Electronic Supplementary Material (ESI) for Analytical Methods

Supporting Information

Label-free and sensitive supersandwich electrochemical biosensor for small molecule detection based on target-induced aptamer displacement

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Experimental Section

Materials and instruments. Adenosine, uridine, cytidine, guanosine, 6-mercapto-1-hexanol (MCH) and hexaammineruthenium (III) chloride (RuHex) were purchased from Sigma (USA). Different concentration of adenosine and 1 mM uridine, cytidine, and guanosine were all prepared in 20 mM Tris-HCl buffer (pH=7.4) containing 0.1 M NaCl and 5 mM $MgCl_2$. DNA Marker and loading buffer were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). SYBR Green I, which is a fluorescent dye that binds directly to double-stranded DNA (dsDNA), was purchased from Invitrogen (USA). All of the chemical reagents were of analytical grade or higher. Ultrapure water (18.2 MΩ·cm) was used throughout.

DNA oligonucleotides were purchased from TaKaRa Biotechnology (Dalian, China) Co., Ltd. Sequences of these DNA were shown in Table1. Capture probe and aptamer were dissolved into 10 mM PBS buffer (pH=7.4) containing 500 mM NaCl and 1 mM $MgCl_2$ before use. Report probe1 and report probe2 were dissolved into 20 mM Tris-HCl buffer (pH=7.4) containing 500 mM NaCl and 20 mM $MgCl_2$ before use.

Chronocoulometry was performed with a CHI660C electrochemical workstation (Shanghai Chenhua Equipment, China). The conventional three-electrode system was employed, which consisted of Au working electrode, platinum wire auxiliary electrode, and KCl saturated calomel reference electrode. Chronocoulometry was carried out at a pulse period of 250 ms and pulse width of 500 mV. The electrolyte was 10 mM Tris-HCl buffer (pH 7.4) containing 50 μM $[Ru(NH_3)_6]^{3+}$.

Surface modification of Au electrodes

Au electrodes was first cleaned in piranha solution (H_2SO_4 / H_2O_2, 3:1(v / v)), followed by a thorough rinsing with water. Then, Au surface was modified with the mixture of 5 μM capture probe and 5 μM aptamer for 24 h at 4 ^oC, followed by a thorough rinsing with 10 mM PBS (pH 7.4). After 20 min of incubation with 1 mM MCH, Au surface was thoroughly rinsed with 10 mM PBS and ready for use. The surface density of capture probe on the Au electrode was measured by chronocoulometry (A. B. Steel, T. M. Herne and M. J. Tarlov, *Anal. Chem.*, 1998, **70**, 4670–4677). It was measured that the surface coverage of capture probe on the Au film was ca. 7.8 x 10^{12} molecules / cm^2.

Characterization of supersandwich

The supersandwich structure was characterized by gel-electrophoresis. 1 μM report probe1 was first incubated with 1 μM report probe2 for 2h at room temperature. After the mixture was diluted 10 times, it can be used for electrophoresis. Next, 10 μL of the above solution was mixed with 2 μL loading buffer and 2 μL SYBR Green 1, and then agarose gel electrophoresis was

performed in 2% gel at 100 V for 1 h.

Detection of adenosine

Detection of adenosine was conducted by exposing the Au electrode to different concentration of adenosine for reacting about 60 min at room temperature, then washing with 10 mM PBS repeatedly. Next, the mixture of 5 μM report probe1 and 5 μM report probe2 was added and incubated for 90 min at room temperature, and then the Au electrode was washed repeatedly with 10 mM PBS. The chronocoulometric signal before and after adenosine treatment was recorded respectively. Hot water (85 ^{o}C) was used to regenerate the Au electrode.

For identifying the target-specificity of this sensing system, 1 μM adenosine analogue solutions of uridine, cytidine, and guanosine were respectively reacted with capture probe which modified on Au film for 60 min at room temperature, then washing with 10 mM PBS thoroughly. Next, the mixture of 5 μM report probe1 and 5 μM report probe2 was added and incubated for 90 min at room temperature, and then the Au electrode was washed repeatedly with 10 mM PBS. The change of chronocoulometric signal was recorded.

