

## Tailoring Dye-sensitized Upconversion Nanoparticles Excitation Bands towards Excitation Wavelength Selective Imaging

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*Materials:* IR-783 (90%), IR-820 (80%), 4-mercaptobenzoic acid (99%), Y<sub>2</sub>O<sub>3</sub> (99.9%), Yb<sub>2</sub>O<sub>3</sub> (99.9%), Er<sub>2</sub>O<sub>3</sub> (99.9%), CF<sub>3</sub>COONa (99.9%), CF<sub>3</sub>COOH, 1-octadecene (90%), oleic acid (90%), oleylamine (90%), nitrosonium tetrafluoroborate (NOBF<sub>4</sub>), N,N-dimethylformamide (DMF, anhydrous, 99.8%), poly(methyl methacrylate) (PMMA) (MW~120,000) were all purchased from Sigma-Aldrich and used without further purification. The lanthanide (Ln) trifluoroacetates, Ln(CF<sub>3</sub>COO)<sub>3</sub>, were prepared as literature described.<sup>[1]</sup>

*Instrumentation:* <sup>1</sup>H NMR spectra were recorded on a Varian AMX400 (400 MHz) using d<sup>6</sup>-DMSO as solvent at room temperature. Transmission electron microscopy (TEM) were performed on a Philips CM10 transmission electron microscope operating at an accelerating voltage of 100 kV, Images were recorded on a Gatan slow-scan CCD camera. UV-Vis absorption spectra were measured on a Perkin/Elmer Lambda 900 UV-Vis-NIR Spectrometer in a quartz cuvette with a path length of 1cm. The NIR photoluminescence measurements of IR-dyes were taken on a Horiba Nanolog Spectrofluorometer with a 5509 PMT detector and 450 W xenon lamp. All dyes were measured at the same concentration in DMF with excitation at 750 nm and

emission measured from 775 nm to 1200 nm. For the up-conversion excitation spectrum measurements, the Ti:sapphire laser was switched to continuous-wave mode and the absence of mode-locking was confirmed by monitoring the laser output using a 400 MHz oscilloscope and fast photodiode. The detection utilized a band pass filter (FF01-524/24-25, Semrock, Rochester, NY) and a PMH100-6 photomultiplier tube module from Becker-Hickl. A pulse generator provided a 5 MHz sync signal to the TCSPC electronics and the photons over the time-to-amplitude converter range were integrated to provide the total signal for each excitation wavelength. In order to use a constant excitation intensity at each wavelength, a 1 mm thick quartz plate was used to pick off part of the excitation beam and directed to a calibrated power meter (corrected for the excitation wavelength, Newport 1830-C with 818 series detector). Excited state lifetime measurements were performed using a Ti:sapphire laser (Mira 900D from Coherent, Palo Alto, CA) as the excitation source and a time-correlated single-photon counting system (SPC150, Becker-Hickl, Berlin, Germany) for detection. The output from the Ti:sapphire, consisting of 150 fs pulses at a repetition rate of 76 MHz was passed through two Glan-Thompson polarizers to rotate the polarization and attenuate the beam. The emitted light was collected using a 35 mm focal length plano-convex lens and focused into a 0.25 m monochromator (CVI Laser Optics, Albuquerque, NM) set between 780 and 840 nm with a 1.1 nm bandwidth. An id100 single-photon avalanche photodiode (idQuantique, Geneva, Switzerland) was used for infrared lifetime measurements. The life time was quantum yield weighted (first moment) averaged. Fluorescence images of the dye-UCNPs were obtained using a custom epifluorescence microscope with a motorized x-y stage (Biopoint 2, Ludl Inc.). The dye-UCNPs in PMMA were loaded into 5 mm deep channels of a poly-urethane and poly-acrylic substrate manufactured with 3D-printing. A 35 mm plano-convex synthetic fused silica

lens focused 790 or 850 nm CW excitation light to an approximately 20 micron spot size. An oscilloscope was used to confirm the absence of mode-locking. The fluorescence was collected using the same lens, passed through dichroic (670 nm low-pass, Semrock Inc.) and band pass filters (550 nm center, 88 nm bandwidth, Semrock Inc.) and focused onto a photomultiplier tube (PMH-6, Becker-Hickl, Berlin). Excitation and emission polarizations were vertical and magic-angle, respectively. Each image corresponded to 70x331 measurements with 0.25 seconds integration time for each point.

