Supporting Information for

Heterogeneous Core/Shell Fluoride Nanocrystals with Enhanced Upconversion Photoluminescence for In Vivo Bioimaging

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Experimental Section.

Chemicals.

Analytical grade Ho_2O_3, Yb_2O_3, Gd_2O_3, YbO_3, $CaCl_2$, NaF, hydrochloric acid (HCl), cyclohexane, ethanol, were obtained from Beijing Chemical Reagents, China. Oleic acid (OA; 90%, Sigma-Aldrich), Octadecene (ODE, 90%, Sigma-Aldrich). These chemicals and solvents were used as received without further purification. Deionized water was used throughout the whole synthetic procedure. Y_2O_3, Gd_2O_3, Yb_2O_3 and Ho_2O_3 were separately dissolved in dilute HNO_3 by heating to prepare the stock solutions of $Ln(NO_3)_3$(Ln= Y, Gd, Yb, Ho).

Synthesis of CaF_2: 20%Yb^{3+}/2%Ho^{3+} NCs. In a typical procedure, 0.78 mmol of calcium chloride, 0.2 mmol ytterbium nitrate salts, 0.02 mmol holmium nitrate salts were added to a mixture of NaOH (1.2 g), ethanol (5 mL), deionized water (3 mL), and oleic acid (18 mL), and the solution was thoroughly stirred. Then 4 mL of sodium fluoride solution (0.5 mol/L) was added with vigorous stirring until a translucent solution was obtained. Subsequently, the milky colloidal solution was transferred to a 50 mL Teflon-lined autoclave, and heated at 180 °C for 24 h. The systems were then allowed to cool to room temperature. The final products were collected by centrifugation at 6000 rpm for 10 minutes, washed with ethanol, and then dispersed in cyclohexane.

Synthesis of (CaF_2: 20%Yb^{3+}/2%Ho^{3+})@ $NaGdF_4$ NCs. In a typical experiment, 0.5 mmol Gd_2O_3 and 2 mmol NaOH were added to a 50 ml flask containing 2 ml

deionized water and 2 ml trifluoroacetic acid (TFA), and then heated at 90 °C till the solution become transparent. After drying with nitrogen purge, 15 ml oleic acid, 15 ml 1-octadecene(ODE), and the CaF_2: 20%Yb^{3+}/2%Ho^{3+} core NCs (1 mmol) in cyclohexane were added. The solution was then vacuum-degassed at 100 °C for 30 min to remove water, oxygen and cyclohexane. Subsequently, the solution was heated at 140°C for 30 min, and heated to 310 °C at a rate of 15 $K \cdot min^{-1}$ under nitrogen protection. After maintaining at 310 °C for 1 h, the reaction was stopped and cooled down to room temperature. The (CaF_2: 20%Yb^{3+}/2%Ho^{3+}) @$NaGdF_4$ NCs were precipitated by using an excess amount of ethanol and then collected after centrifugation. The resulting NCs then were dispersed in cyclohexane for further use.

Synthesis of $NaYF_4$: 20%Yb^{3+}/2%Ho^{3+}NCs. In a typical experiment, 0.39 mmol Y_2O_3, 0.10 mmol Yb_2O_3, 0.01 mmol Ho_2O_3, and 2 mmol NaOH were added to a 50 ml flask containing 2 ml deionized water and 2 ml trifluoroacetic acid (TFA), and then heated at 90 °C till the solution become transparent. After drying the solution with nitrogen purge, 15ml of oleic acid and 15ml of 1-octadecene (ODE) were added, and then degassed at 100 °C for 30 min to remove water and oxygen. The solution was heated to 140°C for 30 min, and then heated to 310 °C at a rate of 15 $K \cdot min^{-1}$ under nitrogen protection. After maintaining at 310 °C for 1 h, the reaction was stopped and cooled down to room temperature. The $NaYF_4$: 20%Yb^{3+}/2%Ho^{3+} NCs were precipitated by using an excess amount of ethanol, and then collected after centrifugation.

