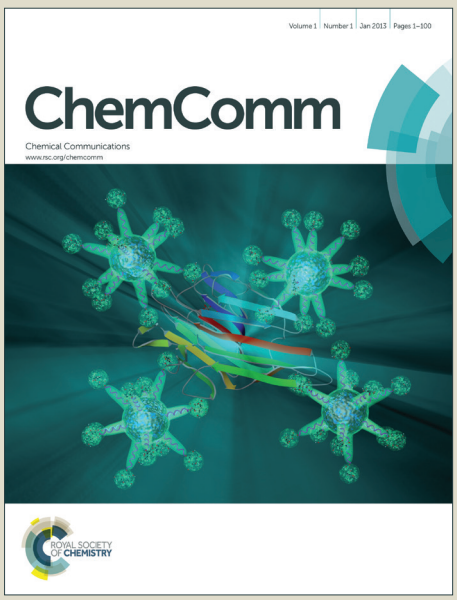
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

COMMUNICATION

Assembling of Functional Cyclodextrin-decorated Upconversion Luminescence Nanoplatform for Cysteine-sensing

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Jingkai Ni,[ab] Chongxin Shan,[a] Bin Li,*[a] Liming Zhang,[a] Heping Ma,[a] Yongshi Luo[a] and Hang Song[a]

A novel rhodamine-oxaldehyde (RHO) functionalized β-$NaYF_4$: Yb^{3+}/Er^{3+} upconversion nanoparticles (UCNPs) for cysteine (Cys) specific detection 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 that the vital role of Cys in organisms, there is a high demand for its selective and sensitive detecting 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 on them should be applied 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, oleic acid ligand on the surface of UCNPs could be inserted into the hydrophobic cavity of α-cyclodextrin (α-CD), improving 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 Förster resonance energy transfer (FRET) process using β-$NaYF_4$:Yb^{3+}/Er^{3+} nanoparticles as donor and ring-opened rhodamine as acceptor respectively. Owing to the excitation of near-infrared region, this system is expected to be used for biological monitoring.

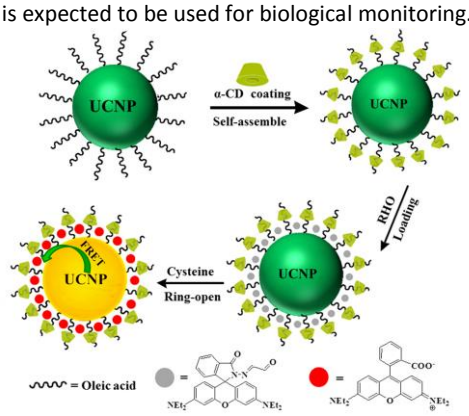


Fig. 1 Schematic illustration of the modification and FRET process based on donor UCNPs and acceptor rhodamine.

Corresponding fabrication strategy and sensing process were illustrated by Fig. 1. According to Zhang's report,[6] β-$NaYF_4$:Yb^{3+}/Er^{3+} were obtained and characterized by X-ray diffraction (XRD). As shown in Fig. S1A, 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, referred to Fig. S1B. For turning our hydrophobic OA-UCNPs into water-dispersible, we employed a method of constructing inclusion complexes between OA and α-CD, as described in Experimental Section. After the self-assembly interaction, OA-stabilized UCNPs were eventually transferred from organic phase to water phase, which

[a] State Key Laboratory of Luminescence and Applications, Changchun Institute of Optics, Fine Mechanics and Physics, Chinese Academy of Sciences, Changchun 130033, P. R. China.
[b] University of Chinese Academy of Sciences, Beijing 100039, P. R. China.
† Tel./Fax: +86 431 86176935. E-mail address: lib020@ciomp.ac.cn
Electronic Supplementary Information (ESI) available: Experimental Section and Characterization. See DOI: 10.1039/x0xx00000x

could be apparently seen in Fig. S2A. The morphology pictures of OA-UCNPs before and after modifying by α-CD were shown by electron microscope 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 referred to Fig. S2B. The successful self-assembly process between OA and α-CD was also confirmed by FT-IR spectroscopy. In Fig. S3, the weak stretching vibration of =C-H group at 3005 cm^{-1} demonstrated the presence of oleic acid on OA-UCNPs surface. After reacting with α-CD, several new bands located at 1156 cm^{-1}, 1080 cm^{-1} and 1034 cm^{-1} were also observed in 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 successfully introducing of α-CD onto UCNPs samples is thus confirmed.

