Förster Resonance Energy Transfer Distance Dependence from Upconverting Nanoparticles to Quantum Dots


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ABSTRACT: Förster resonant energy transfer (FRET) with upconverting nanoparticles (UCNPs) as donors and quantum dots (QDs) as acceptors has been regarded as a promising tool for biosensing applications. In this work, we use time-resolved luminescence spectroscopy to analyze the UCNP-to-QD FRET and we focus on the most relevant parameter of the FRET phenomenon, UCNP-QD distance. This distance is controlled by a nanometric silica shell around the UCNP surface. We theoretically reproduce the experimental results applying FRET theory to the distribution of emitting erbium ions in the UCNP. This simple model allows us to estimate the contribution of every erbium ion to the final FRET response and to explore different strategies to improve FRET efficiency.

INTRODUCTION

Förster resonant energy transfer (FRET) is a physical process whereby the energy of a chromophore in the excited state (donor) can be transferred to a neighbor molecule that is in the ground state (acceptor), over a distance larger than the collisional radii. This phenomenon occurs when the donor and the acceptor are in close proximity, allowing the energy to be transferred without generation of any photons, through long-range dipole–dipole interactions. The rate of the energy transfer depends on many different parameters, such as the extent of the spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor, the quantum yield of the donor, the relative orientation of the donor regarding the acceptor transition dipoles, and the distance between the donor and the acceptor. The FRET’s distance dependency has been crucial to detect two species in close proximity, enabling molecular rulers that can measure the distance between two specific molecules. Such a feature in combination with the wide range of techniques for molecules and biomolecule functionalization with organic dyes has been applied to study the interaction between DNA, antigens, and proteins in vivo at the nanometer scale.

During the last decades, advances in the synthesis of upconverting nanoparticles (UCNPs) with outstanding luminescence properties and higher photochemical stability than that of classical organic dyes have paved the way for analytical platforms using UCNP-based FRET systems. Lanthanide-doped UCNPs are a special type of materials that are able to absorb low energy photons, typically in the near-infrared (NIR) wavelength, and emit UV-vis photons. Samples can be illuminated with high power densities of NIR light, with a high light penetration depth and without the risk of degradation or photobleaching, which is of especial interest for in vivo analyses. Furthermore, the large anti-Stokes shift and the narrow absorption and emission bands of UCNPs simplify the instrumentation for detecting and analyzing FRET processes. However, the quantum efficiency in upconversion processes is rather low, and therefore, it is necessary to use an acceptor with high quantum yield and good spectral overlapping. Such requirements are fulfilled by quantum dots (QDs), which exhibit superb quantum yield and tunable absorption bands that can be adjusted to overlap with the emission band of the donor. Thus, the use of lanthanide-doped nanoparticles as donors and QDs as acceptors can be considered as ideal Förster pairs for bioassays.

Recently, Mattsson et al. have demonstrated a mix-and-measure UCNP-to-QD FRET system for rapid homogeneous bioassays for detecting analytes in aqueous solutions at nanomolar concentrations. A fundamental study of FRET between NaYF$_4$:Er$^{3+}$/Yb$^{3+}$ nanoparticles and CdSe QDs was reported by Bednarkiewicz et
al. They observed an Er\textsuperscript{3+} upconversion luminescence lifetime decrease from 153 to 130 μs because of the presence of QDs. These authors suggested two strategies to increase FRET efficiency: (1) diminishing the size of the UCNPs and (2) distributing the lanthanide active ions only in the outer shell of a core/shell UCNP. In this context, Murh et al. have recently analyzed the efficiency of FRET from UCNPs to organic dyes for UCNPs of different sizes.\textsuperscript{24} As the UCNP size decreases, two competing phenomena take place. On the one hand, an increasing percentage of the UCNP Er\textsuperscript{3+} ions becomes involved in FRET, increasing its efficiency. However, on the other hand, the surface-to-volume ratio increase leads to more luminescence quenching at the UCNP surface, which reduces FRET strength. They indeed found a maximum FRET efficiency for a 21 nm-sized UCNP. In addition, Bhuckory et al. have analyzed the efficiency of FRET between Er\textsuperscript{3+} ions distributed over either the core or the shell of UCNPs and Cy3.5 dye acceptors.\textsuperscript{25} The authors use the FRET efficiency results to estimate parameters such as donor—acceptor (DA) distances and Er\textsuperscript{3+} donor quantum yields. Very recently, Marin et al.\textsuperscript{26} have experimentally demonstrated enhanced FRET from LiYF\textsubscript{4}:Yb\textsuperscript{3+},Tm\textsuperscript{3+} UCNPs to CuInS\textsubscript{2} QDs by following two strategies: reducing the size of the UCNPs and doping the lanthanide active ions in the outer shell of a core/shell particle.

