Electronic Supplementary Information:

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

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1. Experiment Section

Reagents and materials

Rare-earth chlorides used in this work including $YCl_3 \cdot 6H_2O$, $YbCl_3 \cdot 6H_2O$ $Tm(CH_3COO)_3 \cdot xH_2O$, NH_4F, NaOH, oleic acid, 1-octadecene were all purchased from Sigma-Aldrich (U.S.A.). $Hg(NO_3)_2 \cdot 0.5H_2O$, $HAuCl_4 \cdot 4H_2O$, $K_3[Fe(CN)_6]$, $MnCl_2 \cdot 4H_2O$, $Pb(CH_3COO)_2 \cdot 4H_2O$, $FeSO_4 \cdot 7H_2O$, $Cd(NO_3)_2 \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$, $CaCl_2$, $AgNO_3$, cyclohexane and ethanol were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The purified DNA oligonucleotides used in this work (Table S1) were synthesized by Shanghai Sangon Biological Science & Technology Company (Shanghai, China). S1 nuclease (100 units μL^{-1}) was obtained from Thermo Fisher Scientific Inc. The S1 nuclease buffer (20 mM NaAc, 150 mM NaCl, and 1 mM $ZnSO_4$, pH 4.5) was used to dilute S1 nuclease and enzymatic digestion reaction. All the solutions were prepared using ultrapure water (>18.25 MΩ) produced by a Millipore Milli-Q water purification system (Billerica, MA, USA). The real samples including local tap water samples and river water samples obtained from Xiang River (Changsha, China).

Synthesis of oleic acid-coated $NaYF_4$:Yb,Tm@$NaYF_4$ UCNPs

Water-insoluble oleic acid-coated $NaYF_4$:Yb,Tm@$NaYF_4$ nanoparticles (OA-UCNPs) were synthesized according to the method described by literature with slight modification.[S1] First, synthesis of $NaYF_4$:Yb,Tm Core Nanoparticles. Briefly, $YCl_3 \cdot 6H_2O$ (0.695 mmol), $YbCl_3 \cdot 6H_2O$ (0.30 mmol), and $TmCl_3 \cdot 6H_2O$ (0.005 mmol) (1 mmol, Y: Yb: Tm =69.5%: 30%: 0.5%) were added to a 50 mL three-necked flask

containing oil acid (OA) (8 mL) and 1-octadecene (15 mL). The reaction mixture was heated to 100 ℃ under vacuum with stirring for 30 min to remove residual water and oxygen and then heated to 160 ℃ for another 30 min to form a homogeneous solution and then cooled down to room temperature. Then, 10 mL of methanol solution containing NaOH (2.5 mmol) and NH_4F (4 mmol) was added slowly and the resultant solution was stirred for an additional 30 min at 50 ℃. The reaction mixture was heated to 70 ℃ under vacuum to remove methanol and then was rapidly heated to 300 ℃ under stirring and kept at this temperature for 1 h under Ar protection and then cooled down to room temperature. The resulting nanoparticles were precipitated by the addition of ethanol, collected by centrifugation at 8000 rpm for 5 min, washed several times with ethanol and the resulting $NaYF_4$:Yb,Tm Core nanoparticles were obtained. The $NaYF_4$:Yb,Tm@$NaYF_4$ Core-Shell Nanoparticles Synthesis in a similar manner by varying the amount of Y^{3+} ions. YCl_3•$6H_2O$ (0.4 mmol) was added to a 50 mL three-necked flask containing oil acid (OA) (8 mL) and 1-octadecene (15 mL).. The reaction mixture was heated to 100 ℃ under vacuum with stirring for 30 min to remove residual water and oxygen and then heated to 160 ℃ for another 30 min to form a homogeneous solution, then then cooled down to room temperature. $NaYF_4$:Yb,Tm core nanoparticles in 2 mL of cyclohexane were added along with a 5mL methanol solution of NH_4F (2.5 mmol) and NaOH (4 mmol). The resulting mixture was stirred at 50℃ for 30 min, at which time the reaction temperature was increased to 80 ℃ to remove the methanol and cyclohexane. Then the solution was heated to 300 ℃ under an argon flow for 1 h and cooled to room temperature. The

resulting nanoparicles were precipitated out by the addition of ethanol, collected by centrifugation, washed with ethanol for several times, and dried under vacuum for further experiments.

