Supporting Information

Ultralow-Power Near-Infrared Excited Neodymium-Doped Nanoparticles for Long-Term *In Vivo* Bioimaging

Qing-Song Qin, Pei-Zhi Zhang, Ling-Dong Sun,* Shuo Shi, Nai-Xiu Chen, Hao Dong, Xiao-Yu Zheng, Le-Min Li,* and Chun-Hua Yan*

Beijing National Laboratory for Molecular Sciences, State Key Laboratory of Rare Earth Materials Chemistry and Applications, PKU-HKU Joint Laboratory in Rare Earth Materials and Bioinorganic Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China.

E-mail: sun@pku.edu.cn; lilm@pku.edu.cn; yan@pku.edu.cn

Materials.

Rare earth (RE) oxides were purchased from China Rare Earth Online Co., Ltd. Oleic acid (OA; ＞90%, Sigma-Aldrich), oleylamine (OM; ＞80%, J&K), 1-octadecene (ODE; ＞90%, J&K), trifluoroacetic acid (99%, Acros), trifluoroacetic acid lithium salt (99%, Alfa Aesar), ethanol (AR), cyclohexane (AR), dimethylformamide (DMF, AR), nitrosonium tetrafluoroborate ($NOBF_4$; 98%, Alfa Aesar), poly(acrylic acid) (PAA, M.W. 2000, Acros), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC; 99%, Shanghai Mepop Co., Ltd.), N-hydroxysuccinimide (NHS; TCI), Amine poly(ethylene glycol) (NH_2-PEG, M.W.=2000; JenKem Technology). All chemicals were used as received without further purification. $RE(CF_3COO)_3$ (RE = Lu, Nd) were prepared from the corresponding lanthanide oxides and trifluoroacetic acid. Water used in all experiments was obtained using a Milli-Q water system.

Instrumentation.

Low-resolution TEM measurements were operated with a JEOL JEM-2000 TEM operated at 200 kV. HRTEM measurements, energy dispersive spectra (EDS), and EDS spot, line scan, mapping were performed with a JEOL JEM-2100F TEM operated at 200 kV. XRD patterns were operated on a Rigaku D/MAX-2000 diffractometer (Japan), using Cu Kα radiation (λ=1.5406 Å). Photoluminescence spectra, photoluminescence versus excitation map and excitation spectra were carried out with an infrared fluorescence spectrometer (Nanolog FL3-2iHR, HORIBA Jobin Yvon). The absolute QY measurements were obtained with a FLS920 steady-state and time-resolved fluorescence spectrometer (Edinburgh Instruments). FTIR spectra were carried out by fourier transform infrared spectrometer (4000 – 400 cm^{-1}, Vector22, Bruker). The hydrodynamic diameter and zeta potential analysis were performed by a nanoparticle analyzer (ZetaPALS, Brookhaven instruments corporation).

Experimental Section

Synthesis of precursors.

RE oxides were added into the solution of excess trifluoroacetic acid to prepare $RE(CF_3COO)_3$ precursors.

Synthesis of $LiLuF_4:Nd^{3+}$ NPs.

In a typical synthesis, a given amount of CF_3COOLi (1.00 mmol), $Lu(CF_3COO)_3$ (0.90 mmol) and $Nd(CF_3COO)_3$ (0.10 mmol) was added into a mixture of OA, OM and ODE (15 mmol: 5 mmol: 20 mmol) in a three-necked flask (100 mL) at room temperature. The slurry was then heated to 120 °C to remove water and oxygen with vigorous stirring under vacuum for 15 min. The solution was heated rapidly to 320 °C and kept for 30 min under a N_2 atmosphere. Adding excess amount of ethanol into the solution, the NPs were precipitated and collected by

centrifugation. The final product was redispersed in 10 mL of cyclohexane.

