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Synthesis of NaYF₄:Yb/Er/Gd up-conversion luminescent nanoparticles and luminescence resonance energy transfer-based protein detection

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Abstract

High-quality NaYF₄:Yb/Er/Gd up-conversion nanoparticles (UCNPs) were first synthesized by a solvothermal method using rare earth stearate, sodium fluoride, ethanol, water, and oleic acid as precursors. Doped Gd³⁺ ions can promote the transition of NaYF₄ from cubic to hexagonal phase, shorten the reaction time, and reduce the reaction temperature without reducing the luminescence intensity of NaYF₄:Yb/Er UCNPs. X-ray diffraction, infrared spectroscopy, transmission electron microscopy, and luminescence spectroscopy were applied to characterize the UCNPs. The nanoparticles exhibited small size and excellent green up-conversion photoluminescence, making them suitable for biological applications. After the surfaces of NaYF₄:Yb/Er/Gd UCNPs were modified with amino groups through the Stöber method, they could be brought close enough to the analytically important protein called R-phycoerythrin (R-PE) bearing multiple carboxyl groups so that energy transfer could occur. A luminescence resonance energy transfer (LRET) system was developed using NaYF₄:Yb/Er/Gd UCNPs as an energy donor and R-PE as an energy acceptor. As a result, a detection limit of R-PE of 0.5 μg/ml was achieved by the LRET system with a relative standard deviation of 2.0%. Although this approach was first used successfully to detect R-PE, it can also be extended to the detection of other biological molecules.

Keywords

NaYF₄:Yb/Er/Gd nanoparticles; Up-conversion luminescence; Stöber method; Luminescence resonance energy transfer; Phycoerythrin

Up-conversion (UC)¹ is an anti-Stokes process where a longer wavelength radiation, usually near-infrared (NIR) or infrared (IR), is converted to a shorter wavelength such as ultraviolet (UV) or visible (Vis) radiation via a two-photon or multiphoton mechanism [1,2]. In

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¹Abbreviations used: UC, up-conversion; NIR, near-infrared; UV-Vis, ultraviolet-visible; UCP, up-conversion phosphor; UCNP, up-conversion nanoparticle; RE, rare earth; PVP, polyvinylpyrrolidone; R-PE, R-phycoerythrin; FRET, Förster resonance energy transfer; LRET, luminescence resonance energy transfer; DL, detection limit; RSD, relative standard deviation; NaF, sodium fluoride; TEOS, tetraethyl orthosilicate; APTES, 3-aminopropyltrimethoxysilane; EDC, *N*-ethyl-*N'*-[3-(dimethylamino)propyl]carbodiimide hydrochloride; TEM, transmission electron microscope; XRD, X-ray diffraction; FT-IR, Fourier transform infrared; PBS, phosphate buffer solution; EDS, energy dispersive spectroscopy.

addition to applications in laser materials, NIR quantum counters, lighting and display technologies [3–9], there is a growing interest in the application of nano-sized up-conversion phosphors (UCPs) as fluorescent labels for sensitive biological detection [10–12]. When UCPs are used as biolabels, NIR radiation is used to excite UCPs. As a result, very weak autofluorescence can be detected from sample matrix; accordingly, very low background fluorescence from the biological samples can interfere with the specific signal [13]. Therefore, a high signal/noise ratio (S/N) will be obtained.

It has been well accepted that NaYF₄:Yb,Er up-conversion nanoparticles (UCNPs) have the best luminescence property among UC fluorescent materials [14–16]. Several methods for the preparation of NaYF₄:Yb,Er UCNPs have been reported, including the coprecipitation method, the thermal decomposition method, and the hydrothermal/solvothermal method [17–21]. The coprecipitation method was one of the easiest and most convenient approaches, but the UCNPs tended to aggregate into larger sizes. The thermal decomposition method was a good route for synthesizing high-quality rare earth (RE)-doped NaYF₄ UCNPs, but it requires harsh conditions. The hydrothermal/solvothermal method can be used to improve the water solubility and biocompatibility of the UCNPs. During recent years, this method has been used rather widely for preparing NaYF₄:Yb,Er UCNPs. The disadvantage of this method is the long reaction time required to achieve desired UCNPs' morphology; indeed, when the reaction time is 7 h or less, cubic NaYF₄ UCNPs are the main product rather than hexagonal NaYF₄ UCNPs.

