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Excitation power dependent population pathways and absolute quantum yields of upconversion nanoparticles in different solvents[†]

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The rational design of brighter upconversion nanoparticles (UCNPs) requires a better understanding of the radiationless deactivation pathways in these materials. Here, we demonstrate the potential of excitation power density (*P*)-dependent studies of upconversion (UC) luminescence intensities, slope factors, and absolute quantum yields (Φ_{UC}) of popular β -NaYF₄:20% Yb³⁺,2% Er³⁺ UCNPs of different surface chemistries in organic solvents, D₂O, and water as a tool to gain deeper insight into the UC mechanism including population and deactivation pathways particularly of the red emission. Our measurements, covering a *P* regime of three orders of magnitude, reveal a strong difference of the *P*-dependence of the ratio of the green and red luminescence bands ($I_{g/r}$) in water and organic solvents and *P*-dependent population pathways of the different emissive energy levels of Er³⁺. In summary, we provide experimental evidence for three photon processes in UCNPs, particularly for the red emission. Moreover, we demonstrate changes in the excited population dynamics *via* bi- and triphotonic processes dependent on the environment, surface chemistry, and *P*, and validate our findings theoretically.

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Introduction

Upconversion nanoparticles (UCNPs) constitute an emerging class of near-infrared (NIR)-excitable luminescent reporters for bioimaging, fluorescence assays, sensors in bioanalysis, and optical thermometry as well as phosphors for photovoltaic and security applications.¹⁻¹⁰ Typical materials are crystalline fluoride or oxide based hosts like NaYF4 and Gd2O3 exhibiting low phonon energies, which are doped with single trivalent lanthanide (Ln³⁺) ions or mixtures of these ions. Co-doped UCNPs are commonly obtained using Yb³⁺ with its comparatively high absorption cross section as a sensitizer together with luminescent activators Er³⁺, Tm³⁺, or Ho³⁺.¹¹ The advantages include a multitude of characteristic narrow emission bands in the ultraviolet, visible and near infrared (NIR) spectral regions originating from $4f^n$ electron transitions within the localized Ln^{3+} dopants ideal for multiplexing applications, an excellent photostability, lack of blinking,^{2,11} and long luminescence lifetimes in the µs range favorable for time-gated emission, lifetime multiplexing,¹² and time-resolved FRET (luminescence or Förster energy transfer (LRET or FRET)) assays.^{13,14}

Full exploitation and broad use of upconversion (UC) nanotechnology are closely linked to UC efficiency and brightness of UCNPs. Although UCNPs with core-shell structures have been prepared with a narrow particle size distribution, welldefined crystal morphology,¹⁵ control of dopant ion concentration.16 and surface chemistries providing water dispersibility,^{5,17–19} it is not yet completely understood, why the UC efficiencies even of UCNPs with optimized core-shell architectures²⁰⁻²² are typically about one order of magnitude lower than those of the corresponding bulk materials. The fact that smaller particles come at the expense of a weaker UC emission is frequently attributed to the increased surface-tovolume ratio and hence, a higher number of Ln³⁺ ions on the surface prone to quenching by ligands and solvents containing C-H or O-H bonds in addition to deactivation pathways like crystal defects or impurities.^{15,23-31} As UC processes in nanomaterials involve the same mechanisms as in bulk materials, *i.e.*, excited state absorption, sequential energy transfer, cooperative transitions, and photon avalanche, it is expected that the size of the nanophosphors can modify ion-ion and ionlattice interactions to various degrees.32 This can affect population and non-radiative deactivation pathways in UCNPs in different ways as for bulk UC materials. Control of these processes requires a fundamental understanding of the photophysics of commonly used UCNPs.8

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This, together with the relevance of aqueous environments for life sciences applications, has initiated an increasing number of studies on the quenching mechanisms of UC luminescence.^{17,27,33–36} A complete comprehension of the processes controlling population and deactivation pathways in UCNPs requires quantitative luminescence measurements including the determination of the quantum yields of the upconversion processes $(\Phi_{\rm UC})^{37}$ and excitation power density (P)-dependent studies as the sequential absorption of two or more photons³⁸ introduces a *P*-dependence of the UC luminescence, and most likely all luminescence parameters like emission spectra/intensity ratios of emission bands, $\Phi_{\rm UC}$, and decay kinetics. Absolute P-dependent luminescence measurements, regarded as one of the cornerstones of UC nanotechnology,^{8,39} are, however, very challenging. They can be only reliably performed with properly designed and calibrated integrating sphere setups and very stable excitation light sources with a well characterized beam profile. Moreover, precise knowledge of P at the sample position is mandatory $^{40-42}$ and thus, control of the excitation geometry and beam profile. Presently, only a relatively small number of absolute luminescence measurements of UC materials of different sizes using custom-made setups have been reported,^{31,41-46} which were, however, performed under miscellaneous experimental conditions like strongly varying and often rather small P varied over at maximum two orders of magnitude which is often not sufficient to saturate UC luminescence. This complicates the comparability of spectroscopic data in addition to material-related differences such as preparation conditions, size, host material, dopant concentration, and surface chemistry, and hence, mechanistic interpretations. The general importance of P for UC luminescence has been meanwhile recognized not only for mechanistic studies,47,48 but also for other applications like the use of high P for compensating concentration quenching,49 and the switching between UC processes of different photonic orders like two photon and four photon UC in Yb³⁺,Tm³⁺-doped NaYF₄ nanoparticles,⁵⁰ respectively.

Despite the ever increasing use of UCNPs, the population of the two most fundamental emission bands of Yb³⁺-Er³⁺ codoped NC, the green and red bands, is still debated.^{47,48} In addition, influences of surface chemistry, microenvironment, and nanoparticle size are not fully understood. This encouraged us to systematically assess the UC and downconversion (DC) luminescence of a series of 23 nm-sized hexagonal (β-phase) NaYF₄:Yb³⁺,Er³⁺ nanoparticles doped with most commonly used 20% Yb^{3+} and 2% Er^{3+} , which were synthesized in a single large batch and subsequently surface modified to provide stable dispersions in apolar and polar organic solvents, water, and deuterium oxide. Luminescence parameters studied in these solvents for P varied over almost three orders of magnitude included UC emission spectra, slope factors n(P), $\Phi_{\rm UC}$, and the luminescence rise and decay kinetics in the wavelength range from 350 to 1520 nm. In order to directly assess the influence of solubilizing surface ligands and the surrounding medium on the P-dependent UC mechanism, we deliberately chose UCNPs

without a protecting inorganic shell here to derive a population model for the radiative energy levels involved in UC emission. Special attention was dedicated to the population of the ${}^{4}F_{9/2}$ energy level of Er^{3+} from which the red luminescence arises.

