

Supporting information

Synergistic or antagonistic effect of lanthanides on Rose Bengal photophysics in upconversion nanohybrids?

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Materials and methods

Methods

Absorption spectroscopy. Attenuation/absorption spectra were recorded in a 1 cm path length quartz cuvette on the Lambda 1050 UV-vis-NIR spectrophotometer (Perkin Elmer). The absorption of RB in UC_{Er}@RB was obtained by subtracting the scattering using an artificial baseline in the OriginPro software. Briefly, a user-defined baseline created simulating the exponential tendency of the scattering (Peak analyzer function of OriginPro) is created by selecting regions of the spectra where the absorption of the sample is zero ($\lambda > 630$ nm) or close to zero ($390 < \lambda < 460$ nm). A control experiment with RB and increasing concentrations of Ludox in water showed that it was an appropriate approximation to subtract the scattering signal from the attenuation to obtain the absorption of the dye, at least for absorption bands located in the visible region.

Steady state photoluminescence. Steady state photoluminescence spectra were recorded in 1 cm pathlength quartz cuvettes on an FLS1000 photoluminescence spectrometer (Edinburgh Instruments). Two excitation sources were used: the 150 W Xe arc lamp and a 980 nm CW laser (2W, PSU-III-LED, CNI Optoelectronics Technology Co. Ltd.) for UC measurements. The excitation laser irradiance (I) was estimated to be ≈ 2.1 W/cm², based on the laser output power, measured with a thermal sensor (S470C with PM100D reader, Thorlabs Inc.), and the size of the laser spot, measured with a CCD LT665 camera (Ophir Optronics Solutions Ltd.; rectangular profile: D4 σ X= 3.19 mm and D4 σ Y= 6.02 mm). A visible photomultiplier (PMT-980, Edinburgh Instruments) was used to detect emissions in the visible, while a NIR-sensitive photomultiplier (PMT 1700 H10330C-75, Hamamatsu Photonics, K.K.) is used for NIR. Visible phosphorescence and NIR detection measurements by exciting with the Xe lamp are performed in the front-face arrangement and use a standard 645 nm longpass filter in the detection path.

Time-resolved photoluminescence. Time-resolved emission spectra were recorded in 1 cm pathlength quartz cuvettes on the FLS1000 photoluminescence spectrometer (Edinburgh Instruments). Three different excitation sources are used (indicated in each case): nanosecond supercontinuous pulsed laser (SuperK Extreme EXU-6, NKT Photonics), microsecond Xe 60 W flash lamp (μ F2, Edinburgh Instruments) and wave laser 980 nm continuous beam coupled with a pulsed variable electronic laser controller (pulse width: 100 μ s; PM2 box, Edinburgh Instruments). The visible photomultiplier (PMT-980, Edinburgh Instruments) was used to detect emissions in the visible, except to detect instantaneous fluorescence, in which case the visible photomultiplier (MCP-900, Edinburgh Instruments) is used. For NIR emissions, a sensitive NIR photomultiplier (PMT 1700 – H10330C-75, Hamamatsu Photonics, K.K.) is used. The kinetics recorded in the visible and in the NIR exciting at 560 nm with the μ F2 lamp were recorded in a

front-face arrangement with a standard 645 nm longpass filter in the detection path. Fitting of kinetics was done using Fluoracle software. The average lifetime for biexponential fits ($f(x) = y_0 + A_1e^{-x/\tau_1} + A_2e^{-x/\tau_2}$) is obtained from the following equation:

$$\langle \tau \rangle = \frac{\sum_{i=1}^2 A_i \tau_i^2}{\sum_{i=1}^2 A_i \tau_i}$$

Absorption spectroscopy of transient species. Spectral and time-resolved transient species absorption measurements were performed in 1 cm septum-stoppered quartz cuvettes (standard cross-beam geometry) on a laser flash photolysis spectrometer (LP980-KS, Edinburgh Instruments) equipped with a Quanta-Ray INDI Nd:YAG laser (Spectra Physics) with a parametric optical oscillator (primoScan BB, Spectra Physics). The laser output power was 3 and 5 mJ for 560 and 975 nm, respectively. Spectral measurements were made using an ICCD camera (Andor DH320T) that integrates for 500 ns (gate width) with respect to the indicated delay of the laser pulse (pump) and with a 150W Xe pulsed lamp. The kinetics were obtained with a photomultiplier detector and the pulsed Xe lamp. The software used to carry out the measurements and kinetic adjustments is L900 (Edinburgh Instruments). The RB and UC_{Er}@RB solutions/dispersions here used had the same absorbance (0.28) at 560 nm.

