Electronic Supplementary Information

Label-free supersandwich electrochemiluminescence assay for detection of sub-nanomolar Hg^{2+}

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Experimental Section

Apparatus:

Electrochemical and electrochemiluminescent measurements were conducted with an MPI-A CE-ECL system jointly produced by Xi'an Remex Electronics (China) and Changchun Institute of Applied Chemistry (Changchun, China), and a conventional three-electrode cell was used, which consisted of a modified Au working electrode, a platinum wire auxiliary electrode, and a Ag/AgCl reference electrode (saturated with 3 M KCl).

Chemicals and materials:

Tripropylamine (TPA), Ethylenediaminetetraacetic acid (EDTA), mercaptohexanol (MCH), dichlorotris(1, 10-phenanthroline) ruthenium hydrate ($Ru(phen)_3Cl_2 \cdot H_2O$), Tris(Hydroxymethyl)aminomethane (Tris), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), were obtained from Sigma-Aldrich. $Hg(NO_3)_2$, $MgCl_2$, $CaCl_2$, $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Cu(NO_3)_2$, $Ni(NO_3)_2$, $MnSO_4$, $Co(NO_3)_2$, $Pb(NO_3)_2$, $FeCl_3$ was purchased from Beijing Chemical Works. Three oligonucleotides were synthesized by Sangon Biotech (Shanghai) Co., Ltd, and used as received. The sequences of these oligonucleotides were:

A_1: 5'-HS-**TTCT TTCT TCC**-3'

A_2: 5'-*CGTT GTGA CTAG* GC**GG TTGT TTGTT**-3'

A_3: 5'-*CTAG TCAC AACG* CG**TT CTTT CTTCC**-3'

Supersandwich assay sequences:

5'-HS-**TTCT TTCT TCC**-3' (A_1)

3'-**TTGTTTGTTGG**CG*GATCAGTGTTGC*-5' (A_2)

5'-*CTAG TCAC AACG* CG**TT CTTT CTTCC**-3' (A_3)

3'-**TTGTTTGTTGG**CG*GATCAGTGTTGC*-5' (A_2)

5'-*CTAG TCAC AACG* CG**TT CTTT CTTCC**-3' (A_3)

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Three single strand DNA (ssDNA) recorded as A_1, A_2, A_3 were used in the proposed supersandwich assay. A_1 was a 11-mer T-rich oligonucleotide, and A_2 was a 25-mer oligonucleotide, which contained a part of 11-mer T-rich sequences (bold) to hybridize with A_1 and A_3 in the presence of Hg^{2+} by formation of T-Hg^{2+}-T coordination and a part of 12-mer complementary sequences (italic) to A_3. Before using, 1 μM A_2 and A_3 was mixed with equal volume and heated to 90°C and kept for 10 min, then the solution was cooled to room temperature and kept for 1 h. The concentrations of the used ssDNA (A_1, A_2, A_3) were 1 μM.

Fabrication of sensors:

Before using, the gold electrodes (2 mm in diameter) were polished on microcloth with 1 μm and 0.05 μm alumina, followed by sonication in water, ethanol and water for 2 min in each. After electrochemically cleaning in 0.5 M H_2SO_4, a droplet of 10 μL A_1 (1 μM A_1 in 10 mM Tris-HCl, 1 mM EDTA, 1 mM TCEP, 0.1 M NaCl, pH 7.40) was dropped onto the electrode to incubate for 30 min, then the electrode was rinsed with deionized water and passivated with 10 μL MCH (1 mM MCH in 10 mM Tris-HCl, pH 7.40) for 30 min. After that, the prepared electrodes were rinsed with water, dried with N_2, and incubated with a mixture of 5 μL different concentrations of Hg^{2+} and 5 μL partially hybridized A_2&A_3 (1 μM A_2&A_3 in 10 mM Tris-HCl, 0.1 M NaCl, pH 7.40) for 30 min. In order to intercalate ECL probe molecules of $Ru(phen)_3^{2+}$, a droplet of 10 μL 0.2 mM $Ru(phen)_3^{2+}$ was placed on the modified electrode and incubated for 5 h.

