Electronic Supporting Information

Triggered activity of a nicking endonuclease for mercuric (II) ion-mediated duplex-like DNA cleavage

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Experimental Section

Materials. DNA sequences used in the experiment, oligo 1 (5'-NH_2-$(CH_2)_6$-CGGCTTTGATCCCCCCGGATCTTTGCCG-$(CH_2)_6$-SH-3') and oligo 2 (5'-CGGCTTTGATCCCCCCGGATCTTTGCCG-3'), were synthesized by SBS Genetech. Co. Ltd. The nicking endonuclease, Nt.AlwI (containing NE buffer 2) was purchased from NEW England BioLabs Co. Ltd. (Ipswich, 106 MA, USA). 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) was from Jinchun Reagent Co. Ltd. (Shanghai, China). Ferrocenecarboxaldehyde (Fc-CHO) and 2-mercaptoethanol (MCH) were purchased from Sigma-Aldrich. Metal salts used in this study ($Hg(NO_3)_2$, $CoCl_2$, $ZnCl_2$, $MgCl_2$, $CaCl_2$, $Pb(NO_3)_2$, $MnCl_2$, $CuSO_4$, $NiCl_2$, $CdCl_2$, $AgNO_3$) were purchased from Sinopharm Chemical Reagent Co. Ltd. All other reagents were of analytical reagent grade and were used without further purification or treatment. Double distilled water (DDW) was used throughout the measurements.

Instruments. Cyclic voltammetric (CV), differential pulse voltammetric (DPV), and electrochemical impedance spectroscopy (EIS) measurements were performed with a CHI 660D electrochemical analyzer (Shanghai CH Instrument Company, China). A three-electrode system was employed with Pt wire as the auxiliary electrode, saturated calomel electrode as the reference

electrode, and gold electrode or modified gold electrode as the working electrode, respectively.

Labeling a ferrocene (Fc) tag to oligo 1. 1.5×10^{-3} M Fc-CHO in 500 μL DMSO, oligo 1 (3.38 nmol) dissolved in 500 μL 0.1 M sodium borate buffer (pH 8.5), and 500 μL 0.1 M EDC were mixed together and shaken at 25 °C overnight. Then 6 mL ethanol and 3 M acetate buffer (pH 5.2) at the ratio of 20: 1(v/v) was added in the above solution and kept in a refrigerator (< –15 °C) for 20 min. After being centrifuged, the precipitate was washed with ethanol (70%) to remove unreacted labeling reagent. This Fc labeled DNA probe was dissolved in 5 mL TE buffer (10 mM Tris–HCl containing 1 mM EDTA, pH 8.0) and stored in a refrigerator (< –15 °C) before use.

Preparation of the Fc-ssDNA modified gold electrodes. Prior to modification, gold electrodes were polished with 1.0, 0.3, and 0.05 μm alumina slurry, respectively. Then, the electrodes were rinsed thoroughly with DDW between each polishing step and were cleaned by ultrasonication. Then the clean electrodes were incubated in Fc-ssDNA solution for 12 h at 4 °C. After being thoroughly rinsed with DDW, the electrodes were passivated by immersion in 5 mM MCH for 30 min. This is a critical step as it can gradually displace nonspecifically adsorbed oligonucleotides to form an ordered self-assembled monolayer. The Fc-ssDNA modified gold electrodes were soaked in 1 M $NaClO_4$ before use.

Surface hybridization in the presence of Hg^{2+} or other metal ions. The Fc-ssDNA modified gold electrodes were firstly immersed in solutions of metal ions. Then the solutions were heated to 90 °C for 30 s, and allowed for slow cooling down to room temperature for 1 h.

Conditions for cleavage of Hg^{2+} mediated duplexlike DNA by Nt.AlwI. Hg^{2+}-mediated duplexlike DNA (Hg^{2+}-dsDNA) modified electrodes were incubated in a mixture of 48 μL 0.02 M Tris–HCl buffer (containing 5 mM KCl, pH 7.4), 1 μL NE buffer 2 and 1 μL Nt.AlwI at 37 °C for 2 h, and then the electrodes were removed and rinsed with DDW.