*Synthesis of  $\beta$ -NaYF<sub>4</sub>:20%Yb,2%Er nanoparticles (NPs).* Synthesis of  $\beta$ -NaYF<sub>4</sub>:20%Yb,2%Er Core UCNPs: The  $\beta$ -NaYF<sub>4</sub>:20%Yb,2%Er core UCNPs were prepared by a two-step thermolysis method. In the first step, CF<sub>3</sub>COONa (0.5 mmol) and proper Ln(CF<sub>3</sub>COO)<sub>3</sub> (0.5 mmol in total) precursors were mixed with oleic acid (5 mmol), oleyamine (5 mmol) and 1-octadecene (10 mmol) in a two-neck reaction flask. The slurry mixture was heated to 110 °C to form a transparent solution followed by 10 minutes of degassing. Then the flask was heated to 300 °C with a rate of 15 °C/min under dry argon flow, and it maintained at 300 °C for 30 minutes. The  $\alpha$ -NaYF<sub>4</sub>:20%Yb,2%Er intermediate UCNPs were gathered from the cooled reaction solution by centrifugal washing with excessive ethanol. In the second step, the  $\alpha$ -NaYF<sub>4</sub>:20%Yb,2%Er intermediate UCNPs were re-dispersed into oleic acid (10 mmol) and 1-octadecene (10 mmol) together with CF<sub>3</sub>COONa (0.5 mmol) in a new two-neck flask. After degassing at 110 °C for 10 minutes, this flask was heated to 325 °C with a rate of 15 °C/min under dry argon flow, and remained at 325 °C for 30 minutes. Then,  $\beta$ -NaYF<sub>4</sub>:20%Yb,2%Er UCNPs were centrifugally separated from the cooled reaction media and preserved in hexane (10 mL) as stock solution.

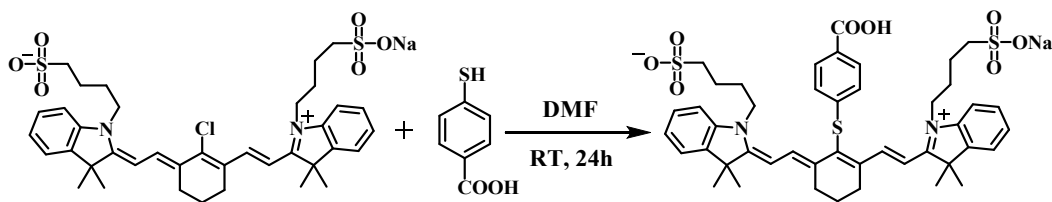
*Preparing hydrophobic ligand free UCNPs.* Oleic acid ligands on the UCNP surface were removed using a published nitrosonium tetrafluoroborate (NOBF<sub>4</sub>) approach [1]. In brief, 10mg of OA-UCNPs were dispersed in hexane and mixed with 0.20g of nitrosonium tetrafluoroborate dissolved in DMF in a sealed vial overnight. Subsequently, UCNPs were precipitated with isopropanol and centrifugally washed once in DMF to obtain the hydrophobic ligand free UCNPs.

*Sample preparation for emission and upconversion excitation spectrum measurements.* The samples for emission spectrum measurements were prepared by adding different amount of prepared nanoparticles to IR-dyes in DMF. The final concentrations of IR-dyes are 5 μmol/L; the final concentrations of NPs are 0, 0.02, 0.04, 0.06, 0.08, 0.1 μmol/L. The individual IR-dye coated nanoparticles for upconversion excitation spectrum measurements were prepared in the aforementioned ratio (IR-dyes vs. NPs: 5 μmol/L: 0.1 μmol/L) in DMF, while the nanoparticles of the two co-coated dyes (IR-783 and IR-845) were prepared by the ratio of 2.5 μmol/L for each dye: 0.1 μmol/L for NPs in DMF, and then the solution was diluted 10 times for measurement. The aforementioned ratio (IR-dyes vs. NPs: 5 μmol/L: 0.1 μmol/L) were isolated by centrifugation respectively, the supernatants derived from NPs and IR-dyes mixture for the absorption spectrum measurement.

*Sample preparation for FTIR measurements.* Mixture of NOBF<sub>4</sub>-treated NaYF<sub>4</sub>:Yb, Er nanoparticles (50 mg) and IR-dyes (0.25 μmol) in DMF (1 mL) was stood for 1 h at room temperature, and then the solution of five samples (NPs, NPs+IR-783, NPs+IR-808, NPs+IR-820, NPs+IR-845) were isolated by centrifuging respectively. The as-precipitated nanoparticles were washed 3 times with hexane and then dried in a vacuum oven at 40 °C overnight for FT-IR measurement.

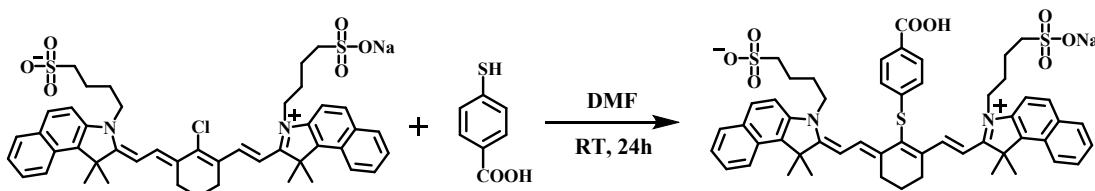
*Prepare Dye-UCNPs in PMMA for orthogonal imaging application.* The UCNPs were first treated with  $\text{NOBF}_4$  and were then mixed with dye solutions. The solutions mentioned above were stood for 1 h at room temperature, and then the samples were isolated by centrifugation; the as-precipitated dye-coated nanoparticles were washed 3 times with DMF and redissolved in small amount of DMF. Meanwhile, 500 mg PMMA was dissolved in 1 mL DMF at  $100^\circ\text{C}$ . This was then cooled to room temperature. Then  $1\ \mu\text{mol/L}$  dye conjugated UCNPs DMF solution was blended with above prepared PMMA. After being dispersed into the PMMA matrix, the dye conjugated UCNP systems were loaded into the letters of the “GNO”. These dye-UCNPs were solidified after being remained overnight at room temperature.

