Electronic Supplementary Information

Target-initiated impedimetric proximity ligation assay with DNAzyme design for *in situ* amplified biocatalytic precipitation

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EXPERIMENTAL SECTION

Materials. Two used single-stranded DNAs (S_1: 5'-SH-AAA AAT CTC TGT GGA GGG-3', S_2: 5'-GGG CAG GGG ACA CA-3') were obtained from Sangon Biotechn. Co., Ltd (Shanghai, China). Hemin was purchased from Tokyo Chem. Inc. (Japan). 4-chloro-1-naphthol (4-CN) and 30% H_2O_2 were purchased from Sinopharm Chem. Re. Co. (Shanghai, China). All the other chemicals were of analytical grade and used without further purification. Ultrapure water obtained from a Millipore water purification system (≥ 18 MΩ, Milli-Q, Millipore) was used in all runs. Before use, DNA stock solution was obtained by dissolving oligonucleotides in 0.01 M phosphate-buffered saline (PBS, pH 7.4) solution. Each oligonucleotide was heated to 90 ºC for 5 min, and slowly cooled down to room temperature before use. Then, the oligonucleotides were diluted with required concentration with HEPES-NaOH buffer (25 mM HEPES-NaOH, pH 7.8, 30 mM KNO_3, 500 mM $NaNO_3$, 0.5 g/L Triton X-100). The stock solution of hemin (5 mM) was prepared in DMSO, stored in the dark at -20 ºC, and diluted to the desirable concentration with the 40 KT buffer (pH 6.2, 100 mM Tris, 50 mM MES, 40 mM KCl, 0.05% Triton X-100, 1% (v/v) DMSO).

Fabrication of Impedimetric Sensor. A gold electrode (3 mm in diameter) was polished repeatedly with 0.3 μm and 0.05 μm alumina slurry, followed by successive sonication in acetone, ethanol and deionized water for 5 min and dried in air. Before modification, the gold electrode was cleaned with hot piranha solution (a 3:1 mixture of H_2SO_4 and H_2O_2, *Cautions*!) for 10 min, and then continuously scanned within the potential range of -0.3 to 1.5 V in freshly prepared deoxygenated 0.5 M H_2SO_4 until a voltammogram characteristic of the clean gold electrode was established. After the pretreatment, the freshly cleaned gold electrode was immediately immersed in the stock solution of 3.0-μL S_1 oligonucleotide solution (1.0 μM). During this process, S_1 oligonucleotide was immobilized on the gold electrode *via* the Au-S bond. After rinsing with distilled water, the modified gold electrode was incubated with 1.0 mM 6-mercaptohexanol in 10 mM Tris-HCl buffer, pH 7.4, for 60 min. Finally, the as-prepared DNA sensor was suspended

over pH 7.4 PBS at 4 ºC for further use.

Measurement Procedure. All electrochemical measurements were carried out with μAutolab III electrochemical analysis system (Metrohm, Switzerland) with a conventional three-electrode system using a modified gold electrode as working electrode, a platinum wire as auxiliary electrode, and a saturated calomel electrode (SCE) as reference electrode. Before measurement, an incubation solution containing 10 μL target Ag^+ standards with various concentrations and 10 μL S_2 oligonucleotide (2 μM) was prepared. To detect target silver ions, the as-prepared DNA sensor was immersed into the incubation solution for 1 h at 37 ºC. During this process, S_2 oligonucleotide was grafted on the surface of the electrode by hybridizing with S_1 oligonucleotide to form quadruplex/duplex DNA structure, and target Ag^+ ions were intercalated between C bases through C-Ag^+-C coordination chemistry. Following that, the electrode was immersed in a solution containing 25 μM hemin for 30 min. Afterwards, the resulting electrode was dipped in the precipitation solution containing 1.0 mM 4-CN and 0.15 mM H_2O_2, and incubation for 10 min at room temperature. Finally, the impedimetric characteristic of the resulting electrode was measured in 0.01 M pH 7.4 PBS containing 2.0 mM $Fe(CN)_6^{4-/3-}$ and 0.1 M KCl. Impedimetric measurement was monitored at frequency 10^{-2} - 10^6 Hz at the formed potential of 220 mV, using alternating voltage of 10 mV. A Nyquist plot (Z_{re} *vs*. Z_{im}) was drown to analyze the impedance results. All calibration plots were calculated relative to zero analyte. Analyses are always made in triplicate.

