Electronic Supplementary Material (ESI) for Nanoscale.

Supporting information

Dye-sensitized Upconversion Nanocomposite for Ratiometric Semi-quantitative Detection of Hypochlotite *in vivo*

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Experimental section

Materials. All reagents and chemicals were obtained from commercial supplies and used as received. Rare-earth oxides Y_2O_3 (99.999%), Yb_2O_3 (99.999%), Er_2O_3 (99.999%), and Nd_2O_3 (99.999%) were purchased from Shanghai Yuelong New materials Co. Ltd. $RECl_3$ (RE^{3+}= Y^{3+}, Yb^{3+}, Er^{3+}, and Nd^{3+}) were prepared by dissolving the corresponding oxides in 10% HCl solution and then evaporating the water completely. Oleic acid (OA), 1-octadecane (ODE 90%), phosphatidylcholine (PC) were obtained from Aldrich.

Characterization. The ^{1}H NMR spectra were recorded on a Brucker spectrometer at 400 MHz. All chemical shifts are reported in the standard δ notation of parts per million. The size and morphology of UCNPs were determined at 200 KV using a JEOL JEM-2010F low to high resolution transmission electron microscope (TEM). The as-prepared samples were dispersed in cyclohexane and dropped on the surface of a copper grid. Powder X-ray diffraction (XRD) measurements were performed on a Bruker D4 diffractometer at a scanning rate of 1°/min in the 2θ range from 10 to 90° (Cu Kα radiation, λ=1.54056 Å). FT-IR spectra were measured using an IR Prestige-21 spectrometer (Shimadzu) from samples in KBr pellets. UV-vis absorption spectra were recorded on a Shimadzu 3000 spectrophotometer. UCL emission spectra were measured on an Edinburgh FLS920 luminescence spectrometer with an external 0-1.5 W adjustable CW semiconductor laser at 808 nm (Shanghai Hi-Tech Optoelectronic Co., China).

Preparation of test solutions. Deionized water were used for spectroscopic studies. Superoxide solution (O_2^-) was prepared by adding KO_2 (1 mg) to dry dimethyl sulfoxide (1 mL) and stirring vigorously for 10 min. Hydroxyl radical (OH•) was generated in situ by the Fenton reaction (to generate OH•, Fe^{2+} was added in the presence of 10 eq of H_2O_2). Nitric oxide (NO) was generated from Sodium Nitroferricyanide (III) Dihydrate (SNP), which was added into degassed deionized water under N_2 then stirred for 30 min at 25 °C. Hypochlorite and hydrogen peroxide solution were prepared by dilution of commercial NaClO solution and H_2O_2 solution in deionized water. m-CPBA and t-BuOOH solutions was prepared by adding commercial compound to to dry dimethyl sulfoxide.

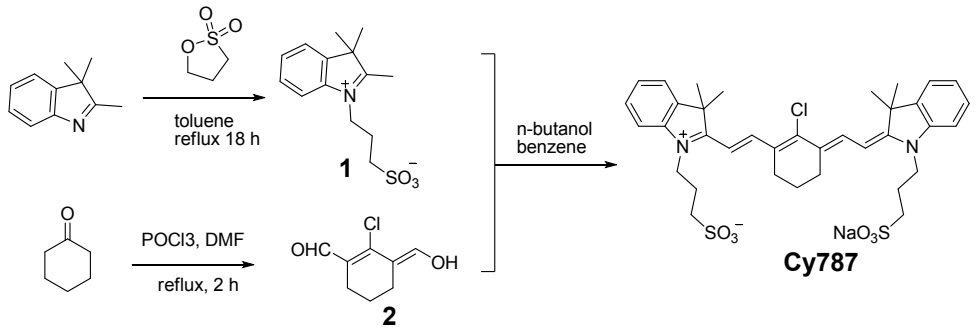
Cell culture. Human cervical carcinoma HeLa cells were provided by the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS). The HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % Fetal Bovine Serum (FBS) and 1% antibiotic/antimycotic solution (penicillin and streptomycin, Invitrogen). All cells were cultured at 37 °C under 5% CO_2.

