Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for

Analyte-Induced Formation of Partial Duplexes for the Preparation of a Label-Free Electrochemiluminescent Aptasensor

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Contents:

Materials and methods

Apparatus. The electrochemical measurements were carried out on a model LK98BII microcomputer-based electrochemical analyzer (Tianjin Lanlike High-Tech Company, Tianjin, China). A traditional three-electrode system was used with Ag/AgCl/KCl (sat) as reference electrode, a 2 mm diameter Au disk electrode modified with DNA as working electrode, and Pt wire as counter electrode. The ECL emission was detected with model MPI-A electrochemiluminescence analyzer (Xi'An Remax Electronic Science & Technology Co. Ltd., Xi'An, China) at room temperature, and the voltage of the PMT was set at -900 V in the detection process.

Chemicals and Materials. Cocaine was obtained from National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. Dichlorotris(1,10- phenanthroline) ruthenium hydrate ($Ru(phen)_3Cl_2 \cdot H_2O$) and tripropylamine (TPA) (Sigma-Aldrich, Shanghai, China) were used as ECL probe and co-reactant with phosphate buffer solution (PBS) as detection electrolyte. 6-Mercapto-1-hexanol, used to block the sensing interface for detection, was obtained from J&K Chemical Ltd., Beijing, China. The 2 mm diameter Au disk electrode was obtained from Tianjin Lanlike High-Tech Company (Tianjin, China). Cocaine was prepared in PBS buffer (100 mmol L^{-1}, pH 7.4). All oligonucleotides were diluted to 5 μmol L^{-1} in TE buffer (10 mmol L^{-1} Tris-HCl, 1 m mol L^{-1} EDTA, pH 7.5). The stock solution of TPA and $Ru(phen)_3^{2+}$ were prepared in doubly distilled water. 30-Mer thiolated anti-cocaine aptamer was prepared by Takara Biotechnology (Dalian, China) with following sequence: 5'-SH-$(CH_2)_6$-GACAA GGAAA ATCCT TCAAT GAAGT GGGTC-3'.

Procedure of the detection of cocaine with the ECL aptasensor. The procedure for determination of cocaine using the ECL aptasensor was illustrated in Scheme 1. After new gold electrode was polished with 0.3 μm aluminum slurry and ultrasonicated with distilled water for 1 min, it was electrochemically cleaned in 1 M H_2SO_4 via potential scanning between 0.0 and +1.6 V until a stable

voltammetric peak was obtained. Further, the electrode was sonicated and cleaned thoroughly with doubly distilled water. After being dried with nitrogen airflow, the gold electrode was soaked in 4 μM thiolated aptamer solution to prepare aptamer-modified electrode for 3 h at 36 °C (Scheme 1A). To block the sensing interface, 10 μL of 0.1 M 6-Mercapto-1-hexanol solutions was dropped onto the electrode for 2 h at 36 °C (Scheme 1B). As shown in Scheme 1B-C, the electrode was then incubated in cocaine solution to capture the cocaine molecule and formed cocaine-binding conformation, where duplex construction formed. In order to insert or intercalate $Ru(phen)_3^{2+}$ molecules into duplexes, the modified electrode was immersed in 20 μM $Ru(phen)_3^{2+}$ solution over night as shown in Scheme 1D. After the doubly distilled water and PBS solutions were used to clean off the unbinding $Ru(phen)_3^{2+}$, the ECL intensity of the resulting functionalized electrode was tested.

Analysis of cocaine in real samples with the ECL aptasensor. To validate the proposed method, urine and plasma samples with and without spike were analyzed. The urine and plasma samples were obtained form healthy volunteers and provided by a local hospital. The plasma was sterile-filtered through 0.25 μm filter before use. Because of the high sensitivity and selectivity of the proposed method, the samples were not subject to other disposal (treatment). The urine and plasma sample were spiked with cocaine at different concentration level for the recovery experiment. The procedure for the analysis of the samples was totally same as that for the standard solution. Briefly, after the gold electrode was modified with anti-cocaine aptamer and was blocked using 6-Mercapto-1-hexanol, and the spiked samples were dropped onto the electrode. The cocaine contained in the samples induced the formation of partial duplex. Then, the electrode was immersed in 20 μM $Ru(phen)_3^{2+}$ solution over night for the intercalation of ECL probe, $Ru(phen)_3^{2+}$. The ECL emission was recorded for the quantification of cocaine in the samples and the recovery test.