Preparation of the DNA-functionalizable UCNPs

The procedure for the preparation of DNA conjugated UCNPs were adapted from the previously reported paper.[S2] A water solution (2mL) containing 0.6 nmol DNAs was slowly added into the oleic acid capped UCNPs (1mg) in 1 mL of cyclohexane, and the solution is vigorously stirred for 18h. Afterward, the UCNPs could be clearly transferred into the lower water layer from the cyclohexane layer due to the DNAs attachment. The water solution was transferred to a microtube. After vigorously sonication, excess DNAs was removed from DNA-UCNPs by centrifugation at 18000 rpm for 16 min and washed several times with ultrapure water. The obtained DNA-functionalizable UCNPs were finally suspended in 0.8 mL of ultrapure water and stored at 4 ℃ for further experiments. The concentration of the DNA-UCNPs was calculated as ~ 1 mg mL^{-1}.

Preparation of the S1 nuclease-treated DNA-modified UCNPs

The procedure for the preparation of the S1 nuclease-treated DNA-modified UCNPs were adapted from the previously reported paper of our group.[S3] 20μL aliquot of reagent solution containing a certain concentration of the DNA-functionalizable UCNPs and S1 nuclease was used to perform the enzymatic digestion reaction. After incubation for 30 min at 37 ℃, the mixture was vigorously vibrate, excess S1 nuclease was removed from bald UCNPs by centrifugation at 18000 rpm for 16 min

and washed several times with ultrapure water. The obtained bald UCNPs were finally suspended in 0.8 mL of ultrapure water and stored at 4 ℃ for further experiments.

Characterization of UCNPs

The morphologies of the nanoparticles were obtained using a JEOL JEM-2100 transmission electron microscope (TEM). Dilute colloid solutions of the OA-coated UCNPs dispersed in cyclohexane and the DNA-UCNPs dispersed in water were drop-cast on thin, carbon formvar-coated copper grids respectively. The X-ray diffraction (XRD) patterns of the the OA-coated UCNPs were performed on a Rigaku D/Max-Ra x-ray diffractometer using a Cu target radiation source (λ=0.14428 nm). The hydrodynamic size distribution and zeta potential distribution of the DNA-UCNPs were determined using a Malvern Zetasizer (Nano-ZS, USA). Fourier-transform infrared (FT-IR) spectrum analysis was performed with a Nicolet 4700 Fourier transform infrared spectrophotometer (Thermo Electron Co., USA) by using the KBr method. X-ray photoelectron spectroscopy (XPS) spectrum was performed with a 180 ° double focal hemisphere analyzer-128 channel detector, using an unmonochromated Al Kα X-ray source (Thermo Fisher Scientific, UK). The UV-vis absorption spectrum was recorded on a UV-2450 UV-vis spectrometer (Shimadzu, Japan).

Determination of Hg^{2+} ions

For the determination of Hg^{2+} ions, a certain concentration of Hg^{2+} ions solution and DNA-functionalizable UCNPs (50 μg mL^{-1} of final concentration) were oscillated with vortex mixing apparatus for 30 seconds and incubated at 25 ℃ for 20 min. The

upconversion fluorescence spectra of the mixture were measured using a FluoroMax-4 Spectrofluorometer (HORIBA Jobin Yvon, Inc., NJ, USA) equipped with an external 980 nm diode CW laser (Changchun New Industries Optoelectronics Tech. Co., Ltd.) as the excitation source instead of the internal excitation source at room temperature.

Detection of Hg^{2+} ions in Local Tap Water, River Water and urine samples

For the detection of Hg^{2+} ions in local tap water, river water and urine samples, the river water samples and urine samples collected were first filtered through a 0.22 μm filter membrane to remove insoluble substance, 20 μL of local tap water, river water or urine samples, a certain concentration of Hg^{2+} ions solution and the DNA-modified UCNPs (50 μg mL^{-1} of final concentration) were oscillated with vortex mixing apparatus for 30 seconds and then incubated at 25 ℃ for 20 min. The fluorescence spectra of the mixture were measured under the excitation of 980 nm at room temperature.

