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Upconversion luminescence turning of $NaREF_4$ (RE=0.4Y+0.4La+0.2 (Yb, Er, Tm)) nanoparticles and their applications for detecting Rhodamine B in shrimp

HU Shigang (胡仕刚)[1], YU Yi (余 意)[1], WU Xiaofeng (吴笑峰)[1], HU Pan (胡 盼)[1], CAO Huiyi (曹会祎)[1], WU Qingyang (吴青杨)[1], TANG Zhijun (唐志军)[1], GUO Yuanjun (郭源君)[2], LIU Yunxin (刘云新)[2,*]

(1. School of Information and Electrical Engineering, Hunan University of Science and Technology, Xiangtan 411201, China; 2. Department of Physics and Electronic Science, Hunan University of Science and Technology, Xiangtan 411201, China)

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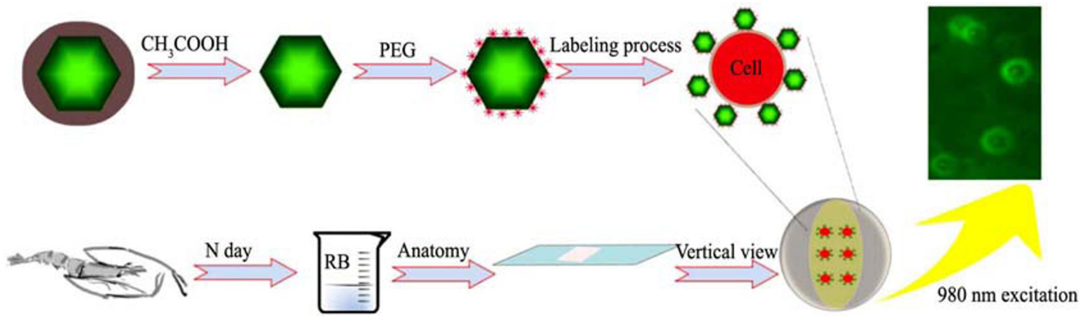
Abstract: Biocompatible $NaREF_4$ (RE=0.4Y+0.4La+0.2(Yb,Er,Tm)(molar ratio)) upconversion nanoparticles (UCNPs) with strong visible fluorescence were synthesized by a solvothermal method and subsequent surface modification. Modulated upconversion luminescence emission spectra were obtained via changing the doping. In vitro and *in vivo* bioimagings were carried out with shrimps. The upconversion nanoprobes with an acidic/PEG hybrid ligand could quickly capture the basic Rhodamine-B (RB) in shrimp cells and formed a close UCNPs@RB system. The residual organic dye RB in shrimps could be detected on the basis of luminescent resonance energy transfer (LRET). It could be rapidly addressed based on LRET detection that RB residue existed in the shrimps after incubating in the aqueous solution of RB higher than 3 μg/mL for 12 h.

Keywords: nanoparticles; upconversion luminescence; *in vivo* detecting; organic dyes; rare earths

In recent years, upconversion nanoparticles (UCNPs) have attracted increasing attention in the fields of bioimaging and cellular nanothermometry, which are expectable to be developed as a novel diagnostic tool, due to their unique advantages such as intense luminescent properties, low toxicity, high chemical stability, great penetration depth of infrared pump light into tissues, and high signal-to-noise ratios[1–6]. It is well known that organic dyes have become a main pollutant released into water and a predominant threat to scarce water sources[7–9]. Detection of organic dyes *in vivo* and their toxicity to biological tissues will become very important and valuable[10–12].

The luminescence resonance energy transfer (LRET) occurs through the nonradiative dipole-dipole interactions between an excited donor molecule (D) and a proximal acceptor molecule (A) when the donor emission and the acceptor absorption are spectrally overlapped. The LRET technique is confirmed to be an important spectroscopic tool in bioanalysis, especially in the detection of molecule binding events and protein[4,13,14]. Here, this LRET process based on upconversion fluorescent donors is developed for detecting organic dyes in opaque shrimps.

In the submitted work, uniform $NaREF_4$ (RE=0.4Y+0.4La+0.2(Yb,Er,Tm)) upconverson nanoparticles (UCNPs) have been successfully synthesized and can be used as a luminescent probe in detecting the residual organic dyes in shrimp based on LRET[15–20] (Scheme 1). The inorganic upconversion nanoparticles can play a role of fluorescent donor which is more stable than conventional organic fluorescent donor, such as fluorescent proteins, under the long-time irradiation of pump light. In addition, infrared light is used as pump light for these



Scheme 1 Schematic illustration of LRET-based detection of organic dyes *in vivo* and fluorescent imaging *in vivo* and *in vitro* using UCNPs as probes

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* **Corresponding author:** LIU Yunxin (**E-mail:** lyunxin@163.com; **Tel:** +86-731-58291433-800)

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upconversion nanoparticles which have much deeper penetration depth to bio-tissues than conventional ultraviolet pump light used for quantum dot and fluorescent dye donors[21–26].

In vitro and *in vivo* bioimaging were carried out with shrimps using $NaREF_4$ (RE=0.4Y+0.4La+0.2(Yb,Er,Tm)) upconversion nanoparticles(UCNPs) as probes. The residual organic dye RB in shrimp could be detected on the basis of luminescent resonance energy transfer (LRET).

