Supplementary material

Electrochemical aptasensor based on cocoon-like DNA nanostrucures signal amplification for detection of *Escherichia coli* O157:H7

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2. Materials and methods

2.1 Chemicals and materials

Tris and EDTA disodium salt were purchased from Amresco Inc. (Solon, OH, USA). Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), MCH, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), hemin, dimethylsulfoxide (DMSO), dithiothreitol (DTT), potassium hexacyanoferrate(III), and potassium ferrocyanide trihydrate were purchased from Sigma–Aldrich (St. Louis, MO, USA). H_2O_2 (30%), ethanol, Na_2HPO_4, NaH_2PO_4 and other metal ion salts were obtained from Beijing Chemical Works Co. Ltd. (Beijing, China). All chemicals were of reagent grade without further purification.

The stock solution of hemin (1 mM) was prepared by dissolving hemin in DMSO and stored at −20 °C for further dilution. Tris-buffer (20 mM, pH 7.4) containing 200 mM NaCl, 50 mM KCl, and 1 mM $MgCl_2$ was used for diluting the stock solution of hemin. Tris–EDTA (TE) buffer (pH 8.0) containing 10 mM Tris and 1 mM EDTA was used for preparing the stock solution of RCA products. Phosphate-buffered solution (PBS, 0.01 M, pH 7.0, 0.1 M KCl) was prepared using NaH_2PO_4, Na_2HPO_4 and KCl in ultrapure water, then filtered and autoclaved (120 °C for 20 min).

Cultures of *E. coli* O157:H7 were incubated in Luria–Bertani broth overnight at 37°C, then centrifuged (4500 rpm, 10 min), washed twice and resuspended in PBS (1 mM, pH 7.4). The bacterial suspension was serially diluted 10-fold in PBS, for cell enumeration and further experiments.

RCA products were verified by 1% agarose gel electrophoresis on DYY-6B electrophoresis cell (Beijing, China) and TFP-M/WL Gel imaging system (Vilber-Lourmat, Marne-la-Vallée, France). Scanning electron microscopy (SEM) images of RCA nanoclusters (CDNs) were obtained on a Zeiss Supra 40 SEM (Oberkochen, Germany) at an accelerating voltage of 15 kV, and equipped with an energy dispersive X-ray spectrometer (EDS, EDAX, USA).

2.4 Pretreatment of the electrode

Previous to the electrode modification, the Au electrode was polished on a suede pad with an alumina slurry (0.05 μm) until a mirror-like surface was obtained. Then, it was treated in a piranha solution (H_2SO_4/H_2O_2, volume ratio of 3: 1) for 30 min, and thoroughly washed with ultrapure water

to remove organic matter on its surface. Then, it was sonicated with absolute ethanol and ultrapure water for 5 min, respectively. After that, the electrode was subjected to potential scanning in H_2SO_4 solution (0.5 M) at a scan rate of 100 mV/s between 0.2 and 1.4 V until a typical stable redox peak of Au was obtained. Finally, the electrode was washed with ultrapure water and dried with high purity nitrogen. The thiolated CP was treated with 1 mM TCEP for 30 min to reduce disulfide bonds. The AP was treated for 3 min at 90 °C, and then quickly incubated on ice to form a functional structure.