Laser-induced fluorescence. Spectral and time-resolved transient species absorption measurements were performed in septum-stoppered 1 cm quartz cuvettes (standard sample holder) in the above laser flash photolysis spectrometer (LP980-KS, Edinburgh Instruments) with a laser Nd:YAG INDI Quanta-Ray (Spectra Physics) and an optical parametric oscillator (primoScan BB, Spectra Physics) but with the Xe lamp turned off. The ICCD camera (Andor DH320T) is used as a detector, which integrates for 100 μ s (gate width) with respect to the indicated delay of the laser pulse.

Quantum yield and upconversion quantum yield. Absolute quantum yield measurements were performed in sealed-tube quartz cuvettes (1 cm optical path length) in a Quantaury QY Plus (C13534-11, Hamamatsu Photonics K.K.) coupled to a NIR photoluminescence measurement unit (C13684-01, Hamamatsu Photonics K.K.). Two excitation sources were used: the built-in Xe lamp and a 2.5 W 980 nm continuous wave laser (MDL-III-980, CNI Optoelectronics Technology Co. Ltd.). The laser power is varied to characterize the quantum upconversion yield using a variable neutral density disk. The reported excitation irradiance was obtained from the laser spot size provided by the manufacturer and the laser power measured with a power meter (PD300-3W, Ophir Optonics Solutions Ltd.) within the integration sphere.

High resolution electron microscopy (HRTEM). HRTEM images were recorded on a TECNAI G2 F20 microscope operating at 200 kV (spot resolution 0.24 nm) and equipped with a GATAN CCD camera. Samples were prepared by drying at room temperature 10 μ L of a 0.5 mg/mL dispersion of oleate-coated UC_{Tm} in chloroform on a 300-mesh copper grid with formvar/carbon film.

Dynamic Light Scattering (DLS). DLS measurements were performed with a Zetasizer Ultra (Malvern, UK). Measurements were made at 5 mg/mL dispersions of UCNP/UCNH in DMF (measurements were performed by triplicate).

Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The ICP analysis was performed at the central research support service (SCSIE) of the University of Valencia using an Agilent 7900 ICP-MS unit.

Materials

The materials used for the synthesis of UCNP/UCNH were: hydrated lanthanide chlorides: YCl₃·xH₂O (REacton AlfaAesar, 99.99 %), YbCl₃·6H₂O (Acros Organics, 99.99 %), and ErCl₃·6H₂O (Sigma Aldrich, 99.9%), 1-octadecene (Alfa Aesar, 90%), oleic acid (Alfa Aesar, 90%), NaOH granules (Scharlau), NH₄F (Sigma Aldrich, 99.99%), sodium salt of rose bengal (Sigma Aldrich).

Synthesis of β -NaYF₄:Yb³⁺(20%), Er³⁺(2%) oleate coated

UCNPs were synthesized according to a previous protocol¹ with some modifications. In a round bottom flask add 15 mL of 1-octadecene and 8 mL of oleic acid. Next, a 2 mL methanol (MeOH) solution containing 1 mmol of lanthanides (0.78 mmol YCl₃·6H₂O, 0.20 mmol YbCl₃·6H₂O and 0.02 mmol ErCl₃·6H₂O) is added. From that moment, a constant stirring and inert atmosphere (N₂) is maintained until the end of the synthesis. The mixture is heated to 160°C and held at that temperature until the salts are dissolved. Subsequently, the solution is cooled to 60°C and a MeOH solution containing NaOH (2.5 mmol) and NH₄F (4 mmol) is added. The solution is heated to 125 °C to remove the MeOH. Finally, the temperature is quickly raised to 305°C and held for 1.5 hours. After that time, let it cool to room temperature. The purification of the UCNPs is carried out by precipitation by centrifugation and redispersion with solvents. First, the reaction mixture is centrifuged at 7000 g for 7 minutes. The precipitate is washed by means of redispersion-centrifugation cycles (7000 g for 7 minutes): three times with a chloroform:ethanol (1:3) mixture and three times with cyclohexane:acetone (1:3). Finally, they are redispersed with 3 mL of cyclohexane, centrifuged at 1000 g for 2 minutes and the supernatant is saved, thus discarding the UCNP aggregates. The UCNP concentration of the final dispersion is calculated by weighing difference, leaving 100 μ L of the dispersion to dry in an oven and it is normally around 60 mg/mL.

The ratio of lanthanides of the final UCNPs, obtained by ICP, agrees well with the lanthanides weighted for synthesis.