UC luminescent nanocrystals can convert an NIR excitation into a visible emission through lanthanide doping [22]. Haase's group developed a method for preparing colloid lanthanide-doped NaYF₄ nanocrystals that were transparently soluble in nonpolar solvent [23]. Zhang and coworkers reported a method for the synthesis of water-soluble and biocompatible polyethylenimine-coated NaYF₄ nanoparticles doped with lanthanide ions [24] as well as biocompatible silica-coated NaYF₄ UC fluorescent nanocrystals [25]. Unfortunately, these nanoparticles cannot be used directly in biological applications because of the presence of some hydrophobic organic ligands as well as the absence of appropriate functional groups on the surface of the nanoparticles. A good example of coating the surface with silica was reported by Li and Zhang [26], who coated polyvinylpyrrolidone (PVP)-stabilized NaYF₄:Yb,Er/Tm UCNPs with a layer of silica via the typical Stöber method.

R-phycoerythrin (R-PE) [27] is a red protein that can serve as an indicator of the presence of cyanobacteria and as a probe for labeling antibodies. R-PE possesses several unique characteristics, such as nontoxicity, strong fluorescence, and small background light interference, making it an attractive candidate for fluorescence labeling in the analysis of biomolecules and cells. It also has potential applications in the polychromatic fluorescence immune detection of animal epidemic mixed infection, bird flu, and other animal epidemics. Therefore, it is important to develop a method for the detection of R-PE.

Förster resonance energy transfer (FRET) is a nonradiative process in which the electronic excitation energy of a donor chromophore is transferred to a nearby acceptor molecule via long-range dipole–dipole interactions [28] and has been widely used for immunoassay [29]. In this work, UCNPs doped with Gd³⁺ ions were synthesized by a solvothermal method and used for luminescence resonance energy transfer (LRET)-based detection of R-PE. The excellent NaYF₄:Yb/Er/Gd UCNPs were synthesized with shorter time and lower temperature through doping of Gd³⁺ ions, which could promote the transition of NaYF₄ from cubic to hexagonal. Then, the NaYF₄:Yb/Er/Gd UCNPs were modified by the typical Stöber method and used as an energy donor to develop an LRET system for detection of R-PE. The detection limit (DL, 3σ) of R-PE of 0.5 μg/ml was achieved with a relative standard

deviation (RSD) of 2.0 (21.9 $\mu\text{g/ml}$, $n = 11$), and this method is expected to be expanded to the detection of other proteins.

Materials and methods

Materials

All chemical reagents used in the experiment, specifically rare earth oxide (RE_2O_3), sodium fluoride (NaF), oleic acid ($\text{C}_{17}\text{H}_{33}\text{COOH}$), stearic acid ($\text{C}_{17}\text{H}_{35}\text{COOH}$), disodium hydrogen phosphate dodecahydrate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$), sodium dihydrogen phosphate dehydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$), tetraethyl orthosilicate (TEOS), and 3-aminopropyltrimethoxysilane (APTES), were of analytical grade and did not undergo any further purification (purchased from National Medicines Corporation, China). *N*-ethyl-*N'*-[3-(dimethyl-amino)propyl]carbodiimide hydrochloride (EDC) was obtained from Acros (USA). R-PE was obtained from H&R Bioscience (China). Triple-distilled water was used throughout the experiments.

