Electronic Supplementary Information

Sub-20 nm sandwich-structured $NaGdF_4$:Yb/Tm@$NaLuF_4$:Yb/Tm@$NaYF_4$ nanocrystals for *in vivo* upconversion luminescence/computed tomography imaging

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Materials and Chemicals

$Gd(CH_3COO)_3{\cdot}xH_2O$ (99.9%), $Yb(CH_3COO)_3{\cdot}xH_2O$ (99.9%), $Tm(CH_3COO)_3{\cdot}xH_2O$ (99.9%), $Lu(CH_3COO)_3{\cdot}xH_2O$ (x≈4, 99.9%), $Y(CH_3COO)_3{\cdot}4H_2O$ (99.9%), oleic acid (technical grade, 90%) and 1-octadecene (technical grade, 90%) were purchased from Alfa Aesar Co., Ltd. 1, 2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-{methoxy-[poly(ethylene glycol)]-2000} (DSPE-PEG 2000) was purchased from Shanghai Advanced Vehicle Technology (China). NH_4F and NaOH were of analytical reagent grade.

Instrumentation and Characterization

Low-resolution transmission electron microscopy (TEM) were performed on a JEOL-100CX-Ⅱ microscope (JEOL, Japan). High-resolution TEM (HRTEM) experiments and energy-dispersive X-ray analysis (EDXA) were performed on Philips Tecnai G^2 F20 microscope (Philips, Eindhoven, The Netherlands) with an accelerating voltage of 200 kV. The X-ray diffraction (XRD) pattern was collected on a Rigaku D/max-2500 X-ray diffractometer (Rigaku, Tokyo, Japan) with Cu Kα radiation (λ = 0.15418 nm). X-ray photoelectron spectroscopy (XPS) data were collected on an Axis Ultra DLD spectrometer (Kratos Analytical, Manchester, UK). Fourier transform infrared (FTIR) spectra in KBr were recorded on a Magna-560 spectrometer (Nicolet, Madison, WI). Thermogravimetric analysis (TGA) experiments were performed on a thermal gravimetric analyzer (Rigaku, Japan) from room temperature to 700 °C. Upconversion fluorescence spectra were recorded on a PTI spectrofluorometer (Photon Technology International, Birmingham, NJ,

USA) with an external and adjustable (0-0.5 W) 980 nm laser excitation source. UV-3600 UV-vis-NIR spectrophotometer (Shimadzu, Japan) was used to collect the near-infrared absorption spectra.

Synthesis of $NaGdF_4$:Yb/Tm(20/1 mol%) Core Nanocrystals (NCs)

In a typical experiment, oleic acid (2.5 mL) and 1-octadecene (7.5 mL) were added to a 50 mL flask containing corresponding rare-earth acetate (total amount, 0.4 mmol). The mixture was heated to 150 °C and kept for 1 h to obtain a transparent solution. After the mixture was cooled down to room temperature, a methanol solution (5 mL) containing NH_4F (1.6 mmol) and NaOH (1 mmol) was added. Subsequently, the mixture was stirred for 10 h at room temperature followed by stirring for another 30 min at 50 °C and increasing the reaction temperature to 100 °C to remove the methanol. The mixture was then heated to 280 °C (~10 °C min^{-1}) and kept for 1.5 h under an argon atmosphere, then cooled down to room temperature. The resulting $NaGdF_4$:Yb/Tm NCs were collected by centrifugation after addition of ethanol (10 mL), washed three times with ethanol and finally re-dispersed in cyclohexane. The $NaGdF_4$ NCs were prepared and collected in parallel.