Results and discussion

Synthesis and surface modification of UCNPs

As a prerequisite for our systematic spectroscopic study with optimum material comparability, we synthesized a single large batch of high quality 23 nm-sized oleate (OA)-coated NaYF₄(Yb³⁺, Er³⁺) UCNPs with a narrow size distribution following a recently described procedure.³⁵ This avoids batch-tobatch-specific deviations in size, shape, crystallinity, and elemental composition, which can affect the luminescence properties of UCNPs. The analytical characterization of these UCNPs with transmission electron microscopy (TEM), X-ray powder diffraction (XRD), dynamic light scattering (DLS), inductively coupled plasma optical emission spectrometry (ICP-OES), and thermal gravimetric analysis (TGA) is provided in the ESI (Fig. S1-S3[†]). Subsequent surface modifications for UCNPs in polar environments like polar and aprotic DMF, water, and D₂O included exchange of OA for BF₄⁻ and citrate and encapsulation by exemplarily chosen amphiphilic DSPE (1,2-distearoyl-sn-glycero-3-phospho-ethanolamine-N-[methoxy] (polythylene glycol)-2000](ammonium salt)), thereby retaining the original OA ligands.³⁵ Citrate stabilization enables direct access of water molecules to the UCNP surface whereas the hydrophobic shell of amphiphilic DSPE surface ligands, where the hydrophobic tails of DSPE interact with the OA ligands via van der Waals forces, while the hydrophilic portion of DSPE provides water dispersibility, is expected to partly protect the particle surface from direct contact with water molecules. Both water solubilization strategies are (citrate functionalization) or can be principally made (DSPE; use of derivatizable surfactant molecules) compatible with further functionalization steps using established conjugation chemistries,⁵¹ e.g., for the design of FRET sensors or targeted optical probes for cellular imaging.^{52–54}

Spectroscopic studies

The spectroscopic properties of the parent OA-stabilized UCNPs and the UCNPs with a hydrophilic surface coating were investigated in cyclohexane, DMF, water, and D₂O as well as in mixtures of water and D₂O, thereby measuring the emission spectra in these solvents as a function of *P* varied over almost three orders of magnitude, using a custom made integrating sphere setup equipped with a high power stability 8 W 976 nm laser diode.⁵⁵ *P*-Dependent $\Phi_{\rm UC}$ ($\Phi_{\rm UC}(P)$) was determined as the ratio of the number of UC photons emitted ($N_{\rm em}$), derived from spectrally corrected⁵⁶ integral emission intensities of all UC emission bands in the wavelength region from 350 nm to 900 nm, and the number of photons absorbed at 976 nm ($N_{\rm abs}$), see eqn (1). This equals the internal upconversion

quantum yield.^{7,57} $\Phi_{\text{UC,sat}}$ values were obtained for high *P* in the saturation power regime (*P*_{sat}) where $\Phi_{\text{UC}}(P)$ levels off.

$$\Phi_{\rm UC} = \frac{N_{\rm em}}{N_{\rm abs}} \text{ for } \lambda_{\rm em} < \lambda_{\rm abs} \tag{1}$$

P-dependent luminescence intensities in organic solvents, H₂O, and D₂O. The *P*-dependence of the UC efficiency, and hence $\Phi_{\rm UC}(P)$, is governed by the competition between the energy transfer upconversion (ETU) rates from the sensitizer ion (Yb³⁺) to the activator ion, here Er³⁺, and the depopulation rates of the intermediate states of the activator ions involved in the UC process as highlighted in Fig. 1 (left panel).

These competitions and changes in the respective population and deactivation rates are also reflected by changes in the intensity ratios of the different UC emission bands. This follows from *P*-dependent measurements of the UC luminescence summarized in Fig. 1 (right panels) and in the ESI (see Fig. S4 and S5†), which reveal a clear dependence on surface chemistry and solvent. The right panel of Fig. 1 highlights the *P*-dependence of the emission spectra of exemplarily chosen DSPE-stabilized UCNP in H₂O and D₂O at low and high *P* (Fig. 1, panels (b) and (c)) and the contributions (in percentage) of the integral photon flux of the different UC emission bands $I(\lambda)$ to the overall integral photon flux $I(\lambda_{all})$ of the UC emission (Fig. 1, panels (d) and (e)). The corresponding data obtained for OA-capped UCNPs in cyclohexane, BF₄⁻-stabilized UCNPs in DMF, and citrate-stabilized UCNPs in water are provided in the ESI (Fig. S4†). As follows from Fig. 1 (right



Fig. 1 Left (panel (a)): Energy scheme of Yb^{3+} , Er^{3+} co-doped NaYF₄; ET: energy transfer, ETU: energy transfer upconversion, CR: cross relaxation, and BET: back energy transfer. The Yb^{3+} ground state absorption (GSA) processes do not generally occur at the same Yb^{3+} ion or at neighboring Yb^{3+} ions, yet the absorbed energy migrates from the absorption site to Yb^{3+} ions adjacent to Er^{3+} UC centers. Thus, the Yb^{3+} energy level diagram represents Yb^{3+} in the NaYF₄ lattice, whereas the Er^{3+} energy level diagram represents a single Er^{3+} ion. Right, top panels: *P*-dependence of the UC luminescence of DSPE-stabilized UCNPs in water and D₂O. Exemplarily shown are spectrally corrected emission spectra of DSPE-capped UCNPs in water (black lines) and D₂O (red lines) at low *P* of *ca*. 16 W cm⁻² (panel (b)) and high *P* of *ca*. 1000 W cm⁻² (panel (c)), respectively. Right, lower panels: Contribution (in percentage) of the integral intensities of the different UC emission bands $I(\lambda)$ to the overall integral intensity $I(\lambda_{all})$ of the UC emission of DSPE-capped UCNPs for the emission bands at panel (d) 850 nm (black) and 810 nm (brown) and panel (e) 655 nm (red) and 545 nm (green) in water (open squares) and in D₂O (solid circles), respectively, for *P* between 10 and 1000 W cm⁻². The arrows indicate the main differences between D₂O and H₂O. In the high *P* region, *i.e.*, for *P* exceeding 300 W cm⁻², the UC luminescence and the intensity contributions of each emission of the Er³⁺ energy levels is high also for UCNPs in water and D₂O. indicating similar population dynamics. Obviously, under these conditions, population of the Er^{3+} energy levels is high also for UCNPs in water due to the increased feeding rates caused by the high pumping power which compensate for the enhanced nonradiative decay rates induced by O–H vibrations. At high *P*, the red Er^{3+} emission is primarily fed *via* a 3-photon process ($^4G_{11/2} \rightarrow ^4F_{9/2$