2. Supplementary Figures and Tables

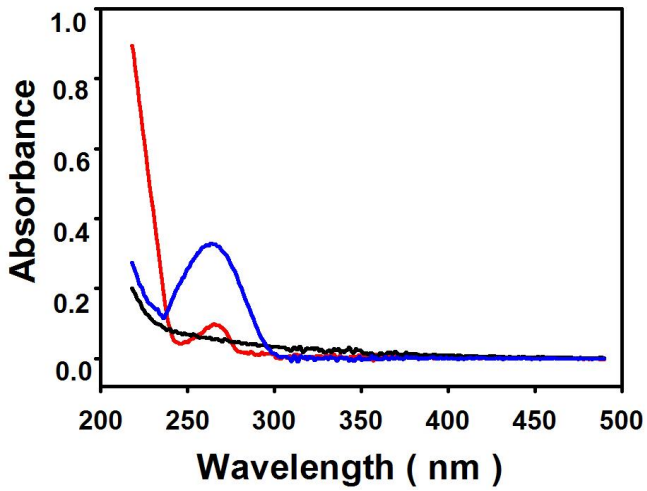


Fig. S1 The UV−vis absorption spectrum of the OA-UCNPs (black line), the DNAs (blue line) and the DNA-UCNPs (red line). A ultraviolet absorption maximum of the DNAs at approximately 260 nm, there no absorption peaks appeared in the UV−vis spectrum of the OA-UCNPs, and a new absorption peak at approximately 260 nm was observed in the spectrum of the DNA-UCNPs as a result of the amount of DNAs combined with UCNPs.

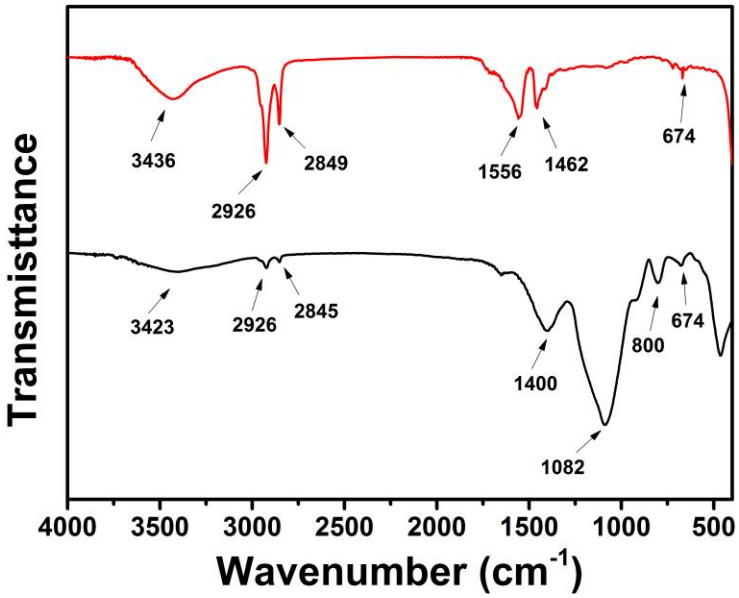


Fig. S2 FT-IR spectra of the as-prepared OA-coated UCNPs (red carve) and DNA-functionalizable UCNPs (black carve). A prominent transmission bands at 1400 and 1082 cm^{-1} for the DNA-functionalizable UCNPs, which were not observed for the UCNPs coated with oleic acid. These bands were ascribed to the stretching vibrations of the glycosidic bond and the stretching vibrations of phosphate diester bond in DNA. Two strong bands centered at 1562 and 1463 cm^{-1} were observed in the OA-coated UCNPs spectrum, these bands were assigned to the asymmetric and symmetric stretching vibrations of the carboxylate anions on the surface of the UCNPs. The absorption bands around 2926 and 2849 cm^{-1} were slightly decreased in the DNA-functionalizable UCNPs as compared to those coated with oleic acid, attributed to the decreased amount of the methylene (–CH2–) in the DNA coating.The UCNPs, either coated with oleic acid or DNA, exhibited a broad band around ~3436 cm^{-1}, corresponding to the asymmetric and symmetric stretching vibrations of the hydroxy (–OH).

