Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Construction of an electrochemical-fluorescent dual-mode sensor with a dual-mode signal AgNCs probe synthesized from cytosine-rich DNA for OTA detection

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Materials and reagents

Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), ochratoxin A (OTA), fumonisin B1 (FB1), and zearalenone (ZEN) were purchased from Sigma Co., Ltd (USA). Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 6-mer-captohexanol (MCH), and ethylenediamine tetraacetic acid (EDTA) were purchased from Yuanye Biological Co. Ltd (Shanghai, China). $AgNO_3$, $NaBH_4$ were purchased from Comio Chemical Reagent Co (Tianjin, China).

All the DNA oligonucleotides were purchased from Sangon Biotech Co. Ltd (Shanghai, China), and the sequences were listed as follows：

Bio-Apt:5'- Bio-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA -3'

C-DNA:5'-CCCCCCCCCCCCCCCCCCCCCCCCCTGTCCGATGCTCCCTTTACGCCCCC-SH-3'

DNA hybridization buffer was made of 20 mmoL L^{-1} PB (pH 7.0) containing 0.2 M Na_2HPO_4 and 1 mM EDTA; CV and EIS buffers of 5 mM $[Fe(CN)_6]^{3-/4-}$ (5 mM $K_3[Fe(CN)_6]$, 5 mM $K_4Fe(CN)_6.3H_2O$, 0.1 M KCl, dissolved in 0.02 M PBS buffer); Magnetic bead washing solution (pH 7.5) was made of 10 mM Tris-HCl, 1 M NaCl, 1 mM EDTA, and 0.05% Tween-20.All these reagents were used without further purification and ultrapure water (18.25 MΩ cm^{-1}, Milli-Q, Millipore) was used in the whole assay.

Instruments

Electrochemical Impedance Spectroscopy (EIS), Cyclic Voltammetry (CV) and Square Wave Voltammetry (SWV) were conducted on a CHI 660E electrochemical workstation (Shanghai Chenhua Instrument Corporation, China). Fluorescence

detection was performed on a Japan Spectroscopy (JASCO, FP-8050) fluorescence spectrometer. Transmission electron microscopy (TEM, JEOL JEM-F200) was used to observe the morphology and microstructure of AgNCs at 200 kV. A three-electrode system was composed of gold electrode (AuE) as the working electrode, Ag/AgCl as the reference electrode, and platinum wire as the counter electrode.

All electrochemical experiments were carried out at CHI660E electrochemical workstation. The three-electrode system used in this experiment was a gold electrode with a working surface of 3 mm as the working electrode, a saturated silver chloride electrode as the reference electrode, and a platinum wire electrode as the counter electrode. The potential used in the AC impedance parameter setting was 0.19 V, the scan rate was 5 mV/s, and the scan range was 0.1 Hz~10^6, and the detection solution was 5 mM $[Fe(CN)_6]^{3-/4-}$ solution. The CV parameters used to characterize the sensor preparation process were a scan rate of 0.1 V/s and a scan range of -0.2 V to 0.6 V, and the detection solution was 5 mM $[Fe(CN)_6]^{3-/4-}$ solution. The SWV method parameters are set to a scan interval of -0.2 ~ 0.4 V, an amplitude of 0.025 V, and a frequency of 15 Hz.

The Electrode Pretreatment

The AuE was soaked in the newly configured piranha solution (H_2SO_4:H_2O_2=7:3) for 20 min, and then ultrasonically cleaned in absolute ethanol and ultrapure water for 5 min, respectively. Next, the AuE was polished with 0.05 μm alumina powder until the surface was smooth, and ultrasonically cleaned in absolute ethanol and ultrapure water for 5 min respectively. It was scanned by cyclic voltammetry (CV) in 0.5 M sulfuric acid solution, then washed with ultrapure water, and dried with nitrogen for further use.