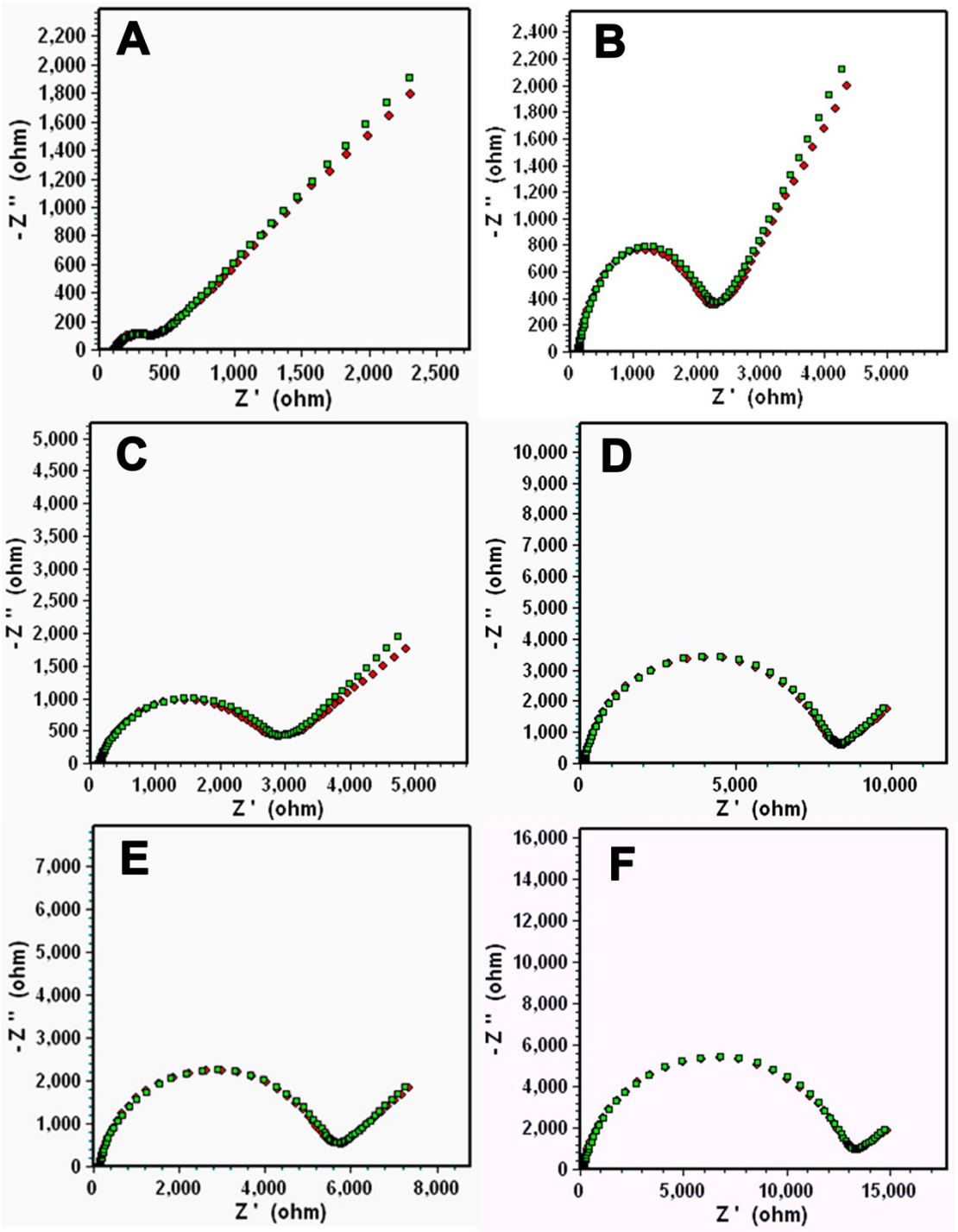

Figure S1 The actual curve (red) obtained by EIS measurement of (A) bare electrode, (B) CP-modified electrode, (C) MCH-modified electrode, (D) aptamer-modified electrode, (E) target-incubated electrode and (F) CDNs modified electrode, and the corresponding fitted curve (green) calculated by ZSimpWin software.

Table S1 Electrical parameter values of fitted equivalent circuit elements from electrochemical impedance spectroscopy

Curve	Rct (Ω)	Rs (Ω·cm^2)	CPE-Yo ($\times10^{-6}$, S·sec^n·cm^{-2})	CPE-n	Zw ($\times10^{-4}$, S·sec^5·cm^{-2})
a	295.9	115.2	0.114	0.9419	6.794
b	2042	133.9	2.691	0.8249	6.786
c	2668	132.8	2.486	0.8073	6.703
d	7825	136.2	0.733	0.9156	7.313
e	5279	142.3	1.123	0.8922	7.061
f	12640	155.7	0.9042	0.9004	6.896

Ret: charge transfer resistance; Rs: resistance of electrolyte; Zw: Warburg impedance; CPE: constant phase capacitance (Yo and n are the two non-zero real number components of the constant phase element corresponding to energy dissipation and surface heterogeneity, respectively.)