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

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Negative-charge change and conformation transition upon DNA immobilization and hybridization on functionalized graphene sheets were monitored by the EIS technique and adopted as the signal for label-free electrochemical DNA hybridization detection.

Graphene, a two-dimensional sheet of sp² conjugated atomic carbon, has stimulated intense research interest because of its unique band structure, massless fermions, and ultrahigh carrier mobility.1,2 These unique properties hold great promise for potential applications in many technological aspects such as nanoelectronics, sensors, nanocomposites, batteries, supercapacitors and hydrogen storage.3 The high specific surface area of 2630 m² g⁻¹ enables it to afford an ultrahigh loading capacity for biomolecules and drugs.4,5 Recently, graphene has been successfully used in many bioassay applications and shows promising potentials.2,5,6

Researches had elucidated that single-stranded DNA (ssDNA) could be stably adsorbed on the graphene sheet, with all nucleobases lying nearly flat on the surface. This was due to π-stacking attraction between the rings in the bases and the hexagonal cells of graphene.7 As a nanoquencher, graphene (graphene oxide) shows super quenching capacity with a wide energy transfer range.8 Both regular and hairpin structured oligonucleotides labeled with fluorophore had been adopted to DNA bioassy.9,10 Graphene could efficiently quench the fluorescence of fluorophore labeled oligonucleotides. When the target reacted with fluorophore labeled oligonucleotides, the formed double-stranded DNA (dsDNA) left the graphene surface resulting in the restoration of fluorescence. Though it is sensitive and selective to the detection of the target molecule, this strategy needs oligonucleotides to be fluorophore labeled. The incorporation of a labeling step into nucleic acid assay has shortcomings of limited labeling efficiency, complex multi-step analysis and contamination to samples.11 Many researches have been made to the development of label-free technologies,12 which detect the hybridization event through changes in the physical and chemical properties of the recognition layer resulting in a change in the optical13 or electrochemical14 signal. Herein, we demonstrate a label-free electrochemical DNA hybridization assay using functionalized graphene as a platform. DNA immobilization and hybridization on the platform caused changes in the interfacial charges and conformation, which could be readily monitored by impedance measurement.

The decoration of graphene with an amine-terminated ionic liquid,15 silane,16 congo red17 and a conjugated polyelectrolyte18 have been achieved in our previous work. Here, 3,4,9,10-perylene tetracarboxylic acid (PTCA) was used to decorate graphene and the procedure was illustrated in Scheme 1. Details of fabrication could be found in the ESI†. There is extensive nuclear magnetic resonance, infrared spectroscopic, and electron diffraction evidence for the presence of –COOH, –OH, and C=O groups at the edge of the graphene oxide sheet, while the basal plane is covered with mostly epoxide and –OH groups.19,20 It would be necessary to decorate the basal plane with –COOH for a covalent binding reaction. The PTCA molecule bears –COOH groups and a π-conjugated structure. The conjugated rings of PTCA could interact with the basal plane of graphene sheets through π–π stacking and hydrophobic forces.21 PTCA separated graphene sheets and decorated graphene with more –COOH, which made the resulting PTCA/graphene composites disperse well in solvents (Fig. 1, inset) and provided more active sites for the immobilization of the 5'-NH₂ modified probe DNA sequence. Transmission electron microscopy (TEM) image of the PTCA/graphene sheet is shown in Fig. 1.

As a comparison, we obtained graphene (chemically reduced graphene oxide) by the same procedure without addition of PTCA. The UV-vis absorption spectra of graphene oxide, graphene and PTCA/graphene are shown in Fig. S1 (ESI†). The spectrum obtained for the graphene oxide dispersion

Scheme 1  Schematic representation of graphene functionalization with PTCA, ssDNA immobilization and hybridization.
exhibited a strong absorption band at 225 nm attributing to π-π* transitions of aromatic C=C bonds. After reduction to graphene, the aromatic C=C bonds red shifted to 261 nm, indicating the restoration of a π-conjugation network. The spectrum of PTCA/graphene contained a strong band at 220 nm and a weak band around 256 nm, elucidating the binding of PTCA with graphene.

The properties of graphene oxide, graphene and the PTCA/graphene modified glassy carbon electrode (GCE) were investigated by electrochemical impedance spectroscopy (EIS) exploiting the solution-based redox probe [Fe(CN)₆]³⁻/⁴⁻ and shown in Fig. 2. In the EIS, the semicircle portion observed at high frequencies corresponds to the electron transfer limiting process. The electron transfer resistance (Rₑ) can be directly measured as the semicircle diameter. When graphene oxide was modified onto a GCE surface, the Rₑ value dramatically increased as compared to the bare GCE, suggesting that graphene oxide acted as an insulating layer which caused the interfacial electron transfer difficult due to their disrupted sp² bonding networks. While at the graphene modified GCE, the Rₑ value decreased distinctively, indicating that graphene had accelerated electron transfer between [Fe(CN)₆]³⁻/⁴⁻ and the electrode, and the main reason was attributed to significantly improved electrical conductivity of graphene sheets, presumably owing to the restoration of a graphic network of sp² bonds. At the PTCA/graphene modified GCE, the Rₑ value increased obviously illustrating the successful functionalization of graphene sheets. The plenty of negatively charged –COOH prevented [Fe(CN)₆]³⁻/⁴⁻ from reaching the electrode. The corresponding cyclic voltammetry characterizations are shown in Fig. S2 (ESI†), which was in agreement with that of EIS studies.

