Electronic Supplementary Material (ESI)

Optimising FRET-Efficiency of Nd³⁺-Sensitised Upconversion Nanocomposites by Shortening the Emitter–Photosensitizer Distance

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1. Experimental Section

Chemicals. Reagent or HPLC grade chemicals used were purchased from Sigma-1.1. Aldrich (St Louis, MO, USA): yttrium acetate hydrate, ytterbium acetate hydrate, erbium acetate hydrate, neodymium acetate hydrate, oleic acid, 1-octadecene, sodium hydroxide, ammounium fluoride, ammounium nitrate, ethyl acetate, tetraethyl orthosilicate (TEOS), chloroform, rose bengal sodium salt (RB), (3-Aminopropyl)triethoxysilane (APTES), nhexadecyltrimethylammonim bromide (CTAB), N-(3-Dimethylaminopropyl)-N'ethylcarbodiimide hydrochloride (EDC), N-Hydroxysulfosuccinimide sodium salt (sulfo-NHS), dimethyl sulfoxide (DMSO), and cell counting kit 8; from Thermo Fisher Scientific (Waltham, MA, USA): n-hexane, acetone, methanol, and ethanol; from Nanocs (New York, NY, USA): PEG-FA-NHS ester (mw 3400). All chemicals were used as received without further purification. Rose bengal hexanoic acid ester (RB-HA) was prepared according to the literature reported method.¹

1.2. Synthesis of the core NaYF₄:Nd(20%),Yb(10%) or NaYF₄:Yb(10%),Er(1%) NPs.

The lanthanide (Ln)-doped nanoparticles (NPs) were prepared according to a literature reported method with minor modifications. To synthesize the core NaYF₄:Nd,Yb NPs, a total of 1 mmol Ln(CH₃COO)₃ precursors in the Y:Nd:Yb = 70:20:10 mol% ratios (or NaYF₄:Yb,Er NPs, a total of 1 mmol Ln(CH₃COO)₃ precursors in the Y:Yb:Er = 89:10:1 mol%) were mixed in a solution containing 10 mL oleic acid (OA) and 15 mL octadecene (ODE). The mixture was heated to 160 °C under an argon flow for 1 h to result in a homogeneous solution. After cooling to room temperature, 10 mL methanol solution

containing 4 mmol (0.15 g) NH₄F and 2.5 mmol (0.1 g) NaOH was slowly added to the solution with stirring for 1 h at 65 °C to evaporate methanol. Subsequently, the solution was heated to 300 °C for 1.5 h and cooled to room temperature. The resulting NaYF₄:Nd³⁺(20%),Yb³⁺(10%) or NaYF₄:Yb³⁺(10%),Er³⁺(1%) NPs were precipitated, washed by ethanol several times, and collected by centrifugation and redispersed in 15 mL n-hexane. ICP Elemental analysis data are listed in Table S1.

1.3. **Synthesis** of the core/shell. core/shell/shell core/shell/shell/shell and NaYF₄:Nd(20%),Yb(10%)@NaYF₄:Yb(10%)@NaYF₄:Yb(10%),Er(1%)@NaYF₄ and NaYF₄:Yb(10%),Er(1%)@NaYF₄:Yb(10%)@NaYF₄:Nd(20%),Yb(10%)@NaYF₄ by the Successive Layer-by-Layer Strategy (SLBL) strategy. The multi-shell nanoparticles were prepared according to the literature reported method using the Successive Layer-by-Layer Strategy (SLBL) with some modifications.² The preparation of the core/shell precusors was performed first: 2.5 mmol $Ln(CH_3COO)_3$ (Ln = Y, Nd, Yb, Er) was added to a solution containing 10 mL OA and 15 mL ODE with stirring. The mixture was then heated to 180 °C for 1 h to obtain a 0.1 M Ln-OA homogeneous precursor solution, which was then cooled down to room temperature. In the meantime, a 0.4 M CF₃COONa (Na-TFA) stock solution in 10 mL OA was prepared under vacuum with stirring to remove residual oxygen and water. Then, 3 mL of the previously synthesized core NaYF₄:Nd,Yb or NaYF₄:Yb,Er NPs in nhexane was added to a solution containing 6 mL OA and 15 mL ODE in a flask. The solution was heated to 65 °C to evaporate n-hexane before heating to 290 °C under an argon flow. Then, 1 mL 0.1 M Yb-OA and 0.5 mL 0.4 M Na-TFA-OA stock solutions were added sequentially at 290 °C, at a 10 min duration time. After cooling to room temperature, the core/shell UCNPs NaYF4:Nd,Yb@NaYF4:Yb or YF4:Yb,Er@NaYF4:Yb was precipitated, washed with ethanol, collected by centrifugation and redispersed in 15 mL n-hexane. The core/shell/shell and core/shell/shell/shell UCNPs can then be prepared according to similar procedures. The amounts of the added lanthanide salt stock solutions could be adjusted according to the molar ratio required. ICP Elemental analysis data are listed in Table S1.