Removal of surface oleate ligands from β -NaYF₄:Yb³⁺ (20%), Er³⁺ (2%) (UC_{Er})

The oleate ligands on the surface of the UCNP were removed by a treatment with NOBF₄ previously described.² To the desired amount of the dispersion of the oleate-functionalized UCNPs is added the same amount of DMF. The phases are then vigorously shaken, a slightly higher quantity of solid NOBF₄ is added than that of UCNP in the starting cyclohexane dispersion and the mixture is stirred for 2 hours at ca. 40°C. Subsequently, the DMF phase is taken, excess chloroform is added and centrifuged at 7000 g for 7 minutes. Two washings are then carried out, which consist of redispersing the precipitate with a small amount of DMF, then adding excess chloroform and centrifuging at 10,000 g for 10 minutes. Finally, the transparent precipitate is redispersed in 5 mL of DMF. The UCNP concentration of the final dispersion is calculated by weighing difference, leaving 100 μ L of the dispersion to dry in an oven.

Functionalization of UC_{Er} with RB (UC_{Er}@RB)

Functionalization with RB is performed following a previously described protocol.³ First, the UC_{Er} dispersion is exposed to excess RB (RB mass is 1.25 times UC_{Er} mass in dispersion) at 0.042 M RB concentration for 24 hours. Subsequently, washings consisting of centrifugation cycles (10,000 g 10 minutes)-redispersion (94x 10 mL) with DMF are performed, until the supernatant does not present RB absorption. Finally, the sample was centrifuged (1000g 2 minutes) twice to remove large aggregates. The UC_{Er}@RB concentration of the final dispersion is calculated by weighing difference, leaving 100 μ L of the dispersion to dry in an oven.

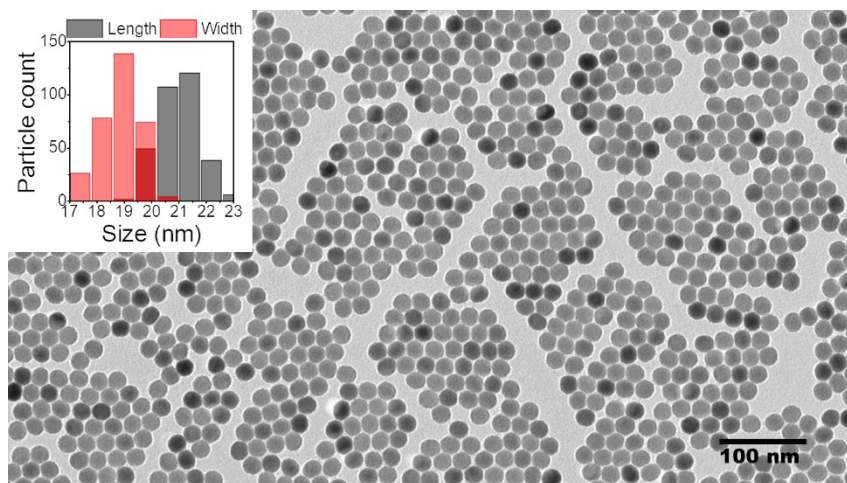


Figure S1. TEM image and size histogram of UC_{Er} for 300 UCNPs.

Table S1. UC_{Er} ICP-MS analysis.

	Elemental composition (%)		
	Y^{3+}	Yb^{3+}	Er^{3+}
UC_{Er}	68.8	28.2	3.0

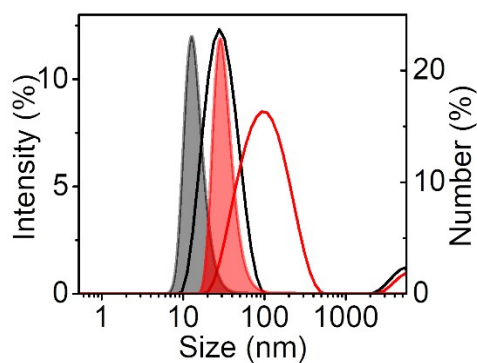


Figure S2. Size distribution by intensity (lines) and number (colored areas) of UC_{Er} (black) and $UC_{Er}@RB$ (red) (5 mg/mL).

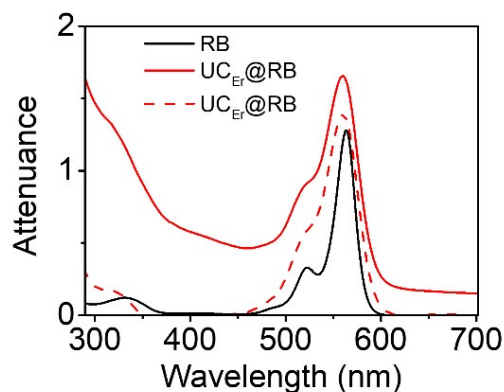


Figure S3. Absorption spectrum of a RB solution ($1.3 \cdot 10^{-5}$ M; black line) and attenuation (red line) and absorption spectra (dashed red line) of a 5 mg/mL $UC_{Er}@RB$ dispersion (red line) in DMF.

