and sodium hydroxide were obtained from Alfa Aesar, and were used without further purification. Rare earth oxides $Y_2O_3$ (99.999%), $Yb_2O_3$ (99.999%), $Er_2O_3$ (99.999%), and $Tm_2O_3$ (99.999%) were purchased from Shanghai Yuelong New Materials Co. Ltd. Octadecene (ODE) and oleic acid (OA) were purchased from Alfa Aesar Ltd. Tetraethyl orthosilicate (TEOS) and hexadecyl trimethyl ammonium bromide (CTAB) were obtained from Sinopharm Chemical Reagent Co. All other chemical reagents of analytical grade were used directly without further purification. Deionized water was used to prepare all aqueous solutions. Solutions of $Cl^-$, $F^-$, $NO_2^-$, and $SO_4^{2-}$ were prepared from their sodium salts; solutions of $NO_3^-$, $Br^-$ and $I^-$ were prepared from their potassium salts. Solution of $HS^-$ was prepared from NaHS.

**Synthesis of Oleic Acid Capped UCNPs [abbreviated as OA-UCNPs]：** OA-UCNPS were prepared by a solvothermal process . 6 mL of OA and 15 mL of ODE were added to 1 mmol (total amounts) of $LnCl_3$ (Ln: 78 mol % Y + 20 mol % Yb + 2 mol % Er + 0.2 mol % Tm) in a 100 mL three-neck round-bottom flask at room temperature. 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 $^oC$)

and were washed three times with ethanol. The obtained purified UCNPs were redispersed in 4 mL of cyclohexane, named OA-UCNPs. The centrifugal machine was carried out by a Sigma 3K30 centrifugal machine.[1]

**Synthesis of UCNPs@mSiO$_2$ nanoparticles：** the synthetic procedure for the mesoporous silica coated upconversion nanoparticles is described as follows. First, 20%$Yb^{3+}$, 2%$Er^{3+}$/0.2%$Tm^{3+}$ co-doped β-$NaYF_4$ nanocrystals were prepared. Then, 2 mL of cyclohexane solution (5 mg $mL^{-1}$) containing the nanocrystals was mixed with 0.1 g of CTAB and 20 mL of water. The mixture was then stirred vigorously to evaporate cyclohexane solvent at room temperature, resulting in a transparent and clear solution (0.5 mg $mL^{-1}$). For mesoporous silica coating, 10 mL of the aqueous CTAB-stabilized nanocrystal solution was added to a mixture of 20 mL of water, 3 mL of ethanol and 150 mL of 0.2 M NaOH solution. The mixture was heated up to 70 $^{o}$C under stirring. 80 μL of tetraethylorthosilicate (TEOS) was then added dropwise and the reaction was continued for 60 min. The nanoparticles were centrifuged and washed with ethanol 3 times. To avoid the nanoparticle dissolution that usually occurs under acidic conditions, the surfactants were removed via a fast and efficient ion exchange method and the nanoparticles were transferred into 50 mL of ethanol containing 0.3 g of $NH_4NO_3$, kept at 45 $^{o}$C for 24 h, and dispersed in ethanol.[2]

**Instrumentation and Methods.** The $^{1}$H NMR spectra were recorded on a Bruker spectrometer at 400 MHz. All chemical shifts are reported in the standard δ notation of parts per million. Mass spectra were measured on a Bruker Autoflex II MALDI-TOF mass spectrometer. UV-Vis absorption spectra were recorded on a Shimadzu 3000 spectrophotometer. Upconversion luminescence (UCL) emission spectra were measured on an Edinburgh FLS920 luminescence spectrometer with an external 3 W adjustable 980 nm semiconductor laser. In our case, all power densities of CW 980 nm excitation for the UCL measurements were fixed at ~ 45 W $cm^2$. FTIR spectra were measured using an IR Prestige-21 spectrometer (Shimadzu) from samples in KBr pellets. X-ray powder diffraction (XRD) measurements were

performed on a Bruker D8 diffractometer at a scanning rate of $1^{o}$ $min^{-1}$ in the 2θ range of 10-$90^{o}$ and 0-$10^{o}$, with graphite monochromated Cu Ka radiation (λ=1.5406 nm). Transmission electron microscope (TEM) images were collected on a JEM 2010 operating at an acceleration voltage of 200 kV.

