Sub-5 nm Lanthanide Doped ZrO_2 Upconversion Nanoparticle for Protein Targeted Biomaging

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Abstract— Lanthanide-doped upconversion nanoparticles (UCNPs) are a promising new generation of imaging agents for bioimaging due to no auto-fluorescence, large penetration depth and non-blinking. In this paper an efficient ultra-small core-shell UCNP was synthesized using a hydrothermal method and a thermal decomposition procedure. A new inorganic host — ZrO_2 was used for its low phonon energy and high absorption coefficient [1]. The as-synthesized core-shell nanoparticles has a diameter smaller than 5 nm. An aptamer was employed as a recognizer for protein recognition to implement the bioimaging. Therefore, the small and efficient core-shell nanoparticles was fabricated to achieve the single-proteins-targeted imaging.

1. INTRODUCTION

Photoluminescence (PL) imaging is a powerful tool for life science studies and biological medical applications due to its high resolution and sensitivity. However, most biological molecules don't have spontaneous fluorescence when excited by light. Thus the molecules of interest need to be labeled by extraneous light-emitting probes for studies. Generally speaking, the cell components and other biological molecules are in a nanoscale size, mainly sub-5-nm. Considering such an ultra-small size, it is necessary to utilize a comparable nanoparticle to label these biological molecules to avoid external interference [2]. But it has been challenging to find probes that are bright enough for imaging and not too large to disrupt the protein's function.

Fluorescent organic dye molecules and most semiconductor quantum dots meet the size requirements but impose other limitations, such as light stability and cytotoxicity. As an alternative, lanthanide-doped upconversion nanoparticles (UCNPs) have been considered as a new choice of luminance probe for biological applications [3–5]. UCNPs utilize sequential absorption of multiple photons through real ladder-like energy levels of trivalent lanthanide ions (Ln^{3+}) which were embedded in an appropriate inorganic host lattice to produce higher energy anti-Stokes luminescence. The excitation light-NIR light has a deeper penetration and also less damage to living body. Compared with traditional luminance probes, UNCPs have many advantages, like deep penetration, autofluorescence free, high resolution, photo-bleaching free, continuous emission and low phototoxicity [6]. These lead UCNPs to attract remarkable *in vitro* and *in vivo* applications including optical bioimaging [7–9] high-sensitive biosensing [10], multimodal imaging [11–13] as well as *in vivo* animal theranostics.

However, the size of commonly used UCNPs is tens of nanometer in diameter, which is not suitable for the single molecules imaging. Although sub-10-nm UCNPs have recently been synthesized, the size is still relatively large for bio-probe [14, 15]. In all, the tradeoff between small size and high luminance still exists. Therefore a sub-5-nm bright probe is in urgent need. We proposed to design lanthanide-doped zirconium oxide (ZrO_2-Ln^{3+}) upconversion nanoparticles (UCNPs). Particularly, ZrO_2 crystal has low phonon energy and high host absorption coefficient [16, 17]. It can be synthesized through a modified solvothermal procedure which results in an ultra-small size (sub-5 nm) [18]. To the best of our knowledge, there are seldom UCNPs based on ZrO_2, neither such small, uniform and monodisperse NPs [19–22].

2. SYNTHESIS AND CHARACTORIZATION

We designed and synthesized an efficient core-shell structure. The uniform and monodisperse ZrO_2:20%Yb^{3+}, 2%Er^{3+} core was synthesized according to a modified solvothermal procedure reported in the literature [23]. This prepared NPs are hydrophobic, because of capped with organic ligand (benzyl alcohol), and dispersed in chloroform to form a clear colloidal solution (Fig. 2(b)). The size and morphology were characterized by transmission electronic microscopy (TEM), which shows uniform spherical NPs with an average diameter of 3.5 nm (Fig. 1(a)). The shell $NaYF_4$:2%Yb^{3+}

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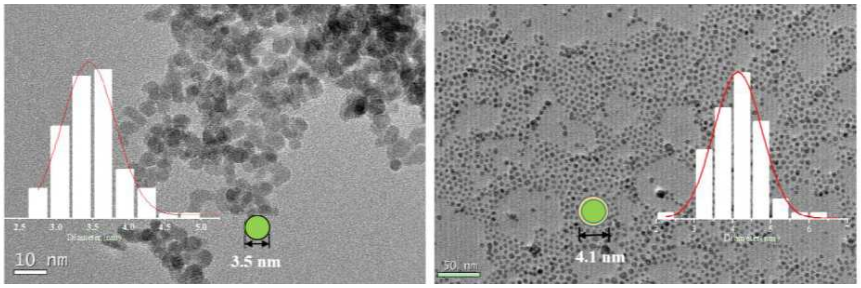

Figure 1: (a) TEM of ZrO_2:20%Yb^{3+}, 2%Er^{3+} (core) and histogram of the size distribution (inset); (b) TEM of ZrO_2:20%Yb^{3+}, 2%Er^{3+}@$NaYF_4$:2%Yb^{3+} (core-shell) and histogram of the size distribution (inset).

