Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting information

Electrochemical Detection of DNA by Using "Pd/GO Label Copper Stain" for Signal Amplification

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1. Experimental Section

1.1 Chemicals and Materials.

Sodium chloride (NaCl), sulfuric acid (H_2SO_4), potassium persulfate ($K_2S_2O_8$), phosphorus pentoxide (P_2O_5), potassium permanganate ($KMnO_4$), and palladium dichloride ($PdCl_2$) were obtained from Shanghai Chemical Reagent Corporation. All chemicals were used as received. Milli-Q water (18.2 $M\Omega.cm^{-1}$) was used in all experiments.

1.2 Synthesis of graphene oxide (GO)

Graphene oxide (GO) was prepared by a modified Hummers method,[1] using native graphite flake as starting material. Briefly, native graphite flake (1 g) was mixed with concentrated H_2SO_4 (1.5 mL), $K_2S_2O_8$ (0.5 g) and P_2O_5 (0.5 g), and then incubated at 80 ^oC for 6 h to preoxidize the graphite. The preoxidized graphite powder (~1 g) was stirred in 98% H_2SO_4 (23 mL) at 0 ºC for 8 h, $KMnO_4$ (3 g) was gradually added while keeping the temperature < 20 ºC. The mixture was then stirred at 35–40 ºC for 2 h, followed by adding 46 mL of water, and kept stirring for 15 min. The reaction was terminated by addition of water (140 mL) and 30% H_2O_2 solution (10 mL). After that, the color of mixture changed to bright yellow. The resulting mixture was washed by repeated centrifugation and filtration, first with 5% HCl aqueous solution, and then distilled water. Finally, water (700 mL) was added to the final product and vortexed well to make a uniform suspension for storage.

1.3 *In situ* Synthesis of Pd/GO Nanoparticles (Pd/GO NPs)

In a typical synthesis of Pd/GO NPs,[2] homogeneous GO suspension (0.2 mg/mL, 5 mL) and Na_2PdCl_4 aqueous solution (15 mL, 2 mM) were kept in a vial under vigorous stirring for 30 min in an ice bath (Chen et al., 2011). Then, the resulting Pd/GO NPs were washed with water by the centrifugation. Before the experiment, the palladium precursor solution (Na_2PdCl_4) was prepared by adding 0.0355 g of $PdCl_2$ and 0.012 g of NaCl to 100 mL of H_2O.