Electrochemical characterization. The electrochemical properties of differently modified electrodes were characterized by CV and DPV, with 0.02 M Tris–HCl (pH 7.4) containing 5 mM KCl and 0.5 M $NaClO_4$ as the supporting electrolyte. EIS measurement was performed in 0.1 M KCl containing 1 mM $Fe(CN)_6^{3-}$ and 1 mM $Fe(CN)_6^{4-}$, with the frequency ranging from 10^{-1} to 10^4 Hz.

Polyacrylamide gel electrophoresis. Samples of oligo 2, Hg^{2+}-dsDNA, and products of Hg^{2+}-dsDNA being digested by Nt.AlwI of the same concentration of 1.35 μM were analyzed by

nondenaturing electrophoresis in 20% polyacrylamide gels. Gels were stained with ethidium bromide, illuminated at 302 nm, and scanned with a CCD camera using the IS-1000 digital imaging system.

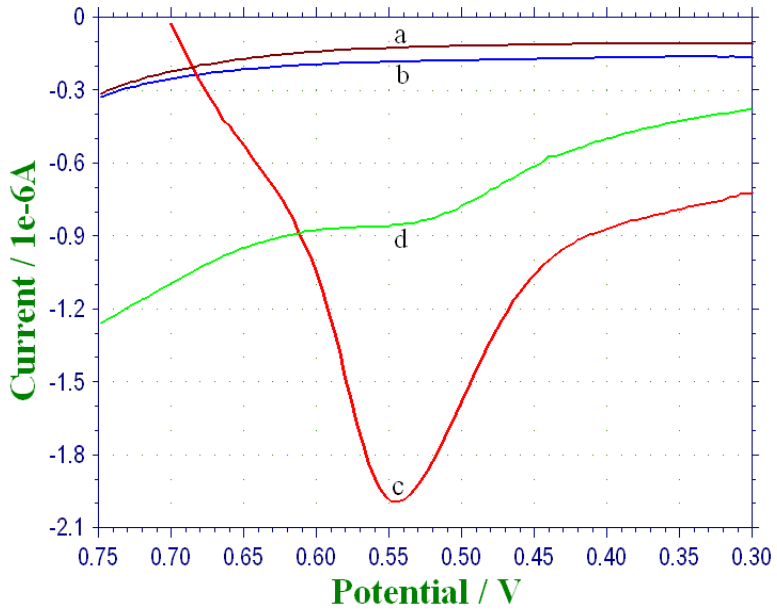

Fig. S1 DPV curves of Fc-ssDNA modified electrode before (a) and after digestion with Nt.AlwI (b); Hg^{2+}-dsDNA modified electrode before (c) and after digestion with Nt.AlwI (d).