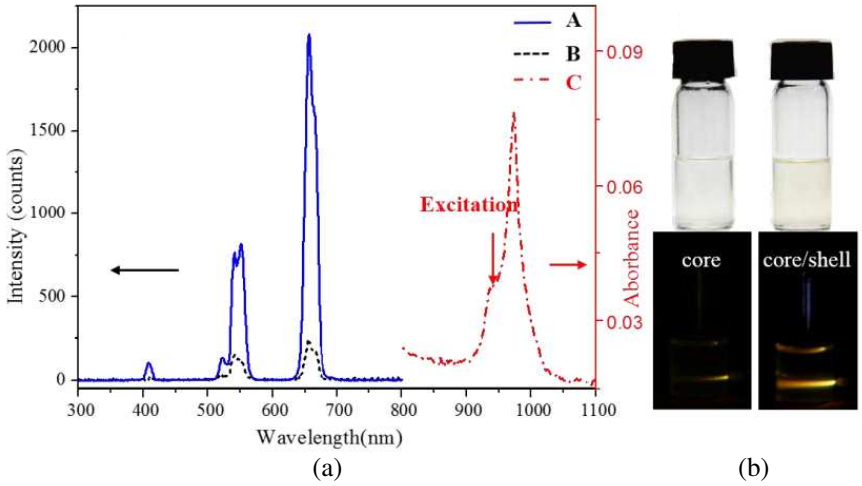

Figure 2: (a) PL emission (left) when excited at 925 nm (red arrow) and PL excitation (right) spectra forZrO_2-Ln^{3+} NPs. A is core/shell NPs PL emission (blue dash dot) and B is core NPs PL emission (black solid line); (b) ZrO_2-Ln^{3+} core NPs (upper left) and core/shell NPs (upper right) dispersed in hexane and luminance photographs were taken with a 2 W laser.

was synthesized through a thermal decomposition procedure which was modified from the procedure of shell of $NaYF_4$ [24]. We controlled the mole ratio of Zr and Y to achieve a thin shell which is about 0.6 nm. The as-prepared core-shell UCNPs was dispersed in organic solvents, like cyclohexane or chloroform, also to form a clear colloidal solution (Fig. 2(b)), which was a little yellowing than the core solution because of the oleic acid (OA). TEM also shows the uniform spherical NDs with an average diameter of 4.1 nm.

3. PL SPECTRA OF CORE AND CORE-SHELL NPS

The UC PL spectra of core and core-shell NPs were measured respectively via the microscope and spectrograph. We use diluted solutions to spin UCNPs onto the coverslips, which can form dispersive particles on the coverslips. A 925-nm laser beam was chosen as the excitation light because of less absorption by water reducing the heating effect when cell and animal experiments were taken [25]. The confocal luminescence images of core and core-shell UCNPs were taken respectively, and single particle was found to implement spot scanning to achieve the corresponding PL spectra. The UC PL of core and core-shell were compared and shown in Fig. 2(a), and obviously the core-shell PL intensity is of one order of magnitude than the core. This is also demonstrated in the luminance photographs (Fig. 2(b)), that the core-shell luminesce is much lighter than the core. Although the core is enough bright, the large surface-to-volume ratios and poor crystallinity that produce many defects which lead to serious energy quenching. A high thermal oil environment can not only produce a thin shell, but also make the crystallinity better.

4. SURFACE MODIFIED AND PROTEIN-TARGETED BIOIMAING

Before biological application, the UCNPs must be transformed into hydrophilia. The scheme below demonstrates the whole process (Scheme 1). The nitrite $NOBF_4$ were used to get rid of the OA capped on the NPs, which rendered the NPs surface positively charged [26]. Then the hydrophilic NPs was capped with water soluble polymers by a layer-by-layer method. The negatively charged PAA was used to encapsulate the hydrophilic UCNPs, which facilitated the successive coating of the positively charged PAH. All the surface zeta potentials were measured by a Zeta-Plus and the results are shown in Fig. 3(b). Surface modification of the nanoparticles was further confirmed by fourier-transform infrared (FTIR) spectroscopy. In the FTIR spectrum of OA-UCNPs (Fig. 3(a)-A), the peak at 3371 cm^{-1} was attributed to the O-H strong wide peak, and peaks at approximately 2923 and 2853 cm^{-1} were attributed to the asymmetric and symmetric stretching vibrations of methylene (CH_2) in the long alkyl chain, indicating the presence of OA on the surface of the nanoparticles (Fig. 3(a)). To be contrastive, peaks at approximately 2926 and 2855 cm^{-1} cannot be obviously observed in the spectrum of the UCNPs free of OA (Fig. 3(a)-B). The peak at 1687 cm^{-1} and 1642 cm^{-1} as well as the inconspicuous peak located around 744 cm^{-1} indicated the existence of additional N-H bonds (Fig. 3(a)-C).

There are many proteins in cell membrane, such as nucleolin or prion protein (PrPC) [27], which can be targeted by the specific aptamer functionalized UCNPs. And the ultra-small UCNPs is suitable to mark single proteins. Considering this, the streptavidin (SA) was linked to the surface of NPs after modified by polymers which was used to specific recognition with the aptamer. We can find there is a high specificity that most cell membranes were marked by the UCNPs when excited under a 925 nm CW (continuous wave) laser in the microscope (Fig. 4(c)). In the control group, when the UCNPs was not cultivated with SA, there were seldom UCNPs labeling the cell membranes (Fig. 4(b)).

