Electronic Supplementary Information

Nuclease cleavage-assisted target recycling for signal amplification of free-label impedimetric aptasensors

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EXPERIMENTAL SECTION

Reagents. Oligonucleotides designed in this study were synthesized by Beijing Dingguo Biotechnol. Co. Ltd. (Beijing, China), which were purified by HPLC and confirmed by mass spectrometry. The sequence of ATP aptamers is 5´-ACCTG GGGGA GTATT GCGGA GGAAG GT-3'. DNA stock solution was obtained by dissolving oligonucleotides in tris-HCl buffer solutions (pH 7.4). Each oligonucleotide was heated to 90 °C for 5 min, and slowly cooled down to room temperature before usage. DNase I (Recombinant DNase I: 1000 U/mL) was purchased from Beijing Dingguo Biotechnol. Co. Ltd. (Beijing, China). All other reagents were of analytical grade and were used without further purification. Ultrapure water obtained from Millipore water purification system (18 MΩ cm resistivity) was used in all runs.

Preparation of Impedimetric Aptasensors. Before modification, glassy carbon electrode (GCE, 2 mm in diameter) was polished with 0.3 μm and 0.05 μm alumina, followed by successive sonication in bi-distilled water and ethanol for 5 min and dried in air. Next, 5 μL of Nafion ethanol solution (0.5%, v/v) was initially cast on the GCE, and then removed to parch with an infrared light for 5 min. After washing with distilled water, the Nafion/GCE was dipped into an aqueous solution of $FeCl_3$ and DMF-dispersed single wall carbon nanotubes (CNTs) in turn, and incubated for 15 min and 30 min at room temperature (RT), respectively (*Note*: The use of $FeCl_3$ was attributed to the following considerations: (*i*) The morphology of Nafion® membrane is represented by hydrated SO_3^- headgroups and counterion clusters are interconnected by short channels immersed in a fluorocarbon backbone network, and some form of phase separation occurs in Nafion® because of the strongly hydrophobic backbone and highly hydrophilic terminal group; (*ii*) Fe^{3+} ions might be diffused into the Nafion® membrane *via* the interaction between sulfonic acid groups with negative charges and Fe^{3+} ions with positive charges; (*iii*) Fe^{3+} ions might serve as an intervening “spacer” matrix to extend the CNTs away from the substrate matrix in the mobile phase, resulting in a good pathway of electron transfer in the complex membrane; and (*iv*) Fe^{3+} ions could act as a bridge between the negatively charged CNTs and the negatively charged Nafion® membrane). The resulting electrode was intermediately washed in alkaline solution to make the CNTs conjugated onto the Fe^{3+}/Nafion-modified GCE.[1] Then, 10 μL of the aptamers (5 μM) was casted onto the surface of the CNTs-functionalized GCE, and incubated for 50 min at RT. Afterwards, the electrode was incubated in 0.25 wt% BSA for 60 min at 37 °C to eliminate non-specific binding effect and

block the remaining active groups. Finally, the resulting electrode was washed with water to remove the unbound proteins, and stored at 4 °C when not in use.

Signal Amplification Strategy and EIS Measurements. Electrochemical impedance spectroscopy (EIS) measurements were performed on a CHI 604D Electrochemical Workstation (Shanghai CH Instruments Inc., China) using a conventional three-electrode system with a modified GCE working electrode, a platinum foil auxiliary electrode, and a saturated calomel electrode (SCE) reference electrode. The detection process of ATP was carried out as follows: (i) *Reaction with the analyte*: 10 μL of mixture solution containing various concentrations of ATP and 50 U/mL DNase I was dropped onto the surface of the aptasensor, and incubated for 80 min at RT; and (ii) *Measurement*: after washing with distilled water, the aptasensor was dipped into 5 mM $Fe(CN)_6^{4-/3-}$ containing 0.1 M KCl. EIS measurement was monitored at frequency 10^{-2} to 10^5 Hz and a Nyquist plot (Z_{re} *vs*. Z_{im}) was drown to analyze the impedance results. Analyses are always made in triplicate.

1:1 Binding Strategy with EIS Measurement. 10 μL of mixture solution containing various concentrations of ATP was dropped onto the surface of the aptasensor, and incubated for 80 min at RT. After washing with distilled water, the aptasensor was dipped into 5 mM $Fe(CN)_6^{4-/3-}$ containing 0.1 M KCl. EIS measurement was monitored at frequency 10^{-2} to 10^5 Hz.

Optimization of Experimental Conditions. To ensure the smooth implementation of target recycling, the concentration of DNase I should be excess. If the level of DNase I was too low, the enzymatic reaction was not adequate, and decreased the dynamic range of the aptasensors. However, the concentration of DNase I was too high, which might decreased the interaction possibility between the target and the aptamers. Based on this consideration, 50 U/mL DNase I was used in the work.

The analytical properties of the aptasensors directly depend on the immobilized amount of the aptamers on the CNT-modified GCE. In this case, this protocol was carried out by dropping 10 μL of 5 μM aptamers on the CNT/Naftion/GCE, and incubating various times. The judgement is based on the change in the resistance. As seen from Fig. S1, the resistance (R_{et}) of the modified electrode increased with the increment of incubation time, and the optimal response was achieved at 50 min. Thus, 50 min was selected for the immobilization of the aptamers on the CNTs-modified GCE.