Before ECL detection, the electrode was rinsed with deionized water and 0.2 M phosphate buffer solution (PBS, pH 7.4). Both cyclic voltammetry (CV) and ECL experiments were conducted by using the MPI-A CE-ECL system in 0.2 M PBS (pH 7.40) containing 50 mM TPA. The CV was conducted from 0 to 1.3 V (versus Ag/AgCl) with the scan rate of 50 mV/s, and the voltage of the PMT was set at -400 V. Unless otherwise mentioned, the experiment was conducted at room temperature.

Comparison of the relative ECL signal increase (ΔI_R) by different modifications of the gold electrode. Some derivatives of Ru^{2+}, for example $Ru(phen)_3^{2+}$, has the capacity to intercalate into the grooves of double-stranded DNA (dsDNA) with high affinity.[s1] Previous fluorescent experiment indicates that the equilibrium constant between $Ru(phen)_3^{2+}$ and dsDNA was 1.24×10^4 M^{-1}, with a site size of 4.[s2] Every four base pairs can capture one $Ru(phen)_3^{2+}$ molecules, therefore, the double strand section of the partially hybridized A_2&A_3 (twelve base pairs included) can capture three $Ru(phen)_3^{2+}$ molecules.

The comparison of ΔI_R of the same gold electrode modified by four different processes was displayed in Fig. S1. Column 1 exhibited the lowest ΔI_R because the complementary base pairs between A_1 and A_2 were too limited, and no consecutive four base pairs were existed, thus the intercalation of $Ru(phen)_3^{2+}$ was inhibited. Column 2 and column 3 showed the medium ΔI_R because the T-Hg^{2+}-T coordination between A_1 and A_2 inhibited the T-Hg^{2+}-T coordination between A_1 and A_2&A_3. Column 4 displayed the highest ΔI_R because A_2 and A_3 were hybridized in the same concentration with a ratio of 1:1 before using, thus no dissociative A_2 existed.

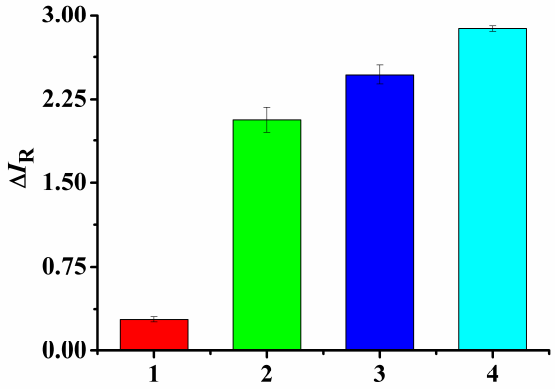

Fig. S1 comparison of 1) $A_1/A_2/Hg^{2+}/Ru(phen)_3^{2+}$ (9 μL A_2+1 μL Hg^{2+}); 2) $A_1/A_2\&A_3$ & $A_2/Hg^{2+}/Ru(phen)_3^{2+}$ (5 μL $A_2\&A_3$+4 μL A_2+1 μL Hg^{2+}); 3) $A_1/A_2\&A_3$ &A_2 &$A_3/Hg^{2+}/Ru(phen)_3^{2+}$ (5 μL $A_2\&A_3$+2 μL A_2+2 μL A_3+1 μL Hg^{2+}); 4) $A_1/A_2\&A_3/Hg^{2+}$/ $Ru(phen)_3^{2+}$ (9 μL $A_2\&A_3$+1 μL Hg^{2+}). The concentrations of Hg^{2+} and DNA (A_1, A_2, A_3) were 500 nM and 1 μM, respectively. 0.2 M PBS (pH 7.40) containing 50 mM TPA was used for ECL study.

Factors concerning the construction of the supersandwich sensor

Interaction between Hg^{2+} and A_1, $A_2\&A_3$. After the gold electrode was modified with the thioled A_1 and passivated with MCH, a mixture of 10 μL Hg^{2+} and partially hybridized $A_2\&A_3$ was dipped onto the electrode, and the incubation time for the formation of T-Hg^{2+}-T coordination was optimized. As shown in Fig. S2, the ECL intensity increases gradually with increasing incubation time and reaches a platform after 30 min. Therefore, the incubation time of T-Hg^{2+}-T coordination was set to 30 min.

