Supporting Information

Efficient Fluorescence Resonance Energy Transfer between Upconversion Nanophosphors and Graphene Oxide: A Highly Sensitive Biosensing Platform

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Experimental Section

Materials and reagents

Poly(acrylic acid) (PAA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), 2-(-N-morpholino)ethanesulfonic acid (MES), 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) and tris(hydroxymethyl) methanamine (Tris) were obtained from Sigma-Aldrich. Graphite powders (325 mesh) were purchased from Alfa Aesar. All of other reagents used in this work were of analytical grade and were used as purchased without further purification. Adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), uridine triphosphate (UTP) and all of the oligonucleotides were supplied by Sangon Biotechnology Co. Ltd. (Shanghai, China).

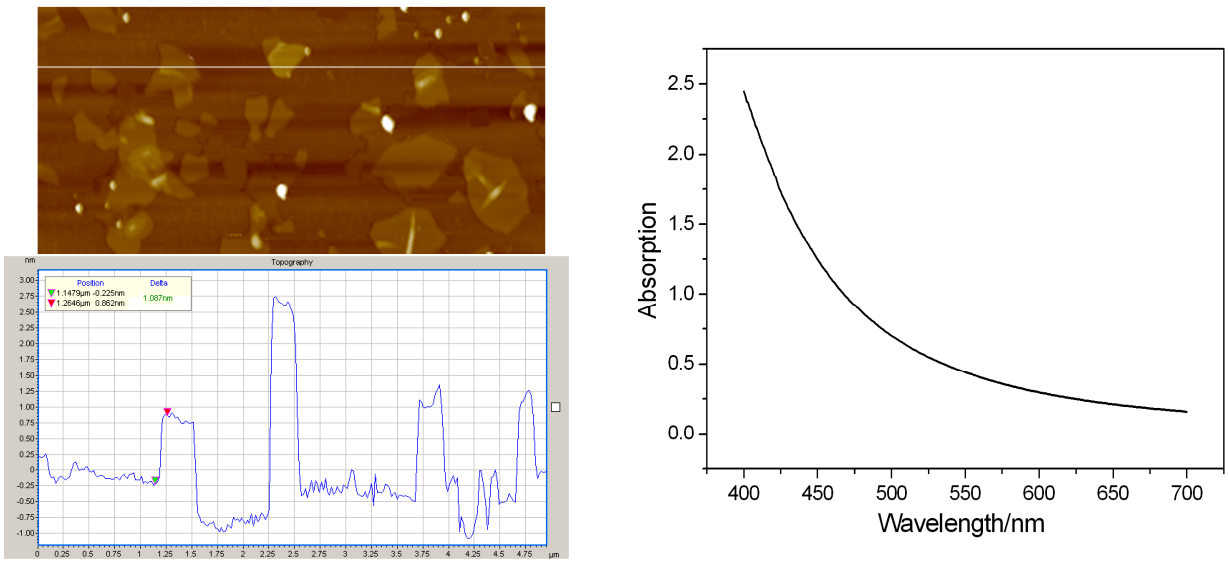
The oligonucleotides used in this work were listed below:

ATP aptamer: 5′-NH_2-TTTTACCTGGGGGAGTATTGCGGAGGAAGGT-3′

Hg^{2+} probe: 5′-NH_2-TTCTTCTTTCTTCCCCTTGTTTGTTG-3′

Synthesis and characterization of graphene oxide

Graphene Oxide (GO) was prepared from graphite powder by a modified Hummer's method.[1, 2] Briefly, 2 g of graphite powder was put into 80 mL concentrated H_2SO_4 and left stirring for 2 h. Afterwards, 10 g $KMnO_4$ was added gradually under stirring by keeping the temperature of the mixture below 20 °C. The mixture was then stirred at 35 °C for 2 h. Successively, distilled water (180 mL) was added and the mixture was stirred at room temperature for 3 h while the temperature was kept below 50 °C. Finally, 450 mL of distilled water and 20 mL H_2O_2 (30%) was added to the mixture to end the reaction. At the end, the mixture was repeatedly washed with 1:10 HCl aqueous solution, and then distilled water for several times. Exfoliation was carried out by sonicating graphene oxide dispersion for 1 h. At last, the resulted product was centrifuged at 12000 rpm for 10 min, and the upper supernatant solution was taken and used for further experiments.