panels, top), in H_2O and D_2O , the emission bands of our UCNPs at 850 nm, 810 nm, 655 nm, and 545 nm clearly differ and reveal a distinguishable P-dependence. This is similarly true for the P-dependences of the 410 nm and 360 nm emission shown in the ESI in Fig. S5.[†] Moreover, the *P*-dependence of the UC luminescence spectra and the intensity ratios of the different emission bands are very similar for D₂O and organic solvents. For UCNPs in D₂O, the green emission is the most intense UC band in the low P regime. With increasing P, the fraction of the green emission decreases whereas that of the red emission steadily increases until $\sim 100 \text{ W cm}^{-2}$ both emissions become equally intense (see the crossing point in panel (e) of Fig. 1). In the high P regime of $P > 100 \text{ W cm}^{-2}$, the red emission becomes eventually the most prominent luminescence. In contrast, for UCNPs in water, the red luminescence is always the most intense emission, also at low P, and rises with increasing P (Fig. 1, panel (e)). Also the P-dependent relative contributions of 850 nm and 810 nm emission bands are strongly affected by solvents (Fig. 1, panel (d)). The blue (410 nm) and UV (360 nm) emissions (see ESI, Fig. S5[†]), which both originate from higher order photonic processes, are reduced in water compared to organic solvents and D_2O . Moreover, the contributions of the integral photon flux of all emission bands of >500 nm to $I(\lambda_{all})$ converge for *P* exceeding 300 W cm⁻² for UCNPs in water and D₂O. This indicates similar population dynamics in water and D₂O at high *P*.

The proposed dominant population pathways of the different UC emission bands in water and in organic solvents and D_2O are highlighted in Fig. 2 (panel (a)), thereby accounting for the efficient coupling of O-H vibrations to the $^4S_{3/2}\!/^2H_{11/2}$ and $^4I_{11/2}$ energy levels of Er^{3+} and the corresponding increase in nonradiative decay rates of these levels. At low P, depopulation of the thermally coupled green emitting energy levels $({}^{4}S_{3/2}; {}^{2}H_{11/2})$ leads to enhanced population of the ⁴F_{9/2} energy level in water, favoring a red emission. Moreover, the radiative transitions ${}^{4}S_{3/2}/{}^{2}H_{11/2} \rightarrow {}^{4}I_{15/2}$ and ${}^{4}S_{3/2} \rightarrow {}^{4}I_{13/2}$ responsible for the green emission and the luminescence at 850 nm are diminished. This effect on the green emission was observed by other groups as well^{29,34} and even recently exploited for the sensitive probing of the water content of organic solvents.58 In addition, coupling of the first resonant level of Er³⁺ (⁴I_{11/2}) to O-H vibrations of water results in the population of the lower 4I13/2 level and an enhanced probability for the ${}^4\mathrm{I}_{13/2} \rightarrow \, {}^4\mathrm{F}_{9/2}$ transition, also enhancing the red luminescence (see Fig. 1, panels (b) and (e)). Moreover, the



Fig. 2 (a) Energy scheme of Yb³⁺-Er³⁺ interactions, with red arrows indicating the dominant population pathways for UCNPs in water at low (left) and high (right) *P*; the latter population pathways closely match with the photophysics of UCNPs in organic solvents and D₂O. Blue arrows indicate a major influence of O-H vibrations. The arrow length represents the energy of the vibrational mode. ET: energy transfer, ETU: energy transfer upconversion, and BET: back energy transfer. Panels (b)–(f) *P*-dependence of the slope factors *n* of the green (520 nm: light green; 545 nm: dark green; *I*_g), red (655 nm; *I*_r) and blue (410 nm; *I*_b) emission bands of the differently surface-functionalized UCNPs dispersed in different solvents. (b) BF₄⁻⁻ capped UCNPs in DMF, (c) OA-capped UCNPs in cyclohexane, (d) DSPE-stabilized UCNPs in D₂O, (e) citrate-stabilized UCNPs in water, and (f) DSPE-stabilized UCNPs in water.

high population of the ${}^{4}I_{13/2}$ energy level induces emission at *ca.* 810 nm (${}^{4}I_{9/2}$); this emission is fed by $Er^{3+}-Er^{3+}$ energy transfer (ET) processes involving the electronic states ${}^{4}I_{13/2}$, ${}^{4}I_{15/2} \rightarrow {}^{4}I_{13/2}$, ${}^{4}I_{9/2}$ (ET1 in Fig. 1, left panel).⁵⁹ Contributions of the inefficient cross relaxation pathway ${}^{4}S_{3/2}$, ${}^{4}I_{13/2} \rightarrow$ ⁴I_{15/2}, ⁴I_{9/2} (CR1), which accounts also for the 810 nm emission,⁶⁰ are further reduced in water due to the quenching of the initial green energy level. The changes of the relative contributions of the photon flux of the blue and UV emissions, which both arise from 3-photon processes, are attributed to efficient quenching of the electronically excited state ${}^{2}F_{5/2}$ of the sensitizer Yb^{3+} (see Fig. 1 and 2, left panels) by water molecules discussed in the next section. The changes of the relative intensities of the Er³⁺ emission bands observed in water arise from its low energy vibrational modes, *i.e.*, the fundamental symmetric $(\nu_1$ -mode) and asymmetric $(\nu_3$ -mode) stretching vibrations and the harmonic (overtone) of the deformation vibration (mode ν_2), which are all located between *ca*. 3300 to 3700 cm^{-1.61} These fundamental vibrations imply excitation from the vibrational ground to the first excited vibrational state and have high oscillator strengths compared to the so-called high energy harmonic transitions of water (for more details on the vibrational modes of the solvents used here see also Fig. S6[†]). In the case of D₂O, these vibrations are shifted to lower energies⁶¹ due to the higher mass of deuterium compared to hydrogen atoms and are hence not resonant anymore with Er^{3+} energy states (ESI, Fig. S6[†]).