Synthesis of $LiLuF_4:Nd^{3+}$@$LiLuF_4$ NPs.

a. By the automatic syringe pump. The as-synthesized $LiLuF_4$:10%Nd^{3+} NPs were selected as the core to prepare core@shell NPs. The 5 mL colloidal solution of $LiLuF_4$:10%Nd^{3+} NPs were added into a mixture of OA and ODE (20 mmol: 20 mmol), the slurry was heated to 120 °C to remove cyclohexane, water and oxygen with vigorous magnetic stirring under vacuum condition. Then the solution was heated to 310 °C with a heating rate of 15 °C /min under a N_2 atmosphere. Meanwhile, the shell precursor of CF_3COOLi and $Lu(CF_3COO)_3$ solids (Table S2) were added into a mixture of OA and ODE (20 mmol: 20 mmol), respectively. The slurry was heated to 120 °C to remove water and oxygen with vigorous magnetic stirring under vacuum condition and formed an optically transparent solution. Afterwards, the as-obtained solution was continuously injected into the three-necked flask slowly by the automatic syringe pump. The speed of injection was 20 min/mmol. After finishing the injection, adding an excess amount of absolute ethanol into the solution, the core@shell structured NPs were collected by centrifugation. The final product was redispersed in 10 mL of cyclohexane.

b. Adding all shell precursor at once. The 5 mL colloidal solution of $LiLuF_4$:10%Nd^{3+} NPs and 3 mmol of CF_3COOLi and $Lu(CF_3COO)_3$ solids were added into a mixture of OA and ODE (20 mmol: 20 mmol), the slurry was heated to 120 °C to remove cyclohexane, water and oxygen with vigorous magnetic stirring under vacuum condition. Then the solution was heated to 310 °C with a heating rate of 15 °C /min under a N_2 atmosphere. The solution was kept for 30 min. Adding excess amount of ethanol into the solution, the resulted NPs were precipitated and collected by centrifugation. The final product was redispersed in 10 mL of cyclohexane.

Preparation of PEGylated Nd@Lu NPs.

In a typical process, 5 mL of Nd@Lu NPs dispersion in cyclohexane was combined with 5 mL of DMF solution of $NOBF_4$ at room temperature. The resulting mixture was vigorous stirred for 15 minutes. After centrifuging at 12000 rpm for 15 min, the precipitated NPs was redispersed in 2 mL of DMF. 50 mg of PAA and 3 mL of H_2O were added into the solution followed by vigorous stirring for 8 h. The solution was centrifuged at 12000 rpm for 15 min, then the product was redispersed in 5 mL of DMF. Afterward, the 50 mg of 2000 molecular weight amine poly(ethylene glycol) (NH_2-PEG), 120 mg of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 12 mg of N-hydroxysuccinimide (NHS) were added into the solution and then stirred for 24 h at room temperature. The solution was centrifuged as above, the product was dispersed in deionized water. Then the water solution was centrifuged without poor solvents to remove the excess organic molecules. The final product was redispersed in deionized water.

Cytotoxicity Assay.

To reveal the biocompatibility of the PEGylated Nd@Lu NPs, the HeLa cells were seeded onto the 96-well plates (5000 cells per well) and cultured at 37 °C overnight. Different concentrations of the PEGylated Nd@Lu NPs were added to the corresponding wells and incubated for 24 h. The medium was discarded and fresh culture medium containing 10% of CCK-8 was added to each well. Then the cell viability was quantified by recording the absorbance at 540 nm by using a microplate reader (Tecan, Durham, NC). The values of cell viability were expressed as a percentage of the control and calculated as $(OD_{experiment} - OD_{blank})/(OD_{control} - OD_{blank}) \times 100\%$ (n = 6).

Measurement of the absolute QY.

The cyclohexane solution of NPs and pure cyclohexane (blank) were excited at 803 nm laser (2 W/cm^2). Due to the low absorption cross-section of lanthanide ions, it is necessary to use a attenuation slice for the excitation light. The emission of 830−1400 nm were integrated for the quantum efficiency determination. The numbers of absorption photons (N_{abs}) and emission photons (N_{em}) were explored to calculate the QY. The absolute QY is defined as the following formula:

$$QY=\frac{N_{em}}{N_{abs}}=\frac{L_{sample}\text{-}L_{reference}}{E_{reference}\text{-}E_{sample}}$$

L_{sample} and $L_{reference}$ are the numbers of emission photons of the sample and the reference, respectively. $E_{reference}$ and E_{sample} are the numbers of the excitation photons not absorbed by the reference and the sample, respectively.

Penetration depth tests.