Factors influencing the construction of the cocaine aptasensor

Interaction between cocaine and its aptamer. A gold-thiol interaction was extensively used to immobilize DNA on the gold electrode, and the incubation time for the formation gold-thiol bond was optimized. The signal intensity increased as the incubation time increased to 10 min and then level off until 40 min (Figure S1). This indicated interaction of thiol group with gold electrode is rapid. Therefore, an incubation time of 30 min was adopted for the subsequent work to keep the stable immobilization of aptamer.

Various works [s2, s3] studied the stability of the cocaine-aptamer complex in terms of their dissociation constant or binding constant. Binding constant is the equilibrium constant of the formation of cocaine-aptamer complex, but dissociation constant is the multiplicative inverse of binding constant, that is, the equilibrium constant of the dissociation of cocaine-aptamer complex. The dissociation constant or binding constant between cocaine and its aptamer is relatively inaccurate. For example, Sun et al [s1] reported the binding constant was $4.6\pm0.3\times10^{9}$ M, corresponding to the dissociation constant of 2.2×10^{-10} M^{-1}. However, Freeman et al [s2] reported the dissociation constant of cocaine-aptamer was 200 μM^{-1}, similar a previous result obtained by Stojanovic et al. [s3]

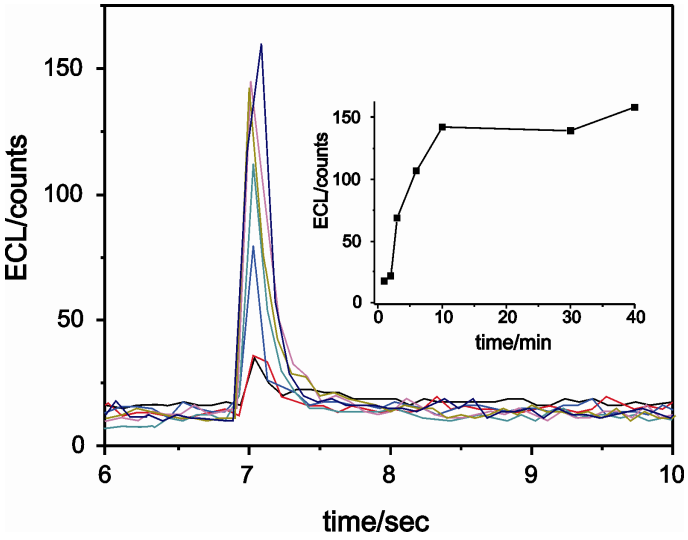

Figure S1. The ECL profiles with different incubation times of the aptamer-modified electrode in cocaine sample for detection of 10 pM cocaine. Inset: the relationship between ECL emission and the

incubation time.

Intercalation of $Ru(phen)_3^{2+}$ into the duplex. The $Ru(phen)_3^{2+}$ concentration was also optimized to maximize the sensitivity. Figure S2 shows the effect of $Ru(phen)_3^{2+}$ concentration on the ECL emission for the detection of 10 pmol L^{-1} cocaine. The ECL emission intensity increased as the concentration increased up to 20 μmol L^{-1} significantly and then slightly until 5 mmol L^{-1}. These results indicated there were two kinds of interactions of $Ru(phen)_3^{2+}$ to the formed duplex. One is the strong intercalation of $Ru(phen)_3^{2+}$, and the other is electrostatic (unspecific) interaction to the negative DNA strands. The results were similar to those of previous fluorescent study. [s4a] Fluorescent titration was used to investigate the intercalation of $Ru(phen)_3^{2+}$ into ds-DNA and gave a binding constant K of 1.24×10^4 mol^{-1} L. [s4a] To ensure complete intercalation of $Ru(phen)_3^{2+}$ and to decrease the unspecific adsorption to $Ru(phen)_3^{2+}$, the concentration of 20 μ mol L^{-1} was employed for further work.