(a) **(b)**

Fig. S1 (a) Tapping mode AFM image (top) of GO sheets on mica substrate and the cross-section profile (bottom) along the white line in the AFM image; **(b)** Absorption spectrum of as-prepared GO (~0.2 mg/mL).

The morphology of the prepared GO was characterized on an Agilent 5500 atomic force microscopy (AFM) using the tapping mode. The sample used for AFM examination was prepared by depositing a droplet of GO dispersion (3 μL, ~10 μg/mL) on a freshly cleaved mica surface and dried at room temperature. Fig. S1a showed the typical AFM image of the prepared GO. From the depth profiles along the white line shown in Fig. S1a, it could be seen that the thickness of single-layer and bilayer GO was about 1.1 nm and 2.8 nm, respectively. According to the cross-section analysis, the average thickness of GO sheet was about 1.0~1.4 nm, which was matched well with the reported thickness of single-sheet graphene oxide.[2-4] The few white areas in the AFM images might be ascribed to the wrinkles of GO sheets with height of 5~10 nm, which were also observed by other groups.[4,5]

Fig. S1b showed the absorption spectrum of the as-prepared GO. It can be seen that the absorption spectrum of GO spans a wide range of wavelengths (approximately 400–700 nm), overlapping the fluorescence spectra of various fluorescent materials including the β-$NaYF_4$:Yb,Er UCNPs, which is in good accordance with the literature results.[3]

Preparation of ssDNA-UCNPs conjugations

Fig. S2 showed the typical transmission electron microscopy (TEM) images of the β-$NaYF_4$:Yb,Er UCNPs synthesized by using our previously reported approach.[6] These UCNPs were spherical, uniform and highly monodisperse with an average diameter of 29 nm. They exhibited the strongest green emission centered at 547 nm under 980 nm laser irradiation, and the fluorescence efficiency was more than 50 times higher than that of α-$NaYF_4$:Yb,Er UCNPs with similar sizes,[6] which indicated that high detection sensitivity should be acquired when using the synthesized β-$NaYF_4$:Yb,Er UCNPs as fluorescent probes for bioassays.

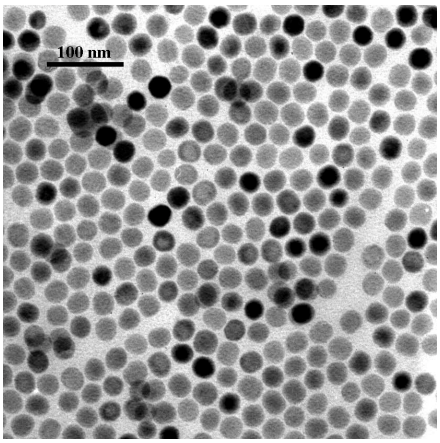


Fig. S2 Typical TEM image of β-$NaYF_4$:Yb,Er UCNPs

Standard procedures[7] were employed with modifications to crosslink carboxylic acid groups on UCNPs with amine-containing ssDNA. Take the preparation of ATP aptamer-UCNPs conjugation as an example. Briefly, EDC (2 mM) and NHS (5 mM) were firstly added to 2 mL of MES buffer (10 mM, pH 5.5) containing 5 mg PAA-UCNPs to activate the surface carboxylic acid group. The mixture was incubated at 30 °C for 2 h with shaking. After centrifugation and washed with distilled water for three times, the precipitate was added to 2 mL of HEPES buffer (10 mM, pH 7.2) containing 2.5 μM ATP aptamer. The linkage reaction was allowed to proceed at 30 °C for 4 h. Afterwards 50 mg Tris was added to block any unreacted NHS. Finally, ATP aptamer-functionalized $NaYF_4$:Yb,Er UCNPs were centrifuged, washed several times with water and dispersed in 1 mL Tris-HCl buffer (pH 7.2) for further applications.