2. Size distribution of Pd NPs on the surface of GO

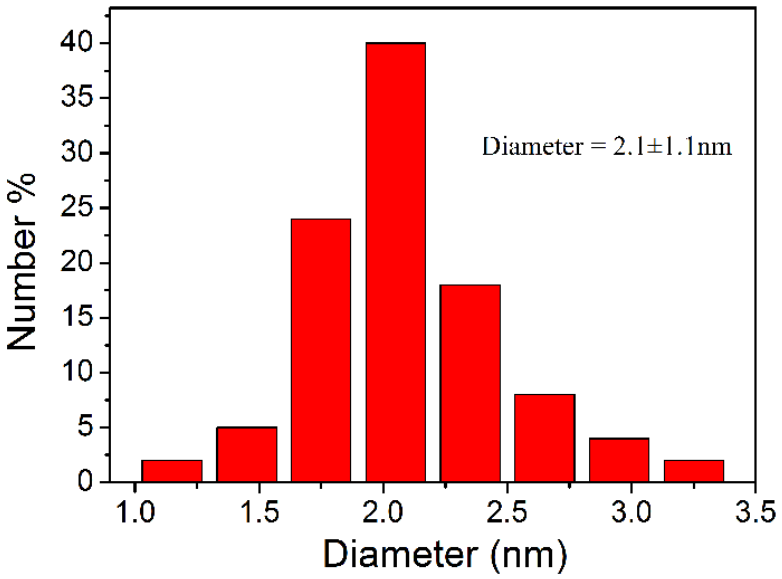

Fig. S1

3. UV-vis spectroscopy of resulting ss DNA_{label}-Pd/GO NPs

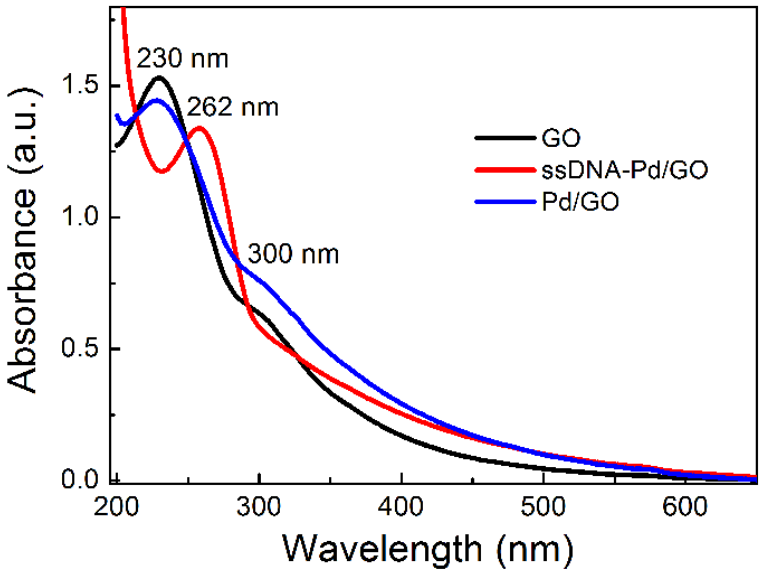

Fig. S2

4. **The time-dependent color changes of copper enhancer solution before and after optimizing its component under the catalysis of same concentration of Pd/GO NPs (10 μL, 2 mM)**

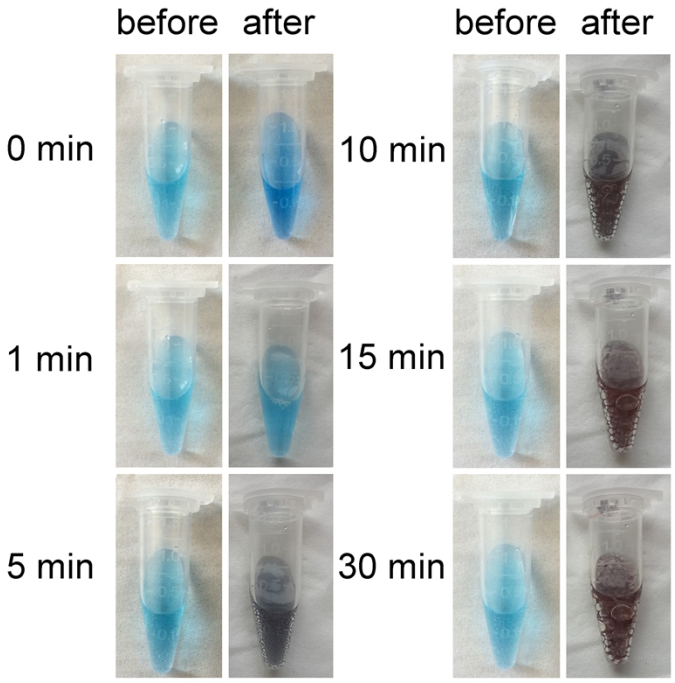

Fig. S3

Before the optimization:

For example:

Solution A: 0.065 g of $CuSO_4 \cdot 5H_2O$, 0.25 g of $KNaC_4H_4O_6 \cdot 4H_2O$, and 0.04 g of NaOH in 10 mL of water; Solution B: the formaldehyde solution (37 wt. % in H_2O).
Solution A/ Solution B=1mL:100 μL

After the optimization:

Solution A: 0.0875 g of $CuSO_4 \cdot 5H_2O$, 0.1693 g of $KNaC_4H_4O_6 \cdot 4H_2O$, and 0.4 g of NaOH in 10 mL of water; Solution B: the formaldehyde solution (37 wt. % in H_2O).
Solution A/ Solution B=1mL:12 μL

5. The electroanalytical performance of silver deposition by DPV measurements

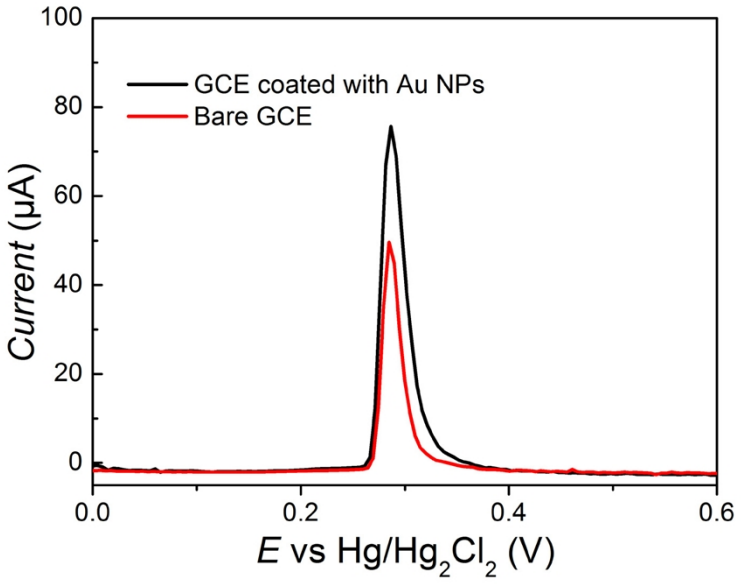

Fig.S4

To further evaluate non-specific silver deposit during silver staining, we comparatively studied the amount of silver deposit on two different GCE after being immersed in silver enhancer solution using DPV measurements. One GCE is the bare one with its surface coated by 5 μL of water. And the surface of another GCE was coated with 5 μL of Au NPs aqueous solution (about 15 nm, 2.27×10^{-8} M). After being dried in air, two GC electrodes were immersed into silver enhancer solution simultaneously for 5 min at room temperature. After that, the resulting electrodes were rinsed with water to remove any residual silver enhancer solution. Finally, the amount of silver atoms deposited on the electrodes was measured by DPV. Prior to use, the silver enhancer solution was prepared by mixing 1 mL of solution A (Sigma-Aldrich) and 1 mL of solution B (Sigma-Aldrich), simultaneously.

6. Electrochemical characterization

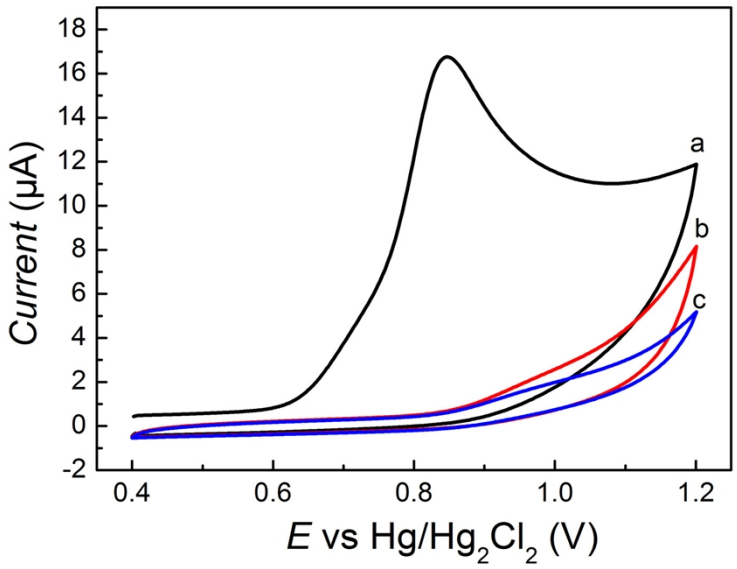

Fig. S5 Cyclic voltammograms of ABA-modified GCE at the 1th, 3th and 6th cycle. Electrolyte: 10 mM phosphate buffer containing 1 mM ABA. Scan rate: 10 mV s^{-1}.

The covalent modification of the GCE with ABA was performed by repeated scanning between in 10 mM phosphate buffer (PBS, pH 7.4) containing 1 mM ABA with a scan rate of 10 mV s^{-1} for 6 cycles. As shown in Fig.S5, for the surface modification with ABA, there is an irreversible oxidation peak around +0.85 V attributed to the formation of amino cation radical and subsequently to chemical bonding of the radical to GCE surface. It was found that, when the potential was repeatedly scanned, the peak gradually diminished, indicating that the successful grafting of ABA to GCE surface.

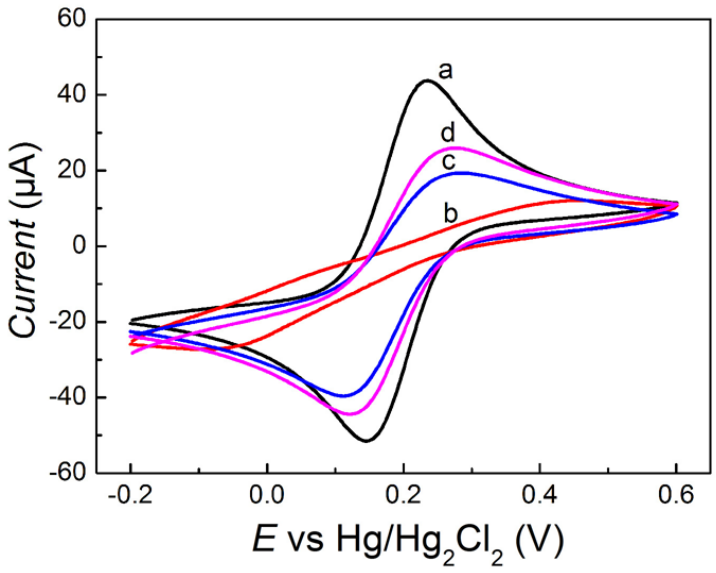

Fig. S6 Cyclic voltammograms of GCE at different stages of modification: (a) blank, (b) modified with ABA, (C) activated by NHS/EDC, (d) the linkage of NH_2-DNA_{probe}. Electrolyte: 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) aqueous solution with 100 mM KCl. Scan rate:100 mV s^{-1}.

To further characterize the surface properties of GCE during the surface modification, the CV behavior of this modified electrode was investigated in 5 mM $K_3Fe(CN)_6$ solution step by step. As shown in Fig. S6, for a bare GCE electrode, a pair of well-defined oxidation and reduction peaks of ferrocene located at about 0.213 V and 0.138 V was observed (Fig. S6a). However, the electron transfer of $Fe(CN)_6^{3-}$ is completely blocked on GCE electrode after the graft of ABA film. It can be explained by the electrostatic interactions between the modified surface and the electroactive probes. In pH 7.4 solution, the carboxyl groups of the modified GCE surface are expected to fully dissociate if we assume that its p*K*a is near to that of ABA (p*K*a 3.24). Thus, on the negatively charged ABA film, the electrostatic repulsion resists access of $Fe(CN)_6^{3-}$ to the electrode surface and blocks its electron transfer on the electrode surface (Fig. S6b). After the EDC/NHS activation, the higher redox response was observed due to the decrease of this density of negative charge on GCE electrode (Fig. S6c). With the attachment of ss DNA_{probe} to the modified GCE surface, the current response increased more because of the improved electrical conductivity of DNA (Fig. S6d), which promoted the interfacial charge transfer.

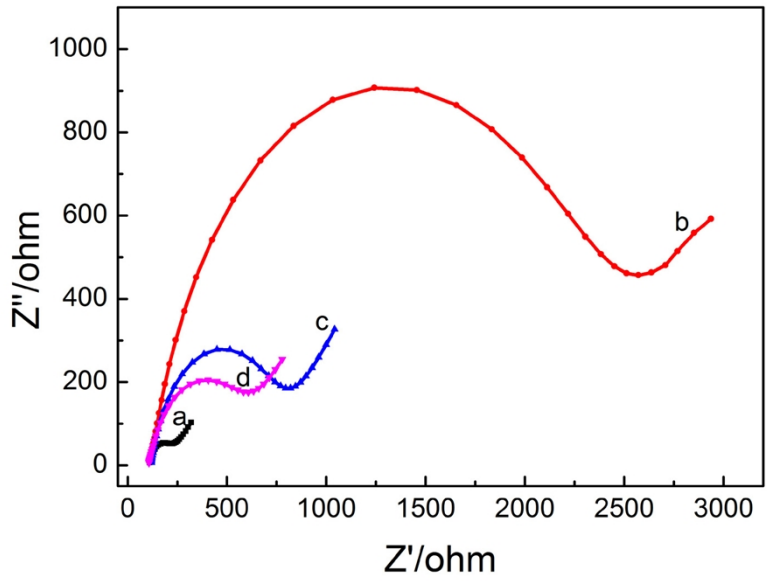

Fig. S7 Nyquist plots corresponding to the GCE at different stages of modification: (a) bare, (b) modified with ABA, (C) activated by NHS/EDC, (d) the linkage of NH_2-DNA_{probe}.

Additionally, electrochemical impedance spectroscopy (EIS) was used to study the surface properties of the modified electrode. In Nyquist diagram, the semicircle portion observed at high frequencies corresponds to the electron transfer limiting process. The electron transfer resistance (R_{et}), can be directly measured as the semicircle diameter. In Fig. S7, when the surface of GCE was modified with ABA, the semicircle dramatically increased to 2570 Ω as compared to bare GCE, suggesting that ABA layer repelled the access of $[Fe(CN)_6]^{3-/4-}$ to the electrode surface for electron communication. For DNA_{probe}-ABA/GCE, the semicircle dramatically decreases suggesting that DNA acted as a conducting layer which can significantly enhance the electron-transfer rate. All results were consistent with these obtained by CVs.

References:

1. Hummers, W.S., Offeman, R.E., 1958. J. Am. Chem. Soc. 80, 1339-1339.
2. Chen, X.M., Wu, G.H., Chen, J.M., Chen, X., Xie, Z.X., Wang, X.R., 2011. J. Am. Chem. Soc. 133, 3693-3695.