1.4. Coating of core/shell/shell UCNPs with amino-functionalized mesoporous silica (mSiO₂). Coating mSiO₂ on the surface of oleate-capped UCNPs was performed according to the previously reported method. The previously prepared core/shell/shell/shell UCNPs (25 mg) was dispersed in 25 mL cetyltrimethylammonium bromide (CTAB, 5.5 mM) solution and sonicated for 30 min. Then 0.1 mL tetraethyl orthosilicate (TEOS), 150 μ L 2 M NaOH and 1.5 mL ethyl acetate were added, and the resulting mixturef was stirred for 3 h at 70 °C. After cooling down to room temperature, the rude product was collected by centrifugation and washed several times with 10 mL ethanol to remove the residual reactants. To remove the remaining CTAB, the rude product was further extracted with NH₄NO₃ (0.3 g) in 25 mL ethanol at 60 °C for 2 h, and cooled to room temperature. The core/shell/shell/shell UCNPs @mSiO₂ product was then obtained by centrifugation and washed.

The functionalization of the synthesized core/shell/shell UCNPs@mSiO₂ with amino groups for further bioconjugation processes follows. In a typical process, 1 mL APTES was added in 20 mL ethanol solution containing core/shell/shell UCNPs@mSiO₂. The container was sealed and kept at 80~90°C for 24 h. Residual reagent was removed by centrfugation and wash by ethanol. The core/shell/shell UCNPs@mSiO₂-NH₂ could be dispersed in H₂O.

1.5. Conjugation of PEG-FA and rose bengal to core/shell/shell/shell UCNPs@mSiO₂-NH₂. The core/shell/shell UCNPs@mSiO₂-NH₂ (10 mg) and 2 mg PEG-FA-NHS were suspended in 2 mL H₂O, followed by sonication and vigorous stirring for 1 h. Residual PEG-FA was removed by centrifugation. Then, 1mg of RB-HA was dissolved in 0.2 mL DMSO together with 1 mg of EDC and 1.5 mg of sulfo-NHS, and incubated at room temperature for 30 min. Then, 1.8 mL of the core/shell/shell/shell UCNPs@mSiO₂-NH₂ was added and stirred for overnight. The NaYF₄:Nd,Yb@Yb@Yb,Er@Y@mSiO₂/RB-PEG-FA (or NdYb-CSs-

UCNCs) or NaYF4:Yb,Er@Yb@Nd,Yb@Y@mSiO₂/RB-PEG-FA (or YbEr-CSs-UCNCs) was collected by centrifuge and washed with DMSO and water. The final product could be dispersed in water.

1.6. Singlet Oxygen Determination under 793 nm NIR Irradiation. ABDA was used to determine the generated singlet oxygen after NIR radiation on the NdYb-CSs-UCNCs and YbEr-CSs-UCNCs according to a published method.³ In a typical experiment, 10 μ M ABDA–DMSO solution was added to 2 mL of NdYb-CSs-UCNCs or YbEr-CSs-UCNCs aqueous solution. The sample was then irradiated using a 793 nm NIR laser with 0.8 W/cm² for 0, 10, 20, and 30 min, and the fluorescence intensities of ABDA at 407 nm were measured in triplicates to correlate with the amounts of singlet oxygen generated.

1.7. *In vitro* NIR induced PDT. MDA-MB-231 cancer cells were seeded in 96-well plates $(1x10^4 \text{ cells/well})$ and incubated in DMEM for 24 h at 37 °C under 5% CO₂. Then appropriate concentration of NdYb-CSs-UCNCs or YbEr-CSs-UCNCs was added into each well and incubated for another 24 h. The cells were irradiated with a 793 nm laser (1 W/cm²) for 30 min, followed by incubation for another 24 h and addition of the CCK-8 reagent (50 µl, 10 times dilution with DMEM) to each well. After incubation for 1.5 h at 37 °C, the cell viability was determined by measuring the absorbance at 450 nm in each well.

