

Upconverting Nanoparticle to Quantum Dot Förster Resonance Energy Transfer: Increasing the Efficiency through Donor Design

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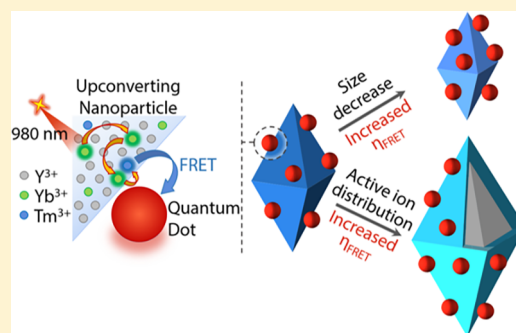
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Supporting Information

ABSTRACT: We propose two effective approaches to enhance the Förster resonance energy transfer (FRET) efficiency from near-infrared excited upconverting nanoparticles (UCNPs, namely, $\text{LiYF}_4:\text{Yb}^{3+},\text{Tm}^{3+}$) to CuInS_2 quantum dots (QDs) upon engineering of the donor's architecture. The study of the particles' interaction highlighted a radiative nature of the energy transfer among the moieties under investigation when in solution. However, analyses performed on dry powders allowed observing clear evidence of a FRET mechanism. In particular, photoluminescence lifetime measurements showed that FRET efficiency could be effectively increased by both reducing the size of the UCNPs and directly controlling the distribution of the active ions throughout the donor's volume, i.e., doping them only in the outer shell of a core/shell system. Both strategies resulted at least in a more than doubled FRET efficiency compared to larger core-only UCNPs. Obtained experimental values were compatible with those predicted from geometrical considerations on the active ions' distribution over the UCNP volume. These results provide a concrete proof of the potential of a UCNP–QD FRET pair when the system is properly designed, hence setting a solid base for the development of robust and efficient all-inorganic probes for FRET-based assays.

KEYWORDS: FRET, energy transfer, upconversion, quantum dots, nanoparticles, core/shell, copper indium sulfide, CuInS_2 , LiYF_4



Förster resonance energy transfer (FRET) is a nonradiative energy transfer (ET) process between two optically active species (donor and acceptor) that is triggered by light:¹ after the radiation is absorbed by the donor, the energy is passed to the acceptor without the involvement of any photon. Overall, this mechanism leads to the donor's emission quenching and possibly to the appearance of the acceptor's luminescence signal. Notably, FRET is a short-range phenomenon whose effects are usually observed in the 0–10 nm donor–acceptor separation range, a fact that directly stems from the dipole–dipole nature of the interaction between the moieties.² Therefore, FRET is a powerful tool to probe events occurring at the nanoscale through an easily detectable optical signal, overcoming the theoretical spatial resolution limit achievable with visible electromagnetic radiation.

FRET has been successfully exploited to investigate the short-scale interaction between chemical species^{3,4} and applied in biology for a number of all-optical assays.^{5–9} “Classical” FRET techniques take advantage of intermolecular interactions,

in particular those occurring among dyes and/or metal complexes.¹⁰ Nonetheless, these techniques suffer from the typical shortcomings that affect organic dye-based methods, e.g., photobleaching, short-lived emission (that makes it hard to clearly discern the dye's signal from the autofluorescence of biological components), spectral cross-talk between moieties, poor chemical stability, and often pH-dependent optical properties.^{11,12} The later advent of green fluorescent protein (GFP) and its derivatives still did not allow properly addressing the drawbacks of short emission lifetime (LT) and broad absorption/emission bands.¹³ Hence, the applicability of FRET-based assays in intracellular milieus, albeit highly attractive and widely spread, remains practically challenging.

More recently, nanoparticles (NPs) have made their appearance in the field as both potential energy donors and

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acceptors, opening a wide spectrum of new possibilities.^{14–21} Among others, upconverting NPs (UCNPs) are alluring energy donors in the frame of FRET.^{22–25} Their appeal resides in a long-lived visible emission triggered by near-infrared (NIR) excitation, which allows overcoming the autofluorescence of biological media.²⁶ Moreover, NIR operational capabilities are of great interest when subcutaneous applications are sought, since in this spectral range light scattering and absorption phenomena in biological tissues are minimized.^{27,28} Although UCNPs' low photoluminescence quantum yield (PLQY) has curbed their applicability in FRET-based assays, the exploitation of Gd³⁺-mediated energy migration throughout the UCNP's volume²⁵ and core/shell engineering^{29–31} were recently proven as effective means to control UCNPs' optical properties and circumvent their limitations in the frame of FRET.

Works proposing UCNPs as FRET donors mainly involve the use of acceptors such as organic dyes,^{32,33} carbon-based systems,³⁴ or gold NPs.^{35,36} Despite the considerable literature on the subject, the combination of UCNPs and quantum dots (QDs) as a donor–acceptor pair is largely unexplored. This pair has the potential to become a robust all-inorganic FRET system widely applied in optical assays; indeed, the combined advantages of UCNPs' NIR excitation and long-lived emission, along with QDs' broadband absorption, are paramount assets. Moreover, these features come bundled with the superior photo- and chemical stability granted by both inorganic species.

The study of this type of system is still in its infancy, leaving plenty of room for investigation at the level of both material optimization and application. Following the path initiated by the first studies on the subject,^{32,37,38} the seminal report on FRET between UCNPs and QDs was published by Bednarkiewicz et al. in 2010.³⁹ The authors studied the ET from NaYF₄:Yb³⁺,Er³⁺ UCNPs to CdSe QDs, reporting a FRET efficiency of approximately 15%. To the best of our knowledge, few papers on similar systems were published since then. These studies confirmed the potential of the UCNP–QD FRET pair applied in, for example, biotin⁴⁰ and oligonucleotide detection⁴¹ or as an effective NIR detection system.⁴² Although these are promising observations, there is a lack of careful investigation about the fundamental physical aspects governing FRET among UCNPs and QDs, while a thorough optimization of the architecture/geometry of the pair is currently missing.