Despite these interesting works in UCNP-based FRET systems, to the best of our knowledge, no exhaustive studies of the FRET efficiency dependence on distance have been carried out. These systems are rather complex because the potential donors are distributed inside the UCNPs, which sizes are comparable to the length scale of FRET. This leads to a distribution of DA distances, a crucial key to the energy transfer phenomena. In this work, we present an in-depth study of the distance dependence of FRET in UCNP—QD systems with the aim of developing more efficient UCNP—QD FRET biosensors. To control the UCNP—QD distance, we cover the UCNPs with a nanometric silica shell. By varying the shell thickness, we analyze the change of the upconversion luminescence lifetime and, therefore, the FRET efficiency. Upconversion process involves many transitions and competing mechanisms, which makes it very difficult to isolate the real contribution of FRET to the energy transfer process. Here, we assume that energy transfer is most probably due to FRET. Then, the experimental results are interpreted by using FRET theory and taking into account the multiple distances between individual donor ions inside the UCNP and the FRET acceptors on its surface.

**EXPERIMENTAL SECTION**

**Chemicals.** ErCl\textsubscript{3}·6H\textsubscript{2}O 99.9%, YbCl\textsubscript{3}·6H\textsubscript{2}O 99.998%, YCl\textsubscript{3}·6H\textsubscript{2}O 99.99%, oleic acid (OA) technical grade 90%, NH\textsubscript{4}F 98%, NaOH 98%, methanol 99.9%, anhydrous N,N-dimethyl formamide 99.8%, 1-octadecene (1-ODE) technical grade 90%, n-hexane 97%, tetraethyl orthosilicate (TEOS) 98%, succinic anhydride 99%, NH\textsubscript{4}OH ACS reagent 28° C and kept at this temperature for 60 min. After this time, the reaction was quickly cooled down to room temperature. The UCNPs were separated by centrifugation: we split the product in four different centrifuge tubes and filled them with a solution 1/1 of H\textsubscript{2}O and ethanol. Then, we centrifuged the tubes at 8000 rpm for 10 min. The supernatant was discarded and the nanoparticles were resuspended in 0.5 mL of n-hexane and then reprecipitated with ethanol and centrifuged again at 8000 rpm for 10 min. Finally the UCNPs were resuspended in 10 mL of n-hexane with a concentration of 12 g/L and stored for further experiments.

**Preparation of NaY\textsubscript{0.78}F\textsubscript{4}:Yb\textsubscript{0.2},Er\textsubscript{0.02}@SiO\textsubscript{2}—NH\textsubscript{2}.** The UCNPs were covered with different thicknesses of SiO\textsubscript{2}, following the reverse microemulsion method.\textsuperscript{26,29} IGEPAL CO-520 (240 μL) were mixed with 4 mL of n-hexane, 1 mL of UCNPs dispersed in the previous solution (12 g/L), and 40 μL of NH\textsubscript{4}OH. The resulting solution was submitted to sonication until a transparent emulsion was formed. Different volumes of TEOS ranging from 10 to 30 μL were added depending on the desired SiO\textsubscript{2} shell thickness. The reaction was stopped after 18 h with the addition of methanol to disrupt the microemulsion, and then, it was centrifuged at 9000 rpm for 10 min, and the supernatant was discarded. This process was repeated three times to remove the excess of IGEPAL CO-520. Finally, the SiO\textsubscript{2} surface was functionalized with the amino groups by the addition of APTES (15 μL, 0.068 mmol) to the synthesized UCNPs@SiO\textsubscript{2} dispersed in 5 mL of ethanol. The heterogeneous solution was stirred overnight at room temperature, and the next day, the UCNPs@SiO\textsubscript{2}—NH\textsubscript{2} were recovered by centrifugation at 9000 rpm for 10 min. The supernatant was discarded and the nanoparticles were redispersed in ethanol. This process was repeated three times.