Synthesis of ($NaYF_4$: 20%Yb^{3+}/2%Ho^{3+}) @ $NaYF_4$ NCs. 0.195 mmol Y_2O_3 and 1 mmol NaOH were added to a 50 ml flask containing 2 ml deionized water and 1 ml

trifluoroacetic acid (TFA), and then heated at 90 °C till the solution become transparent. After drying with nitrogen purge, 15 ml oleic acid, 15 ml 1-octadecene(ODE), and the prepared $NaYF_4$: 20%Yb^{3+}/2%Ho^{3+} core NCs (1 mmol) in cyclohexane were added. The solution was then vacuum-degassed at 100 °C for 30 min to remove water, oxygen and cyclohexane. Subsequently, the solution was heated at 140°C for 30 min, and heated to 310 °C at a rate of 15 $K \cdot min^{-1}$ under nitrogen protection. After maintaining at 310 °C for 1 h, the reaction was stopped and cooled down to room temperature. The core/shell NCs were precipitated by using an excess amount of ethanol and then collected after centrifugation. The resulting NCs then were dispersed in cyclohexane for further use.

Synthesis of $NaGdF_4$: 20%Yb^{3+}/2%Ho^{3+}NCs. The $NaGdF_4$: 20%Yb^{3+}/2%Ho^{3+} Yb^{3+} NCs were prepared using the same procedure for the synthesis of $NaYF_4$: 20%Yb^{3+}/2%$Ho^{3+}$$Yb^{3+}$ NCs.

Ligand Exchange of the Oleic Acid with the Polyacrylic Acid. The ligand exchange procedure is adapted from a literature work.S1 In a typical procedure, a mixture of DEG (8.0 mL) and PAA (0.1 g) was heated to 110 ° C with vigorous stirring under N_2 flow. Then, 2 mL chloroform solution of CaF_2: 20%Yb^{3+}/2%Ho^{3+} @$NaGdF_4$ NCs (50 mg/mL) were injected and the system was heated to 240 ° C for 10 min. During this process, the initial milky solution gradually became clear. After cooling to room temperature, an excess of diluted hydrochloric aqueous solution was added in into the solution until a white precipitation is seen. The precipitation was

collected by centrifugation, washed three times with pure water, and neutralized with a diluted solution of sodium hydroxide. Finally, the (CaF_2: 20%Yb^{3+}/2%Ho^{3+} @$NaGdF_4$) NCs are dispersed in deionized water.

Cytotoxicity Assay. Cell viability was measured using a 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) proliferation assay. THP-1 Macrophage cells were seeded in a 96-well flat-bottomed microplate (5000 cells/well) and cultured in 100μL growth medium at 37℃ and 5% CO^2 for 24 h. Then, the culture medium was discharged and the cells were washed with 1 mL PBS and fresh culture medium, respectively. Subsequently, cells were incubated in 1 mL fresh culture medium for another 24 h at 37℃ under 5% CO^2. Incubated with PAA-coated (CaF_2:20%Yb^{3+}/2%Ho^{3+})@$NaGdF_4$ UCNCs at different concentrations ranging from 0 to 90μg mL^{-1} and 0 to 240μg mL^{-1} for 24 h, the cells were washed with 1 mL fresh culture medium (3 times) and PBS (3times). Finally, the number of viable THP-1 Macrophage cells was calculated by measuring the activity of NADH-dependent cellular oxidoreductase in cells.

Instruments.

The powder X-Ray diffraction (XRD) pattern was carried out on a Rigaku D/max-γB diffractometer equipped with a rotating anode and a Cu Kα source (λ=0.154056 nm). The 2θ angle of the XRD spectra was recorded at a scanning rate of 5 °/min. The size and morphology of colloidal CaF_2: 20%Yb^{3+}/2%Ho^{3+}, (CaF_2: 20%Yb^{3+}/2%Ho^{3+})@$NaGdF_4$, $NaYF_4$: 20%Yb^{3+}/2%Ho^{3+} and $NaGdF_4$: 20%Yb^{3+}/2%Ho^{3+} powders were characterized by means of transmission electron