P-dependence of slope factors *n*. The *P*-dependences of the slope factors ((n(P)) of the red $({}^{4}F_{9/2} \rightarrow {}^{4}I_{15/2})$, green $({}^{4}S_{3/2}, {}^{2}H_{11/2} \rightarrow {}^{4}I_{15/2})$, and purple $({}^{2}H_{9/2} \rightarrow {}^{4}I_{15/2})$ emissions of OA-capped UCNPs in cyclohexane, BF_{4}^{-} -stabilized UCNPs in DMF, citrateand DSPE-stabilized UCNPs in water and DSPE-stabilized UCNPs in D₂O, are summarized in panels (b)–(f) of Fig. 2. In the low *P* regime, *n* values of the green, red, and purple emissions are the highest and provide the number of photons required for the generation of each emission band $(I_{UC} \sim P^{n})$. With increasing *P*, n(P) decreases as a consequence of the enhanced population and onset of saturation of the different emissive states of the Er³⁺ ions. Particularly interesting is the rather similar shape of n(P) of the purple, green, and red emissions in cyclohexane, DMF, and D₂O (Fig. 2, panels (b)–(d)), which considerably deviates from the shape of n(P) of the green and red emissions of DSPE- and citrate-stabilized UCNPs in water (Fig. 2, panels (e) and (f)).

In the low P regime, n of the red emission of UCNPs in cyclohexane, DMF, and D₂O amounts to values between two and three, underlining the contribution from at least one 3-photon channel to this emission. Apparently, the red emission can be fed from the 3-photon ${}^4G_{11/2} \rightarrow {}^4F_{9/2}$ transition in addition to the two biphotonic transitions ${}^{4}I_{13/2} \rightarrow {}^{4}F_{9/2}$ and ${}^{4}S_{3/2} \rightarrow {}^{4}F_{9/2}$. Here, it should also be considered that the ${}^{4}G_{11/2} \rightarrow {}^{4}F_{9/2}$ transition is accompanied by back energy transfer (BET; see Fig. 1(a) and 2(a)) from Er³⁺ to Yb³⁺ which can lead to a re-excitation of Er^{3+} and a slope factor of n < 3.62Moreover, as can be derived theoretically (see the section Model of the population dynamics in Yb³⁺-Er³⁺ co-doped UCNPs and Fig. S14[†]), for very small *P* below the *P* used in this study, n of the red emission approaches two, indicating a threshold for the 3-photon process in UCNPs. For citrateand DSPE-capped UCNPs in H_2O , however, *n* of the red emission equals only about two. This underlines the population of ${}^4F_{9/2}$ from the ${}^4S_{3/2}$ \rightarrow ${}^4F_{9/2}$ and ${}^4I_{13/2}$ \rightarrow ${}^4F_{9/2}$ trans sitions in this solvent as low energy O-H vibrations can prevent the population of higher energy levels and support the ${}^{4}I_{11/2} \rightarrow {}^{4}I_{13/2}$ transition. This effect compensates the influence of Yb³⁺ quenching on the slope of the red emission. In the low P regime, n values of the blue and green emissions of citrateand DSPE-capped UCNPs in H₂O are, however, enhanced due to the high quenching rates of Yb³⁺.³⁴

P-Dependence of the green and red intensity ratios. Fig. 3 (left panel) summarizes the influence of *P* and the microenvironment on the intensity ratios of the green (545 nm; I_{g}) and



Fig. 3 *P*-Dependence of $l_{g/r}$ (left) and Φ_{UC} (right) for OA-capped UCNPs in cyclohexane, citrate-stabilized UCNPs in water, and DSPE-stabilized UCNPs in water and D₂O, respectively, for *P* varied between 10 and 1000 W cm⁻².

red (655 nm; I_r) emission bands ($I_{g/r}$) of our UCNPs in cyclohexane, DMF, water, and D₂O. These dependences clearly reflect the different population dynamics of the green and red emissive states of the UCNPs. The *P*-dependence of $I_{\alpha/r}$ is especially pronounced for OA- and BF₄-capped UCNPs in organic solvents and for DSPE-stabilized UCNPs in D₂O, where $I_{\rm r}$ increases much more strongly than $I_{\rm g}$ with increasing P, reaching a ratio of one ($I_{g/r} = 1$) at *ca*. 100 W cm⁻² (see Fig. 1, panels (b), (c), and (e) and Fig. 3). This underlines the rather similar population dynamics of the emissive states for UCNPs in cyclohexane, DMF, and D₂O, independent of the different surface ligands, which obviously control mainly only UCNP dispersibility. In these solvents, at low P, the ${}^{4}F_{9/2}$ -level (red emission) of Er^{3+} is mostly, but inefficiently populated from the green $({}^{4}S_{3/2})$ and the lower ${}^{4}I_{13/2}$ energy levels (biphotonic processes), and contributions from the higher energy level ${}^{4}G_{11/2}$ (3-photon process) are small, accounting for the dominance of the green emission. On the contrary, for citrate- and DSPE-stabilized UCNPs in water, which reveal a closely matching *P*-dependence of $I_{g/r}$, this intensity ratio varies only between *ca*. 0.4 and 0.7 for *P* values ranging from 5 W cm⁻² up to 3000 W cm⁻². For low P, as applied by most studies of UCNPs in water, $I_{g/r}$ is small, rising with increasing P, and decreasing again for high P. This trend is attributed to efficient population of the ${}^{4}F_{9/2}$ level (red emission) from the lower ${}^{4}I_{13/2}$ level at low *P*. This level is directly fed from the ${}^{4}I_{11/2}$ level of Er³⁺ by non-radiative relaxation initiated by coupling to O-H vibrations after the first energy transfer from the ${}^{2}F_{5/2}$ state of Yb^{3+} as detailed in the previous sections. With increasing P, the probability of populating the ${}^{2}H_{11/2}$ and ${}^{4}S_{3/2}$ states of Er^{3+} increases (see Fig. 2, panel (a)) and therefore, the intensity of the green emission, as reflected by the increase of $I_{g/r}$. The fact that for high P, $I_{g/r}$ of all UCNP samples converge at a value of about 0.4, indicates similar relaxation dynamics of the UC emission independent of the respective microenvironment. Under high P excitation, population of the red emissive state originates mainly from higher energy levels of Er^{3+} (see Fig. 2, panel (a)).