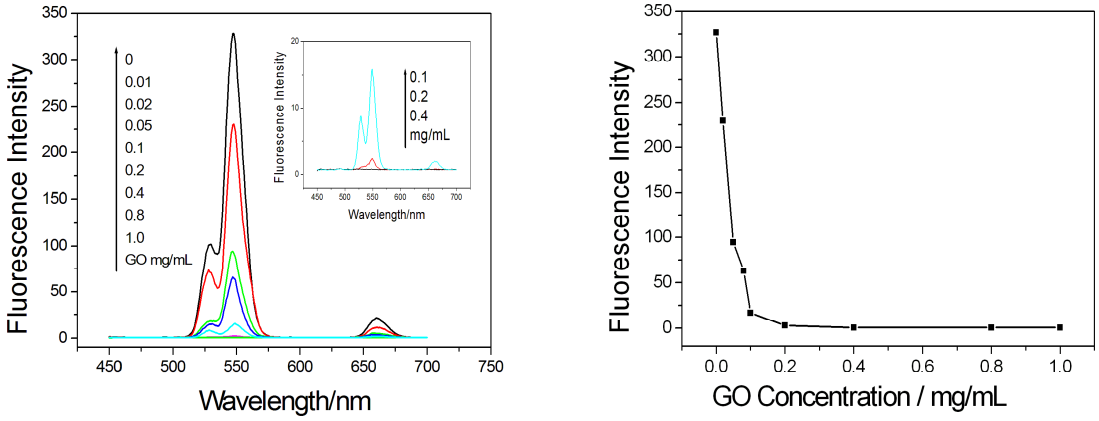
The cross-linking of Hg^{2+}-probe with UCNPs was performed following the similar procedures.

Procedures for ATP and Hg^{2+} detection

For ATP detection, a certain concentration of ATP and ATP aptamer-functionalized UCNPs (final concentration of 0.38 mg/mL) were mixed in Tris-HCl (10 mM containing 150 mM NaCl, pH 7.2) buffer. The mixture was first heated at 90 °C for 2 min and then kept at 30 °C for 1 h. Afterwards GO (final concentration of ~0.8 mg/mL) was added to the mixture and further incubated at 30 °C for 80 min. Then the upconversion fluorescence spectra of the final mixture were measured using an F-4500 fluorescence spectrometer (Hitachi, Japan) with an external 500 mW 980 nm laser diode with 1 m fiber (Beijing Viasho Technology Co.) as the excitation source in place of the xenon lamp in the spectrometer.

The detection of Hg^{2+} was carried out following the similar procedures except that Hg^{2+} probe-functionalized UCNPs were first incubated with different concentration of Hg^{2+} at 90 ºC for 2 min and 30 ºC for 15 min, and then mixed with GO for 80 min before the fluorescence measurements.

Effect of GO concentration on the upconversion fluorescence quenching efficiency



(a) **(b)**

Fig. S3 (a) Upconversion fluorescence spectra of ssDNA-UCNPs (0.38 mg/mL) after incubation with different concentrations of GO. **Inset:** Enlarged fluorescence emission spectra of ssDNA-UCNPs upon addition of 0.1, 0.2, and 0.4 mg/mL GO; **(b)** the plot of upconversion intensity recorded at 547 nm vs GO concentration. The incubation of ssDNA-UCNPs and GO was carried out at 30 °C for 80 min.

The final concentration of ssDNA-UCNPs used throughout this work was 0.38 mg/mL. When the concentration of ssDNA-UCNPs was fixed, the quenching efficiency could be strongly influenced by GO concentration. As shown in Fig. S3, with the increasing concentration of GO, the upconversion fluorescence intensity of ssDNA-UCNPs decreased obviously and tended to be completely quenched when GO concentration was higher than 0.4 mg/mL. For this upconversion fluorescence-enhanced detection assay, the sensitivity was strongly dependent on the initial quenching efficiency of GO on UCNPs. Therefore, 0.8 mg/mL GO was employed for analytical purpose in this work to ensure high quenching efficiency.

