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Assembling of a functional cyclodextrin-decorated upconversion luminescence nanoplatform for cysteine-sensing†

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A novel rhodamine-oxaldehyde (RHO) functionalized β-$NaYF_4$:Yb^{3+}/Er^{3+} upconversion nanoparticle (UCNP) for specific detection of cysteine (Cys) in aqueous solution was achieved through a Förster resonance energy transfer (FRET) process. Based on self-assembling interaction, hydrophobic upconversion nanoparticles could be modified with α-cyclodextrin to make them water dispersible.

As an important biological thiol, cysteine is highly relevant to a variety of physiological and pathophysiological processes in organisms. Generally, alternations in the level of cellular cysteine have been associated with various diseases, such as slow growth, liver damage, skin lesions, hair depigmentation and neurotoxicity.[1] Considering the vital role of Cys in organisms, there is a high demand for its selective and sensitive detection method.[2]

Currently, there are an increasing number of reports on the organic chemosensors for responding to Cys.[3] However, these fluorophores are generally excited with ultra violet or visible light which induces autofluorescence and interferences. Upconversion nanoparticles are excited by near-infrared (NIR) light (usually 980 nm) which is situated in biological "optical transparency windows" of 700–1100 nm. Moreover, these nanoparticles show merits including multicolor emission, large Stokes shift, long lifetime, low cytotoxicity, remarkable photostability and deep tissue penetration, which endow them with superior availability in biological sensing, labeling, imaging and therapy.[4] However, due to their hydrophobic surface, oleic acid-capped UCNPs (OA-UCNPs) couldn't be dispersed in aqueous solutions or biological buffers, limiting their practical applications. As a consequence, surface modification should be applied on them to render them water-dispersible. Cyclodextrin has been extensively used for phase transfer of nanoparticles in host–guest chemistry. Owing to the suitable size and self-assembly interaction, the oleic acid ligand on the surface of UCNPs could be inserted into the hydrophobic cavity of α-cyclodextrin (α-CD), improving the water solubility of UCNPs and enabling its further applications.[5]

In the present work, a novel rhodamine functionalized upconversion sensing system was developed for sensing Cys in aqueous solution. The optical probe was excited by the Förster resonance energy transfer (FRET) process using β-$NaYF_4$:Yb^{3+}/Er^{3+} nanoparticles as donors and ring-opened rhodamine as an acceptor, respectively. Owing to the excitation of the near-infrared region, this system is expected to be used for biological monitoring.

The corresponding fabrication strategy and the sensing process are illustrated in Fig. 1. According to Zhang's report,[6] β-$NaYF_4$:Yb^{3+}/Er^{3+} particles were obtained and characterized by X-ray diffraction (XRD). As shown in Fig. S1A (ESI†), all diffraction peaks matched well with those of pure hexagonal-phase crystals (JCPDS stand card no. 28-1192) without any other impurities. Its energy-dispersive X-ray analysis (EDX) result confirmed the presence of F, Na, Y, Yb and Er elements in nanocrystals, as shown in Fig. S1B (ESI†). For turning our hydrophobic OA-UCNPs into water-dispersible, we employed a method for constructing inclusion complexes between OA and α-CD, as described in the Experimental Section. After the self-assembly

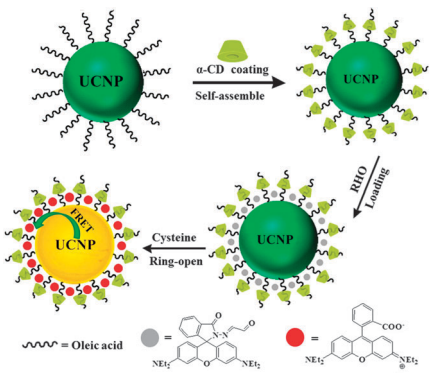

Fig. 1 Schematic illustration of the modification and FRET process based on donor UCNPs and acceptor rhodamine.

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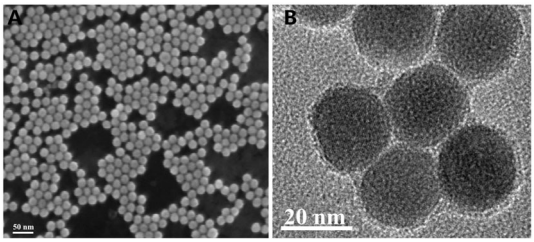

Fig. 2 SEM and TEM images of OA-UCNPs.