P-dependence of Φ_{UC} in organic solvents, H₂O, and D₂O. Although there are several reports on the quenching of the UC luminescence in water,^{27,29,34,36,58} especially for core-only systems, very little quantitative data have been reported yet,^{17,45} and no measurements of *P*-dependent $\Phi_{\rm UC}$ in water, despite their importance for an improved mechanistic understanding of water quenching of the UC luminescence of UCNPs. Such measurements are particularly challenging as contrary to most organic solvents, D₂O and water absorb at 980 nm. This affects the actual P and leads to heating of the sample, which can affect UC luminescence spectra and $\Phi_{\rm UC}$. As detailed in the ESI,[†] in order to account for such effects, P was rescaled for all measurements in water and the excitation induced temperature increase was estimated. Comparison of the shape of $\Phi_{\rm UC}(P)$ and the values of $\Phi_{\rm UC,sat}$ of the UCNPs in organic solvents and water shown in the right panel of Fig. 3 reveals that water affects only slightly the slope of $\Phi_{\rm UC}(P)$, yet clearly the size of $\Phi_{\rm UC}$, accounting for a reduction of the UC emission by a nearly constant factor of *ca*. 3 compared to cyclohexane, DMF, and D_2O at low *P* and *ca*. 2.5 at high *P*, respectively.

The drastic changes in the intensity contribution of the different Er^{3+} emission bands as well as $I_{g/r}$ and the corresponding *n* values detailed in the previous sections indicate that the decrease of $\Phi_{\rm UC}$ is mainly caused by Yb³⁺ quenching by water molecules. The high Yb³⁺ concentration favors efficient energy transfer by $Yb^{3+}-Yb^{3+}$ interactions to Yb^{3+} ions at the UCNP surface, which are quenched by coupling to O-H vibrations of water. The $^2\mathrm{F}_{5/2}$ energy level of Yb^{3^+} and the corresponding ${}^{4}I_{11/2}$ energy level of Er^{3+} are both resonant with the second harmonic of the fundamental vibration (overtone) of water and an energy transfer can occur directly to one of these resonant vibrations of water or to combinations of low energy vibrations that have a higher oscillator strength. Whereas the population rates of the emissive states of Er³⁺ are obviously much higher at high P than the respective quenching rates (see Fig. 1), in the case of Yb^{3+} , even at the highest P provided here, water quenching cannot be compensated. Hence, for applications in water, typically used dopant concentrations of Yb³⁺ and Er³⁺ in the NaYF₄ host should be revisited for UCNPs without and maybe even for UCNPs with very thin shells as used e.g. for FRET applications. A reduction of the Yb³⁺ concentration and an increase in the Er³⁺ concentration may be more favorable. The only slightly different $\Phi_{\rm UC}(P)$ of the citrate- and DSPE-stabilized UCNPs and the considerable enhancement of $\Phi_{\rm UC}$ in D₂O provide clear hints that the ligand shell of DSPE-stabilized UCNPs is not dense or close enough to efficiently shield the lanthanide ions at the particle surface from water molecules. Such a protection can be achieved only with tight inorganic shells of a sufficient thickness made from e.g., the host material, CaF2 or SrF2, yielding UC enhancement factors of at least three or even more, depending on the size of the core particles.23

Luminescence decay behavior

We subsequently assessed the luminescence decay kinetics of all UC and DC emission bands of Er³⁺ and the DC emission of Yb³ (excitation at 976 nm and emission at 930 nm) of our differently surface functionalized UCNPs in organic solvents, D_2O_2 , and water. This included the lifetimes of the ${}^4H_{9/2}$ (410 nm), ${}^{4}S_{3/2}$ (545 nm), and ${}^{4}F_{9/2}$ (655 nm) radiative energy levels of Er³⁺, excited at 378 nm, 490 nm, and 976 nm, respectively, in the latter case involving ETU from initially excited Yb^{3+} to Er^{3+} . In addition, also the lifetimes of the ${}^{4}I_{9/2}$ (810 nm) and ${}^{4}I_{13/2}$ (1520 nm) energy levels of Er³⁺ were studied. Here, it needs to be kept in mind that the lifetimes of all Yb³⁺ and Er³⁺ energy levels participating in energy migration processes contribute to the overall lifetimes of the energy levels populated via UC, whereas for the DC processes, only the lifetimes and energy transfer rates of higher lying energy levels determine the overall lifetimes of the different emissive states. The decay curves of the UC and DC luminescence of DSPE-stabilized UCNPs dispersed in water (blue) and D_2O (black) are representatively shown in Fig. 4 (panels (a)-(d)).



Fig. 4 Luminescence decay kinetics arising from UC (symbols) and DC (lines) processes in DSPE-stabilized UCNPs dispersed in water (blue) and D₂O (black) for (a) $\lambda_{em} = 930$ nm, (b) $\lambda_{em} = 1530$ nm, (c) $\lambda_{em} = 545$ nm, and (d) $\lambda_{em} = 655$ nm, respectively.

The corresponding luminescence lifetimes resulting from fits detailed in the ESI[†] are summarized in Table 1; the decay kinetics of other emission bands of these UCNPs and the respective particles in organic solvents as well as in mixtures of D₂O and water are provided in the ESI (see Fig. S9-S12 and Tables S1-S3[†]). The lifetime of the main emission component of the directly excited (λ_{ex} = 976 nm) $^{2}F_{5/2}$ level of Yb³⁺ (930 nm emission) is decreased by a factor of four in water (ca. 40 µs) compared to D_2O (160 µs) due to efficient coupling of the sensitizer ion Yb³⁺ to O-H vibrations. This factor is also reflected by $\Phi_{\rm UC}(P)$ measurements shown in Fig. 3 (right panel), revealing that a four times higher P is needed for UCNPs in water to reach the same $\Phi_{\rm UC}$ as found in D₂O. Moreover, time-resolved luminescence measurements reveal a new long lived decay component of the 976 nm Yb³⁺ luminescence in aqueous dispersions (Fig. 4, panel (a) and Table 1). In the case of Er^{3+} , the lifetime of the directly excited green emission $({}^4S_{3/2})$ is diminished in water and UC excitation (see Fig. 1, panel (a) and Fig. 2, left panel) also reveals a clear reduction of the lifetime of the main decay component of the green luminescence compared to non-aqueous environments together with the appearance of a new decay component with an effectively prolonged lifetime. The latter is also observed in D₂O and organic solvents, yet is much less pronounced (see e.g., Fig. 4 and ESI, Fig. S12[†]). In contrast, the lifetimes of the directly excited red emission (⁴F_{9/2}) of DSPE-stabilized UCNPs in water and D₂O differ only slightly (see Fig. 4 and Table 1), underlining inefficient coupling of O-H vibrations to this Er³⁺ energy level. Analogously to the green Er³⁺ luminescence and the Yb³⁺ emission at 976 nm, also for the red Er³⁺ emission, a new long lived decay component appears. We tentatively attribute this long lifetime component to the slow population dynamics of the different emissive states. The lifetime of the DC emission of Er^{3+} at 1520 nm arising from the ${}^{4}\text{I}_{13/2}$ energy level is shortened in water compared to D₂O but is nevertheless still in the ms range (Fig. 4, panel (b)). In water, this long lived excited state is effectively populated from the next higher ${}^{4}I_{11/2}$ energy level of Er^{3+} which is quenched by O–H vibrations (see Fig. 1 and 2).