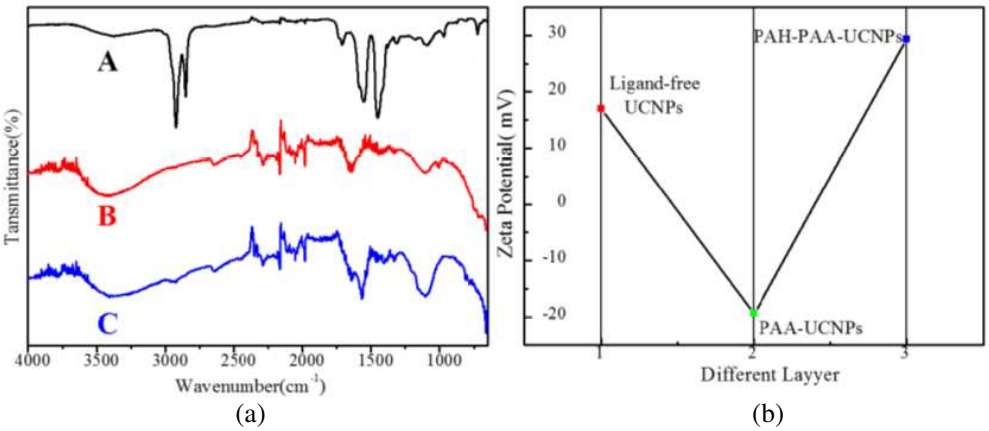

Figure 3: (a) FTIR spectra of (A) OA-UCNPs, (B) ligand-free UCNPs, (C) PAH-UCNPs. (b) Zeta potential data of different layers on UCNPs.

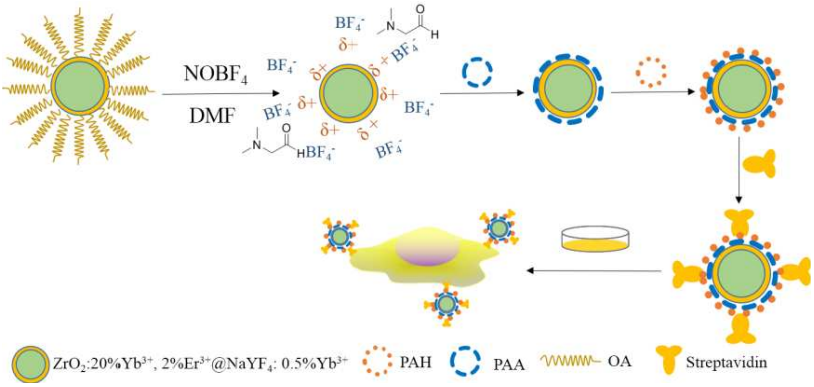

Figure 4: Scheme 1. Schematic illustration showing the hydrophilization and coating of PAA and PAH, the bioconjugation between PAA-PAH-UCNPs, and the incubation of SA-UCNPs with Hepatoma cells.

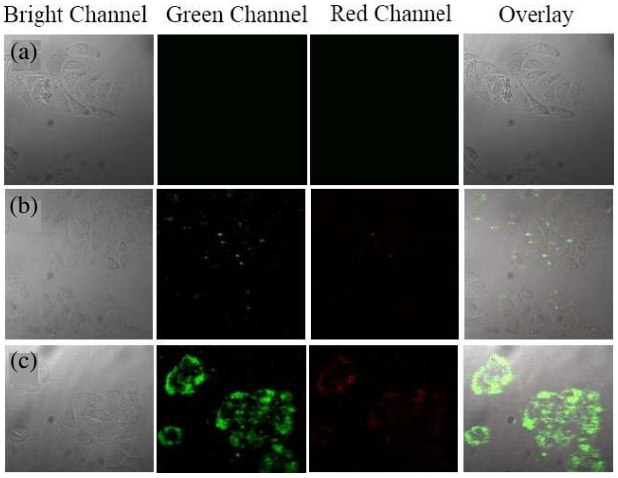

Figure 5: In vitro cancer cell imaging (right) using 925 nm laser excited UCNPs: images of Hepatoma cells separately incubated with (a) nothing as blank, (b) bio-aptamer pre-incubated and then PAH-ZrO_2-Ln^{3+}@ $NaYF_4$:Yb^{3+}, (c) bio-aptamer pre-incubated and then SA-ZrO_2-Ln^{3+}@ $NaYF_4$:Yb^{3+}. Bright channel (first column), green channel (second column), red channel (third column) and overlay image.

5. CONCLUSION

A type of 4-nm lanthanide-doped core-shell UCNPs was designed and sythnsized. Owing to the efficient host and shell, it has a remarkable brighter luminance. This small as well as brilliant UCNPs has a significant application in bioimaging. It would contribute a lot in the single-molecules imaging, which has become increasingly important because it can greatly help the researchers to understand the activities of human cells at the molecular level.

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