Synthesis of $NaGdF_4$:Yb/Tm(20/1 mol%)@$NaLuF_4$:Yb/Tm(20/0.5 mol%) NCs

The shell stock solution was obtained by mixing $Ln(CH_3COO)_3$ (0.4 mmol) (Ln: 79.5 mol% Lu + 20 mol% Yb + 0.5 mol% Tm) in a 50 mL flask containing oleic acid (3 mL) and 1-octadecene (7 mL). The mixture was heated to 150 °C and kept for 1 h to obtain a transparent solution, then cooled down to 50 °C. $NaGdF_4$:Yb/Tm core NCs (0.4 mmol) in cyclohexane (3 mL) were added and stirred for 10 min. Subsequently, a methanol solution (5 mL) containing NH_4F (1.6 mmol) and NaOH (1 mmol) was added followed by stirring at 50 °C for 30 min and increasing the reaction temperature to 100 °C to remove the methanol. The mixture was heated to 300 °C and kept for 1 h under an argon atmosphere, then cooled to room temperature. The resulting NCs were collected by centrifugation after addition of ethanol (10 mL), washed three times with ethanol and finally re-dispersed in cyclohexane. The $NaGdF_4$@$NaLuF_4$:Yb/Tm(20/0.5 mol%) NCs were prepared and collected in parallel.

Synthesis of $NaGdF_4$:Yb/Tm(20/1 mol%)@$NaLuF_4$:Yb/Tm(20/0.5 mol%)@$NaYF_4$ (GdLuY) NCs

The synthesis procedures for shell coating of $NaYF_4$ on $NaGdF_4$:Yb/Tm@$NaLuF_4$:Yb/Tm NCs were similar to those for shell coating of $NaLuF_4$:Yb/Tm on $NaGdF_4$:Yb/Tm NCs except that $Y(CH_3COO)_3$ (0.4 mmol) was used to prepare the shell stock solution. The $NaGdF_4$@$NaLuF_4$:Yb/Tm(20/0.5 mol%)@$NaYF_4$ NCs were prepared and collected in parallel.

Synthesis of $NaYF_4$:Yb/Tm(20/0.5 mol%) NCs

Typically, oleic acid (3 mL) and 1-octadecene (7 mL) were added to a 50 mL flask containing corresponding rare-earth acetate (total amount, 0.4 mmol). The mixture was heated to 150 °C and kept for 1 h to obtain a transparent solution. After the mixture was cooled down to room temperature, a methanol solution (5 mL) containing NH_4F (1.6 mmol) and NaOH (1 mmol) was added. Subsequently, the mixture was stirred at 50 °C for 30 min followed by increasing the reaction temperature to 100 °C to remove the methanol. The mixture was heated to 300 °C and kept for 1 h under an argon atmosphere, then cooled down to room temperature. The resulting NCs were collected by centrifugation after addition of ethanol (10 mL), washed three times with ethanol and finally re-dispersed in cyclohexane.

Surface Modification of Oleic Acid Capped GdLuY NCs

DSPE-PEG 2000 capped GdLuY NCs (DPGLY NCs) were prepared according to Li et al.[1] Dispersion of oleic acid capped GdLuY NCs in chloroform (10 mL, 10 mg mL^{-1}) was added into DSPE-PEG 2000 solution in chloroform (10 mL, 10 mg mL^{-1}), followed by stirring for 10 min. The chloroform was slowly evaporated at room temperature and then the mixture heated to 60 °C and kept for 1 h under vacuum. Thereafter, water (10 mL) was added. Finally, DPGLY NCs were purified by filtration (0.22 μm membrane filter) and centrifugation.

Cytotoxicity Assay of the DPGLY NCs

In vitro cytotoxicity of the DPGLY NCs on the NIH 3T3 cells was evaluated by a standard methyl thiazolyl tetrazolium (MTT) assay. Cells were seeded into 96-well plates with a concentration of 5000 cells/well and cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine plasma at 37°C and under 5% CO_2 overnight. Subsequently, the medium was replaced by fresh DMEM in the presence of DPGLY NCs (0, 100, 200, 400, 600 and 800 μg mL^{-1}). After incubation for another 24 h, the cell viability was measured by standard MTT assay.