The high population together with a comparably long lifetime enables Er³⁺-Er³⁺ energy transfer (ET1 in Fig. 1, $^4\mathrm{I}_{13/2},~^4\mathrm{I}_{15/2} \rightarrow ~^4\mathrm{I}_{13/2},~^4\mathrm{I}_{9/2}\text{)}.$ This enhances the commonly weak 810 nm emission from the ${}^{4}I_{9/2}$ energy level, which shows also a lifetime in the low ms range (see Table 1 and Fig. S10[†]) and the biphotonic ${}^{4}I_{13/2} \rightarrow {}^{4}F_{9/2}$ transition feeding the red emission which is detailed in the next section. The fact that the long lived decay components of the red and green emissions of Er³⁺ clearly differ in H₂O and D₂O (see Fig. 4, panels (c) and (d)) and are still present even when the ${}^{2}F_{5/2}$ level of Yb³⁺ is completely depopulated (except for the long lived component in water) indicates that these decay components arise from relaxation processes of the ⁴I_{13/2} and ⁴I_{9/2} energy levels coupled *via* the ${}^{4}I_{13/2}$, ${}^{4}I_{15/2} \rightarrow {}^{4}I_{13/2}$, ${}^{4}I_{9/2}$ energy transfer pathway (ET1) and an additional Er³⁺-Yb³⁺/Yb³⁺-Er³⁺ energy transfer. The latter is suggested by the long lived decay component of the Yb³⁺ emission at 930 nm in water. This Er³⁺-Yb³⁺/Yb³⁺-Er³⁺ energy migration is facilitated in water by the effective coupling of the ${}^{4}I_{11/2}$ and ${}^{4}I_{13/2}$ energy levels *via* O–H vibrations. These findings were confirmed by decay measurements of citrate-stabilized UCNPs in D2O/H2O mixtures and OA- and BF₄⁻-capped UCNPs in organic solvents (see ESI, Fig. S11 and S12†).

Model of the population dynamics in Yb³⁺-Er³⁺ co-doped UCNPs

The population of the most fundamental emission of Yb^{3+} - Er^{3+} co-doped UCNPs and UC materials, the green and red

Table 1 Fit results of luminescence decays of DSPE-stabilized UCNPs in water and D_2O . n.d.: can not be determined, since the derivation of the decay curve shows no convergence, (m): main component

Solvent	Lifetime	Upconversion wavelength [nm]				Downconversion wavelength [nm]				
		410	545	655	810	410	545	655	930	1520
H ₂ O	τ_1 (µs)	40	69 (m)	185 (m)	1100	16	45 (m)	140 (m)	42 (m)	2100 (m)
D_2O	$\tau_2 (\mu s) $ $\tau_1 (\mu s)$	74	120 (m)	180 (m)	2800	15	65 (m)	110 (m)	1200 160 (m)	1400
	$\tau_2(\mu s)$		3000	3100	_		n.d.	n.d.	2900	4400 (m)



Fig. 5 Calculated data for (a) the *P*-dependent relative emission intensities of the red and green emission bands in water (open circles) and D_2O (solid squares), respectively (top), and $I_{g/r}$ (bottom), (b) n(P), and (c)–(f) the luminescence decay kinetics arising from UC processes in solvents with (blue) and without (black) O–H vibrations for the Yb³⁺ emission (c)) and different Er^{3+} emissions, *i.e.*, (d) green, (e) 1520 nm, and (f) red, respectively.

bands, is expected to involve a biphotonic process. Especially for the red emission band of Er^{3+} originating from ${}^{4}F_{9/2}$, two different population pathways are typically proposed, i.e., (1) population via nonradiative relaxation from the green emitting energy level (see Fig. 1) and (2) relaxation after the first energy transfer step followed by direct excitation via a second energy transfer $({}^{4}I_{13/2} \rightarrow {}^{4}F_{9/2}$ transition).^{2,5,6,8,9,12,14-16,24,28,29,32-34} Different cross relaxation (CR) processes are also proposed dependent on the size, local structures of the emitting ions or ion concentration which can also influence the red-togreen intensity ratio.16,33,63 The mathematical description of this two photonic population model relies on rate equations which take into account the different Er³⁺ energy levels up to ⁴I_{7/2}.^{12,28} This is in contrast to recent publications from the Berry and May group, concluding that the ⁴I_{13/2} is not a feeding state for UC luminescence.⁴⁸ Instead, a three photon population process was proposed and a rate equation model was developed including the Er³⁺-levels up to ${}^{4}G_{11/2}$.

In order to verify our interpretation of the population dynamics of Yb^{3+} – Er^{3+} co-doped UCNPs, we modelled our experimental data with the coupled rate equations recently published by the Berry group.⁶⁰ This implied estimation of the corresponding radiative and non-radiative rates from lifetime measurements and simulation of the relative emission intensities, slope factors, and decays (see Fig. 5). A drawback of this model is that the energy level ${}^{4}I_{9/2}$ is treated as being nonemissive although it is responsible for the 810 nm emission.

