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Energy transfer in diiodoBODIPY-grafted upconversion nanohybrids

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Steady-state and time-resolved emission studies on nanohybrids consisting of NaYF₄:Yb,Er and a diiodo-substituted Bodipy (UCNP-IBDP) show that the Yb³⁺ metastable state, formed after absorption of a near-infrared (NIR) photon, can decay via two competitive energy transfer processes: sensitization of IBDP after absorption of a second NIR photon and population of Er³⁺ excited states.

Understanding resonant energy transfer (RET) processes between emissive nanoparticle donors and organic dye acceptors is of paramount importance for both fundamental photophysics and application oriented studies. Detailed studies on the RET phenomenon can be found in literature mostly regarding Quantum Dots (QDs) donors. By contrast, only few examples of time-resolved studies have been reported for upconversion nanoparticles (UCNPs) acting as RET donors.

Whereas QDs undergo down-conversion after excitation, the UCNPs emit in the visible spectral range upon near infrared (NIR) excitation due to multiple intra-configurational 4f electronic transitions. This up-conversion process paves the way to initiate photodynamic (PDT) reaction with deeply penetrating NIR light and shall enable more efficient PDT at larger tissue volumes. There are however two challenges to make this possible. Since the PDT may occur only on efficient ET from UCNPs to photosensitiser, anchoring photosensitisers (PS) directly to UCNPs and finding PSs of appropriate excitation spectrum (i.e. overlapping the luminescence of up-converting lanthanides) is of critical importance.

In particular, ytterbium and erbium co-doped sodium yttrium fluoride (NaYF₄: Er³⁺,Yb³⁺) nanoparticles have been used as energy donors for different PSs, such as Ru(bpy)₃²⁺, zinc phthalocyanine,₅ merocyanine S40, hypericin,₁ᵣ methylene blue,₁₂,₁₃ rose bengal,₁₄ pyropheophorbide a,₁₄ chlorin e₆,₁₅ and a diiodo-bodipy compound.₁₆ These UCNPs have also proved successful energy donors to QDs and other fluorescent dyes such as rhodamine 6G.₂ In all these systems, the spectral overlap of the UCNP emission spectrum with the PS absorption spectrum is a pre-requisite for the efficient energy transfer (ET). Moreover, for organic/inorganic fluorophores the ET yield (η) depends on the donor-to-acceptor separation distance (r_D) with an inverse 6th power law due to the dipole-dipole coupling mechanism following simple relation

$$\eta = \frac{1}{1 + (r_D/R_0)^6}$$

where $R_0$ is Forster distance ($R_0<10nm$).

For upconverting nanoparticles, there are numerous donors within single nanoparticle, which display a whole range of effective distances to acceptors anchored at the surface, and the photophysics behind UC-RET is a more complex phenomenon. Different strategies have been used to anchor the PS molecules as dense and as close to the surface as possible, by grafting or adsorption to the surface, or by covalent linkage to the nanoparticle functional capping.
In this context, we have recently designed a nanohybrid made of NaYF₄:Yb³⁺, Er³⁺ UCNP and a diiodo-substituted Bodipy derivative (namely, 3-(2,6-dioiodo-1',3',5',7'-tetramethyl-4',4'-difluoro-4'­bora-3'-a,4'-a-diaza-s-indacen-8'-yl) propanio acid; IBDP), which is anchored to the nanoparticle surface directly and embedded in the organic capping (a polyethylene glycol, PEG, derivative). This nanohybrid was dispersible in water due to its polymeric coating.

Now, to determine the efficiency of the energy transfer process between IBDP and the UCNP core in UCNP-IBDP nanohybrids, both steady-state and time-resolved emission measurements were carried out with water- or organic-dispersible nanohybrids (Figure 1). Nanohybrids were prepared from the same batch of oleate-capped UCNPs (UCNP@OA), and their photophysical properties were compared with those of the UCNP@OA and naked UCNPs (UCNPnaked), which also originated from the same UCNP@OA batch.

This made it possible to compare the influence of the nanoparticle capping and the solvent itself on the optical properties of the nanohybrids.

Upconversion nanoparticles (NaYF₄: Yb³⁺ (16%), Er³⁺ (4%)) capped with oleate (UCNP@OA) were synthesised following a protocol described in the literature with some modifications (see ESI Fig. S1-S3). Transmission electron microscopy (TEM and high resolution TEM, HRTEM) showed that the UCNPs were uniform hexagonal prisms (38.4 ± 1.7 and 13.6 ± 1.4 nm, height and side, respectively, Fig. S1). Then, naked UCNPs (UCNPnaked) and UCNP@PEG were synthesised by removing the oleate ligand of UCNP@OA by acidification with HCl²², whereas UCNP@PEG were synthesised by exchange of the oleate with HS-PEG-NH₂ (See Fig. S4 and ESI for further details).