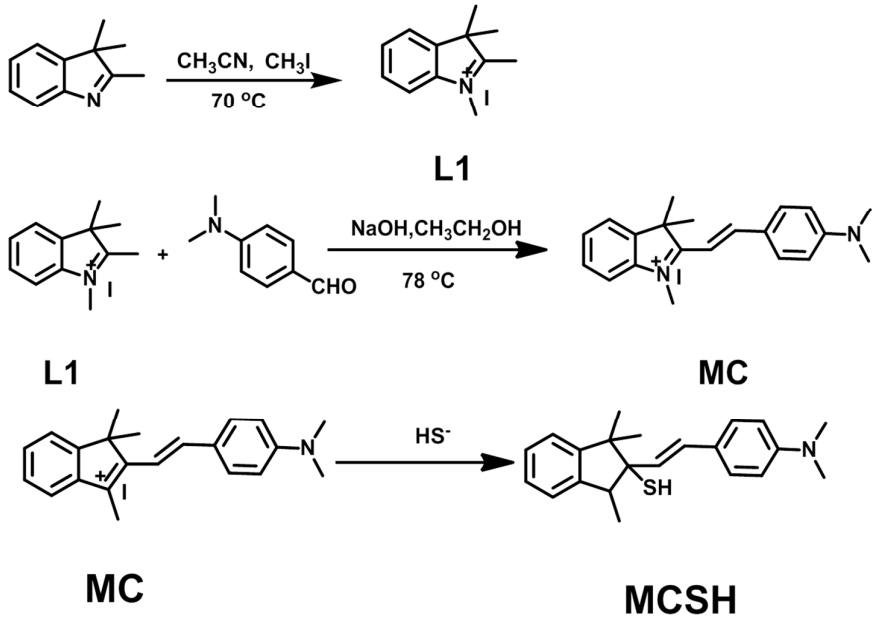
Nitrogen adsorption–desorption measurements were performed on a V-Sorb 2800 surface area analyzer using the volumetric method and samples were degassed. Particle size was measured with a nanoparticle size analyzer Brookhaven 90Plus. Pore size distributions were estimated from adsorption branches of the isotherms by using the Barrett–Joyner–Halenda (BJH) method.

The cell lines HeLa were provided by the Institute of Biochemistry and Cell Biology, SIBS, CAS (China). The HeLa cells were grown in MEM (modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) at 37 $^{o}$C and 5% $CO_2$. HeLa cells were planted on 14 mm glass coverslips and allowed to adhere for 24 h. Laser-scanning upconversion luminescence microscopy (LSUCLM) imaging was performed with an OLYMPUS FV1000 scanning unit. Experiments to assess $HS^-$ uptake were performed over 20 h in the same medium supplemented with 10 μM $HS^-$. Before the experiments, HeLa cells were washed with PBS buffer, and then the cells were incubated with 5 μM UCNPs@m$SiO_2$-MC in PBS for 2 h at 37 $^{o}$C. Cell imaging was then carried out after washing the cells with PBS. Cells loaded with OA-Ir1-UCNPs were excited by a CW laser at 980 nm (Connet Fiber Optics, China) with the focused power of ~14 mW. Emission was collected by green UCL channel from 500-555 nm and red channel from 620-670 nm.

The in vitro cytotoxicity was measured using a standard methyl thiazolyl tetrazolium (MTT, Sigma Aldrich) assay in HeLa cells lines. Briefly, cells growing in log phase were seeded into 96-well cell culture plate at $1 \times 10^4$/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 °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 690 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.




**Scheme S1.** Reaction routes for synthesis of target compound MC and the $HS^-$ adduct of MC[3]

**Synthesis of L1**: For the preparation of **L1**, 2,2,3-trimethyl-3H-indole (1.6 g, 10 mmol) and iodomethane (1.56 g, 11 mmol) was mixed in acetonitrile (5 mL) under an argon atmosphere. The solution was stirred for 24 h at 70 °C. After the reaction was completed, the resultant mixture was washed with petroleum ether and dried under vacuum at room temperature overnight. Yield, 70%. $^1$H NMR (400 MHz, DMSO) δ = 7.89 (d, J = 5.9, 1H), 7.81 (d, J=5.41, 1H), 7.65 – 7.57 (dd, 2H), 3.55 – 3.27 (s, 3H), 2.74 (s, 3H), 2.53 – 2.43 (s, 6H).