**Morphological Characterization.** Transmission electron microscopy (TEM) images were acquired using a JEOL JEM 1010 microscope operated at 80 kV (JEOL, Japan; 80 kV) equipped with a digital camera Gatan MegaView II. High-resolution TEM (HRTEM) and low-angle annular dark field scanning TEM images were acquired using a JEOL JEM 3000F operated at 300 kV and a Gatan ADF detector.

**Optical Characterization.** Absorbance spectra of QDs water solutions at different concentrations were measured using a UV—vis spectrometer (Ocean Optics, Red Tide 650) with 3 mm path length cuvettes.

Upconversion luminescence spectra were recorded with a fluorescence home-built system. The excitation beam comes from a pigtailed 10 W CW laser (JDSU, L4-9897603) working at 976 nm and provided with a current and temperature controller (ILX Lightwave, LDX-36025-12 and LDT-5525B, respectively). The laser beam is transmitted through a long-pass dichroic filter.
The excited level lifetime, and half-maximum) is around 150 μs. In our measurements, we use laser excitation powers around 2 kW/cm². This allows us to ensure that the laser is directly connected (without using a pre-amplifier).

Upon excitation pulses of 40 μs, the luminescence emission at 540 nm is detected by the PMT, which operates below the excitation saturation intensity of the NaYF₄:Yb,Er@SiO₂ nanoparticles. This code simulates the discriminator and the multichannel counter. Upon analysis of more than 5000 trigger signals, we obtain a luminescence decay curve. Decay curve measurements were repeated at least three times for each sample under the same experimental conditions.

**RESULTS AND DISCUSSION**

To study the distance dependence of energy transfer between UCNPs and QDs, we covered the surface of the as-synthesized donors (UCNPs) and the acceptors (QDs) used in the FRET system under consideration. The upconversion photoluminescence coming from the sample is reflected by the dichroic mirror toward the short-pass filter which blocks the IR reflected radiation. Then, it is focused into an optical fiber connected to a monochromator (HORIBA Jobin Yvon, iHR320). The monochromator is equipped with a photomultiplier tube (PMT) (HAMAMATSU, R928) and uses a 1200 g/mm grating blazed at 900 nm. A similar ratio has been used by Marin et al.²⁶ Negatively charged QDs were electrostatically adsorbed on the surface of positively charged UCNPs, as shown in the TEM and HRTEM images of Figure 1E,F, respectively. Single drops of this mixture were placed on a filter paper and allowed to dry at room temperature in air. The filter paper containing the mixture was set between two microscope glass slides for optical characterization.

As a reference, we prepared a filter paper with a drop of the ethanol solution containing UCNPs@SiO₂−NH₂ and CdTe−COOH QDs. First, the UCNPs@SiO₂−NH₂ were dispersed in ethanol at 5 g/L (1 × 10⁻⁷ M), while the CdTe−COOH QDs were dispersed in distilled water at 2.5 g/L (5 × 10⁻⁶ M). Then, we mixed equal volumes of the ethanol solution with the UCNPs@SiO₂−NH₂ and the aqueous solution with the CdTe−COOH QDs, so that an excess of QDs was used (QDs−UCNPs ratio of 478). A similar ratio has been used by Marin et al.²⁶ Negatively charged QDs were electrostatically adsorbed on the surface of positively charged UCNPs, as shown in the TEM and HRTEM images of Figure 1E,F, respectively. Single drops of this mixture were placed on a filter paper and allowed to dry at room temperature in air. The filter paper containing the mixture was set between two microscope glass slides for optical characterization.