Therefore, we focused here on the red and green emissions and $I_{g/r}$. The good agreement of the modelled data with our experimental results summarized in Fig. 1-3 confirms our assumption that the transition ${}^{4}I_{13/2} \rightarrow {}^{4}F_{9/2}$ is favored in water by vibrational coupling of O-H groups and is unlikely in D₂O. As follows from Fig. 5, summarizing the modelled data, the trend of $I_{g/r}(P)$ for UCNPs in water including the slight increase and decrease indicating the different contributions of the biphotonic transitions ($^4I_{13/2}$ \rightarrow $^4F_{9/2},~^2H_{11/2}/^4S_{3/2}$ \rightarrow $^4F_{9/2})$ and triphotonic transition ($^4G_{11/2}$ \rightarrow $\,^4F_{9/2})$ to the population of the red Er^{3+} energy level with increasing *P* can be well described. This trend is also reflected for $I_{\alpha}(P)$. In addition, all decays including that of the Yb³⁺ emission at 930 nm show in principle the long lifetime components originating from the metastable ⁴I_{13/2} energy level. For the simulation of the time resolved measurements we generated a pulsed excitation with a pulse length equaling the experimental conditions of ca. 1 ms. Also the calculated n values are in good agreement with the ones experimentally determined for UCNPs in H₂O, D₂O, and organic solvents. A detailed comparison is shown in the ESI.† For the evaluation of the agreement of modelled and measured data, it needs to be kept in mind that we used a simplified model and did not attempt to develop a new set of rate equations taking into account the number of lanthanide ions per UCNP and at least the ${}^{4}I_{9/2}$ level. This energy level is of considerable importance for UCNPs due to its high population by ET1 and strong emission for cases where other radiative UC pathways are quenched.

Conclusion and outlook

Based upon a systematic study of the excitation power density (P)-dependent upconversion (UC) luminescence intensities, slope factors (n), and absolutely measured luminescence quantum vields (Φ_{UC}) of popular β -NaYF₄:Yb³⁺,Er³⁺ UCNPs of different surface chemistries in organic solvents, D₂O, and water, we could demonstrate the potential of (P)-dependent luminescence measurements to derive population processes and radiative and non-radiative deactivation pathways of UCNPs in various environments. Our results confirm that the observed overall luminescence quenching and reduced Φ_{UC} in solvents with O-H groups results mainly from inefficient energy transfer from Yb^{3+} to Er^{3+} , although also the ${}^{4}I_{13/2}$ energy level of Er³⁺ is quenched and contributes to nonradiative energy losses. Moreover, we show experimentally and theoretically that in Yb³⁺-Er³⁺ co-doped UCNPs, the population dynamics of the Er³⁺ energy levels strongly differ for UCNPs dispersed in water and in organic solvents in the low P regime due to the efficient coupling of the fundamental vibrational modes of water to Yb^{3+} and Er^{3+} energy levels. At low P, applied for most spectroscopic studies and analytical or bioanalytical applications of UCNPs in aqueous media, the population of the red emitting level of Er³⁺ via the biphotonic processes ${}^4I_{13/2} \rightarrow {}^4F_{9/2}$ and ${}^2H_{11/2}/{}^4S_{3/2} \rightarrow {}^4F_{9/2}$ is responsible for a small green-to-red intensity ratio $(I_{g/r})$, whereas in the high *P* regime, the population dynamics of Er^{3+} are similar for water and organic solvents. This is advantageous for all applications involving high P like microscopic studies and single particle imaging. In organic solvents, the green emission is dominantly fed via the ${}^{4}I_{11/2} \rightarrow {}^{4}F_{7/2}$ transition and the red emission originates from inefficient biphotonic processes and a three photon process. These different population dynamics render $I_{g/r}$ strongly dependent on the UCNP environment and P. Furthermore, our results suggest that the typically used doping concentrations of 20% Yb^{3+} and 2% Er^{3+} of $NaYF_4$ host matrices may have to be optimized for applications in water, possibly also for different UCNP sizes. This is of interest e.g., for the construction of FRET-based nanosensors which requires a close distance between the UCNP donor and the acceptor, typically a stimuli-responsive organic dye or a molecular fluorophore incompatible with thick protecting shells as mandatory for the efficient shielding of surface lanthanide ions from interaction with surrounding solvent molecules.

In addition, our results and the derived population models demonstrate that for UCNPs for all cases involving strong luminescence quenching like the presence of solvent molecules or surface ligands with O–H vibrations and small surface to volume ratios, the contribution of three photon processes *e.g.*, to the overall population of the red emissive Er^{3+} energy level decreases due to the reduced lifetimes and accelerated decays of the excited energy levels involved in these processes. Our results can also be exploited for deriving easily accessible parameters for the screening of the UC efficiency of newly designed UC materials and material comparison. Best suited for Yb³⁺–Er³⁺ co-doped UCNPs are measurements of the luminescence lifetime of Yb³⁺ and Er^{3+} (1520 nm) and measurements of the intensity of the 810 nm emission band of Er^{3+} , which increases with increasing quenching of the red and green UC emissions due to $Er^{3+}-Er^{3+}$ energy transfer.

Materials and methods

Chemicals

DMF, cyclohexane and D_2O were of spectroscopic grade and purchased from Sigma Aldrich and Milli Q water (Millipore Corporation) (typically 18.2 M Ω cm at 25 °C) was used.

Yttrium(III) chloride hexahydrate (99.99%) and ytterbium(III) chloride hexahydrate (99.9%) were purchased from Treibacher Industrie AG (Althofen, Austria), Ammonium fluoride (ACS) (reagent grade, \geq 98.0%), erbium(III) chloride hexahydrate (99.99%), sodium hydroxide (reagent grade, \geq 98.0%), and nitrosyl tetrafluoroborate (95%) were purchased from Sigma-Aldrich (Steinheim, Germany). Oleic acid (technical grade, 90%) and 1-octadecene (technical grade, 90%) were from Alfa Aesar (Karlsruhe, Germany). DSPE-mPEG(2000) (1,2 distearoylsn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000](ammonium salt)) was purchased from Avanti Polar Lipids (Alabaster, Alabama). Tri-sodium citrate dihydrate was purchased from Merck (Darmstadt, Germany). N,N-Dimethylformamide (DMF) (99.5%) and cyclohexane (99.5%) were from Acros Organics (Geel, Belgium). All other reagents and organic solvents were of the highest grade available. Unless otherwise noted, all chemicals were used as received without any further purification.