MTT assay [1]. Hela cells were incubated in RPMI 1640 (high glucose) medium containing 10% FBS. All cells were harvested and subcultured in 96-well plates at a density of 4×10^4 cells per well for 24 h in a humidified atmosphere containing 5% CO_2 at 37 °C. Cy787@PC with varying concentrations were added into the wells, respectively, and further cultured for another 12 h. After that, the culture media

containing Cy787@PC were discarded with fresh cell growth medium (200 μL). The cells were allowed to continue growing for 24 h and 48 h. Then MTT (5 mg/mL, 10 μL per well) was added to the wells and the cells were incubated at 37 °C for another 4h. DMSO (200 μL per well) was added to dissolve the produced formazan after discarding the supernatant. The plates were shaken for 10 min and the absorbance values of the wells were then read with microplate reader at 570 nm. The cell viability rate (VR) was calculated according to the the following equation: VR = (Aexperimental group/Acontrol group) × 100%

Luminescence imaging in vitro and in vivo. Animal procedures were in agreement with the guidelines of the institutional Animal Care and Use Committee. UCL imaging was performed with a modified upconversion luminescence imaging system designed by our group. External 0-3.5 W adjustable CW 808 nm semiconductor laser. A cooled electron-multiplying charge-coupled device (EMCCD, Andor DU897) was used as the signal collector. Imaged of luminescence signals were analyzed with Kodak Molecular Imaging Software. UCL signal were collected at 540 nm ± 12 nm.

Synthesis of NIR dye Cy787 [2]



Scheme S1. Synthetic route of the NIR dye Cy787

Synthesis of compound 1. 2,3,3-trimethylindolenine (6.3 g, 62 mmol) and 1,3-propane sultone (8.2 mL, 94 mmol) were dissolved in toluene (50 mL), and the solution was heated under reflux for 18h. The reaction mixture was allowed to cool to room temperature and the resulting pink crystals were filtered and washed with acetone. The filtered product was recrystallized from a solution of MeOH and Et_2O. The crystals were collected and dried under vacuum.

Synthesis of Compound 2. A solution of $POCl_3$ (37 mL, 397 mmol) in DCM (35 mL) was slowly added to an ice-cooled solution of DMF (40 mL, 516 mmol) in DCM (40 mL). After the addition was finished, cyclohexanone (10 g, 100 mmol) was added in via syringe. The resulted reaction mixture was refluxed for 2 h. The mixture was then cooled in ice. Water (200 mL), pre-cooled to 0 °C was added slowly while the mixture was stirred. Then the mixture was stirred for 30 min. DCM layer was collected and the water layer was extracted with additional DCM. The DCM solutions were combined, passed through the $MgSO_4$ columm, concentrated on a rotavapor and treated with pentane (200 mL) to give compound 2 as yellow crystalline solid to reserve in the cool temperature.

Synthesis of Compound Cy787. Into a flask attached with Dean-Stark trap and a condenser were added compound 1 (7.9 g, 20 mmol), freshly prepared compound 2 (1.7 g, 10 mmol), n-butanol (200 mL) and benzene (20 mL). The mixture was heated to 120 ^oC for 24 h, resulting in a green solution. Solvents were removed on a rotavapor. ^1H NMR (400 MHz, MeOD) δ 8.47 (d, J = 14.1 Hz, 2H), 7.54 (d, J = 7.4 Hz, 2H), 7.47

– 7.43 (m, 4H), 7.33 – 7.23 (m, 2H), 6.49 (d, J = 14.1 Hz, 2H), 4.47 – 4.36 (m, 4H), 2.99 (t, J = 6.8 Hz, 4H), 2.80 (t, J = 6.0 Hz 4H), 2.33 – 1.91 (m, 8H), 1.93 (dd, J = 11.0, 4.5 Hz 2H), 1.76 (s, 12H). MS-ESI $(M)^+$ m/z calcd for $C_{36}H_{42}ClN_2O_6S_2$: 699.3, found 699.3.

