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Efficient Sub-25 nm Highly Tm^{3+}-Doped Upconversion Nanoparticles for Microscopic Imaging †

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Abstract: Lanthanide-doped upconversion nanoparticles (UCNPs) are capable of converting multiple near-infrared (NIR) photons into shorter-wavelength one by utilizing real long-lived, ladder-like energy levels of lanthanide ions. Such unique anti-Stokes emissions are immune to the auto-fluorescence background interference and derive from moderate irradiation compared to conventional multi-photon fluorescence, ideally suitable for imaging applications, coupled with improved imaging depth by NIR excitation light, excellent photostability, multicolor sharp-band emissions and tunable long emission lifetimes. However, the critical concentration quenching on upconversion luminescence (UCL) generally observed in UCNPs sets barriers to obtain bright high doping UCNPs at low excitation power density, which hampers this promising luminescence probe applying to long-term live cell imaging. We explore the mechanism of Tm^{3+} concentration quenching from ensemble and single-particle levels to design the new generation sub-25-nm ultra-bright highly Tm^{3+}-doped UCNPs for high-contrast imaging at limited excitation power density condition. This achievement provides a new strategy for developing small-sized bright highly activator-doped UCNPs and will broaden the applications of UCNPs in microscopic imaging.

Keywords: lanthanide; upconversion; concentration quenching; microscopic imaging

1. Introduction

Optical upconversion uses two or more NIR photons to generate emissions at shorter wavelength in a multiple-step excitation process [1]. This luminescence facilitates background-free imaging in deep tissue and many other applications, such as optical sensing, photovoltaics and anti-counterfeiting [2,3]. However, the weak UCL caused by concentration quenching of highly lanthanide activator ion doping remains one major thorny problem in the application of UCNPs as single-molecule bio-imaging probes, especially for frequently-used Tm^{3+} [4].

Here, we elaborately devised a core-shell-shell-shell structure UCNPs, $NaYbF_4$@$NaYF_4$:x% Tm^{3+}@$NaYbF_4$@$NaYF_4$, highly sensitized by Yb^{3+} and realized that the optimal doping concentration of Tm^{3+} boosts to 70 mol% at 100 kW/cm^2 of 980 nm excitation.

2. Methods

The designed UCNPs were synthesized following previously reported protocols with some modifications [5]. We first prepared 5 nm radius β-$NaYbF_4$ seed crystals [6] and then epitaxial grew desired thickness and component layer by layer until getting the final product without changing crystal phase.

Transmission electron microscopic (TEM) measurements to determine the size and morphology of the UCNPs were done with a Tecnai G2 Spirit Twin 12 microscope. Crystal phase was examined by powder X-ray diffraction (XRD) a BRUKER D8 ADVANCE diffractometer with a Cu Kα radiation source (λ = 1.5405 Å) operated at 40 kV and 40 mA. The scan was performed in the 2θ range from 10° to 80° with a scan speed of 0.4 °/s in steps of 0.02°.

Emission spectra were recorded on a monochromator (Princeton Instruments, HRS-300) equipped with an Electron-Multiplying CCD camera (Andor, Newton 970P). Spectral resolution was about 1 nm. Wide-field fluorescence image, corrected for the laser illumination profile, was taken by scientific CMOS camera with 40X NA0.95 air objective (Olympus, UPLXAPO). Above optical measurements were done at room temperature with 100 ng/mL UCNPs dispersed in hexane that were spin-coated onto a coverslip and a fiber coupled laser diode with tunable output power at 980 nm was used as the pump source.

3. Results and Discussion

As shown in Figure 1a, the designed structure contains a 5 nm radius $NaYbF_4$ active core and a 4 nm thick $NaYbF_4$ active layer, between which the $NaYF_4$:x% Tm^{3+} monoatomic layer can be sufficiently sensitized under the irradiation of a 980 nm laser. Besides, a 3 nm thick $NaYF_4$ inert outermost layer partly inhibits energy loss caused by surface quenching centers during energy transfer process across the network of rare earth ions [7].