Penetration depth tests were measured in chicken tissue. By covering with different depth chicken layers above the pellet of Nd@Lu NPs (0.1 mg/mm^2), the NIR luminescence was recorded using a home-made *in vivo* imaging system coupled with InGaAs camera. A 732 nm or 803 nm fiber-coupled CW laser was used as the excitation source. The excitation intensity was kept the same (100 mW/cm^2) and exposure time was 0.2 s. The 830 nm filter was used to prevent the interference of the excitation. All the parameters were set the same for clarity.

NIR luminescence *in vivo* imaging.

BALB/c nude mice were bought from Vital River Co. Ltd., Beijing. The InGaAs CCD camera (Princeton Instruments) was used to acquire NIR images. The 732 nm excitation light was provided by a fiber-coupled diode laser (1 W, Hi-Tech Optoeletronics Co., Ltd.) with a laser extender counter. The emitted light passed through a suitable filter (830 nm longpass, Semrock). The 100 μL of the PEGylated Nd@Lu NPs solution (10 mg/mL) was intravenously injected into the nude mice. The excitation intensity of 732 nm irradiation was kept 10 mW/cm^2 and the

exposure time was 1 s. All animal procedures were in agreement with the guidelines of the Institutional Animal Care and Use Committee.

Table S1. The size of $LiLuF_4$:n%Nd^{3+} NPs (n=1, 3, 5, 7, 10, 15, 20).

Nd^{3+}	1%	3%	5%	7%	10%	15%	20%
length (nm)	21.4±2.1	23.4±2.4	26.5±2.0	25.3±2.4	35.6±2.4	41.6±4.2	40.6±3.9
width (nm)	19.4±2.0	21.4±2.3	22.8±2.5	23.2±2.4	31.9±2.2	36.4±2.7	34.0±3.0

Table S2. Detailed dosage of shell precursors for core@shell NPs.

Dosage of shell precursors	Size		
	length (nm)	width (nm)	Shell thickness (nm)
CF_3COOLi (1 mmol) + $Lu(CF_3COO)_3$ (1mmol)	43.8±3.5	37.2±2.7	4.1
CF_3COOLi (2 mmol) + $Lu(CF_3COO)_3$ (2mmol)	52.3±3.7	44.1±2.3	8.3
CF_3COOLi (3 mmol) + $Lu(CF_3COO)_3$ (3mmol)	60.9±4.3	41.6±3.3	12.6