Kinetic characterization of the upconversion fluorescence quenching reaction

To further understand the interaction between GO and ssDNA-UCNPs and the ATP induced upconversion fluorescence recovery, time dependent fluorescence quenching experiments were conducted for ssDNA-UCNPs and ssDNA-UCNPs-ATP complexes in the presence of GO. As shown in Fig. S4, when GO (0.8 mg/mL) was added to

ssDNA-UCNPs solution, the upconversion fluorescence intensity reduced rapidly at the beginning and was completely quenched after 80 min. However, upon the addition of GO (0.8 mg/mL), the ssDNA-UCNPs solution after incubation with ATP (0.5 mM) at 90 °C for 2 min and then 30 °C for 1 h showed a slow decrease in the fluorescence intensity, indicating a decrease in the adsorption rate of ssDNA-UCNPs-ATP complexes on GO. After addition of GO for 70 min, the fluorescence still remained at ~40% of the original intensity of ssDNA-UCNPs in Tris-HCl buffer, and would kept stable for several hours. Based on these phenomena, 80 min incubation time for GO and ssDNA-UCNPs or ssDNA-UCNPs-ATP complexes was selected for further analytical applications.

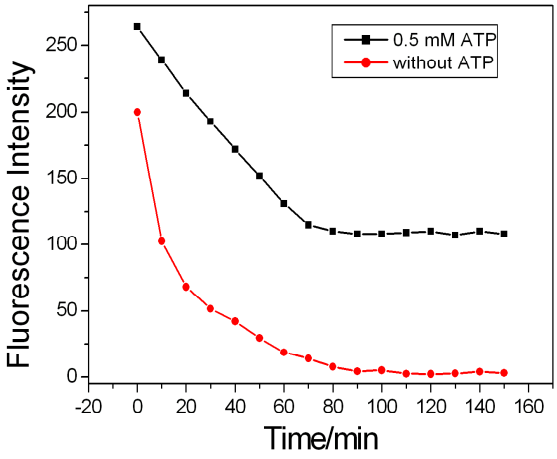


Fig. S4 Fluorescence quenching of ssDNA-UCNPs (0.38 mg/mL) by GO (0.8 mg/mL) before and after being incubated with ATP (0.5 mM) as a function of time.

Specificity evaluation for ATP detection

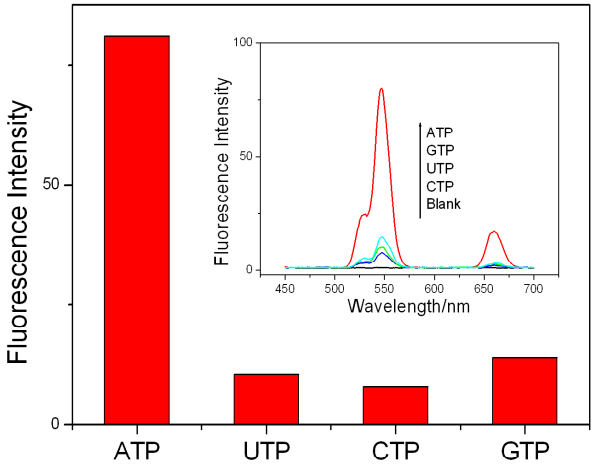


Fig. S5 Fluorescence intensity (rescorded at 547 nm) of the UCNPs-GO FRET aptasensor in the presence of 100 μM ATP, UTP, CTP, or GTP. The inset was the corresponding fluorescence spectra.