*Synthesis of IR-808:* The dye was similarly synthesized according to the method described in the literature [2]. A mixture of IR-783 (100 mg, 0.12 mmol) and 4-mercaptobenzoic acid (80 mg, 0.24 mmol) in DMF (5 mL) was stirred at room temperature for 24 hours. After reaction, the solution was filtered through a  $0.45\ \mu\text{m}$  PTFE syringe filter and then diethyl ether (50 mL) was added slowly to precipitate the product. The precipitate was collected by centrifugation, washed with diethyl ether, and dried under vacuum to afford 86 mg (0.1 mmol, 83%) of gold-colored crystals.  $^1\text{H}$  NMR (400 MHz,  $\text{d}^6\text{-DMSO}$ ):  $\delta$  8.51(d, 2H,  $J=14$ ), 7.84(d, 2H,  $J=8.4$ ), 7.51(d, 2H,  $J=7.2$ ), 7.42(d, 2H,  $J=8$ ), 7.36(d, 2H,  $J=8.4$ ), 7.36(t, 2H,  $J=8$ ), 7.20(t, 2H,  $J=7.2$ ), 6.39(d, 2H,  $J=14$ ), 4.16(t, 4H,  $J=6.8$ ), 2.78(t, 4H,  $J=6$ ), 2.50(t, 2H,  $J=5.2$ ), 1.93(t, 2H,  $J=5.2$ ), 1.74(m, 10H), 1.36(s, 12H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{d}^6\text{-DMSO}$ ):  $\delta$  172.58, 167.39, 147.62, 145.29, 143.62, 142.71, 141.76, 133.96, 131.13, 129.24, 128.45, 126.00, 125.68, 123.07, 112.26, 102.72, 51.35, 49.42, 44.40, 27.81, 26.81, 26.54, 23.16. HR-MS: calculated for  $\text{C}_{45}\text{H}_{51}\text{O}_8\text{S}_3\text{N}_2$ : 843.2808; found: 843.2795.



The synthesis of IR-808

*Synthesis of IR-845:* (Method same as above)  $^1\text{H}$  NMR (400 MHz,  $\text{d}^6\text{-DMSO}$ ): 12.89(s, 1H), 8.66(d, 2H,  $J=14.4$ ), 8.19(d, 2H,  $J=8.4$ ), 8.02(t, 2H,  $J=8$ ), 8.01(t, 2H,  $J=8.8$ ), 7.89(d, 2H,  $J=7.2$ ), 7.76(d, 2H,  $J=9.2$ ), 7.59(t, 2H,  $J=8.4$ ), 7.46(t, 2H,  $J=8$ ), 7.45(d, 2H,  $J=8.8$ ), 6.42(d, 2H,  $J=14.4$ ), 4.29(t, 4H,  $J=7.2$ ), 2.82(t, 4H,  $J=5.6$ ), 2.50(t, 2H,  $J=5.6$ ), 1.8(m, 24H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{d}^6\text{-DMSO}$ ):  $\delta$ : 173.73, 167.41, 147.51, 144.35, 143.57, 140.39, 134.27, 133.95, 132.05, 131.18, 131.06, 130.54, 128.61, 128.36, 128.04, 126.51, 125.59, 122.94, 112.53, 102.29, 51.34, 51.16, 44.56, 27.41, 27.08, 26.58, 23.14. HR-MS: calculated for  $\text{C}_{53}\text{H}_{55}\text{O}_8\text{S}_3\text{N}_2$ : 943.3121; found: 943.3125.