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**RESULTS AND DISCUSSION**

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luminescence emission spectrum of NaYF₄:Yb,Er@SiO₂ nanoparticles under a 976 nm CW excitation laser is shown in Figure 2A (blue curve, right axis). Two green emission peaks near 525 and 540 nm are observed. These peaks correspond to 2H₁₁/₂ and ₄S₃/₂ transitions of the Er³⁺ ions, respectively (see in Figure 2B, the energy transfer upconversion (ETU) mechanism populating these two levels). A red emission, with similar intensity, near 655 nm is also observed because of ⁴I₁₅/₂ → ⁴I₁₃/₂ transition (see Figure 2B). Figure 2A also shows the absorbance of a CdTe QDs water solution with 0.5 g/L in a 3 mm path length cuvette (red curve, left axis). Notice that the size of the QDs can be inferred from the position of their absorption peak. In our case, we found an absorption peak around 529 nm, that gives us an estimation of the QDs size of 3 nm, in good agreement with that obtained from HRTEM images. Therefore, because the green emission bands of the UCNPs (donors) perfectly overlap with the absorption peak of the QDs (acceptors), the possibility of an efficient nonradiative energy transfer from UCNPs to QDs is ensured provided that both are close enough. Additionally, the QDs fluorescence emission spectrum under excitation by a LED radiation at 455 nm is shown in Figure 2A (green curve, right axis). The QDs orange emission band whose peak is around 572 nm does not overlap with the emission bands of the Er³⁺ ions avoiding mixed fluorescent emissions.

Now, let us test the interaction between UCNPs and QDs. Figure 2A shows the luminescence emission spectrum from the dry sample containing UCNPs with QDs electrostatically adsorbed on their surface (black curve, right axis). There, a new emission peak around 600 nm and a reduction of the green emission bands of the UCNPs are observed. Both features reveal that QDs are being excited by the ⁴S₃/₂ → ⁴I₁₁/₂ transitions of the Er³⁺ ions. Note that QDs cannot be excited with the IR radiation that excites the UCNPs. The emission peak arising from the QDs fluorescence appears as red-shifted respect to that obtained for the QDs solution excited under UV light. This is due to the inner filter effect that becomes relevant when increasing the QDs concentration during the drying process (see Figure S1). A similar shift was previously reported in UCNP–QD dried samples.

Because the reduction of the UCNPs green luminescence intensity is due to both nonresonant energy transfer and re-absorption, to characterize in detail the Er³⁺ to QD FRET mechanism, luminescence lifetime studies are needed. Thus, luminescence decay signals for Er³⁺ ions at 540 nm (⁴S₃/₂ → ⁴I₁₃/₂) were measured. As an example, Figure 3 shows the luminescence intensity decay of UCNPs with a 3 nm silica shell, in the presence (black) and absence (green) of QDs. Fluorescence signals show an initial increase that is a signature of the upconversion process energy transfer from Yb³⁺ to Er³⁺ ions within the UCNPs, followed by an exponential decay. The luminescence decay time was obtained by fitting the decay curve to a single exponential function. For the fitting, we considered a time window from tini to tfinal where the final fitting time was set to tfinal = 1.2 ms. For each experimental decay curve, we calculated around 25 fits by changing the initial fitting time tini within the range where the luminescence intensity varies from 70 to 30% of its maximum value. This fitting procedure gives us an average lifetime with its standard error. For the particular curve shown in Figure 3, the presence of the QDs reduces the luminescence lifetime from 104.7 ± 0.3 to 93.7 ± 0.2 μs. This decrease confirms the occurrence of nonradiative energy transfer from UCNPs to QDs. We have also corroborated that this energy transfer process produces a slow component (<100 μs) in the decay of the QD fluorescence at 590 nm, which otherwise would typically be in the nanosecond range (see the example in Figure S2 upper panel).