Here, we have sought to investigate the effect that the UCNP size, as well as the distribution of the active ions in the UCNP volume, has on the FRET efficiency to QDs. LiYF₄:Yb³⁺,Tm³⁺ and CuInS₂ (CIS) were the selected materials to prepare UCNPs (donors) and QDs (acceptors), respectively. The rationale underlying the choice of the upconverting material is the strong ultraviolet (UV)–blue upconversion luminescence (UCL) stemming from LiYF₄ doped with Yb³⁺ (sensitizer) and Tm³⁺ (activator) ions, which considerably overlaps with ternary CIS QD broadband absorption. Moreover, newly emerging CIS QDs were preferred over “traditional” binary QDs for two reasons: CIS QDs' optical properties can be tuned with ease to ensure NIR operational capabilities, and they are free of toxic heavy metal ions (contrary to most high-quality binary QDs). Both features are extremely valuable when foreseeing the possibility of using the system in biological environments. Overall, we give comprehensive insight into the most relevant factors determining FRET from UCNP to QD, in order to provide a solid background for new advancements in the field.

■ EXPERIMENTAL SECTION

Chemicals. Yttrium oxide (Y₂O₃, 99.99+%), ytterbium oxide (Yb₂O₃, 99.99+%), thulium oxide (Tm₂O₃, 99.99+%), trifluoroacetic acid (TFA, 99%), lithium trifluoroacetate (LiTFA, 97%), indium acetate (In(OAc)₃, 99.99%), oleic acid (OA, 90%), 1-octadecene (ODE, 90%), and hexane (99%) were purchased from Alfa Aesar. Copper (I) iodide (CuI, 98%), oleylamine (OLAm, 70%), 1-dodecanethiol (DDT, 98+%), toluene (99%), and acetone (99%) were purchased from Sigma Aldrich. Ethanol (99%) was obtained from Commercial Alcohols. All chemicals were used as received.

Nanoparticle Synthesis. *Large LiYF₄:Yb³⁺,Tm³⁺ UCNPs (L-UCNPs).* UCNPs were synthesized according to the thermal decomposition method outlined by Mahalingam et al.⁴³ Lanthanide trifluoroacetates were prepared from the corresponding oxides: 219.3 mg (0.93125 mmol) of Y₂O₃, 123.2 mg (0.3125 mmol) of Yb₂O₃, and 2.4 mg (0.00625 mmol) of Tm₂O₃, corresponding to a nominal composition LiYF₄: 25% Yb³⁺, 0.5% Tm³⁺. They were mixed in a 100 mL three-necked round-bottom flask along with 5 mL of distilled water and 5 mL of TFA. The slurry was refluxed under vigorous stirring at 80 °C until a clear solution was obtained. Then, the temperature was decreased to 60 °C, and the residual TFA and water were allowed to slowly evaporate overnight. A 2.5 mmol amount of LiTFA was added to the dried lanthanide trifluoroacetates together with 20 mL of OA and 20 mL of ODE. The mixture was degassed at 110 °C for 30 min and backfilled with Ar. The temperature was then raised to 330 °C, and the reaction was allowed to proceed for 1 h. After this time, the heating was stopped and the flask was allowed to naturally cool to 60 °C. The reaction product was split in two centrifuge tubes, and the UCNPs were precipitated by the addition of ethanol. They were centrifuged (20 min at 6000g) and the supernatant was discarded. Eventually, the UCNPs were dispersed in hexane and washed three times with ethanol before being redispersed in toluene for further characterization.

Small LiYF₄:Yb³⁺,Tm³⁺ UCNPs (s-UCNPs). The procedure followed for the synthesis of smaller LiYF₄: 25% Yb³⁺, 0.5% Tm³⁺ crystals was identical to the one described above, with the only exception of substituting 5 mL of OA with an equal volume of OLAm. The purification process of the UCNPs was the same as for L-UCNPs. The sample was eventually dispersed in toluene for further characterization.

Core/Shell LiYF₄:Yb³⁺/LiYF₄:Yb³⁺,Tm³⁺ UCNPs (CS-UCNPs). The trifluoroacetates for the synthesis of inactive-core/active-shell UCNPs were prepared respectively starting from (i) *core*: 220.8 mg (0.9375 mmol) of Y₂O₃ and 123.2 mg (0.3125 mmol) of Yb₂O₃; (ii) *shell*: 219.3 mg (0.93125 mmol) of Y₂O₃, 123.2 mg (0.3125 mmol) of Yb₂O₃ and 2.4 mg (0.00625 mmol) of Tm₂O₃. These quantities correspond to a nominal composition LiYF₄: 25% Yb³⁺/LiYF₄: 25% Yb³⁺, 0.5% Tm³⁺. Core UCNPs (C-UCNPs) were synthesized according to the procedure described for the synthesis of L-UCNPs. After 30 min of core growth, 2.5 mmol of LiTFA, 7.5 mL of OA, and 7.5 mL of ODE were added to the dried shell precursors, and the mixture was heated stepwise to 125 °C in 30 min under vacuum. After 1 h of core growth, 5 mL of the reaction mixture was taken, and the shell solution was injected at a flow rate of 1.5 mL·min⁻¹. Once the injection was completed, the reaction was allowed to proceed for 1 h more. The purification steps were the same as reported for the other UCNP samples. CS-

UCNPs were eventually dispersed in toluene for further characterization.

CIS QDs. Copper-deficient CIS QDs were synthesized according to a simple thermal decomposition method. A 9.5 mg amount of CuI (0.05 mmol), 58.4 mg of In(OAc)₃ (0.2 mmol), 10 mL of ODE, 3.9 mL (16 mmol) of DDT, and 0.708 mL (2 mmol) of OA were introduced into a 50 mL three-necked round-bottom flask. The solution was stirred at 100 °C under vacuum for 45 min and backfilled with Ar. Then, the temperature was raised to 120 °C and maintained at this stage for 5 min. The temperature was further raised to 150 °C and maintained for 5 min more. Eventually, the temperature was set to 230 °C. Upon heating, the solution became completely clear and slightly yellow. Subsequently, the color changed from deep yellow to orange, then red, and eventually dark red, indicating the nucleation and growth of the QDs. After 45 min the flask was quenched in cold water. The QDs were transferred to a centrifuge tube and precipitated by the addition of acetone. They were centrifuged (20 min at 6000g) and the supernatant was discarded. The QDs were dispersed in toluene and washed twice with acetone, before being redispersed in toluene (approximately 0.15 mM) for further characterization.