The synthesis of IR-845

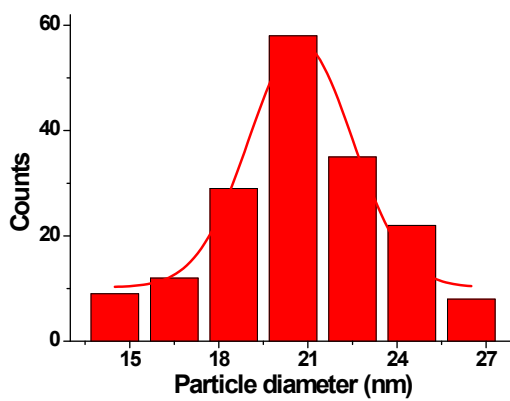
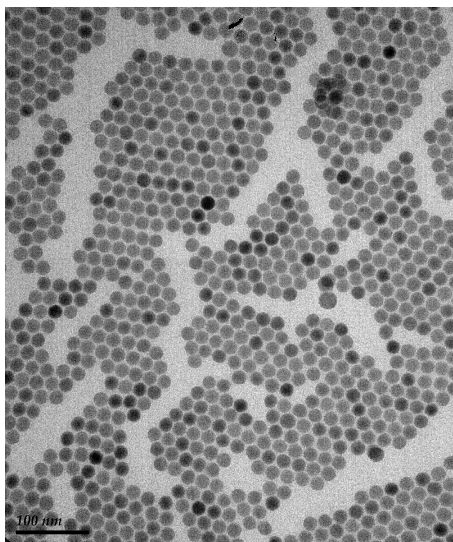
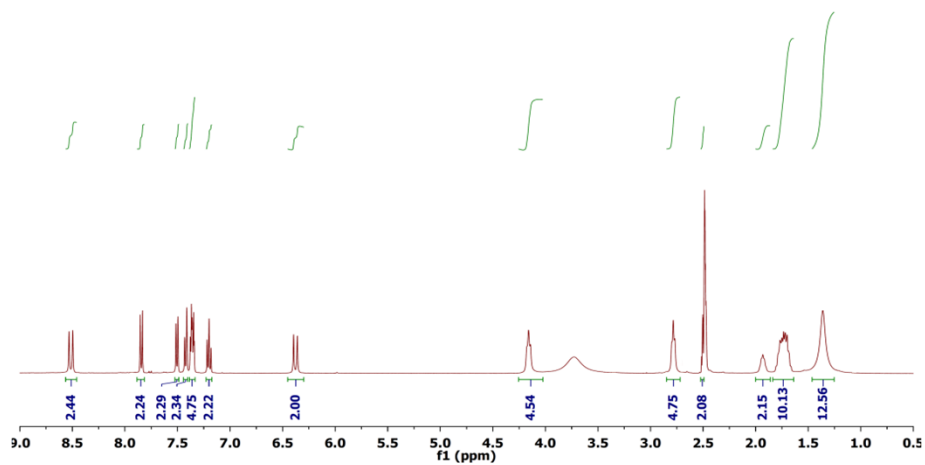
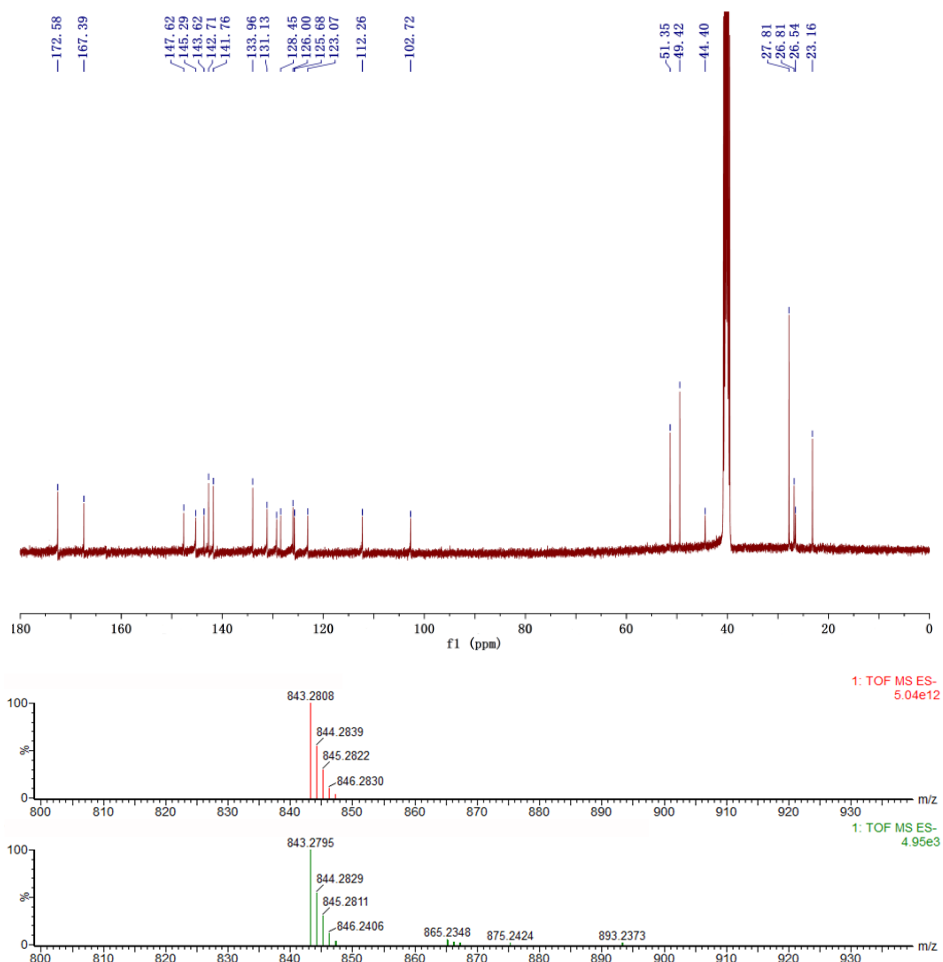
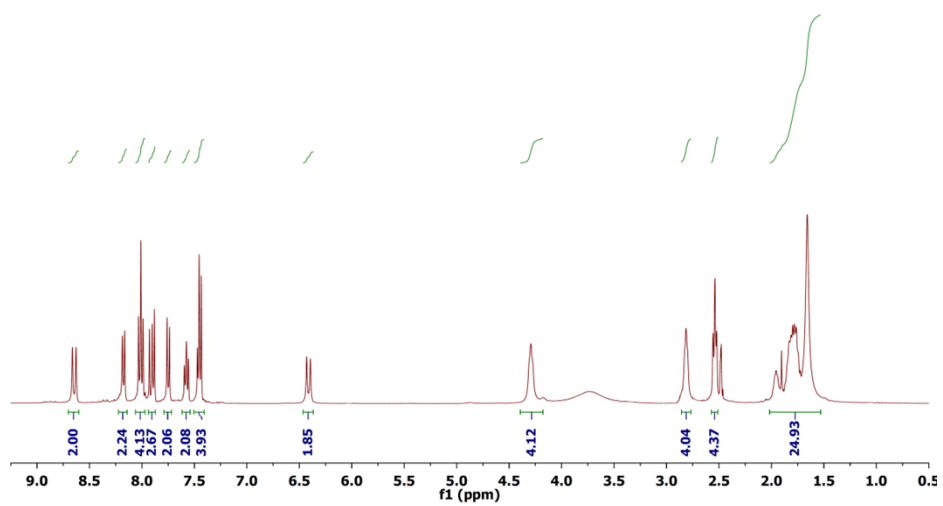