Once the FRET mechanism has been verified, its characterization is in order. FRET efficiency (E) can be computed from the experimental luminescence decay curves (as the ones shown in Figure 3) as

$$ E = 1 - \frac{r_D}{r_{DA}} $$

(1)

where r_D and r_{DA} are the donor excited-state lifetime in the absence and in the presence of acceptor, respectively. For the particular case shown in Figure 3, an efficiency of E = (10.5 ± 0.4)% was achieved with a SiO₂ shell of 3 nm, which is quite high considering the relatively large diameter of the UCNPs (33 nm). Note that while luminescence emission comes from the Er³⁺ ions
distributed within the entire nanoparticle, ions far from the particle surface cannot participate in FRET.²²

To analyze the behavior of FRET with the distance between the UCNPs and the QDs, we measured the luminescence decay times of UCNPs with different SiO₂ shell thicknesses, with and without QDs. The FRET efficiency as a function of shell thickness is plotted in Figure 4. The general trend is a fast decrease of the FRET efficiency for DA distances up to 12 nm, above which the energy transfer is negligible. A maximum FRET efficiency of around 10% is obtained, in agreement with the values reported in previous works.²³ We obtained similar results on UCNPs with a diameter of 33 nm. Laser intensity: 1.7 kW/cm²

![Figure 4](image)

**Figure 4.** FRET efficiency, $E_J$ (eq 1) for different silica shell thicknesses on UCNPs with a diameter of 33 nm. Laser intensity: 1.7 kW/cm² (triangles), 2.4 kW/cm² (circles). Theoretical prediction of FRET efficiency (eq 7) for a Förster distance of $R_0 = 5.5$ nm and a number of QDs of 24 (solid line). Dashed lines correspond to the theoretical prediction under a variation of ±6 QDs.

Theoretical FRET Efficiency. After analyzing the experimental data, let us now have a look at the theoretical counterpart for further interpretation of our results. The rate of energy transfer from a single donor to a single acceptor separated by a distance $R$ is given by²

$$k_{ET} = \frac{9(ln 10) \eta_D k^2}{128 \pi^3 N A^4 \eta_A^2 R^6 J},$$

$$J = \int_{\Delta \lambda} F_D(\lambda) \epsilon_A(\lambda) \lambda^4 d\lambda,$$

where $N_A$ is the Avogadro’s number, $n$ is the refraction index of the medium surrounding the FRET pair, $r_0$ is the donor excited-state lifetime, and $\eta_D$ is the intrinsic quantum yield of the donor in the absence of acceptor, that is, the quantum yield of the Er³⁺ ion excited state.²⁵ Note that $\eta_D = r_D/\tau_D^{rad}$, being $r_D^{rad}$ the radiative donor lifetime. Furthermore, $k^2$ is a factor describing the relative orientation of the donor and the acceptor dipole moments, and the integral $J$ measures the overlap between the donor emission spectrum and the acceptor absorption spectrum within the selected spectral donor emission region $\Delta \lambda$. Last, $F_D(\lambda)$ is the luminescence intensity of the donor normalized to the total intensity (i.e., $\int_{\Delta \lambda} F_D(\lambda) d\lambda = 1$), and $\epsilon_A(\lambda)$ is the molar extinction coefficient of the acceptor. In addition, the rate of energy transfer can be rewritten as

$$k_{ET} = \frac{1}{\tau_D} \left( \frac{R_0}{R} \right)^6$$

where $R_0$ is the Förster distance defined as the distance at which half of the donor ions decay by transferring their energy to the acceptors, that is, the distance at which the energy transfer rate is equal to the donor decay rate in the absence of acceptor. By comparing eqs 2 and 3, one can obtain a closed expression for $R_0$ as follows

$$R_0 = 0.0211 (k^2 \eta_D n^4 J)^{1/6} [\text{nm}]$$

To estimate the Förster distance $R_0$, we calculate the overlap integral $J$ from the experimental measurements of $\epsilon_A(\lambda)$ and $F_D(\lambda)$. The molar extinction coefficient of the QDs is obtained through the Beer–Lambert’s law by measuring the absorbance at different concentrations and taking into account the cuvette path length ($L = 3$ mm). The result is shown in Figure 5. From the experimental data shown in Figure 5, the overlap integral results to be $J = 9.6 \times 10^{15}$ M⁻¹ cm⁻¹ nm⁴. Note that this value can be roughly estimated by considering a nearly constant value of $\epsilon_A$ in the green region of the spectrum around $\lambda \approx 540$ nm, which gives $J \approx \epsilon_A \lambda^4 \approx 10^{16}$ M⁻¹ cm⁻¹ nm⁴.