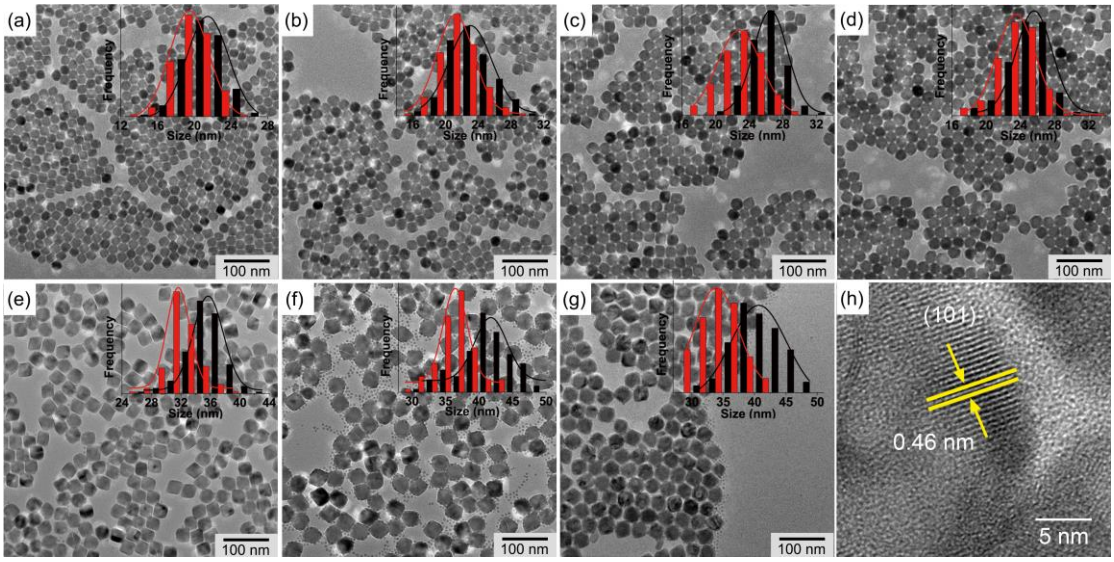

Figure S1. (a-g) TEM images of $LiLuF_4$:n%Nd^{3+} NPs, n=1, 3, 5, 7, 10, 15, 20. Inset: size distribution, black bars represent the length and red bars represent the width of the NPs. The size of NPs grew larger with the increase concentration of Nd^{3+}, which was also consistent with the previous report.[S1] Once the precursor concentration of Nd^{3+} ions was over 10%, a small amount of small NdF_3 NPs would form. The segregation of lanthanide fluorides was also noticed in Gd^{3+}-doped $LiYF_4$ NPs.[S2] (h) The HRTEM image of $LiLuF_4$:10%Nd^{3+} NPs. It shows clear lattice fringes with d spacing of 0.46 nm for the (101) plane of tetragonal $LiLuF_4$, which revealed the high crystallinity of the NPs.

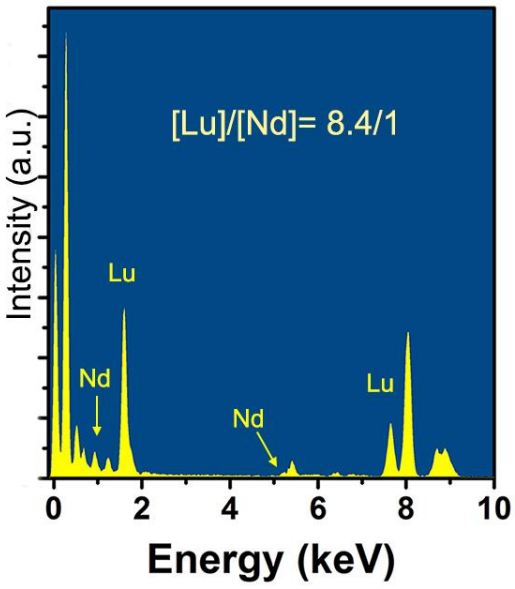

Figure S2. The energy-dispersive X-ray analysis (EDS) of $LiLuF_4$:10%Nd NPs

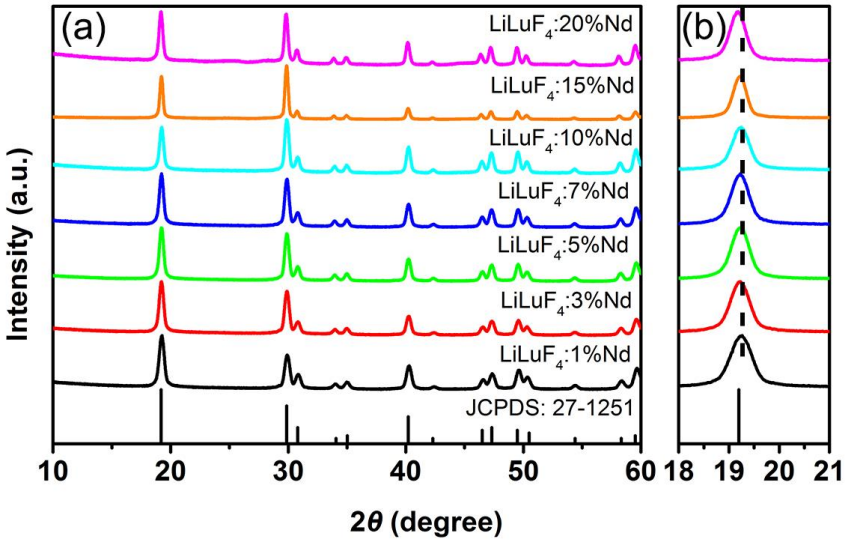

Figure S3. (a) XRD patterns of the $LiLuF_4$:n%Nd^{3+} NPs, n=1, 3, 5, 7, 10, 15, 20. All diffraction peaks match well with the standard pattern of tetragonal $LiLuF_4$ (JCPDS No.027-1251). (b) The diffraction peaks shifted toward smaller angles with the enhancement of Nd^{3+} ion due to the radius of Nd^{3+} is larger than that of Lu^{3+} ion.

