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Supporting Information

Yb/Er/Ho-engineered rare earth fluoride nanoparticles to unlock multimodal *in vivo* medical imaging

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Additional Experimental Section

Characteristic of the Quantum Yield. The quantum yield of samples was measured in an Edinburgh FLS 980 fluorescence spectroscope, which was used to monitor the 980 nm excitation (0.5 W/cm^2) and emission (visible light (300-700 nm) and NIR light (1100-1700 nm), respectively) photons. The emission and reflected excitation photons were scattered and collected by an integrating sphere (Figure S3) and then detected by a photomultiplier (PMT). The samples were dispersed in cyclohexane and pure cyclohexane was used as the reference. The absolute quantum yield (QY) is determined by QY = P sample /(Sref-Ssample), where P sample is the emission intensity, Sref and Ssample are the intensities of the scattered light in the presence of the reference and sample, respectively.

***In vitro* biocompatibility.** The biocompatibility of PEG-modified $NaGdF_4$:15%Yb, 15%Er, 15%Ho NPs against L929 cells were measured. L929 cells were seeded onto 96-well plates in 5% CO_2 at 37 °C for 24 h to allow the attachment of cells. A sample solution of volume 100 μL and of different concentrations of (15.6, 31.5, 62.5, 125, 250, and 500 μg mL^{-1}) was added to each well, and then, the cells were incubated for 24 h. Then, 20 μL of MTT solution (5 mg mL^{-1}) was added into each well, and the cells were incubated for another 4 h. The absorbance at 490 nm was obtained to calculate the cell survival rate using the plate reader.

***In vitro* cellular uptake.** The cellular uptake was evaluated in HeLa cells by using a CLSM. 4T1 cells were seeded in a 6-well plate containing 28-mm cover glass at 1×10^5 cells each well and cultured overnight for adherence. 1 mL of FITC-labeled samples

(100 μg mL^{-1}) was incubated into the 6-well plate for various time intervals (0.5, 1, and 3 h). Then, the cells were stained with DAPI for 15 min by following the manufacturer's protocol. After incubation, the HeLa cells were washed with PBS three times and fixed with glutaraldehyde (2.5%). At last, the fluorescence images were recorded employing a CLSM.

***In vitro* and *in vivo* T_1/T_2-weighted MRI.** MRI was measured by an animal MRI scanner (BioSpec 94/20USR 9.4 T). We put samples aqueous solutions with different Gd concentrations (0, 0.05, 0.1, 0.2, 0.4, and 0.8 mmol L^{-1}) for T_1-weighted MRI and different Ho concentrations (0, 0.0125, 0.025, 0.05, 0.1 and 0.2 mmol L^{-1}) for T_2-weighted MRI at in 1.5 mL centrifuge tubes. T_1 and T_2 signal intensity at various sample concentrations was measured. The *in vivo* T_1/T_2 -weighted MRI experiments were obtained by intravenously injecting 100 μL of PEG-modified $NaGdF_4$:15%Yb, 15%Er, 15%Ho NPs ([Gd] = 5 mg kg^{-1}) and ([Ho] = 5 mg kg^{-1}) solution. MR imaging was acquired at preinjection, 0, 3, 6, 9, and 12 h post-injection of samples. All animal experiments were approved by the Drug Safety Evaluation Center of Harbin Medical University, Harbin, China. Female BALB/c mice (18~22 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The procedure of animal experiments was implemented in accordance with the Guidelines for Care and Use of Laboratory Animals of the Drug Safety Evaluation Center of Harbin Medical University. Female BALB/c mice were subcutaneously transplanted with 4T1 cancer cells (100 μL), and the tumors grew to 60 mm^3 before the experiment was started.

***In vitro* and *in vivo* X-ray CT imaging.** *In vitro* and *in vivo* CT images were captured by a Quantum FX microCT scanner under photon energy of 90 kVp. For CT imaging *in vitro*, the PEG-modified $NaGdF_4$:15%Yb, 15%Er, 15%Ho NPs were dispersed in saline with various concentrations (0, 1, 2, 4, 8, and 16 mg mL^{-1}) and then placed in a succession of 1.5 mL tubes. To acquire the CT imaging *in vivo*, firstly, the tumor-bearing BALB/C mice were anesthetized with 1% pentobarbital sodium (dosage: 50 mg kg^{-1}) by intraperitoneal administration. Subsequently, PEG-modified $NaGdF_4$:15%Yb, 15%Er, 15%Ho NPs were intratumorally (50 μL) injected for CT imaging. The mice were scanned before and after the injection of the nanoparticles.

