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Supporting information

A sensitive and specific Electrochemiluminescent sensor for lead based on DNAzyme

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Materials and Chemicals

DNA oligonudeotides (1) and (2) were synthesized by Sangon Inc. (Shanghai, China). Their sequences are shown below:

5'-HS-$(CH_2)_6$-TTT TTC ATC TCT TCT CCG AGC CGG TCG AAA TAG TGA GT-3' (1)

3'-GTA GAG AAG G rA TAT CAC TCA GTG CTC AAC TGT-$(CH_2)_6$-NH_2-5' (2)

Where (1) is the DNAzyme and (2) is the substrate strand. The $Ru(bpy)_3^{2+}$ N-hydroxyl-succinimide ester (TBR-NHS ester), Tripropylamine (TPA), 6-mercaptohexanol (MCH) and Tris(2-carboxyethyl) phosphinehydrochloride (TCEP) were purchased from Sigma, USA. All other chemicals were of analytical grade.

All solutions were prepared with Milli-Q water (Milli-Q, Millipore, 18.2-MΩ resistivity). DNA immobilization buffers contain 300 mmol/L NaCl, 10 μM TCEP and 25 mmol/L Tris-acetate (TAE) (pH 8.2). Targeted Pb^{2+} buffers were composed of 0.5 mol/L NaCl and 50 mmol/L Tris-acetate (pH 8.2). Buffer for ECL detection was 5 mM Tris-acetate buffer (pH 8.2).

Sensor Preparation

The TBR labels were incorporated into the 5' end of DNA (2) using the phosphoramidite method by previous literature[1]. 1 OD DNA(2) was dissolved in 250 μL of TAE buffers, and then 200 μL of 6.0×10^{-4} mol/L $Ru(bpy)_2(dcbpy)NHS$ and 10 μL of 0.10 mol/L TAE buffer was added to the above DNA(2) solution, respectively, allowing to shaking at low speed overnight at room temperature. Then, by addition of 100 μL of 3 mol/L NaAc and 2 mL of ethanol to the mixture, the precipitate reaction was carried out in refrigerator over 12 h. The mixture was centrifuged in a micro-centrifuge for 30 min. The precipitate was preserved and rinsed with cold 70% ethanol twice and dried in air. The dried precipitate was re-dissolved in TAE buffer (pH 8.2) and stored under -16 ℃ in refrigerator to obtain target DNA-TBR solution.

Gold electrodes (2 mm in diameter, CH Instruments, Shanghai, China) were polished with aqueous slurries of alumina powders (average particle diameters: 1.0 μm and 0.3 μm α-Al_2O_3) on a polishing microcloth and rinsed with water for 5 min. They were then electrochemically cleaned in 0.5 mol/L sodium hydroxide and 0.5 mol/L sulfuric acid in turn. The cleaned gold surfaces were immersed in the immobilization buffer containing 1 μmol/L DNAzyme for 90 min. The DNAzyme-modified electrodes were then passivated with 1.0 mmol/L MCH for 60 min to obtain a well-aligned DNAzyme monolayer, followed by washing with distilled water to remove unspecific adsorbed DNA. The resulting DNAzyme monolayer-functionalized surfaces were immersed into stirred Tris-acetate (pH 8.2) solution containing target DNA-TBR in a 65 ℃ water bath for 10 min. The water bath

was then allowed to cool to room temperature over 1 h. The hybridization was performed for 16 h at room temperature. After this, the electrode was incubated in buffer at 37 ℃ for 2 h to reduce the physical adsorption. The obtained sensor was then allowed to react with various concentrations of target Pb^{2+} in the buffered (50 mmol/L Tris-acetate + 0.5 mol/L NaCl) (pH 8.2) for 1 h in a 37 °C water bath to obtain the maximum cleavage of substrate strand on the gold surface.

ECL Measurements

The ECL detection system consisted of a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China) and a CHI 660a electrochemical system (CH Instruments, Shanghai, China). A three-electrode electrochemical cell with an optically flat bottom was used. The DNA modified electrode label with TBR was used as the working electrode. Platinum wire and Ag/AgCl (saturated with KCl) were used as counter and reference electrodes, respectively. Buffers were purged with highly purified nitrogen for 20 min before measurement and kept in the cell during determination. The electrochemical cell was placed directly in front of a photomultiplier (PMT, operated at -850 V) and the PMT window was only opened to the working electrode to reduce the interference of ECL from the counter electrode.

Optimization of Self-Assembly Time

In this study, ECL response and sensitivity are dependent on a molecular recognition element and an ECL signal-producing compound on the surface. While the ECL response of the DNA biosensor was strongly affected by the probe density of DNAzyme on electrode, which was related to the self-assembly time of the probe onto the gold electrode and the concentration of the DNAzyme used. For obtaining the optimization of self-assembly time, we studied on the relationship between self-assembly time and the density of DNAzyme on the gold electrode at a certain concentration of the DNAzyme by EIS measurement. The concentration of DNAzyme was chosen as 1 μmol/L in this experiment.