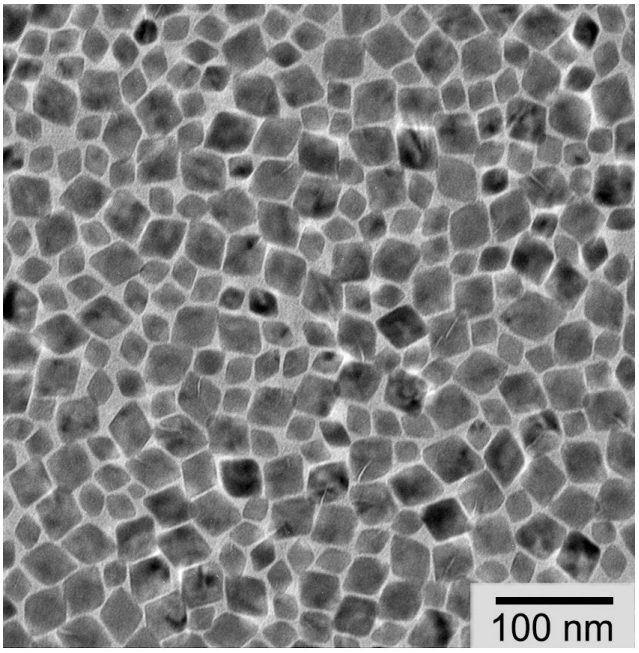

Figure S4. TEM image of the core@shell NPs preparing by adding all of shell precursor at once. The as-prepared NPs were inhomogenous.

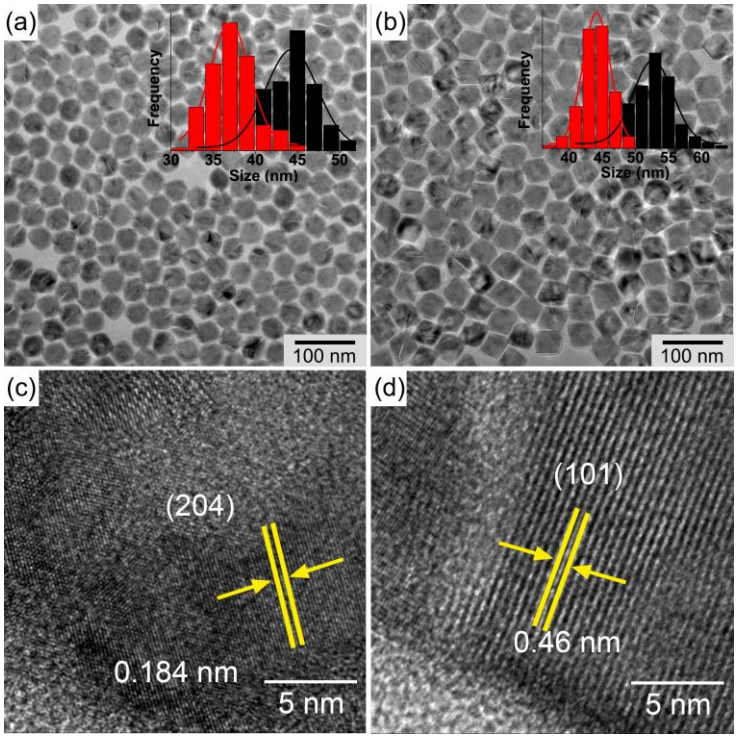

Figure S5. The TEM and HRTEM images of NPs with different shell thickness of 4.1 nm (a,c) and 8.3 nm (b,d), respectively. HRTEM images show clear lattice fringes with d spacing of 0.46 nm for the (101) plane and 0.184 nm for the (204) plane of tetragonal $LiLuF_4$, which revealed the high crystallinity of the NPs.