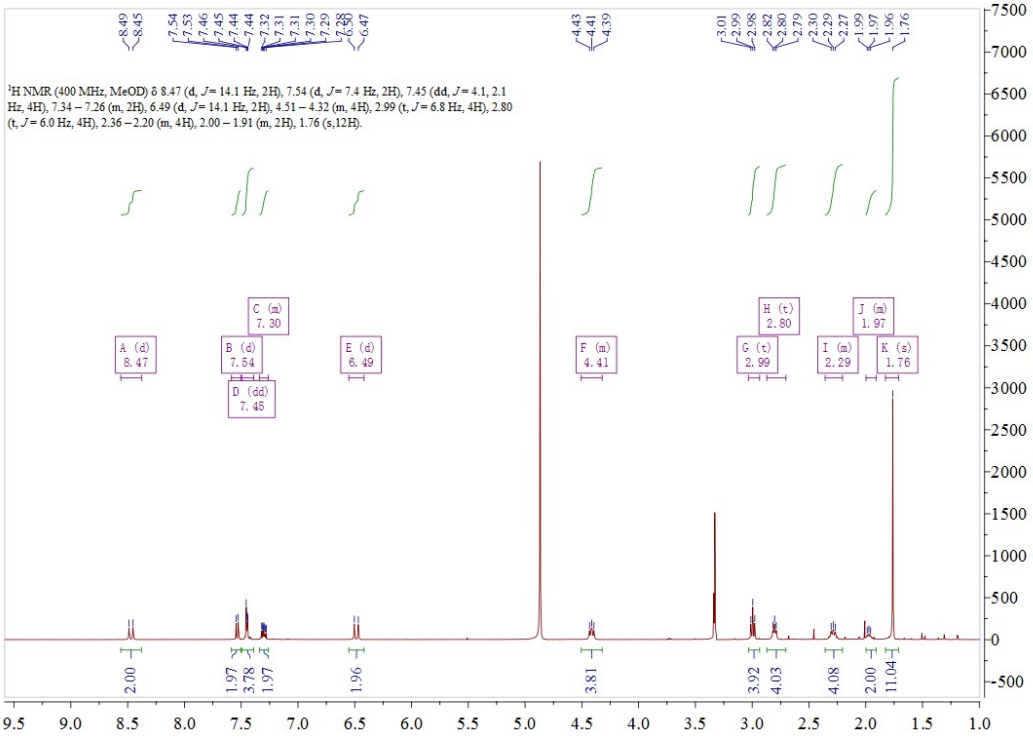


Figure S1. [1]H NMR spectra of Cy787 in the MeOD.

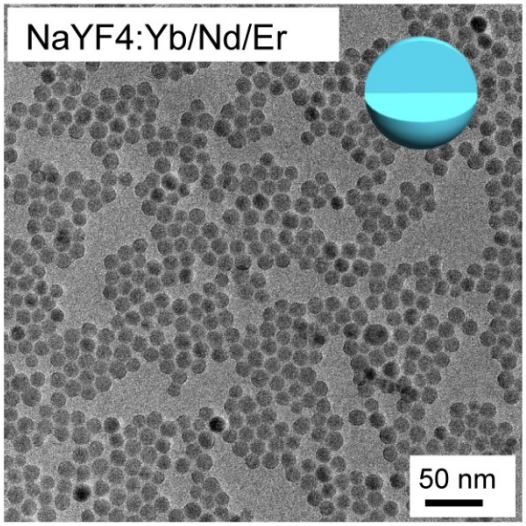


Figure S2. TEM images of $NaYF_4$:30%Yb/1%Nd/0.5%Er.

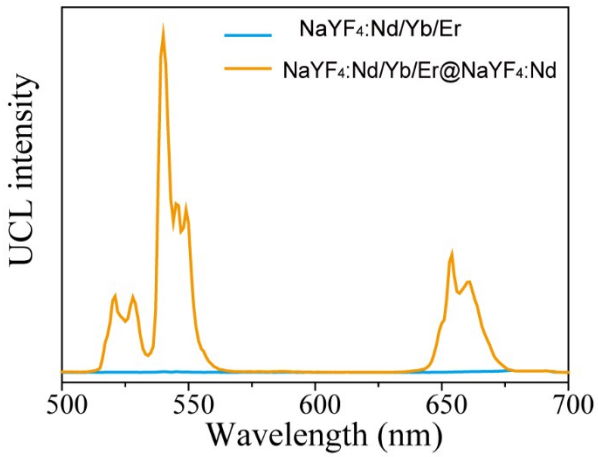


Figure S3. The upconversion luminescence spectra of the $NaYF_4$:30%Yb/1%Nd/0.5%Er and $NaYF_4$:30%Yb/1%Nd/0.5%Er@$NaYF_4$:20%Nd. λ_{ex} = 808 nm