Characterization Techniques. Structure and Morphology. The crystalline structure of the samples was probed by means of X-ray powder diffraction (XRPD) with a Bruker D8 Advance diffractometer (Bruker, USA) using Cu K α radiation. The samples were prepared by depositing a few drops of the toluene sol of purified QDs or UCNPs on a glass slide and letting the solvent slowly evaporate in air. The morphology of the two structures was analyzed by means of transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) using a JEOL JEM 3010 microscope (1.7 Å point-to-point resolution at Scherzer defocus) (JEOL, Japan). Before the imaging, each sample was further diluted in hexane to an approximate concentration of 0.1 mg/mL and sonicated for 2 min. One drop of the solution was deposited on a carbon-coated nickel grid, and the solvent was allowed to slowly evaporate in air.

Optical Properties. Absorption spectra were acquired on a Varian Cary 5000 spectrophotometer (Agilent, USA), diluting the QD sol in toluene to an optical density below 1 throughout the whole measurement range. PL and UCL spectra were collected at room temperature using a lens at an angle of 90° from the excitation beam and recorded with an Avaspec-ULS2048L spectrometer (Avantes, The Netherlands). QD and UCNPs emission were obtained under LED excitation at 450 nm (Thorlabs, USA) and laser diode excitation at 980 nm (BTW, China), respectively. In order to remove any stray light from the excitation sources, a short-pass 785 nm filter and long-pass 500 nm filter (Semrock, Inc., USA) were used in the two cases. The optical density of the QD sol was kept below 0.1 at the excitation wavelength. The UCL spectra were recorded using sols of UCNPs at a concentration of 0.1 mg/mL. The relative PLQY of QDs was calculated using an aqueous solution of Ru(bpy)₃Cl₂ as a standard (emission peak: 613 nm, PLQY = 0.040 ± 0.002⁴⁴). For the optical measurements of dried samples, a homemade confocal fluorescence microscope was employed. A single-mode fiber-coupled laser diode (980 nm) was used as the excitation source and focused on the sample with a microscope objective lens. Power densities on target varied between 1.4 × 10⁴ and 7.7 × 10⁴ W/cm². The sample emission was collected through the same microscope objective. Emission and excitation light were discriminated by means of a

dichroic mirror and a short-pass filter (FESH0950, Thorlabs, USA). The luminescence of the sample was collimated into the entrance of a high-resolution spectrometer (Horiba i320, Japan), and the signal was detected by a CCD detector (Synapse, Horiba, Japan). For LT measurements, samples were prepared casting a drop of sol of each UCNP batch on a glass slide and letting it dry in air. Concurrently, different amounts of CIS QDs were mixed with the UCNPs in toluene. A drop of the obtained sols was deposited on a glass slide. Each sample was excited by an optical parametric oscillator source (Spectra Physics, USA) at 960 nm (corresponding to the maximum absorption of Yb³⁺ ions in LiYF₄; see Figure S1). The UCL signal at 450 nm was filtered spectrally (FESH0750, Thorlabs, USA) and collimated into the entrance of a high-resolution spectrometer. The emission was amplified by a photomultiplier tube (R2949, Hamamatsu, Japan), and the UCL decay curves were recorded by means of a digital oscilloscope (LT372, LeCroy, USA).

Mathematical Modeling of UCNP-QD FRET. The distinctive feature of the FRET mechanism is its nonradiative nature, meaning that no emission of photons from the donor is involved, but rather the energy is transferred according to a dipole–dipole type interaction between the two species.⁴⁵ One major requirement is that donor and acceptor should be in close proximity. The general expression to determine the Förster radius (R_0 , i.e., the donor–acceptor separation R at which the FRET efficiency is 50%) is the following:

$$R_0^6 = \frac{9 \ln 10 PLQY \kappa^2 J}{128 \pi^5 n^4 N_A} \quad (1)$$

where N_A is the Avogadro constant ($6.2 \times 10^{23} \text{ mol}^{-1}$), κ^2 is the dipole orientation factor, $PLQY$ is the photoluminescence quantum yield of the donor, n is the refractive index of the medium, and J is the spectral overlap integral:

$$J = \frac{\int f_D(\lambda) \epsilon_A(\lambda) \lambda^4 d\lambda}{\int f_D(\lambda) d\lambda} \quad (2)$$

where f_D is the spectral profile of the donor emission and ϵ_A is the molar extinction coefficient of the acceptor. Both acceptor and donor species in this study have been carefully designed in order to maximize the value of the latter parameter, specifically choosing UCNPs whose emission largely overlaps with QD absorption. While J was obtained experimentally, two critical parameters in eq 1 are the $PLQY$ value and the dipole orientation factor κ^2 . The effect of both parameters was therefore simulated (see next sections).

The efficiency of FRET scales with R^{-6} according to the following expression:

$$\eta = \frac{R_0^6}{R_0^6 + R^6} = 1 - \frac{\tau'_D}{\tau_D} \quad (3)$$

As can be noticed, FRET efficiency can be experimentally determined from the donor's LT values in the presence or absence of an energy acceptor (respectively τ'_D and τ_D). Using this approach, the average donor–acceptor distance was indirectly determined. Although we have until now referred to UCNPs when speaking about donors, it is important to mention here that the actual donor species are the optically active Tm³⁺ ions (activators). For this reason, when taking into account the donor–acceptor distance R , we will not refer to the UCNP–QD interparticle distance. More correctly, we will

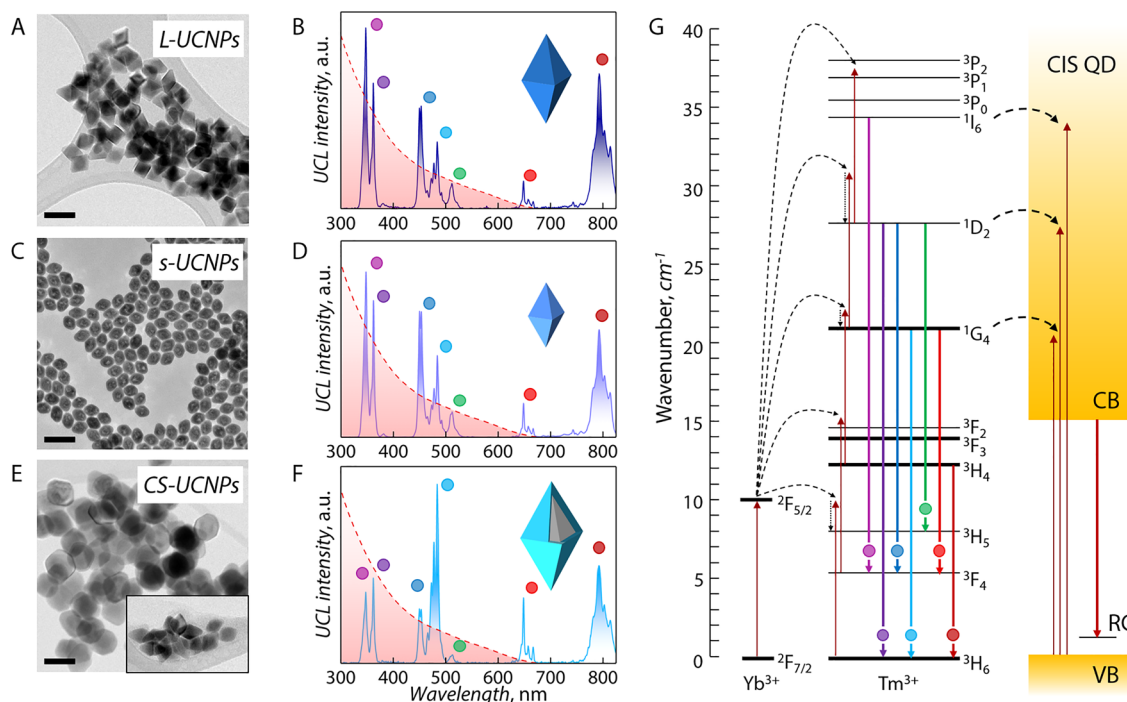


Figure 1. TEM micrographs show the three different UCNP architectures for *L*-UCNPs (A, core only), *s*-UCNPs (C, small core only), and *CS*-UCNPs (E, core/shell). Scale bars correspond to 100 nm. The different architecture is mirrored by the different UCL spectra of the three UCNP batches (B, D, and F, respectively; sketch of each UCNP architecture is illustrated as an inset) since smaller and core/shell UCNPs show less intense higher photon-order transitions, centered at shorter wavelengths. The absorption of CIS QDs (shaded red area in each UCL spectrum) largely overlaps with the UCL of the donors, setting the ground for an efficient FRET process. UCL spectra were recorded exciting at 980 nm, power density = $327 \text{ W}\cdot\text{cm}^{-2}$. The electronic level diagram of the Yb^{3+} – Tm^{3+} system is proposed (G), where the radiative transitions responsible for each emission band are depicted as solid colored arrows and correspondingly marked with a circle. The QD's valence and conduction bands (VB and CB, respectively) are shown along with the energy transfer processes (dashed arrows) that are expected to take place from the UCNP to the QD (RC is a recombination center). Legend: solid arrow, electron transition; dashed arrow, energy transfer; dotted arrow, nonradiative relaxation.

consider the distance between the QD surface and Tm^{3+} ions that are doped in the crystalline lattice of LiYF_4 , which represent only a small fraction of the overall cations composing the crystal.

RESULTS AND DISCUSSION

Material Characterization. CIS QDs were synthesized intentionally introducing a substoichiometric amount of Cu^+ in the reaction environment. Although it is not clear yet how the presence of copper vacancies plays a role in the emission mechanism, Cu^+ -deficient QDs display a higher PLQY with respect to the stoichiometric counterparts.⁴⁶ Synthesized QDs have a PLQY of 11% as obtained from the optical characterization (Figure S2A), which is in line with state-of-the-art CIS QDs.⁴⁷ Their crystalline structure is the typical tetragonal chalcopyrite, as confirmed by the XRPD pattern (PDF #00-047-1372, Figure S2B). The morphology of the sample cannot be clearly distinguished from TEM observations, due to the poor contrast that this material gives under the electronic beam (Figure S2C). However, crystalline fringes are clearly observed from the micrographs, confirming the good crystallinity of the sample. From these images the diameter of the QDs is estimated to fall between 3 and 4 nm.

L-UCNPs, *s*-UCNPs, and *CS*-UCNPs have a tetragonal crystalline lattice (PDF #01-078-2179), as confirmed from their XRPD patterns (Figure S3A, D, G). They have a bipyramidal habitus, which is characteristic of LiYF_4 (Figure 1A, C, E),^{43,48} and are well crystallized, as can be inferred from the presence of extended crystalline fringes in HRTEM micro-

graphs and strong diffraction spots in their electron diffraction patterns (Figure S3B, C, E, F, H, I and insets). The core/shell structure of *CS*-UCNPs is confirmed by the observation that this sample is composed of a single population of particles larger than parent core *C*-UCNPs (Figure 1E and inset, respectively). In order to geometrically describe the UCNPs, they were modeled as perfect square-based bipyramids, and their characteristic lengths (a and l) were obtained from TEM micrographs (Figure S4). Statistical analysis shows that the growth of the shell takes place preferentially along the a axis rather than longitudinally along l . For ease of modeling, also the shape of sample *CS*-UCNPs has been considered as bipyramid with sharp vertexes.

The dopant ions and the doping amount of UCNPs have been carefully chosen in order to maximize the overlap of their emission with the QDs' absorption, so as to promote FRET. For this reason, LiYF_4 and $\text{Yb}^{3+}/\text{Tm}^{3+}$ were selected respectively as the host material and the sensitizer/activator couple. All three batches of UCNPs display the characteristic emission lines of Tm^{3+} in LiYF_4 , but the relative intensities of the bands vary considerably. In particular, the higher n^{th} photon order emissions (fifth: $^1\text{I}_6 \rightarrow ^3\text{F}_4$; fourth: $^1\text{D}_2 \rightarrow ^3\text{H}_6$, $^1\text{D}_2 \rightarrow ^3\text{F}_4$) are markedly less intense in *CS*-UCNPs compared with the second ($^3\text{H}_4 \rightarrow ^3\text{H}_6$) and third photon order transitions ($^1\text{G}_4 \rightarrow ^3\text{H}_6$, $^1\text{G}_4 \rightarrow ^3\text{F}_4$). This behavior stems from the prevalently superficial distribution of Tm^{3+} ions throughout the particle volume. In *L*-UCNPs and *s*-UCNPs these optically active centers are homogeneously distributed over the entire crystal lattice, while in *CS*-UCNPs a larger percentage occupies sites close to

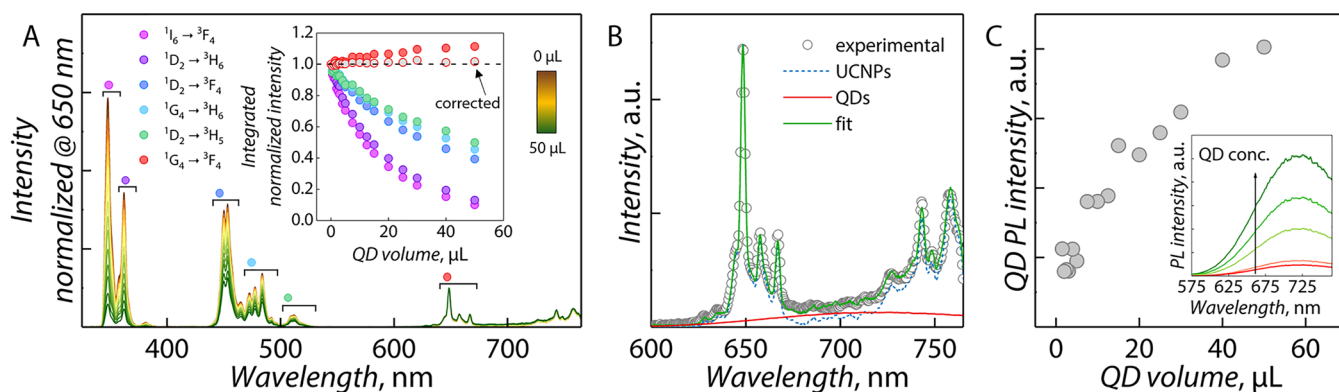


Figure 2. Upon titrating the *L*-UCNPs sol with the CIS QD sol (A), the UCL signal decreases proportionally to the acceptor's concentration, as can be appreciated from the trend of the integrated normalized UCL intensity versus the QD sol volume added (inset in A). Nonetheless, the $\text{Tm}^{3+}: {}^1\text{G}_4 \rightarrow {}^3\text{F}_4$ transition intensity increases, due to the appearance of the QD emission in that region. Deconvolution of the luminescence signal of the sols containing both UCNPs and QDs (B, highest QD sol volume tested) allowed obtaining the QDs' contribution to the spectrum of each mixed sol (C and inset). This information was used to correct the UCL $\text{Tm}^{3+}: {}^1\text{G}_4 \rightarrow {}^3\text{F}_4$ transition intensity from the mixed sols (circles indicated by the arrow in the inset of A). As could be anticipated from the minimal overlap of the $\text{Tm}^{3+}: {}^1\text{G}_4 \rightarrow {}^3\text{F}_4$ line with the QDs' absorption, the UCL intensity in this region remained constant upon addition of an increasing acceptor amount. UCL spectra were recorded under excitation at 980 nm, power density = $327 \text{ W}\cdot\text{cm}^{-2}$.

the surface. This makes electron trapping in surface defect states more likely and fosters the interaction with solvent molecules, whose phonons promote nonradiative electron de-excitation from higher energy levels.^{49,50} Nevertheless, the larger fraction of superficial Tm^{3+} ions granted by the core/shell architecture represents a major benefit in the frame of FRET: there is a higher chance that an active center is close enough to an acceptor in contact with the UCNP's surface so that the nonradiative ET can take place. The same advantage is expected for small core-only UCNPs, since the higher surface-to-volume ratio of *s*-UCNPs with respect to *L*-UCNPs directly translates to an overall higher percentage of activators occupying a site in the proximity of the UCNP surface.

Study of Transfer in Sol. Initially, we investigated the possibility of observing the ET process in a homogeneous sol of UCNPs and QDs. The NPs composing the sol are subjected to the random phenomenon of Brownian motion.⁵¹ The purpose of this study was to understand if and to what extent the ET mechanism takes place when the contact between the moieties is not favored (e.g., via surface modification) but is rather governed by stochastic events. Built on the observations made by Hong et al.⁴² for the $\text{NaYF}_4:\text{Yb}^{3+},\text{Er}^{3+}/\text{NaYF}_4\text{-CuInS}_2/\text{ZnS}$ system, this investigation aimed to elucidate the mechanism of the observed ET. To do so, a 0.1 nM sol of *L*-UCNPs was prepared in toluene, and the UCL signal was recorded exciting at 980 nm (power density = $327 \text{ W}\cdot\text{cm}^{-1}$), increasingly adding CIS QD sol in toluene or pure solvent (Figures 2A and S5, respectively). While the titration with the pure solvent did not lead to variations of the integrated UCL signal intensity, increasing the QD amount in the sol led to a steep decrease of the UCL intensity of the high-order transitions (insets of Figures 2A and S5, respectively). This behavior could be explained in light of the spectral overlap between the UCNP emission and the QD absorption (Figure 1B). Even more interestingly, the integrated intensity of the $\text{Tm}^{3+}: {}^1\text{G}_4 \rightarrow {}^3\text{F}_4$ line experienced an increase of approximately 15%, which followed from the appearance of the QD emission in the same wavelength range (Figure S2A). It is worth mentioning here that a similar behavior was observed for the *CS*-UCNPs sample (Figure S6). Although the UCL quenching below 600 nm was comparable to that observed in the case of *L*-UCNPs, the

above-mentioned integrated intensity increase was barely appreciable (less than 4%) likely due to lower PLQY featured by *CS*-UCNPs. To confirm the postulated origin of the observed UCL intensity increase, a signal deconvolution was conducted⁵² (refer to the SI for a thorough mathematical discussion). Specifically, the emission spectrum of each mixed sol (an example is shown in Figure 2B) was fitted, thus retrieving the QD emission in each case (Figure 2C and inset). The QD PL intensity integrated in the wavelength range of the $\text{Tm}^{3+}: {}^1\text{G}_4 \rightarrow {}^3\text{F}_4$ transition was used to correct the UCL signal (inset of Figure 2A). Upon subtracting the QD contribution, the intensity of the mentioned transition remains unchanged irrespective of the volume of acceptor sol added. The ET mechanism was interpreted combining the quantitative information about the QD emission and the estimation of the NP nearest neighbor distribution, as obtained from the sols' concentrations (Figure S7; a detailed mathematical discussion is reported in the SI). For each point, the integrated QD PL intensity was plotted against the first moment of the distribution (Figure 3). It was already mentioned that the typical FRET efficiency, and thus the acceptor's emission intensity, follows a R^{-6} dependence.

Instead, the efficiency of a radiative ET phenomenon decreases with the square of the distance and depends on the decay of the electromagnetic radiation in a medium. The mathematical model used to simulate these behaviors relies on the equation having the form

$$I = \frac{B}{(R - C)^n} \quad (5)$$

where R is the interparticle distance, n is an exponent that can take a value of 2 (radiative ET) or 6 (FRET), and C is a geometrical factor that accounts for the steric hindrance, thus the impossibility of the NPs to spatially overlap (the choice of this value is discussed in the SI, Figure S8). B is a scaling parameter that was left free to vary during the fitting procedure. Equation 5 was used to fit the experimental data, observing that the radiative ET model better described the behavior of the system under study. This result is a strong indication of the nature of the ET process, although it does not completely

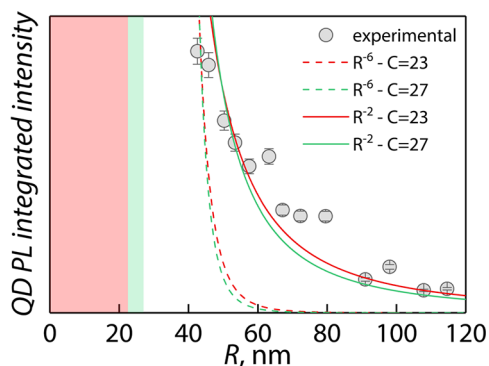


Figure 3. QD emission intensity was plotted versus the mean interparticle distance (R) that is ultimately the average donor–acceptor distance. The experimental points were fit using R^{-6} (dashed lines) and R^{-2} (solid lines) dependencies, respectively, typical for nonradiative and radiative ET processes. The better match obtained with the latter model confirms the radiative nature of the ET phenomenon from UCNPs to QDs in a sol. Shaded areas in the graphs represent the zones that are physically inaccessible for the acceptors when $C = 23$ (red) and $C = 27$ (green).

exclude the possibility that a small fraction of the transferred energy could indeed be passed to the acceptors nonradiatively. This may occur in particular at the higher concentrations tested, where the NPs are statistically closer. Final evidence for the occurrence of FRET would come from the measurements of the donor's LT. However, such experiments failed to show any LT decrease, thus substantially ruling out the presence of FRET (Figure S9). It is to be mentioned that at high QD concentration (when FRET is expected to possibly become a competing transfer mechanism), the UCL signal was heavily quenched (up to 50–60%; see Figure 2B). This led to a poor signal-to-noise ratio, making the expected small—if any—FRET-induced LT changes not meaningful.

Study of Transfer in Dry Powders. In order to establish the effect of different UCNPs architectures on the mechanism of ET to QDs, the behavior of different combinations of the two species as dry powders was studied. To increase the likelihood of FRET events, the closest proximity between donors and acceptors was fostered by increasing the surface-to-volume ratio and preferentially segregating the active centers in the outer volume of the donors. Hence, three sets of samples were obtained, one for each type of UCNPs mixed with different CIS QD amounts. In all three cases, the lifetime of the $\text{Tm}^{3+}: ^1\text{D}_2 \rightarrow ^3\text{F}_4$ transition experienced an appreciable shortening that allowed us to postulate a FRET-like mechanism taking place

in this configuration (Figure 4A, B, C). At first inspection of the decay curves, *CS-UCNPs* and *s-UCNPs* both feature a slower decay rate than *L-UCNPs*. This difference between samples likely stems from a difference in the samples' geometry, but is not relevant for the interpretation of the FRET mechanism (a thorough discussion on the subject is presented in the SI). The mean LT for each combination of UCNPs–QD was evaluated from the integration of the normalized decay curves (Table 1). The curves were recorded for samples featuring a QDs-to-UCNPs ratio approximately up to 600. Experimental data suggest that it is possible to increase the efficiency of FRET by acting both on the size of the UCNPs and more directly on the distribution of the active ions throughout the donor's volume (Figure 4D). Specifically, in the presence of the highest amount of QDs, *s-UCNPs* and *CS-UCNPs* respectively experience a lifetime decrease of 32% in both cases, roughly double the highest value (14%) observed for *L-UCNPs*. These values correspond to the FRET efficiency of each system according to eq 3. In the case of *CS-UCNPs*, the LT decrease was steeper compared to the more gradual LT shortening in *L-* and *s-UCNPs*, and it could be observed starting from a lower acceptor concentration.