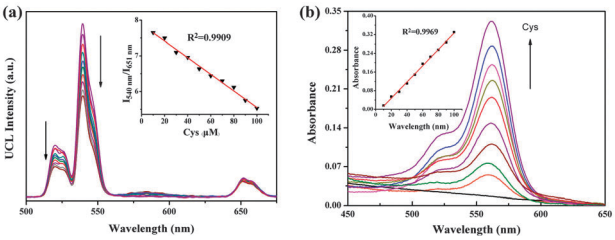

Fig. 3 (a) Under 980 nm excitation, UCL emission changes (inset: the variation of the relative UCL emission intensity at 540 nm to 651 nm ratio upon addition of different amounts of Cys) and (b) UV-Vis absorption titration spectra (inset: the linear response of the absorption peak intensity at 562 nm and Cys concentration) of RHO functionalized UCNPs (0.5 mg mL^{-1} in PBS, pH = 7.0) with gradual increment of Cys (10–100 μM).

interaction, OA-stabilized UCNPs were eventually transferred from the organic phase to the water phase, which could be apparently seen in Fig. S2A (ESI†). The morphology of OA-UCNPs before and after modification by α-CD is shown in electron microscopy images. OA-UCNPs dispersed in cyclohexane were spherical ones with an average diameter of 22 nm and narrow size distribution, as shown in Fig. 2. After ligand self-assembly with α-CD, α-CD modified UCNPs (α-CD-UCNPs) could be monodispersed in water without shape change or aggregation, as shown in Fig. S2B (ESI†). The successful self-assembly process between OA and α-CD was also confirmed by FT-IR spectroscopy. In Fig. S3 (ESI†), the weak stretching vibration of the =C–H group at 3005 cm^{-1} demonstrated the presence of oleic acid on the OA-UCNP surface. After reaction with α-CD, several new bands located at 1156 cm^{-1}, 1080 cm^{-1} and 1034 cm^{-1} were also observed in the FT-IR spectrum, which are attributed to anti-symmetric glycosidic vibration ν_a(C–O–C) and coupled stretch vibration ν(C–C/C–O), respectively. The successful introduction of α-CD onto UCNP samples is thus confirmed.

We designed a Cys sensing platform by performing the FRET process from donor UCNPs to acceptor Cys-triggered RHO in phosphate buffer (PBS, pH = 7.0). RHO, as a highly sensitive probe for Cys (Fig. S4, ESI†), could be non-covalently loaded onto the surface of UCNPs by a hydrophobic interaction with the oleic acid layer.[7] The X-ray photoelectron spectra (XPS) of the as-prepared sensing platform were also measured to examine the composite of the crystal surface (Fig. S5, ESI†). Sensing unit RHO was selected as a FRET acceptor owing to the following factors. (I) Rhodamine derivatives have virtues including long absorption and emission wavelength, high excitation coefficient and high fluorescence quantum yield (QY).[8] (II) In the presence of Cys, RHO excitation shows large spectral overlap with the green up-conversion luminescence (UCL) of Er^{3+} peaking at 521 nm and 540 nm, which makes an effective FRET detection process possible (Fig. S6, ESI†). (III) There are apparent differences between UCNPs and Cys-triggered RHO in their emission wavelength position and bandwidth, which enable us to distinguish and individually study the two spectra.

The FRET process was achieved by irradiating a solution that contained RHO-loaded α-CD-UCNPs (0.5 mg mL^{-1}) in PBS, pH = 7.0. Based on the UV-Vis spectra, the loading amount was estimated to be 1.13 mmol g^{-1} (Fig. S7, ESI†). Under 980 nm excitation, UCL emission spectra obtained at various concentrations of Cys (10–100 μM) are presented in Fig. 3(a), and all the spectral tests were performed 45 min after the addition of Cys (Fig. S8, ESI†). The emission intensity of green UCL centered at 521 nm and 540 nm decreased progressively with the increasing Cys concentrations. Since the red UCL emission at 651 nm had no overlapping with absorption of Cys-triggered RHO, almost no change was observed for this band. Therefore, it could be used as an internal reference for getting rid of any disturbance from environmental effects. The dynamic light scattering (DLS) analysis revealed that these nanoparticles did not show any significant aggregation and were mostly monodispersed in testing solution (Fig. S9, ESI†). We measured the UCL intensity of the system in the presence of Cys at different times, which were almost unchanged (Fig. S10, ESI†). The inset of Fig. 3(a) exhibited the variation of the green emission intensity ($^2H_{11/2}$ + $^4S_{3/2}$ → $^4I_{15/2}$) to red emission intensity ($^4F_{9/2}$ → $^4I_{15/2}$) ratio upon gradual Cys addition. The UCL intensity quenching increased linearly with the added Cys concentration in the range of 10–100 μM (R^2 = 0.9909). The detection of limit was calculated to be 1.1 μM.

The sensing property of RHO-functionalized UCNPs was further testified by UV-Vis absorption spectroscopy. The absorbance values at 562 nm at various Cys concentrations are shown in Fig. 3(b). In the range of 10–100 μM, a good linearity was obtained with a coefficient of determination R^2 of 0.9969, which also verified the effective FRET process from UCNPs to RHO. At the same time, a gradual colorimetric change from colourless to deep pink was also observed as shown in Fig. S11 (ESI†).