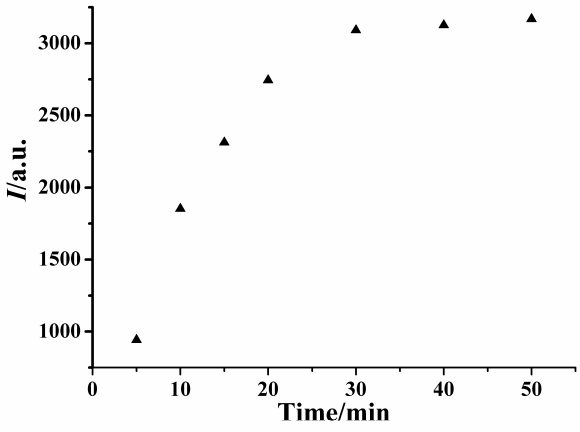

Fig. S2 The ECL profiles with the detection of 10 nM Hg^{2+} as a function of incubation time of Hg^{2+}.

Intercalation of $Ru(phen)_3^{2+}$ into the double-strand DNA. The intercalation time of $Ru(phen)_3^{2+}$ into the dsDNA among the supersandwich DNA sequences was optimized. With the increase in the incubation time, much more $Ru(phen)_3^{2+}$ can intercalate into the dsDNA, thus a gradual increase of ECL signals from one to five hours was observed in Fig. S3. However, a platform was obtained after five hours. So the incubation time of 5h was used for the intercalation of $Ru(phen)_3^{2+}$ during the experiment.

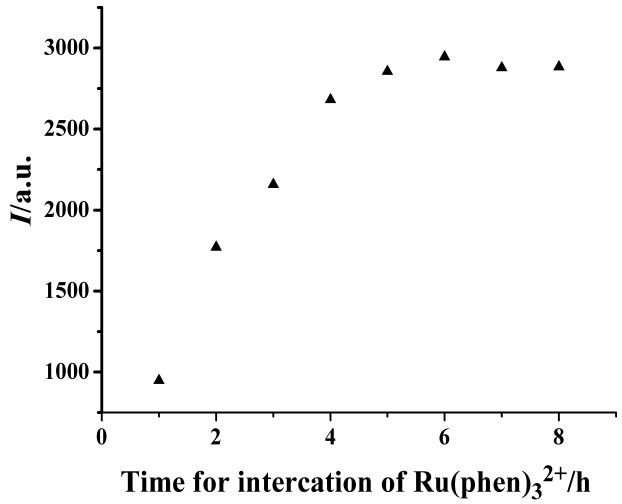

Fig. S3 The effect of the incubation time on intercalation of $Ru(phen)_3^{2+}$.

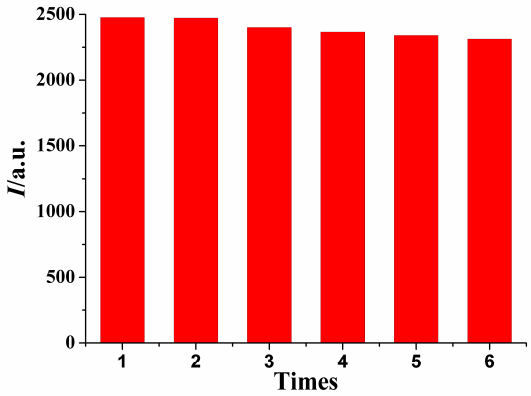

Fig. S4 The reproducibility of the supersandwich sensor for six parallel detection of 5 nM Hg^{2+} with RSD of 2.86%.

Table S1. Comparison of different methods for Hg^{2+} detection.

Detection technique	Liner range	Detection limit (nM)	reference
Colorimetry	0.2-6.0 μM	50	3
Fluorescence	0-66.43 nM	1.33	4
Electrochemistry	-	10	5

ECL	5-250 nM	5	6
ECL	5-500 nM	2.3	7
ECL	0.5-2500 nM	0.25	Present work

Supporting References

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