Effect of dye aggregation in the UCNP functionalization

The dye concentration in the dispersion during functionalization protocol plays a fundamental role in determining the final dye aggregation state. To illustrate this result six UCNP samples, named 1-6, were functionalized starting with the same UC_{Er} concentration (34 mg/mL) and varying the RB concentration in steps of an order of magnitude from $4 \cdot 10^{-2}$ to $4 \cdot 10^{-7}$ M, respectively. Figure S4 shows the attenuation spectrum of the samples 1-6 at 1 mg/mL after the functionalization and purification (centrifugation-redispersion cycles with DMF until the supernatant shows no RB absorption). As shown, the highest RB loading on the UC_{Er} occurs in the sample 2 (which corresponds to $4 \cdot 10^{-3}$ M RB in functionalization process) instead of the sample 1, the one with the higher RB concentration in the functionalization. In addition, the A_{560}/A_{522} ratio of sample 1 might indicate that dye is aggregated.

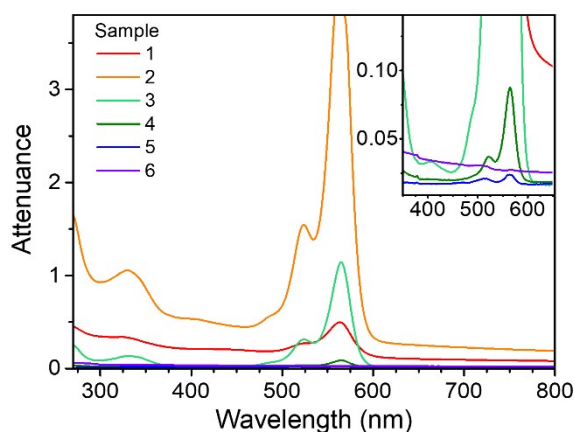


Figure S4. (a) Attenuance spectra of samples 1-6 (1 mg/mL, DMF).

Calculation of RB molecules/single UCNP

The procedure to calculate the number of RB molecules in UCNH is based on calculating the number of RB molecules and the number of UCNP in 1 mg of UCNH and dividing it. Several approximations are made for the calculation and are detailed below:

1) Calculation of unit cells (CU) by UCNP

TEM images show a monodisperse population of UCNP in the shape of a 21 nm diameter hexagonal prism. With the volume of a UCNP and knowing that the volume of a unit cell is 107.44 \AA^3 ,⁴ the amount of CU for each UCNP (CU_{UCNP}) is calculated. It is found that there are 45132.6 CU/UCNP.

2) Calculation of UCNP in 1 mg of UCNH

First, the amount of CU in 1 mg of UCNH is calculated. For this, it is approximated that 1 mg of UCNH is equivalent to 1 mg of UCNP, therefore, by means of the weight of a unit cell ($\text{PCU}_{\text{NaYF}_4} = 187.84 \text{ u}$) and Avogadro's number, this data is calculated. Subsequently, this data is divided by CU_{UCNP} and thus the amount of UCNP in 1 mg ($\text{UCNP}_{\text{UCNH}}$) is obtained. It is obtained that there are $7.1 \cdot 10^{13}$ UCNP/mg UCNH.

3) RB molecules in 1 mg UCNH

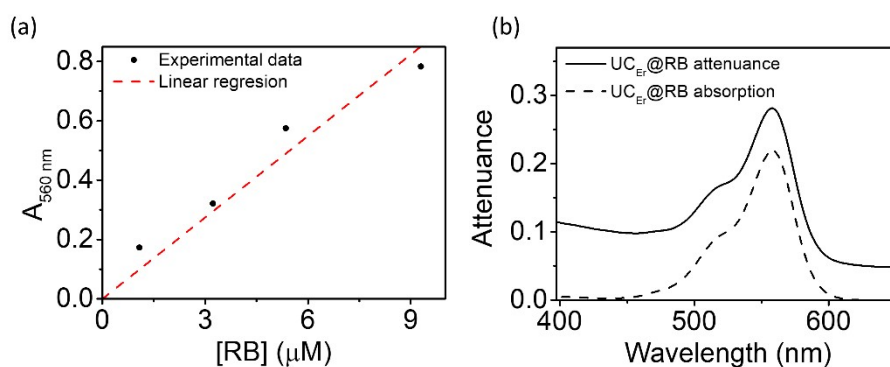


Figure S5. (a) Calibration curve and linear regression of increasing concentration RB solutions. (b) Attenuance spectrum of 1 mg/mL UC_{Er}@RB dispersion (solid line) and absorption spectrum of RB in the UCNH (dashed line) once subtracted manually the scattering.