Synthesis and modification of RE-doped NaYF_4 UCNPs

$\text{NaYF}_4\text{:Yb/Er/Gd}$ UCNPs were synthesized according to a previously reported procedure [21] in which rare earth stearate [$(\text{C}_{17}\text{H}_{35}\text{COOH})_3\text{RE}$] was used as the precursor and oleic acid was used as the stabilizing agent. At first, RE stearate (1 mol), NaF (0.2011 g), ethanol (15 ml), water (10 ml), and oleic acid (4 ml) were mixed together and stirred to form a homogeneous solution. Then, the mixed solution was treated at 150 °C for 7 h with a high-pressure nitrifying pot. After reaction, the product was separated by centrifugation at 8500 rpm and then dried at 60 °C for 10 h. A series of $\text{NaYF}_4\text{:Yb/Er/Gd}_x$ UCNPs were obtained by tuning the amount of $(\text{C}_{17}\text{H}_{35}\text{COOH})_3\text{Gd}$.

Surface modification of NaYF_4 UCNPs was completed using the typical Stöber-based method reported in our previous work [14]. Briefly, 20 mg of NaYF_4 UCNPs was dispersed in isopropyl alcohol (70 ml) and treated by ultrasonication at room temperature for 40 min. Then, 2.5 ml of 15% $\text{NH}_3 \cdot \text{H}_2\text{O}$ and 10 ml of triple-distilled water were added to the mixture, followed by the dropwise addition of TEOS (20 μl) and APTES (200 μl). After the reaction reached completion, the product was washed with ethanol, centrifuged, and finally dried at 60 °C.

Characterization

The size and morphology of as-prepared nanoparticles were observed on a JEM-2100HR transmission electron microscope (TEM, JEOL, Japan) using an accelerating voltage of 200 kV. X-ray diffraction (XRD) measurements were performed on a D/max 2500/PC diffractometer (JEOL) at a scanning rate of 8°/min with graphite monochromatized Cu K α radiation ($\lambda = 0.15406$ nm). UC luminescence spectra of the dried and powdered nanoparticles were measured on an LS-55 fluorescence spectrophotometer (PerkinElmer, USA) attached to an external 980-nm laser (Beijing Hi-Tech Opto-electronics, China) instead of the internal excitation source. The maximum power of the laser was 1200 mW. Fourier transform infrared (FT-IR) spectra of the silica-coated UCNPs were measured on a Spectrum One (B) spectrometer (PerkinElmer) by using the KBr method.

Pretreatment of R-PE

The R-PE solution obtained from H & R Bioscience was treated by centrifugation at 3000 rpm at 4 °C. After the supernatant was completely removed, the deposit was dissolved in phosphate buffer solution (PBS, pH 7.0). Then, the mixture was dialyzed with dialysis tubing for 10 h under constant stirring. Finally, it was separated by centrifugation, where the

deposit was removed and the supernatant was reserved for the UV–Vis absorption and fluorescence spectrometric detection.

Preparation of P-RE/NaYF₄:Yb,Er,Gd LRET systems

Here, 0.8 mg of amino-modified NaYF₄:Yb/Er/Gd UCNP was dispersed in 1 ml of PBS (pH 7.0) by ultrasonication for 30 min. Then, 1 ml of 0.8 mg/ml NaYF₄/NH₂ and 0.2 ml of 0.2 mg/ml EDC were added into a series of tubes containing different volumes (0.002–0.13 ml) of 0.875 mg/ml R-PE. The final solution volume was then adjusted to 2 ml with PBS. This series of solutions was incubated for 15 min with low shaking at room temperature. After that, the solutions were reactivated for 2 h at 4 °C. The visible light emission of the resultant system can be detected by 980 nm excitation.

Results and discussion

Influence of doped RE Gd³⁺ ions

Fig. 1A shows the UC luminescence intensity of the NaYF₄:Yb/Er/Gd UCNP with different reaction times and amounts of doped RE Gd³⁺. It can be seen that with increasing reaction time, the luminescence intensity of the NaYF₄:Yb/Er/Gd UCNP became much stronger for reaction time shorter than 10 h. When the reaction time was invariant, the luminescence intensity of the UCNP first increased and then decreased along with the increasing molar ratio of the doped RE Gd³⁺ ions, but all of them were better than the case without doping Gd³⁺ ions. By increasing reaction time and gradually decreasing the molar ratio of the doped RE Gd³⁺ from 7% to 3%, the luminescence intensity reached a maximum. Because the reaction was more complete as the reaction time increased, the luminescence intensity of the NaYF₄:Yb/Er/Gd UCNP became more sensitive to the doped RE Gd³⁺. Meanwhile, it can be seen in Fig. 1B that from NaYF₄:Yb/Er (20/2 mol%) to NaYF₄:Yb/Er/Gd (20/2/3 mol%), the luminescence intensity of the UCNP changes over different reaction times (4, 6, 7, 8, and 10 h). These results show that the maximum luminescence intensity change was obtained with the reaction time of 7 h.