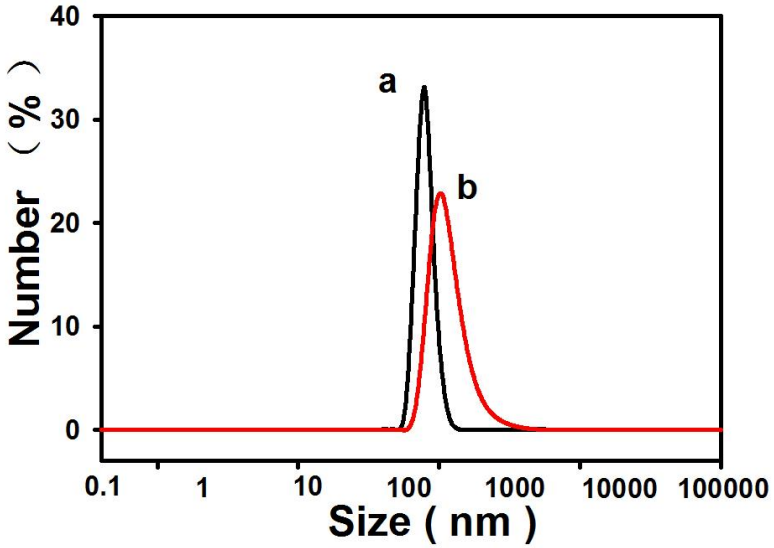


Fig. S3 The hydrodynamic size distribution of (a) the OA-coated UCNPs in cyclohexane and (b) the DNA-functionalizable UCNPs in water, determined by the dynamic light scattering. The DNA-functionalizable UCNPs were well-dispersed in water with a mean hydrodynamic diameter of about 97 nm. In comparison with the OA-coated UCNPs dispersed in cyclohexane (ca. 75 nm), the DNA-functionalizable UCNPs increase of approximately 22 nm in diameter was in agreement with the layer of the DNA stretch in the water.

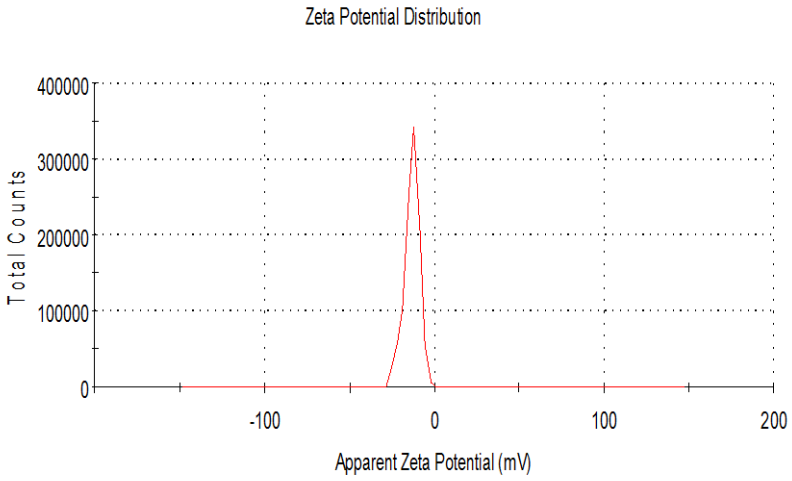


Fig. S4 Zeta potential experiments of the as-prepared DNA-functionalizable $NaYF_4$:Yb,Tm@$NaYF_4$ UCNPs. The zeta potential of the resulting DNA- modified UCNPs was -13.3 mV.

