Electronic Supplementary Information (ESI)

Label-free electrochemical impedance sensing of DNA hybridization based on functionalized graphene sheets

Experimental Section

Chemicals. Graphite powders (320 mesh) were of spectroscopically pure reagent and obtained from Shanghai Chemicals, China. 3,4,9,10-perylenetetracarboxylic dianhydride (PTCDA, 97%), tris(hydroxymethyl)amminomethane (Tris), *N*-hydroxysulfosuccinimide (NHS) and *N*-(3-dimethylamino) propyl-N'-ethyl carbodiimidehydrochloride (EDC) were obtained from Sigma-Aldrich, USA. Hydrazine solution (50% in water) was purchased from Beijing Yili Chemicals, China. Ammonia solution (25% in water) was purchased from Beijing Chemicals, China. Sodium dodecylsulfate (SDS) was purchased from Shanghai Reagent Company, China. Other reagents were of analytical grade and were used as received. All aqueous solutions were prepared with ultrapure water (>18 MΩ) from a Milli-Q Plus system (Millipore).

The 20-base synthetic oligonucleotides probe (ssDNA), its cDNA (target DNA, conserved sequence of the pol gene of human immunodeficiency virus 1 (HIV-1)), single-base mismatched, double-base mismatched, four-base mismatched and non-complementary DNA were all synthesized in Sangon Biotechnology Inc. (Shanghai, China). All oligonucleotide stock solutions (1.0×10^{-4} M) were prepared by using Tris–HCl solution (pH 7.0), which were stored at 4 °C. More diluted solutions were obtained by diluting an aliquot of the stock solution with ultrapure water prior to use. The hybridization solution was diluted with 2×SSC (pH 7.0), which consisted of NaCl (0.30 M) and sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7\cdot2H_2O$; 0.030 M).

Instruments. UV-vis absorption spectra were recorded using a Cary 500 UV/vis/near-IR spectrometer. Transmission electron microscopy was conducted using a JEOL 2000 transmission electron microscopy operating at 200 kV. Electrochemical impedance spectroscopy (EIS) measurements were carried out with Solartron 1255B Frequency Response Analyzer (Solartron Inc., UK). Cyclic voltammetry (CV) measurements were performed using a conventional three-electrode cell with a platinum wire as the auxiliary electrode and an Ag/AgCl (saturated KCl) as reference in a CHI 660 Electrochemical Workstation (CHI). Working electrodes were bare or modified glassy carbon electrodes (GCE) (d = 3 mm). Before use, GCEs were carefully polished to a mirror finish with 1.0, 0.3, and 0.05 μm alumina slurries, successively.

Functionalization of graphene. The functionalization of graphene with 3,4,9,10-perylene tetracarboxylic acid (PTCA) was conducted according to our previous work.[1] Namely, graphene oxide was synthesized from natural graphite powder by a modified Hummers method as originally presented by Kovtyukhova and colleagues.[2] The PTCA solution was made by hydrolyzing PTCDA in a minimal volume of 1.0 M sodium hydroxide. Red deposits appeared in the yellow-green solution and were collected by centrifugation and dried under vacuum at room temperature. 21.2 mg graphene oxide and 5.2 mg PTCA were dissolved in 20.0 mL water by ultrasonication, and then stirred at 40 °C overnight. Subsequently, 26.8 μL hydrazine solution and 0.30 mL ammonia solution were added to the above solution and the resulting mixture was held at 95 °C for 30 min under vigorous agitation. The product was subsequently filtered through a Nylon membrane with 0.22 μm pores, thoroughly washed with ultrapure water and dried under vacuum at room temperature. Then the PTCA/graphene product was ready to use.

