Supplementary information

Breaking the Nd³⁺-sensitized upconversion nanoparticles myth about the need of onion-layered structures

Nestor Estebanez,^a Juan Ferrera-González,^a Laura Francés-Soriano,^a Raul Arenal,^{b,c} María González-Béjar^a* and Julia Pérez-Prieto^a*

^a Instituto de Ciencia Molecular (ICMol), University of Valencia, Spain
^b Laboratorio de Microscopias Avanzadas (LMA), Instituto de Nanociencia de Aragon (INA), U. Zaragoza, 50018 Zaragoza – Spain
^c ARAID Foundation, 50018 Zaragoza - Spain

INDEX

Materials and methods	
Methods and equipment	
Scheme S1. Schematic representation of the energy transfer from Nd ³⁺ to Yb ³⁺ and Er ³⁺	
Figure S1. Low-resolution TEM images of UC _{C,Er} and UC _{C,Tm}	
Figure S2. Low-resolution TEM images of UC _{CS,Er} and UC _{CS,Tm}	
Figure S3. High-resolution TEM image of UC _{CS,Tm} @CB	
Figure S4. XRD spectra of UC _{CS,Er} and UC _{CS,Tm}	
Figure S5. EDS spectra of UC _{CS} @CB and UC _{CS} @CB@Nd	
Table S1. Composition of UC _{CS} @CB and UC _{CS} @CB@Nd from EDS	
Figure S6. FTIR spectra of UC _{CS} @oleate, UC _{CS} -naked and UC _{CS} @CB	
Figure S7. Thermogravimetric analyses of UC _{CS,Er} @CB and UC _{CS,Tm} @CB	
Figure S8. Emission spectra a of UC _{CS,Tm} @CB and UC _{CS,Tm} @CB@Nd	
Figure S9. Multiphoton microscopy images of UC _{CS,Er} @CB and UC _{CS,Er} @CB@Nd (λ_{ex} =785 nm)	S12
Figure S10. Kinetic profile from multiphoton time-resolved microscopy	
Figure S11. Emission spectra of UC _{CS,Er} and UC _{CS,Tm}	
Figure S12. Multiphoton microscopy images of UC _{CS,Er} @CB (λ_{ex} =980)	
Figure S13. Multiphoton microscopy images of $UC_{CS,Er}@CB$ ($\lambda_{ex}=800$)	
Figure S14. Multiphoton microscopy images of UC _{CS,Er} @CB@Nd (λ_{ex} =980 nm)	
Figure S15. Multiphoton microscopy images of UC _{CS,Er} @CB@Nd (λ _{ex} =800 nm)	
Figure S16. Multiphoton microscopy images of UC _{CS,Tm} @CB ($\lambda_{ex.}$ =980 nm)	
Figure S17. Multiphoton microscopy images of UC _{CS,Tm} @CB@Nd (λ _{ex} =980 nm)	
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References	

Experimental

Materials and methods

Chemicals: The chemicals used for the nanoparticles syntheses were: lanthanide chlorides (YCl₃· $6H_2O$, YbCl₃· $6H_2O$, ErCl₃· $6H_2O$, TmCl₃· $6H_2O$ and NdCl₃· $6H_2O$ (>99.9%, all of them)), Cucurbituril[7] hydrate (>99%), 1-octadecene (95%), oleic acid (70%), NaOH and NH₄F (99.99%). All these chemicals were purchased from Sigma-Aldrich and used as received without previous purification.

Synthesis

Synthesis of oleate-capped $NaYF_4$: Yb^{3+} , Er^{3+} nanoparticles (UC_{C,Er}@OA)

NaYF₄:Yb,Er nanoparticles were synthesized by following a previously reported protocol with some modifications.¹ In a 50 mL round-bottom flask, oleic acid (8 mL) and octadecene (15 mL) were added. Then, a solution containing YCl₃· $6H_2O$ (0.80 mmol), YbCl₃· $6H_2O$ (0.20 mmol) and ErCl₃· $6H_2O$ (0.02 mmol) dissolved in methanol (2 mL) was added to the flask and the mixture was stirred at 160 °C under N₂ until everything was dissolved. Next, the solution was cooled to 100°C and 4 mL of a methanol solution containing NaOH (2.5 mmol) and NH₄F (4.0 mmol) were slowly added into the flask during 5 min. Then the solution was heated until 125 °C under N₂ flow and continuous stirring to remove completely methanol and water traces. Finally, the reaction was heated at 305 °C under N₂ flux during one hour. After that, the solution was cooled to room temperature and the nanoparticles were precipitated by centrifugation (9000 rpm, 15 min, 25 °C). Later on, the oleate-capped UCNPs were washed three times with (2.7:2.5:1 v/v) hexane/acetone/methanol solution.