From Fig. 1, it can also be seen that in a short reaction time (<10 h), the luminescence intensity of NaYF₄:Yb/Er/Gd_x ($x = 0–15$ mol%) UCNP is stronger than that of NaYF₄:Yb/Er UCNP under the same reaction conditions. The intensity of the NaYF₄:Yb/Er/Gd_x ($x = 3$ mol%) with the reaction time of 7 h is roughly the same as that of the NaYF₄:Yb/Er UCNP with the reaction time of 10 h. Namely, we can get the same intensity with reduced reaction time by doping Gd³⁺ ions.

The XRD patterns of the prepared nanoparticles with and without Gd³⁺ doping are shown in Fig. 2. All of the samples were prepared for 7 h at 150 °C. It can be seen that, without Gd³⁺ ions, the NaYF₄:Yb/Er (20/2 mol%) UCNP are mainly the cubic phase of NaYF₄ (JCPDS no. 77-2042), whereas those doped with 3 mol% of Gd³⁺ are mainly the hexagonal phase of NaYF₄ (JCPDS no. 28-1192). Namely, there is an evident transformation from cubic to hexagonal due to the doping. When the Gd³⁺ concentration is further increased to 15 mol%, the cubic phase of NaYF₄ does not change significantly because of the formation of cubic phase of NaGdF₄, whereas the luminescence intensity declines somewhat. It can be concluded that the cubic-to-hexagonal phase conversion is promoted only at the proper level of doped Gd³⁺. When the products were prepared without doped Gd³⁺, the structure of UCNP was mainly the cubic phase of NaYF₄, which is unable to emit UC luminescence and, thus, cannot be used for biological applications. After being doped with a small amount of the Gd³⁺ (3 mol%), the structure of UCNP is mainly the hexagonal NaYF₄, which can be used in biological application. With further increased Gd³⁺ ion concentration, the cubic-to-hexagonal phase transformation process is not further improved due to the formation of the NaGdF₄.

TEM was used to observe the morphology and size of the Yb/Er and Yb/Er/Gd-doped nanoparticles. As shown in Fig. 3, there is no obvious difference in the shape and size of nanoparticles between NaYF₄:Yb/Er and NaYF₄:Yb/Er/Gd_x ($x = 3$ mol%). The Gd³⁺-doped UCNPs have an average size of 30 nm and are spherical in shape. Those doped with Gd³⁺ concentrations up to 15 mol% have a primarily rod-like shape rather than spherical shape due to the formation of NaGdF₄. This result is in agreement with the XRD results (Fig. 2).

It is well known that NaYF₄:Yb/Er UCNPs synthesized by the solvothermal method, which needs longer reaction time, show well-established, efficient UC luminescence and are suited for biological application. When the reaction time is shorter than 7 h, the products are cubic NaYF₄ crystal, which cannot emit UC luminescence and cannot be used in biolabeling applications. After the addition of a suitable amount of RE Gd³⁺, the transformation from cubic to hexagonal structure of NaYF₄ in these samples occurred, leading to the production of UCNPs mainly in the hexagonal structure of NaYF₄. This result can be attributed to the small structural difference between the hexagonal phase NaYF₄ and NaGdF₄. It can be seen that the peak shifts toward lower diffraction angles because Y³⁺ ions were replaced by the larger Gd³⁺ ions in the host lattice from Fig. 2 [30]. By further increasing the concentration of Gd³⁺ ions, the crystal phase changed back to cubic and the nano-particles took a rod-like shape due to the formation of the cubic phase of NaGdF₄ instead of the cubic phase of NaYF₄. It can be seen that through doping with a suitable amount of Gd³⁺, the reaction temperature can be lower (150 °C) and the reaction time can be reduced to 7 h while keeping the luminescence intensity strong enough to be used in biological applications. As an additional benefit, the use of paramagnetic Gd³⁺ dopant ions may be able to provide the nanocrystals with a second functionality, namely a magnetic capability for magnetic resonance imaging probes [31].