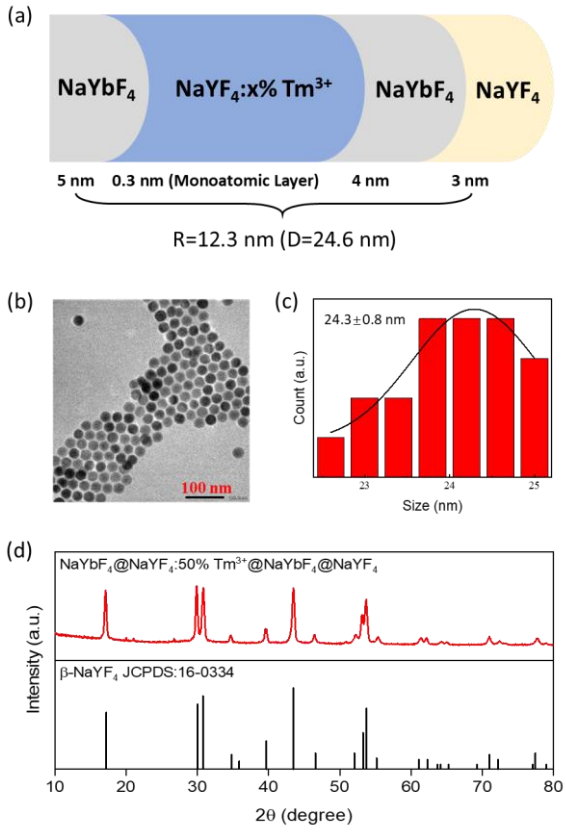

Figure 1. Structural characterizations of core–shell–shell-shell $NaYbF_4$@$NaYF_4$:x% Tm^{3+}@$NaYbF_4$@$NaYF_4$ (x = 10, 30, 50, 70) UCNPs. (**a**) The structural diagram of designed

$NaYbF_4$@$NaYF_4$:x% Tm^{3+}@$NaYbF_4$@$NaYF_4$ nanoparticle; (**b**) TEM image of $NaYbF_4$@$NaYF_4$:50% Tm^{3+}@$NaYbF_4$@$NaYF_4$ nanoparticles; (**c**) The corresponding size distributions of nanoparticles in (**b**) with Gaussian fits.; (**d**) XRD pattern of $NaYbF_4$@$NaYF_4$:50% Tm^{3+}@$NaYbF_4$@$NaYF_4$ nanocrystal and the standard data of hexagonal phase $NaYF_4$ (JCPDS 16-0334).

$NaYbF_4$@$NaYF_4$:50% Tm^{3+}@$NaYbF_4$@$NaYF_4$ nanoparticles with uniform diameter, ~24.3 nm, are successfully synthesized as designed (Figure 1b,c). Crystal structure of the as-prepared sample was characterized by XRD. The diffraction peaks are indexed to the standard data of hexagonal phase $NaYF_4$ (Figure 1d), which is consistent with the shape of nanoparticles in TEM image.

As illustrated in Figure 2a,b, under the excitation by a 980 nm laser, the intensity of each emission peak in ensemble UCL spectra of $NaYbF_4$@$NaYF_4$:50% Tm^{3+}@$NaYbF_4$@$NaYF_4$ enhance with the increase of power density. Moreover, the 455 nm emission peak raise faster than 800 nm due to more photons involved in this upconversion process (Figure 2c) [8]. Particularly, the strong 455 nm emission peak indicates the decent sensitization effect of designed Yb-rich structure.

