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COMMUNICATION

DNA-functionalized upconversion nanoparticles as biosensor for rapid, sensitive, and selective detection of Hg^{2+} in complex matrices

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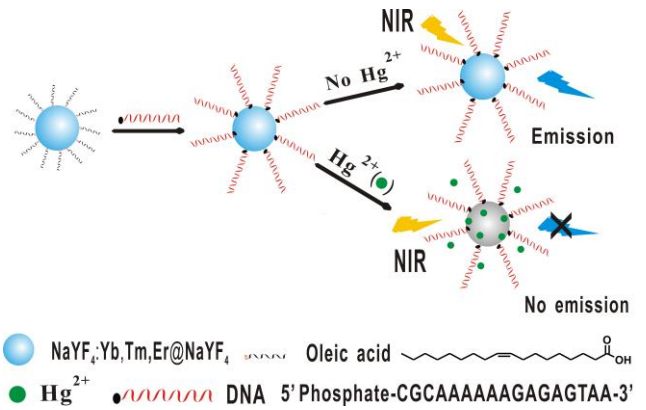
We have developed a facile one-step approach to make the hydrophilic and DNA-functionalizable upconversion nanoparticles (UCNPs), which are used to act as a biosensor for determining Hg^{2+} in complex matrices. The proposed approach is simple and exhibits low background interference, high sensitivity and rapid response.

Mercury (Hg^{2+}) has been widely deemed as one of the most hazardous pollutants and dangerous elements by reason of its toxic properties and accumulation in the environment, and it causes serious and permanent damage human health problems even at low concentrations.[1-2] Hence, the development of methods for the rapid, sensitive and selective detection of Hg^{2+} ions is of profound importance for environment and human health. Toward this goal, plenty of sensitive and selective Hg^{2+} sensors have been developed based on small molecule fluorophores,[3-5] proteins,[6-8] and metal nanoparticles.[9-10] Although these approaches have made the substantial progress toward the detection of Hg^{2+} ions, each of them still exhibits some defects, such as the poor aqueous solubility of some fluorophores, cross-sensitivity toward other metal ions, and the complicated synthesis of probe. Especially, these fluorescence probes based on traditional downconversion nanomaterials or organic dyes are usually excited using ultraviolet or blue light, which tends to give rise to strong background fluorescence from the endogenous chromophores in biological or environmental samples, thus limiting their applications in complex biological samples. Therefore, the highly sensitive and low background interference methods for the detection of Hg^{2+} remain extremely desirable.

Lanthanide-doped upconversion nanoparticles (UCNPs), which are capable of emitting strong visible fluorescence under the excitation of near-infrared (NIR) light (typically ca. 980 nm), have attracted considerable attention. These UCNPs have shown significant advantages over the traditional downconversion nanomaterials due to their superior optical and chemical features, such as improved quantum yield, minimal photobleaching, reenforced light penetration depth in tissue, and low toxicity.[11] More importantly, under the excitation of NIR light, the effect of autofluorescence from complex samples and scattering light becomes negligible. These merits make UCNPs an ideal candidate as the fluorescence probe. Up to now, many research works have been reported to detect ions,[12-14] small molecules,[15-18] proteins,[19-20] and nucleic acids[21-22] based on UCNP fluorescence probes. Recently, Qu's group has reported utilizing photochromics conjugated upconversion nanophosphors for biphasic enantioselective biocatalysis[23] and protein harvesting and near-infrared light triggered release[24].

A major challenge in the development of the UCNPs-based biosensors is to make water-dispersible, biocompatible and functionalizable UCNPs, because they are normally prepared in organic solvents and capped with hydrophobic ligands.[25] Many approaches such as one-step solvothermal synthesis,[26-27] silica coating[28] and phospholipid coating[29] have been developed to solve this problem. Our group has also prepared phospholipid-modified UCNPs for the detection of phospholipase D[30] and human immunodeficiency virus antibody[31]. Recently, Lu's group has reported an approach to prepare DNA-functionalized UCNPs based on ligand exchange process,[32] which not only converts hydrophobic UCNPs into water-dispersible and biocompatible ones, but also avoids the extra steps of bioconjugations using cross-linkers.

Here we prepared water-dispersible and DNA-functionalized UCNPs based on a facile one-step ligand exchange approach and proposed a novel UCNPs-based biosensor for sensitive and selective determination of Hg^{2+} ions. Scheme 1 illustrated the principle of the biosensor toward Hg^{2+} ions. The hydrophobic



Scheme 1 Schematic illustration of the upconversion biosensor for Hg^{2+} ions detection.

