Supporting Information

Experimental Section

Materials and chemicals

DNA oligonucleotides were synthesized by Sangon Inc. (Shanghai, China). Their sequences are shown below:

Capture ssDNA: 5'-HS-$(CH_2)_6$-CTCGCCTCTGGCCC-3' (1)

Probe ssDNA: 5'-NH_2-GGGCCACAGGCGAG-3' (2)

There is a C-C mismatched base pair (in underline) in the DNA. 6-mercaptohexanol (MCH) and ferrocene acetic acid were purchased from Sigma, USA. N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) and N-Hydroxysuccinimide (NHS) were received from Shanghai Medpep Co., Ltd. All other chemicals were of analytical grade.

All solutions were prepared with using Milli-Q reagent water (Milli-Q, Millipore, 18.2-MΩ resistivity). DNA buffer solutions (the concentrations of oligonucleotides are 1 μmol/L) were obtained by dissolving oligonucleotides with a 40 mmol/L Tris-acetate buffer solution (pH 7.6) containing 100 mmol/L NaCl. Metal ion solutions were prepared from nitrate salts. All work solutions were prepared with Tris-acetate buffer solution (pH 7.6).

Apparatus

All electrochemical measurements were carried out using CHI 660D electrochemical system (CH Instruments, Shanghai, China) at room temperature. A

three-electrode electrochemical cell was used. Gold electrode (2 mm in diameter, CH Instruments, Shanghai, China) was used as the working electrode. Platinum wire and Ag/AgCl (saturated with KCl) were used as counter electrode and reference electrode, respectively.

Preparation of Fc-Labeled ssDNA

The Fc-labeled ssDNA was synthesized according to the previously reported procedure[1]. Firstly, 1 mg of ferrocene acetic acid was added to 2 mL of Tris-acetate buffer solution containing EDC/NHS (0.1 mol/L each) for 2 h to activate the COOH group of ferrocene acetic acid. Then, 50 μL of activated ferrocene acetic acid solution was mixed with 50 μL probe ssDNA solution and reacted at room temperature overnight. After dialyzing against Tris-acetate buffer solution for 24 h (to remove excessive ferrocenecarboxylic acid), the mixture was then stored in refrigerator at 4 ^{o}C for the following experiments.

Preparation of the modified electrode

A gold electrode was polished with aqueous slurries of 1.0 μm, 0.3 μm and 0.05 μm α-Al_2O_3 powders on a polishing microcloth and sonicated with deioned water, ethanol for 3 min, respectively. Finally, the gold electrode was rinsed with deioned water, and then electrochemically activated in by consecutively cycling in the potential range of 0 ~ +1.6 V in 0.5 mol/L sulfuric acid until a cyclic voltammogram reached, which means a clean gold electrode was obtained. The activeted gold electrodes were then interacted with 0.1 μmol/L capture ssDNA solution for 90 min, and capture ssDNA can be immobilized on the gold electrode through thiol-Au

interaction. And the surfaces of the gold electrodes were passivated with 1 mmol/L MCH. And then capture ssDNA-modified electrode was immersed into the 0.1 μmol/L Fc-labeled ssDNA solution for 2 h in 37 ^{o}C water bath. Thus dsDNA-modified electrode was obtained for following experiment. (Note: After each modification step, the electrode should be rinsed with deioned water or buffer solution to eliminate the physical adsorption)

Electrochemical experiment

The above treated gold electrode, as the working electrode, was immersed into the electrochemical cell containing 2 mL of Tris-acetate buffer solution. Here differential pulse voltammetry (DPV) was used as scan mode. The parameter was set as follow: the potential interval from 0 to +0.5 V vs. Ag/AgCl, modulation amplitude 0.05 V, pulse width 0.06 s, and sample width 0.02 s, and further incubation in different concentration of Cys for 3 h. The current intensity at about 0.23 V was used for quantification. Electrochemical impedance experiments were performed in the solution containing 5 mmol/L $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol/L KCl. The biased potential was 0.214 V (vs. Ag/AgCl) and the amplitude was 5.0 mV, and the electrochemical impedance spectra were recorded in the frequency range of 10 kHz to 1 Hz.