***In vitro* and *in vivo* NIR-II fluorescence imaging.** *In vitro* and *in vivo* NIR-II images were captured by a liquid-nitrogen-cooled, small animal imaging system (In Vivo Master, Wuhan Grand-imaging Technology Co., LTD). For NIR-II fluorescence imaging (centred at 1525 and 1155 nm) *in vitro*, the PEG-modified $NaGdF_4$:15%Yb, 15%Er, 15%Ho NPs with different Er and Ho concentrations were dispersed in saline then placed in a succession of 1.5 mL tubes under 980 nm irradiation. *In vivo* NIR-II fluorescence images of 4T1 tumor-bearing BALB/c mice were obtained after intratumoral injection of PEG-modified $NaGdF_4$:15%Yb, 15%Er, 15%Ho NPs (50 μL) at the tumor sites under 980 nm irradiation with [Er] = 5 mg kg^{-1} and [Ho] = 5 mg kg^{-1}, respectively. The mice were scanned before and after the injection of the nanoparticles.

Biodistribution of PEG-modified $NaGdF_4$:15%Yb, 15%Er, 15%Ho NPs. The biodistribution of PEG-modified $NaGdF_4$:15%Yb, 15%Er, 15%Ho NPs *in vivo* was studied after injecting it (200 μg mL^{-1}, 100 μL) into tumor-bearing mice intravenously.

Main organs (including heart, lungs, liver, spleen, and kidneys) were collected of the mice after different injection times (3, 6, 9, 12, 15 and 24 h). The samples were weighed and dissolved in a mixture of HNO_3 and H_2O_2 solution at 70 °C to achieve clear solutions. The mixture was then centrifuged to obtain the supernatant for the ICP-OES. The samples concentrations in the supernatants were presented as the percentage of injected dose pergram of tissue (%ID g^{-1}).

Histological examination. Histological analysis was conducted after 7 days of injection. Representative mice from treatment groups were sacrificed. Then, main organs were collected for histopathological analysis. These last stained sections were observed using Leica TCS SP8 instrument.

Statistical analysis. Quantitative data are indicated as mean ± S.D. Means were compared using the student's t-test. Statistical significance was assumed at a value of ***$p < 0.001$, **$p < 0.01$, or *$p < 0.05$.

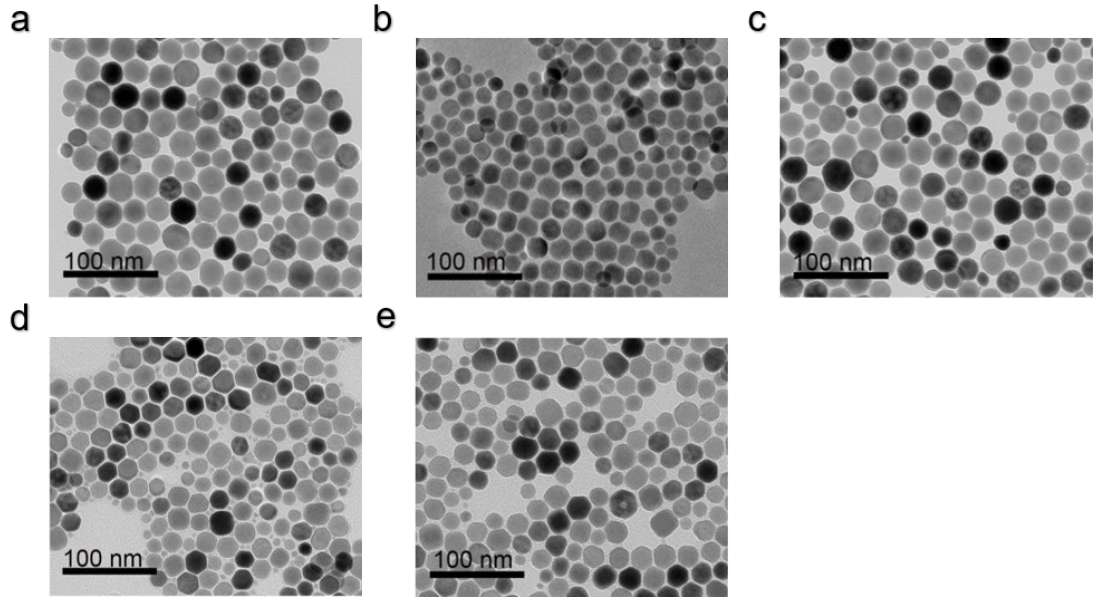

Figure S1 TEM images of $NaGdF_4$: 15%Yb, x%Er NPs: (a) 5% Er, (b) 10% Er, (c) 15% Er, (d) 20%Er, (e) 25% Er.

