Reusable, Label-free Electrochemical Aptasensor for Sensitive Detection of Small Molecules

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Experimental Section

Materials. HPLC purified Oligonucletides designed according to the literature [1] were synthesized by the TaKaRa Biotechnology (Dalian) Co., Ltd. The sequence of the adenosine-binding aptamer was 5' AAC TGG GGG AGT ATT GCG GAG GAA GGT CTGTA-3' (ABA) and the one of part complementary strand was 5'HS-$(CH_2)_6$-TACAGACCTTCC-3' (PCS). Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and cytidine were from BBI and Fluka respectively. Adenosine and uridine were purchased from Sigma. All other chemicals were of analytical grade.

Buffer for hybridization was 25 mM Tris-HCl, 300 mM NaCl, 44.5 μM TCEP, pH 8.23 (H-buffer) and the one for washing the modified electrodes was 25 mM Tris-HCl, 300 mM NaCl, pH 8.23 (I-buffer). Before detecting, 5.05 mM adenosine, mixture of 3.6 mM uridine and 3.2 mM cytidine mixture were diluted to required concentrations using 25 mM Tris-HCl, 150 mM NaCl, pH 8.23 (B-buffer). H-buffer has been sterilized before use.

All the buffers used and the experiment temperatures were according to the works of Lu's group[1], with a little modification.

Instrumentation. All electrochemical experiments (Cyclic voltammetry (CV) and

electrochemical impedance spectroscopy (EIS)) were done on an Autolab PGSTAT30 (Utrecht, The Netherlands, controlled by GPES4 and Fra software) using a conventional three electrode test cell with Ag-AgCl electrode as reference electrode, Pt coil as counter electrode and gold disk (1.2 mm in diameter) as working electrode. The cell was housed in a homemade Faraday cage to reduce stray electrical noise. All the measurements with the Autolab were carried out at room temperature (~17 to 18 ºC). EIS was performed under an oscillation potential of 5mV over the frequency range of 10 KHz to 0.1 Hz and in the solution of 5 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$, 67 mM PBS buffer, pH 7.0.

Fabrication of the sensing interface. The gold electrode (1.2 mm in diameter) was polished with 1.0 and 0.3 μm α-Al_2O_3 and then washed ultrasonically with pure water for 3 times, subsequently electrochemically cleaned in 0.1 M H_2SO_4 by potential scanning between -0.2 V to 1.55 V until a reproducible cyclic voltammogram was obtained. Then it was sonicated and rinsed with copious amount of pure water, finally blown dry with nitrogen before assembly.

Part DNA duplex (PCS-ABA) were prepared as follows: The mixture of the PCS (1.25 μM) and ABA (1.4 μM) in H-buffer was heated and kept at 84 ºC for 10 min, then the solution was slowly cooled to room temperature of ~17 to 18 ºC and kept at this temperature for about 30 min.

The sensing interface (Au/PCS-ABA) was prepared by placing prepared 15μL PCS-ABA solution on the gold electrode held upside-down. Then the end of the electrode was fitted with a plastic cap to protect the solution from evaporation. The assembly was kept over 20 h at room temperature and then rinsed with pure water and I-buffer for several times. At last the modified electrode was immersed in B-buffer for 3 h (22$\pm$1 ºC) to make sure the formation of a stable and high-density layer.

Adenosine binding was performed by immersing the Au/PCS-ABA in required concentrations of adenosine solutions (B-buffer). After 3 hour' s interaction at 22-23 ºC, the electrode was rinsed with pure water and I-buffer, followed by EIS measurement.

In the control experiments, Au/PCS-ABA was immersed in the solution of 0.36 mM uridine and 0.32 mM cytidine (B-buffer) for 3 h (22±1 ºC), followed by rinsing with pure water and I-buffer. After detection, the electrode was immersed in 0.3 mM adenosine (B-buffer) for 3 h (22±1 ºC).

Regeneration of the sensing interface used Au/PCS-ABA, which was already interacted with 500 μM adenosine. The electrode was first covered by 15 μL 2.4 μM ABA solution for 2 h at 4 ºC. Then the electrode was covered by 15 μL 2.4 μM ABA solution at room temperature (~17 to 18 ºC) for 2 h, 1 h, 1 h and 1 h successively.

Finally, the regenerated electrode was immersed in the solution of 0.36 mM uridine and 0.32 mM cytidine (B-buffer) for 3 h (22±1 ºC) for another control experiment. At last, the regenerated electrode was immersed in 30 μM adenosine (B-buffer) for 3 h to realize the reusing ability.

The sensing ability of the regenerated electrode.

To test the sensing ability, the regenerated electrode was used to detecting adenosine again. Meanwhile, control experiment was done as well. As shown in Figure S1**,** after the regenerated electrode was immersed in the solution of 0.36 mM uridine and 0.32 mM cytidine (B-buffer) for 3 h, a little increase of the Ret was observed, which was consistent with the control experiment done using the original Au/PCS-ABA system. While after binding with the 30 μM adenosine, the Ret of the regenerated electrode decreased obviously, which illustrated that the sensing system could be used again after regeneration.

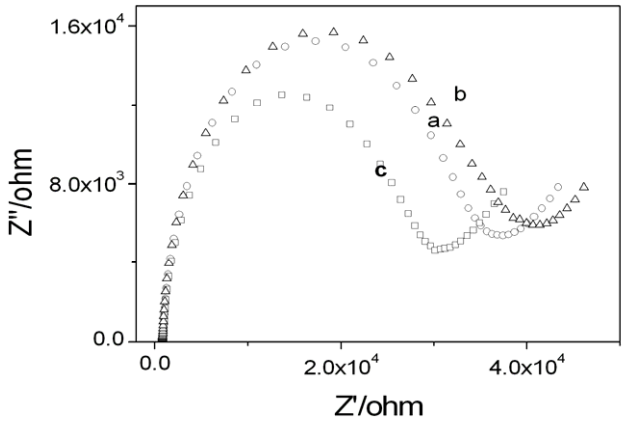

Figure S1 (a) Nyqiust plots of the regenerated sensing surface; (b) Nyqiust plots after interacting with 0.36 mM uridine and 0.32 mM cytidine. (c) Nyqiust plots after interacting with 30μM adenosine.

Reference

1. J. W. Liu and Y. Lu, *Nat. Protocols*, 2006, **1**, 246. ; J. W. Liu, D. Mazumdar and Y. Lu, *Angew. Chem. Int. Ed*., 2006, **45**, 7955.