In Vivo UCL Imaging

Nude mice (BALB/c) (~ 16 g) were obtained from HFK bioscience Co., Ltd. (Beijing, China). All animal procedures were in agreement with the guidelines of the regional ethic committee for animal experiments. A NightOWL LB 983 Imaging System (Berthold, Bad Wildbad, Germany) equipped with an external and adjustable continuous-wave excitation source (980 nm, BWT Beijing Ltd, China) was used to collect the in vivo UCL images. A normal nude mouse was anesthetized with chloral hydrate through intraperitoneal injection (400 mg kg^{-1}) and administered

with DPGLY NCs (100 μL, 3 mg mL^{-1}) via tail vein injection, followed by in vivo UCL imaging. UCL signals were collected at 790 ± 20 nm by the CCD camera with constant exposure time.

X-ray Attenuation Measurements and In Vivo CT Imaging of Kunming Mice

Water dispersions of the DPGLY NCs were placed in a series of 1.5 mL tubes for X-ray attenuation measurements using a clinical GE discovery CT750 HD scanner. Kunming mice (~ 20 g) were also obtained from HFK bioscience Co., Ltd. (Beijing, China). For *in vivo* CT imaging, the DPGLY NCs (500 μL, 30 mg mL^{-1}) were administered to a Kunming mouse via tail vein injection under isoflurane anesthesia. *In vivo* CT imaging was carried out on Siemens Inveon CT system, imaging parameters were as follows: 80 kV, 400 μA; exposure time, 800 ms; field of view, 50 mm × 70 mm.

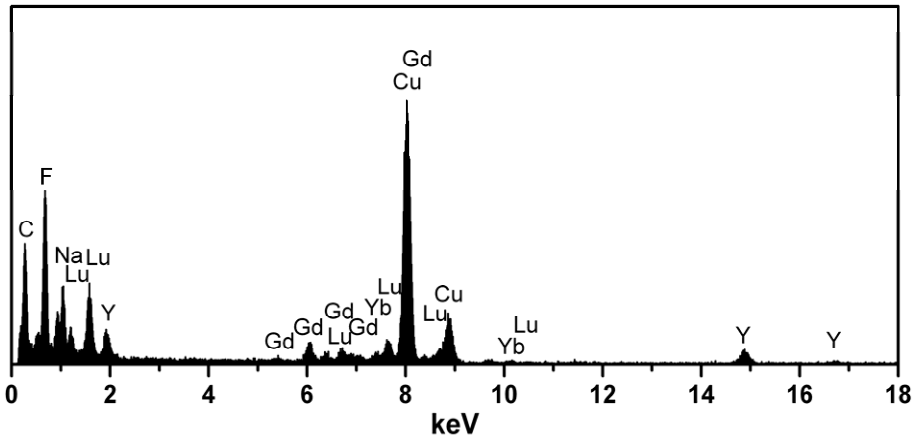

Fig. S1. EDXA of elemental composition of oleic acid capped GdLuY NCs.