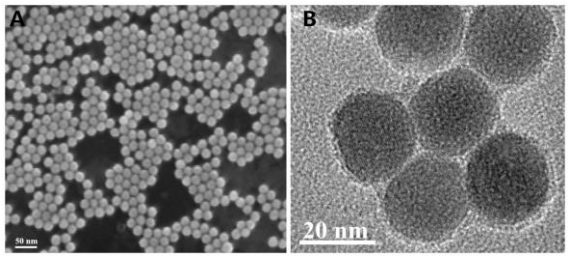


Fig. 2 SEM and TEM images of OA-UCNPs

We designed a Cys sensing platform by constructing 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), 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).Sensing unit RHO was selected as FRET acceptor owing to the following factors. (I) Rhodmaine derivatives has 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). (III) There are apparent differences between UCNPs and Cys-triggered RHO in their emission wavelength position and band width, which enables distinguish and individual study of the two spectra.

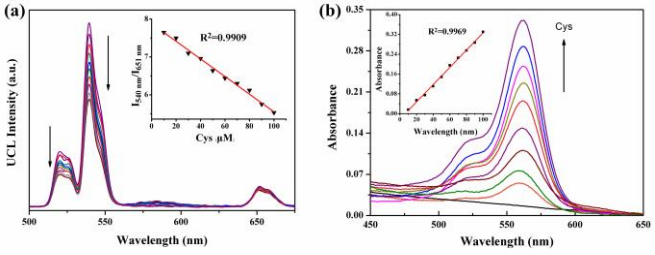


Fig. 3 (a) Under 980 nm excitation, UCL emission changes (Inset: the variation of relative UCL emission intensity at 540 nm to 651 nm ratio upon different amount of Cys.) and (b) UV-Vis absorption titration spect (Inset: the linear response of the absorption peak intensity at 562 nm ar Cys concentration.) of RHO functionalized UCNPs (0.5 mg/mL in PB pH=7.0) with gradual increment of Cys (10-100 μM).

The FRET process was achieved by irradiating a solution th contained RHO-loading α-CD-UCNPs (0.5 mg/mL) in PBS, pH=7.0 Based on the UV-vis spectra, the loading amount was estimate to be 1.13 mmol/g (Fig. S7). Under 980 nm excitation, UC emission spectra with various concentrations of Cys (10-100 μM were presented in Fig. 3(a), and all the spectral tests were operated 45 min later when Cys added (Fig. S8). Emission intensity of green UCL centered at 521 nm and 540 nm decreased progressively with the increasing Cys concentrations. Since the red UCL emission of 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 getting rid of any disturbance from environmental effect. Th dynamic light scattering (DLS) analysis revealed that, these nanoparticles did not show any significant aggregation and were mostly monodispersed in testing solution (Fig. S9). We measured the UCL intensity of the system in the presence of Cys at different times, which were almost unchanged (Fig. S10). Inset of Fig. 3 (a) exhibited the variation of green emission intensity ($^2H_{11/2}$+$^4S_{3/2}$→$^4I_{15/2}$) to red emission intensity ($^4F_{9/2}$→$^4I_{15/2}$) ra upon gradual Cys addition. The UCL intensity quenching increase linearly with the added Cys concentration in the range of 10-10 μM (R^2= 0.9909). Detection of limit was calculated as 1.1 μM.

Sensing property of RHO-functionalized UCNPs was furth testified by UV-Vis absorption spectroscopy. The absorbance values at 562 nm upon various Cys concentrations were shown 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, whic also verified the effective FRET process from UCNPs to RHO. At the same time, gradual colorimetric change from colourless deep pink was also observed as shown in Fig. S11.

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

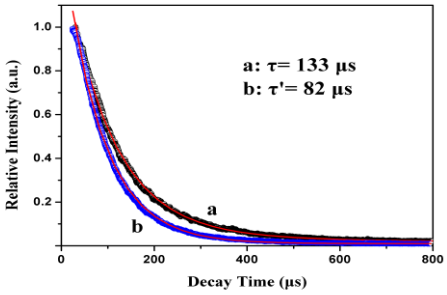


Fig. 4 Luminescence decay curve of the Er^{3+} with the absence (black, R^2= 0.9993, τ = 133 μs) and presence (blue, R^2= 0.9993, τ' = 82 μs) of Cys at 540 nm.

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

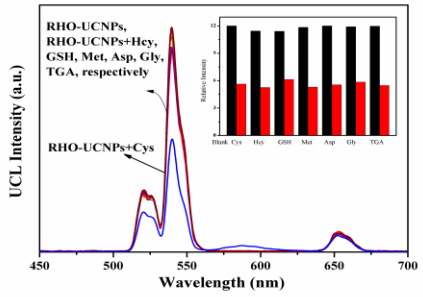


Fig. 5 UCL spectra variation of RHO functionalized UCNPs (0.5 mg/mL) before and after addition of 100 μM Cys and other amino acids and thiols, respectively. Inset: the relative UCL intensity ($I_{540\ nm}/I_{651\ nm}$) changes upon addition of different analytes (black bars represent the adding of other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

Sensing mechanism of above Cys detection was carried out as follows. According to previous reports, N-terminal Cys reacts chemoselectively with unsaturated aldehyde to form thiazolidines,[9] and this reaction has been extensively used for the design of probes.[10,11] Once reacted with α, β-unsaturated aldehyde, 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 shown in Fig. S12: –SH and $–NH_2$ groups would firstly participate cyclization with unsaturated aldehyde of RHO. Then, the obtained structure experienced a ring-opening process to give an unstable intermediate M_1. Following that, 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 ESI-MS spectrum (positive ion mode), as shown in Fig. S13A and B. As for GSH, no above cyclization reaction could happen due to its relative large separation between $-NH_2$ and –SH.