We have previously reported that the ligand exchange reaction of UCNP@PEG with IBDP led to water-dispersible nanohybrids (UCNP-IBDP@PEG)²¹, see Fig. S5 and experimental details in ESI. Similar methods were applied to UCNP@OA nanoparticles in order to produce lipophilic hybrid (UCNP-IBDP@OA) nanoparticles. Briefly, a mixture of IBDP and the UCNP@OA was sonicated in water in the presence of triethyamine for 15 min to ensure the deprotonation of the IBDP carboxylic group and, consequently, its grafting to the UCNP surface. Then, the mixture was stirred for 24 h in an orbital shaker at room temperature. Then, the nanoparticles were centrifuged and washed five times in acetonitrile in order to remove the excess of IBDP. Finally, the UCNP-IBDP@OA nanohybrid (pink powder) was re-suspended in toluene (See experimental details in the ESI).

The UV-Vis absorption spectra (Figure 2) and TGA analyses (not shown) of UCNP-IBDP@OA and UCNP-IBDP@PEG revealed an IBDP load of 40% and 10%, respectively. Figure 2 shows the comparison between the emission spectra of the water-dispersible nanoparticles, i.e., UCNPnaked, UCNP@PEG, and UCNP-IBDP@PEG on the left panel, while right panel compares the spectra of the toluene-dispersible nanoparticles, i.e., UCNP@OA and UCNP-IBDP@OA, after 975 nm excitation.

The UV-Visible absorption spectrum of IBDP has been included in both solvents to compare the overlap between the absorption spectrum of IBDP (the energy acceptor) and the emission of the UCNP (energy donor) in both water and toluene, as such overlap is a prerequisite for RET energy transfer to occur. The overlap is quantified with the spectral overlap integral (J) calculated as J=∫σD(λ)σA(λ)λ4δλ, where σA is the normalized donor emission spectrum, σD is the acceptor molar extinction coefficient, and λ is the wavelength of light.

Upon excitation at 975 nm (where Yb³⁺ absorbs), the emission spectra of all the UCNPs exhibited three bands owing to Er³⁺ emission: an intense band at 540 nm (¹S₄/₂→³I₄/₂), another close emission at 520 nm (¹S₄/₂→³I₄/₂), and a red band at longer wavelengths (at ca. 670 nm, ⁴F₄/₂→³I₄/₂). Power dependence measurements showed that emission was proportional to the 2nd power of excitation intensity, which is consistent with previously reported values (Figure S8). As expected, all the bands of UCNPnaked were less intense than those of the PEG-capped UCNP, which is consistent with the capacity of PEG to passivate the UCNP surface (Figure 1, left).

The comparison between the emission spectrum of each nanohybrid, UCNP-IBDP@PEG and UCNP-IBDP@OA, and its precursor, i.e., UCNP@PEG and UCNP@OA, respectively, revealed that the IBDP-induced quenching of the green UCNP emission band, where IBDP absorption was present. The efficiency of the RET was calculated by using simple formula based on either emission intensity or emission lifetimes, η = (I-D-A/I-D) = (1 − τ_A/τ_D), where I_D and I_A are the integrated emission of D-A nanohybrid (UCNP-IBDP@PEG and UCNP-IBDP@OA) and the respective nanohybrid precursor emission (UCNP@PEG and UCNP@OA), while τ_A and τ_D are the luminescence lifetimes of D-A and D alone species, at the D emission wavelength. The efficiencies in the 513-560 nm range, decreased by ca. 50% and 30% for UCNP-IBDP@OA and UCNP-IBDP@PEG, respectively (similar results were obtained when the intensities at 546 nm were compared). In quantitative terms, the J overlap of UCNP-IBDP@OA in toluene was larger than that of UCNP-IBDP@PEG in water (9.948·10⁻⁶ nm²).
The decay lifetimes of the UCNP emission were in the tens to time scale. Note that intensities are not real. To get insight into the nature of the intermolecular quenching the UCNP emission was presumed to be due to filter effects. As a control experiment, small volumes of a toluene solution of a diiodobodipy lacking the carboxylic acid group (specifically, 4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene), IBDPnc, 5·10^{-5} M) were added to an oleate-capped UCNP dispersed in toluene (1 mg·ml^{-1}) up to a final IBDPnc concentration of 0.45 mM. Figure S9 shows the decrease of the green emission with increasing concentrations of IBDPnc. It was expected that this substance would not anchor the UCNP surface and, consequently, the quenching of the UCNP emission was presumed to be due to filter effects.