Assay Principle of Impedimetric Sensor. The strategy of on-line enzymatic biocatalytic precipitation (BCP) for Ag^+ detection is illustrated in Fig. 1. One split part of hemin/G-quadruplex-based DNAzyme is initially immobilized on the gold electrode through the thiol-Au affinity, and then treated with 6-mercaptohexanol to block the non-specific sites on the surface. When the sensing interface is incubated with incubation solution containing target Ag^+ and another split parts, two split parts containing probe S_1 and probe S_2 can easily assemble to form the G-quadruplex. Meanwhile, target silver ions can intercalate into the C bases of the appended

duplex on the 5' terminals of the G-quadruplex. The formation C-Ag^+-C base pairs can strengthen the G-quadruplex and thus promote the binding of G-quadruplex to hemin. The incorporation of hemin into the hemin/G-quadruplex-based DNAzyme is catalytically active in accelerating oxidation of 4-CN by H_2O_2 to yield the insoluble and insulating product benzo-4-chlorohexadienone on the transducer surface. Thus, the resistance of the sensor dramatically increased. Conversely, in the absence of Ag^+, the G-quadruplex structure is unstable and without the ability to bind hemin, thus exhibiting no catalytic activity in the precipitation of insoluble products. By monitoring the change in the resistance, we can indirectly determine the concentration of target Ag^+ in the sample.

Optimization of Experimental Conditions. To acquire an optimal analytical performance, some experimental conditions, such as hybridization time for target-induced proximity ligation assay and deposition time of 4-CN for the BCP, should be investigated. In this work, the signal amplification of the impedimetric sensor mainly derived from the biocatalytic precipitation through the formed split DNAzyme. So, the hybridization time of S_1 oligonucleotides interaction with S_2 oligonucleotides and silver ions should be firstly optimized. During this process, 10 nM Ag^+ was used as an example. As shown in Fig. S1-a, the electron-transfer resistances increased with the increasing hybridization time, and tend to level off after 60 min. Hence, 60 min was used for S_1 hybridization with the complementary strand and target analyte.

The biocatalytic precipitation process is also very crucial to achieve the optimal performance. After the hemin/G-quadruplex-based DNAzyme was formed on the gold electrode, the sensor was incubated with 4-CN in the presence of H_2O_2 for different times at 37 ºC (10 nM Ag^+ was used as an example). As seen from Fig. S1-b, the optimal impedimetric response was obtained after 10 min, and longer deposition times did not cause the significant change in the resistance. To save the time, 10 min was selected as the deposition time for the development of BCP.

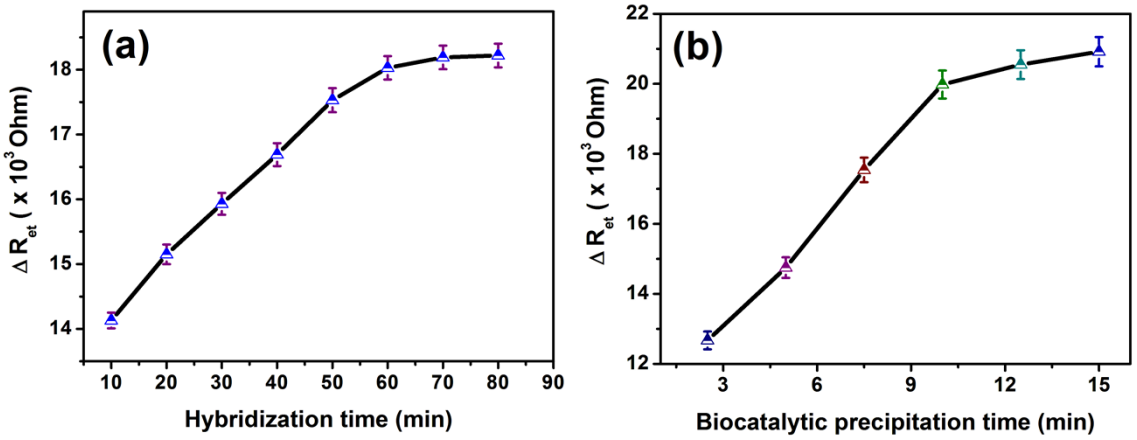

Fig. S1 The effect of (a) hybridization time of target-initiated proximity ligation assay and (b) biocatalytic precipitation time on the impedimetric signal of the developed assay protocol by using 10 nM Ag^+ as an example.

Table S1 Comparison of analytical properties of PLA-based impedimetric sensor with other Ag^+ assay methods.

Method	Linear range (nM)	LOD (nM)	Ref.
Fluorogenic and chromogenic probe based sensor	100 – 5000	130	S1
Surface plasmon resonance sensor	50 - 2000	10	S2
Carbon-nanotube-based fluorescent sensor	0-150	1	S3
DNAzyme-based colorimetric assay	50-3000	64	S4
Tween 20-stabilized gold nanoparticles-based colorimetric assay	400-1000	-	S5
Impedimetric sensor	100-800	10	S6
Solid-contact potentiometric polymer microelectrodes based assay	1-10000	-	S7
Potentiometric sensor	-	1	S8
Peptide-coated CdS quantum dots based optical assay	-	~500	S9
PLA-based amplified strategy	0.1-25	0.08	This work

Notes and references

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