Figure S1: TEM image of  $\beta$ -NaYF<sub>4</sub>:20%Yb,2%Er (left), and the size distribution (right).

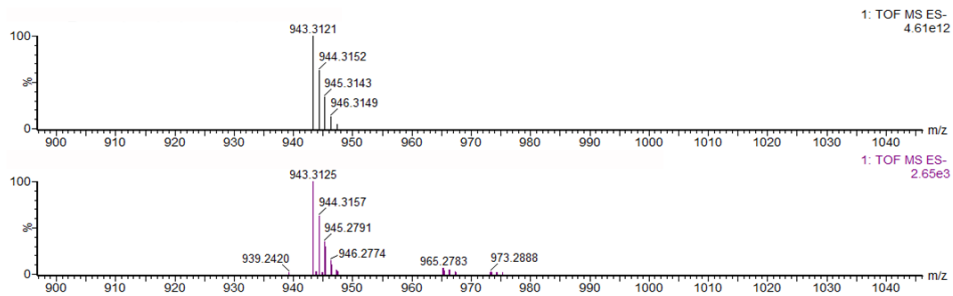
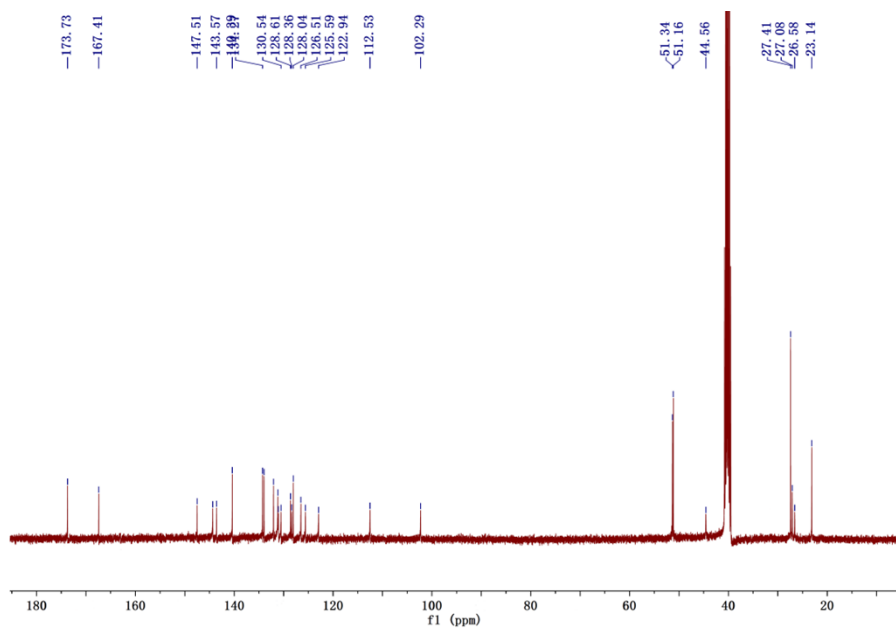