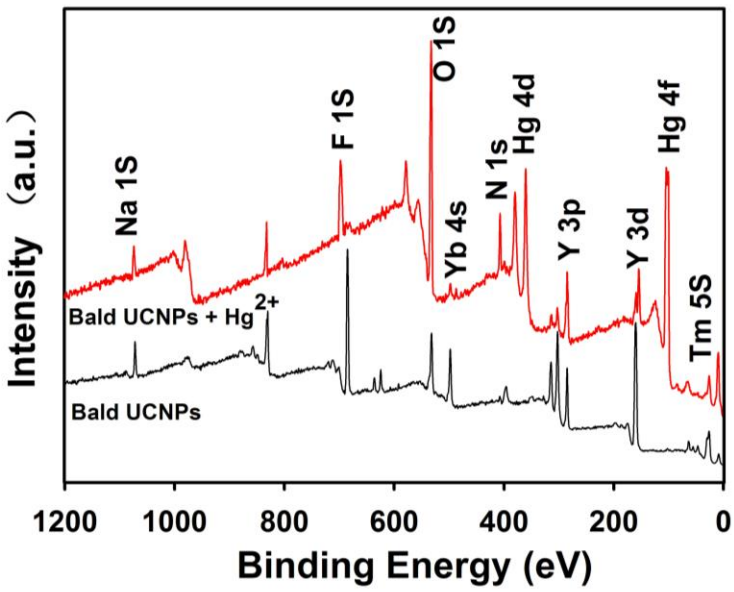


Fig. S5 X-ray photoelectron spectroscopy (XPS) spectra of the S1 nuclease-treated DNA-modified UCNPs (bald UCNPs) in the presence (red curve) and absence (black curve) of $Hg(NO_3)_2$.

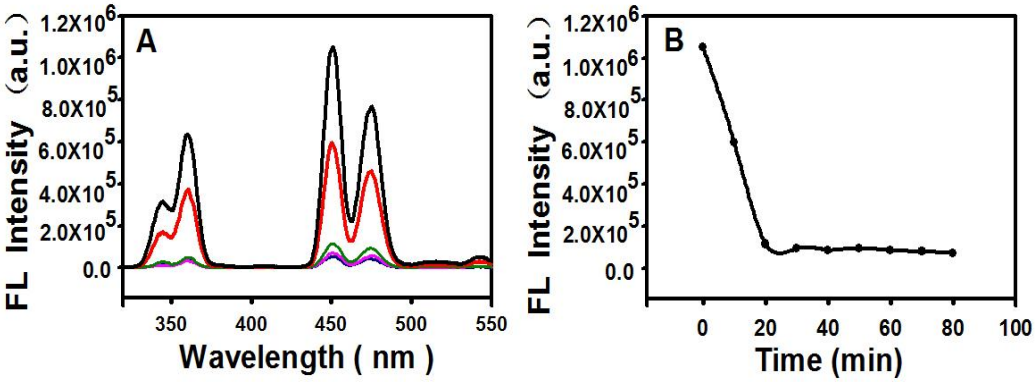


Fig. S6 Effect of the incubation time of the DNA-UCNPs with Hg^{2+} ions on the upconversion fluorescence quenching. The fluorescence intensity decreased rapidly with the increase in the reaction time before 20 min, and then reached a fixed value after 20 min. The concentrations of DNA-UCNPs and Hg^{2+} ions were 50 μg mL^{-1} and 10 uM, respectively.