Other important parameters necessary to evaluate eq 4 are $k^2$, $n$, and $\eta_D$. The orientation factor of DA dipoles $k^2$, is usually assumed to be 2/3, which corresponds to the dynamic random averaging. The material through which FRET mainly takes place is silica whose index of refraction is $n = 1.46$. Finally, the intrinsic quantum yield $\eta_D$, which measures the probability that donor de-excites radiatively in the absence of acceptor, has been estimated as around 20–30% by Bhuckory et al. from FRET measure-
ments of UCNPs with different core/shell architectures. Note that this value is much larger than the one corresponding to the overall UCNP quantum yield. We estimate the intrinsic quantum yield by considering that the radiative lifetime for the excited-donor level (S_{E2}) is in the millisecond range, \( \tau_D^{rad} = 0.5 \) ms and by using our experimental luminescence decay rate \( \tau_D \approx 105 \) ms. This leads to \( \eta_D = \tau_D / \tau_D^{rad} \approx 0.21 \). For the sake of simplicity, we assume the quantum yield to be the same for all the UCNPs@SiO_2. This is reasonable because the donor lifetime obtained without QDs was quite similar throughout the range of silica shell thicknesses used in our experiments. Finally, by evaluating eq 4 with these parameters, our theoretical prediction for the Förster distance is \( R_0 \approx 5.5 \) nm.

With this estimation in mind, to understand the observed dependency of the FRET efficiency on distance, we must consider the distribution of multiple DA pairs. First, let us express the transfer efficiency (i.e., the probability of de-excitation via energy transfer) of a single DA pair as

\[
E_{Er-QD} = \frac{k_{ET}^{DA}}{1/\tau_D + k_{ET}^{DA}} = \frac{1}{1 + (R/R_0)^6}
\]

From eq 5, we see how an Er^{3+} ion close to the UCNP external surface is able to transfer its energy to the QD for small silica shell thicknesses \( R < R_0 \), whereas an ion close to the UCNP center will not be able to transfer its energy to the QD even in the absence of silica shell \( R = R_0 \) (see Figure 5).

We consider that the energy transfer rate from each Er^{3+} ion inside the UCNP to all the QDs adsorbed onto its surface results from the sum of the single pair transfer rates:

\[
k_{ET}^{DA} = \sum_{A=1}^{N_QD} k_{ET}^{DA}(A),
\]

where \( N_QD \) is the total number of QD adsorbers coupled onto the UCNP surface. Therefore, the FRET efficiency for each single Er^{3+} ion is

\[
E_{Er-QD} = \frac{\sum_{A=1}^{N_QD} k_{ET}^{DA}(A)}{1/\tau_D + \sum_{A=1}^{N_QD} k_{ET}^{DA}(A)}
\]

Here, \( k_{ET}^{DA}(A) \) results from the evaluation of eq 3 at the particular DA distance \( R \) for every case. Although we take \( N_QD \) as a fitting parameter in our simulations, we can roughly estimate this value from the TEM images as \( N_QD \approx 20 \). FRET efficiency is then calculated by averaging the efficiency of each Er^{3+} ion inside the nanoparticle

\[
E = \langle E_{Er-QD} \rangle_{N_{Er}}
\]

Here, the number of Er^{3+} ions inside the nanoparticle is \( N_{Er} = f_{Er} m_{N_{Er}} N_A / W = 4405 \), where \( f_{Er} = 0.019 \) is the fraction of Er^{3+} ions, \( W = 205.3 \) g/mol is the molar weight of NaYF_4:Yb/Er, and \( m_{N_{Er}} = 7.9 \times 10^{-17} \) g is the mass of the UCNP.

In our calculations, we generate a uniform random distribution of the Er^{3+} ions inside the UCNP, and we compute the average FRET efficiency using eq 7 with the Förster distance calculated above, that is \( R_0 = 5.5 \) nm, and a number of QDs around the particle of 24. Our simulations result presented in Figure 4 (solid line) shows a very good agreement with the experimental data. We have also included in Figure 4 (dashed lines) theoretical curves with a different number of QDs, to account for the observed variation of the number of acceptors per UCNP in TEM images.