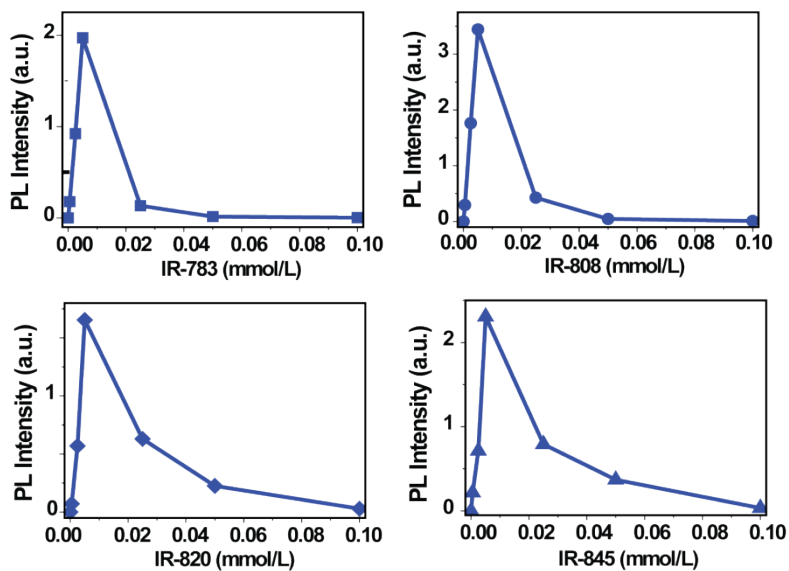
**Figure S2A:**  $^1\text{H}$ -NMR (400 MHz,  $\text{d}^6$ -DMSO),  $^{13}\text{C}$ -NMR (100 MHz,  $\text{d}^6$ -DMSO) and HRMS spectrum of IR-808 (top: calculated; bottom: experimental result).



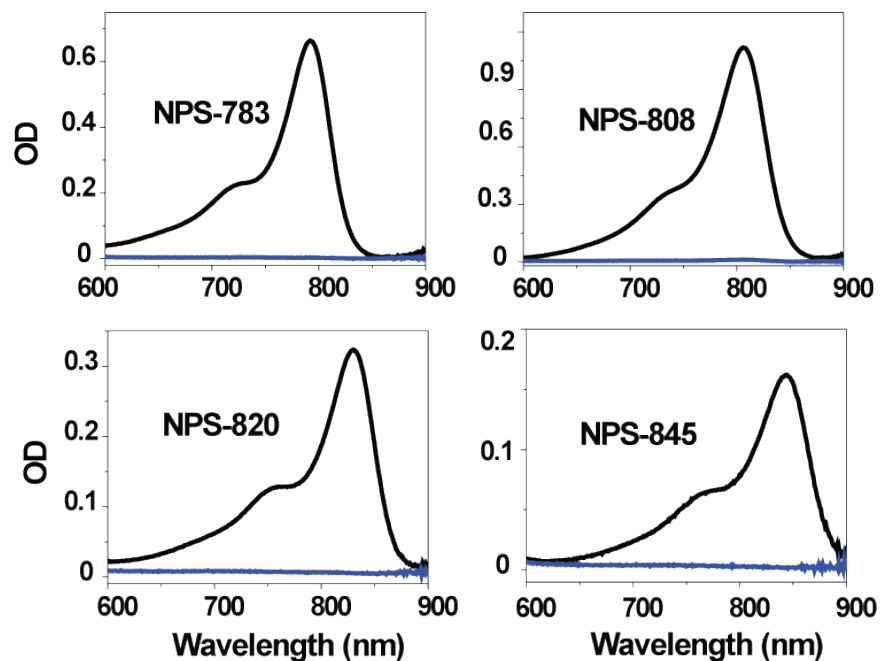




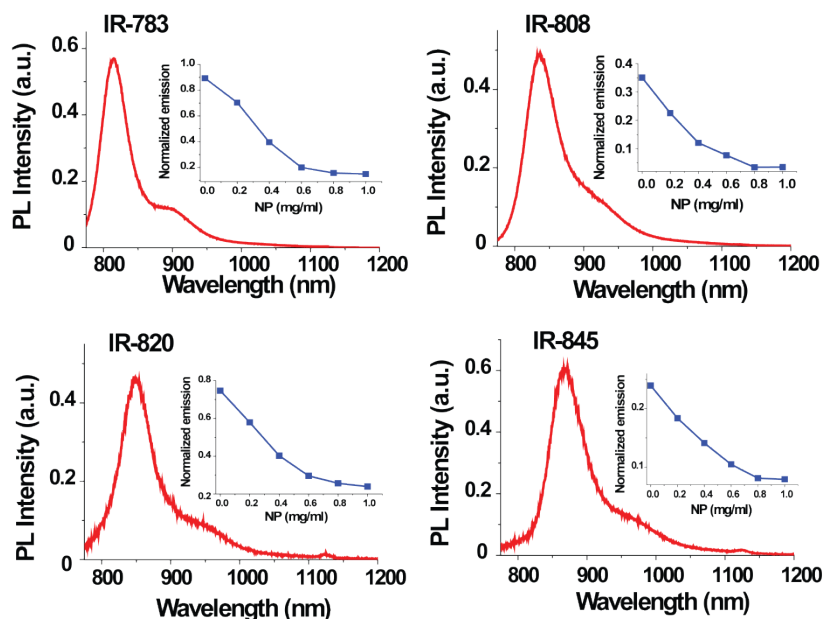
**Figure S2B:**  $^1\text{H-NMR}$  (400 MHz,  $\text{d}^6\text{-DMSO}$ ),  $^{13}\text{C-NMR}$  (100 MHz,  $\text{d}^6\text{-DMSO}$ ) and HRMS spectrum of IR-845 (top: calculated; bottom: experimental result).