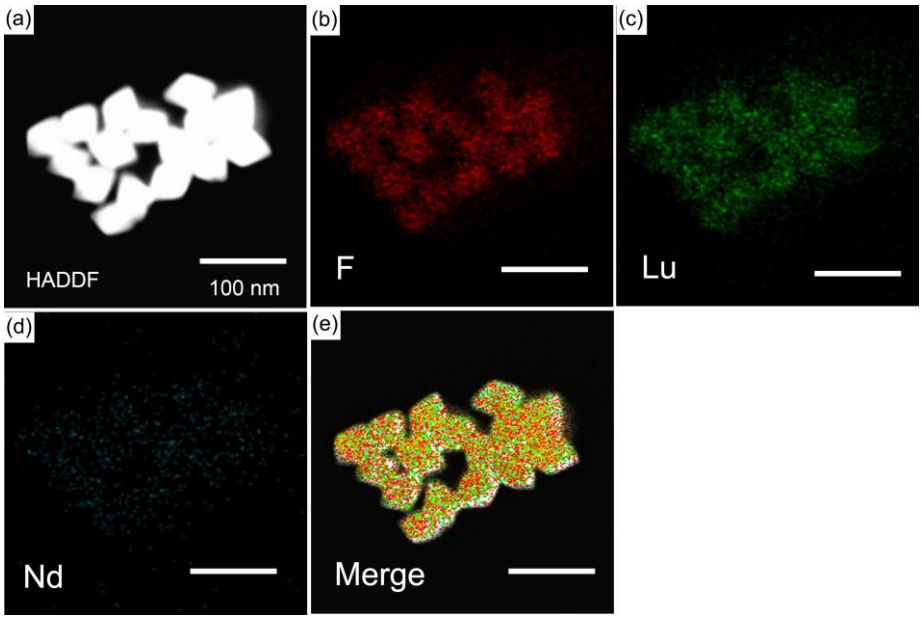

Figure S6. (a-e) HAADF-STEM images of the Nd@Lu (thickness of 12.6 nm) NPs. (a) The HAADF image of Nd@Lu NPs. The element maps showed the distribution of F (red, b), Lu (green, c), Nd (blue, d) and their merge one (e) (scale bar = 100 nm).

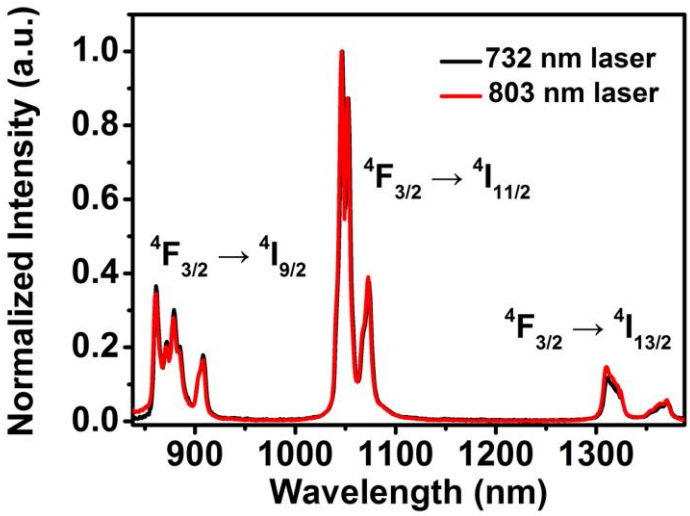

Figure S7. The normalized emission spectra of Nd@Lu NPs under 732 nm and 803 nm laser excitation, respectively.

