Supporting Information

(α-NaYbF$_4$:Tm^{3+})/CaF$_2$ Core/Shell Nanoparticles with Efficient Near-Infrared to Near-Infrared Upconversion for High-Contrast Deep Tissue Bioimaging

Guanying Chen,[†‡⊥◊] *Jie Shen*,[§◊] *Tymish Y. Ohulchanskyy*,[‡] *Nayan Patel*,[‖] *Artem Kutikov,*[#] Zhipeng Li,[□] *Jie Song,*[#] *Ravindra K. Pandey*,[‖] *Hans Ågren*,[⊥] *Paras N. Prasad*,[†‡♀]* and *Gang Han*[§]*

[†]School of Chemical Engineering and Technology, Harbin Institute of Technology, 150001 Harbin, People's Republic of China

[‡]Institute for Lasers, Photonics, and Biophotonics, University at Buffalo, State University of New York, Buffalo, New York 14260

[⊥]Department of Theoretical Chemistry and Biology, Royal Institute of Technology, S-10691 Stockholm, Sweden

[§]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605

[‖]Photodynamic Therapy Center, Roswell Park Cancer Institute, Buffalo, New York 14263

[#]Department of Orthopedics and Department of Cell Biology, University of Massachusetts Medical School, Worcester, Massachusetts 01655

[□]Department of Physics and Astronomy, University of North Carolina, Chapel Hill, NC 27599

[♀]Department of Chemistry, Korea University, Seoul, Korea, 136-701

◊Equal contributions

*Corresponding Authors. E-mail: pnprasad@buffalo.edu, gang.han@umassmed.edu

I. Supporting Image

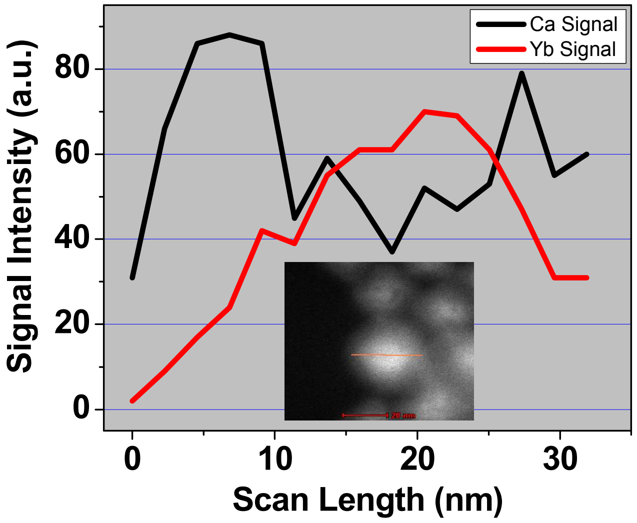

Figure S1. Energy dispersive x-ray spectroscopic (EDX) line scan conducted with scanning transmission electron microscopic (STEM) imaging (inset) on a ($NaYbF_4$: Tm0.5%) @CaF_2 nanoparticle. A higher Ca concentration is observed in the peripheral region of the crystal, while a most intense Yb signal is distinguished in the center of the nanoparticle; this result gives a direct evidence of the formation of a core–shell structure.

II. Determination of Upconversion Quantum Yield of Photoluminescence

The quantum yield (QY) of photoluminescence is defined as the ratio of the number of the emitted photons to the number of the absorbed photons.[S1] QY can be measured either by an absolute method or on a relative basis using a standard with a known QY as a reference. Here, we determined the NIR UC PL QY of our core/shell nanocrystals by referencing to the standard: IR 26 dye dissolved in 1,2-dichloroethane (DCE), with a known QY of 0.05%.[S2] The QY of the nanoparticles, QY_X, was calculated according to the following equation:[S1]

$$QY_X = QY_R \cdot \frac{E_X}{E_R} \cdot \frac{A_R}{A_X} \cdot \frac{I_R}{I_X} \cdot \frac{N_X^2}{N_R^2} \qquad \text{(S1)}$$

where QY_R and QY_X are the quantum yields of the referenced standard sample and the sample to be determined, respectively; E_R and E_X are the numbers of the emitted photons for referenced standard sample and measured sample, respectively; A_R and A_X are the numbers of the photons absorbed by referenced standard sample and measured sample, respectively, I_R and I_X indicate the relative intensity of the exciting light for referenced standard sample and measured sample, respectively; N_R and N_X are the average refractive indices of the solvents used for dissolving referenced standard sample and the measured sample, respectively. Subscripts R and X refer to the referenced standard sample and the sample to be measured, respectively.