Ag^+ detection

Before and after incubation of modified electrode in different concentrations of Ag^+ for 2 h, DPV was carried out in Tris-acetate buffer solution (pH 7.6) and the current was recorded.

Cys detection

After incubation in 200 nmol/L Ag^+, the modified electrode was immersed into different concentrations of Cys for 3 h, DPV was carried out in Tris-acetate buffer solution (pH 7.6) and the current was recorded. Additionally, the modified electrode was only immersed into Cys (or mixture of Ag^+ and Cys) for 3 h, and then DPV was carried out in same buffer solution again, and current was recorded.

Characterization of the modified electrode

Faradic electrochemical impedance spectroscopy (EIS) is employed to investigate the interface properties of the gold electrode surface during stepwise modifications. Fig. S1 shows Nyquist plots of different modified electrodes in 5 mmol/L $Fe(CN)_6^{3-/4-}$ solution containing 0.1 mol/L KCl. The equivalent circuit, as the model for the working electrode, (shown in the inset of Fig. S1), is used to fit the EIS data. This equivalent circuit consists of the electrolyte solution resistance R_s, the surface electron transfer resistance R_{ct} (R_{ct} reflects the surface condition of the gold electrode surface), the Warburg impedance Z_w and the constant phase element related to double layer capacitance C_{dl}.[2] For bare gold electrode (curve a, 45.7 Ω), the EIS exhibits a very small semicircular domain, indicating a fast electron-transfer process at such electrode. Immobilization of capture ssDNA results in a larger R_{ct} value (curve b, 168.4Ω), the reason lies in that the negatively charged phosphate backbone of the oligonucleotides immobilized on the gold electrode prevented the negatively charged redox probe $Fe(CN)_6^{3-/4-}$ from reaching the gold electrode and inhibited interfacial charge transfer.

Using MCH to block the left bare sites on gold electrode surface resulted in the further increase of Ret (curve c, 501.4Ω). When capture ssDNA hybridized with probe ssDNA, there is a remarkable increasment of the negative charges on the gold electrode surface, which results in much larger enhancement of R_{ct} (curve d, 698.9Ω). These results show that DNA can well immobilize and hybridize on the gold electrode.

References

1 S. Takenaka, Y. Uto, H. Kondo, T. Ihara and M. Takagi, *Anal. Biochem.*,1994, **218**, 436.

2 F. Patolsky, A. Lichtenstein and I. Willner, *J. Am. Chem. Soc.*, 2001, **123**, 5194.

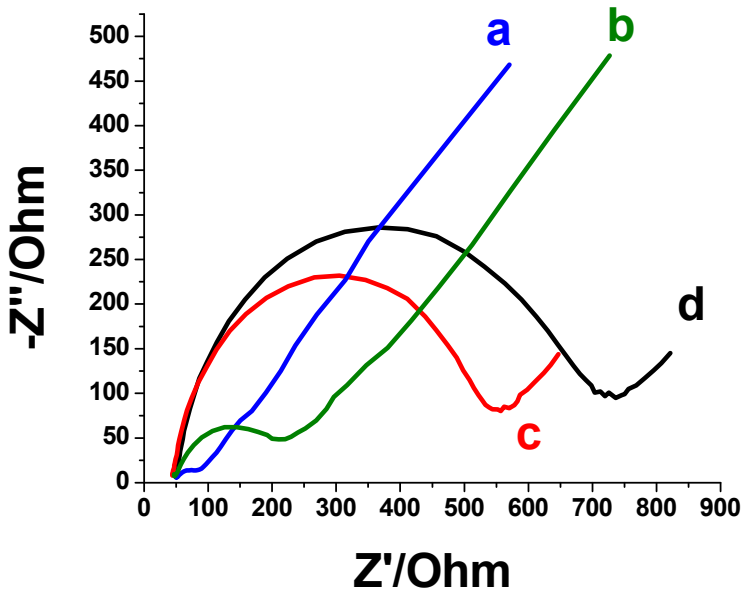

Fig. S1 Nyquist plots of the different electrodes in the 5.0 mmol/L $[Fe(CN)_6]^{3-/4-}$ solution containing 0.5 mol/L KCL. (a) Bare gold electrode; (b) Capture ssDNA-modified electrode; (c) Capture ssDNA/MCH-modified electrode; (d) Mismatch dsDNA/MCH-modified electrode. The biasing potential is 0.218 V with 5 mV alternative voltage in the frequency range of 1Hz-10kHz. Inset shows the equivalent circuit.