microscope (TEM, Tecnai G2 Spirit Twin 12) operating at 80 kV. High resolution transmission electron microscopic (HRTEM) images were obtained on the microscope of Spirit Twin Tecnai G2 D339 operating at 300 kV. One drop of diluted colloidal nanocrystals solution dispersed in cyclohexane was allowed to be dried on the surface of the carbon-coated copper grid. Upconversion (UC) photoluminescence (PL) spectra were recorded using a lens-coupled monochromator (Zolix Instruments Co. Ltd., Beijing) with a slit width defining spectral resolution of 2 nm. The emissions were excited at 980 nm using a fiber-coupled laser diode (Hi-Tech Optoelectronics Co. Ltd., Beijing). All measurements were performed at room temperature, preserving the same geometry for the upconversion luminescence recording. Photographic UC PL images of colloidal nanocrystals of CaF_2:Ho^{3+}/Yb^{3+} core, CaF_2:Ho^{3+}/Yb^{3+}@$NaGdF_4$ core/shell, and $NaYF_4$:Ho^{3+}/Yb^{3+} were taken by a digital camera (Canon Power Shot SX100 IS) without adding any filter. *In vivo* UC PL images of mouse injected with water-dispersed CaF_2:Ho^{3+}/Yb^{3+}core, (CaF_2:Ho^{3+}/Yb^{3+})@$NaGdF_4$ core/shell, $NaYF_4$:Ho^{3+}/Yb^{3+} and ($NaYF_4$: 20%Yb^{3+}/2%Ho^{3+}) @ $NaYF_4$ nanocrystals were also taken by the digital camera (Canon Power Shot SX100 IS).

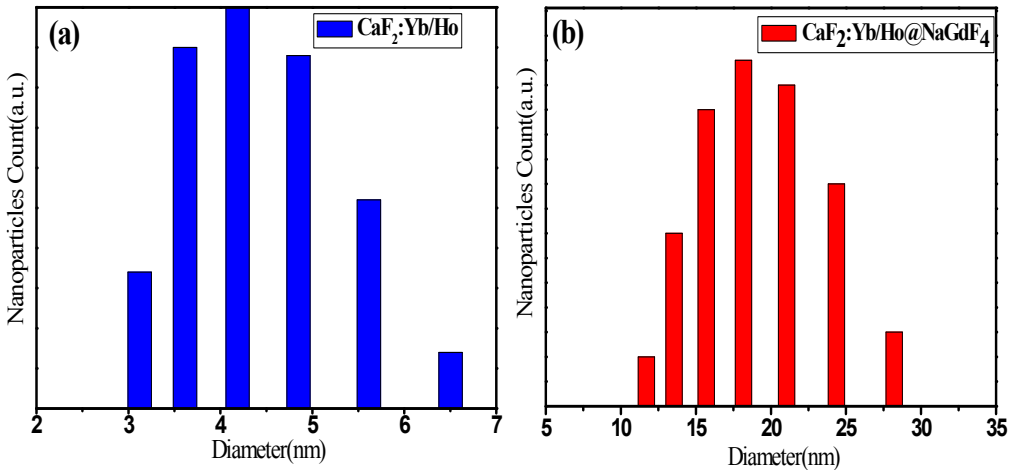

Figure S1. Histograms of the particle sizes of (a) CaF_2: 20%Yb^{3+}/2%Ho^{3+} and (b) (CaF_2: 20%Yb^{3+}/2%Ho^{3+}) @$NaGdF_4$. The average size is found to be around 4 and

17 nm for the core, and core/shell nanocrytals.

