Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Target recycling amplification for sensitive and label-free impedimetric genosensing based on hairpin DNA and graphene/Au nanocomposite

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Experimental Section

Materials: Tris-HCl, 6-mercapto-1-hexanol (MCH) and $HAuCl_4$ were purchased from Sigma (St. Louis, MO). Spectroscopically pure graphite powder was received from Shanghai Chemicals Co., Ltd. (Shanghai, China). Disposable SPCE, comprised of a carbon working electrode (3 mm in diameter), a carbon counter electrode, and a silver pseudoreference electrode, were obtained from Zensor R&D Co., Ltd (Taichung, Taiwan). All oligonucleotides[1] were ordered from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China), and the sequences of these oligonucleotides were as follows:

Hairpin DNA probe: 5'-SH-$(CH_2)_6$-AGGAAGACGTACGTATCTTCCTTTTGTTC-3';

Complementary DNA: 5'-GAACAAAAGGAAGATACGTACAAAATC-3';

One base-mismatch DNA: 5'-GAACAAAAGGAAG<u>T</u>TACGTACAAAATC-3';

Three base-mismatch DNA: 5'-GAACAAAAGGAACTAACGTACAAAATC-3';

Non-complementary DNA: 5'-ACCTGGGGGAGTATTGCGGAGGAAGGT-3'

All reagents were analytical grade and solutions were prepared using ultrapure water (specific resistance of 18 MΩ-cm).

Preparation of graphene oxide (GO): GO was prepared according to modified Hummers' methods.[2,3] In brief, the graphite powder (10 g) was added to a mixture of concentrated H_2SO_4 (15 mL), $K_2S_2O_8$ (5 g), and P_2O_5 (5 g) at 80 °C. The resulting dark blue mixture was thermally isolated and allowed to cool to room temperature over a period of 6 h, followed by dilution with water, filtration and washing with water until pH=7.0. The pellet was dried overnight in air at ambient temperature to obtain the oxidized graphite powder. Next, the oxidized graphite powder (10 g) was added to cold concentrated H_2SO_4 (0 °C, 230 mL). Then, $KMnO_4$ (30 g) was gradually added to the mixture with the temperature lower than 20 °C. The mixture was stirred at 35 °C for 2 h, followed by the addition of water (460 mL). In next 15 min, water (1.4 L) and 30% H_2O_2 (25 mL) was added to the mixture to obtain a bright yellow solution. The mixture was filtered and washed with 1:10 HCl solution (2.5 L) and dialysed against water to completely remove metal ions and acids to obtain GO.

Electrochemical deposition of graphene/AuNPs on the electrode: The SPCE was first preconditioned in Tris-HCl buffer (20 mM, pH 7.4) by cycling the potential between -0.6 and 0.6 V at 0.5 V s^{-1}. Next, the N_2 bubbled mixture (50 μL) of GO (0.5 mg mL^{-1}) and $HAuCl_4$ (1 mM) in carbonate buffer solution (100 mM, pH 9.0) was applied to the electrode surface, followed by cycling the potential between -1.5 and 0.6 V at a rate of 25 mV s^{-1} for five cycles to obtain the graphene/AuNP modified SPCE.[4]

Electrochemical impedance spectroscopy (EIS) sensing protocol: The hairpin probe (10 μL, 1 μM) in PBS buffer (20 mM K_2HPO_4/KH_2PO_4, 0.15 NaCl, pH 7.0) was applied to the working electrode of the freshly prepared graphene/AuNPs modified SPCE and incubated at room temperature (25 °C) for 12 h, followed by washing with 0.2% SDS to remove any non-specifically adsorbed DNA probe. Next, 10 μL of 2 mM MCH was dropped on the electrode surface for 1 h. The electrode was then washed with Tris-HCl buffer (20 mM Tris-HCl, 100 mM NaCl, 5mM $MgCl_2$, pH 8.0), and the target DNA at various concentrations together with Exo III (5 U) were added and incubated with the electrode at 37 °C for 1 h. After washing with Tris-HCl/SDS (20mM Tris-HCl, 100 mM, 5mM $MgCl_2$, 0.2% SDS, pH 8.0), 50 μL of KCl solution (0.1 M) containing $[Fe(CN)_6]^{3-/4-}$ (5 mM, 1:1) was dropped to cover all three electrodes and EIS measurements were performed on a CHI660D EC workstation (CH Instruments Inc., Shanghai, China) with the frequency range from 10 kHz to 50 MHz and an alternate voltage of 5 mV.

SEM images of the SPCE before and after electrochemical deposition of graphene/AuNPs:

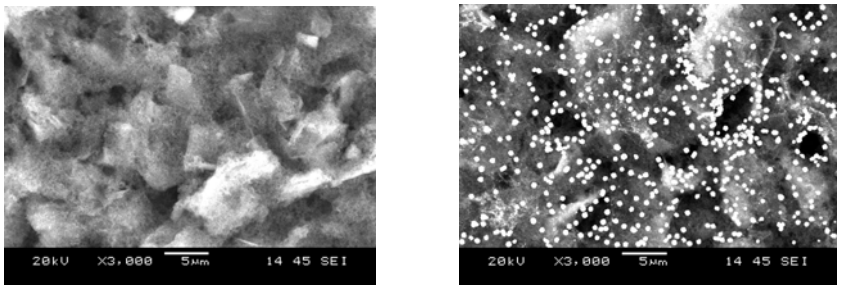

Fig. SI1 SEM images of different electrodes: (left) bare SPCE and (right) graphene/AuNPs/SPCE. SEM images were taken by a S4800 scanning electron microscope (HITACHI Co., Japan).

Cyclic voltammograms of the sensor at different stages:

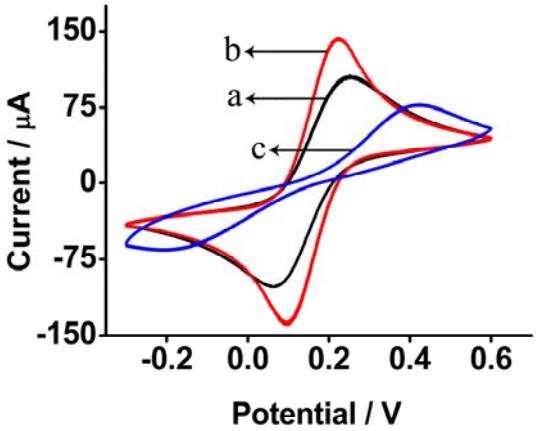

Fig. SI2 Cyclic voltammograms corresponding to (a) bare SPCE electrode, (b) graphene/AuNP modified SPCE and (c) hairpin DNA and MCH self-assembled graphene/AuNPs/SPCE. The voltammograms were recorded in 0.1M KCl solution containing 1 mM $[Fe(CN)_6]^{3-/4-}$ at a scan rate of 50 mV s^{-1}.

References:

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