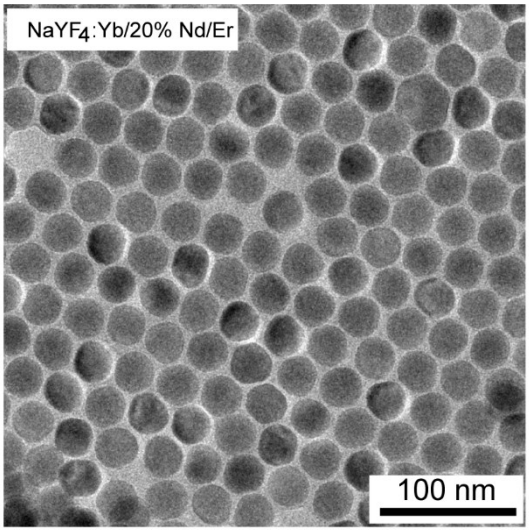


Figure S4. TEM images of $NaYF_4$:30%Yb/20%Nd/0.5%Er.

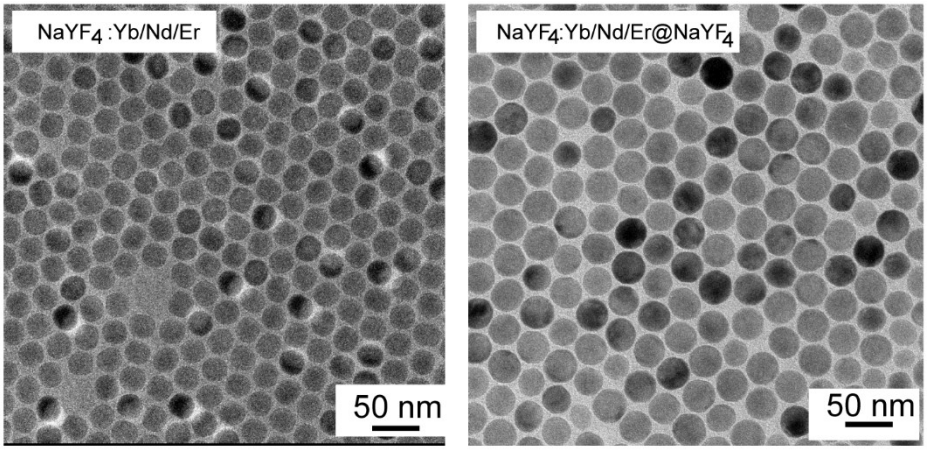


Figure S5. TEM images of $NaYF_4$:30%Yb/0.5%Er/1%Nd and $NaYF_4$:30%Yb/0.5%Er/1%Nd@$NaYF_4$.

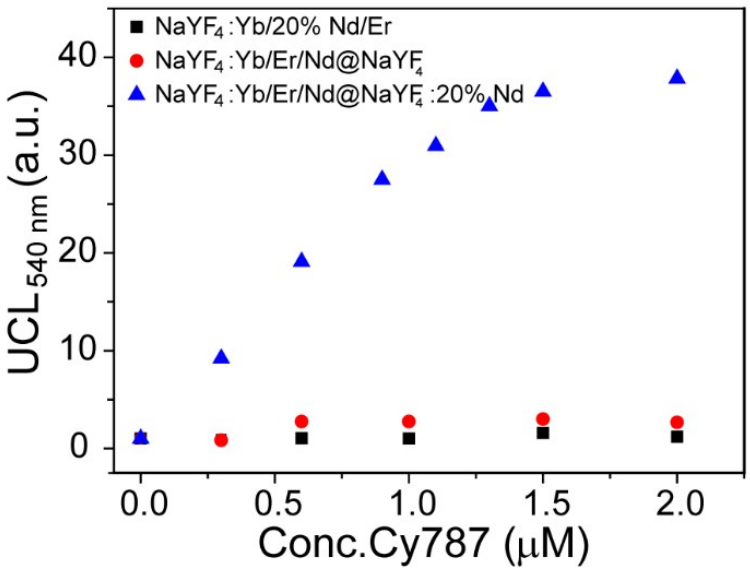


Figure S6. The UCL intensities of the different upconversion materials versus varied Cy787 dye concentration. λ_{ex} = 808 nm.