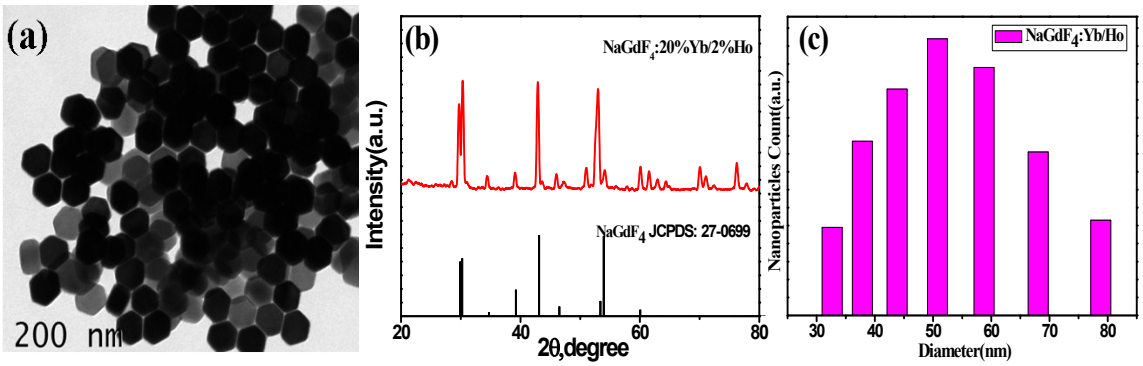

Figure S2. (a) Transmission electron microscope (TEM) images of for hexagonal $NaGdF_4$: 20%Yb^{3+}/2% Ho^{3+} nanocrystals; (b) x-ray diffraction (XRD) pattern of $NaGdF_4$: 20%Yb^{3+}/2% Ho^{3+} nanocrystals, (c) the size distribution of $NaGdF_4$: 20%Yb^{3+}/2% Ho^{3+} nanocrystals.

The resulting $NaGdF_4$: 20%Yb^{3+}/2% Ho^{3+} nanocrystals are of hexagonal shape and have an average size of 50 nm. The XRD pattern in (b) indicates that the resulting $NaGdF_4$: 20%Yb^{3+}/2% Ho^{3+} nanocrystals have a hexagonal crystal phase.

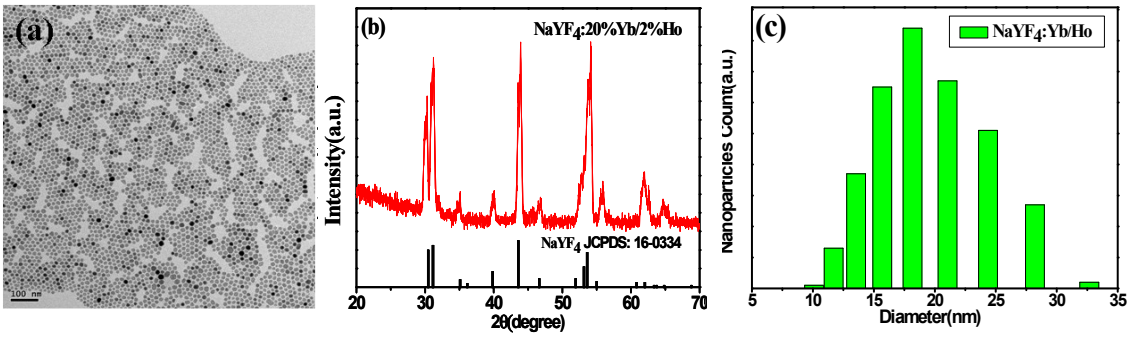

Figure S3. (a) TEM image of for $NaYF_4$: 20%Yb^{3+}/2% Ho^{3+} nanocrystals; (b) x-ray diffraction (XRD) pattern of $NaYF_4$: 20%Yb^{3+}/2% Ho^{3+} nanocrystals, (c) the size distribution of $NaYF_4$: 20%Yb^{3+}/2% Ho^{3+} nanocrystals.

As shown in Figure S3, the resulting $NaYF_4$: 20%Yb^{3+}/2% Ho^{3+} nanocrystals are spherical with an average size of 18 nm. The XRD pattern in (b) indicates that the resulting $NaYF_4$: 20%Yb^{3+}/2% Ho^{3+} nanocrystals are of hexagonal structure.