Exactly the same geometry was utilized to excite the reference standard sample (IR 26 dye, Exciton, Inc.) dissolved in 1,2-dichloroethane (DCE) and the α-($NaYbF_4$:0.5% Tm^{3+}) @CaF_2 UCNPs dispersed in hexane, and to detect their PL spectra. A calibrated SPEX 270M spectrometer (Jobin Yvon), equipped with an InGaAs TE-cooled photodiode (Electro- Optical Systems, Inc.), was used for recording NIR PL. During the QY measurement, the laser at 975 nm from a fiber end was collimated to perform the excitation; the absorbance of the referenced standard sample and the measured core/shell samples has been matched at 975 nm by adjusting the concentration of these two samples. To avoid the effect of reabsorption, an "optically thin" absorbance of 0.09 at 975 nm is utilized. We confirmed that the intensity across the laser beam follows a Gaussian profile; the beam diameter was defined to be 5 mm, using $1/e^2$ of the peak power. The average power density employed was determined to be about 0.3 W/cm^2 by dividing laser output power (59 mW) with the area of laser point (0.196 cm^2). The QYs of colloidal UC nanocrystals have been measured through either absolute integrating sphere or reference standard method employing a Gaussian laser and an average laser power density.[27-29,42,S3] Therefore, we follow these previously established protocol on reference standard method, using the average laser power density and neglecting the intensity variation across the laser beam. This allows for a direct comparison of QYs of designed nanoparticles in this work with QYs values in previous reports.

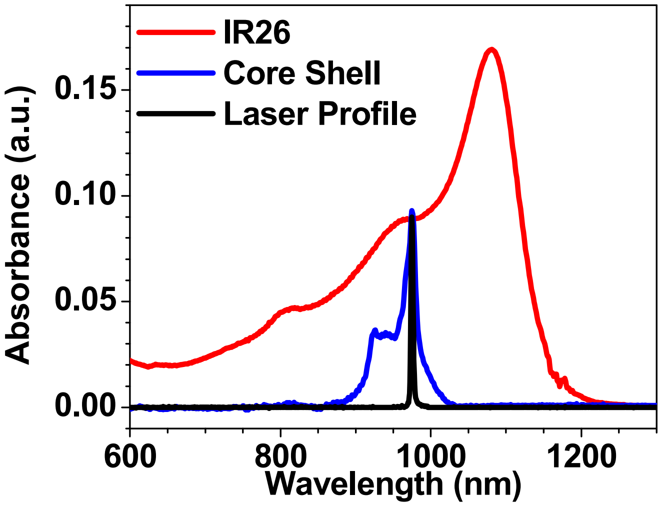

Figure S2. Absorbance of the referenced standard IR 26 dissolved in 1,2-dichloroethane (DCE) and the measured α-($NaYbF_4$:0.5% Tm^{3+}) @CaF_2 core/shell UCNPs dissolved in hexane; they have been matched at 975 nm by adjusting the concentration of these two samples.

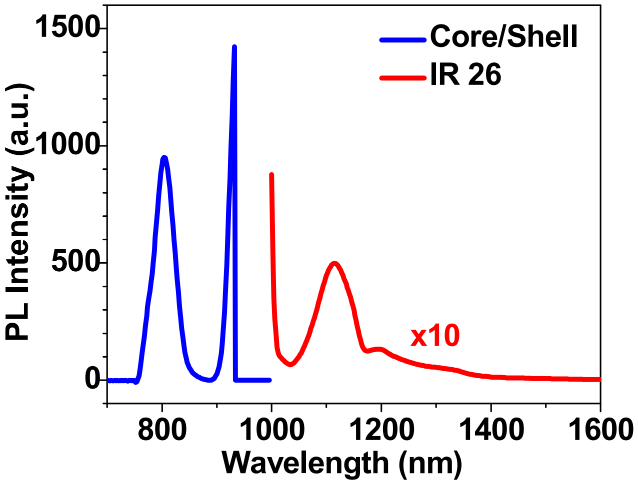

Figure S3. Anti-stokes NIR UC PL spectra of α-($NaYbF_4$:0.5% Tm^{3+}) @CaF_2 core/shell dispersed in hexane and stokes NIR PL of referenced standard IR 26 dye dissolved in DCE under 975 nm excitation of 0.3 W/cm^2. The PL intensity of IR 26 dye in DCE was magnified by 10 times.

Since the laser excitation intensity and the absorbance are same, the ratio of A_RI_R/ A_XI_X in Equation (S1) equals 1. It is known that hexane has a refractive index of 1.38, while DCE has a refractive index of 1.44. The parameter of N_X^2/ N_R^2 in Equation (S1) was, therefore, determined to be 0.92, The equation (S1) can thus be simplified to,

$$QY_X = QY_R \cdot \frac{E_R}{E_X} \cdot 0.92 \tag{S2}$$

As illustrated in Figure S3, the integrated intensity of NIR UC PL in the wavelength range of 700-900 nm for α-(NaYbF$_4$:0.5% Tm^{3+}) @CaF$_2$ core/shell nanoparticles is about 13±2 times higher than the integrated NIR PL intensity of the standard IR 26 dye in the range of 1050-1600 nm. We would like to mention that the ratio value of E_R/E_X in the PL intensity in Figure S3 has been evaluated using four sets of core/shell UC nanoparticles and IR 26 at different concentrations. According to Equation (S2), this intensity ratio corresponds to a QY of 0.6±0.1 % for α-(NaYbF$_4$:0.5% Tm^{3+}) @CaF$_2$ core/shell under low excitation density of 0.3 W/cm^2 at 975 nm.

