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Electronic Supplementary Information (ESI)

Mercury Nanoladders: A New Method for DNA Amplification, Signal Identification and Its Application on the Detection of Hg(II) Ions

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1 Experimental section of our work:

1.1 Materials and methods

All the synthetic DNA sequences listed in Table S1 were commercially synthesized at KareBay Biochem Inc. In detail, FAM-P1 were labeled with a fluorophore, FAM (fluorescein amidite) at the end of 5' with a length of 30 nt. The sequences of FAM-P1 and P2 are complementary apart from some T-T mismatches between them. This configuration guarantees that the two oligonucleotides hybridize to each other and produce a long DNA duplex only when Hg(II) is added.

GO aqueous solution was purchased from Nanjing XFNANO Materials Tech Co., Ltd. GO thickness as-received was ~1.6 nm, characteristic of a fully exfoliated GO sheet, with average lateral size of about 50-200 nm. $Hg(NO_3)_2$ was purchased from Sigma and dissolved in 0.5% HNO_3. All other reagents were of analytical grade and used without further purification or modification. Ultrapure water used throughout was obtained from a Milli-Q water purifying system.

The GO sheets were observed under a tapping mode atomic force microscope (AFM) SPA300HV equipped with a SPI-3800 controller (Seiko Instruments Industry Co., Japan). Tapping mode AFM was performed with a nanoscope multimode scanning probe micro-scope (Bruker/Digital Instrument, Santa Barbara, CA). The samples for AFM measurement were prepared by depositing a drop of aqueous GO solution on a freshly cleaved mica surface and drying at room temperature. We used a Chirascan™-plus CD Spectrometer (Applied Photophysics, UK) to collect CD measurements.

1.2 Fabrication of Hg(II) nanoladders and HNIG biosensor.

The commercially synthetic DNA sequences FAM-P1 and P2 were dissolved in ultrapure water to a final concentration of 10 μM. Different concentrations of Hg(II) were then incubated with 50 nM FAM-P1 and 100 nM P2 in Tris-HCl buffer B1(10 mM Tris, 1 mM $MgCl_2$, pH 7.4) at 37 °C for 30 min to trigger the self-assembly of the Hg(II) nanoladders. Different concentrations of FAM-P1 and P2 were exploited to improve the binding ratios of the fluorescent probe, and ultimately to ensure the accuracy of the HNIG system. We then added 25 μg/mL GO to the reaction system and incubated the mixture at room temperature for 5-10 min. The HNIG biosensor was established during this step. The final volume of the reaction solution was 100 μL. The same procedure without Hg(II) was used as a blank control.

1.3 Optimization of reaction conditions.

The optimization of DNA-GO adsorption system were carried out based on calculation and experimental verification. The following buffer solutions were prepared: PBS buffer (8.1 mM Na_2HPO_4, 1.9 mM NaH_2PO_4, 120 mM NaCl, pH 7.4), Tris-HCl buffer (10 mM Tris, 500 mM NaCl, 1 mM $MgCl_2$, pH 7.4), HEPES buffer (10 mM HEPES, 200 mM $NaNO_3$, pH 7.4), and 4×SSC buffer (containing 2% BSA and 0.05% Tween-20, pH 7.4). In a typical experiment, 50 nM FAM-P1, 100nM P2 or T1, 25μg/mL GO with or without 1μM Hg(II) were mixed in varying buffers. After approximately 10 min mixture of DNA and GO, the fluorescence was read by a real-time PCR thermocycler in the FAM channel at 25 ℃. After that, the ionic strength and organic components in Tris-HCl buffer were farther optimized using the same procedure above.

Table S1. Experimental DNA Probe Sequences

Name	Sequence（5'→3'）
FAM-P1	FAM-*TACG**TT**GC**TT**C**T**CTG*CCTG**TT**GC**T**C**TT**CTT
P2	*CAG**T**G**TT**GC**TT**CGTA*AAG**TT**G**T**GC**TT**CAGG
T1	AAGAAGAGCAACAGGCAGAGAAGCAACGTA
P1(27-1)	*TAG**TT**GCC**TTT**CTG*CTG**TT**GCC**TTT**CT
P2(27-1)	*CAG**TTT**GGC**TT**CTA*AG**TTT**GGC**TT**CAG
P1(24-1)	*TG**TT**GCC**TTT**CG*T**T**C**T**G**T**C**T**C**T**CT
P2(24-1)	*CG**TTT**GGC**TT**CA*AG**T**G**T**G**T**C**T**G**T**A

*Boldface type presents T-T mismatches in Hg(II) nanoladders. Italic and underlining type indicate complementary characteristics. T_1 is the complementary strand of FAM-P_1.