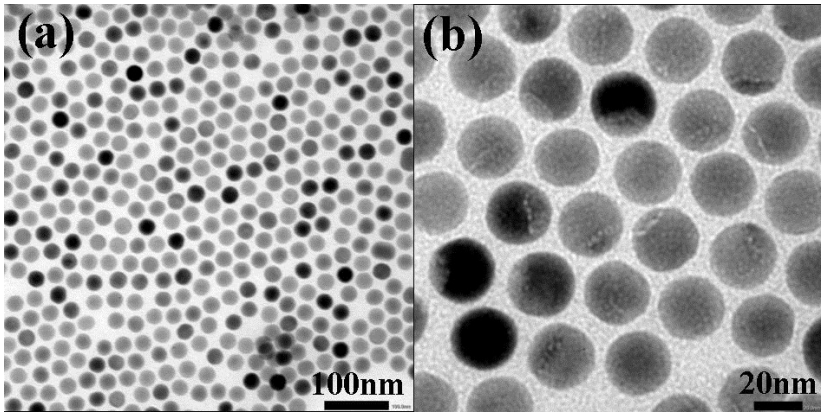

Figure S4. (a) TEM image and (b) magnified TEM image of prepared $NaYF_4$: ($NaYF_4$:20%Yb^{3+}/2%Ho^{3+})@$NaYF_4$ core/shell nanocrystals.

As shown in Figure S4, the resulting $NaYF_4$: ($NaYF_4$:20%Yb^{3+}/2%Ho^{3+})@$NaYF_4$ nanoparticles are spherical with an average size of 28 nm. The increase of the size suggests that the $NaYF_4$ shell with a thickness of 5 nm has been successfully grown on the $NaYF_4$: 20%Yb^{3+}/2% Ho^{3+} nanoparticles.

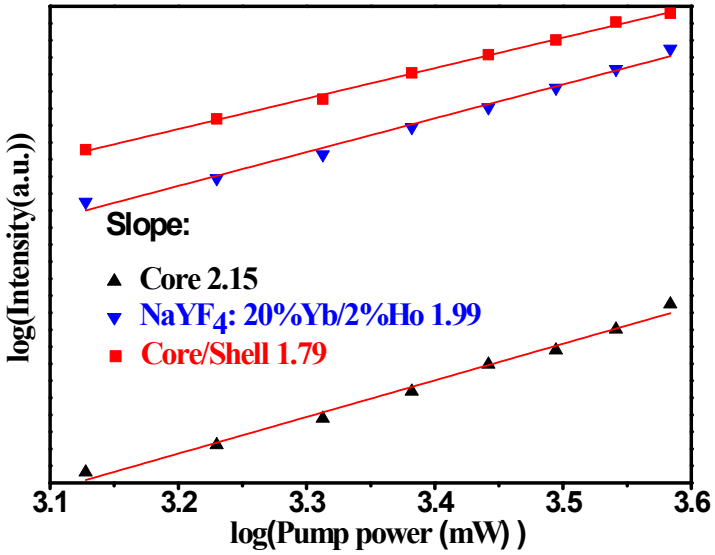

Figure S5. The dependence of the intensity of UC PL at 540 nm on the excitation power in colloidal NCs of CaF_2: 20%Yb^{3+}/2%Ho^{3+} (core), $NaYF_4$: 20%Yb^{3+}/2%Ho^{3+} NCs, and (CaF_2: 20%Yb^{3+}/2%Ho^{3+}) @ $NaGdF_4$(core/shell).

The number of photon process can be derived from the relation, $I_f \propto P^n$, where I_f is the fluorescent intensity, P is the pump laser power and n is the number of the laser

photons required to populate the upper emitting state under unsaturated condition and its value can be obtained from the slope of the line in the plot of log I_f versus log p. As one can see in Figure S5, a slope value of 2.15, 1.99, and 1.79 are observed for the CaF_2: 20%Yb^{3+}/2%Ho^{3+} (core), $NaYF_4$: 20%Yb^{3+}/2%Ho^{3+} , and (CaF_2: 20%Yb^{3+}/2%Ho^{3+}) @ $NaGdF_4$ (core/shell) NCs, illustrating involvement of the two-photon process in the generation of the UCPL peaked at 540 nm. It is noted that the slope value of the core/shell NCs is smaller than that of the core NCs, which clearly shows that the epitaxial growth of undoped $NaGdF_4$ shell can produce a larger UC rate at the intermediate state.