DNA immobilization and hybridization. 5 mg PTCA/graphene product was dispersed in 10 mL ultrapure water under ultrasonication for 5 min. A uniform, wine PTCA/graphene solution was obtained. An aliquot of 10 μL PTCA/graphene solution was coated on the clean GCE surface with a microsyringe and dried in air for 2 h before use. After the modification of GCE with PTCA/graphene sheets, it was transferred into 50 mM phosphate buffer solution (pH 7.4) containing 8 mM EDC and 16 mM NHS for 1 h at 4 °C. Then, a 5 μL drop of ssDNA solution (1.0×10^{-6} M) was uniformly deposited on the activated PTCA/graphene/GCE surface to form recognition layer. After air-dryness, the recognition layer was washed with 0.2% SDS solution and then rinsed with ultrapure water. DNA hybridization reaction was conducted by dropping 5 μL appropriate concentration of target DNA solution onto the recognition surface and allowed reaction for 30 min. Then the electrode was washed with 0.2% SDS solution to remove the un-hybridized DNA. The same procedure as mentioned above was applied to the probe-modified electrode for hybridization with single-base mismatched, double-base mismatched, four-base mismatched and non-complementary DNA sequences.

Electrochemical Measurements. The following parameters were employed for CV: scan rate 100 mV/s, potential scanning range +0.6 ~ -0.2 V. The EIS experimental parameters: the AC voltage amplitude was 5 mV and the voltage frequencies ranged from 0.1 Hz to 10^4 Hz. And it was carried out under open-circuit conditions. Both of the supporting electrolyte were 2.0 mM $K_3[Fe(CN)_6]$ and 2.0 mM $K_4[Fe(CN)_6]$ (1:1) solution containing 0.1M KCl.

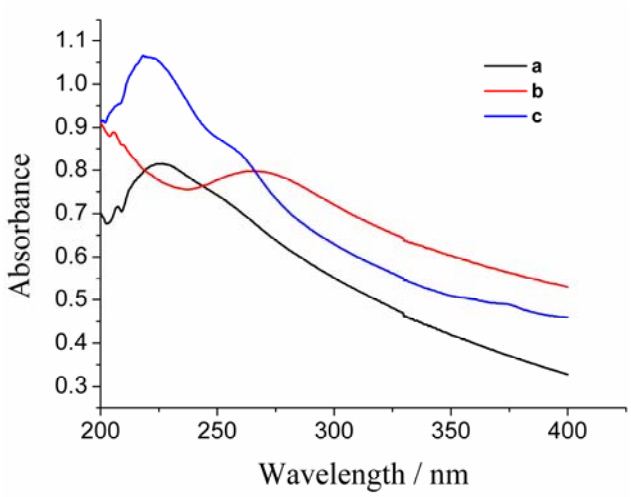

Fig. S1 UV-vis absorption spectra of graphene oxide (a), graphene (b) and PTCA/graphene (c).

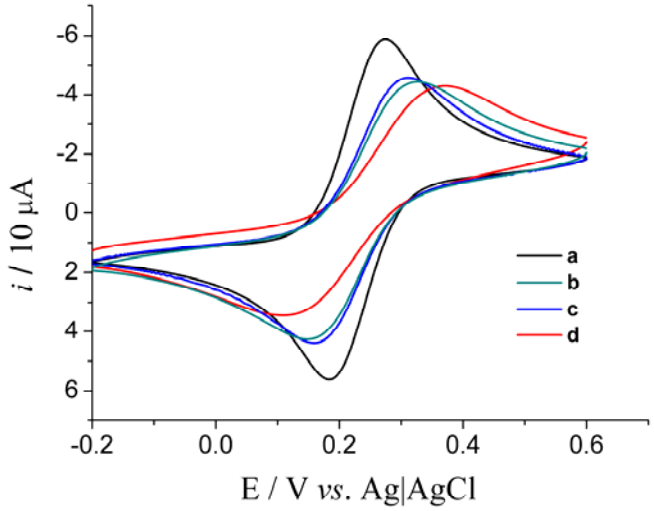

Fig. S2 CVs of 2.0 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) containing 0.1 M KCl recorded at bare GCE (a), graphene oxide modified GCE (b), graphene modified GCE (c), and PTCA/graphene modified GCE (d).

