Supporting information

A Portable Polymeric Electrochromic-Based Visual Biosensing Device with Distance Readout

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1. Experimental procedures

1. 1 Materials and Apparatus

Aniline ($C_6H_5NH_2$, AR, ≥99.5%), sulfuric acid (H_2SO_4, AR, 98%), gold chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, ≥99.9%), sodium dihydrogen phosphate (NaH_2PO_4, AR), and dibasic sodium phosphate (Na_2HPO_4, AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Tris (2-carboxyethyl) phosphine (TCEP, AR） and 6-mercaptohexanol (MCH, AR) were purchased from Aladdin Chemical Reagent Co., Ltd. (China). Fluorine-doped tin oxide (FTO) conducting glass was purchased from Shenzhen Huanan Xiang cheng Technology Co., Ltd. (China) The designed OTA toxin aptamer sequence[1] is as follows: 5'-SH-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA-3' and TE buffer solution was purchased from Sangon Biotech (China). Ochratoxin A (OTA), aflatoxin B1 (AFB1) were purchased from Sigma-Aldrich (USA). Other interferents including ochratoxin B (OTB), ochratoxin C (OTC) and T-2 toxin were purchased from Puhuashi Technology Company, (China). The deionized water used has been treated by the Milli-Q system. All chemical reagents were received without further purification.

The pre-designed patterns are etched on FTO using Portable fiber laser marking machine (RUIZHIYILASER RZY-GX-20, China). Energy-dispersive X-ray spectroscopy (EDS) and morphologies of nanomaterials were observed by scanning electron microscopy (SEM, JSM-7800F, Japan), X-ray diffraction (XRD) spectra were obtained through the Bruker D8 ADVANCE (German), and Infrared spectrum (IR) were got from Nicolet Nexus 470 FTIR (America). And, in this work, all electrochemical tests in this work use CHI 760E electrochemical workstation, Chen Hua Instruments Co., Shanghai, China. The EIS measurements were conducted under an oscillation potential of 5 mV in a frequency range covering 10 kHz–0.01 Hz, in phosphate-buffered saline (PBS) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl at room temperature.

1.2 Device Modification

Firstly, FTO glasses were cut into 2 cm × 5 cm size pieces using a glass knife. Subsequently, they were ultrasonically cleaned with toluene, acetone, ethanol and deionized water in sequence for 15 minutes each and dried at 60 ℃ for 1 hour. The pre-designed working area on the dried glass surface was etched using laser etching techniques. Ag/AgCl was selected as the reference electrode while Pt served as the counter electrode for electrochemical polymerization of Polyaniline on the glass surface. This process took place in an electrolyte containing 0.1 M aniline and 0.5 M sulfuric acid under galvanostatic conditions with a density of 0.2 mA/cm^2 for a duration of 800 seconds, followed by overnight drying at room temperature. Finally, gold nanolayers were prepared in the detection area through cyclic voltammetry (CV) utilizing a scanning range of -0.2 to 1.2V and a scanning speed of 20 mV/s over ten cycles in a solution containing chloroauric acid at a concentration of 2 mM. Ag/AgCl was employed as the reference electrode while Pt served as the counter electrode.

1.3 Activation of thiol aptamers

The purchased thiol modified aptamer is in the form of SH Oligo, which is an exposed thiol group that can undergo spontaneous oxidation during transportation or storage to form a dimer structure with disulfide bonds.[2] Therefore, restoration processing is therefore necessary prior to utilization. The newly acquired aptamer was subjected to centrifugation at a speed of 13,000 rpm for a duration of ten minutes, followed by the addition of TE buffer solution to prepare a stock solution with an aptamer concentration of 10 μM. The aptamer reserve solution was supplemented with a specific volume of 1mM TCEP solution, maintaining a volume ratio of 1:25 between the TCEP solution and the aptamer reserve solution. The mixture was thoroughly mixed and incubated at room temperature for 60 minutes.