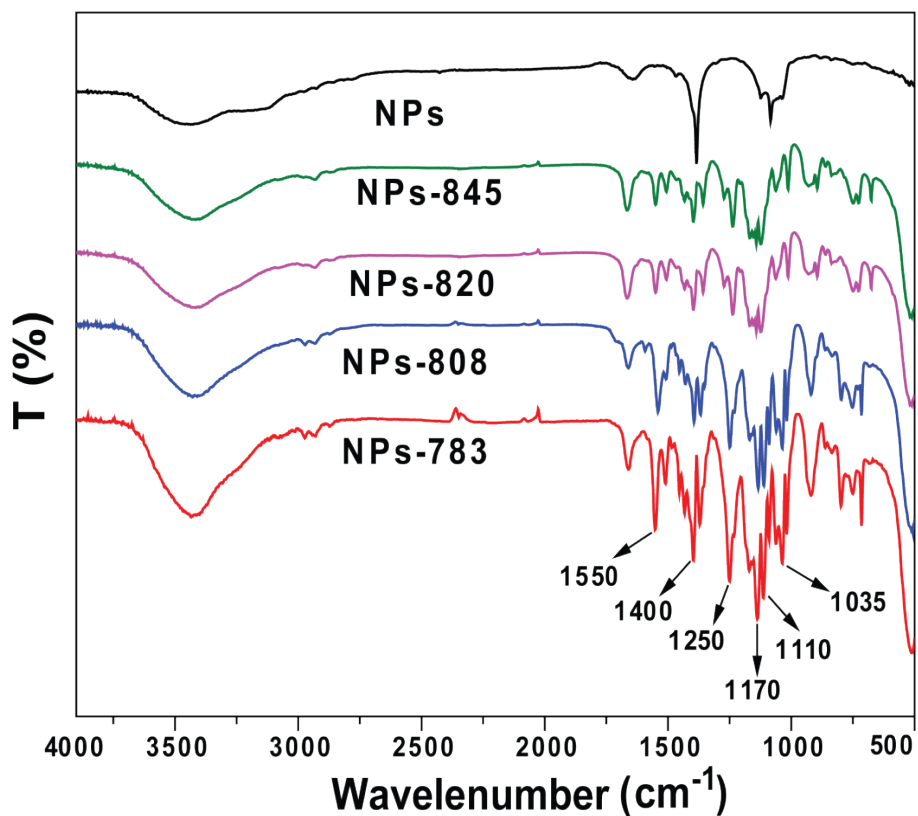
**Figure S3:** Emission intensity integrated in the range 500–700 nm of  $\beta$ -NaYF<sub>4</sub>:20%Yb,2%Er NPs (0.1  $\mu$ mol/L) as a function of a series of IR-dyes 5  $\mu$ mol/L (0.005 mmol/L) (IR-783, IR-808, IR-820, IR-845) content excited by 790 nm, 810 nm, 830 nm, 850 nm, 2 mW, c.w. lasers respectively.



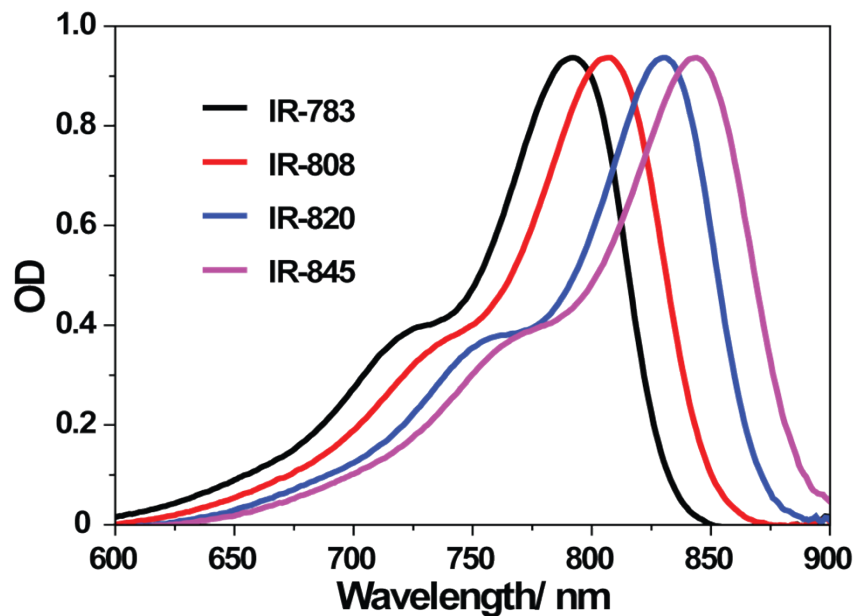
**Figure S4:** The absorption spectra of 5  $\mu$ mol/L IR-dyes (black line) and the supernatant of centrifuged NPs and IR-dyes mixture (5  $\mu$ mol/L: 1 mg/mL) (blue line). The spectrum revealed that comparing to 5  $\mu$ mol/L IR-dyes, the supernatant of each solution did not show noticeable absorption from 600 nm to 900 nm, suggesting the IR-dyes nearly quantitatively conjugate on the UCNP surface.