Fluorescent spectra, XRD, and TEM results show that doping a suitable amount of Gd³⁺ ions can promote the phase transition of the NaYF₄ UCNPs from cubic to hexagonal, whereas the shape and size of the nanoparticles are almost unchanged. It is well known that hexagonal-phase (β -phase) NaYF₄ is a more efficient UC host material than the cubic phase under 980 nm excitation [14]. As a result, after being doped with Gd³⁺ ions, UC luminescent intensity of the NaYF₄ UCNPs was largely enhanced due to the formation of hexagonal phase.

Surface features of NaYF₄:Yb/Er/Gd nanoparticles before and after surface modification

Fig. 3D shows the TEM image of NaYF₄:Yb/Er/Gd (20/2/3 mol%) UCNPs prepared in 7 h at 150 °C. It shows that the UCNPs are spherical in shape and fairly uniform, but some UCNPs tend to be rod-like because of the doped Gd³⁺. It can be seen that the average size of the UCNPs is roughly 30 nm, which can meet the requirements of biological applications. Fig. 3E shows that surface modification of UCNPs yielded a successful coating of silica around NaYF₄:Yb/Er/Gd (20/2/3 mol%) UCNPs. After being modified by the Stöber method, the NaYF₄:Yb/Er/Gd UCNPs once capped by a layer of hydrophobic oleic molecules displayed hydrophilic functional groups (–NH₂) on their surfaces. The resultant amino-functionalized NaYF₄:Yb/Er/Gd UCNPs could be easily dispersed in water and used for biological detections.

The existence of NaYF₄ is indicated by the peaks for Na, Y, and F as well as other peaks from the energy dispersive spectroscopy (EDS) analysis results shown in Fig. S1a of the supplementary material. Unlike Fig. S1a, the peaks of C, O, and Si can be seen in Fig. S1b. Silicon and oxygen peaks can be attributed to the silica shell, and the carbon signal can be assigned to the methylene in the hydrolysate of APTES. It confirms the formation of the SiO₂ layer, which proves the successful amino modification through the typical Stöber method.

To further confirm the surface modification of NaYF₄:Yb/Er/Gd UCNP, FT-IR analysis was carried out to characterize the UCNP before and after modification (see Fig. S2 in supplementary material). Before surface modification, there was a layer of oleic acid that is confirmed by the two peaks at 2914 and 2849 cm⁻¹, which are attributed to the asymmetric and symmetric stretching vibrations of the methylene group in the oleic acid, respectively. The peak at 1469 cm⁻¹ is the characteristic peak of a COO⁻ bond. The oleic acid's characteristic peaks disappeared after surface modification. The peak at 1097 cm⁻¹ results from the symmetrical stretching vibration of the Si-O bond, which confirms the existence of a layer of silica. The amine group can be proved by peaks at 3432 and 1637 cm⁻¹ collectively. Besides, the two peaks at 2929 and 2857 cm⁻¹ are attributed to the substance of the APTES. The FT-IR and EDS analysis results show that the surface of the NaY-F₄:Yb/Er/Gd UCNP has been successfully amino functionalized.

Pretreatment of R-PE

The R-PE contained amino materials that would have affected the energy transfer between amino-modified NaYF₄:Yb/Er/Gd UCNP and R-PE. Therefore, R-PE should be pretreated to remove ammonium salt prior to use. Here, we used a dialysis bag to remove the ammonium salt from R-PE. It can be seen that the baseline of the UV-Vis absorption spectrum of R-PE exceeds zero by more than 0.2 cm⁻¹, whereas it tends to be zero after pretreatment (see Fig. S3 in supplementary material). This indicates that the ammonium salt was eliminated completely.