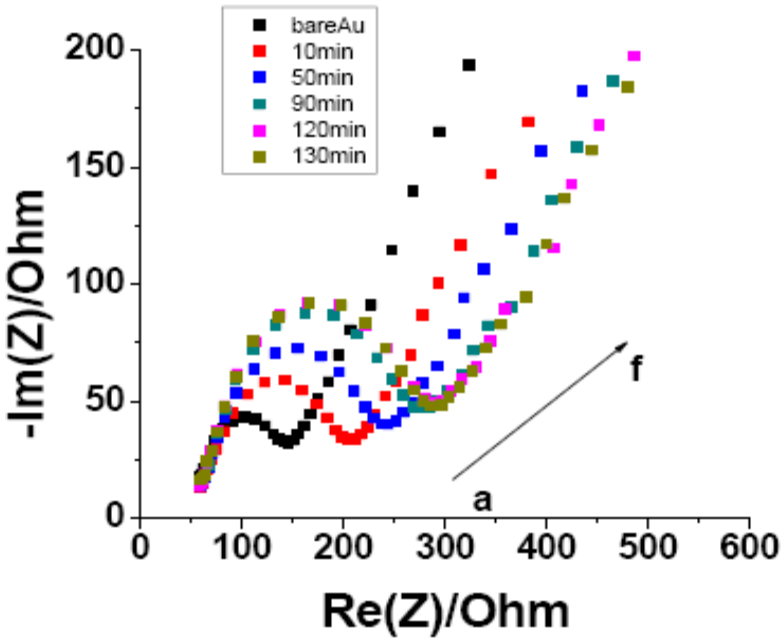

Fig. S1 Nyquist diagrams record on ss-DNA modified gold electrode at different time (a) 0; (b) 10; (c) 50; (d) 90; (e) 120; (f) 130 min. Supporting electrolyte solution is 5.0 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 mol/L KCL.

The Nyquist diagrams of different self-assembly time under the same concentration of DNAzyme were illustrated in Fig. S1. At beginning, the diameter of the semicircle increases rapidly in the 50 min. However, it increases slowly between 50 and 90 min, and with further increase in the self-assembling time, the diameter of the semicircle is almost unchanged. This is attributed to the steric and electrostatic hindrance arising from the more tightly packed probe and some interstitial space between the probes, which are necessary for high hybridization efficiency. When self-assembly time reach 90 min, the DNAzyme has been assembled on the gold electrode completely. Therefore, the self-assembly time of 90 min was employed for the assemblage of the DNAzyme on the gold electrode.

We also study on the effect of the time of target DNA hybridized with the ssDNA by the EIS measurement. With the increase of the assemble time, the diameter of the semicircle increase rapidly in 12 h, however, when the hybridization time is over 16 h, the diameter of the semicircle almost no longer changes. So 16 h has been chosen as the optimize hybridization time.

The stability of the modified electrode

The stability of the TBR modified electrode had been tested. If 1.15 V potential had been applied on the working electrode. A constant ECL intensities can be detected for several minutes. The result was shown in Fig. S2.

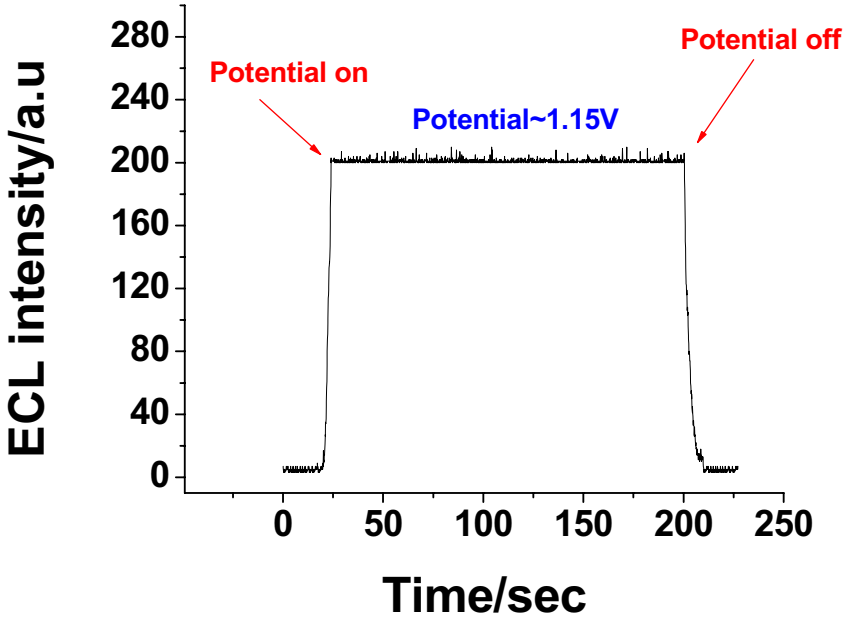

Fig. S2 ECL intensity curves from the modified ECL DNAsensors under continuous potential at 1.15 V for 3 min

References

1. J. Zhang, H. L. Qi, Y. Li, J. Yang, Q. Gao, C. X. Zhang, *Anal. Chem*. 2008, **80**, 2888-2894.