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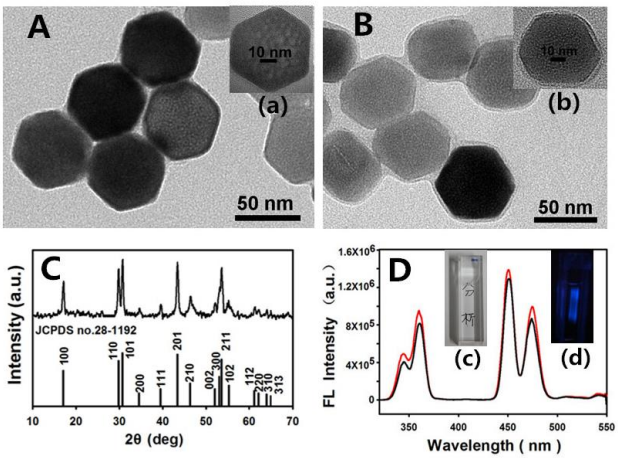

Fig. 1 TEM images of the (A) OA-UCNPs in cyclohexane and (B) DNA-modified UCNPs in water. (The inset shows the high-resolution TEM images of the respective samples). (C) X-ray diffraction pattern of $NaYF_4$:Yb,Tm@$NaYF_4$ nanocrystals. (D) Upconversion fluorescence spectra of the OA-UCNPs (red curve) and DNA-UCNPs (black curve). (The inset shows a photograph of the DNA-UCNPs in water under ambient light (c) and irradiated by 980 nm light (d)).

OA-coated UCNPs were first converted into water-dispersible DNA-functionalized UCNPs by using the 5'-end phosphate-modified DNA oligonucleotides through the interaction between the negatively charged phosphates of the DNA with the surface of lanthanide ions. In the absence of Hg^{2+}, the DNA-UCNPs exhibit intense blue fluorescence under an excitation wavelength of 980 nm. However, in the presence of Hg^{2+}, the DNA-UCNPs fluorescence could be effectively quenched due to facilitating non-radiative electron/hole recombination annihilation through an effective electron transfer process.[33-35] As a result, by taking advantage of the quenching effect, a facile upconversion-based biosensor can be fabricated for the detection of Hg^{2+} ions.

Highly efficient upconverting $NaYF_4$:Yb,Tm@$NaYF_4$ nanoparticles were synthesized using oleic acid (OA) as the stabilizing agent. The size and morphology of the oleic acid-capped UCNPs were characterized by transmission electron microscopy (TEM). The monodisperse nanohexagons of the as-prepared $NaYF_4$:Yb,Tm@$NaYF_4$ samples with approximate diameter of 58 nm suggested that the long-chain oleic acid ligands on the crystal surface prevented aggregation in cyclohexane (Fig. 1A). The X-ray diffraction (XRD) analysis (Fig. 1C) indicated that the peak positions and intensities of the nanocrystals agreed well with the calculated values of the pure hexagonal-phase $NaYF_4$:Yb,Tm@$NaYF_4$ nanocrystals (JCPDS no. 28-1192). After the DNAs conjugated onto the upconversion nanoparticle surfaces by ligand exchange at the liquid−liquid interface, The TEM image of the resulting DNA-modified UCNPs indicated that they remained monodisperse without obvious change in size, shape and crystallinity after modification with DNA and confirmed a uniform, approximately 3 nm thick, hydrophilic DNA layer around the surface the resulting DNA-UCNPs (Fig. 1B). UV−vis absorption spectroscopy also demonstrated the conjugation of DNA oligonucleotides with UCNPs (Fig. S1, ESI†). In addition, the assembly of the DNA oligonucleotides on the UCNP surface was further confirmed by FT-IR (Fig. S2, ESI†). Compared with the spectrum of OA-coated UCNPs, new peaks at 1400 and 1082 cm^{-1} appearing on the DNA-modified UCNPs, were ascribed to the stretching vibrations of the glycosidic bond and phosphate diester bond in DNA, indicating that the DNA oligonucleotides had been assembled successfully on the UCNP surface. Dynamic light scattering (DLS) measurements indicate that the DNA-modified UCNPs are well-dispersed in water, with a mean hydrodynamic diameter of about 97 nm (Fig. S3, ESI†). The zeta potential of the resulting DNA- modified UCNPs was -13.3 mV (Fig. S4, ESI†). Furthermore, the upconversion fluorescence spectrum of DNA-modified UCNPs in water was similar to that of the OA-coated UCNPs in cyclohexane with a slight decrease owing to the surface quenching effect of water molecules (Fig. 1D). The DNA-modified UCNPs showed excellent water solubility with long-term stability in water and resistance to aggregation over several weeks (Fig. 1D, inset). Upon continuous excitation at 980 nm, the fluorescence of the DNA-modified UCNPs in water appeared blue (Fig. 1D, inset). These results strongly indicated that the characteristic upconversion property of the nanoparticles was unaffected by the DNA coating.