Conjugation of NaYF₄:Yb,Er,Gd UCNP and R-PE

There are two prerequisites for FRET; one is that the UV-Vis absorption spectrum of the acceptor overlaps well with the UC luminescent emission spectrum of the donor, and the other is that the donor and acceptor are in proximity to each other. Luminescence of RE ions caused by the f-f transition [32] does not belong to the fluorescence category. So, the energy transfer that occurred in our system has been referred to as LRET instead of FRET in the field of RE luminescent materials.

It is known that R-PE can emit strong fluorescence and has a good absorption performance. Moreover, R-PE contains multiple carboxyl groups, making it possible to conjugate R-PE to amino-modified NaYF₄:Yb/Er/Gd UCNP. It can be seen from spectrum a in Fig. 4 that the pretreated P-RE shows a strong absorption band around 550 nm that can be used as an acceptor in LRET-based assays. Spectrum b in Fig. 4 shows the luminescence spectrum of NaYF₄:Yb/Er/Gd UCNP, which have a strong emission bands at 543 nm. The green emission of the UCNP is suitable for use as an energy donor in the LRET-based assays. From Fig. 4, we can see that the UV-Vis absorption spectrum of R-PE overlaps with the luminescence spectrum of NaYF₄:Yb/Er/Gd UCNP very well, and, thus, fulfills the first requirement of the LRET.

It can be seen from Fig. 5 that the luminescence intensity of the NaYF₄:Yb/Er/Gd UCNP decreased obviously after R-PE was added and decreased still further after EDC was added to the system. From these data, it can be determined that the electrostatic attractions between nanoparticles and R-PE aid in shortening the distance between donor and acceptor. Furthermore, the addition of EDC to the system enables chemical conjugation between the donor and acceptor, further shortening the distance between them. As a result, the second requirement for energy transfer to occur is satisfied. The preconditions shown in both Figs. 4 and 5 indicate that the system can be used for energy transfer successfully.

After R-PE of different volumes, ranging from 0.002 to 0.13 ml (0.875–56.9 µg/ml), was added to the system, the green emission (543 nm) of UCNP was obviously quenched. In the

meantime, the luminescence of R-PE was enhanced somewhat, whereas the red emission (659 nm) of UCNP was almost unchanged, as shown in Fig. 6A, because the UV-Vis absorption spectrum of R-PE does not overlap with the luminescence spectrum of NaYF₄:Yb/Er/Gd UCNP at 659 nm. It can be seen from Fig. 6A that the luminescence intensity of the NaYF₄ UCNP at 543 nm gradually decreases and the luminescence intensity of R-PE at 575 nm increases little by little with the increase of the R-PE concentration. We can see that the extent to which the luminescence intensity decreases at 543 nm is much greater than the extent to which it increases at 575 nm.

Two linear relationships were developed in the system. One of them is between the Napierian logarithm of the UCNP's luminescence intensity at 543 nm ($\ln I_{UC}$) and the concentration of R-PE (C), whereas the other is between the Napierian logarithm of the R-PE's luminescence intensity ($\ln I$) and the Napierian logarithm of the R-PE's concentration ($\ln C$). In Fig. 6B, a good linear relationship can be established between $\ln I_{UC}$ and the concentration of R-PE in the range of 0.875 to 56.9 $\mu\text{g/ml}$ that follows the equation $\ln I_{UC} = 5.947 - 0.016C$, with a square of the related coefficient of 0.9992. In Fig. 6C, there is a good linear relationship between the Napierian logarithm of the R-PE's luminescence intensity and $\ln C$ in the R-PE concentration range of 0.875 to 56.9 $\mu\text{g/ml}$. This relationship can be fitted by the equation of $\ln I = 1.499 + 0.442 \ln C$, with a square of the related coefficient of 0.9991. The R-PE DL of 0.5 $\mu\text{g/ml}$ was obtained from the data in Fig. 6B, and the RSD of the detection is 2.0% (21.9 $\mu\text{g/ml}$, $n = 11$). These results demonstrate the successful energy transfer between amino-modified NaYF₄:Yb/Er/Gd UCNP and P-RE, which can be exploited for sensitive protein detection. When the concentration of R-PE exceeded 56.9 $\mu\text{g/ml}$, the luminescence signal no longer increased because the amount of the donor (UCNP) was depleted and, thus, could not interact with the increasing acceptor (P-RE) concentration.