1 Experimental

1.1 Materials

All rare earth oxides were of 99.99% purity. Rare earth chloride $RE(Cl)_3$ (RE=La, Y, Yb, Er, and Tm) solutions were prepared by dissolving the corresponding rare earth oxides in hydrochloric acid at a high temperature. All other chemicals were of analytical grade and used without further purification.

1.2 Synthesis of $NaREF_4$ (RE=0.4Y+0.4La+0.2(Yb, Er, Tm)) nanoparticles

Synthesis of $NaREF_4$ (RE=0.4Y+0.4La+0.2(Yb, Er, Tm)) nanoparticles was conducted according to a previously reported procedure as shown in Refs. [4] and [21].

1.3 Characterization

The shape, size, and uniformity of synthesized UCNPs were measured with a transmission electron microscope (H-7650c) and a high-resolution transmission electron microscope (JEM 3010). Upconversion luminescence spectra were recorded with a fluorescence spectrometer (Hitachi F-2700), which has a 980 nm laser as the excitation source. A multiple CCD camera (Sony) was used to take pictures of the upconversion luminescence.

1.4 Surface modification of OA-UCNPs with PEG

PEG-UCNPs were synthesized followed by a previous protocol. First, 1 mL of cyclohexane solution of upconversion nanoparticles was reacted with acetic acid and ethanol solutions (volume ratio=1:1) stocked for 30 min, and subsequently collected by centrifugation at 10000 r/min for 5 min. Then, the obtained nanoparticles were dispersed in 5 mL of aqueous solutions with 0.6 mL of PEG. After a vigorous stirring for 1 h at 20 ºC, the precipitates were separated by centrifugation, rinsed with ethanol three times to remove the excess PEG, and readily dissolved in water.

1.5 *In vivo* animal experiments

The shrimps were cultured in ordinary organic dyes with different concentrations for a certain time. Then, a mixture of PEG-UCNPs in 0.9% NaCl saline was added into the shrimps' gastro. After 30 min, the *in vivo* imaging was obtained under infrared excitation using a Sony multiple CCD camera. The laser power density was –0.2 W/cm^2 during imaging, which was a safe power.

1.6 *In vitro* cells experiments

After washing with phosphate buffer solutions (PBS), the slices of shrimps were incubated with PEG-UCNPs conjugates for 2 h under a temperature of 20 ºC, and then any superfluous reagents were removed during the washing of PBS before cell imaging. The pictures of the PEG-UCNPs uptook by shrimps tissues were performed using Olympus BX43 fluorescence microscopy. The tissues were excited by a CW infrared laser operating at 980 nm.

2 Results and discussion

2.1 Upconverison fluorescence of $NaREF_4$ (RE=0.4Y+0.4La+0.2(Yb,Er,Tm)) nanoparticles

The morphology and microstructure of the synthesized nanoparticles were characterized by TEM and high-resolution TEM (HRTEM). From Fig. 1, it can be seen clearly that the $NaREF_4$ (RE=0.4Y+0.4La+0.2(Yb, Er, Tm)) nanoparticles possess a uniform size of 20 nm, pure hexagonal structures and good crystallinity. These uniform samples have good dispersibility in hexane.

The upconversion fluorescent spectra of the Yb^{3+},Er^{3+}/Tm^{3+} co-doped $NaREF_4$ under the excitation of a 980 nm laser diode are shown in Fig. 2. From Fig. 2(a), it is clear that the green emission centered at 546 nm increases gradually with increasing the concentration of Er^{3+} (from 0% to 2%) when the content of Tm^{3+} is fixed at 0.5 mol.%. Fig. 2(b) shows that the green emission centered at 540 nm decreases gradually with increasing the concentration of Tm^{3+} (from 0% to 0.5 mol.%) when the content of Er^{3+} is fixed at 2 mol.%. This indicates that the codoping of Tm^{3+} ion can enhance Er^{3+} ion emission and the $NaREF_4$ (RE=La/Y, La^{3+}:Y^{3+}=1:1) host is helpful to the energy transfer from Er^{3+} to Tm^{3+}.

The simplified energy diagram is shown in Fig. 3. The sharp blue emission band centered at 474 nm originates from Tm^{3+}-$4f^n$ electronic transitions $^1G_4\rightarrow{}^3H_6$, while the green emission peaks at 520 nm and 546 nm are attributed to $^2H_{9/2}\rightarrow{}^4I_{15/2}$ and $^4S_{3/2}\rightarrow{}^4I_{15/2}$ transitions of Er^{3+} ions, respectively. The red emission band centered at 657 nm is assigned to both Er^{3+}-$4f^n$ electronic transitions $^4F_{9/2}\rightarrow{}^4I_{15/2}$ and Tm^{3+}-$4f^n$ electronic transitions $^1G_4\rightarrow{}^3F_4$. For $NaREF_4$ (RE=0.4Y+0.4La+0.175Yb+0.02Er+0.005Tm), there is a direct transition of Tm^{3+} from $^1G_4$ to $^3F_4$ for emitting red light. Meanwhile, it may excite Er^{3+} from $^4I_{15/2}$ to $^4F_{9/2}$.

Fig. 4 shows the spectra of $NaREF_4$ (RE=0.4Y+0.4La+0.19Yb+0.005Er+0.005Tm) nanoparticles, which are used to indicate the existence of energy transfer between Er^{3+} and Tm^{3+}. The excitation power dependence