RB molecules are calculated by applying the Lambert-Beer law. For this, it is approximated that the molar absorption coefficient ($\epsilon(\lambda)$) of the free RB is the same as that for the functionalized RB on the surface of the UCNP. The experimentally calculated $\epsilon(560)$ turns out to be $(9.1 \pm 0.7) \cdot 10^4 \text{ M}^{-1}\text{cm}^{-1}$ (Figure S4) and the absorption at 560 nm of a 1 mL dispersion of 1 mg/mL UC_{Er}@RB is 0.22. With these data it is possible to calculate the amount of RB molecules per mg of UCNH (RB_{UCNH}). Thus, it is obtained that on average there are $1.44 \cdot 10^{15}$ molecules of RB/mg UCNH.

4) RB molecules by UCNP

The number of average RB molecules per UCNP is obtained by dividing RB_{UCNH} by UCNP_{UCNH} and results in *ca.* 20 RB/UCNP molecules. By approximating the volume of an RB molecule to that occupied by a 1.5 nm diameter sphere and calculating the surface area of a spherical UCNP, one can calculate the number of RB molecules that could be anchored to the surface of the UCNP which turns out to be 784. Therefore, only *ca.* 3% of the UC_{Er} surface would be covered by RB. Even assuming that the molar absorption coefficient of RB is half, as has been described for RB aggregates on the surface of SiO₂@Al₂O₃ colloids,⁵ the surface coating would only reach *ca.* 5 %.