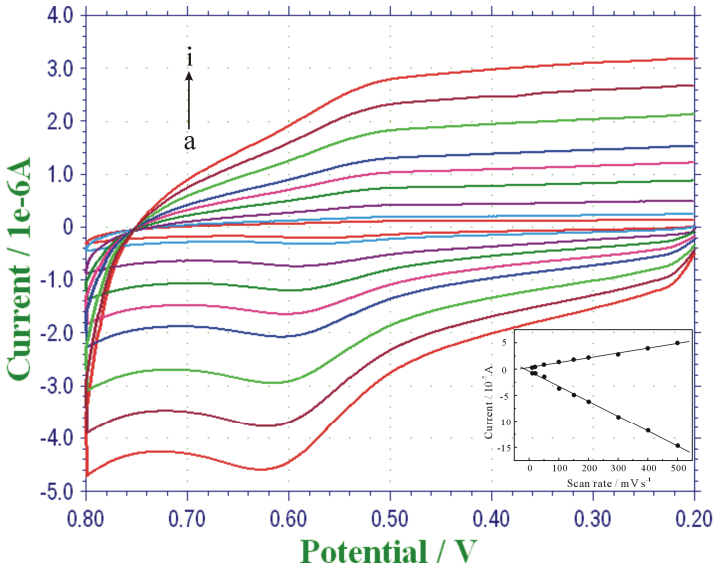

Fig. S2 CVs of Hg^{2+}-dsDNA modified gold electrode in 0.02 M Tris–HCl containing 5 mM KCl and 0.5 M $NaClO_4$ (pH 7.4) at different scan rates of (a) 10, (b) 20, (c) 50, (d) 100, (e) 150, (f) 200, (g) 300, (h) 400, (i) 500 mV s^{-1}. Inset shows the plots of peak current versus the scan rate.

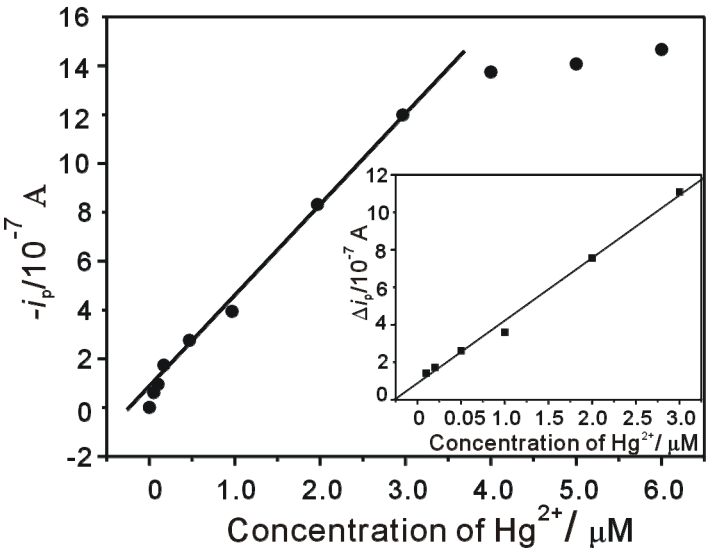

Fig. S3 Plot of DPV peak currents versus Hg^{2+} concentrations. Inset: current signal change of Hg^{2+}-dsDNA modified electrode after being digested by 10 units Nt.AlwI versus Hg^{2+} concentration ranged from 0.1 to 3 μM.

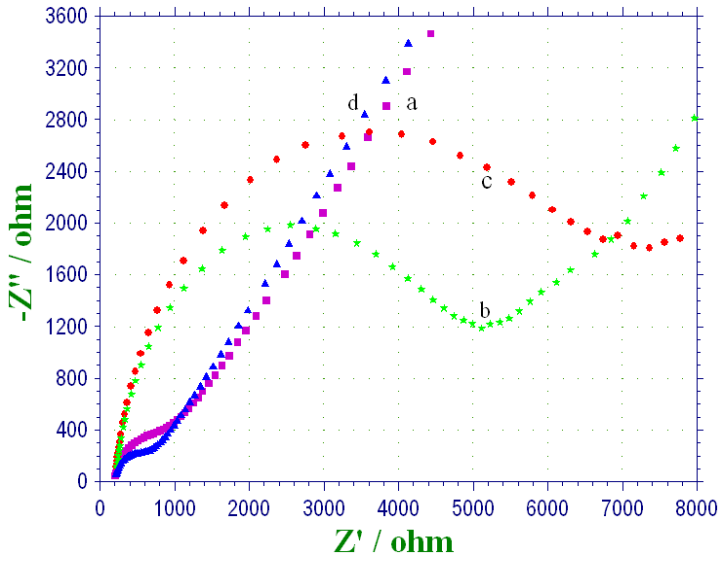

Fig. S4 EIS for (a) the bare gold electrode, (b) Fc-ssDNA modified electrode, (c) Hg^{2+}-dsDNA modified electrode, and (d) Hg^{2+}-dsDNA modified electrode treated with Nt.AlwI in a solution of 0.1 M KCl containing 1.0 mM $Fe(CN)_6^{3-}$ and 1.0 mM $Fe(CN)_6^{4-}$.