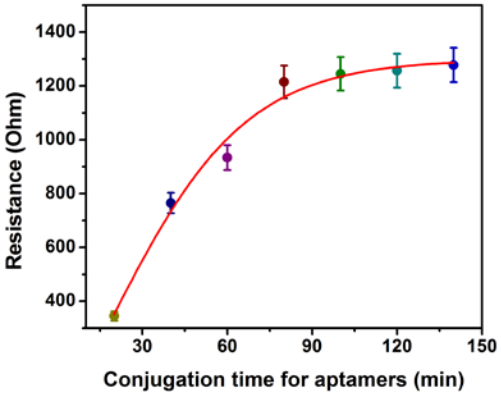

Fig. S1 Influence of conjugation time of the aptamers with the CNT/Nafion/GCE on the response of the impedimetric aptasensors.

To achieve an optimal EIS response, incubation time of the as-prepared aptasensors with the targets should be optimized. In this step, 5 nM ATP was used as an example. Initially, 10 μL of mixture solution containing 5 nM ATP and 50 U/mL DNase I was dropped onto the surface of the aptasensor, and incubated for various times at RT. Following that, EIS measurement was carried out in 5 mM $Fe(CN)_6^{4-/3-}$ containing 0.1 M KCl. As indicated from Fig. S2, the resistance decreased with the increase of incubation time from 10 to 80 min, and tended to level off at 50 min. Longer incubation time did not obviously change of the resistance. Therefore, 50 min was chosen as incubation time for the detection of ATP in the work.

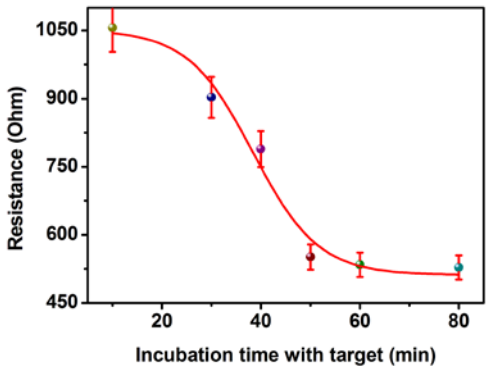

Fig. S2 Influence of incubation time of the aptasensors with the target and DNase I on the response of the impedimetric aptasensors.

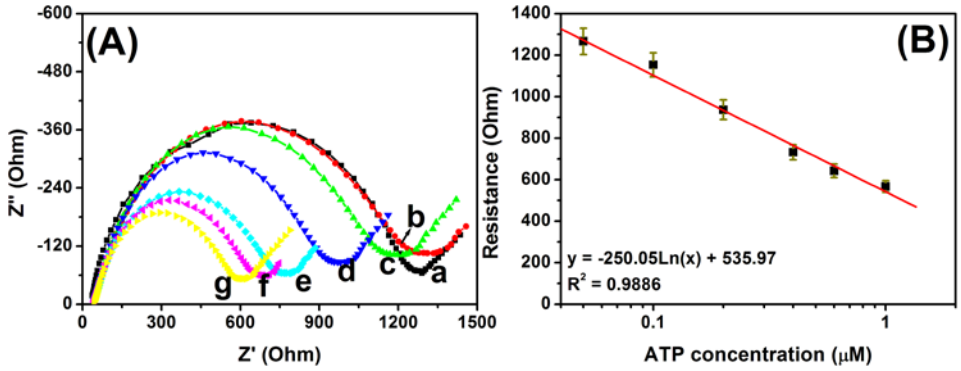

Fig. S3 (A) Nyquist diagrams for the 1:1 binding strategy after incubation with various ATP standards: (a) zero analyte, (b) 0.05 μM, (c) 0.1 μM, (d) 0.2 μM, (e) 0.4 μM, (f) 0.6 μM, and (g) 1.0 μM, in 5 mM $Fe(CN)_6^{4-/3-}$ + 0.1 M KCl. (B) Calibration plots of the aptasensors (log*C vs.* R_{et}) for the target ATP from 0.05 μM to 1.0 μM (error bars: SD, n = 3).

Table S1. Analytical Properties of Different ATP Aptasensors

Method	Amplified strategy	Linear range	Detection limit	Selectivity (CTP, GTP, and UTP)	Ref.
Chemiluminescence	Hemin/G-quadruplexes	-[b]	10 μM	-[b]	[2]
Electrochemiluminescence	Quantum dots	0.018-90.72 μM	6 nM	No interference	[3]
Amperometry	Silver microspheres	0.05-56.5 nM	23 pM	No interference	[4]
Electrochemiluminescence	$[Ru(bpy)2dppz]^{2+}$	0-1.0 μM	100 nM	No interference	[5]
Impedimetry	-	<10 mM	10 nM	No interference	[6]
Fluorescence	Coupper nanoparticles	0.05-500 μM	28 nM	-[b]	[7]
Amperometry	Target recycling	1.0 pM – 500 μM	0.1 pM	No interference	[8]
Amperometry	Ferrocence	1.0 nM-300 μM	0.1 nM	No interference	[9]
Amperometry	Quarntum dots	0.01-100 nM	0.01 nM	No interference	[10]
Impedimetry	Target recycling	0.5 pM-1.0 μM	0.1 pM	No interference	This work

[a] CV: coefficient of variation; [b] No discussion

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