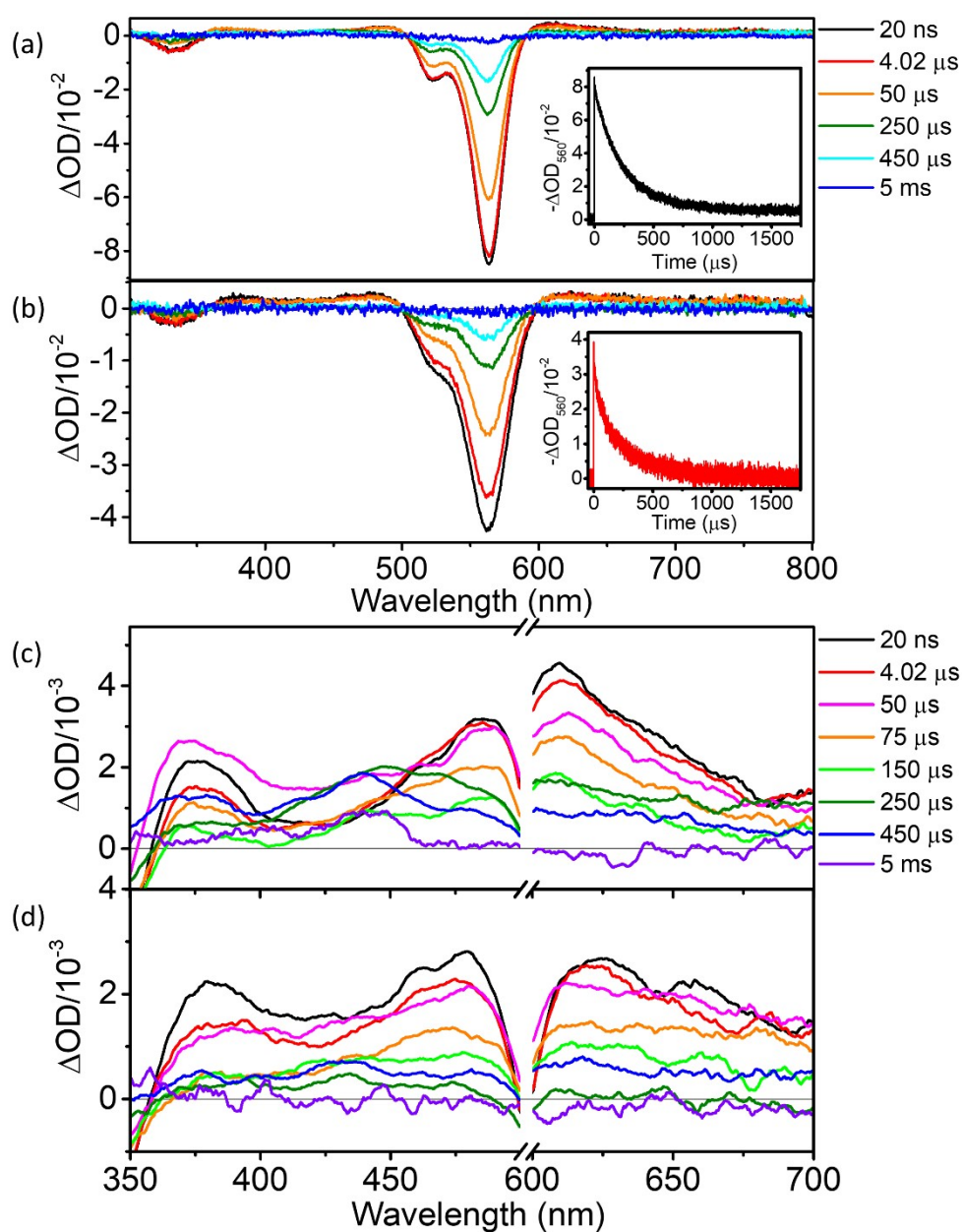


Figure S6. Transient absorption spectra of (a,c) RB and (b,d) $UC_{Er}@RB$ under N_2 in DMF ($\lambda_{exc,pump} = 560$ nm). The insets (a) and (b) show the bleaching kinetic at 560 nm. (c,d) Detailed transient absorption spectrum (20 points adjacent-averaging smoothing).

Table S2. Transient absorption kinetics fitting parameters obtained for a RB solution and a UC_{Er}@RB dispersion (both absorbances were 0.28 A at 560 nm).

λ_{exc}/nm (Power)	λ_{abs}/nm	Sample	Atmosphere	$\tau/\mu s$	SD*	χ^2
560	560	RB	N ₂	244	2	0.778
			air	0.92	0.01	1.802
		UC _{Er} @RB	N ₂	46 (8 %)	2	0.744
			air	265 (92 %)	3	
560 (3 mJ)			air	2.409	0.05	3.211
610	610	RB	N ₂	241	7	0.695
			air	1.06	0,01	5.241
		UC _{Er} @RB	N ₂	258	16	0.727
			air	2.96	0.03	3.728

*SD: fitting standard deviation.

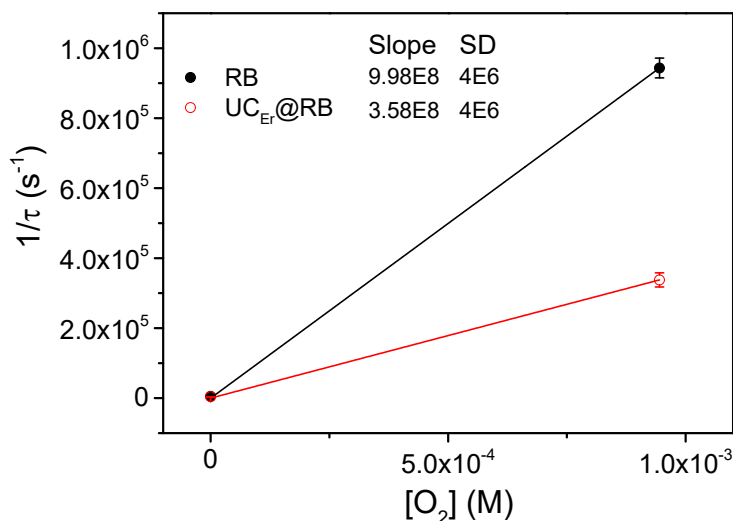


Figure S7. Stern-Volmer plot and fitting of the oxygen quenching for RB and UC_{Er}@RB. The O₂ concentration in air was calculated assuming Henry Law is valid from ⁶.

Table S3. NIR maximum emission intensities of UC_{Er} (1 mg/mL) and UC_{Er}@RB (1 mg/mL).

λ_{exc} (nm)	Sample (atmosphere)	Emission intensity (a. u.)	
		980 nm	1525 nm
560	UC _{Er} (N ₂)	12	90
	UC _{Er} (O ₂)	31	41
	UC _{Er} @RB (N ₂)	2.66 · 10 ⁴	3.90 · 10 ⁴
	UC _{Er} @RB (O ₂)	1.22 · 10 ⁴	1.79 · 10 ⁴
655	UC _{Er} (N ₂)	389	642
	UC _{Er} (O ₂)	366	604