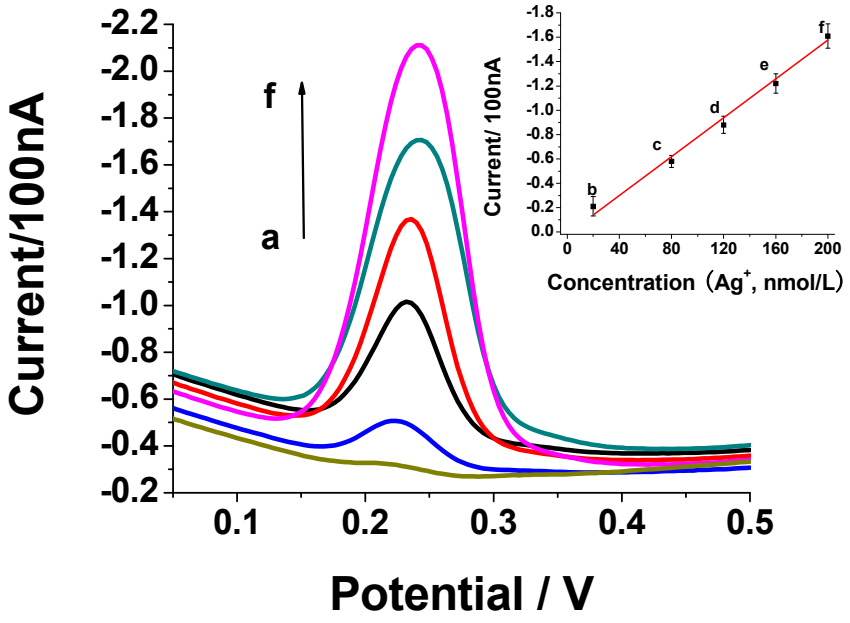

Fig. S2 DPVs upon interaction of mismatched dsDNA-modified electrode with different concentrations of Ag^+ in Tris-acetate buffer solution (pH 7.6). Concentrations of Ag^+ are (a) 0; (b) 20, (c) 80; (d) 120; (e)160; (f) 200 nmol/L, respectively. Inset: plot of current intensity vs concentrations of Ag^+.

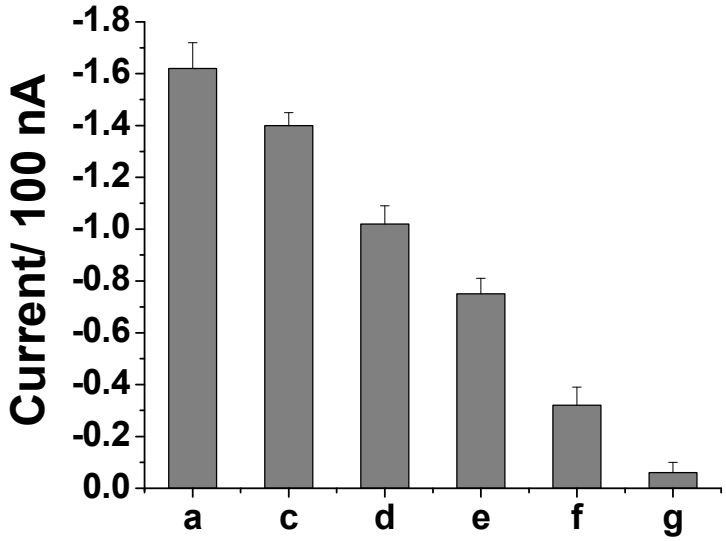

Fig. S3 Cys concentration-dependent change in current intensity of 200 nmol/L Ag^{+}-mediated dsDNA-modified electrode in Tris-acetate buffer solution (pH 7.6). Concentrations of Cys are (a) 0; (b) 35, (c) 60; (d) 80; (e)130; (f) 200 nmol/L, respectively.