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# An optical sensor for Cu(II) detection with upconverting luminescent nanoparticles as an excitation source†

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**The first optical sensor for Cu(II) detection, with upconverting luminescent nanoparticles as an excitation source, showing high selectivity and good linear Stern–Volmer characteristics, has been achieved through a fluorescence resonance energy transfer (FRET) process between NaYF<sub>4</sub>:Yb<sup>3+</sup>/Er<sup>3+</sup> and RB-hydrazide. The sensing mechanism is then discussed.**

Cu(II) is an essential trace element for many biological processes and has been regarded as a significant environmental pollutant due to its widespread use.<sup>1,2</sup> Since excessive Cu(II) intake can cause serious human diseases, the detection and quantification of Cu(II) in environmental and biological monitoring are thus of importance. Recently, there have been increasing number of reports on alternative methods using colorimetric sensors and fluorescence sensors, with varying degrees of success regarding sensitivity, selectivity and stability.<sup>3</sup> In this case, fluorescence resonance energy transfer (FRET) is widely adopted in bioassays,<sup>4</sup> biosensing and bioimaging,<sup>5</sup> as an efficient spectroscopic molecular ruler.<sup>6</sup> An efficient FRET process requires: (i) the donor emission spectrum overlaps with the acceptor absorption spectrum; and (ii) the distance between a donor and an acceptor must be close enough.<sup>7</sup> Organic dyes and quantum dots have been most frequently used for FRET detection and imaging as donors or acceptors.<sup>8</sup> However, these probes need photo-excitation in the UV/blue region, in which region the endogenous chromophores or acceptors can also be excited, leading to background light interference and thus limiting their applications in biological samples.

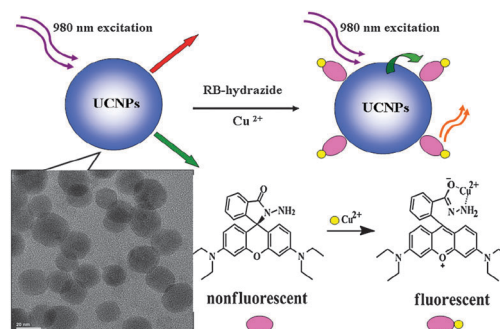
Recently, research focusing on near-infrared (NIR) to visible upconversion nanoparticles (UCNPs) has gathered considerable attention due to their potential use as bio-probes for

imaging and detection.<sup>9</sup> Such UCNPs have shown many advantages, including large Stokes shifts, higher chemical stability, improved quantum yields, reinforced light penetration depth in tissue, lower toxicity, and slight background light.<sup>10</sup> As a result, there is increasing interest in sensing and bioimaging based on UCNPs as luminescent labels. Wolfbeis and co-workers have developed several optical chemosensors based on UCNPs, in sensing pH, NH<sub>3</sub>, O<sub>2</sub>, respectively.<sup>11</sup> A later work on a sensitive UCNPs system to detect Hg(II), CN<sup>-</sup> was reported by Li *et al.*<sup>12</sup> However, to the best of our knowledge, no Cu(II) fluorescence probe based on UCNPs has been reported to date.

In the present study, a novel Cu(II) sensor is achieved by choosing β-NaYF<sub>4</sub>:Yb<sup>3+</sup>/Er<sup>3+</sup> as the energy donor and RB-hydrazide as the energy acceptor. This system is expected to be used for selective and sensitive quantification of Cu(II) in environmental and biological monitoring.

The schematic drawing of the FRET process is shown in Fig. 1. β-NaYF<sub>4</sub>:Yb<sup>3+</sup>/Er<sup>3+</sup> was synthesized according to a reported procedure.<sup>13</sup> The X-ray diffraction (XRD) pattern of this sample agrees well with that of pure hexagonal phase NaYF<sub>4</sub> nanocrystals, as reported in the JCPDS card no. 28-1192 (see ESI†, Fig. S1). The transmission electron microscopy (TEM) image shows that the nanocrystals were uniform in size with a very narrow size distribution (see Fig. 1, inset). The energy-dispersive X-ray (EDX) confirms the presence of F, Y, Na, Yb, Er in the nanocrystals (see ESI†, Fig. S2).