Synthesis of oleate-capped $NaYF_4$: Yb^{3+} , $Tm^{3+}nanoparticles$ ($UC_{C,Tm}@OA$)

NaYF₄:Yb,Tm nanoparticles were synthesized by following a previously reported protocol with some modifications.¹ In a 50 mL round-bottom flask, YCl₃·6H₂O (0.80 mmol),YbCl₃·6H₂O (0.20 mmol), TmCl₃·6H₂O (0.002 mmol), oleic acid (12 mL) and octadecene (15 mL) were stirred at 160 °C under N₂ until complete dissolution of lanthanides salts. Then, the solution was cooled to 110°C and 10 mL of a methanol solution containing NaOH (2.5 mmol) and NH₄F (4.0 mmol) were slowly added into the flask. Methanol was removed at 100 °C under N₂ flow and continuous stirring. Finally, the reaction was heated at 305 °C under N₂ flux during one hour. After that, the solution was cooled to room temperature and the nanoparticles were precipitated by centrifugation (9000 rpm, 15 min, 25 °C). Later on, the oleate-capped UCNPs were washed three times with (2.7:2.5:1 v/v) hexane/acetone/methanol solution.

Synthesis of oleate-capped NaYF₄: Yb^{3+} , $Er^{3+}/NaYF_4$ core/shell nanoparticles ($UC_{CS,Er}@OA$) or $NaYF_4$: Yb^{3+} , $Tm^{3+}/NaYF_4$ core/shell nanoparticles ($UC_{CS,Tm}@OA$)

In a 50 mL round-bottom flask, oleic acid (4 mL) and octadecene (8 mL) were added. Then, $YCl_3 \cdot 6H_2O$ (0.25 mmol) was dissolved in methanol (1 mL) and added to the flask. The mixture was stirred at 160 °C under N₂ until methanol was evaporated.

Next, the solution was cooled to 80°C and a solution of UC_C@OA (100 mg in 5 mL) of chloroform was slowly added and the mixture was heated at 100°C while 4 mL of a methanol solution containing NaOH (1.3 mmol) and NH₄F (2.0 mmol) were slowly added into the flask during 5 min. Methanol was removed at 125 °C under N₂ flow and continuous stirring. Finally, the reaction was heated at 325 °C under N₂ flux during 25 minutes. After that, the solution was cooled to room temperature and the nanoparticles were precipitated by centrifugation (9000 rpm, 15 min, 17 °C). Later on, the oleate-capped UCNPs were washed three times (3x15 mL) with (2.7:2.5:1 v/v) hexane/acetone/methanol solution.

Synthesis of ligand-free $UC_{CS,Er}$ or $UC_{CS,Tm}$

Oleate ligand was removed from the nanoparticle surface by following a previously described protocol.^{2, 3} Briefly, 150 mg of UC_{CS,Er}@OA or UC_{CS,Tm}@OA were dispersed in 15 mL of HCl aqueous solution at pH 3 and stirred for 2 h. After that, three extractions with diethyl ether (3x15 mL) were carried out in order to remove the free oleic acid. Then, ether phases were recombined and re-extracted with water. Ligand-free nanoparticles were recovered by precipitation with acetone and subsequent centrifugation at 9000 rpm for 15 min (17°C). Finally, the as-prepared UC_{CS,Er} or UC_{CS,Tm} were redispersed in MilliQ water.

Synthesis of CB[7]-capped nanohybrids (UC_{CS}@CB)

These nanohybrids were prepared by following our previously reported protocol.⁴ Briefly, in a centrifuge tube 0.012 mmol (14 mg) of CB[7] were added to 30 mg of ligand-free UCNPs (UC_{CS,Er} or UC_{CS,Tm}) 15 mL of MilliQ water. Then, the mixture was sonicated for 15 minutes. The solution was stirred during 48 h in an orbital shaker at 350 ppm and kept in the dark. In order to remove the excess of CB[7], 2 extra cycles of centrifugation at 9000 rpm x 15 minutes (17°C) were carried out: firstly with 10 mL of water and secondly with 10 mL of acetonitrile. Both UC_{CS}@CB (UC_{CS,Er}@CB or UC_{CS,Tm}@CB) were easily resuspended in MilliQ water.