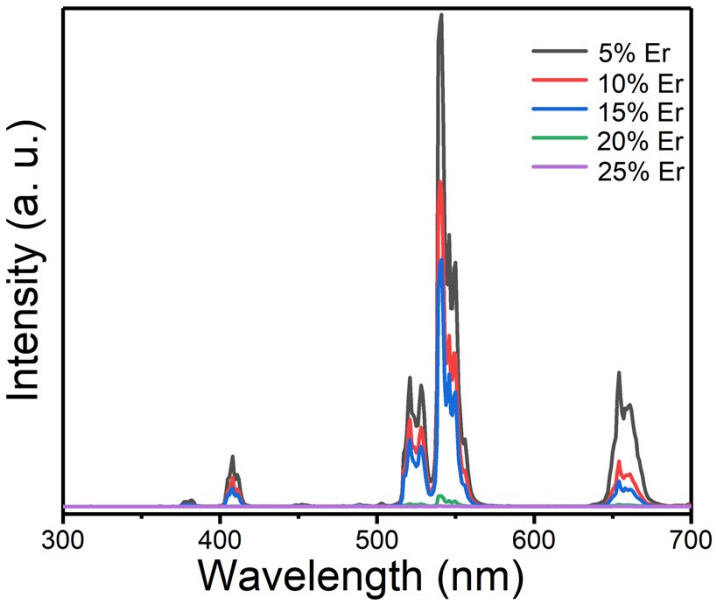

Figure S2 Upconversion emission spectra of $NaGdF_4$:15%Yb, x%Er.

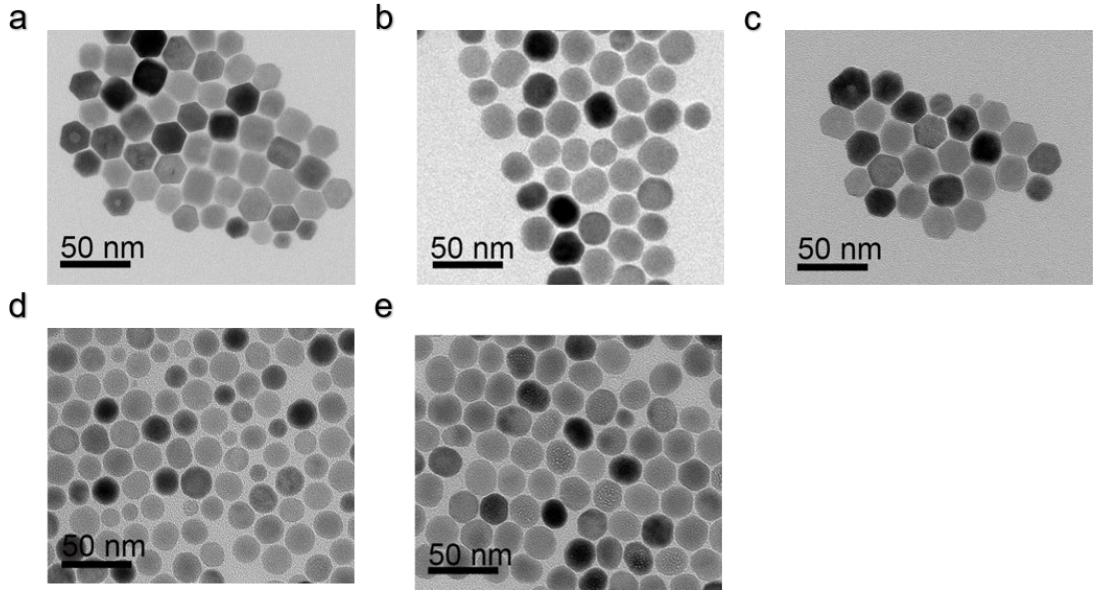

Figure S3 TEM images of $NaGdF_4$: 15%Yb,15%Er, x%Ho NPs: (a) 5% Ho, (b) 10% Ho, (c) 15% Ho, (d) 20% Ho, (e) 25% Ho.