Under 980 nm excitation, the UCNPs give dual emission bands in green and red parts of the visible region, as shown in Fig. 2.

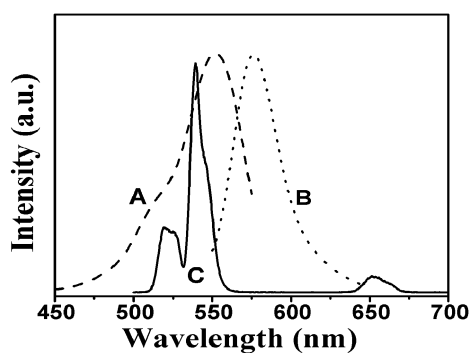


**Fig. 1** Schematic drawing of FRET based on UCNPs (NaYF<sub>4</sub>:Yb<sup>3+</sup>/Er<sup>3+</sup>) and RB-hydrazide. The inset shows a typical TEM image of the nanocrystals.

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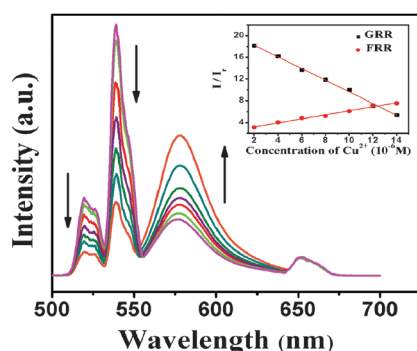
† Electronic supplementary information (ESI) available: Experimental details, XRD pattern and EDX spectra of sample NaYF<sub>4</sub>:Yb<sup>3+</sup>/Er<sup>3+</sup>, schematic energy level diagrams of Yb<sup>3+</sup> and Er<sup>3+</sup> ions following 980 nm excitation, the relative fluorescence intensity of RB-hydrazide in the presence of Cu(II) for different times, the reference experiments for luminescence emission of UCNPs when only RB-hydrazide or Cu(II) is present and fluorescence responses of RB-hydrazide to various metal ions. See DOI: 10.1039/c2cc31642k



**Fig. 2** Excitation (A,  $\lambda_{\text{em}} = 578 \text{ nm}$ ) and emission spectra (B,  $\lambda_{\text{ex}} = 540 \text{ nm}$ ) of RB-hydrazide in the presence of Cu(II); luminescence emission of NaYF<sub>4</sub>:Yb<sup>3+</sup>/Er<sup>3+</sup> under 980 nm excitation (C).

The emission bands centered at 521 nm, 539 nm and 651 nm can be assigned to  ${}^2\text{H}_{11/2} \rightarrow {}^4\text{I}_{15/2}$ ,  ${}^4\text{S}_{3/2} \rightarrow {}^4\text{I}_{15/2}$ ,  ${}^4\text{F}_{9/2} \rightarrow {}^4\text{I}_{15/2}$  transitions of Er<sup>3+</sup>, respectively (see ESI†, Fig. S3). RB-hydrazide is here chosen as the FRET acceptor owing to: (i) in the presence of Cu(II), RB-hydrazide's excitation spectrum overlaps well with the two shortwave emissions (521 nm and 539 nm) of UCNP, leading to an efficient energy transfer to the acceptor; (ii) the emission spectra of the donor and the acceptor are quite different in wavelength position and band width, making it possible to compare the two spectra and thus study the energy transfer process; and (iii) the FRET donor of UCNP can be excited using a NIR laser at 980 nm where RB-hydrazide cannot be photoexcited, thereby avoiding RB-hydrazide excitation by external light and eliminating luminescence background interference.