To get insight into the nature of the intermolecular quenching process in the nanohybrids and corroborate the filter effects in the UCNP@OA/IBDPnc mixtures, we carried out time-resolved measurements of all the UCNPs. The emission of UCNP@OA, recorded at 546 and 654 nm, showed no time-dependence in the presence of increasing IBDPnc concentrations up to the highest concentrations tested. This confirmed that the energy transfer in the intermolecular system occurred via a trivial emission-reabsorption mechanism. The kinetic traces at \( \tau_{\text{exc}} \) = 975 nm excitation for the UCNP@OA, UCNP@PEG, UCNP@IBDP@PEG, and UCNP-IBDP@OA showed characteristic rise and decay phases (Figure 3, Table 1, and ESI).^{26} The rise and decay lifetimes (\( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) respectively) were determined by fitting the data and these two components correspond to the sensitisation and subsequent decay of the Er^{3+} excited state. All the fittings exhibited good quality match and the \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) decays were estimated by triplicate.

The decay lifetimes of the UCNP emission were in the tens to hundreds \( \mu \)s range and those for the red emission were longer (ca. 250-320 \( \mu \)s) than those of the green (65-125 \( \mu \)s), see Table 1. The fitted kinetics for each of the UCNPs is shown in the ESI (Fig S10-S20). In this study, we used the same batch of UCNP@OA for the preparation of the other four samples and no change in the crystalline phase, size, or shape of the UCNP core was detected upon hybrid formation.

Therefore, the observed differences in the decay lifetimes shall be attributed to non-radiative processes due to the interaction of the UCNPs with the solvent, the capping ligand, and, in the case of the nanohybrids, the diiodobodipy acceptor molecules. As expected, taking into account that O-H vibrations deactivate lanthanide exited states due to multiphonon non-radiative interactions,^{27} the decay of the green and red emissions were longer for the UCNPs dispersed in toluene than in water (Table 1). This impact of the solvent was less pronounced for the red emission (ca. 300 vs 280 \( \mu \)s) as compared to green (ca. 80 vs 120 \( \mu \)s), which suggests that the population of higher energy levels and non-radiative deactivation of these levels are more susceptible to the presence of O-H vibrations.\(^2\) Larger discrepancies can be observed for the luminescence risetimes, where the toluene dispersed UCNP@OA exhibited almost twice as long values (50 and 122 \( \mu \)s for green and red, respectively) as compared to water soluble UCNP@PEG (44 and 69 \( \mu \)s for green and red, respectively).

This observation demonstrates the fast saturation of intermediate excited state levels of Er^{3+} ions in water dispersible UCNP@PEG NPs and most probably supports the hypothesis of critical role of Yb^{3+} energy migration network in releasing the absorbed energy through the surface Yb^{3+} ions. In addition, the presence of IBDP in UCNP-IBDP@PEG led to a shortening of \( \tau_{\text{decay}} \) (from 82 to 67 \( \mu \)s, \( 1 - \tau_{\text{OA}}/\tau_{50} = 18\% \)). A similar behaviour was observed for the green emission of the lipophilic nanohybrid (\( \tau_{\text{decay}} \) of 119 \( \mu \)s and 96 \( \mu \)s, \( 19\% \), for UCNP@OA and UCNP-IBDP@OA, respectively, Table 1).

The presence of IBDP in both UCNP-IBDP@PEG and UCNP-IBDP@OA demonstrated faster rise-times of red emission band kinetics, in comparison to non-IBDP NPs. This behavior indicates the IBDP plays a role in the up-conversion and changes the balance between ETU and depopulation phenomena during the intermediate steps despite the evident energy mismatch between the excited states of Er^{3+} donors and energy levels of IBDP acceptor.

**Table 1. Luminescence lifetimes for the UCNPs excited at 975 nm.**

<table>
<thead>
<tr>
<th>UCNP</th>
<th>( \tau_{515} \rightarrow \tau_{552} ) green emission</th>
<th>( \tau_{654} \rightarrow \tau_{670} ) red emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>water dispersible</td>
<td>( \tau_{\text{rise}} ) = 45 ( \pm ) 7 ( \mu )s</td>
<td>( \tau_{\text{rise}} ) = 94 ( \pm ) 4 ( \mu )s</td>
</tr>
<tr>
<td>UCNP@PEG</td>
<td>82 ( \pm ) 2 ( \mu )s</td>
<td>119 ( \pm ) 1 ( \mu )s</td>
</tr>
<tr>
<td>UCNP-IBDP@PEG</td>
<td>67 ( \pm ) 2 ( \mu )s</td>
<td>122 ( \pm ) 18 ( \mu )s</td>
</tr>
<tr>
<td>UCNP@OA</td>
<td>96 ( \pm ) 6 ( \mu )s</td>
<td>61 ( \pm ) 6 ( \mu )s</td>
</tr>
<tr>
<td>UCNP-IBDP@OA</td>
<td>58 ( \pm ) 10 ( \mu )s</td>
<td>317 ( \pm ) 4 ( \mu )s</td>
</tr>
</tbody>
</table>