Here, the energy transfer between NaYF₄:Yb/Er/Gd UCNP and R-PE occurred when the amino groups on the surface of the NaYF₄:Yb/Er/Gd UCNP and the carboxyl groups of the R-PE interacted to bring them close enough. Surface modification of NaYF₄ UCNP was completed by using a typical Stöber-based method, whereas there were multiple carboxyl groups of R-PE itself. The fact that amino-modified NaYF₄:Yb/Er/Gd UCNP and R-PE can be conjugated without adding other materials can explain the existence of electrostatic attraction between NaYF₄/NH₂ and R-PE. In addition, they will be well conjugated under the activation of EDC, and the transfer efficiency was better. After energy transfer, the luminescence of the NaYF₄:Yb/Er/Gd UCNP can be transferred to R-PE, so the luminescent spectrum of R-PE can be detected by NIR (980 nm) excitation. These results demonstrate that the LRET system consisting of amino-modified NaYF₄:Yb/Er/Gd UCNP and R-PE, which are brought closer to allow LRET to occur, can be used for sensitive protein detection.

This LRET system uses UCNP instead of quantum dots (QDs) or organic dyes as an energy donor that can convert NIR light to visible light. The LRET system can be detected under NIR irradiation, so the UCNP-based LRET system is an ideal approach for biological detection. The detection of R-PE with such a low DL of 0.5 $\mu\text{g/ml}$ has never been reported. The UCNP-based LRET system may also be used to detect other biomolecules such as peptides and target biomolecules [33,34].

Conclusion

After being doped with Gd³⁺ ions during preparation, phase transformation of NaYF₄ UCNP from cubic to hexagonal was greatly enhanced, so UCNP with the same excellent UC properties were prepared with a shorter reaction time and lower reaction temperature. Our approach is more energy efficient and improves the reaction efficiency greatly. The

synthesized NaYF₄:Yb/Er/Gd UCNP were amino modified and successfully used for the detection of R-PE through an LRET system, which was established with NaYF₄:Yb/Er/Gd UCNP as the donor and R-PE as the acceptor. Experimental results suggest that the LRET system is simple and effective for the detection of the technologically important protein R-PE. Our method can find potential applications in the detection of other biological molecules due to its higher sensitivity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:
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Fig. 1.

(A) Tendency chart of luminescence intensity of UCNPs at 543 nm (980 nm excitation, 0.42 watt) affected by both the reaction time and doped molar ratio Gd^{3+} . (B) Tendency chart of luminescence enhancement from $\text{NaYF}_4:\text{Yb/Er}$ (20/2 mol%) to $\text{NaYF}_4:\text{Yb/Er/Gd}$ (20/2/3 mol%) affected by reaction time.

Fig. 2.
X-ray powder diffraction patterns of the NaYF₄:Yb/Er/Gd_x ($x = 0, 3$, and 15 mol%) UCNPs.

Fig. 3. (A–C) TEMs of the NaYF₄:Yb/Er/Gd_x doped with different concentrations of Gd³⁺: (A) $x = 0$ mol%; (B) $x = 3$ mol%; (C) $x = 15$ mol%. (D,E) TEMs of UCNP before (D) and after (E) surface modification.

Fig. 4. UV-Vis absorption spectrum of R-PE (a) and emission spectrum of NaYF₄:Yb/Er/Gd UCNP (b).

Fig. 5. Emission spectra of UCNPs (a), UCNPs + R-PE (b), and UCNPs + R-PE + EDC (c).

Fig. 6.
 (A) Luminescence spectra of UCNPs at different volumes of R-PE. (B) Linear relationship between the Napierian logarithm of the UCNPs' fluorescent intensity at 543 nm and the concentration of R-PE. (C) Linear relationship between the Napierian logarithm of the R-PE's fluorescent intensity and the Napierian logarithm of the R-PE's concentration.