III. Molecular Weight Calculation

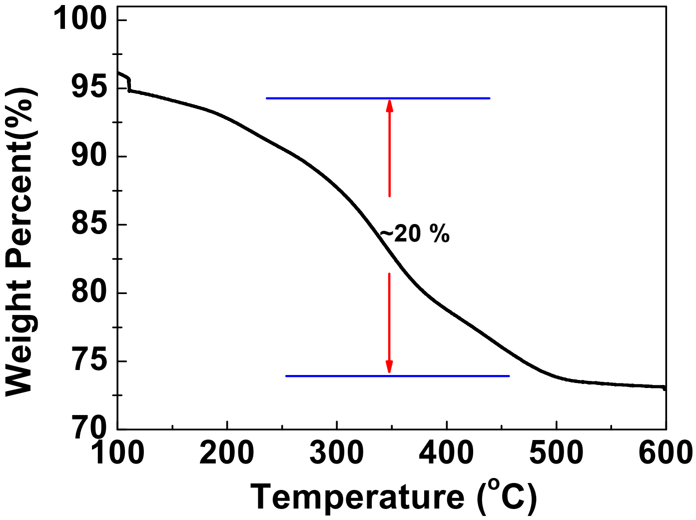

Figure S4. Thermogravimetic test of HA-coated UCNPs was carried out on SDT 600 instrument, which revealed a 21.3% (=20%/94%) weight loss corresponding to the organic surface layer on UCNPs. The test was implemented using a calefaction program as follows, 10 ^{o}C/min to 110 ^{o}C and hold for 5 min under helium atmosphere, then switch helium to air and increase at a speed of 10 ^{o}C/min to 600 ^{o}C.

The cubic $NaYbF_4$ unit cell has a lattice constant as a = 0.5448 nm, and its chemical formula is $Na_2Yb_2F_8$ in a cell unit. The diameter of spherical α-$NaYF_4$:Yb,Tm core is *ca*. 20 nm, thus, the "molecular weight" of core portion can be calculated as follows:

$$M_{core} = Mr(Na_2Yb_2F_8) \times V_{core} \div V_{unit} = Mr(Na_2Yb_2F_8) \quad \times ((4\pi R^3/3) \div a^3)$$

$$=1.41 \times 10^7 \text{ g/mol}$$

The cubic CaF_2 unit cell has a lattice constant as *a'* = 0.545 nm, and its chemical formula is Ca_4F_8 in a cell unit. The α-$NaYF_4$:Yb,Tm@CaF_2 UCNP has a cubic profile with edge length d = 27.1 nm, so the "molecular weight" of shell portion can be calculated as follows:

$$M_{shell} = Mr(Ca_4F_8) \times V_{shell} \div V_{unit} = Mr(Ca_4F_8) \quad \times ((V_{UCNP} - V_{core}) \div a'^3)$$

$$= Mr(Ca_4F_8) \quad \times (d^3 - 4\pi R^3/3) \div a'^3$$

$$= 3.03 \times 10^7 \text{ g/mol}$$

Therefore, the "molecular weight" of UCNP inorganic portion is

$$M_{inorganic} = M_{core} + M_{shell} = 4.44 \times 10^7 \text{ g/mol}$$

The TGA test of UCNPs revealed a 21.3 % weight loss due to the decomposition of organic substances (Fig S4), so the "molecular weight" of UCNP is 5.64× 10^7 g/mol. The "pmol" appeared in the text refers to the number of nanoparticles, defined by the mass of nanoparticles divided by the "molecular weight" of single nanoparticle.

Supporting References

S1. Rhys Williams, A. T.; Winfield, S. A.; Miller, J. N. *Analyst*, **1983**, *108*, 1067-1071.

S2. Semonin, O. E.; Johnson, J. C.; Luther, J. M.; Midgett, A. G.; Nozik, A. J.; Beard, M. C. *J. Phys. Chem. Lett.* **2010**, *1*, 2445-2450.

S3. Ostrowski, A. D.; Chan, E. M.; Gargas, D. J.; Katz, E. M.; Han, G.; Schuck, P. J.; Milliron, D. J.; Cohen, B. E. *ACS Nano*, **2012**, *6*, 2686-2692.