The FRET process is achieved by irradiating a solution containing UCNP (2.0 wt%) and RB-hydrazide ( $2 \times 10^{-5} \text{ M}$ ) at pH = 7 (NaAc–HAc buffer). The RB-hydrazide molecules could be noncovalently adsorbed to the surface of UCNP upon simple mixing, *via* hydrophobic interactions with the oleic acid layer on the inorganic nanoparticle surface.<sup>14</sup> Upon 980 nm excitation, the emission spectra under different concentrations of Cu(II) are presented in Fig. 3. It can be seen that the intensity of green upconverting emission at 521 nm and 539 nm decreases gradually with increasing Cu(II) concentration. A new emission appears at 578 nm, corresponding to



**Fig. 3** Fluorescence spectra of NaYF<sub>4</sub>:Yb<sup>3+</sup>/Er<sup>3+</sup> with RB-hydrazide in the presence of increasing concentration of Cu(II) ( $10^{-5}$  to  $10^{-6} \text{ M}$ ) upon 980 nm excitation. Inset: the variation of both green to red emission ratio (GRR) of Er<sup>3+</sup> and RB-hydrazide fluorescence emission to red emission ratio (FRR) upon different concentrations of Cu(II).

the fluorescence emission from RB-hydrazide and thus confirming the successful FRET process. Free RB-hydrazide of spiroactam structure is nearly non-fluorescent. However, in the presence of Cu(II), a delocalized xanthene moiety of the RB group is generated, resulting in a strong fluorescence emission.<sup>15</sup> Among the more relevant transition metal ions, Cu(II) has a particularly high thermodynamic affinity for typical N,O-chelate ligands and fast metal-to-ligand binding kinetics, which is responsible for the high selectivity for Cu(II).<sup>16</sup> We measured the fluorescent intensity of RB-hydrazide in the presence of Cu(II) different times, which was nearly unchanged (see ESI†, Fig. S4). Upon the addition of increasing concentrations of Cu(II), the fluorescence intensity of RB-hydrazide increases dramatically as shown in Fig. 3, with the red emission of UCNP at 651 nm nearly unchanged. Since RB-hydrazide has no absorption for the red emission of UCNP at 651 nm, the red emission intensity can thus be used as a reference as follows to evaluate the energy transfer process between the donor and the acceptor. The inset of Fig. 3 exhibits the variation of both green ( ${}^2\text{H}_{11/2} + {}^4\text{S}_{3/2} \rightarrow {}^4\text{I}_{15/2}$ ) to red ( ${}^4\text{F}_{9/2} \rightarrow {}^4\text{I}_{15/2}$ ) emission ratio (GRR) of Er<sup>3+</sup> and dye fluorescence emission to red ( ${}^4\text{F}_{9/2} \rightarrow {}^4\text{I}_{15/2}$ ) emission ratio (FRR) at different concentrations of Cu(II). The GRR ratio behavior supports the UCNP emission/RB-hydrazide absorption mechanism hypothesis, whereas FRR changes suggest resonant energy transfer to be present as well. In other words, the energy transfer between UCNP and RB-hydrazide should be responsible for the decreased intensity of UCNP green emission and the increased intensity of RB-hydrazide fluorescence, indicating the successful energy transfer between the donor and the acceptor. Simultaneously, reference experiments were researched (see ESI†, Fig. S5). In the absence of Cu(II), the emission of UCNP was nearly not affected upon addition of RB-hydrazide, the same for Cu(II) (no RB-hydrazide).

The Forster radius ( $R_0$ ) is defined as a distance of this pair of donor and acceptor at which the energy transfer efficiency is 50%. The  $R_0$  depends on fluorescence quantum yield of the donor in the absence of the acceptor ( $Q_0$ ), the overlap integral ( $J$ ) of the donor emission spectrum with the acceptor absorption spectrum and their mutual molecular orientation ( $\kappa^2$ ) and index of refraction ( $n_d$ ) as expressed by the following equation

$$R_0^6 = \frac{9Q_0\kappa^2J(\ln 10)}{128\pi^5n_d^4N_A}$$

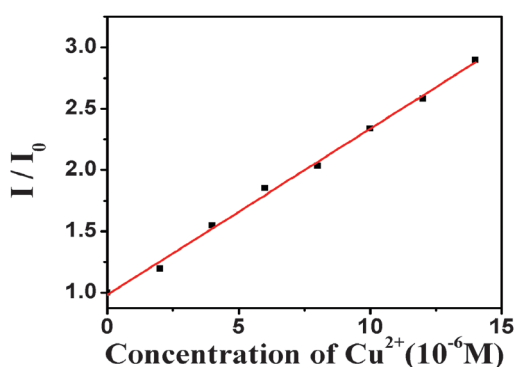
$N_A$  is the Avogadro number.

