Supporting Information for

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

Shuwei Hao, a Liming Yang, b Hailong Qiu, a Rongwei Fan, d Chunhui Yang*, a, e and Guanying Chen*, a, c

a School of Chemical Engineering and Technology, Harbin Institute of Technology, Heilongjiang 150001, People’s Republic of China

b Institute for Lasers, Photonics and Biophotonics, State University of New York at Buffalo, Buffalo, New York 14260, USA

c Department of Pathophysiology, Harbin Medical University, 150081 Harbin, China

d National Key Laboratory of Tunable Lasers, Institute of Optical-Electronics, Harbin Institute of Technology, 150001 Harbin, People’s Republic of China

e Harbin Huigong Technology Co., Ltd.

Corresponding Emails: chenguanying@hit.edu.cn, yangchh@hit.edu.cn
Experimental Section.

Chemicals.

Analytical grade Ho$_2$O$_3$, Yb$_2$O$_3$, Gd$_2$O$_3$, 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$_2$O$_3$, Gd$_2$O$_3$, Yb$_2$O$_3$ and Ho$_2$O$_3$ 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$_2$O$_3$ 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·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$_2$O$_3$, 0.10 mmol Yb$_2$O$_3$, 0.01 mmol Ho$_2$O$_3$, 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·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$_2$O$_3$ 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 NaYF₄: 20%Yb³⁺/2%Ho³⁺ 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·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 NaGdF₄: 20%Yb³⁺/2%Ho³⁺ NCs.** The NaGdF₄: 20%Yb³⁺/2%Ho³⁺ Yb³⁺ NCs were prepared using the same procedure for the synthesis of NaYF₄: 20%Yb³⁺/2%Ho³⁺ Yb³⁺ 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 N₂ flow. Then, 2 mL chloroform solution of CaF₂: 20%Yb³⁺/2%Ho³⁺ @NaGdF₄ 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°C 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°C 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](image-url)  
**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 nanocrystals.

Figure S2. (a) Transmission electron microscope (TEM) images of 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 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 S4](image)

**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.

![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.](image)

**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 $^2F_{5/2}(Yb^{3+}) + ^5I_7(\text{Ho}^{3+}) \rightarrow ^2F_{7/2}(Yb^{3+}) + ^5F_5(\text{Ho}^{3+})$, (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.

![Graph](image)

**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 °C 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: