Supporting Information for

A Sensitive, Non-Damaging Electrochemiluminescent Aptasensor with DNA-Modified Gold Electrodes Operated at Low Potential

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Contents

1. ECL mechanisms

In the general ECL procedure, the ECL emission is generated via a potential sweep or an applied potential in the presence of a co-reactant, such as tripropylamine (TPA) via the followed reactions. [S1-S3]

Scheme 1.

$$Ru(bpy)_3^{2+} - e^- \rightarrow Ru(bpy)_3^{3+} \quad (1)$$

$$Ru(bpy)_3^{3+} + TPA \rightarrow Ru(bpy)_3^{2+} + TPA^{\bullet+} \quad (2)$$

$$TPA^{\bullet+} \rightarrow (C_3H_7)_2N(^{\bullet}CHC_2H_5) + H^+ \quad (3)$$

$$Ru(bpy)_3^{3+} + (C_3H_7)_2N(^{\bullet}CHC_2H_5) \rightarrow Ru(bpy)_3^{2+*} + [(C_3H_7)_2N{=}CHC_2H_5)]^+ \quad (4)$$

$$Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{2+} + hv \quad (5)$$

In this process, the electrochemical oxidation of $Ru(bpy)_3^{2+}$ is necessary for ECL emission. The oxidized product, $Ru(bpy)_3^{3+}$, reacts with TPA to produce the highly reducing radical, $(C_3H_7)_2N(^{\bullet}CHC_2H_5)$. The radical further reacts with $Ru(bpy)_3^{3+}$ to generate the excited-state $Ru(bpy)_3^{2+*}$ for the ECL emission. [S1-S3] However, because of the potential of ca 1.10 V for the oxidation of $Ru(bpy)_3^{2+}$, the electrochemical oxidative damage to the biomolecules occurs possibly in the case of probing DNA sequence or protein assays. Therefore, lowering the applied potential for ECL emission is critical to extend the sensing application of ruthenium complex-based ECL. Under some conditions, if TPA is oxidized to produce electrochemically the highly active radical $(C_3H_7)_2N(^{\bullet}CHC_2H_5)$. [S4-S7] The radical reduces $Ru(bpy)_3^{2+}$ into $Ru(bpy)_3^{+}$. The reaction between $Ru(bpy)_3^{+}$ and $TPA^{\bullet+}$ can generate the excited-state $Ru(bpy)_3^{2+*}$ for ECL emission with the following route. [S4-S7]

Scheme 2.

$$TPA \rightarrow TPA^{\bullet+} \rightarrow (C_3H_7)_2N(^{\bullet}CHC_2H_5) + H^+ \quad (6)$$

$$Ru(bpy)_3^{2+} + (C_3H_7)_2N(^{\bullet}CHC_2H_5) \rightarrow Ru(bpy)_3^{+} + products \quad (7)$$

$$Ru(bpy)_3^{+} + TPA^{\bullet+} \rightarrow TPA + Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{2+} + hv \quad (8)$$

The emission showed in Scheme 2 is generally termed low potential ECL because the oxidation of TPA occurs at ca 0.85 V.

2. Validation of the direct oxidation of TPA at ds-DNA modified electrode

To investigate the role of the DNA film in TPA oxidation, the oxidation of TPA at bare gold electrode was tested as a comparison (Figure S1). We found a very small oxidation current in PBS (pH 7.5) containing 20 mM TPA at about 1.0 V (Figure S2). As shown in Figure S1b, TPA can be directly oxidized in PBS solution at pH 11 and the oxidation potential is at about 0.8 V vs. Ag/AgCl. [S8] The oxidation current was obviously lower than that obtained using the ds-DNA-modified electrode at pH 7.5, even if PBS at pH 7.5 was used as electrolyte (Figure S2). Previous work indicated that TPA oxidations were characterized as an irreversible electron transfer. [S3] Kanoufi et al [S8] studied TPA oxidation in PBS at different pH levels and found that the TPA oxidation peak observed by cyclic voltammetry decreased in intensity and shifted towards a more positive potential as the pH decreased from 11 to 7.5. Figure S1 validated the results. The oxidation of TPA in the $Ru(bpy)_3^{2+}$/TPA system (pH 7.5) was mainly achieved through the catalytic homogeneous electron transfer between $Ru(bpy)_3^{3+}$ and TPA because of the high TPA oxidation peak potential (about 1.15 V vs. Ag/AgCl). [S8]

Figure S2 shows the CVs of ds-DNA composite film electrode in 100 mM PBS (pH 7.5) before and after being immersed in 50 mM PBS containing 20 mM TPA solution. The increased oxidation peak in Figure S2b validates that backbone of DNA can adsorb $TPAH^+$ via electrostatic interaction between $TPAH^+$ and phosphate ions in the backbone.