1.8. *In vivo* NIR imaging. The animal experiments in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of National Yang-Ming University. The BALB/c nude mice were purchased from BioLASCO Taiwan Co., Ltd. All in vivo experiments were carried out in compliance with the Animal Protection Act of Laws and Regulations of the Republic of China and the guidelines for the Care and Use of IACUC of National Yang-Ming University. *In vivo* down-conversion NIR images employing the newly designed nanocomposites were obtained by a homemade equipment with an InGaAs CCD camera (NIRvana 640 Camera, Princeton Instruments). The 793 nm excitation light source was provided by DPSS laser (125 mW, Changchun New Industries Optoelectronics

Technology Co., Ltd.) with a diffuser. The emitted photons passed through an 850 nm longpass filter (Schott RG-850, 50.8 mm Sq.) were measured as the photon signals. A volume of 100 µL of the NaYF4:Nd,Yb@Yb@Yb,Er@Y@mSiO2/RB-PEG-FA solution (10 mg/mL) was administered by intratumoral injection for the imaging of the 4T1 tumor-bearing mouse according to a published procedure.⁴

1.9. Characterization. The morphology image measurements of nanoparticles were carried out on a JEM-2000EX II transmission electron microscope (TEM) that operating at an acceleration voltage of 100 kV. High-resolution transmission electron microscope (HR-TEM) images and energy-dispersive X-ray spectroscopy (EDS) data were recorded on a JEOL-JEM 1400PLUS operated at an acceleration voltage of 100 kV. Elemental analysis of the lanthanide ion content was determined by using the Agilent 725 inductively coupled plasma optical emission spectrometry (ICP-OES), by the Instrument Center at National Tsing Hua University, Hsinchu, Taiwan. Photoluminescence spectra were recorded with a FSP-920 steady-state and phosphorescence lifetime spectrometer (Edinburgh Instruments) equiped with a 40W 793 nm adjustable laser. The 793 nm NIR laser with a power density of 1W/cm² was used in this study, which is lower than the safety insurance (2.4 W/cm^2) for laser therapy application.⁵ The UV-Vis spectra were recorded in 1 cm quartz cuvettes with a Hewlett-Packard/Agilent 8453 diode-array uv-vis spectrophotometer. The size distributions of nanoparticles were determined by SZ-100 zetasizer for dynamic light scattering (DLS) measurement (HORIBA Scientific). All the nanoparticles under current study were prepared by procedures described above and characterized by similar experimental conditions and instrumentation settings using in-house standard operation procedures (SOPs) including careful calibrations.



Figure S1. DLS analyses of various UCNPs. Upper ones, (a) NaYF₄:Nd,Yb, (b) NaYF₄:Nd,Yb@Yb, (c) NaYF₄:Nd,Yb@Yb@Yb,Er, (d) NaYF₄:Nd,Yb@Yb@Yb,Er@Y 1.9 nm, (e) NaYF₄:Nd,Yb@Yb@Yb,Er@Y 5.4 nm, (f) NaYF₄:Nd,Yb@Yb@Yb,Er@Y 12.1 nm. Lower ones, (g) NaYF₄:Yb,Er, (h) NaYF₄:Yb,Er@Yb, (i) NaYF₄:Yb,Er@Yb@Nd,Yb, (j) NaYF₄:Yb,Er@Yb@Nd,Yb@Y 2.2 nm, (k) NaYF₄:Yb,Er@Yb@Nd,Yb@Y 6.8 nm, (l) NaYF₄:Yb,Er@Yb@Nd,Yb@Y 12.0 nm.



Figure S2. EDS data of the core and core/shell/shell UCNPs for both NaYF₄:Nd,Yb @Yb@Yb,Er (upper ones) and NaYF4:Yb,Er@Yb@Nd,Yb (lower ones).



Figure S3. DLS analyses of various UCNPs coated with mesoporous silica. Upper ones, (a) NaYF₄:Nd,Yb@Yb@Yb,Er@Y@mSiO₂ Y = 2.9 nm, (b) NaYF₄:Nd,Yb@Yb@Yb,Er@Y @mSiO₂ Y = 4.8 nm, (c) NaYF₄:Nd,Yb@Yb@Yb,Er@Y@mSiO₂ Y =11.5 nm. Lower ones, (d) NaYF₄:Yb,Er@Yb@Nd,Yb@Y@mSiO₂ Y = 2.1 nm, (e) NaYF₄:Yb,Er@Yb@Nd,Yb@Y @mSiO₂ Y = 6.2 nm, (f) NaYF₄:Yb,Er@Yb@Nd,Yb@Y@mSiO₂ Y =11.3 nm.