Analytical performance for Hg^{2+} detection

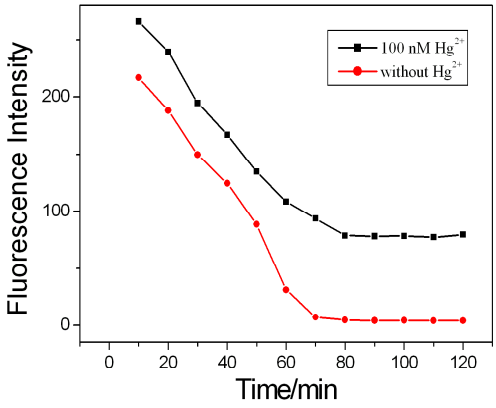


Fig. S6 Fluorescence quenching of Hg^{2+} probe-functionalized UCNPs (0.38 mg/mL) by GO (0.8 mg/mL) before and after being incubated with Hg^{2+} (100 nM) as a function of time. 80 min incubation time for GO and Hg^{2+} probe-UCNPs or Hg^{2+} probe-UCNPs-Hg^{2+} complexes was selected for Hg^{2+} detection.

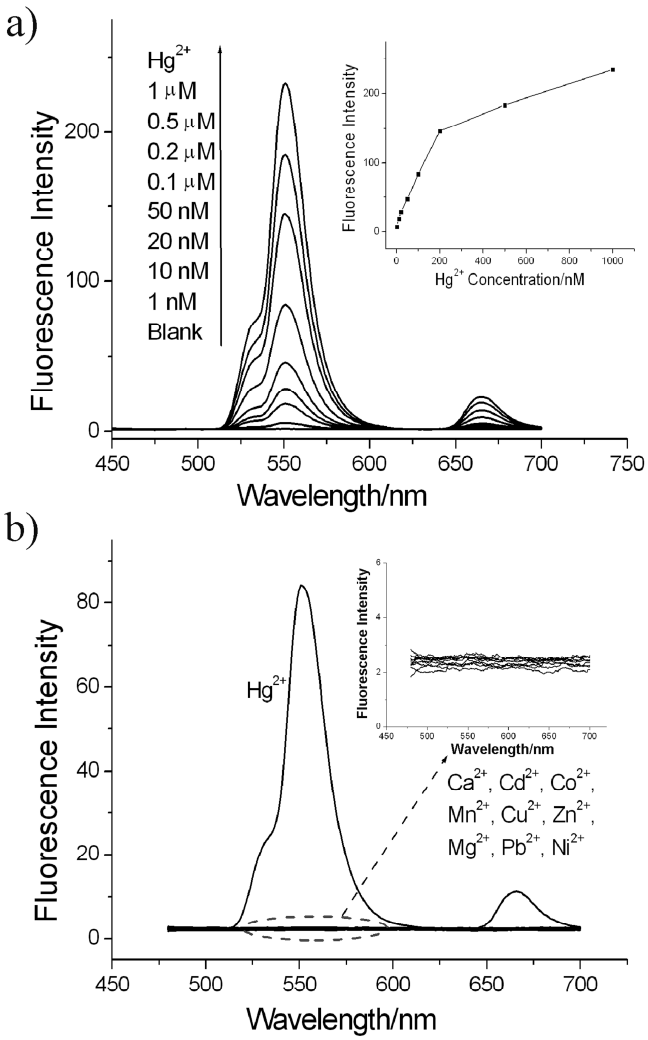


Fig. S7 (a) Upconversion fluorescence spectra of the UCNPs-GO FRET sensor in the presence of 0 ~ 1 μM Hg^{2+}. The inset is the plot of fluorescence intensity at 547 nm vs Hg^{2+} concentration. A good linear relationship is observed between the

upconversion fluorescence intensity and Hg^{2+} concentration in the range of 1 nM to 200 nM (R=0.9978). **(b)** Interference evaluation of various divalent cations on Hg^{2+} detection with the same concentration of 100 nM. Experimental conditions: GO, 0.8 mg/mL; Hg^{2+} probe-UCNPs, 0.38 mg/mL; λ_{EX}=980. Hg^{2+} probe-UCNPs are first incubated with different concentration of Hg^{2+} at 90 ºC for 2 min and 30 ºC for 15 min, and then mixed with GO for 80 min before the fluorescence measurements.

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