1.4 Fabrication of the biosensing device

The detection area was equipped with frame tapes to prevent solution overflow. Subsequently, 20 μL of activated aptamer was applied onto the detection area and incubated for several hours at room temperature. Following this, the detection area was

gently rinsed with ultrapure water to eliminate any unbound aptamer. Then, 20 μL of 1 Mm MCH was added dropwise and allowed to react for 1 hour in order to block the active site. Finally, a slow flush with ultrapure water was performed to remove any unconjugated MCH, completing the preparation of the biosensing device.

1.5 The detection of OTA toxin

The detection area was exposed to varying concentrations of OTA toxin, followed by incubation at room temperature for 1 hour. Subsequently, an external voltage was applied to the biosensing device and maintained for a specific duration to observe the extent of polyaniline line discoloration in the visualization area, which directly correlated with the concentration of OTA toxin.

1.6 Pretreatment of actual samples

OTA toxin in the various maize samples were extracted by suspending two grams of maize flour in 10 mL of 70% (v/v) methanol. The suspensions were subsequently homogenized by shaking for approximately five minutes at room temperature. One hundred microliter (100 μL) aliquot from each suspension was diluted with 600 μL of distilled water to reduce the concentration of methanol to 10% (v/v).[3] Subsequently, the OTA toxin levels were measured by the use of a novel electrochemical biosensor chip.

2. Results and discussion

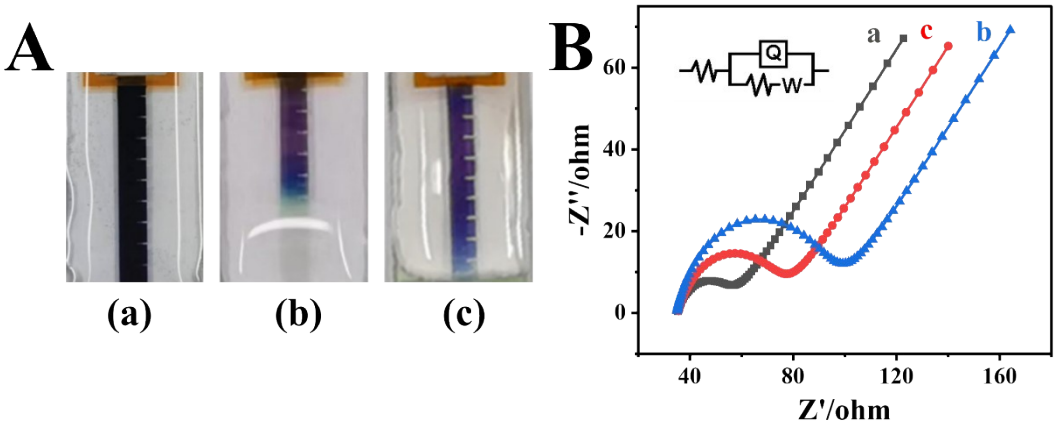

Fig. S1 (A) Photographs of the visual area and (B) EIS spectra with different modification of the detection area: Au/FTO (a), aptamer/Au/FTO (b), and OTA/aptamer/Au/FTO (c)