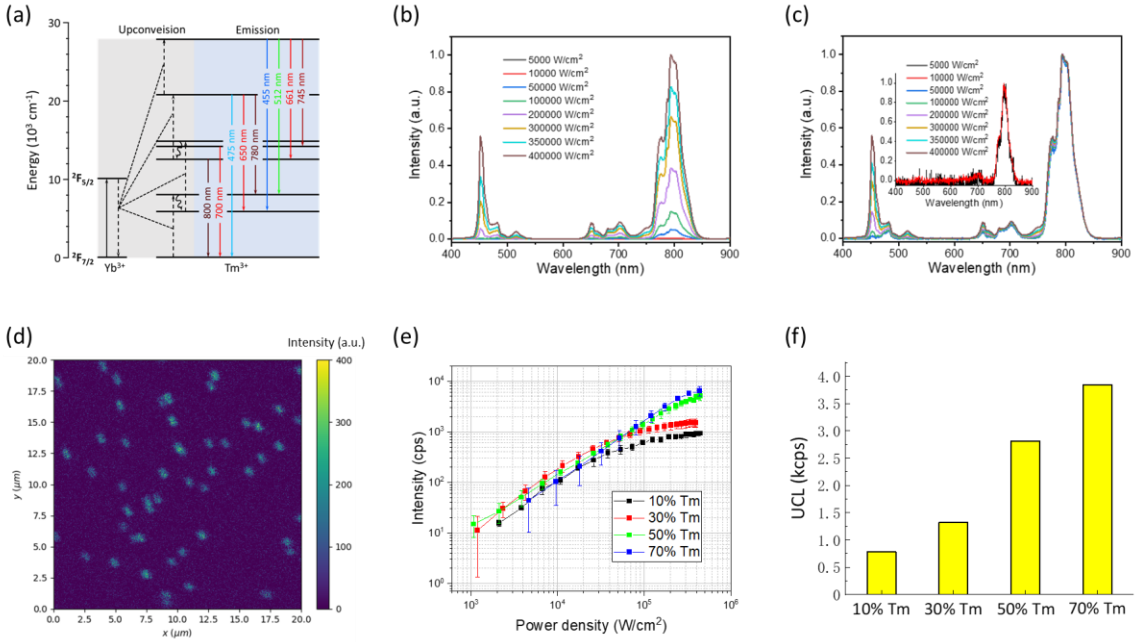

Figure 2. UCL spectra and wide-field fluorescence microscopy study of core–shell–shell-shell $NaYbF_4$@$NaYF_4$:x% Tm^{3+}@$NaYbF_4$@$NaYF_4$ (x = 10, 30, 50, 70) UCNPs. (**a**) Energy level diagram of Yb^{3+}/Tm^{3+} doped UCNPs. Solid arrows, excitation and emission; curved arrows, non-radiative relaxation; dashed arrows connected by dotted lines, energy transfer processes; Ensemble UCL spectra of $NaYbF_4$@$NaYF_4$:50% Tm^{3+}@$NaYbF_4$@$NaYF_4$ under the excitation by a 980 nm laser diode at a series of power densities. (**b**) UCL intensity normalized with the strongest emission peak; (**c**) UCL intensity normalized with the respective 800 nm emission peak (inset improve the display quality considering the signal-to-noise ratio at relative low power density); (**d**) Wide-field image taken at 100 W/cm^2 for $NaYbF_4$@$NaYF_4$:50% Tm^{3+}@$NaYbF_4$@$NaYF_4$; (**e**) UCL intensity saturation curves for UCNPs with different Tm^{3+} doping concentrations at power densities varying from 1 kW/cm^2 to 500 kW/cm^2 obtained with wide-field microscopy The error bars represent the standard deviation from the mean (n > 30); (**f**) Comparison of the average integrated UCL emission intensities of single UCNPs with different Tm^{3+} doping concentrations at 100 kW/cm^2.

Figure 2d shows a representative UCL wide-field image of $NaYbF_4$@$NaYF_4$:50% Tm^{3+}@$NaYbF_4$@$NaYF_4$ UCNPs taken at 100 W/cm^2 with the signal to noise ratio over 5, exhibiting their ability for high-contrast imaging at limited excitation condition. To systematically compare the UCL brightness of the different Tm^{3+}-doped UCNPs, we measured the power density-dependent UCL saturation curves of single particles over a power density range of nearly three orders of magnitude from 1 kW/cm^2 to 500 kW/cm^2 (Figure 2e). Each curve was obtained by averaging data

measured for 33 single particles within a field of view (FOV). Unlike the past experiment phenomenon observed in canonical core-only UCNPs [9,10], the optimal doping concentration of Tm^{3+} for UCL brightness apparently increases (at least to 30 mol%) in designed multi-shell UCNPs at 10 kW/cm^2. What's more, saturation curves further diverge before 100 kW/cm^2. The UCNPs doped with 70%Tm^{3+} are found to be the brightest one at 100 kW/cm^2 (Figure 2f), which intuitively demonstrates the validity of our designed core–shell–shell-shell structure for suppressing the UCL concentration quenching effect existing in Tm^{3+} ions.

4. Conclusions

We successfully synthesized the designed sub-25 nm hexagonal phase core–shell–shell-shell $NaYbF_4$@$NaYF_4$:x% Tm^{3+}@$NaYbF_4$@$NaYF_4$ (x = 10, 30, 50, 70) UCNPs highly sensitized by Yb^{3+}. This structure achieves restraint of the UCL concentration quenching effect existing among Tm^{3+} ions under 980 nm irradiance, which provides a new sight of producing small-sized bright high activator doping UCNPs for microscopic imaging at life science research level.

Conflicts of Interest: The authors declare no conflict of interest.

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