The strong fluorescence of DNA-functionalizable UCNPs was sufficiently quenched (~94%) after incubation with Hg^{2+} ions. In contrast, when other metal ions such as Cu^{2+} and Ca^{2+} were added instead of Hg^{2+}, no obvious fluorescence decreases were observed under the identical conditions (Fig. 2). To investigate the quenching mechanism, the DNA oligonucleotides modified on the surface of UCNPs were enzymatically digested using S1 nuclease through incubation at 37 ℃ for 30 min to exclude the influence of DNA. The X-ray photoelectron spectroscopy (XPS) experiments verified the presence of Hg^{2+} on the UCNPs (Fig. S5, ESI†), indicating that the quenching was due to the non-radiative electron/hole recombination annihilation through an effective electron transfer process. The effect of the DNA sequence modified at the UCNP surface on the quenching efficiency was also studied (Table S1, ESI). It could be concluded that the DNA sequence which can form T-Hg-T structure resulted in relatively low quenching efficiency, which may be attributed to the binding of target Hg^{2+} on the DNA. Therefore, the DNA1 oligonucleotide was selected in the subsequent experiments. The effect of incubation time of the DNA-modified UCNPs with Hg^{2+} ions on the quenching efficiency was first investigated. The fluorescence intensity decreased rapidly with the increase in the reaction time within 20 min, and then reached a fixed value after 20 min (Fig.

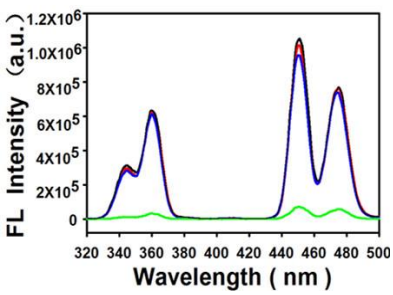

Fig. 2 Fluorescence spectra of DNA-modified UCNPs before (black curve) and after incubation with Hg^{2+} ions (green curve), Cu^{2+} ions (blue curve) and Ca^{2+} ions (red curve).

S6, ESI). So, an incubation time of 20 min was chosen as the appropriate time in the following experiments. We also studied the effect of incubation temperature between DNA-modified UCNPs and Hg^{2+} ions on the fluorescence intensity, and found that no obvious changes in the fluorescence signal could be observed in the range from 25 ℃ to 50 ℃ (Fig. S7, ESI). Therefore, an incubation temperature of 25 ℃ was selected for further experiments.

Under the optimal conditions, the fluorescence intensity of the DNA-modified UCNPs gradually decreased with the increasing concentration of Hg^{2+} ions (Fig. 3A), and the fluorescence quenching efficiency was linearly correlated to the Hg^{2+} ions concentration (correlation coefficient R^2 = 0.993 and 0.956) in the range from 10 nM to 10 μM (Fig. 3B). The detection limit of Hg^{2+} ions was calculated to be 5 nM according according to the 3σ rule. The UCNPs-based Hg^{2+} biosensor provided a lower detection limit and a wider dynamic range than those provided by existing colorimetric and down-conversion based fluorescence

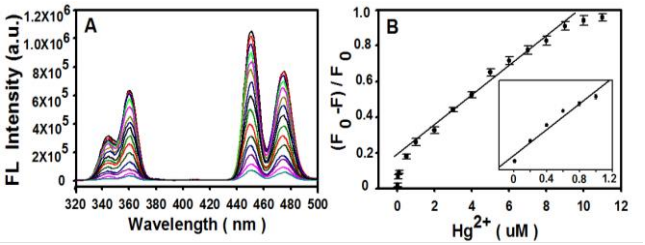

Fig.3 (A) Upconversion fluorescence spectra of the biosensor with varying concentrations of Hg^{2+} ions. (B) Linear relationship between the fluorescence quenching efficiency and the concentrations of Hg^{2+} ions within the range of 10 nM-10 μM. The error bars represented the standard deviations of three independent experiments.

methods (Table S2, ESI†). These results indicated that the UCNPs-based Hg^{2+} biosensor could be applied for sensitive Hg^{2+} ions analysis in a wide concentration range.