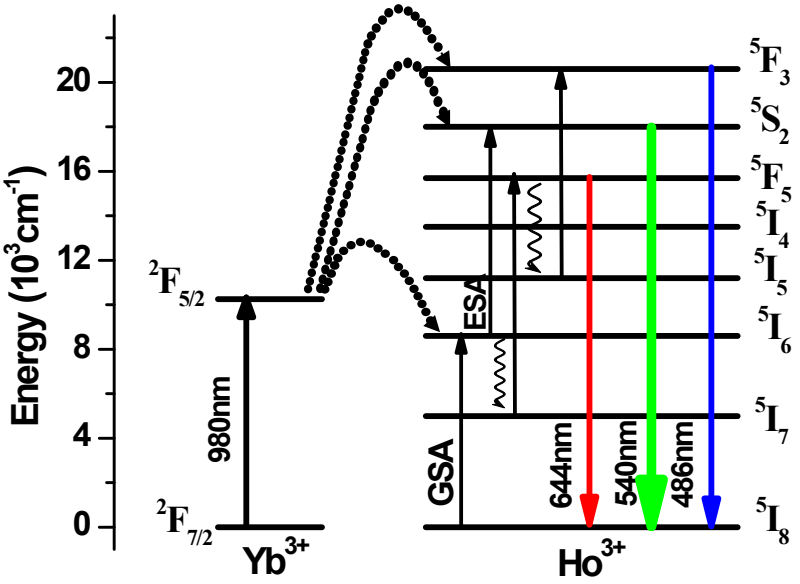

Figure S6. Energy level diagram of the Ho^{3+} and Yb^{3+} ions as well as the UC mechanism proposed to produce the green, red and blue emission under a 980 nm laser excitation.

Yb^{3+} ion absorbs one laser photon and thus, is excited from the ground $^2F_{7/2}$ state to the $^2F_{5/2}$ state. Then, the Yb^{3+} ion transfers its absorbed energy to the neighboring Ho^{3+} ions to populate the $^5I_6$ excited level. The energy difference between the two levels was abridged by the vibration energy of the host lattice. The population in the

$^5I_6$ level can be promoted to the $^5S_2$ levels either by excited state absorption (ESA) or by energy transfer (ET) from another excited Yb^{3+} ion. Once the $^5S_2$ level is populated, the excited electron can release its energy by emitting green emissions. The red emission at 644 nm can be produced by radiative decay to the ground state from the $^5F_5$ state, which can be populated by two possible ways. (i) Excited from the intermediate $^5I_7$ using the energy transfer process of $^2F_{5/2}(Yb^{3+})$ $+^5I_7$ (Ho^{3+}) $\rightarrow ^2F_{7/2}(Yb^{3+})$ + $^5F_5$ (Ho^{3+}), (ii) multiphonon-assisted relaxations from the $^5S_2$ excited levels to the $^5F_5$ level. Some ions at the $^5I_5$ level can be promoted to the $^5F_3$ level, from which weak emission at 486 nm is produced.