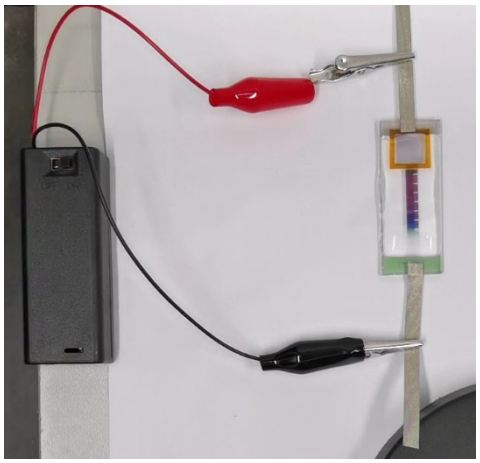

Fig. S2 Photo of the biosensing device

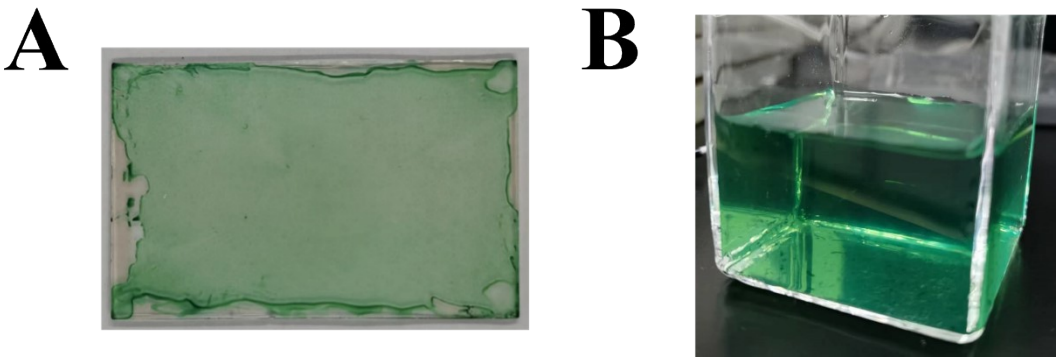

Fig. S3 Polystyrene sulfonic acid doped polyaniline on the surface of FTO (A) and Polystyrene sulfonic acid doped polyaniline aqueous solution (B)

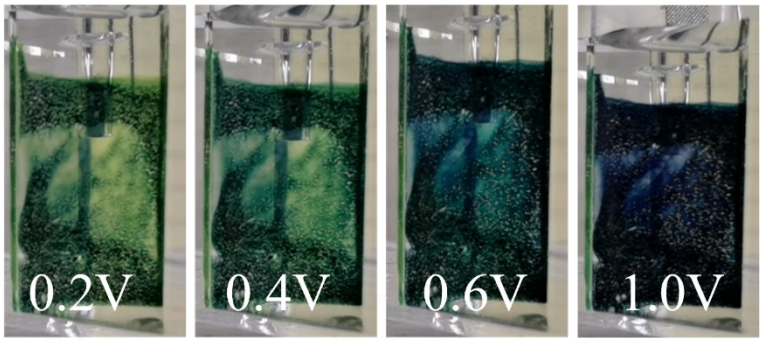

Fig. S4 Color changes of polyaniline doped with dodecyl benzene sulfonic acid at different potentials