We conclude that the averaging process carried out when considering multiple DA configuration is needed to explain the FRET efficiency found in the experiments. For further analysis of this phenomenon, Figure 6 shows the distribution of FRET efficiency for each Er^{3+} ion, \( E_{Er-QD} \), as a function of its radial distance to the particle center (blue points). Significant energy transfer, larger than 10%, occurs only for those ions placed farther than 11 nm from the UCNP center. This means that only the ions in close proximity to the UCNP external surface (\( \leq 5.5 \) nm) are susceptible to efficiently transfer their energy to the QDs. However, we observe a wide distribution on FRET efficiencies even for these superficial ions because the QDs are randomly distributed on the silica shell without completely covering the UCNP surface (see the inset in Figure 6).

**Optimal Design of UCNPs for FRET Performance.** As we mentioned in the Introduction, two different strategies have been used to increase the FRET efficiency in UCNP systems: reducing the UCNP size and developing inert-core/active-shell UCNP architectures. Marin et al. have experimentally reported a two-fold FRET efficiency increase by applying these strategies to square-based bipyramidal shape UCNPs and QDs.

Let us use our theoretical approach to estimate the FRET efficiency that could be achieved under these two enhancement strategies for our particular UCNP–QD system. We consider UCNPs as the ones used in the experiments but with a tunable size inert-core, that is, particles with 33 nm of diameter, a silica shell of 3 nm, and we consider 24 QDs adsorbed onto the particle surface (see the particle scheme in Figure 7A). The quantum yield of particles with different inert-core diameters is expected to change. However, determining this magnitude is a challenging experimental problem, and as a first approximation, we consider here that all particles have roughly the same quantum yield, that is, the same Förster distance \( R_0 = 5.5 \) nm. Figure 7A shows the FRET efficiency and the number of Er^{3+} ions per nanoparticle as a function of the inert-core radius. We observe that to obtain a notable increase of FRET efficiency, an inert-core radius larger than half of the particle radius is required (see blue symbols in Figure 7A, left axis).

Note that the reduction in the total number of Er^{3+} ions in a particle with an inert core is not significant until the inert core takes half of the particle radius (see black symbols in Figure 7A, right axis). We obtain a two-fold FRET efficiency increase when the inert radius is almost as large as the particle radius, that is, when the active shell is around 2 nm thick.

Finally, we now analyze the effect of the UCNP size on the FRET response. We consider UCNPs with diameter from 32 to...
yield of the Er\(^{3+}\) ions in the excited level \(^{4}S_{3/2}\) of 0.21. This is a total number of Er\(^{3+}\) ions inside each UCNP, \(N_{\text{Fo}}\). From the acceptor absorption spectrum, we calculated a value for the overlap between the donor emission spectrum and the acceptors. In our system, several small CdTe QDs were adsorbed on each larger UCNP@SiO\(_2\) surface by electrostatic forces, positively charged NaYF\(_4\):Yb,Er@SiO\(_2\) (B2017/BMD-3867 RENIM-CM) co-funded by Comunidad de Madrid and Santander Bank (PR26/16-12B), Ministerio de Economía y Competitividad-MINECO (MAT2016-75955S, MAT2017-83111R), The European Upconversion Network COST Action CM1403 (2014-2018), and Comunidad de Madrid (B2017/BMD-3867 RENIM-CM) co-funded by European Structural and Investment Fund. D.M.-G. thanks