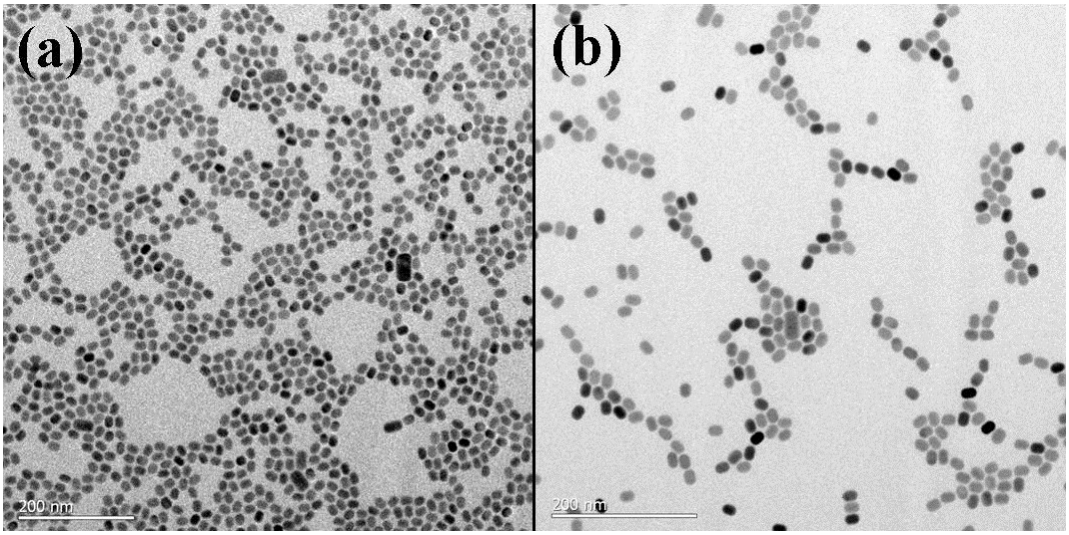

Figure S7. TEM images of (CaF_2: 20%Yb^{3+}/2%Ho^{3+}) @ $NaGdF_4$ (core/shell) NCs (a) before and (b) after ligand exchange.

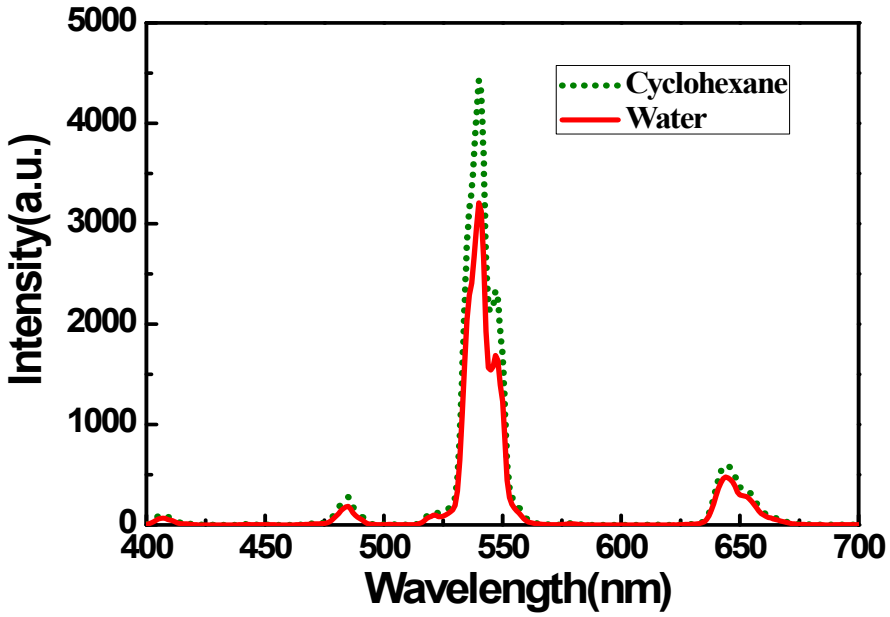

Figure S8. The UC PL of (CaF_2: 20%Yb^{3+}/2%Ho^{3+}) @ $NaGdF_4$ NCs dispersed in cyclohexane (dashed green line) and in water (solid red line) upon NIR laser diode excitation at 980 nm. Excited at 980 nm of 70 W/cm^2.

The UC PL of (CaF_2: 20%Yb^{3+}/2%Ho^{3+}) @ $NaGdF_4$ NCs dispersed in water (after ligand exchange) shows a slight reduction, compared with that of core/shell NCs prior to ligand exchange (dispersed in cyclohexane).