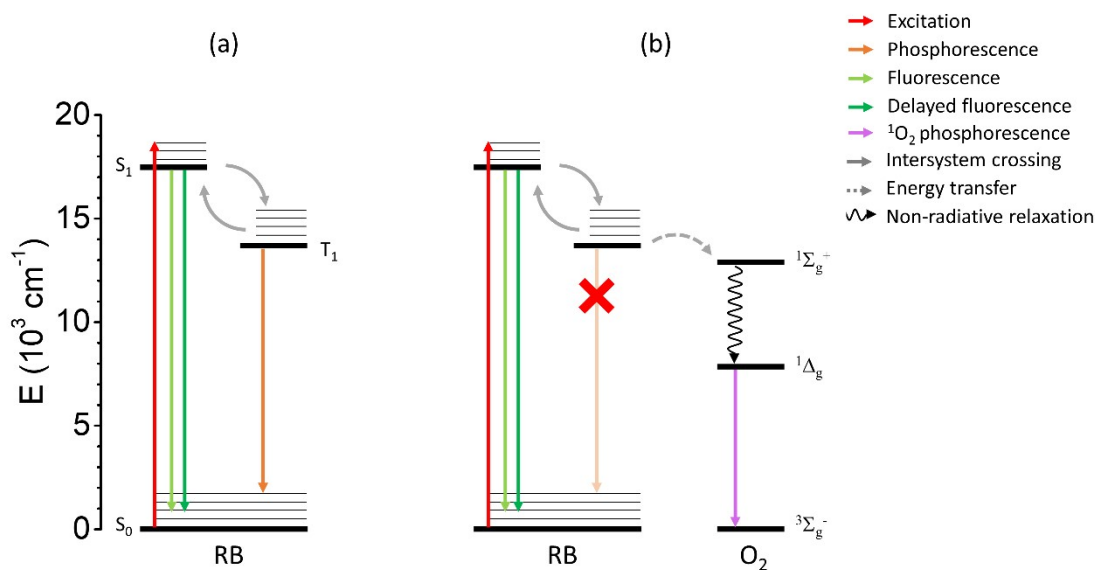


Figure S8. Energy diagram of photophysical processes observed for RB under (a) N_2 and (b) air atmosphere ($\lambda_{exc}=560 \text{ nm}$).

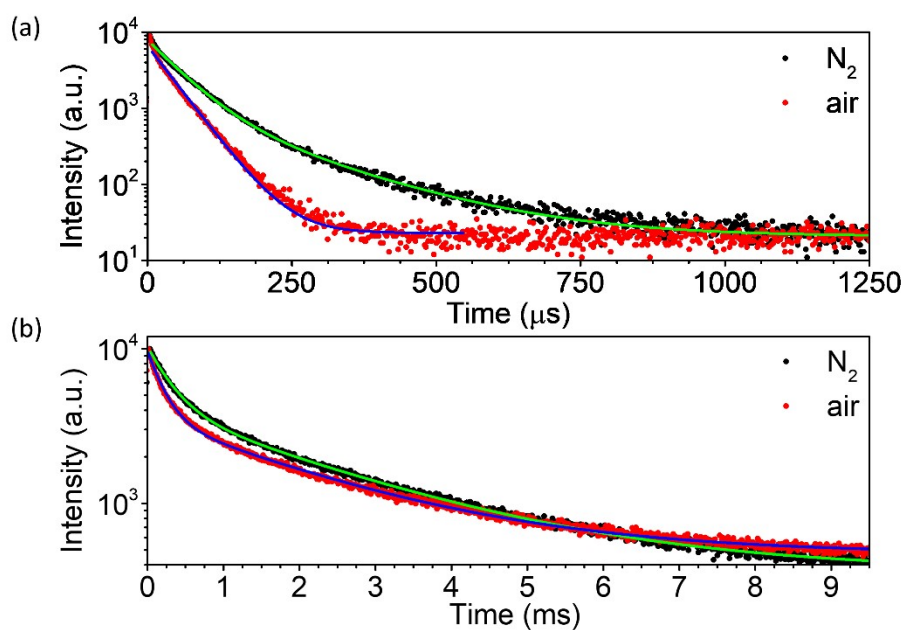


Figure S9. Kinetic time profiles of $UC_{Er}@RB$ (5 mg/mL) emissions at (a) 975 nm and (b) 1550 nm DMF under N_2 and air ($\lambda_{exc}=560 \text{ nm}$, μF2 lamp).