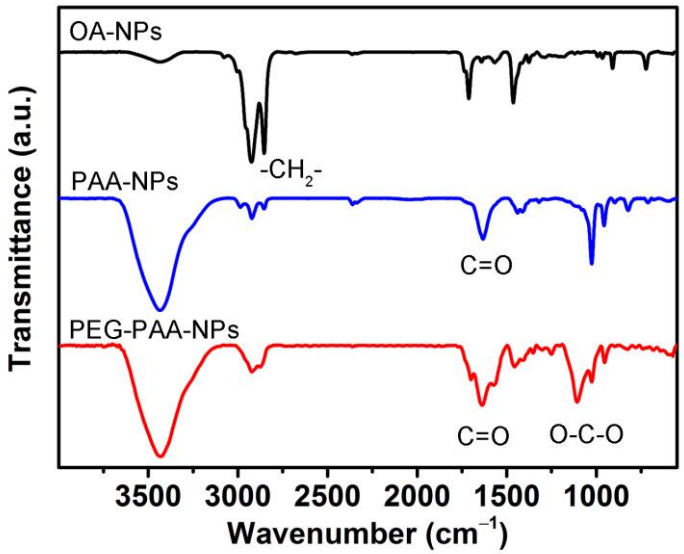

Figure S8. IR spectra of OA coated Nd@Lu NPs (a), PAA coated Nd@Lu NPs (b) and PEG-PAA coated Nd@Lu NPs (c). The OA-capped NPs showed characteristic antisymmetric and symmetric alkyl CH_2 stretching bands at 2925 and 2852 cm^{-1}, respectively. A strong characteristic carboxylic acid C=O stretch was observed at near 1700 cm^{-1}, and a broad O—H stretching band at around 3450 cm^{-1} which confirmed that PAA successfully coated at the surface of NPs. The strong characteristic ether C—O—C stretching band at 1100 cm^{-1} which indicated PEG successfully coated the surface of NPs.

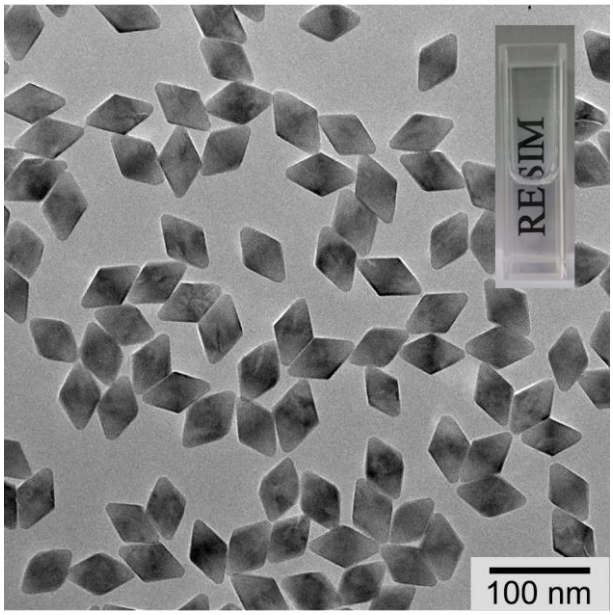

Figure S9. TEM image of the PEGylated Nd@Lu NPs, the inset was digital photograph of aqueous solution of PEGylated Nd@Lu NPs.

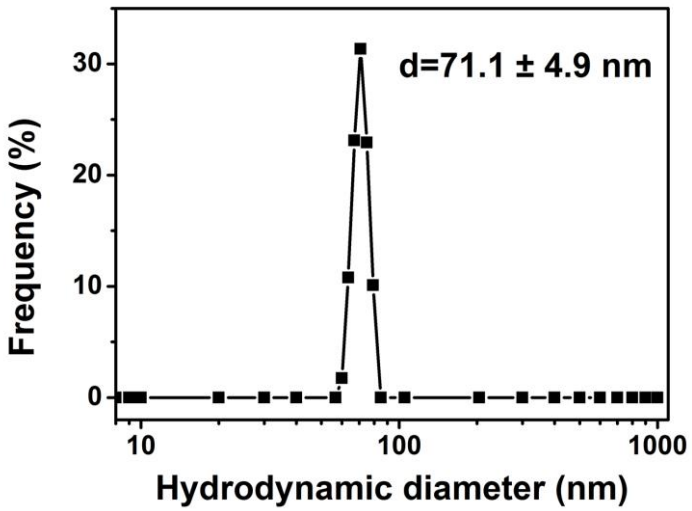

Figure S10. Hydrodynamic diameter distribution of the PEGylated Nd@Lu NPs.

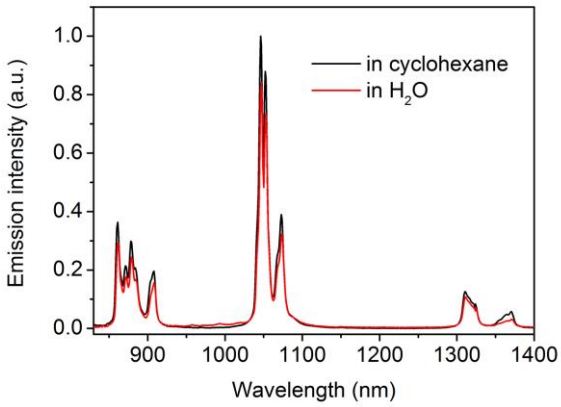

Figure S11. The NIR emission spectra of Nd@Lu NPs in cyclohexane solution and water, respectively.