To assess the specificity of the upconversion biosensor for target Hg^{2+} ions, the responses of the biosensor toward some other metal ions were investigated. As shown in (Fig. 4), only weak fluorescence decrease signals could be detected for the other metal ions, even if they are 10-folds of Hg^{2+} ions. The results clearly demonstrated that the present upconversion biosensor is highly selective to Hg^{2+} ions.

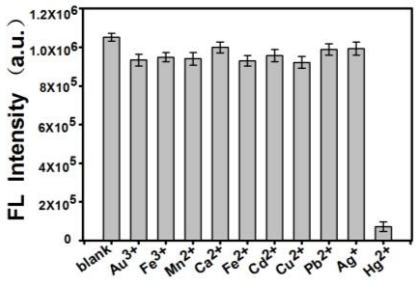

Fig. 4 Selectivity of the analysis of Hg^{2+} ions by the method depicted in Scheme 1 with the following metal ions: Au^{3+}; Fe^{3+}; Mn^{2+}; Ca^{2+}; Fe^{2+}; Cd^{2+}; Cu^{2+}; Pb^{2+}; Ag^{+}; Hg^{2+}. The concentration of Hg^{2+} was 10 μM. The concentrations of the other metal ions were 100 μM. The error bars represented standard deviation of three repetitive experiments.

To investigate the ability of the upconversion biosensor to overcome the interference from background fluorescence and scattering light, we applied the developed biosensor to local tap water samples. With local tap water as the assay medium, the same Hg^{2+} ions-dependent fluorescence changes as that in aqueous were observed (Fig. S8A, ESI†), except for a slightly high background signal, which may be due to the influence of the scattered exciting light. A linear range from 15 nM to 10 uM was also obtained in the local tap water (Fig. S8B, ESI†). In addition, the recovery experiment was also performed. Local tap water, river water samples obtained from Xiangjiang River and urine samples obtained from healthy people spiked with different concentrations of Hg^{2+} ions were determined, and the results were shown in Table 1. The recoveries of the local tap water were from 99% to 101% with RSD around 5%, of river water were from 97% to 102% with RSD around 6% and of urine samples were from 102% to 104% with RSD around 6.7%. The environmentally tolerable level of Hg^{2+} is 10^{-8} M (or 2 ng / mL as claimed by the US environmental protection agency), the proposed assay procedure is capable for monitoring the Hg^{2+} level to confirm whether the water tested satisfy the environmental standards.

Table 1 Analytical results of the Hg^{2+} ions in local tap water and river water using the upconversion biosensor.

sample	added(μM)	found(μM)	recovery	RSD(n=3)
Tap water 1	0.08	0.081	101%	4.8%
Tap water 2	0.5	0.498	99%	5.2%
River water 1	3	2.91	97%	5.4%
River water 2	8	8.16	102%	6.1%
Urine sample1	5	5.20	104%	5.8%
Urine sample2	9	9.22	102%	6.7%

In summary, we developed a facile one-step approach to make water-dispersible DNA-modified UCNPs through ligand exchange at the liquid−liquid interface, which can avoid the extra step of bioconjugations using cross-linkers and directly convert hydrophobic UCNPs into biocompatible and water-dispersible ones. The nanoparticles can be used for Hg^{2+} ions sensing with a lower detection limit, a wider dynamic range, and good selectivity, and applied to determine Hg^{2+} ions concentration in local tap water river water and urine samples with satisfactory results. Compared with the known fluorescence-based Hg^{2+} detection strategies,[36-37] our upconversion nanosystem based method has several advantages: first, the NIR-excitation technique offers non-autofluorescence assays, which enables the detection of Hg^{2+} in complex matrices; second, because of the low background signal by NIR excitation, the proposed method has a lower detection limit than UV excitation; third, as the fluorescence report element, the high photostability of UCNPs can ensure ideal signal output. Therefore, this approach provides a sensitive, selective and highly desirable Hg^{2+} detection platform.

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Notes and references

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