**Synthesis of MC:** Ligand MC was synthesized according to the previous report.[1] L1 (0.9 g, 3 mmol) and 4-(dimethylamino)benzaldehyde (0.89g, 6 mmol) were added to a solution of sodium hydroxide (40 mg, 1 mmol) in ethanol (10 mL) under an argon atmosphere. The solution was stirred for 24 h at 78 $^{o}$C, and then evaporated in vacuo. The residue was purified by column chromatography on neutral alumina to give MC (540 mg) as dark red solid. Yield, 60%. $^{1}$H NMR (400 MHz, DMSO) δ 8.30 (d, J = 15.7 Hz, 1H), 8.07 (d, J = 8.6 Hz, 2H), 7.76 (d, J = 7.3 Hz, 1H), 7.69 (d, J = 7.9 Hz, 1H), 7.53 (t, J = 7.4 Hz, 1H), 7.46 (t, J = 7.4 Hz, 1H), 7.25 (d, J = 15.8 Hz, 1H), 6.87 (d, J = 9.0 Hz, 2H), 3.95 (s, 3H), 3.15 (s, 6H), 1.73 (s, 6H). MS (positive mode, m/z): Calcd. 303.44, found 305.392 for $[M]^{+}$.

**Adsorption of MC on the mesopores of UCNPs@mSiO$_2$:** 10 mg of UCNPs@mSiO$_2$ was dissolved in ethanol(5 mL), and then 10 mg of MC were added, and the mixture was stirred for 24 h at room temperature. After that, the product was separated by centrifugation at 12000 rpm for 10 min. The collected solid was washed several times with ethanol and deionized water by a repetitive dispersion/precipitation cycle to rinse away any excess of MC. The final product was re-dispersed in 5 mL PBS (pH 7.40) solution.[4]