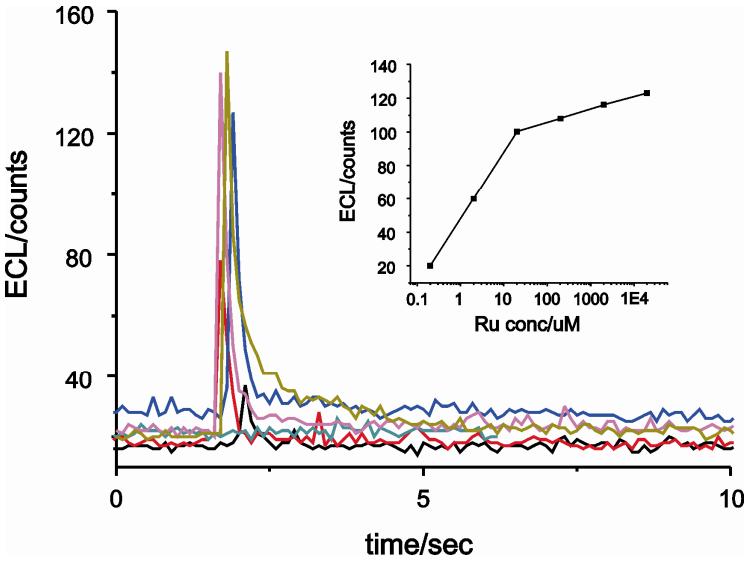

Figure S2. The ECL profiles with different $Ru(phen)_3^{2+}$ concentrations for detection of 10 pM cocaine with the proposed aptasensor. Inset: the relationship between ECL emission and the $Ru(phen)_3^{2+}$ concentration used for intercalation.

In our previous work, [s4a] the incubation time for the intercalation of $Ru(phen)_3^{2+}$ into double stranded DNA was investigated. As shown in Figure S3, the ECL emission increased from two to seven hours

and then level off. That means the incubation time can be shortened as 7 h and the increased incubation time does not affect the stability of the intercalation of $Ru(phen)_3^{2+}$ into double strand. To facilitate the experiment, we incubated the cocaine-aptamer complex-modified electrode with $Ru(phen)_3^{2+}$ solution at evening and then determined the cocaine content next morning.

Comparing with the fast interaction between aptamer and its target (less than half of hour as shown in Figure S1 for cocaine and its aptamer), the intercalation of $Ru(phen)_3^{2+}$ into double stranded DNA takes a much long period of time. On the other hand, $Ru(phen)_3^{2+}$ can be intercalated into almost any double strand part. Artificially formed double stranded DNA (ds-DNA) with different sequences was used to develop DNA-based biosensors in our previous works by the use of the intercalation of $Ru(phen)_3^{2+}$ into ds-DNA. [s4]

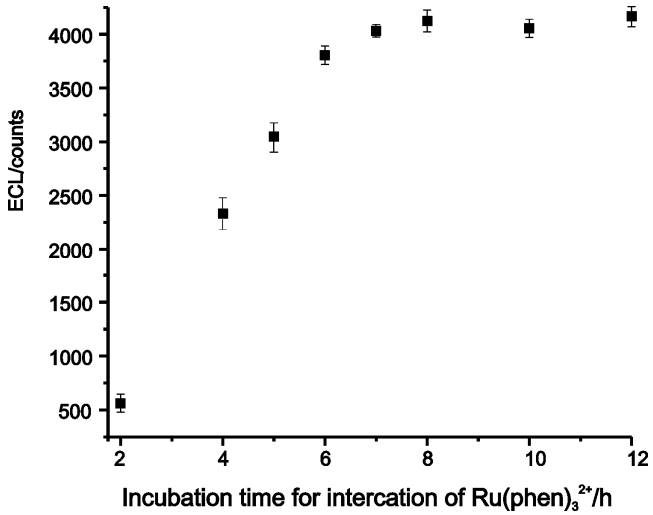

Figure S3. The dependence of ECL signal of ds-DNA on the incubation time for intercalation of $Ru(phen)_3^{2+}$ for the hybrid of 20-mer ss-DNA and the anti-thrombin apatamer. [s4a]