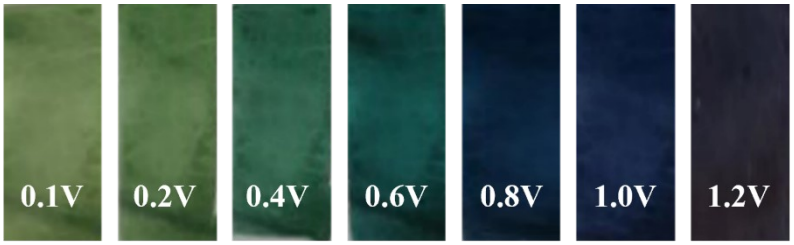

Fig. S5 Color change of sulfuric acid doped polyaniline at different potentials

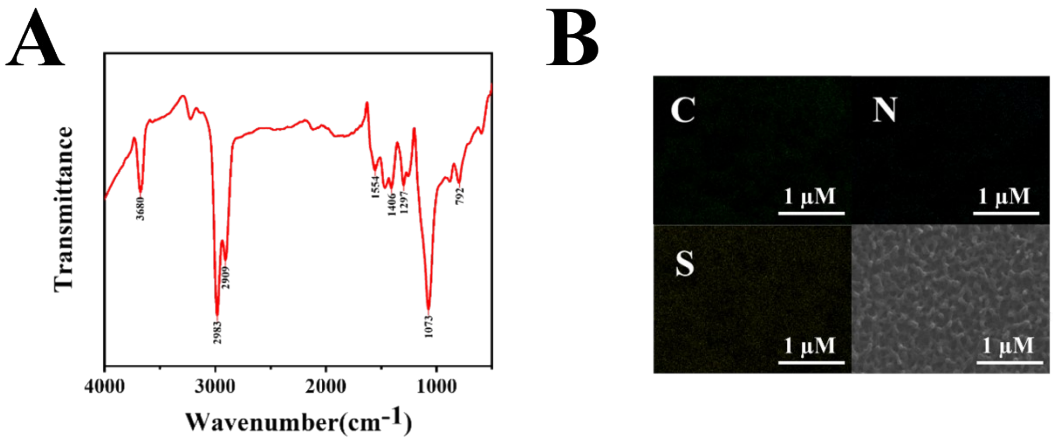

Fig. S6 FTIR analysis (A) and The EDS spectra (B) of synthesized PANI.

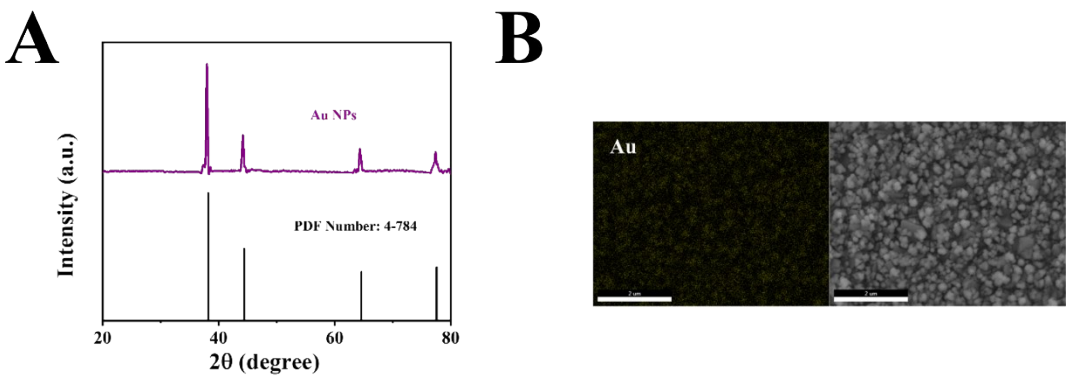

Fig. S7 XRD analysis (A), The EDS spectra and SEM (B) of synthesized Au NPs.

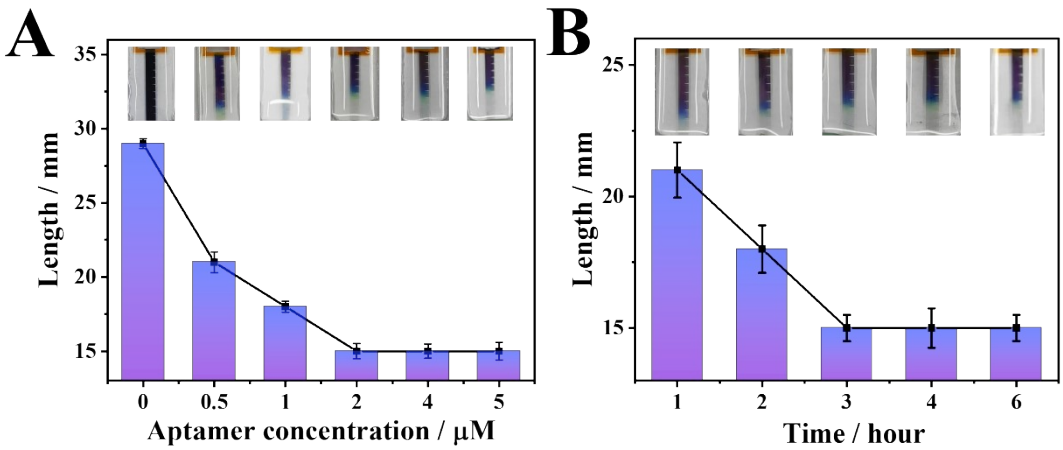

Fig. S8 Photographs of the visual area and respective electrochromic lengths (A) modified with different concentrations of aptamers (0.5 μM, 1 μM, 2 μM, 4 μM, and 5 μM) and (B) combined with different time between aptamers and AuNPs (1, 2, 3, 4, and 6 hours)