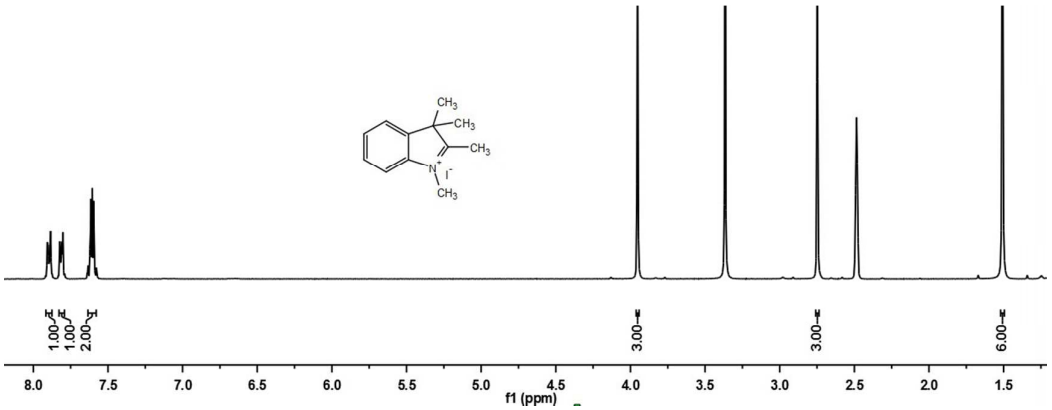



**Figure S1.** $^{1}$H NMR spectrum of **L1** in DMSO-$d_6$.

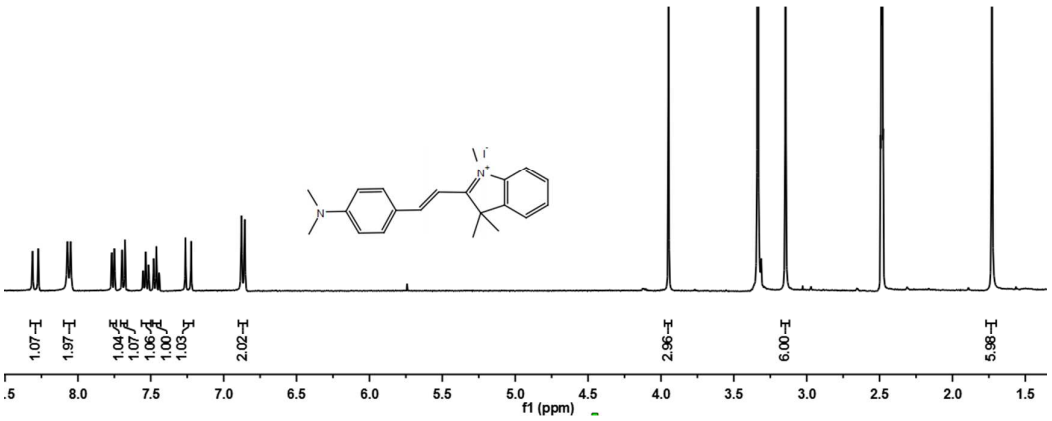



**Figure S2.** $^{1}$H NMR spectrum of MC in DMSO-$d_6$.

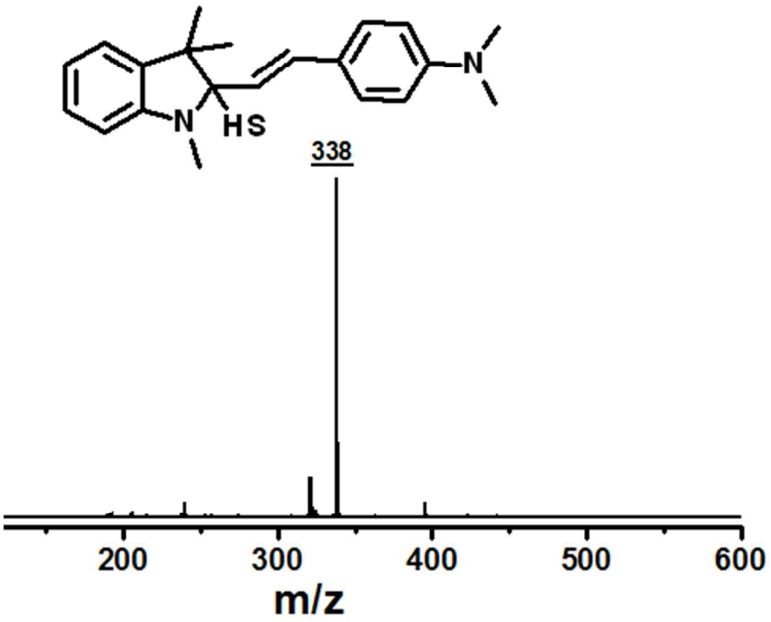



**Figure S3.** MS spectrum of MCSH calcd. for 337.522 [M $^{+}$]; found for 338 [M+H]$^{+}$.

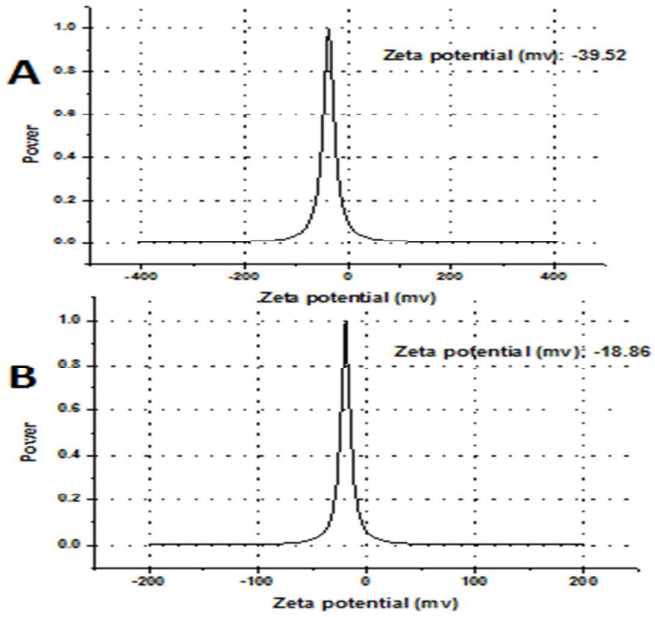



**Figure S4.** Zeta potential of the UCNPs@mSiO$_2$ nanoparticles (A) and UCNPs@mSiO$_2$-MC (B).

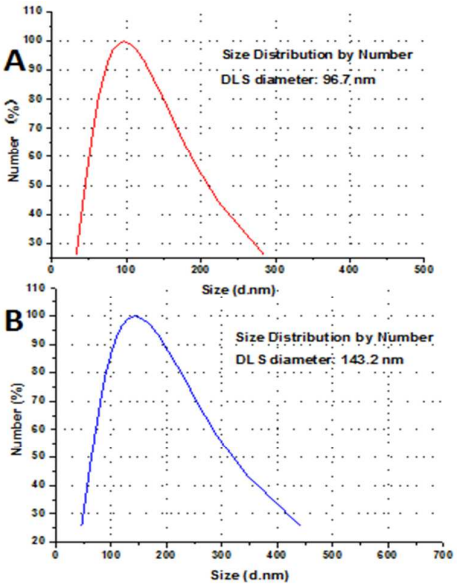