No.	NdYb UCNPs	Photon counts at 540 nm	Size TEM (DLS), nm	No.	YbEr UCNPs	Photon counts at 540 nm	Size TEM (DLS) nm
1	NaYF4:Nd,Yb @Yb,Er	7.606×10^2	23.4 ± 1.1 (24.6 ± 5.8)	7	NaYF ₄ :Yb,Er @Nd,Yb	1.359×10^4	$\begin{array}{r} 26.4 \pm 1.2 \\ (28.7 \pm 6.9) \end{array}$
2	NaYF ₄ :Nd,Yb @Yb,Er@Y 2.0 nm	3.435×10^3	$27.4 \pm 1.8 \\ (28.9 \pm 6.2)$	8	NaYF ₄ :Yb,Er @Nd,Yb@Y	1.537 x 10 ⁵	$\begin{array}{r} 30.8 \ \pm \ 1.4 \\ (31.8 \pm 7.3) \end{array}$
3	NaYF ₄ :Nd,Yb @Yb@Yb,Er	9.609 x 10 ²	26.7 ± 0.6 (28.7 ± 6.1)	9	NaYF ₄ :Yb,Er @Yb@Nd,Yb	2.016 x 10 ⁴	$\begin{array}{rrr} 30.7 & \pm & 1.1 \\ (31.3 \pm 7.1 \) \end{array}$
4	NaYF ₄ :Nd,Yb @Yb@Yb,Er@Y	1.268 x 10 ⁵	$\begin{array}{c} 32.5 \pm 1.7 \\ (32.9 \pm 3.5) \end{array}$	10	NaYF ₄ :Yb,Er @Yb@Nd,Yb@Y	3.830 x 10 ⁵	$\begin{array}{rrr} 34.9 & \pm & 3.2 \\ (36.5 \pm 7.8) \end{array}$
5	NaYF ₄ :Nd,Yb @Yb@Yb,Er @Y 4.8 nm	1.661 x 10 ⁵	36.3 ± 1.9 (40.2 ± 8.9)	11	NaYF ₄ :Yb,Er @Yb@Nd,Yb@Y 6.2 nm	5.447 x 10 ⁵	$\begin{array}{rrr} 43.1 & \pm & 1.8 \\ (46.3 \pm 8.0) \end{array}$
6	NaYF ₄ :Nd,Yb @Yb@Yb,Er@Y 11.5 nm	2.573 x 10 ⁵	$49.7 \pm 3.2 \\ (55.3 \pm 5.5)$	12	NaYF ₄ :Yb,Er @Yb@Nd,Yb@Y 11.3 nm	6.104 x 10 ⁵	$53.3 \pm 2.3 \\ (57.0 \pm 6.1)$

Table S1. Photon counts at the highest emissive 540 nm peak and TEM and DLS diameters (sizes) of the various UCNPs of interest. The thickness data of the Y shell for UCNPs designated as 2, 4, 5, 6, 8, 10, 11 and 12 are listed after their formulas.