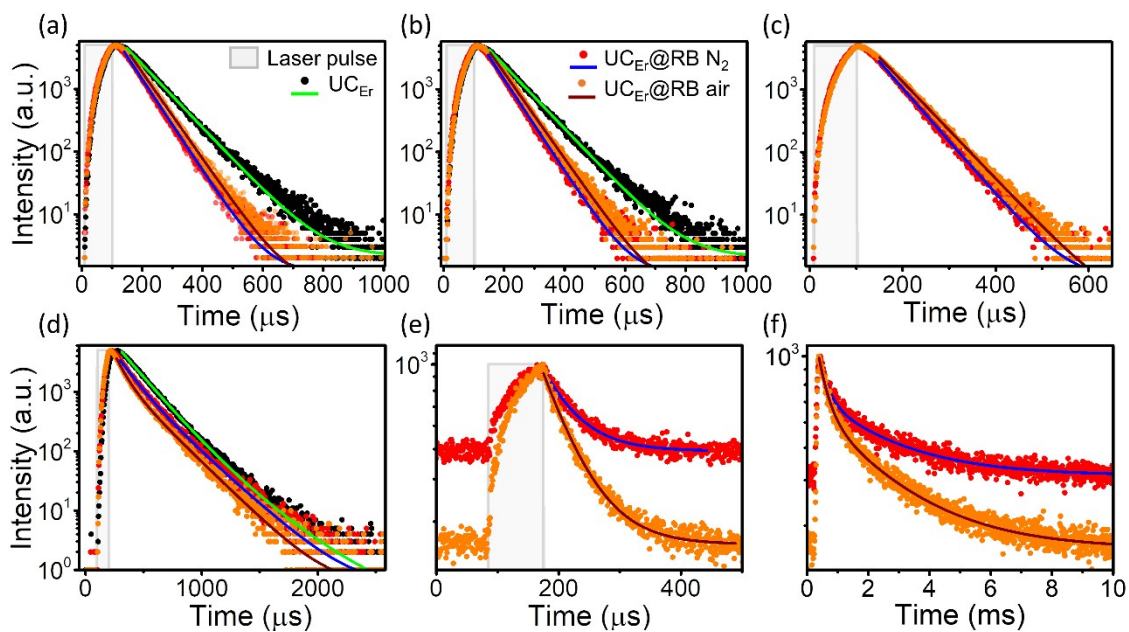


Figure S10. Kinetic time profiles and fittings of UC_{Er} (5 mg/mL) and $UC_{Er}@RB$ (5 mg/mL) dispersions in DMF under N_2 and air atmosphere at (a) 520, (b) 540, (c) 600, (d) 655, (e) 1050 and (f) 1550 nm ($\lambda_{exc} = 980$ nm; CNI laser). Grey area represents the laser pulse.

Table S4. Measurement conditions and exponential fitting parameters for RB solutions and UC_{Er}@RB dispersions at different concentrations.

$\lambda_{\text{exc}}/\text{nm}$ (Source)	$\lambda_{\text{em}}/\text{nm}$	Sample	Conc.	Atm.	$\tau/\mu\text{s}$ (%)	SD*	$\langle\tau\rangle^{**}/\mu\text{s}$	SD*	χ^2	
560 (NKT laser 5.5 MHz)	589	RB	$2.7 \cdot 10^{-7}$ M	N ₂	$2.2 \cdot 10^{-3}$	$0.1 \cdot 10^{-3}$			1.107	
		UC _{Er} @RB	<1 mg/mL	N ₂	$1.5 \cdot 10^{-3}$	$0.1 \cdot 10^{-3}$			1.244	
560 (μF2)	750	RB	$1.6 \cdot 10^{-5}$ M	N ₂	250	2			1.014	
				air	< 8					
	975	UC _{Er} @RB	5 mg/mL	N ₂	52.3 (65 %)	0.5	94	2	1.476	
				air	171 (35 %)	3				
	1550	UC _{Er} @RB	5 mg/mL	N ₂	305 (17 %)	3	1956	13	1.104	
					2284 (83 %)	13				
				air	209 (17 %)	2	1799	12	1.387	
	1275	RB	$1.6 \cdot 10^{-5}$ M	air	23.4	0.2			1.002	
		UC _{Er} @RB	5 mg/mL	air	18.8	0.2			1.166	
	980 (CNI CW laser)	525	UC _{Er}	5 mg/mL	N ₂	85.9	0.2			1.698
			UC _{Er} @RB	5 mg/mL	N ₂	59.5	0.1			1.298
		540	UC _{Er} @RB	5 mg/mL	air	62.9	0.1			1.078
N ₂					85.7	0.1			1.504	
600		UC _{Er} @RB	5 mg/mL	N ₂	59.3	0.1			1.310	
				air	63.3	0.1			1.234	
655		UC _{Er}	5 mg/mL	N ₂	51.3	0.1			1.019	
				air	56.0	0.1			1.056	
		UC _{Er} @RB	5 mg/mL	N ₂	185 (87 %)	3	205	9	1.108	
					339 (13%)	32				
				N ₂	127 (39 %)	5	202	5	1.025	
					251 (61 %)	5				
1050	UC _{Er} @RB	1 mg/mL	air	79 (31 %)	2	182	2	1.003		
			N ₂	228 (69 %)	2					
1550	UC _{Er} @RB	1 mg/mL	N ₂	51	1			1.108		
			air	52.1	0.7			1.134		
			N ₂	286 (10 %)	25	2170	92	1.004		
				2368 (90 %)	92					
			air	317 (11 %)	19	2154	62	0.961		
				2381 (89 %)	62					

*SD: standard deviation; ** $\langle\tau\rangle$: average lifetime.

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