Synthesis of Nd^{3+} -capped $UC_{CS}@CB$ nanohybrids ($UC_{CS}@CB@Nd$)

 $UC_{CS}@CB$ nanohybrids (30 mg) were dissolved in 15 mL of MilliQ water. Then, NdCl₃·6H₂O was added in excess (4 mol of Nd per mol of CB in the shell for $UC_{CS,Er}@CB$ or $UC_{CS,Tm}@CB$) and, the mixture was stirred in an orbital shaker for 24 h at 400 rpm in the dark. Later on, nanoparticles were separated by centrifugation (9000 rpm, 15 min, 17° C). Finally, the $UC_{CS}@CB@Nd$ nanohybrids were washed with MilliQ water (10 mL) followed by centrifugation.

Methods and equipment

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) images were obtained using a Jeol 1010 microscope operating at 100 kV equipped with a digital camera AMT RX80 (8Mpx). For the preparation of the samples, 10 μ L of a 0.5 mg·mL⁻¹ solution of the UCNPs was left to dry under vacuum at room temperature on a formvar/carbon film supported on a 300 mesh copper grid. High-resolution transmission electron microscopy (HRTEM) images were recorded using a

TECNAI G2 F20 microscope operating at 200 kV (point resolution of 0.24 nm) and equipped with a CCD GATAN camera.

Scanning Electron Microscopy (SEM)

The top and cross-sectional SEM images were obtained using a HITACHI S-4800, with a spotlight of field emission (FEG). Images were acquired at 20 kV.

The EDS analysis was carried out in HITACHI S-4800, equipped with XFlash 5030 Bruker detector and acquisition software QUANTAX 400.

High-resolution scanning transmission electron microscopy (HRSTEM)

The TEM samples were prepared by dispersing a drop of the ultrasonicated suspension in ethanol onto copper carbon holey grids.

TEM studies have been developed using a FEI Titan Low-Base microscope, working at 120 kV. This microscope is equipped with a Cs probe corrector, a monochromator and ultra-bright X-FEG electron source. The convergence angle was 25 mrad. HRSTEM imaging was performed by using high-angle annular dark field (HAADF) detector. The inner and outer angles for the micrographs recorded with this detector were 48 and 200 mrad, respectively.

X-ray power diffraction (XRD)

XRD analyses were performed on a Bruker D8 Advance A25 diffractometer using CuK α (λ =1.54060 Å) radiation at a voltage of 40 kV and 30 mA, and a LynxEye detector. The powder diffraction pattern was scanned over the angular range of 2-80° (2 θ) with a step size of 0.020°, at room temperature.

Fourier-transform infrared spectra measurements (FTIR)

All FTIR spectra were performed using a FTIR Thermo Nicolete Nexus spectrophotometer at room temperature with 64 scans and a resolution of 4 cm⁻¹ between 400 and 4000 cm⁻¹.

Thermogravimetric analysis (TGA)

The TGA analyses were carried out using a TG-TGA Pyris Diamond system with an operative temperature range 25-1100 °C and 0.1 microgram sensitivity. The samples were heated from 25 to 800 °C, with an increase of 5°C \cdot min⁻¹ and under air flux of 50 mL \cdot min⁻¹.

Centrifugation

Centrifugation was carried out in an Eppendorf Centrifuge 5804 R.

Steady-state photoluminescence

Steady-state photoluminescence spectra were obtained at room temperature with a 2-nm slit width and 5 nm \cdot s⁻¹ speed scan using a SLM Amingo Bowmann series 2 (AB2) fluorometer (Microbeam, S.A.). The AB2 software (v. 5.5) was used to register the data. All data were acquired in a front face set up. Upconversion emission spectra were

recorded by excitation at 975±10 nm using a CW 975 nm diode laser (Thorlabs L975P1WJ) as excitation source coupled to the fluorometer.