Synthesis of UCNPs

The large-batch synthesis of oleate-coated UCNPs was performed as published previously.35 Briefly, YCl₃·6H₂O (15.6 mmol), YbCl₃·6H₂O (4.0 mmol), and ErCl₃·6H₂O (0.4 mmol) were dissolved in ~40 mL of methanol by sonication and transferred into a 1 L three-necked flask. Under a nitrogen atmosphere 160 mL of oleic acid and 300 mL of 1-octadecene were added and the mixture was heated to 160 °C for 30 min. After cooling to room temperature 200 mL of methanol containing NaOH (0.25 M) and NH₄F (0.4 M) were added at once. The resulting colloidal suspension was stirred for 30 min at 120 °C under a gentle flow of nitrogen and then heated to reflux at ~320 °C for ~22 min. Subsequently, the mixture was cooled to 200 °C. An additional heating step (>300 °C for ~5 min) was applied. Oleate-coated hexagonalphase UCNPs were precipitated by addition of ~400 mL of ethanol after cooling to room temperature and were isolated via centrifugation at a relative centrifugal force (RCF) of 1000g for 5 min. The pellet was washed three times, which involved different steps of dispersing of the UCNPs in ~10 mL of chloroform and cyclohexane and precipitation by addition of ~150 mL of ethanol and acetone. Finally, the purified oleatecoated (OA) UCNPs were dispersed in a total volume of 160 mL of cyclohexane.

Surface modifications of oleate-coated UCNPs

The procedures for the surface modifications of oleate-coated UCNPs were published recently,³⁵ *i.e.*, (i) modification with DSPE-mPEG (2 kDa); (ii) modification with BF_4^- ; and (iii) modification with citrate.

Instrumentation

Transmission electron microscopy (TEM) was performed using a Philips CM12 microscope (120 kV; Hillsboro, Oregon). The size distributions of the UCNPs were evaluated from the TEM images using ImageJ software (http://rsbweb.nih.gov/ ij/). A Zetasizer Nano ZS from Malvern (Worcestershire, UK) was used for dynamic light scattering (DLS) experiments. X-ray powder diffraction (XRD) patterns with a resolution of 0.005° (2 θ) were collected using a Huber Guinier G670 diffractometer (Rinsting, Germany) with a Cu source (Ka radiation, $\lambda = 1.54060$ Å; 40 kV; 30 mA). A Spectro Flame-EOP (Kleve, Germany) inductively coupled plasma optical emission spectrometer (ICP-OES) was used for the determination of the amount of rare-earth ions in the UCNPs. All centrifugation steps were carried out using a Hettich Universal 320 centrifuge (Tuttlingen, Germany). A Sonorex Digitech DT255H ultrasonic bath from Bandelin (Berlin, Germany) was used.

Steady-state absorption and fluorescence spectroscopy

Absorption spectra were recorded on a Cary 5000 spectrometer (Varian). Fluorescence emission spectra were measured with a previously described calibrated spectrofluorometer FSP 920 (Edinburgh Instruments) in a $0^{\circ}/90^{\circ}$ measurement geometry, which was equipped with a high stability 2 W 976 nm laser diode in addition to the conventional continuous xenon lamp. In order to cover the wavelength region from 300 nm to 1700 nm, three different detectors with overlapping detection windows were used. For temperature-dependent measurements of the UC emission in the temperature range of 20 °C to 80 °C a thermostated cell holder was used.

Absolute determination of *P*-dependent $\Phi_{\rm UC}$

For this purpose, a previously described integrating sphere setup⁶⁴ was equipped with a high stability 8 W 976 nm laser diode, the spectral radiance of which could be attenuated with the aid of a set of eight neutral density filters of known transmittance. In order to guarantee precise matching between the excitation wavelength of the laser diode and the very narrow absorption bands of the activator ions, the intensity-weighted average emission wavelength of the laser was adjusted with a Peltier cooler in resonance to the Yb³⁺ absorption peak at 976.4 nm. The beam profile and the excitation power density at the position of the measurement cell were recorded with a powermeter. The area of the excitation spot was determined by integration of the image after subtracting the background caused by scattered laser light. All measurements were per-

formed with a Gaussian beam profile. For the detection of the scattered excitation light required for the calculation of the number of absorbed photons, the very intense laser light was attenuated with a calibrated absorptive neutral density (ND) filter (attenuation factor of *ca.* 5600).

Calibration of the integrating sphere setups

The calibration of the wavelength scale and the range of linearity of both integrating sphere setups was performed following previously described procedures.^{55,64} The detection channel including the spectral response of the UV/vis/NIR integrating sphere used for measurements of the UC luminescence was calibrated from 350 nm–1050 nm with a certified quartz halogen lamp mounted inside a sphere (Gigahertz-Optik GmbH; PTB-calibrated wavelength dependence of the spectral radiance ($L_{\lambda}(\lambda)$) for different short-pass filters and validated with the BAM spectral emission standards F003– F005.^{55,64}

Measurement of UC luminescence

In order to minimize reabsorption and indirect excitation, a small sample volume was used. UCNP dispersions were measured in a 10 mm × 4 mm quartz cuvette (filling height of 30 mm; volume of 1200 mm³). For minimal attenuation/distortion of the laser beam profile, we chose the particle concentration such that the absorbance was minimized to ca. 0.02. Under these conditions, an attenuation of the excitation power density due to scattering by UCNPs could be excluded. The time for a single measurement was set to ca. 30 s to reduce temperature effects. For this reason, also a delay time was implemented between single $\Phi_{\rm UC}$ measurements to further minimize temperature effects and to exclude thermal effects due to the high incident laser light. The *P*-dependence of $\Phi_{\rm UC}$ was determined in duplicate in two cycles with increasing and decreasing P, thereby also ensuring the prevention of temperature effects.

Time-resolved fluorescence measurements

Luminescence decay kinetics. Lifetime measurements were carried out with a commercial Edinburgh Instruments spectro-fluorometer FSP-920 equipped with an electrically pulsed 1 W 978 nm laser diode with a 950 ms long square pulse width recorded with a red extended PMT (R2658P) and a NIR PMT (R5509P) from Hamamatsu. The time-dependent changes in the UC emission intensities and the 980 nm emission of Yb³⁺, directly excited at 930 with a xenon lamp, were determined with the phosphorescence accessory of FSP 920. For the decays measured at 1520 nm, we used a multi-exponential fit for the rise and decay.

Data analysis. All fits of the measured decay curves were performed with a tail-fit instead of a deconvolution procedure of the recorded decay curves with the instrument response function (IRF) which is usually mandatory for accurate analysis of fluorescence lifetimes from time-correlated single-photon counting measurements.

P-dependent slope factors *n*(*P*)

n(P) are calculated from energetically neighboring *P*-dependent intensity values $I_{\text{em},i}(\lambda_{\text{em}},P_i)$ and $I_{\text{em},j}(\lambda_{\text{em}},P_j)$, see eqn (2):

$$n(P) = \frac{\ln(I(x_j)) - \ln(I(x_i))}{\ln(x_j) - \ln(x_i)}$$
(2)

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