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

Notes and references

1 X. F. Yang, Y. X. Guo and R. M. Strongin, *Angew. Chem. Int. Ed.,* 2011, **50**, 10690 –10693.
2 (a) W. H. Wang, O. Rusin, X. Y. Xu, K. K. Kim, J. O. Escobedo, S. O. Fakayode, K. A. Fletcher, M. Lowry, C. M. Schowalter, C. M. Lawrence, F. R. Fronczek, I. M. Warner, and R.M. Strongin, *J. Am. Chem. Soc.,* 2006, **127**, 15949-15958; (b) X. F. Wang and M. S. Cynader. *J. Neurosci*., 2001, **21**, 3322-3331.
3 (a) X. Wang, J. Z. Lv, X. Y. Yao, Y. Li, F. Huang, M. M. Li, J. Yang, X. Y. Ruan and B. Tang, *Chem. Commun.,* 2014, **50**,15439-15442; (b) H. L. Wang, G. D. Zhou, H. W. Gai and X. Q. Chen, *Chem. Commun.,*2012, **48**, 8341-8343; (c) S. Y. Lim, K. H. Hong, D. Kim, H. Kwon and H. J. Kim, *J. Am. Chem. Soc*., 2014, 136, 7018-7025.
4 (a) C. X. Li and J, Lin, *J. Mater. Chem*., 2010, **20**, 6831-6847 (b) J. Zhou, Z. Liu and F. Y. Li, *Chem. Soc. Rev*., 2012, **41**, 1323-1349.
5 (a) J. Wang, D. Y. Wang, N. S. Sobal, M. Giersig, M. Jiang and H. Möhwald, *Angew. Chem. Int. Ed*., 2006, **45**, 7963–7966; (b) A. Harada, Y. Takashima and M. Nakahata, *Acc. Chem. Res*., 2014, **47**, 2128 –2140; (c) Q. D. Hu, G. P. Tang and P. K. Chu, *Acc. Chem. Res*., 2014, **47**, 2017–2025; (d) L. Peng, M. X. You, C. C.Wu, D. Han, I. öcsoy, T. Chen, Z. Chen and W. H. Tan, *ACS. Nano*., 2014, **8**, 2555-2561;
6 Z. Q. Li, Y. Zhang and S. A. Jiang, *Adv. Mater*., 2008, **20**, 4765–4769.
7 (a) Q. Liu, M. Chen, Y. Sun, G. Y. Chen, T. S. Yang, Y. Gao, X. H. Zhang and F. Y. Li, *Biomaterials*., 2011, **32**, 8243-8253; (b) L. Cheng, K. Yang, M. W. Shao, S. T. Lee and Z. Liu, *J. Phys. Chem. C*., 2011, **115**, 2686–2692; (c) G. Tian , W. L. Ren , L. Yan , S. Jian, Z. J. Gu, L. J. Zhou, S. Jin,W. Y. Yin, S. J. Li and Y. L. Zhao, *Small*., 2013, **9**, 1929-1938.
8 (a) H. N. Kim, M. H. Lee, H. J. Kim, J. S. Kim and J. Yoon, *Chem. Soc. Rev*., 2008, **37**, 1465–1472; (b) X. Q. Chen, T. Pradhan, F. Wang, J. S. Kim and J. Yoon, *Chem. Rev*., 2012, **112**, 1910-1956.
9 (a) X. Q. Chen, Y. Zhou, X. J, Peng and J. Yoon, *Chem. Soc. Rev*., 2010, **39**, 2120 –2135; (b) T. J. Tolbert and C. H. Wong, *Angew. Chem. Int. Ed*., 2002, **41**, 2171-2174.
10 (a) O. Rusin, N. N. St. Luce, R. A. Agbaria, J. O. Escobedo, S. Jiang, I. M. Warner, F. B. Dawan, K. Lian and R. M. Strongin, *J. Am. Chem. Soc*., 2004, **126**, 438-439; (b) P. Wang, J. Liu, X. Lv, Y.L. Liu, Y. Zhao and W. Guo, *Org. Lett*., 2012, **14**, 520 -523; (c) S. Lim, J. O. Escobedo, M. Lowry, X. Y. Xu and R.Strongin, *Chem. Commun*., 2010, **46**, 5707–5709.
11 H. L. Li, J. L. Fan, J. Y. Wang, M. Z. Tian, J. J. Du, S. G. Sun, P. P. Sun and X. J. Peng, *Chem. Commun*., 2009, 5904–5906.