Time-resolved photoluminescence

Measurements for the up-conversion luminescence were performed using an Olympus FV1000MPE laser scanning confocal coupled to an Olympus BX61WI upright microscope equipped with a 25x water immersion objective (1.05 NA). This confocal was provided with a Mai Tai HP Deep See multiphoton laser with a pulse width of 100 fs as excitation source. The laser focused over the sample was tuned to 980 nm with an excitation power of 30 mW. The excitation power density was determined with a focused spot of about a 1 μ m radius. The image was acquired by means of a 25x 1.05 N.A. Olympus dipping lens, appropriated emission filters and dwell time of 2 μ s·pixel⁻¹ and a resolution of 1024x1024 pixels. Emission is detected in a 4 channel visible-range detector (420-500/515-580/590-650/660-740). Samples were prepared by drop-casting a water dispersion of the corresponding NPs onto a 25×75 mm microscope glass slide. Then, solvent was evaporated and the sample was covered with a 22×22 mm glass slide.

Upconversion quantum yield measurements

For the measurement of the upconversion quantum yield a Quantaurus-QY Plus UV-NIR absolute quantum yield spectrometer (C13534-11) was used. The nanohybrids were excited with an internal 980 nm (laser power 0.5W) or a CW 808 nm laser (808 ± 5 nm; laser power 1.6W) coupled to the spectrometer as excitation source. The spot size was 1 mmx0.1 mm and an exposure time of 145.6 ms.



Scheme S1. Schematic representation of energy transfer from Nd^{3+} to Yb^{3+} and Er^{3+} .



Figure S1. Representative low-resolution TEM images for UC_C together with size distribution histograms constructed from these TEM particle measurements: (A) NaYF₄:Yb,Er (UC_{C.Er}) and (B) NaYF₄:Yb,Tm (UC_{C.Tm}). Figure S1A illustrates that NaYF₄:Yb,Er cores are hexagonal nanoprisms⁵ with a narrow particle size distribution 35 ± 2 nm (hexagon width) × 41±1 nm (rod length). After the anisotropic shell growth of the undoped NaYF₄ shell, the as-synthetized UC_{CS,Er} (NaYF₄:Yb,Er/NaYF₄) display an average size of 38 ± 2 nm (width) × 66 ± 3 nm (length) indicating as expected, a slight increase along one preferential direction (Figure S2A). ⁶⁻⁹

Similarly figure S1B illustrates NaYF₄:Yb,Tm cores are hexagonal nanoprisms with a size distribution 17 ± 1 nm (hexagon width) × 24±1 nm (rod length). After the anisotropic shell growth of the undoped NaYF₄ shell, the as-

synthetized UC_{CS,Tm} (NaYF₄:Yb,Tm/NaYF₄) display an average size of 21 ± 1 nm (width) × 31 ± 2 nm (length) indicating as expected,⁶⁻⁸ a slight increase along one preferential direction (Figure S2B).



Figure S2. Representative low-resolution TEM images for UC_{CS} together with size distribution histograms constructed from these TEM particle measurements: (A) NaYF₄:Yb,Er/NaYF₄ (UC_{CS,Er}) and (B) NaYF₄:Yb,Tm/NaYF₄ (UC_{CS,Tm}).



Figure S3. High-resolution TEM image of UC_{CS,Tm}@CB.



Figure S4. XRD spectra of: UC_{CS}: NaYF₄:Yb,Tm/NaYF₄ (blue), NaYF₄:Yb,Er/NaYF₄ (red) and hexagonal NaYF₄ standard (JCPDS PDF number 16-0334). The extra peaks are due to NaCl (DB card number 01-070-2509 Halite, syn; 20: 31.64, 45.39, 56.31).



Figure S5. EDS spectra of (A) UC_{CSEr}@CB; (B) UC_{CS,Tm}@CB; (C) UC_{CS,Er}@CB@Nd; (D) UC_{CS,Tm}@CB@Nd (20 kV).

Table S1.	Composi	tion of	samples	from	EDS.
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	Atom. C[at%]							
Nanohybrid	Yttrium	Ytterbium	Erbium	Thulium	Neodymium			
UC _{CS,Er} @CB	89.5 ± 0.1	9.5 ± 0.1	1.0 ± 0.04					
UC _{CS,Er} @CB@Nd	88.5 ± 0.6	9.1 ± 0.5	1.0 ± 0.7		1.4 ± 0.05			
UC _{C,Tm} @OA	80.9 ± 1.0	19.0 ± 0.7		<lod* (<1)<="" td=""><td></td></lod*>				
UC _{CS,Tm} @CB	88.6 ± 0.4	11.4 ± 0.4		<lod* (<1)<="" td=""><td></td></lod*>				
UC _{CS,Tm} @CB@N d	88.7 ± 0.6	9.5 ± 0.1	—	<lod* (<1)<="" td=""><td>1.7 ± 0.2</td></lod*>	1.7 ± 0.2			

* Limit of detection of the method

The presence of CB7 was corroborated by Fourier transform infrared (FTIR) spectroscopy and thermogravimetric analyses (TGA) (Figures S6-S7).



