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Electronic Supporting Information

Gold Nanorods-based FRET Assay for Ultrasensitive Detection of Hg^{2+}

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Experimental Details.

Chemicals. Unless otherwise indicated, all reagents and solvents were purchased in their highest available purity and used without further purification or treatment. $HAuCl_4 \cdot 4H_2O$ was purchased from Sigma (USA). Fluorescein-tagged thymine-rich DNA (FDNA) (5'-TTCTTTCTTCCCCTTGTTTGTT-FAM-3'), and T-rich DNA (5'-TTCTTTCTTCCCCTTGTTTGTT-3') were synthesized by Shanghai Sangon Biotechnology Co. (Shanghai, China) and used without further purification. The oligonucleotide stock solutions were prepared with a Tris-HCl buffer (10 mM Tris-HCl, pH 7.4, 0.1 M NaCl and 5 mM KCl) and kept frozen. Millipore Milli-Q (18.2 MΩ.cm) water was used in all experiments.

Instrumentation. Fluorescence spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer. CD spectra were measured on a Chirascan Circular Dichroism Spectrometer (Applied Photophysics Ltd, England). UV-visible adsorption spectra were recorded on U-3900H UV-Vis spectrophotometer (Tokyo, Japan). TEM images were obtained using a JEM-2100 transmission electron microscope.

Synthesis of Gold Nanorods. Gold nanorods were synthesized by using a seed-mediated, surfactant-assisted growth method in a two-step procedure [1, 2]. Briefly, colloidal gold seeds were first prepared by mixing aqueous solutions of hexadecylcetyltri-methylammonium bromide (CTAB, 0.1 M, 7.5 mL) and hydrogen tetrachloroaurate (III) hydrate (1%, 0.098 mL). Freshly prepared aqueous solution of sodium borohydride (0.01M, 0.6 mL) was then added. The colloidal gold seeds solution (0.215 mL) were then injected into an aqueous growth solution of CTAB (0.1 M, 47.6 mL), hydrogen tetrachloroaurate (III) hydrate (1%, 0.788 mL), silver nitrate (0.01 M, 0.3 mL) and freshly prepared ascorbic acid (0.1 M, 0.32 mL). The nanorods were purified by several cycles of suspension in ultrapure water, followed by centrifugation. They were isolated in the precipitate, and excess CTAB was removed in the supernatant. Then, it was stored in a refrigerator at 4 °C before being used. The concentration of GNRs (12.4 nM) was estimated by UV/vis spectroscopy based on an extinction coefficient of 4.6×10^{8} $M^{-1}\cdot cm^{-1}$ at λ=782.5 nm for gold nanorods. [3]

Fluorescence Experiments. First, the fluorescence spectra of FAM-labeled DNA were recorded on a fluorometer (F-7000, Hitachi) with excitation at 480 nm and an emission range from 500 to 600 nm. Then, a certain volume of GNRs solution was added into the FAM-labeled DNA solution. For Hg^{2+} detection, appropriate concentrations of Hg^{2+} solution was added into the above mixture for fluorescence detection.

CD Spectroscopy. The CD spectra of DNA oligonucleotides were measured for 2 μM DNA total strand concentration using a Chirascan Circular Dichroism Spectrometer (Applied Photophysics Ltd, England). CD spectra were recorded using a quartz cell of 1-mm optical path length and an instrument scanning speed of 100 nm/min with a response time of 2 s at room temperature. CD spectra were obtained by taking the average of three scans made from 200 to 320 nm. All DNA samples at a final concentration of 2 μM were dissolved in Tris-HCl buffer and heated to 90 °C for 5 min, gradually cooled to room temperature, and incubated at 4 °C overnight.

UV Measurements. Typically, the UV absorb spectra of GNRs were recorded on UV-Vis spectrophotometer (U-3900H, Tokyo, Japan). Then, a certain quantity of DNA was added into GNRs suspension solution. After thorough mixing, a suitable Hg^{2+} was added to the mixture. The solution is vortexed thoroughly, and used for the UV–Vis absorption spectra measurements experiment.

Analysis of Water.

The applications of the proposed method were evaluated for determination of Hg^{2+} in tap water samples. For tap water, the sample was collected after discharging tap water for about 20 min and boiled for 5 min to remove chlorine. All the water samples were spiked with Hg^{2+} at different concentration levels. The concentrations of Hg^{2+} were determined by our new approach and the cold vapor atom fluorescence spectroscopy (CVAFS). These data showed no obvious difference between the results of our new approach and that of the standard methods. The results are summarized in Table 2 and

shown good agreement with the expected and found values. This exhibited the practicality our method for detecting Hg^{2+} in real water samples.