**Figure S5.** Particle size distribution of UCNPs@mSiO$_2$ (A) and UCNPs@mSiO$_2$-MC (B).

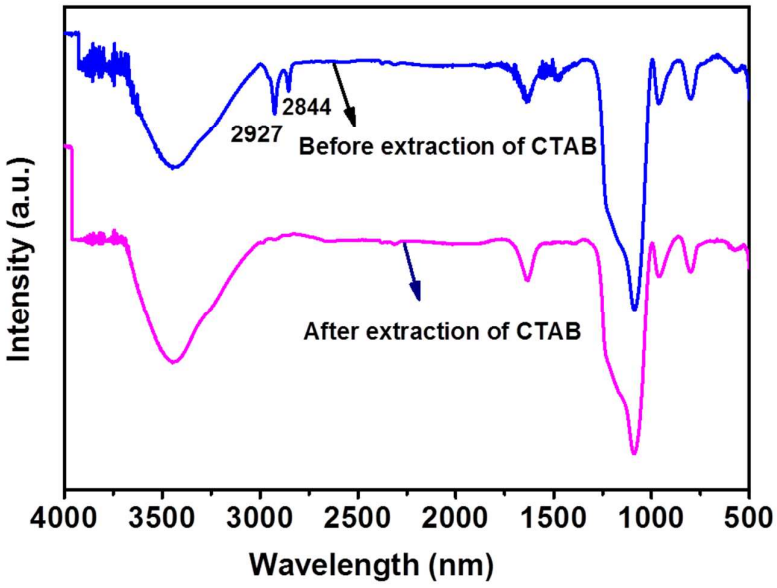



**Figure S6.** FTIR spectra of the UCNPs@mSiO$_2$ nanoparticles before and after the removal of CTAB molecules.

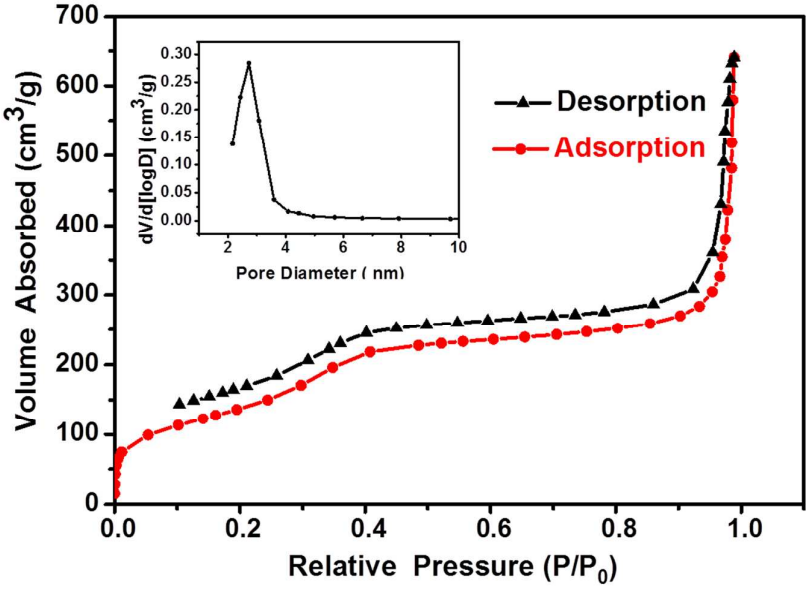



**Figure S7.** The nitrogen adsorption/desorption isotherm and pore size distribution of the UCNPs@mSiO$_2$ mesoporous nanoparticles.