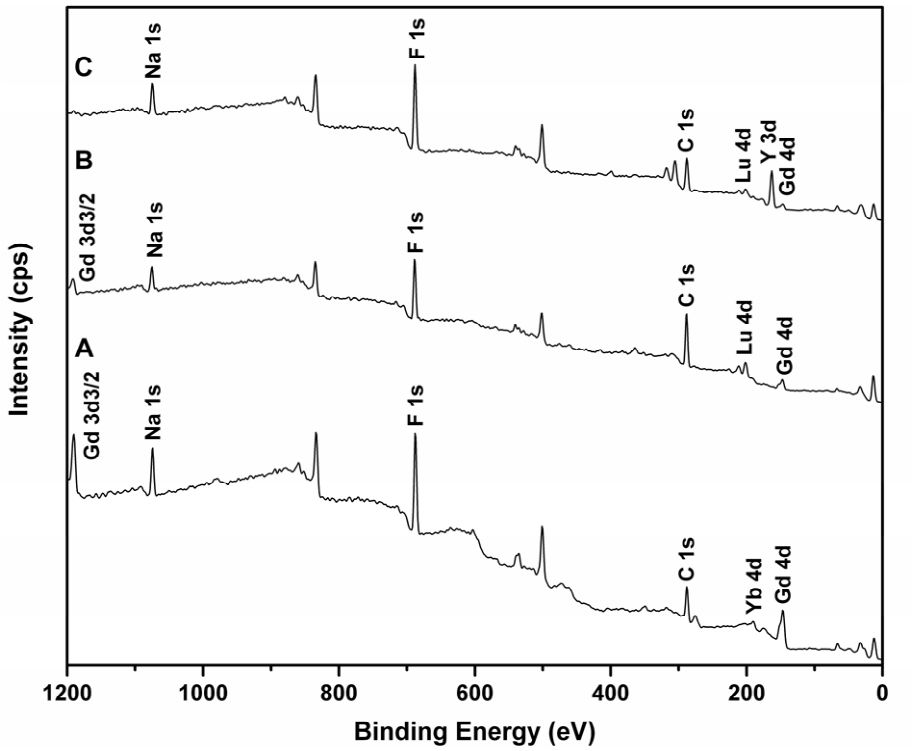

Fig. S2. XPS spectra: (A) $NaGdF_4$:Yb/Tm; (B) $NaGdF_4$:Yb/Tm@$NaLuF_4$:Yb/Tm; (C) GdLuY NCs. The shell composition changes were reflected by the variation in the characteristic peaks of Gd^{3+}, Yb^{3+}, Lu^{3+} and Y^{3+} in the XPS spectra. Shell coating of $NaLuF_4$:Yb/Tm results in a new Lu4d peak and weaker Gd4d(3d3/2) peaks. A new strong Y3d appears and peaks of Lu4d and Gd4d(3d3/2) become much weaker after coating $NaYF_4$ on the surface of $NaLuF_4$:Yb/Tm. The above results demonstrate the sandwich structure of the as-prepared GdLuY NCs.

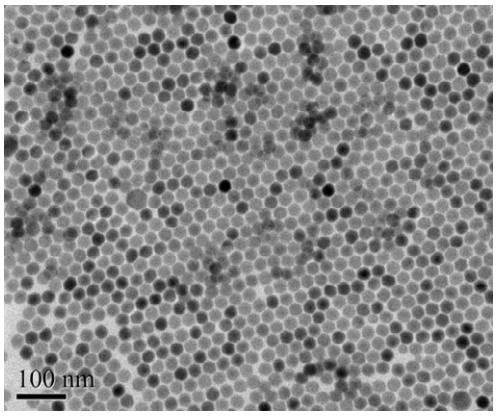

Fig. S3. TEM image of the as-prepared $NaYF_4$:Yb/Tm NCs (~25 nm).

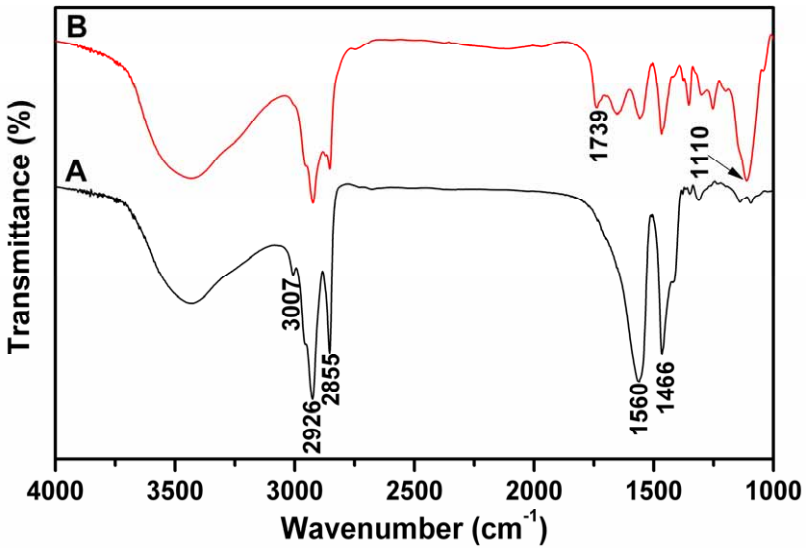

Fig. S4. FTIR spectra: (A) Oleic acid capped GdLuY NCs; (B) DPGLY NCs. The DPGLY NCs show the characteristic absorption bands of C-O-C (1110 cm^{-1}) and COO (1739 cm^{-1}) in corresponding spectrum, demonstrating successful DSPE-PEG 2000 functionalization.

