Electronic Supplementary Material (ESI) for Chemical Communications

An Ultrasensitive Electrochemical DNA Sensor based on the ssDNA-assisted Cascade of Hybridization Reaction

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SUPPORTING INFORMATION

Chemicals and Reagents. Oligonucleotides were synthesized and purified by Sangon Inc. (Shanghai, China). The sequences of these oligonucleotides are listed in Table S1. The 5' terminus of signaling probe (**P1**) was labeled with thiol group with a 6-carbon spacer for immobilization onto the gold electrode. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Alfa Aesar (Ward Hill, MA) and directly used as received. 6-mercaptohexanol (MCH), methylene blue (MB), bovine serum albumin (BSA), adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), uridine triphosphate (UTP) and thymidine triphosphate (TTP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals used in this work were of analytical grade and directly used without additional purification, purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All solutions were prepared with ultrapure water (18.2 MΩ·cm) from a Millipore Milli-Q system (Bedford, MA). The buffers employed in this work were as follows. DNA immobilization buffer (I-buffer) was 10 mM Tris-HCl, 0.1 M NaCl, and10 μM TCEP (pH 7.4). MCH incubation buffer (M-buffer) was 2 mM MCH and 10 mM Tris-HCl (pH 7.4). The staining buffer (S-buffer) was 10 mM PBS, 10 mM NaCl, and 2 mM MB (pH 7.0). The electrochemical detection buffer (D-buffer) was 10 mM PBS and10 mM NaCl (pH 7.0).

Table S1. Sequences of oligonucleotides used in this work.

Name	Sequence*
P1	5'-SH-$(CH_2)_6$ ACCTGGGGGAGTAT -3'
S1	5'- CGGCACCTGGGGGAGTATTGCGGAGGAAGGTGCCG-3'
S2	5'-TACTCCCCCCAGGTGCCGACGGCACCTTCCTCCGCA-3'
T1	5'- ATACTCCCCCAGGTGCCGATACTCCCCCAGGT-3'
T2	5'- ATACTCCCCCAGGTGCCGTGCGGAGGAAGGT-3'
M1	5'- *G*TACTCCCCCAGGTGCCGATACTCCCCCAGGT-3'
M2	5'- *G*TACTCCCCCAGGTGCCG*C*TACTCCCCCAGGT-3'
M3	5'- *G*TACTCCCCCAGGTGCCG*CG*ACTCCCCCAGGT-3'
M4	5'- *GC*ACTCCCCCAGGTGCCG*CG*ACTCCCCCAGGT-3'

M5	5'- ATACTCCCCCAGGTGCCG*CG*ACTCC*AA*CA*C*GT-3'
M6	5'- ATACTCCCCCAGGTGCCG*C*TACTCCCCCAGGT-3'

* The ATP aptamer fragments are represented in underlined portions. The complementary sequences are represented in bold portions in the same color. Italic portions represent the mutation base in target DNAs.

Instrumentation. The electrochemical characterizations including cyclic voltammetry (CV) and square wave voltammograms (SWV) measurements were carried out on a CHI 660D electrochemical workstation (Chenhua Instrument Company, Shanghai) with a conventional three-electrode cell. The three-electrode mode consists of a bare gold working electrode (2 mm in diameter), a platinum counter electrode, and a Ag/AgCl reference electrode (saturated with 3.0 M KCl). All electrochemical measurements were carried out at laboratory ambient room temperature (RT, 22−25°C).

E-DNA Sensor Fabrication. The E-DNA sensor was fabricated according to the reported protocols.[1] Briefly, bare gold electrodes (2.0 mm diameter) were polished with two micropolish deagglomerated alumina suspensions (0.3 and 0.05 μm diameter) in sequence for 5 min each, followed by ultrasonic cleaning with absolute ethanol and Milli-Q water for 5 min each to remove the residual alumina powder. Then the electrodes were electrochemically cleaned to remove the remaining impurities by performing consecutive CV measurements in a fresh 0.5 M H_2SO_4 solution with following parameters: potential range from −0.3 to 1.5 V at a scan rate of 0.1 V/s versus Ag/AgCl. A cyclic voltammogram characteristic of a gold electrode was clearly observed with typical single sharp reduction peak located at ~0.88 V and multiple overlapping oxidation peaks in the range of 1.1−1.5 V (Figure S1). After that, the electrodes were washed thoroughly with Milli-Q water and dried in a nitrogen stream to obtain a clean gold surface. Then the electrodes were ready for DNA immobilization.

Prior to immobilization onto the gold electrode, 1 μM thiolated oligonucleotide (**P1**) was dissolved in I-buffer and incubated for 30 min at RT to reduce disulfide bonds. Then an aliquot of **P1** solution was dispensed on the freshly cleaned electrode for 4 h at RT. After that the gold electrode was washed thoroughly with Milli-Q water and dried in a nitrogen stream. Then the gold electrode was incubated in M-buffer for 1 h at RT to prevent nonspecific DNA adsorption on the gold surface in the following experiments. Subsequently, the electrode was rinsed thoroughly with Milli-Q water and dried with a stream of nitrogen gas again, which was ready for the electrochemical experiments. For the control experiments, the MCH-modified gold electrode was fabricated as above procedures just skipping the immobilization step of thiolated oligonucleotides.

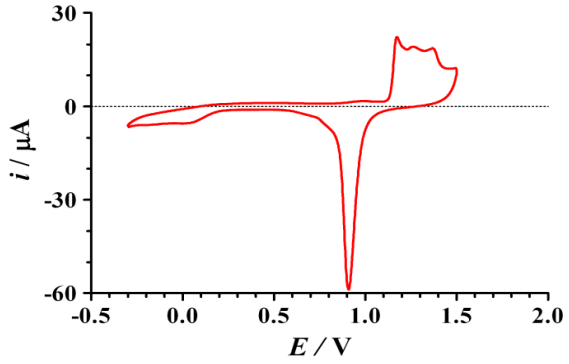

Figure S1. A typical CV curves for a clean, bare gold electrode in a 0.5 M H_2SO_4 solution.

Electrochemical Detection. Prior to target detection, a series of 10 μL S-buffer were dispensed on the fabricated **P1**-modified electrodes for 40 min, respectively. Then the electrodes were washed for three times with D-buffer to remove non-specific MB adsorption, and transferred to a single-compartment cell filled with 8-mL D-buffer to gain the initial redox currents from intercalating MB by SWV measurement. SWV measurement was performed with following parameters: initial potential of -0.6 V, final potential of 0.2 V, frequency of 25 Hz. After the initial measurement, the electrodes were washed thoroughly with Milli-Q water, and dried in a nitrogen stream. The targets (such as **T1** or ATP) with known concentrations were mixed with **S1** (1 μM) and **S2** (1 μM) solution in D-buffer, respectively. Subsequently, a series of 10 μL mixture were dispensed on the electrodes for 1 h at RT, respectively. After that, the electrodes were washed thoroughly with Milli-Q water for 10 s, and dried in a nitrogen stream. Then the electrodes were dispensed in 10 μL S-buffer again for 40 min. Following that, the electrodes were washed thoroughly with D-buffer, and then transferred to a single-compartment cell filled with 8-mL D-buffer to obtain the final redox currents by SWV measurement.

Reference

1 (a) Y. Xiao, R. Y. Lai and K. W. Plaxco, *Nat. Protocols.*, 2007, **2**, 2875; (b) J. Zhang, S. Song, L. Wang, D. Pan and C. Fan, *Nat. Protocols.*, 2007, **2**, 2888.