To further verify the FRET process from UCNPs to Cys-triggered RHO, luminescence decay lifetimes of the donor (540 nm) were determined in the absence and presence of Cys. As seen from Fig. 4, the donor lifetime decreased from (τ) 133 μs to (τ') 82 μs in the presence of Cys, which firmly proved the energy transfer from Er^{3+} to the ring-opened RHO. Meanwhile, the corresponding energy transfer efficiency (η) was calculated to be 38.35% according to equation $\eta = 1 - \tau'/\tau$. This value seemed to be unsatisfactory and could be explained as follows: the upconversion luminescence was obtained from Er^{3+} distributed in the volume of each nanosphere. However, the FRET process occurred only from parts of superficial ions. Furthermore, due to the increased surface defects of small particles and the applied polar water medium, additional luminescence quenching processes would take place, compromising the efficiency of the energy transfer process.

High selectivity is necessary for an excellent sensor. As depicted in Fig. 5, upon addition of competing amino acids and thiols of the same concentration (100 μM), including homocysteine (Hcy) and glutathione (GSH), as compared to the blank sample, only

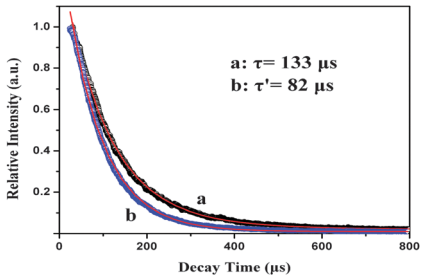

Fig. 4 Luminescence decay curve of the Er^{3+} in the absence (black, R^2 = 0.9993, τ = 133 μs) and presence (blue, R^2 = 0.9993, τ' = 82 μs) of Cys at 540 nm.

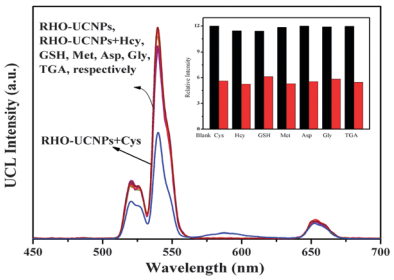

Fig. 5 UCL spectral variation of RHO functionalized UCNPs (0.5 mg mL^{-1}) before and after addition of 100 μM Cys and other amino acids and thiols, respectively. Inset: the relative UCL intensity (I_{540} nm/I_{651} nm) changes upon addition of different analytes (black bars represent the addition of other amino acids and thiols (100 μM) individually; red bars represent the subsequent addition of equal amounts of Cys to the above solutions).

Cys induced apparent quenching of UCL. Other competing amino acids and thiols such as methionine (Met), aspartic acid (Asp), glycine (Gly) and thioglycolic acid (TGA) caused no obvious influence on the UCL intensity. Upon adding Cys (100 μM) into the above solution containing Hcy, GSH, Met, Asp, Gly and TGA, respectively, an apparent decrease of UCL intensity was observed as in the case when only Cys existed (red bars in the inset in Fig. 5). Therefore, RHO-UCNPs could serve as specific Cys sensors with high selectivity.

The sensing mechanism of the above Cys detection was carried out as follows. According to previous reports, N-terminal Cys reacts chemoselectively with unsaturated aldehydes to form thiazolidines,[9] and this reaction has been extensively used for the design of probes.[10,11] Once reacted with α, β-unsaturated aldehydes, Cys tended to form generally favored 5-menbered ring heterocycles as compared to Hcy (6-menbered ring formation). A possible mechanism was thus proposed and is shown in Fig. S12 (ESI†): –SH and $–NH_2$ groups would firstly participate in cyclization with unsaturated aldehyde of RHO. Then, the obtained structure experienced a ring-opening process to give an unstable intermediate M_1. Following that, the hydrolysis process was promoted to release lactam-opened molecular M_2 and M_3, which induced corresponding fluorescence and colour changes. This hypothesis was tentatively proved by the ESI-MS spectrum (positive ion mode), as shown in Fig. S13A and B (ESI†). As for GSH, no above cyclization reaction could occur due to its relatively large separation between $–NH_2$ and –SH.

In conclusion, a novel cysteine nanosensor based on the FRET process between UCNPs and ring-opened RHO was fabricated. It possessed excellent ability to discriminate Cys from other common amino acids in aqueous solution, especially Hcy. The NIR-triggered mode enabled this nanosensor to effectively avoid background fluorescence interference and penetrate even deeper in biosamples, which endowed this system with further potential applications in biological and analytical fields. Since no covalent interaction occurs between hydrophobic molecules, other appropriate sensing molecules or fluorophores may be loaded onto the α-CD modified UCNPs as well, extending the capability of this system to other sensing or imaging processes.

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