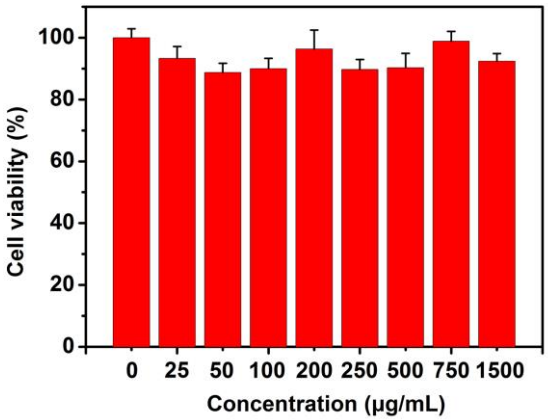

Figure S12. In vitro cytotoxicity test of PEGylated Nd@Lu NPs using HeLa cell line after incubation for 24 h. Cell viability was determined by CCK-8 assay. Data represents mean ± SD (n = 6).

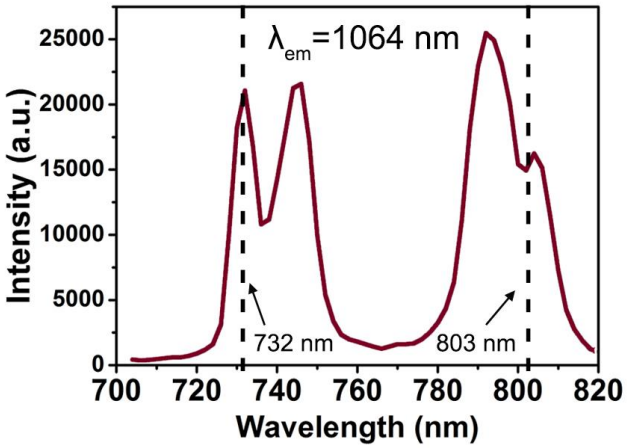

Figure S13. The excitation spectrum (λ_{em} = 1064 nm) of $LiLuF_4$:Nd NPs. The dashed lines are guides for the eyes which represent 732 nm and 803 nm, respectively.

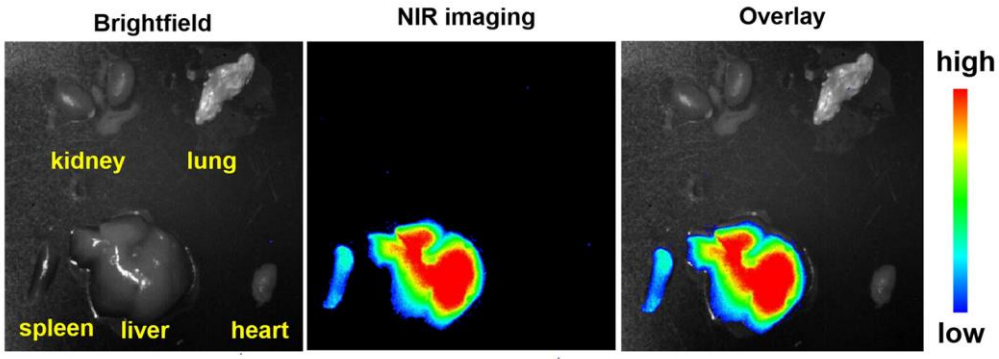

Figure S14. The ex vivo NIR imaging of organs under excitation at 732 nm (1 h post injection). The spleen and liver had clear NIR signal, while other organs, such as kidney, showed no NIR signal.

References

[S1] X. Jiang, C. Cao, W. Feng, F. Y. Li, *J. Mater. Chem. B* 2016, **4**, 87-95.

[S2] H. Na; J. S. Jeong; H. J. Chang; H. Y. Kim; K. Woo; K. Lim; K. A. Mkhoyan; H. S. Jang, *Nanoscale* 2014**, 6**, 7461-7468.