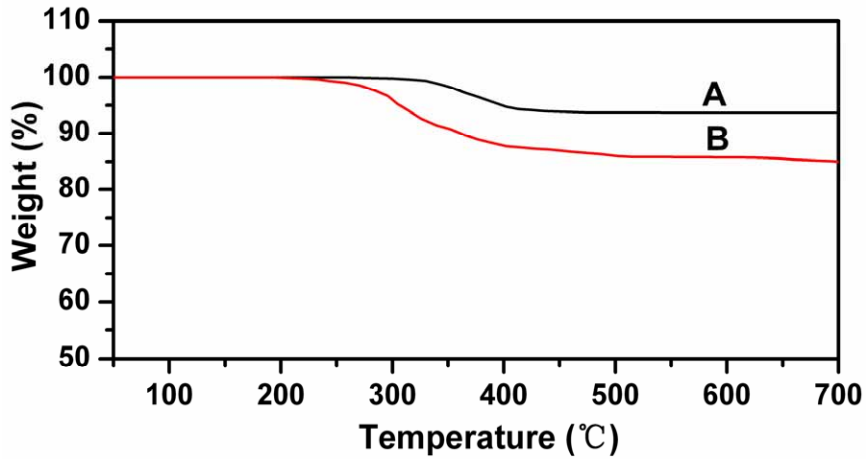

Fig. S5. TGA curves: (A) oleic acid capped GdLuY NCs; (B) DPGLY NCs. TGA curves demonstrate the content of the oleic acid ligands in the GdLuY NCs is 6.3% (wt). DSPE-PEG 2000 is successfully capped on the as-prepared GdLuY NCs. The content of DSPE-PEG 2000 in the DPGLY NCs is 8.7% (wt).

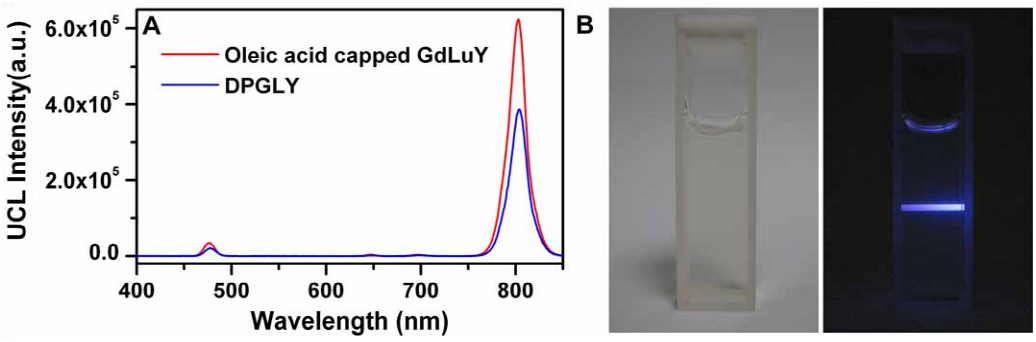

Fig. S6. (A) UCL spectra of DPGLY NCs dispersed in pure H_2O and oleic acid capped GdLuY NCs dispersed in cyclohexane at identical concentrations. (B) The bright field (left) and UCL photos (right) of the DPGLY NCs dispersed in pure H_2O. The UCL photo, taken by a digital camera, only displayed bright blue UCL visually.

References

1. L. L. Li, R. B. Zhang, L. L. Yin, K. Z. Zheng, W. P. Qin, P. R. Selvin, Y. Lu, *Angew. Chem. Int. Ed.*, 2012, **51**, 6121.