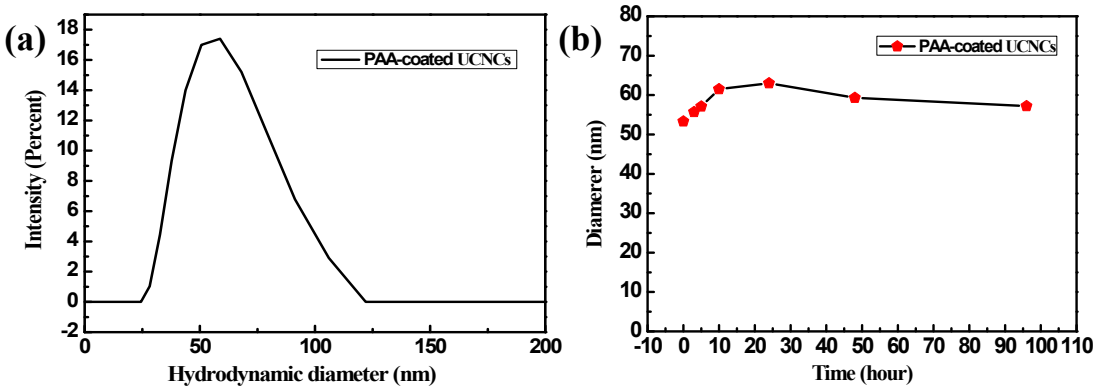

Figure S9. Hydronamic size (a) and stability (b) of PAA-coated (CaF_2:20%Yb^{3+}/2%Ho^{3+})@$NaGdF_4$ UCNCs with a dispersion concentration of 2 mg/mL in phosphate buffered saline (PBS). The dynamic light scattering (DLS)

measurements were performed on a Malvern Nano S90.

Figure S9a shows that PAA-coated UCNCs have a hydrodynamic diameter of about 53 nm. Compared with the size of (CaF_2:20%Yb^{3+}/2%Ho^{3+})@$NaGdF_4$ shown in TEM images (~ 24 nm), the significant size increase can be attributed to the carboxyl groups in PAA that interact with aqueous environment. We found that the hydrodynamic size remained almost unchanged for around ~ 96 hours, indicating the high stability of PAA-coated UCNCs (Figure S9b) that makes them suitable for bioapplications.

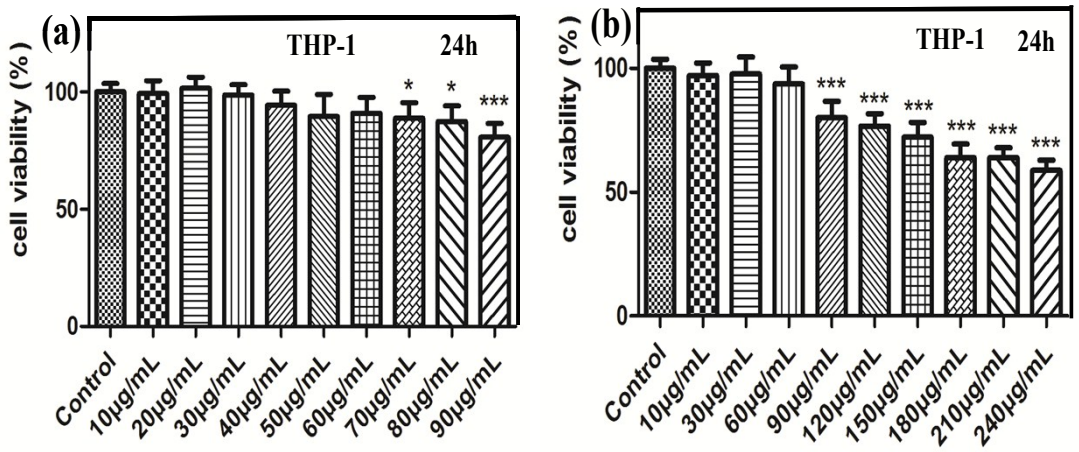

Figure S10. Cell viability of two groups of THP-1 macrophage cells incubated with PAA-coated (CaF_2:20%Yb^{3+}/2%Ho^{3+})@$NaGdF_4$ UCNCs at 37 ℃ for 24 h, using different concentrations (a: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 μg/mL) and (b: 0, 10, 30, 60, 90, 120, 150, 180, 210, 240 μg/mL).

Methyl thiazolyl tetrazolium (MTT) assay have been employed to test the in vitro toxicity of PAA-coated (CaF_2:20%Yb^{3+}/2%Ho^{3+})@$NaGdF_4$ UCNCs using two groups of THP-1 macrophage cells, and the results are shown in Figure S10. A nearly 100% cellular viability was observed at a concentration of 60 μg mL^{-1}, and the cellular viability can be higher than 80% even at a concentration as high as 120 μg mL^{-1}.

However, the cellular viability decreased ~ 60-70%, showing a low toxicity of these PAA-coated core/shell NCs at a higher doses of 240 μg mL^{-1}.

Reference:

S1. T. Zhang, J. Ge, Y. Hu and Y. D. Yin, Nano Lett., 2007, 7, 3203.