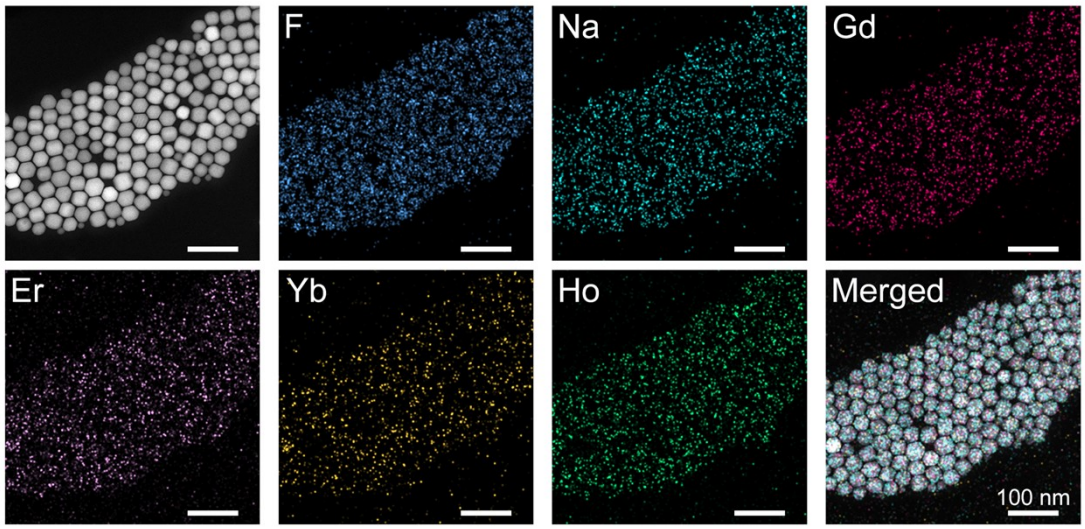

Figure S4 The HAADF and elemental mapping images of $NaGdF_4$: 15%Yb,15%Er, 15%Ho NPs.

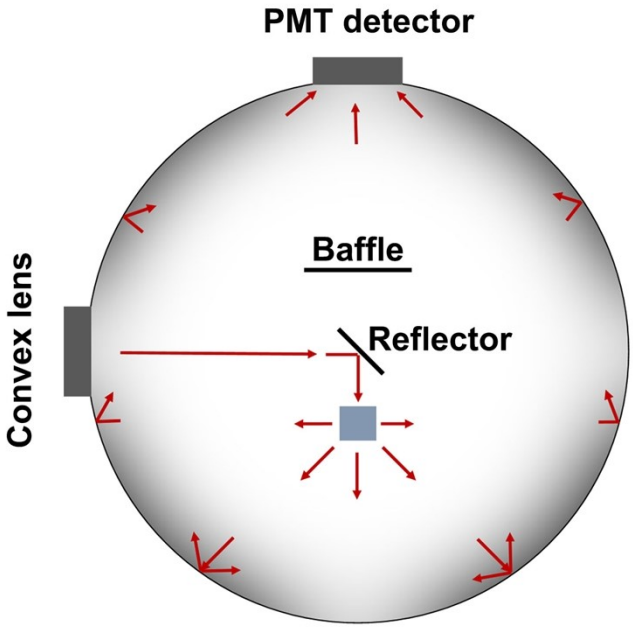

Figure S5 Experimental set-up for the measurement of quantum yield and the light scatter, catch process.

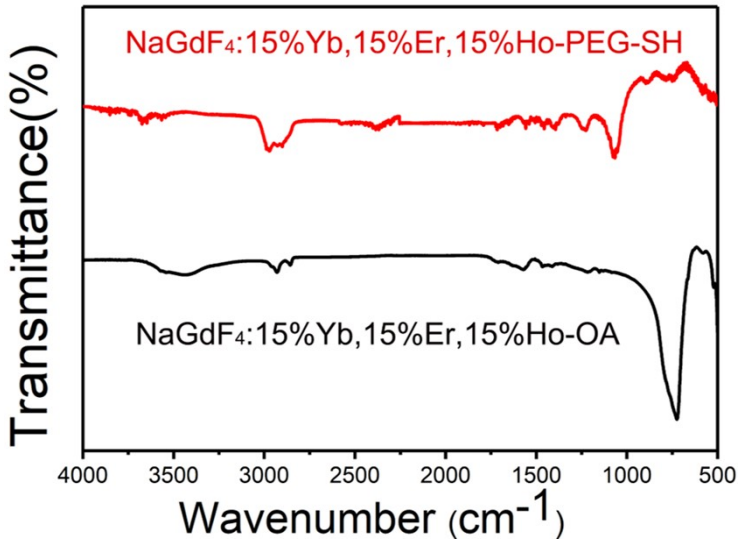

Figure S6 FTIR spectra of NaGdF4:15%Yb, 15%Er, 15%Ho before and after hydrophobic modification.

Figure S7 *In vivo* NIR-II fluorescence images of 4T1 tumor-bearing BALB/c mice under (a) 980 nm irradiation: bright field, (b) downconversion luminescence, and (c) merged images.