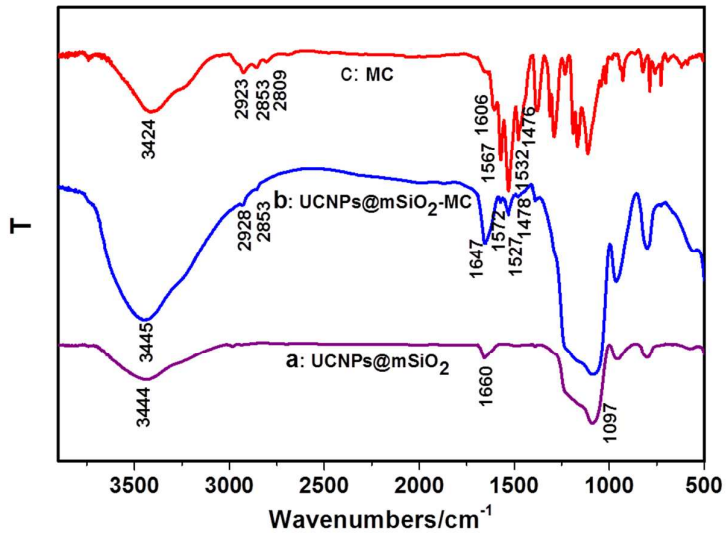



**Figure S8.** FTIR spectra of UCNPs@mSiO$_2$, MC and UCNPs@mSiO$_2$-MC.

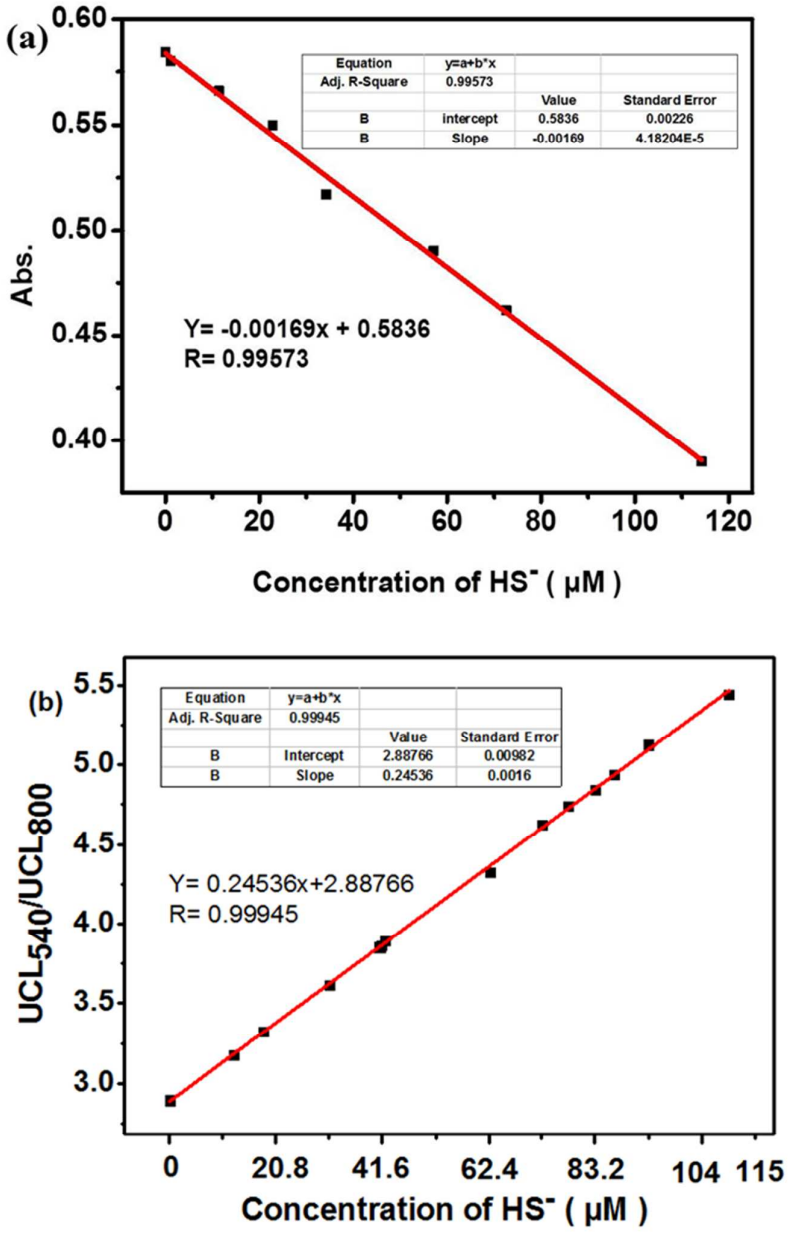



**Figure S9.** The absorption spectrum and UCL emission spectrum of 22.84 μM UCNPs@mSiO$_2$-MC in PBS buffer (pH = 7.40) was collected for 10 times to determine the background noise. Then the solution was treated with NaHS of concentration from 1 to 115 μM, and all spectra were collected after mixing for 30 s. A linear decline curve was then fitted according to the $A_{548\ nm}$ and $UCL_{540}/UCL_{800}$ in the range of [$HS^-$] from 0 to 115 μM, and the slope of the curve was obtained. the detection limit (LOD) was given by the (3σ/slope) where 3 is the factor at the 