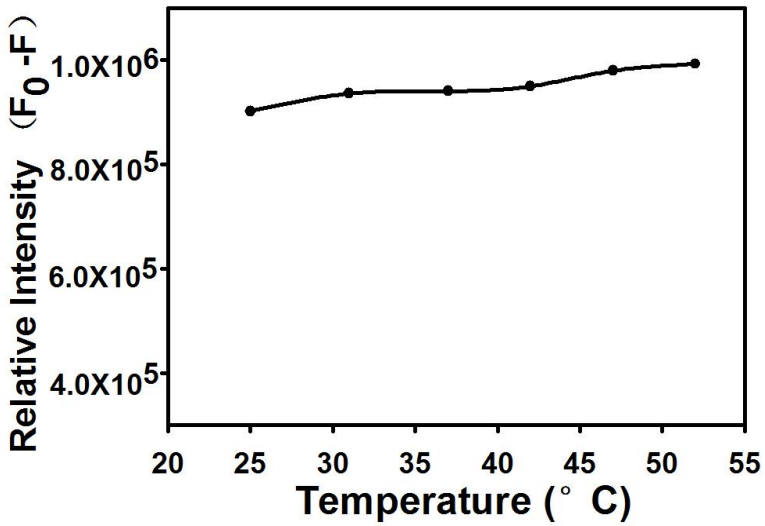
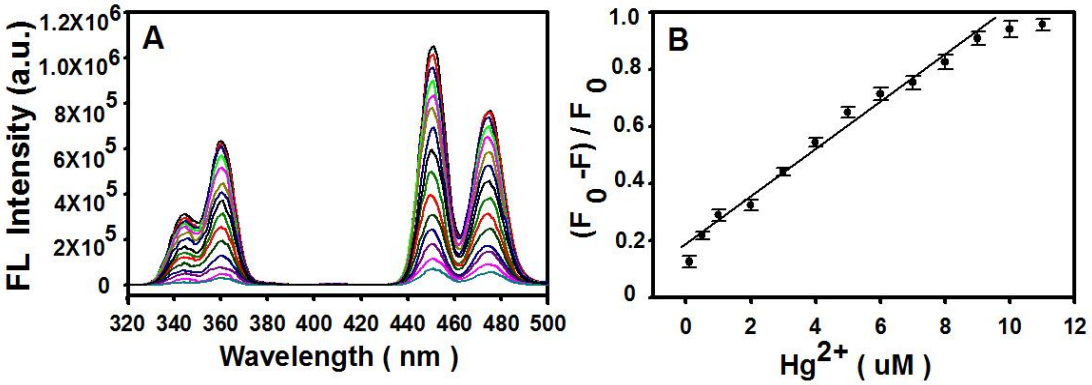


Fig. S7 Effect of temperature for the DNA-UCNPs with Hg^{2+} ions on the upconversion fluorescence quenching. There no obvious changes in the fluorescence signal could be observed in the range from 25 ℃ to 50 ℃. The concentrations of DNA-UCNPs and Hg^{2+} ions were 50 μg mL^{-1} and 10 uM, respectively.



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243 **Fig. S8** (A) Upconversion fluorescence spectra of the biosensor with varying

244 concentrations of Hg^{2+} ions. (B) Linear relationship between the fluorescence relative

245 intensity and the concentrations of Hg^{2+} ions within the range of 10 nM-10 uM. All

246 experiments were performed in local tap water. The slightly increased background in

247 the assay for local tap water was attributed to some other metal ions on UCNPs

248 surface, which might affect the interaction between the Hg^{2+} ions and the

249 DNA-UCNPs.

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Table S1 The comparison of different sequences of the DNA-UCNPs for the determination of Hg^{2+} ions (DNA4 and DNA5 can form the T–Hg–T structure).

DNA	Added (uM)	The quenching effciency	RSD (n=3)
Bald UCNPs (the S1 nuclease-treated DNA-modified UCNPs)	10	95.8%	6.2%
DNA1 (5'-CGCAAAAAAGAGAGTAA-3')	10	95.2%	5.8%
DNA2 (5'-CCCCCCCCCCCC-3')	10	94.5%	4.5%
DNA3 (5'-ACCTGGGGGAGTATTGCGGA GGAAGGT-3)	10	93.4%	6.7%
DNA4 (5'-GGTCTTCCTTTTGTTCC-3')	10	89.5%	4.9%
DNA5 (5'-TTCTTTCTTCCCCTTGTTTGTT-3')	10	87.2%	5.8%

Table S2 The comparison of sensors for the determination of Hg^{2+} ions.

Method	Target(s)	LODa	Ref
Colorimetric	Hg^{2+}	200 nM	S4
	Hg^{2+}	100 nM	S5
	Hg^{2+}	500 nM	S6
	Hg^{2+}	50 nM	S7
Fluorescence	Hg^{2+}	40nM	S8
	Hg^{2+}	32 nM	S9
	Hg^{2+}	15 uM	S10
the proposed assay	Hg^{2+}	5 nM	

3. References

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