Figure S6. FTIR spectra of (A) $UC_{CS,Er}$ @OA (blue, left) and $UC_{CS,Tm}$ @OA (blue, right); (B) $UC_{CS,Er}$ (green, left) and $UC_{CS,Tm}$ (green, right); (C) $UC_{CS,Er}$ @CB (red, left) and $UC_{CS,Tm}$ @CB (red, right) nanohybrids. (D) CB[7] has been added for comparison purposes.



Figure S7. Thermogravimetric analyses (TGA) of $UC_{CS,Er}@CB$, (left) $UC_{CS,Tm}@CB$ (right), expressed as weight (%) as a function of applied temperature together with their first derivative (red lines).

Thus, the FTIR spectra of UC_{CS}@CB nanohybrids exhibited a strong band at 1735 cm⁻¹, which corresponds to the stretching vibration of CB[7] carbonyl groups (C=O). As expected, this band shifted as compared to 1720 cm⁻¹ of free CB[7] due to the interaction between CB[7] and the NP. Moreover, the TGA of UC_{CS}@CB nanohybrids showed a weight loss of 5.3% for UC_{CS,Er}@CB and 6.2% for UC_{CS,Tm}@CB at ca. 430°C (peak of the first derivate) characteristic of CB[7] (Figure S7).



Figure S8. Normalized emission spectra at 475 nm of: $UC_{CS,Tm}@CB$ (black line) and $UC_{CS,Tm}@CB@Nd$ (red line).



Figure S9. Multiphoton microscopy images of UC_{CS,Er}@CB and UC_{CS,Er}@CB@Nd under the same excitation and optical setup (λ_{ex} =785 nm; 0.497 µm/pixel; 2.0 µs/pixel).



Figure S10. Example of kinetic profile obtained from an intensity profile using multiphoton timeresolved microscopy. All kinetic traces obtained by this method showed the characteristic rise and decay phases of the UCNPs.



Figure S11. Emission spectra of $UC_{CS,Er}$ (left) and $UC_{CS,Tm}$ (right). Detection channels are depicted in colored rectangles.





 515-560nm 590-650nm 660-740nm

 Figure S12. Multiphoton microscopy images of UC_{CS,Er}@CB (λ_{ex} =980 nm; 0.497 µm/pixel; 2.0 µs/pixel).



Figure S13. Multiphoton microscopy image of UC_{CS,Er}@CB. The image is the overlapping of all detection channels at sensitivities >400V (λ_{ex} =800 nm; 0.497 µm/pixel; 2.0 µs/pixel).

UC_{CS,Er}@CB@Nd



515-560nm

590-650nm

660-740nm

Figure S14. Multiphoton microscopy images of UC_{CS,Er}@CB@Nd (λ_{ex}=980 nm; 0.497 μm/pixel; 2.0 μs/pixel).



515-560nm

590-650nm

660-740nm

Figure S15. Multiphoton microscopy images of UC_{CS,Er}@CB@Nd (λ_{ex}=800 nm; 0.497 μm/pixel; 2.0 μs/pixel).

$UC_{CS,Tm} @CB \\$



420-500nm590-650nmFigure S16. Multiphoton microscopy images of UC_{CS,Tm}@CB (λ_{ex} =980 nm; 0.124 µm/pixel; 8.0 µs/pixel).



UC_{CS,T}m@CB@Nd

Figure S17. Multiphoton microscopy images of UC_{CS,Tm}@CB@Nd (λ_{ex} =980 nm; 0.159 µm/pixel; 8.0 µs/pixel).



420-500nm590-650nmFigure S18. Multiphoton microscopy images of UC_{CS,Tm}@CB@Nd (λ_{ex} =800 nm; 0.497 µm/pixel; 8.0 µs/pixel).

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