Fig. S1 The sequences of supersandwich structure

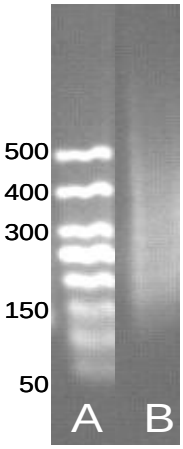

Fig. S2 The gel-electrophoresis of the supersandwich structure

(A)marker; (B) supersandwich structure

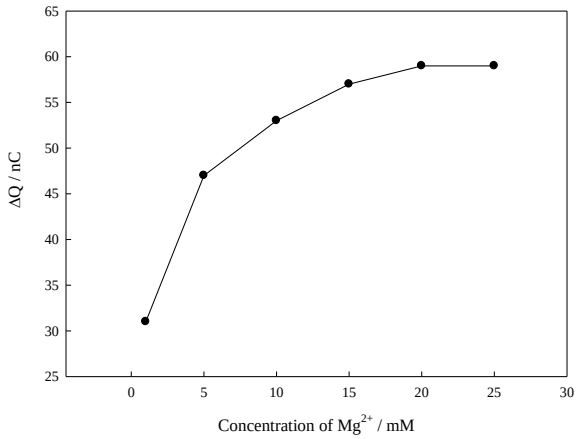

Fig. S3 The change of charges corresponding to different concentration of Mg^{2+} ion. Concentration of report probes: 5 μM; concentration of adenosine: 1 μM; the reaction time of adenosine: 60 min; supersandwich-formation time: 90 min.

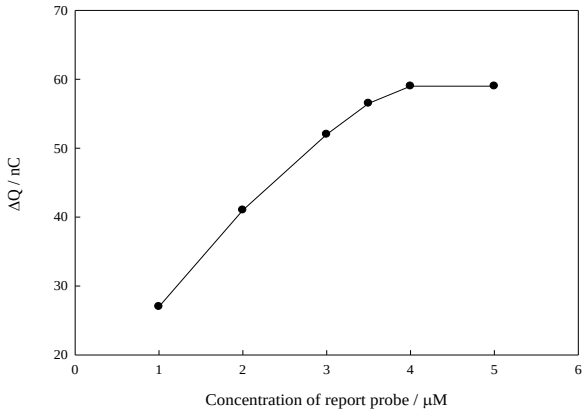

Fig. S4 The change of charges corresponding to different concentration of report probes. Concentration of Mg^{2+} ion: 20 mM; concentration of adenosine: 1 μM; the reaction time of adenosine: 60 min; supersandwich-formation time: 90 min.

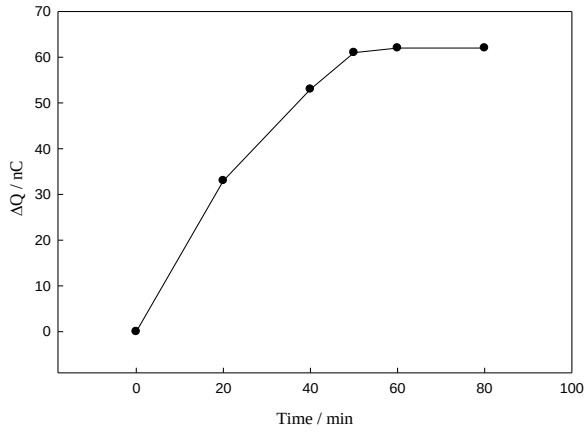

Fig. S5 The change of charges corresponding to different reaction time of adenosine. Concentration of Mg^{2+} ion: 20 mM; concentration of adenosine: 1 μM; concentration of report probes: 5 μM; supersandwich-formation time: 90 min.

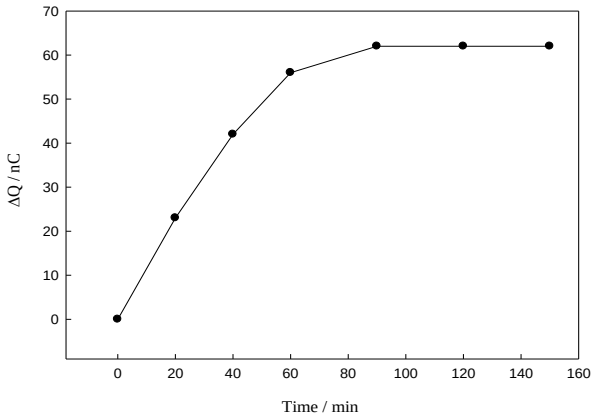

Fig. S6 The change of charges corresponding to different supersandwich-formation time. Concentration of Mg^{2+} ion: 20 mM; concentration of adenosine: 1 μM; concentration of report probes: 5 μM; the reaction time of adenosine: 60 min.