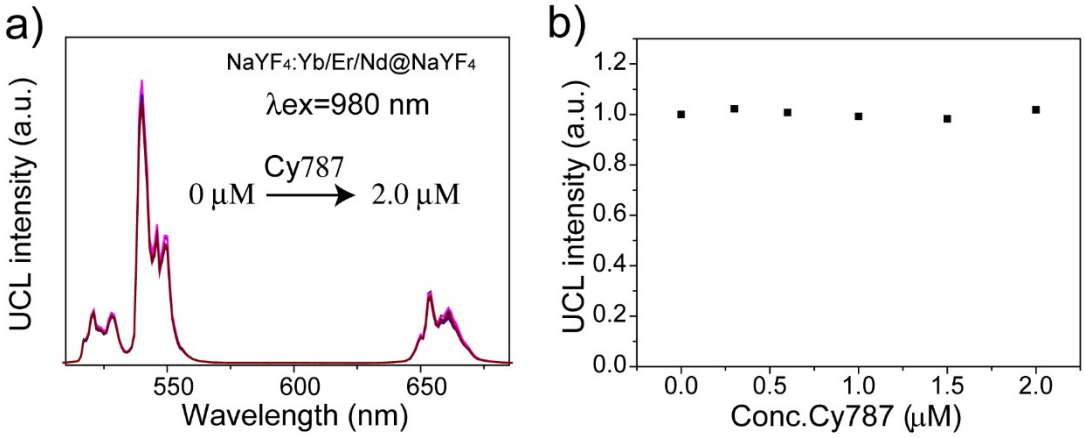


Figure S7. UCL spectra from ligand free UCNPs $NaYF_4$:30%Yb/0.5%Er/1%Nd@$NaYF_4$ with varied concentration of Cy787 dye in EtOH excitation at 980 nm.

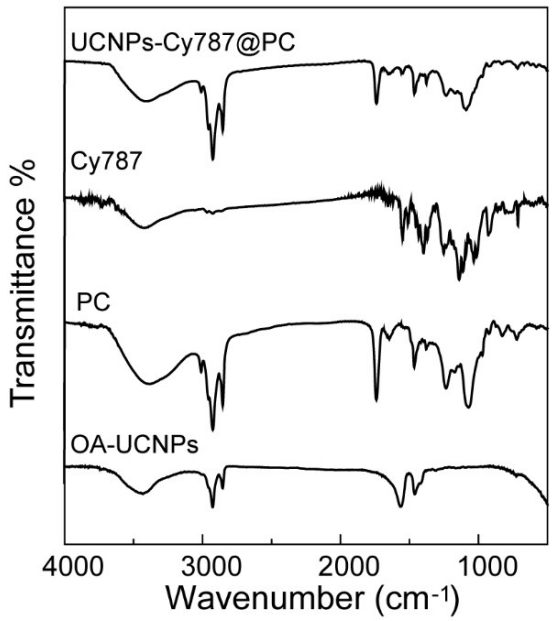


Figure S8. FTIR spectra of OA-UCNPs, PC, Cy787, and UCNPs-Cy787@PC.