In the above table, \( \tau_{\text{rise}} \), \( \tau_{\text{decay}} \), \( \tau_{\text{exc}} \) refers to the lifetime, the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively. \( \tau_{\text{exc}} \) is the excitation lifetime. \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are the lifetimes for the rise and decay, respectively.

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Moreover the red emission decays in UCNP-IBDP@PEG was ca. 10% shorter than that of UCNP@PEG, while UCNP-IBDP@OA exhibited the red decay to be slightly longer than the non IBDP nanohybrid. The interpretation of such relations is not straightforward and requires further investigations.

Regarding the risetimes (τ_rise of the green and red emission donor hybrids, again the hydrophilic UCNP@PEG NPs exhibited shorter lifetimes than UCNP@OA. Remarkably, the τ_rise of the UCNP-IBDP@OA green emission decreased almost two-fold as compared to that of UCNP@OA, and similar shortening was also detected for τ_rise of the red emission. Such consistent behaviour was not observed for PEG coated NPs. A likely interpretation for the lipophilic UCNP-IBDP@OA could be that the presence of IBDP makes a competitive decay possible for the metastable state of Yb^{3+} (Yb^{3+*}). Specifically, Yb^{3+*} could be involved in the population not only of Er^{3+} excited states but also in that of IBDP (IBDP*) after absorption of a second photon (Figure 4). As a consequence, the risetime of both red and green emissions would reflect the decrease of the contribution of Yb^{3+*} to populate the Er^{3+} excited states involved in those emissions.

To corroborate this hypothesis we prepared the lipophilic Yb^{3+}(IBDP)_3 complex (see ESI for experimental details) which was excited at 975 nm. Remarkably, the τ_rise of the UCNP-IBDP fluorescence (Figure 5 left, in blue) was detected in spite of the considerably low absorption cross section of the Yb^{3+} ions (as compared to Stokes excitation of IBDP) at the NIR and the low fluorescence of the diiodo-Bodipy. This suggested that the Yb^{3+}(IBDP)_3 complex can up-convert following the proposed scheme

\[
\begin{align*}
\text{Yb}^{3+} \text{-IBDP} + h\nu_1 & \rightarrow \text{Yb}^{3+*} \text{-IBDP} \\
\text{Yb}^{3+*} \text{-IBDP} + h\nu_1 & \rightarrow \text{Yb}^{3+} \text{-IBDP}^* \\
\text{Yb}^{3+} \text{-IBDP}^* & \rightarrow \text{Yb}^{3+} \text{-IBDP} + h\nu_2 (h\nu_2 > h\nu_1)
\end{align*}
\]

It is known that Bodipy compounds are capable to undergo two-photon absorption processes.²⁸ Therefore, control experiments were carried out to confirm the involvement of the lanthanide in the emission of IBDP in the Yb^{3+}(IBDP)_3 when being excited at 975 nm. Figure 5 left shows the much more efficient emission of the Bodipy in the complex. Moreover, time-resolved measurements evidenced that the emission lifetime of Yb^{3+}-IBDP^* was ca. 5 µs (Figure 5 right), a great deal longer than that of IBDP*, whose emission lifetime was but a few ns. Finally, power-dependence measurements corroborated that the IBDP emission after NIR excitation on Yb^{3+}(IBDP)_3 was proportional to the 2nd power of the 975 nm excitation intensity (inset Figure 5 right). The latter observation evidenced the need to involve two low energy 532 nm photons, to get visible emission of IBDP.

Conclusions

We have prepared hydrophobic and hydrophilic UCNP-IBDP nanohybrids, which showed a decrease in their green to red emission compared to that of their precursors. Time-resolved experiments demonstrated that the quenching of the green emission was due to energy transfer from the UCNP to the anchored IBDP. Furthermore, the decrease in the efficiency of green and red emissions in UCNP-IBDP was attributed to the competitive decay of Yb^{3+*} via energy transfer to IBDP after absorption of a second photon. This process was more competitive in the organic solvent. Therefore, the comparison between the emission risetimes, as well as between the emission decay lifetimes, of the nanohybrids and those of UCNP_naked, UCNP@PEG, and UCNP@OA, give valuable information about the excited states involved in UCNP emission kinetics.

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Notes and references