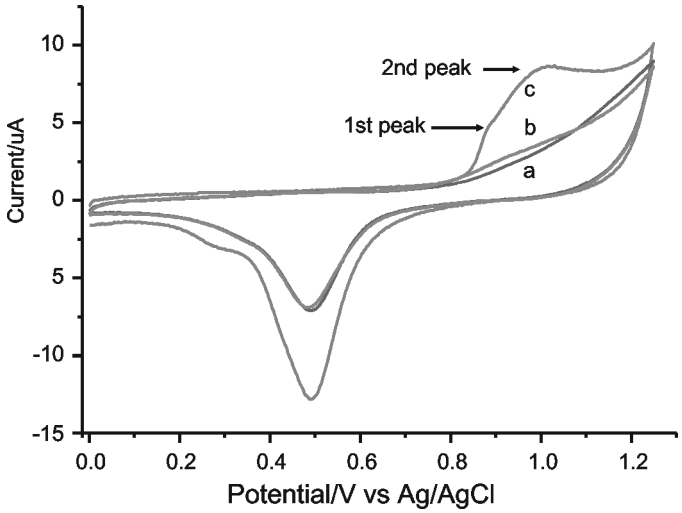

Figure S4. Cyclic voltammograms at (a) the anti-cocaine aptamer-modified Au electrode; and (b) the (a) electrode incubated with 500 pmol L^{-1} cocaine; (c) the (b) electrode intercalated with $Ru(phen)_3^{2+}$. Electrolyte: 0.1 M phosphate buffer saline (pH 7.5); Scan rate: 50 mV s^{-1}.

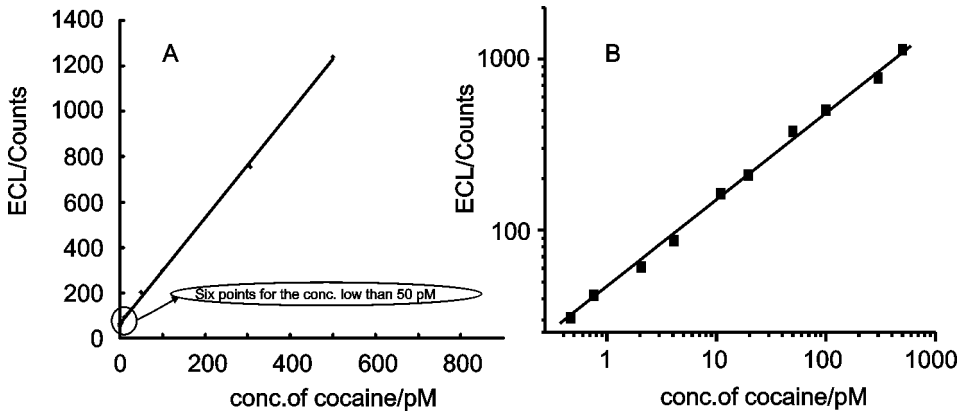

Figure S5. Linear ranges (R^2=0.997) with (A) linear scale and (B) Log-log scale for cocaine determination with the aptasensors using the analyte-induced formation of duplex for the intercalation of the ECL probe, $Ru(phen)_3^{2+}$.

Figure S5 shows the linear range for cocaine determination with the proposed aptasensors with linear scale and Log-log scale. This linear range is three orders of magnitude (R^2=0.997). We can find six data points for the concentrations low than 50 pmol L^{-1} were stacked together by the use of linear scale mode. Log-log scale is often used in the case with a wide linear range. As shown in Figure S5B, the log-log scale gives a good resolution for each point. The detection limit (3σ) of 0.2 pmol L^{-1} was achieved. This detection limit is more than two orders of magnitude lower than that obtained with previous aptasensors as shown in Table S1. The results in Table S1 also validated that ECL is more sensitive than the other detection techniques.