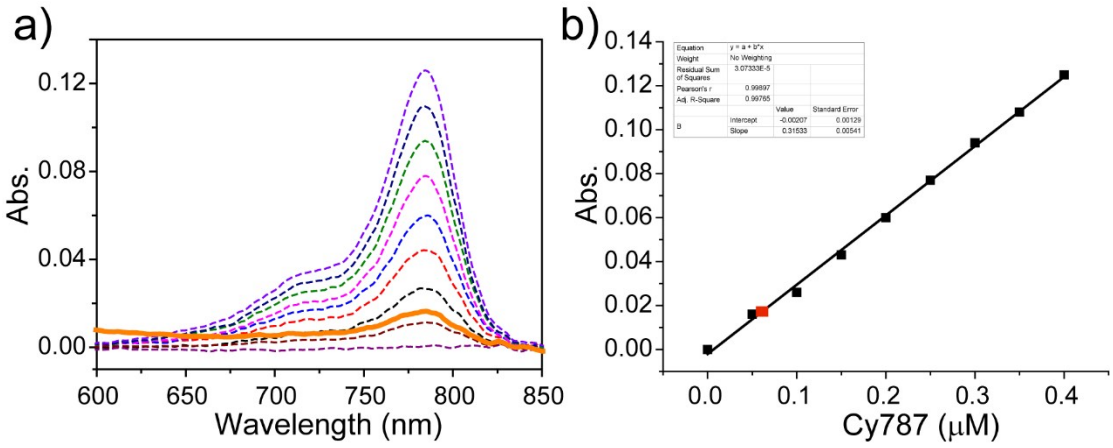


Figure S9. The concentration of Cy787 loaded in the UCNPs-Cy787@PC was calculated according to the unloaded dye measured by the absorption spectroscopy technique. (a) Absorption spectra of the Cy787 with different concentrations of 0-0.5 μM in EtOH/H_2O (v/v, 1:1, dash line) and the absorption of unloaded dye in different samples (solid line). (b) the absorbance at 785 nm as a function of Cy787 concentration.

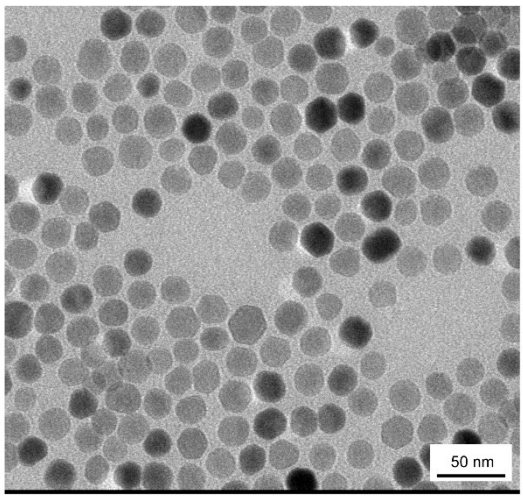


Figure S10. TEM image of UCNPs-Cy787@PC

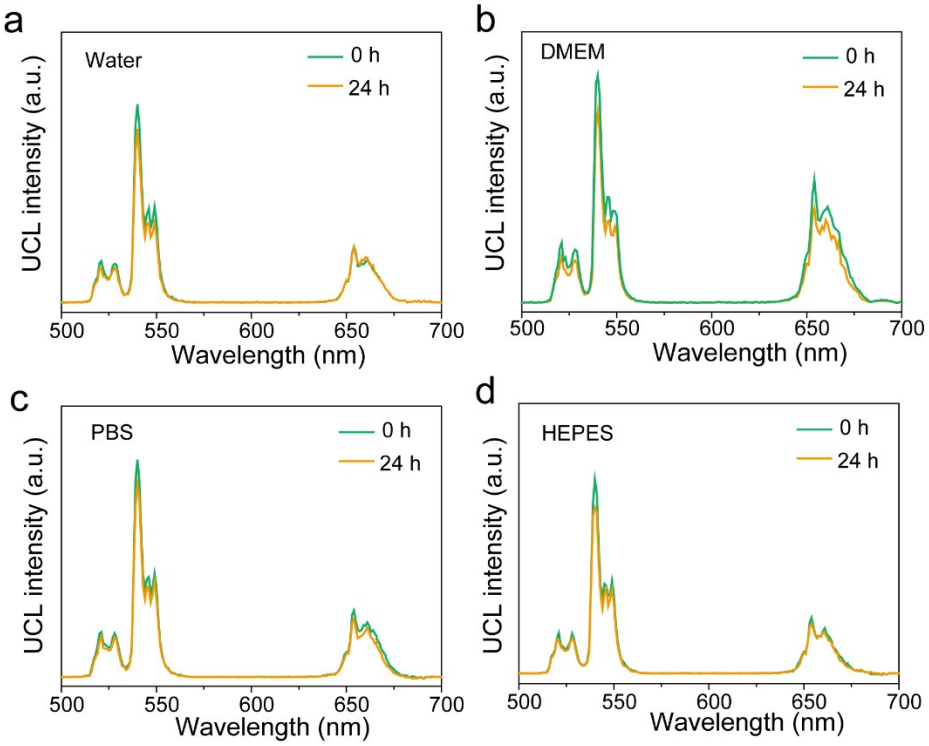


Figure S11. The changes of UCL intensity of UCNPs-Cy787@PC dispersed in different media for 24 h. (a) in water, (b) DMEM, (c) PBS, (d) HEPES. λ_{ex} = 808 nm