99% confidence level, σ the standard deviation of the blank measurements， The limit of detection (LOD) was determined to be 1.14, 0.58 μM of $HS^-$ respectively, for UCNPs@mSiO$_2$-MC (a) using UV/Vis absorption technique and for UCNPs@mSiO$_2$-MC (b) using UCL emission technique.

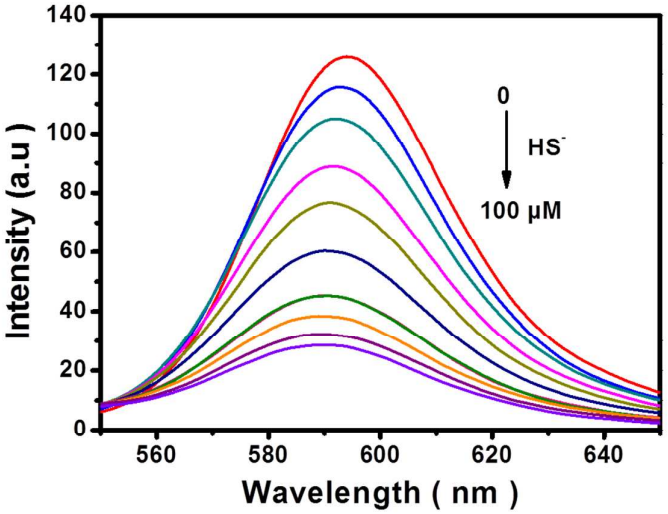



**Figure S10.** Fluorescence spectra of 10 μM MC in PBS buffer (pH=7.40) determined at different time after treat with NaHS of concentration from 0 to 100 μM of $HS^-$. All spectra were collected upon excitation at 540 nm.

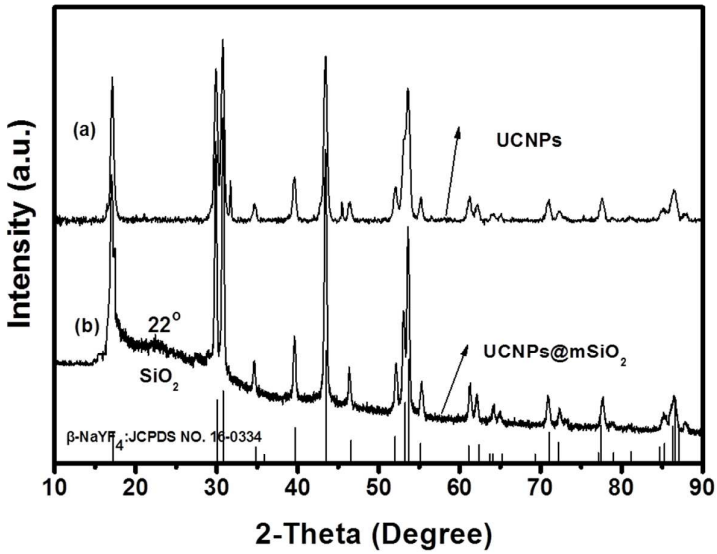



**Figure S11.** XRD patterns of UCNPs (a) and UCNPs@$mSiO_2$ (b).The standard card of $\beta$-$NaYF_4$ has also been given as a reference.