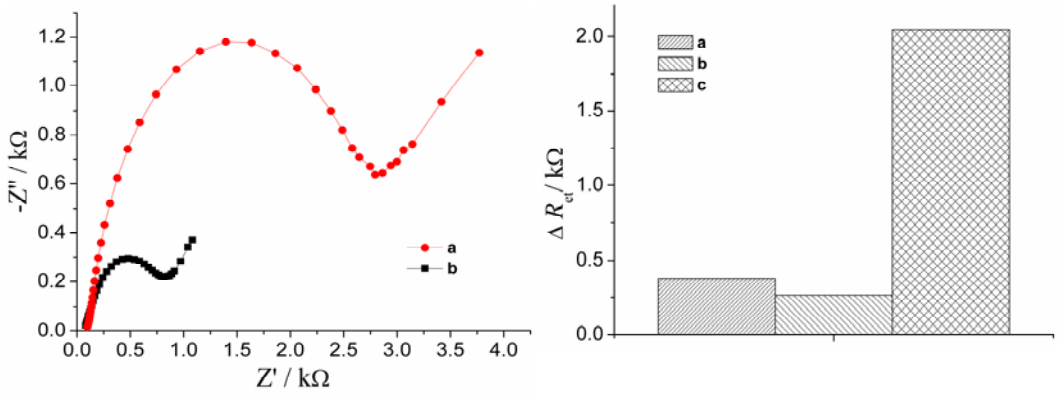

Fig. S3 Left: Nyquist diagrams recorded at PTCA/graphene/GCE before (a) and after (b) activation by EDC and NHS. Right: Comparison of ΔR_{et} value observed at graphene oxide/GCE (a), graphene/GCE (b) and PTCA/graphene/GCE (c) before and after activation.

There would be –COOH groups existed at the edges of graphene oxide and graphene sheets. We needed PTCA decoration to introduce more active sites to increase the immobilization amount of probe DNA and enhance hybridization signal. Fig. S3 (left) was the Nyquist plots of PTCA/graphene/GCE before and after activation by EDC and NHS. And the difference of R_{et} value (ΔR_{et}) was 2046.5 Ω (Fig. S3 right, c), which was much higher than these calculated from graphene oxide/GCE and graphene/GCE, to be 375.8 and 263.8 Ω, respectively. The larger ΔR_{et} value elucidated that PTCA/graphene sheets contained more –COOH groups, which was beneficial to DNA immobilization and hybridization.

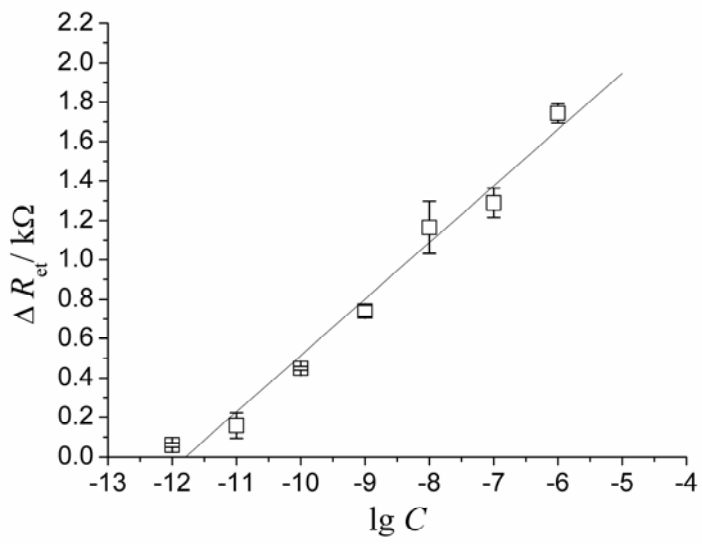

Fig. S4 The plot of ΔR_{et} *vs.* the logarithm of HIV-1 pol gene sequences concentrations.

References

1. F. Li, H. Yang, C. Shan, Q. Zhang, D. Han, A. Ivaska, L. Niu, *J. Mater. Chem.*, 2009, **19**, 4022.
2. N. I. Kovtyukhova, P. J. Ollivier, B. R. Martin, T. E. Mallouk, S. A. Chizhik, E. V. Buzaneva, A. D. Gorchinskiy, *Chem. Mater.*, 1999, **11**, 771.