# Supporting Information

# Robust and Ultrasensitive Electrochemical Detection of Ochratoxin A Using Highly Reactive DNAzyme Wired via Primer Exchange Reaction

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## Experiment section

### Materials and reagents

All oligonucleotides utilized in the detection were obtained from Sangon Biotech. Co., ltd. (Shanghai, China) with HPLC purification. Ochratoxin A (OTA), dimethyl sulfoxide (DMSO), zearalenone (ZEN), tris (2-carboxyethyl), phosphine (TCEP), anhydrous magnesium chloride ($MgCl_2$), 6-mercapto-1-hexanol (MCH) were purchased from Aladdin Biochemical Technology Co., ltd. (Shanghai, China). Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), hydrogen peroxide ($H_2O_2$), ochratoxin B (OTB), ochratoxin C (OTC), aflatoxin B1 (AFB1) were purchased from Sigma-Aldrich Trading Co., ltd. (Shanghai, China) KF DNA polymerase (KF), NEBuffer 2 (pH=7.9), deoxynucleotide dATP, dCTP. and dGTP mixture (dHTPs) were purchased from New England Biolabs Ltd; (Beijing, China). All the chemical reagents used in the experiment are analytically pure, and Ultrapure water was obtained using a Millipore water purification system (18.2 MΩ cm resistivity).

### Instruments

The equipment used in this experiment includes CHI 660D Electrochemical Workstation (Shanghai, China) for detecting cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV). Electrophoresis instrument power supply (Beijing, China) and WD-9405F decolorisation shaker (Beijing, China) and Bio-Rad Gel Electrophoresis Imaging System (USA): polyacrylamide gel electrophoresis characterization.

## Figures and tables

**Table S1.** Oligonucleotide sequences used in this work

| Oligonucleotide name | sequence (5' to 3') description |
|---|---|
| Aptamer (Apt) | GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA |
| Primer 1 (P1) | TGCCGATG CAAA G ACA CCGA |
| Hairpin 1 (H1) | **CGAAGAGCCGC** TTTGGGG AAAAA CCCCAAA **GCGGCTCTTCG** TCCGGCTCGG-inverted-dt |
| Hairpin 2 (H2) | **CACCCGAGCCG** TTTGGGG AAAAA CCCCAAA **CGGCTCGGGTG** TCTTTGCGGC-inverted-dt |
| DNAzyme | AGACA CCC GAGCCGGACGA AGAGCC |
| H-AgNCs | ***CCCCCCCCCCCC*** **CCGGC** GGCTCTrAGGGTGTCT **GCCGG** |
| Capture DNA (cDNA) | **CCGCCGG** AAAAAA **CCGCCGG** AAAAAA **CCGCCGG** AAAAAA-SH |

Table S1. shows the nucleic acid probes and their sequences involved in this work. The aptamer (Apt) is underlined with promoter 1 (P1) as the region where they hybridize. bold red bases in P1 are the region that hybridizes with hairpin 1 (H1). bold in H1 and hairpin 2 (H2) is the region that hybridizes itself, to be extended, and is underlined as the stop site after hybridization. the middle of the DNAzyme is its active center and is underlined as the region that can be designed to hybridize with hairpins in H-AgNCs. The italicized C-rich sequence in H-AgNCs is the DNA template for the synthesis of AgNCs, the blue bold is the part of the hairpin that hybridizes itself, and the blue region in the 3' direction can be used as a primer loop for a new round of PER. the bold in capture DNA (cDNA) captures the binding region of AgNCs and the A-rich base is the spacer region. the 3' ends of H1 The 3' ends of H1 and H2 are modified with inverted-dt to prevent non-specific extension. The 3' end of cDNA is modified with a sulfhydryl group to anchor to the gold electrode.

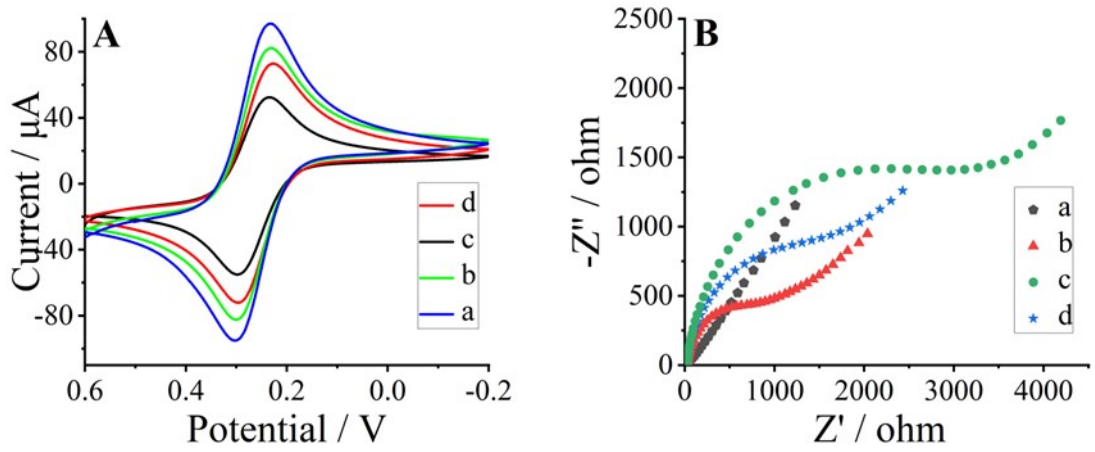


**Figure S1.** Electrochemical characterization curves of the reaction process on the electrode surface. CV change (A) and EIS change (B) on the electrode surface. Bare gold electrode (a); gold electrode with modified cDNA (b); gold electrode with modified MCH and cDNA (c); gold electrode with positive sample added and modified MCH and cDNA (d).

**Table S2** Application of the method for OTA determination in actual samples and comparison with the HPLC.

| Samples | Spiked amount（ng/mL） | Measured（ng/mL） | | Recovery (%)±SD, n=3 | |
|---|---|---|---|---|---|
| | | Our method | HPLC | Our method | HPLC |
| water | 15.00 | 15.283±0.047 | 14.997±0.067 | 100.35±4.3 | 101.7±1.2 |
| | 10.00 | 10.25±0.053 | 9.998±0.026 | 100.24±4.5 | 96.2±4.5 |
| | 5.00 | 4.897±0.088 | 4.9893±0.018 | 99.32±5.2 | 99.53±3.5 |
| | 2.00 | 2.367±0.068 | 2.5030±0.006 | 99.94±0.8 | 97.2±0.4 |
| | 1.00 | 1.023±0.073 | 0.957±0.009 | 100.26±2.4 | 101.1±1.6 |