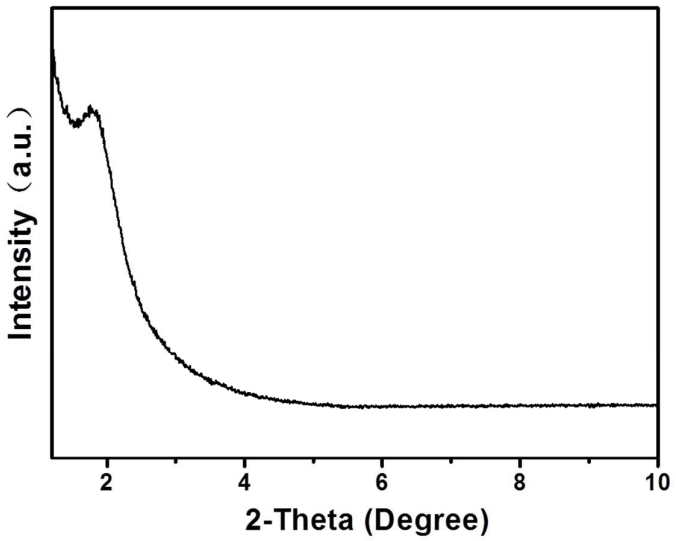



**Figure S12.** XRD patterns of UCNPs@$mSiO_2$ in the 2θ range of 0-10$^o$.

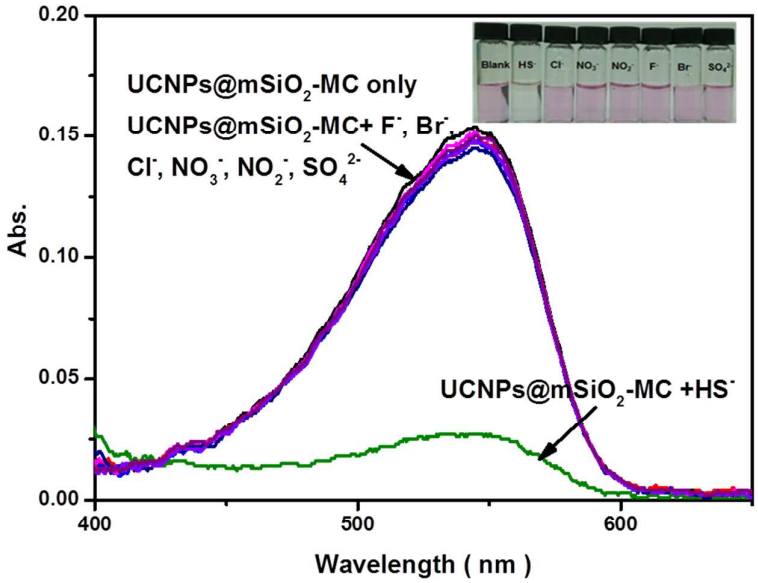



**Figure S13.** UV/Vis absorption spectra of MC (6 μM) in PBS (pH 7.40) solution upon addition of 500 μM of different anions. Inset shows the photos of the MC in PBS (pH 7.40) solution with or without addition of different anions.

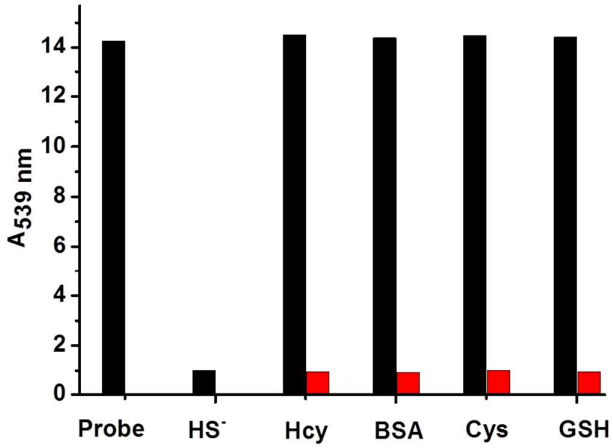



**Figure S14.** Absorption of MC (30.7 μM) in the same buffer in the presence of $HS^-$ (110 μM), Cys (2 mM), GSH (1 mM), Hcy (160 μM), and bovine serum albumin (BSA; 200 μM). Black bars correspond to free MC, or MC with $HS^-$ or MC with marked biological thiols; white bars to MC in the presence of both $HS^-$ (110 μM) and the marked biological thiols.

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