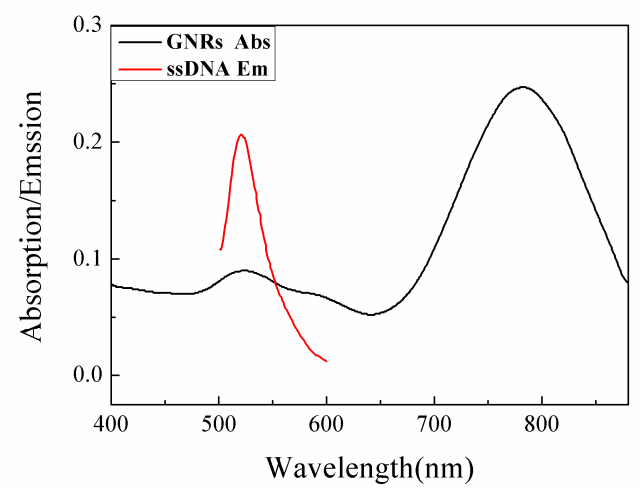

Figure S1 Absorption spectrum of GNRs and emission spectrum of F-DNA.

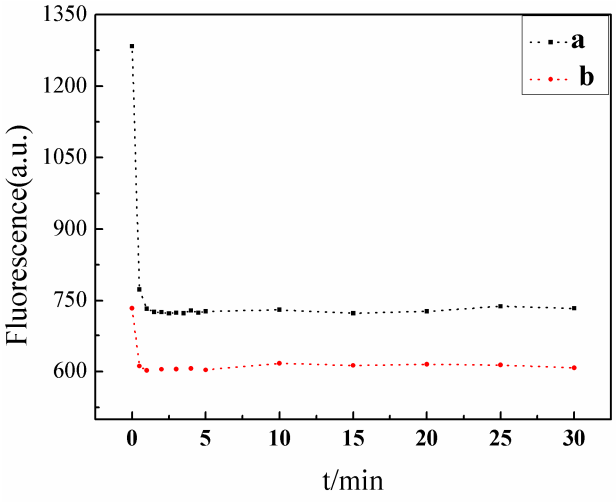

Figure S2 Fluorescence quenching of F-DNA (20 nM) in Tris–HCl buffer by GNRs as a function of time (a) in the absence of Hg^{2+} and (b) in the presence of Hg^{2+}.

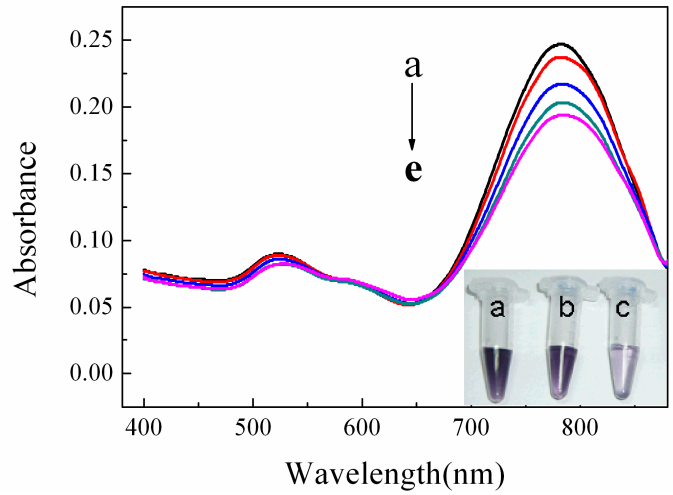

Figure S3 UV-vis absorption of GNRs and GNRs/ssDNA complex in the presence of Hg^{2+}. (a) GNRs, (b) GNRs/ssDNA, (c-e) GNRs/ssDNA + Hg^{2+}, from curve c to curve e, the concentration of Hg^{2+} is 30 nM, 60 nM, 90 nM, respectively. The inset shows visual detection Hg^{2+} based on the color change of GNRs. As follows: GNRs (tube a), GNRs + ssDNA (tube b), GNRs + ssDNA + 90 nM Hg^{2+} (tube c).