Indeed, the preferential segregation of Tm^{3+} ions at more superficial crystallographic sites in the core/shell architecture grants QDs an easier accessibility to this fraction of ions. Thus, the emission from these donors is masked less by the unquenched signal stemming from nonsuperficial Tm^{3+} ions, which do not directly participate in the transfer mechanism. Therefore, in *CS-UCNPs* the optical information regarding LT values of Tm^{3+} ions involved in FRET to QDs is more readily accessible. In addition to the time-resolved measurements described above, spectral confirmation of FRET was also obtained. The UCL spectra of UCNPs alone and mixed with the highest amount of QDs investigated (Figures S10) showed that *s-UCNPs* and *CS-UCNPs* experience a dramatic UCL quenching in the short-wavelength side, which is accompanied by the appearance of the broad PL signal from QDs in the red spectral range.

A more complete picture was obtained considering how the above-mentioned parameters are related to the geometry of the system and, specifically, the average distance between donor and acceptor (R). To do so, we explored how the Förster radius (R_0) varied depending on the value taken by each parameter of eq 1. Here, we report the case of *L-UCNPs*, but the same procedure was applied to all types of UCNPs (results are reported in Figure S11). The intensity of the $^1\text{D}_2 \rightarrow ^3\text{F}_4$ transition was normalized and the overlap integral (J) was

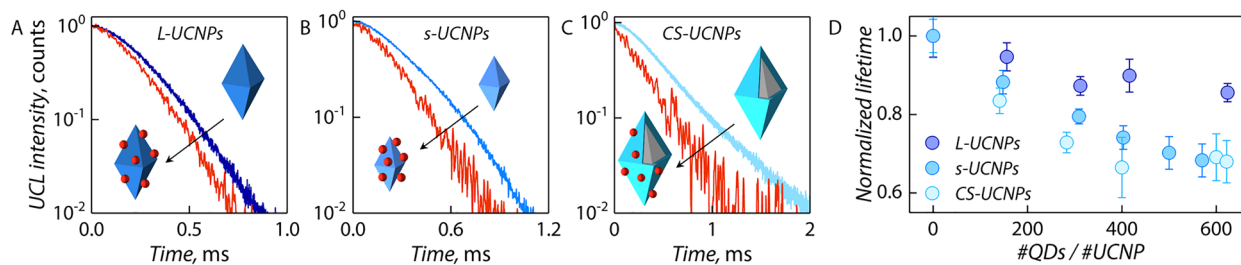


Figure 4. Mean $\text{Tm}^{3+} ^1\text{D}_2 \rightarrow ^3\text{F}_4$ transition LT in all UCNPs experienced a shortening in the presence of CIS QDs (A, *L-UCNPs*; B, *s-UCNPs*; C, *CS-UCNPs*). The UCNPs LT monitored in the presence of increasing amounts of QDs and normalized to that recorded in the absence of acceptors showed a gradual shortening (D). The trend observed is not the same for the three batches, *L-UCNPs* being the UCNPs that showed the lesser degree of quenching considering comparable $\#QD/\#UCNP$ values. This confirms that FRET efficiency can be increased with a proper UCNPs design. Measurements were performed exciting at 960 nm.

Table 1. LT Values in the Absence (τ) and in the Presence of the Higher Amount of QDs Investigated (τ') Used to Evaluate the FRET Efficiency (η); Förster Radius (R_0) and Average Donor–Acceptor Distance (R) Evaluated Considering Two κ^2 Values;^a Percentage of Tm³⁺ Ions Present within the First 10 and 25 Å in the Most Superficial UCNP Volume as a Fraction of Donors That Can Effectively Give Rise to FRET to Nearby QDs^b

	τ , μs	τ' , μs	η , %	$\kappa^2 = 0.67$		$\kappa^2 = 4$		Tm ³⁺ (10–25 Å), %
				R_0 , Å	R , Å	R_0 , Å	R , Å	
L-UCNPs	276 ± 15	237 ± 6	14 ± 2	13.7	18.5	18.5	25.0	(13 ± 4)–(29 ± 9)
s-UCNPs	325 ± 18	222 ± 14	32 ± 5	14.2	15.7	19.2	21.1	(18 ± 3)–(40 ± 7)
CS-UCNPs	477 ± 20	324 ± 26	32 ± 5	13.8	16.1	18.6	21.8	

^a0.67 is the usually accepted value used for randomly oriented dipoles, while 4 was chosen to compare the results with those obtained in the reference work of Bednarkiewicz et al.³⁹ ^bThe errors associated with these values were derived via standard error propagation calculations using the uncertainties on the crystals' size as reported in Figure S4. This range of values is not reported for CS-UCNPs due to limitations on the calculation imposed by the particular morphology of the sample.

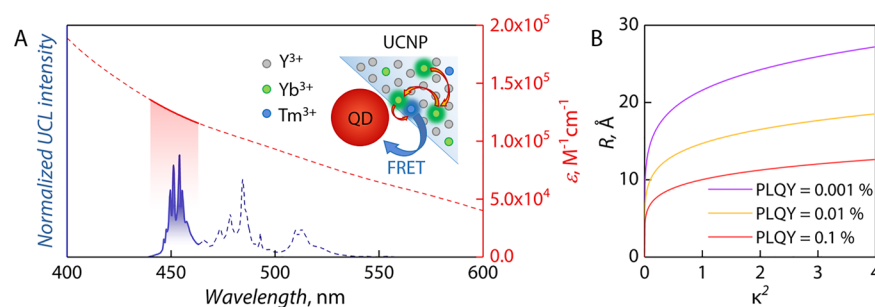


Figure 5. Normalized UCL of UCNPs and the molar extinction coefficient of QDs (A) were used to calculate the overlap integral (J); this value in turn was used to simulate the dependence of the Förster radius on the donor PLQY (B). The plots were obtained considering a refractive index $n = 1.454$, a value between 1.459 (OA and DDT) and 1.449 (LiYF₄). The inset in A depicts the suggested FRET mechanism from UCNP to QD prompted by NIR excitation of the donor (red/orange curved arrows represent energy migration events between Yb³⁺ ions and eventually to the emitting Tm³⁺ center).