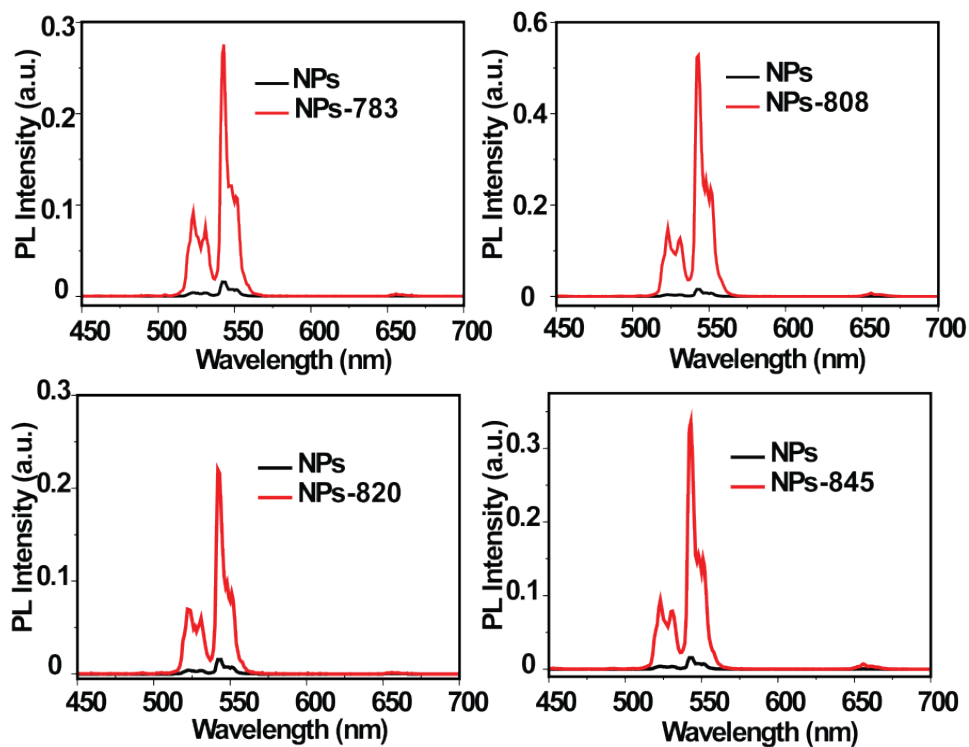
**Figure S5:** Emission spectrum of IR-dyes Final conc. (5  $\mu\text{mol/L}$  in DMF) the insert depicts indicate that upon increasing concentration of  $\beta\text{-NaYF}_4\text{:20\%Yb,2\%Er}$  by adding NPs volume from 0 ml to 1 ml, (Final conc. 0, 0.02, 0.04, 0.06, 0.08, 0.1  $\mu\text{mol/L}$ ) the fluorescence intensity of IR-dyes gradually decreased. All of the samples excited at 750 nm.



**Figure S6.** FT-IR spectra of NPs (black line), IR-783-coated NPs (red line), IR-808-coated NPs (blue line), IR-820-coated NPs (pink line) and IR-845-coated NPs (green line).



**Figure S7:** Normalized absorption spectrum of IR-dyes.



**Figure S8.** The emission spectrum of Nanoparticles (excited at 980 nm), IR-783/Nanoparticles (excited at 790 nm), IR-808/Nanoparticles (excited at 810 nm), IR-820/Nanoparticles (excited at

830 nm) and IR-845/Nanoparticles (excited at 850 nm), All the samples excited by a 2 mW, c.w. laser.

1. J. E. Roberts, Lanthanum and Neodymium Salts of Trifluoroacetic Acid. *J. Am. Chem. Soc.* **1961**, *83* (5), 1087-1088.
2. W. Zou, C. Visser, J. A. Maduro, M. S. Pshenichnikov, J. C. Hummelen, Broadband dye-sensitized upconversion of near-infrared light. *Nature Photon.* **2012**, *6*, 560–564.