The detection area was gently coated with 20 μL of solution containing OTA aptamers at various concentrations (0, 0.5 μM, 1 μM, 2 μM, 4 μM, and 5 μM) using a dropwise application method. The binding was occurred at room temperature for 6 hours and then carefully rinse the detection area with PBS (pH 7.4) to eliminate any residual bound aptamers. Subsequently, a voltage of 1.5V was applied to the device for a duration of 60 seconds, resulting in a gradual decrease in electrochromic distance with increasing OTA aptamer concentration. however, once the concentration reached 2.0

μM, further changes in electrochromic length became negligible. Consequently, 2.0 μM was selected as optimal concentration for constructing a distance-based readout electrochromic visualization biosensing device.

To further shorten the reaction time, we investigated the effect of binding time of OTA aptamer on the color change distance. A volume of 20 μL containing a concentration of 2 μM OTA aptamer was carefully dispensed onto the surface of AuNPs in the designated detection area, allowing for an appropriate incubation period. The binding of gold-thiol bonds occurred at room temperature. The experimental results demonstrate a decrease in the color change distance with increasing binding time; however, once the binding time exceeds 3 hours, the color change distance reaches a plateau. This observation can be attributed to the progressive binding of aptamers to AuNPs on the substrate over time, resulting in an elevation in impedance within the detection area. Hence, a binding time of 3 hours was chosen to establish the interaction between the aptamer and the substrate.

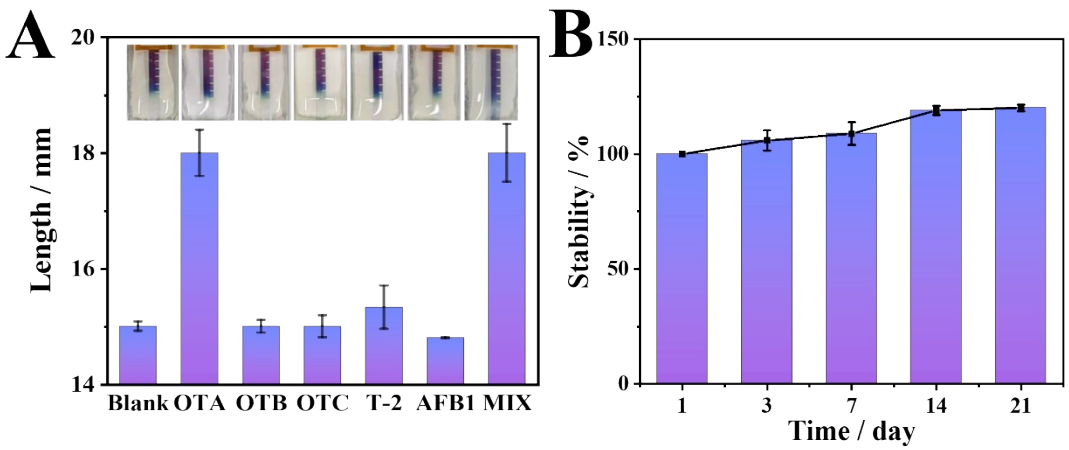

Fig. S9 (A) The selectivity and (B) the stability test of the electrochromic biosensing device

Table S1. Comparison with other detection methods for detecting OTA toxin

Methods	Liner range (μg/L)	Detection limit (ng/L)	Refs.
ECL aptasensor	0.1-320	30	4
Colorimetric method	0.1–500	41.9	5
fluorescence probe	0.02-2.00	8	6
fluorescent aptasensor	0.01-500	2.7	7
electrochemical aptasensor	0.000100-50	0.081	8
electrochromic-based biosensor platform with distance readout	0.000010-50	0.00495	This work

Table S2. Results of OTA Toxin Detection in corn flour Samples (n = 3)

Sample	Added concentration (ng/L)	Found (ng/L)	Recovery (%)	RSD (%)
1	0.1	0.09958	99.58	3.14
2	50	47.54	99.05	5.44
3	300	326.51	108.83	8.5

3. Reference

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