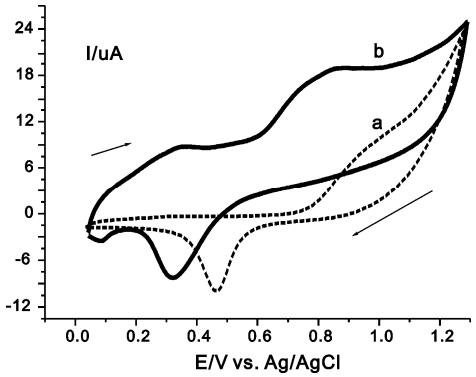

Fig. S1 Cyclic voltammograms of the bare gold electrode in phosphate buffer solution containing 20 mM TPA at (a) pH 7.5 and (b) pH 11. Scan rate: 50 mV/s.

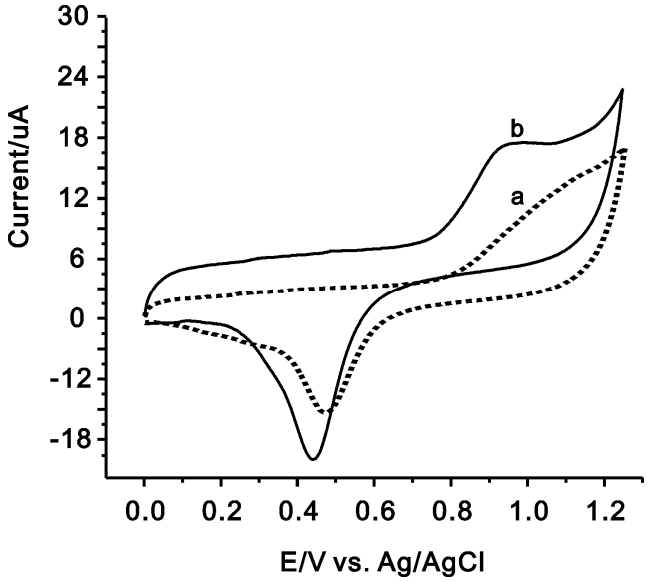

Fig. S2 Cyclic voltammograms of ds-DNA-modified electrode in 50 mM PBS (pH 7.5) before (a) and after (b) being immersed in 50 mM PBS containing 20 mM TPA solution. Scan rate: 50 mV/s.

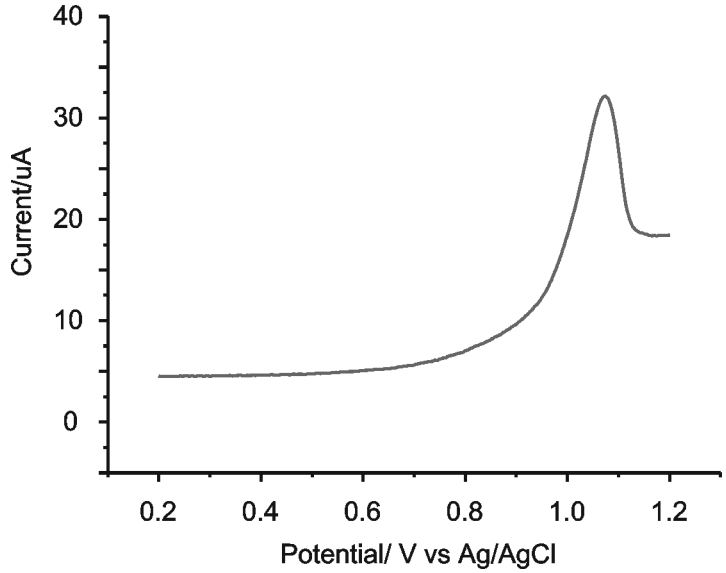

Fig. S3 The linear sweep voltammogram of intercalated $Ru(phen)_3^{2+}$ into ds-DNA-modified electrode in 100 mM phosphate buffer solution (pH 7.5). Scan rate: 50 mV s^{-1}.

Table S1. Method detection limits (DL) for lysozyme using different aptamer-based techniques.

Method	Notes	DL/pM	Ref.
ECL[a]	$Ru(phen)_3^{2+}$ as intercalator into ds-DNA with low potential ECL	0.45	This work
ECL	Quantum dot as ECL probe		S9
ECL	$Ru(bpy)_2(dcbpy)$ labeling with Au nanoparticle amplification	0.1	S10
EC [a]	layer-by-layer assembled multilayers with ferrocene-appended poly(ethyleneimine), carbon nanotubes (CNTs) and aptamer	0.14 ng ml^{-1}	S11
EC	Au nanoparticle amplification with surface-bound $Ru(NH_3)_6^{3+}$ as probe	0.01 μg mL^{-1}	S12
EC	Faradic impedance spectroscopy change before and after the hybrid of aptamer and lysozyme	70	S13
EC	The oxidation guanine and adenine	18 nM	S14
EC	aptamer-coated magnetic beads and chronopotentiometric stripping measurements of the captured lysozyme	7 nM	S15
FL [a]	fluorophore-labeling for imaging array	5 pM	S16

[a] ECL: electrochemiluminescence; EC: electrochemistry; FL: fluorescence method.

Supplementary References

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