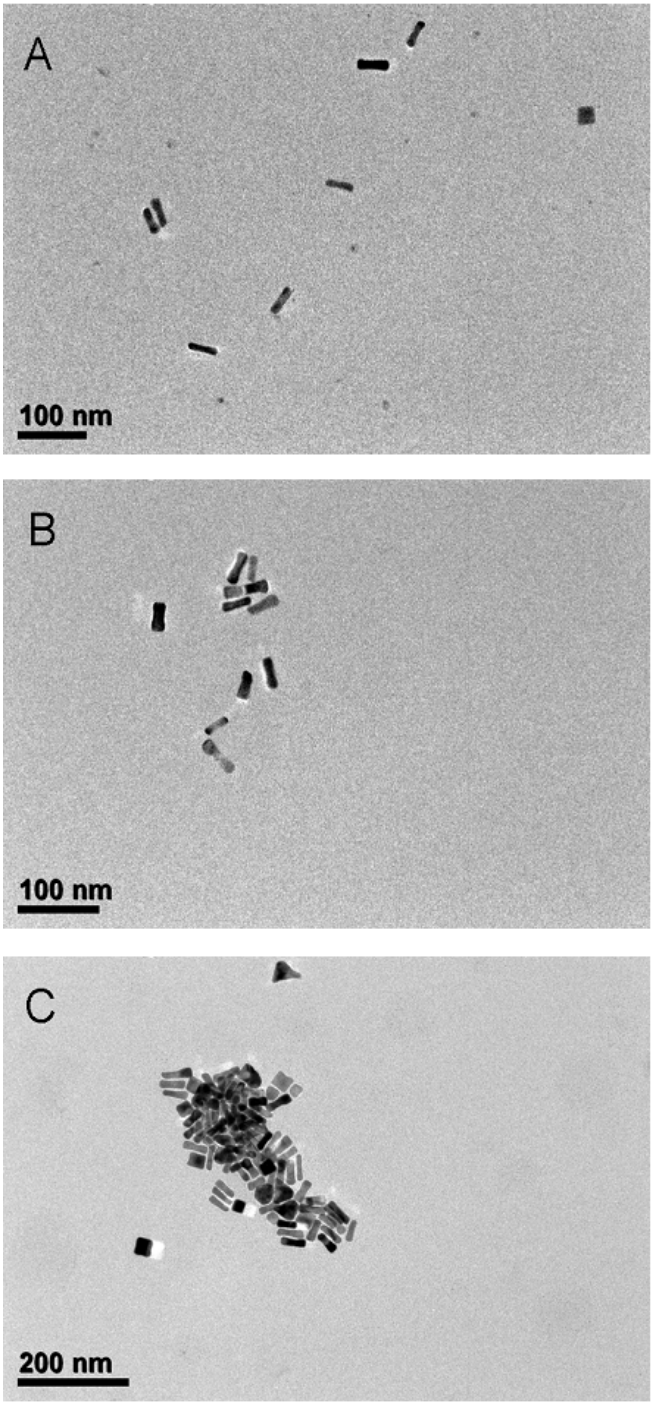

Figure S4 TEM images of GNRs (A) in the presence of ssDNA (B) and the mixture of ssDNA and Hg^{2+} (C).

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Table 1 Comparison of different optical methods for specific Hg^{2+} detection.

Method	Transducer	Operation	Detection limit	Response time	Selectivity
FRET(this study)	Turn-off	Simple	2.4 pM	2 min	good
FRET(labeled) [4]	Turn-off	Simple	40 nM		good
FRET(labeled) [5]	Turn-on	Simple	40 nM		good
FRET(labeled) [6]	Turn-on	Simple	3.2 nM	3 min	good
FRET(labeled) [7]	Turn-off	Simple	5.0 nM	15 min	moderate
FRET(labeled) [8]	Turn-on	Complex	2.1 nM	5 min	good
FRET(labeled) [9]	Turn-on	Simple	14.5 nM	20 min	good
FRET(labeled) [10]	Turn-on	Simple	10 nM		moderate
Fluorescence[11]	Turn-on	Simple	1.33 nM	5 min	good
Fluorescence[12]	Turn-off	Simple	5 nM	10 min	good
Colorimetric [13]	Color	Complex	100 nM		moderate
Colorimetric [14]	Color	Complex	3 μM		moderate
Colorimetric [15]	Absorbance	Simple	30 nM		good
Colorimetric [16]	Color	Complex	0.1 nM	20 min	good
Colorimetric [17]	Absorbance	Simple	500 nM	1 min	good
Colorimetric [18]	Absorbance	Simple	10 nM	20 min	moderate
Fluorescence[19]	turn-off/on	Simple	5 nM /1.5 nM	5 min	good
Fluorescence[20]	Turn-on	Complex	2.4 nM	5 min	good
Colorimetric[21]	Absorbance	Complex	50 nM		good
Fluorescence[22]	Turn-on	Simple	2.8 nM	2 min	moderate
Electrochemical [23]	Turn-on	Complex	0.02 nM	30 min	moderate
Gel-based[24]	Color	Complex	75 nM	1 h	moderate

Table 2 Determination of Hg^{2+} in water samples using the proposed method and CVAFS.

Samples	Added(nM)	The proposed method mean±SD[a](nM)	CVAFS mean[b] (nM)
Tap water 1	5	5.31±0.31	5.52±0.69
Tap water 2	8	8.09±0.12	7.91±0.06

[a] Mean of three determinations ± SD; SD, standard deviation.

[b] Cold vapor atom fluorescence spectroscopy.

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