20 nm, all particles with a 3 nm silica shell. The number of QDs adsorbed onto the UCNP surface and the donor quantum yield are properties that depend on the UCNP size; however, for the sake of simplicity, we consider the values previously used, that is, \(N_{\text{QD}} = 24\) and \(R_0 = 5.5\) nm. Figure 7B shows the FRET efficiency as a function of the UCNP size in a log–log plot. The linear fit shown in this figure (dashed line) indicates that the FRET efficiency follows a power law behavior with the UCNP diameter \(d_{\text{UCNP}}\) with an exponent close to minus 2, that is, \(E \propto d_{\text{UCNP}}^{-2}\). Under these assumptions, the change of FRET efficiency is proportional to the nanoparticle area. For example, by reducing the UCNP diameter from 33 to 23 nm, therefore reducing the nanoparticle volume by 1/3, we calculate a FRET efficiency of around 20\%, which is similar to our previously calculated efficiency for a 33 nm particle consisting of a 2 nm active-shell and a 31 nm inert-core and doubles the value measured in our experiments for the 33 nm diameter UCNPs. Similar increases in efficiency with reductions in the particle diameter have been experimentally reported in a previous work.\(^{26}\)

**CONCLUSIONS**

We have studied the FRET phenomenon from UCNPs to QDs, which is a system with several advantages. The CdTe QDs exhibit a strong absorption band that perfectly overlaps with the luminescence emission band of the UCNPs. Furthermore, cross-excitation is avoided because the IR UCNP donor excitation does not produce fluorescence in the QD acceptors. We experimentally verified FRET between 33 nm diameter positively charged \(\text{NaYF}_4:Yb,Er@SiO}_2–\text{NH}_2\) particle donors and 3 nm diameter negatively charged CdTe–COOH QDs acceptors. In our system, several small CdTe QDs were adsorbed on each larger UCNP@SiO\(_2\) surface by electrostatic interaction. This multiple acceptor configuration enhances the FRET response. The QD adsorption leads to a significant reduction of the upconversion luminescence lifetime, which clearly demonstrates that an additional decay pathway for the excited UCNPs takes place because of non-radiative energy transfer to the QDs. FRET is limited by the fact that the donor and acceptor must be in extreme close proximity. Considering the overlap between the donor emission spectrum and the acceptor absorption spectrum, we calculated a value for the Förster distance of \(R_0 \approx 5.5\) nm, assuming an intrinsic quantum yield of the Er\(^{3+}\) ions in the excited level \(^{8}S_{3/2}\) of 0.21. This is a large value compared to the Förster radius characteristic of organic fluorophores of a few angstroms to 2 nm, but still small compared to the UCNP radius. We experimentally analyzed the effect of the UCNP–QD distance in the FRET efficiency by varying the thickness of the UCNPs silica shell from 3 to 16 nm. A maximum FRET efficiency of around 10\% is achieved for the 3 nm silica shell, which is a remarkably large value if we take into account the distribution of all the potential donors, that is, Er\(^{3+}\) ions, inside the relatively large UCNP in comparison with the Förster distance. The UCNP-to-QD nonradiative interaction vanishes for distances above 12 nm.

We have theoretically explained the experimental results by calculating the FRET efficiency of each single Er\(^{3+}\)–QD pair and averaging the FRET response of every Er\(^{3+}\) ion inside the UCNP. Therefore, to theoretically obtain accurate values of the FRET efficiency in UCNP-based FRET systems, this averaging process is required. Indeed, we were able to establish that only Er\(^{3+}\) ions placed in close proximity to the UCNP surface, in a shell of around 5 nm (similar to the Förster distance), are able to participate in an energy transfer process with \(E > 10\%\).

In summary, our work supports that the main physical characteristics governing the UCNP-to-QD energy transfer process is the relative spatial configuration of donors and acceptors. Our results show that the as-proposed UCNP–QD FRET system is a good potential platform for biosensing short biomolecules whose length of interaction is below the estimated Förster distance (\(\leq 6\) nm). Nanoparticle systems with alternative architectures, as the ones discussed in this work, can be used to improve the distribution of DA distances for more efficient FRET-based biosensing applications.

**ASSOCIATED CONTENT**

Supporting Information

Fluorescence spectrum of CdTe QDs excited at 455 nm: water solution and dried sample; FRET analysis at the acceptor QDs emission channel (590 nm); experimental FRET efficiency calculation from lifetimes; and theoretical FRET efficiency of a single Er\(^{3+}\) ion to a single QD (PDF)

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**Notes**

The authors declare no competing financial interest.

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