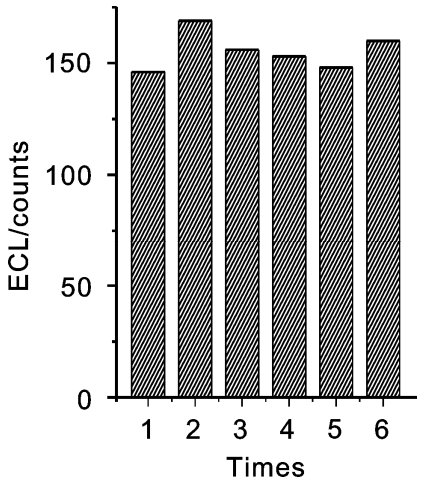

Figure S6. The reproducibility of the aptasensor for detection of 10 pmol L^{-1} cocaine using the analyte-induced formation of duplex for the intercalation of ECL probe, $Ru(phen)_3^{2+}$ with six replicate cocaine determinations.

Table S1. The comparison of detection limits for cocaine with different aptasensors.

Method	Notes	Sensing mode	DL [b]	Ref.
ECL [a]	Analyte-induced formation of duplex for label-free electrochemiluminescent Aptasensor	Turn-on	0.2 pmol L^{-1}	This work
ECL	$Ru(bpy)_3^{2+}$-tagged aptamer was covalently coupled onto paraffin-impregnated graphite electrode	Turn-on	10 pmol L^{-1}	[s1]
ECL	Electrogenerated chemiluminescence aptamer-based biosensor for the determination of cocaine	Turn-on	1 nmol L^{-1}	[s5]
cascade activity	Supramolecular cocaine-aptamer complexes activate biocatalytic cascades	Turn-on	0.5 μmol L^{-1}	[s2]
EC [a]	Electrochemical sandwich assays based on single aptamer sequences	Turn-on	1 μmol L^{-1}	[s6]
EC	Splitting ampater for sandwich assay of Cocaine	Turn-on	10 μmol L^{-1}	[s7]
EC	Electrochemical aptasensor incorporating gold nanoparticles modification	Trun-on	0.5 μmol L^{-1}	[s8]
FL [a], EC	Self-assembly of supramolecular aptamer structures for optical or electrochemical sensing	Turn-on	1 μmol L^{-1}, 10 μmol L^{-1}	[s9]
FL	Fluorescence aptameric sensor for strand displacement amplification detection of cocaine	Turn-on	2 nmol L^{-1}	[s10]
FL	Spotlighting of cocaine by an autonomous aptamer-based machine	Turn-on	5 μmol L^{-1}	[s11]
CL [a]	DNA aptamer folding on magnetic beads for sequential detection of adenosine and cocaine by substrate-resolved chemiluminescence technology	Turn-on	3.2 nmol L^{-1}	[S12]
Colorimetry	G-quadruplex-based DNAzyme with magnetic nanoparticles as separation and amplification element	Turn-on	50 nM	[s13]
Visual	Gold nanoparticles and rationally engineered aptamer structures	Turn-off	2 μmol L^{-1}	[s14]
HPLC [a]	Solid-phase extraction using an aptamer-based sorbent	Turn-on	0.1 μg mL^{-1}	[s15]
EC, SPR [a]	Electrochemical, photoelectrochemical, and surface plasmon resonance detection using supramolecular aptamer complexes and nanoparticles		1 μmol L^{-1}, 10 μmol L^{-1}	[s16]

[a] ECL: electrochemiluminescence; EC: electrochemistry; FL: fluorescence; CL: chemiluminescence; HPLC: high performance liquid chromatography; SPR: surface plasmon resonance method. [b] DL: detection limits.

Table S2. The concentration and recovery of cocaine for urine and serum samples

	Added conc. (pM)	Determined conc. (pM)	Recovery (%)
Urine sample	0	0	
	50.0	45.8	91.6
	100.0	94.1	94.1
	500	427	85.4
Plasma sample	0	0	
	5	4.22	84.4
	10.0	9.40	94.0
	500	394	78.8

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