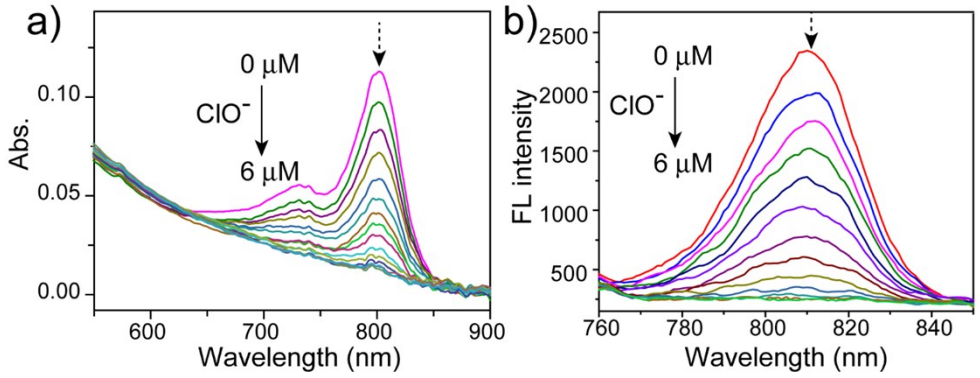


Figure S12. (a) The UV-vis absorption spectra and (b) photoluminescence UCNPs-Cy787@PC in aqueous solution upon gradual addition of NaClO. λ_{ex} = 730 nm.

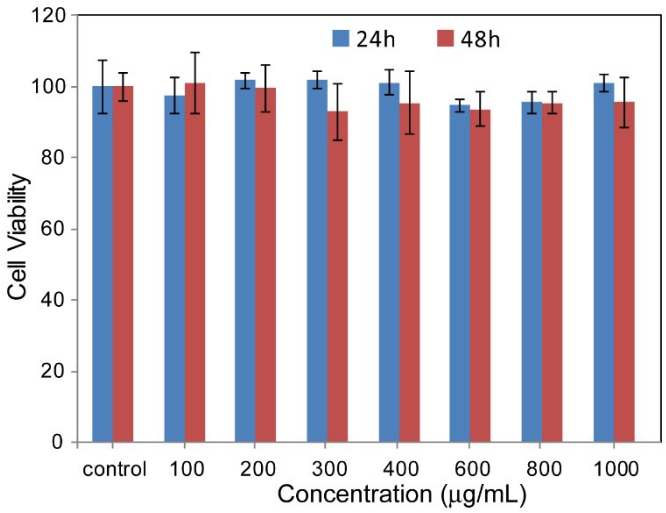


Figure S13. In vitro cell viability of Hela cells incubated with UCNPs-Cy787@PC at different concentration for 24 h and 48 h, respectively.

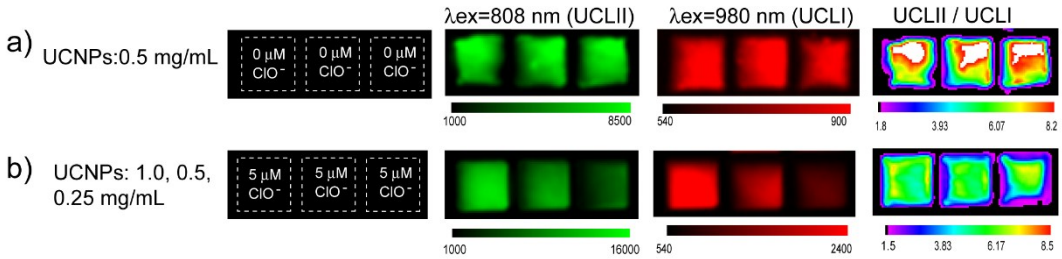


Figure S14. Ratiometric imaging and detection of ClO^- in different environment and different concentration. (a) 0.5 mg/mL UCNPs-Cy787@PC with 0 uM ClO-. (b) 5.0 uM ClO^- with 0.25, 0.5, 1.0 mg/mL UCNPs-Cy787 respectively.

Reference

1 H. Yang, C. Han, X. Zhu, Y. Liu, K. Y. Zhang, S. Liu, Q. Zhao, F. Li and W. Huang, *Adv. Funct. Mater.,* 2016, **26**, 1945-1953.

2 X. Zou, M. Xu, W. Yuan, Q. Wang, Y. Shi, W. Feng and F. Li, *Chem. Commun.,* 2016, **52**, 13389-13392