The spectral overlap integral  $J$  is calculated as

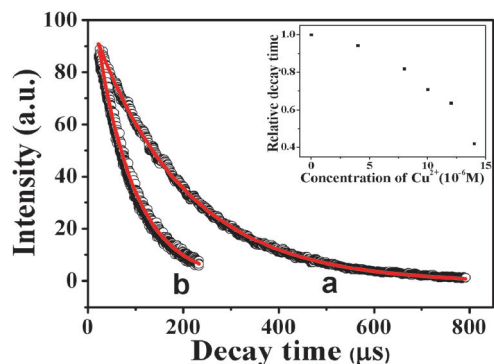
$$J = \int f_D(\lambda)\epsilon_A(\lambda)\lambda^4d\lambda$$

The fluorescence quantum yield in the range of 0.005% to 0.3% was measured for the green emission of several hexagonal-phase NaYF<sub>4</sub>:2% Er<sup>3+</sup>, 20% Yb<sup>3+</sup> nanoparticles with particle sizes ranging from 10 to 100 nm, whereas a quantum yield of 3% was measured for bulk samples with the same dopant levels.<sup>17</sup> The  $R_0$  value is equal to  $\sim 17 \text{ \AA}$ .

The plot of sample fluorescence intensity against Cu(II) concentration is depicted in Fig. 4. A good linear response in the range  $10^{-5}$  to  $10^{-6} \text{ M}$  is obtained with a linearly dependent coefficient  $R^2$  of 0.99652, and the detection limit



**Fig. 4** The plot of normalized fluorescence response  $I/I_0$  of RB-hydrazide upon additions of Cu(II) following 980 nm excitation.



**Fig. 5** Luminescence decays of the  $^4S_{3/2}$  level of a  $Er^{3+}$  donor without (a:  $R^2 = 0.99889$ ,  $\tau = 191.82 \mu s$ ) or with (b:  $R^2 = 0.99739$ ,  $\tau' = 79.48 \mu s$ ) the presence of RB-hydrazide and Cu(II). Inset: in the presence of RB-hydrazide, luminescence decays of excited  $Er^{3+}$  upon addition of Cu(II) under 980 nm excitation.

for Cu(II) is  $10^{-6}$  M. High selectivity is highly desired for an excellent fluorescence sensor. Fig. S6 (see the ESI†) shows the fluorescence responses of RB-hydrazide to various metal ions and its selectivity for Cu(II) ions under 980 nm excitation. It can be seen that interfering ions result in weak change in fluorescence intensity at 578 nm. However, the addition of Cu(II) causes a prominent fluorescence enhancement, showing an obvious difference from the cases for other metal ions, which indicates the high selectivity for Cu(II).

To confirm the FRET process from UCNP to RB-hydrazide, luminescence lifetime of donor alone as well as those of the mixed system with both donor and acceptor are determined (see Fig. 5). In the presence of acceptor admixture, excited state  $Er^{3+}$  luminescence lifetime ( $\lambda_{mon} = 539$  nm) gradually decreased upon the addition of Cu(II) under 980 nm excitation, which confirms the FRET process from UCNP to RB-hydrazide and the FRET system quenching is a dynamic quenching process (see Fig. 5, inset). The energy transfer efficiency ( $\eta$ ) of FRET can be calculated relating the donor luminescence lifetimes with ( $\tau'$ ) and without ( $\tau$ ) the acceptor by the formula of  $\eta = 1 - \tau'/\tau$ . The FRET efficiency is then calculated to be as high as 58.57%, and this high value suggests that the energy

transfer between UCNP and RB-hydrazide is efficient, favoring the improvement of sensing performance.

In summary, a new type of Cu(II) sensor based on efficient FRET between UCNP and RB-hydrazide is achieved, which overcomes the lack of NIR-excitable probes for Cu(II). It brings us the advantages of quick response towards Cu(II), no luminescence background, good selectivity, and ease of fabrication, which endows the system with further potential applications for Cu(II) detection in biological and analytical fields.

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