In order to covalently immobilize 5'-NH₂ modified probe DNA, the PTCA/graphene sheets needed to be activated previously by N-hydroxysulfosuccinimide (NHS) and N-(3-dimethylamino) propyl-Ν'-ethyl carbodiimidehydrochloride (EDC) (details in ESI†). The Nyquist plots of PTCA/graphene/GCE before and after activation by EDC and NHS were recorded (Fig. S3, ESI†).

To verify the bioassay capability of the PTCA functionalized graphene sheets, the conserved sequence of the pol gene of human immunodeficiency virus 1 (HIV-1) was selected as the target DNA, and the sequences were listed in Table 1.

The DNA immobilization and hybridization were verified by EIS, which is very sensitive to changes in interfacial impedance upon bio-recognition events occurring at the surface/electrolyte interface. When the ssDNA was covalently immobilized on activated PTCA/graphene sheets, the Nyquist plot was shown as curve a (Fig. 3). An increase of the Rₑ value was observed after ssDNA immobilization comparing to that of activated PTCA/graphene/GCE (Fig. S3, ESI†). As mentioned above, ssDNA lies on the graphene basal plane via π-stacking attractions between the bases of ssDNA and the hexagonal cells of graphene. Then, the negatively charged phosphate backbones of ssDNA were exposed to the electrolyte, which repelled [Fe(CN)₆]³⁻/⁴⁻. When ssDNA hybridized with its complementary DNA (cDNA) sequence, a double-helix structure was formed and more negatively charged phosphate backbones were introduced. Meanwhile, the “lying” ssDNA became “standing” dsDNA. It was much more difficult for [Fe(CN)₆]³⁻/⁴⁻ to reach the electrode surface through the channels among “standing” dsDNAs. Hence, the Rₑ value increased following the formation of dsDNA. That was to say negative-charge change and conformation transition caused the interfacial property changes. With the increase of cDNA concentration, the Rₑ value increased correspondingly (Fig. 3b–h). The difference (namely ΔRₑ) between the Rₑ value of the ssDNA immobilized electrode and that after hybridization with cDNA was adopted as the measurement signal. The ΔRₑ value was linear with the logarithm of the HIV-1 pol gene sequence concentrations (Fig. S4, ESI†). The dynamic detection range for the sequence-specific DNA was from 1.0 × 10⁻¹² to 1.0 × 10⁻⁶ M with the regression equation ΔRₑ = 286.51 log C + 3379.5 and the regression coefficient (r) 0.9880. The detection limit was estimated to be 5.0 × 10⁻¹³ M with 3σ (where σ was the relative standard deviation of 11 parallel measurements of the blank solution). The result indicated that the label-free electrochemical strategy based on functionalized graphene exhibited high sensitivity.

To test whether the label-free impedance bioassay of DNA hybridization based on functionalized graphene sheets was...
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Table 1 Lists of DNA sequences (mismatch underlined)

<table>
<thead>
<tr>
<th>Sequences</th>
<th>From 5’ to 3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>ssDNA (probe DNA)</td>
<td>NH₂-C₆-GCT TGC CAA TGA TCT GTC CA</td>
</tr>
<tr>
<td>Target DNA (complementary DNA)</td>
<td>TGG ACA GAT CAT TGG CAA GC</td>
</tr>
<tr>
<td>Single-base mismatched DNA</td>
<td>TGG ACA AAT CAT TGG CAA GC</td>
</tr>
<tr>
<td>Double-base mismatched DNA</td>
<td>TGG ACA AAT CAT CGG CAA GC</td>
</tr>
<tr>
<td>Four-base mismatched DNA</td>
<td>TGT ACA AAT CAT CGG CAG GC</td>
</tr>
<tr>
<td>Non-complementary DNA</td>
<td>CAT CTC ATG GCC GAT TCG TG</td>
</tr>
</tbody>
</table>

In conclusion, graphene sheets were functionalized with PTCA, which introduced plenty of –COOH groups. 5’-NH₂-ssDNA was covalently immobilized on the functionalized graphene sheets. After hybridization with its complementary sequence, the “lying” ssDNA became “standing” dsDNA. Negative-charge change and conformation transition upon DNA immobilization and hybridization were monitored by EIS and adopted as the hybridization signal. Without any label, the HIV-1 pol gene sequence was satisfactorily detected via this strategy.

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Notes and references