evaluated considering the molar extinction coefficient of QDs (Figure 5A). The effective refractive index, n , in the space separating the donor and the acceptor was considered to be 1.454, a value between that of OA and DDT molecules covering the UCNPs' surface (1.459 for both compounds) and that of LiYF₄ (1.449). However, the effect exerted over the Förster radius (R_0) by the refractive index in this range is negligible (Figure S12). The variation of R_0 was eventually plotted as a function of PLQY (Figure 5B; κ^2 has little influence on R_0 in this system, as shown in Figure S11D). A realistic range of PLQY value for the UCNPs was considered to be 0.001–0.1%,⁵³ while the dipole orientation parameter was allowed to take values from 0.67 to 4. The above-reported results are in line with what has been shown by Bednarkiewicz et al.,³⁹ although the R_0 values they obtained are slightly larger. This difference is tentatively ascribed to the better overlap between the selected UCL band and the QD's absorption realized here, thanks to the use of strongly blue-emitting Tm³⁺ instead of green-emitting Er³⁺. As anticipated, the small R_0 values calculated for all UCNPs suggest that FRET takes place to the QDs from Tm³⁺ ions occupying the very first few nanometers of the crystal surface. Considering core only UCNPs (*L*-UCNPs) the FRET efficiency is similar to that observed in the NaYF₄:Yb³⁺,Er³⁺-CdSe QDs pair (14.8%). It has to be noted that Tm³⁺ is doped in the crystal lattice at 4-fold dilution with respect to Er³⁺.^{39,43} However, remarkably we could more than double the FRET efficiency increasing the surface-to-volume ratio (*s*-UCNPs) and/or the active centers' distribution over the UCNP volume (*CS*-UCNPs).

It was argued that the dominant FRET mechanism is the transfer from one donor to one acceptor (QD).³⁹ On the basis

of this assumption, it was possible to estimate the fraction of Tm³⁺ ions in our system that can take part in the FRET mechanism. LiYF₄ has a tetragonal lattice where the ratio between the long and short edges of the unit cell (c/a) is close to 2 and each unit cell contains four formula units (i.e., 4 Li, 4 Y, 16 F). So one could consider the unit cell as composed of two cubic subcells of edge a , each containing two formula units. Taking a cell edge $a = 5.2$ Å (according to PDF #01-078-2179) it is possible to estimate the number of subcells in a single UCNP using the dimensions determined from TEM micrographs (values in Figure S4). Considering the case of *L*-UCNPs, the volume of a single UCNP is 7.39×10^7 Å³, which gives 5.25×10^5 subcells per UCNP, corresponding to 5253 Tm³⁺ ions. Therefore, in the 10 to 25 Å of *L*-UCNPs' outermost volume 13–29% of the donors can be found. This is the fraction of Tm³⁺ ions that is expected to predominantly contribute to FRET. This means that the majority of Tm³⁺ ions (from 87% to 71%) will not experience a LT shortening in the proximity of QDs and their emission will display the usual decay rate.

In smaller UCNPs (*s*-UCNPs) the fraction of active centers capable of effectively transferring the energy to nearby QDs is increased, compared to nonsuperficial Tm³⁺ ions. Following the same reasoning, a percentage of superficial Tm³⁺ ions ranging from 18% to 40% was estimated. These numbers are in good agreement with the efficiencies calculated from the UCL decay measurements. In the case of the core/shell (*CS*-UCNPs) architecture, similar calculations could not be satisfactorily carried out due to the rather irregular morphology that prevented obtaining meaningful results. Reported results were obtained considering a PLQY of 0.01% for all the UCNPs. This is one of the most critical parameters in the considered system.

Here, it is reasonable to assume that *L*-UCNPs feature somewhat higher emission efficiency with respect to *s*-UCNPs. This would translate to a smaller Förster radius in the latter UCNPs, thus a smaller fraction of superficial Tm^{3+} involved in the transfer mechanism (inset in Figure 5A shows the proposed FRET mechanism).

All considered, the results presented above unequivocally demonstrate that a rational design of the UCNP (donor) architecture allows obtaining a remarkable improvement of FRET performance in pairs composed of UCNPs and QDs. Specifically, the UCNP size reduction and the localization of the activators preferentially in the outer UCNP volume have a comparable effect.

CONCLUSION

In this work, we studied the FRET phenomenon between $\text{LiYF}_4\text{:Yb}^{3+},\text{Tm}^{3+}$ UCNPs and CuInS_2 QDs. In particular, we investigated the effect of donor architecture on the process by either reducing the surface-to-volume ratio or segregating the dopant ions to the outer shell in a core/shell architecture. Initial studies in solution showed that the ET process was mostly radiative in nature. However, in the dry form, LT measurements demonstrated that FRET efficiency could be effectively increased, acting both on the size of the UCNPs and more directly on the distribution of the active ions throughout the donor's volume. Both small and core/shell architectures featured a FRET efficiency (both 32%) more than doubled with respect to the reference UCNP sample (14%). Geometrical modeling of the donors showed a good match between the experimental results and the estimated fraction of superficial Tm^{3+} ions able to transfer their energy to nearby acceptors (QDs). These results highlight that FRET from UCNPs to QDs is indeed obtainable with a good efficiency when the energy donors are properly designed. These observations pave the way for the development of optical bioassays that rely on the use of robust all-inorganic FRET pairs featuring NIR operational capabilities combined with a convenient long-lived emission.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsp Photonics.8b00112.

Description of squared residual minimization procedure; mathematical model for average interparticle distance in sol; interpretation of UCNPs' decay rate difference; absorption spectrum of UCNPs; characterization of UCNPs and CIS QDs; steady-state UCL of UCNPs mixed with CIS QDs; effect of PLQY, κ^2 , and refractive index on FRET efficiency (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

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