Table S2. The Components of Different Tris Buffers Used in the Experiment

	Tris	**NaCl**	**$MgCl_2$**
A_1	0 mM		
A_2	1 mM		
A_3	5 mM	500 mM	1mM
A_4	10 mM		
A_5	20mM		
B_1		0 mM	
B_2		100 mM	
B_3	10 mM	200 mM	1mM
B_4		300 mM	
B_5		600 mM	
C_1			0 μM
C_2			200 μM
C_3	10 mM	500 mM	500 μM
C_4			2 mM
C_5			5 mM

Table S3. Hg(II) Recovery Experiment from Drinking Water Samples

Sample	**Hg(II) added (nM)**	**Hg(II) detected (nM)***	**Recovery (%)**
1	3	$3.1^{a}\pm0.03^{b}$	103.3 ±0.1
2	6	$5.8^{a}\pm0.05^{b}$	96.67±0.8

*The value of Hg(II) detected consists of two parts: [a] represents the mean value of three individual determinations, and [b] demonstrates the standard deviation.

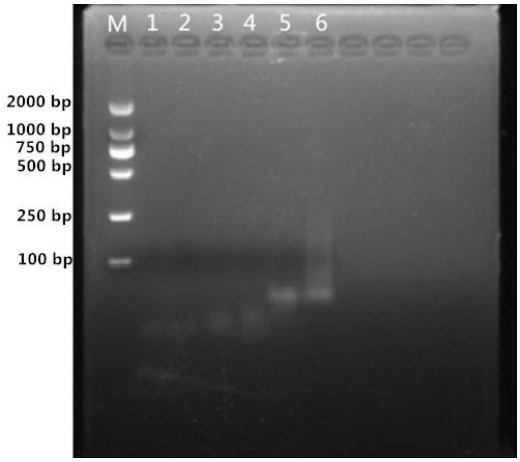

Figure S1. The confirmation of Hg(II) nanoladders

M：marker D2000；1：P1(24-1)+P2(24-1)；2：P1(24-1)+P2(24-1)+ Hg(II)；3：P1(27-1)+P2(27-1)；4：P1(27-1)+P2(27-1) + Hg(II)；5：P1+P2；6:P1+P2+ Hg(II)

The stability of T-Hg(II)-T is key to the formation of Hg(II) nanoladders. In this part, agarose gel electrophoresis were used to describe the formation of the sandwich struncture of nanoladders. Agarose gel electrophoresis of Hg(II) nanoladders generated by P1-P2, P1 (27-1) -P2 (27-1), P1 (24-1) -P2 (24-1) is shown in figure S1. The highest signal/noise ratio was obtained using P1-P2 as a template, which means DNA sandwich can not grow without Hg(II), and the addition of Hg(II) can effectively promote the generation of Hg(II) nanoladders. Therefore, we chose P1-P2 as the template sequence of Hg(II) nanoladders.

Table S4. Comparison of analytical parameters resulting from different methods for the detection of Hg(Ⅱ) ions.

Method	Dynamic range	LOD	Time	Reference
Elisa	2.5 -49.9 nM	2.99 nM	few weeks	1
Fluorescent Rhodamine-Thioamide-Alkyne Chemosensor	0.5 nM-4μM	39 nM	2 days	2
Colorimetric/Fluorescent dual-mode sensor	1.0 - 10 nM	0.1 nM	75 min	3
Gold-dendrimer with Glucometer Readout Sensor	10.0 pM-100 nM	4.2 pM	＞200 min	4
Colorimetric Sensor based on G-Quadruplex	250-1250 nM	50 nM	240 min	5
HNIG biosensor	0-8 nM	1.5 nM	Less than 40 min	This work

Reference

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