NdYt UCN) Ps	NaYF4	4:Nd,Yb	NaYF4 @Yb	:Nd,Yb	NaYF4 @Yb@	:Nd,Yb Yb,Er	NaYF4 @Yb@ @Y 1.9	:Nd,Yb)Yb,Er 9 nm	NaYF4 @Yb@ @Y 5.4	:Nd,Yb Yb,Er 1 nm	NaYF4 @Yb@ @Y 12	:Nd,Yb Yb,Er .1 nm
Theo Cal		wt %	mol %	wt %	mol %	wt %	mol %	wt %	mol %	wt %	mol %	wt %	mol %
	Y	57.4	70.0	69.2	80.0	72.9	83.0	82.9	89.8	90.2	94.3	94.7	97.0
	Nd	26.6	20.0	14.0	10.0	9.5	6.7	6.0	4.0	3.4	2.2	1.9	1.2
	Yb	16.0	10.0	16.8	10.0	17.1	10.0	10.8	6.0	6.2	3.3	3.4	1.8
	Er	0.0	0.0	0.0	0.0	0.6	0.3	0.3	0.2	0.2	0.1	0.1	0.1
Exp ICP													
	Y	57.8	70.3	68.7	79.7	72.2	82.6	81.2	88.8	91.3	95.0	94.1	96.7
	Nd	26.9	20.2	14.3	10.2	9.1	6.4	5.4	3.6	2.8	1.8	2.0	1.3
	Yb	15.3	9.6	17.0	10.1	18.0	10.6	13.2	7.4	5.7	3.0	3.8	2.0
	Er	0.0	0.0	0.0	0.0	0.7	0.4	0.2	0.1	0.2	0.1	0.1	0.1
YbEr UCN	Ps	NaYF4	4:Yb,Er	NaYF4 @Yb	:Yb,Er	NaYF4 @Yb@	:Yb,Er Nd,Yb	NaYF4 @Yb@ @Y 2.2	:Yb,Er Nd,Yb 2 nm	NaYF4 @Yb@ @Y 6.8	:Yb,Er Nd,Yb 3 nm	NaYF4 @Yb@ @Y 12	:Yb,Er Nd,Yb .0 nm
Theo Cal		wt %	mol %	wt %	mol %	wt %	mol %	wt %	mol %	wt %	mol %	wt %	mol %
	Y	80.7	89.0	81.4	89.5	72.9	83.0	82.9	89.8	90.2	94.3	94.7	97.0
	Nd	0.0	0.0	0.0	0.0	9.5	6.7	6.0	4.0	3.4	2.2	1.9	1.2
	Yb	17.6	10.0	17.7	10.0	17.1	10.0	10.8	6.0	6.2	3.3	3.4	1.8
	Er	1.7	1.0	0.9	0.5	0.6	0.3	0.3	0.2	0.2	0.1	0.1	0.1
Exp ICP													
	Y	80.2	88.7	81.0	89.2	73.1	84.2	84.2	90.6	90.0	94.3	95.1	97.2
	Nd	0.0	0.0	0.0	0.0	9.5	5.5	5.5	3.6	3.2	2.1	1.7	1.1
	Yb	17.9	10.2	18.0	10.2	16.9	10.0	10.0	5.5	6.5	3.5	3.1	1.6
	Er	1.9	1.1	1.0	0.6	0.5	0.3	0.3	0.2	0.3	0.2	0.1	0.1

Table S2. Theoretical calculated (on the basis of starting reaction mol% data) andexperimental (on the basis of ICP data) wt% and mol% compositions of Y, Nd, Yb and Eu inthe core, core/shell, core/shell/shell and core/shell/shell UCNPs of bothNaYF4:Nd(20%),Yb(10%)@NaYF4:Yb(10%)@NaYF4:Yb(10%),Er(1%)@NaYF4andNaYF4:Yb(10%),Er(1%)@NaYF4:Yb(10%)@NaYF4:Nd(20%),Yb(10%)@NaYF4.

No.	NdYb UCNPs	Photon	Photon	No.	YbEr UCNPs	Photon	Photon
		counts	counts			counts	counts
		at 975 nm	at 1525 nm			at 975 nm	at 1525 nm
3	NaYF ₄ :Nd,Yb	5.266 x 10 ⁴	6.187 x 10 ³	9	NaYF ₄ :Yb,Er	7.923 x 10 ³	5.710 x 10 ³
4	@Yb@Yb,Er NaYF ₄ :Nd,Yb	6.319 x 10 ⁴	6.812 x 10 ³	10	@Yb@Nd,Yb NaYF ₄ :Yb,Er	4.701 x 10 ⁴	6.292 x 10 ³
5	@Yb@Yb,Er@Y 1.9 nm NaYF ₄ :Nd,Yb	7.163 x 10 ⁴	7.667 x 10 ³	11	@Yb@Nd,Yb@Y 2.2 nm NaYF ₄ :Yb,Er	5.876 x 10 ⁴	6.874 x 10 ³
<i>.</i>	@Yb@Yb,Er @Y 5.4 nm	0.005 104		10	@Yb@Nd,Yb@Y 6.8 nm	- 446 104	
6	NaYF ₄ :Nd,Yb @Yb@Yb,Er@Y 12.1 nm	8.205 x 10 ⁴	6.770 x 10 ³	12	NaYF ₄ :Yb,Er @Yb@Nd,Yb@Y 12.0 nm	7.446 x 10 ⁴	6.768 x 10 ³

Table S3. Photon counts at the downconversion 975 nm and 1525 nm peaks of the variousUCNPs of interest. The numerical designations of UCNPs are the same as those in Table S2.

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