Optimizing Label-free DNA electrical detection on graphene platform

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Supporting information

Experimental set-up – Electrochemical system

Figure S1: (a) Scheme of the experimental set-up and (b) its 3D representation.
**DNA-hybridization detection on anodized HOPG electrodes: Nyquist plot – comparison with anodized EG**

Figure S2: Nyquist plot before (blue solid square), and after (red solid triangle) DNA-probe immobilization by covalent grafting on (a) anodized HOPG and (b) anodized EG; after incubating the electrodes in a hybridization buffer containing different concentrations of DNA-target DNA for 40 min at 42°C: (a) 50fM, 1pM, 50pM and 1nM and (b) 50fM, 1pM, 50pM, 1nM, 50nM and 1µM. (a) Insert: zoom in and plot of ∆Rct against the concentration of DNA-target. (b) Insert: Plot of ∆Rct against the concentration of DNA-target.

**DNA-hybridization detection on anodized EG electrodes – capacitance measurements**

The influence of bio-affinity events (DNA-probes immobilization and DNA hybridization) on the total capacitance was also investigated for covalent grafting (Figure S3a) and π-π stacking (Figure S3b). Both DNA-probes immobilization led to a shift of the peak frequencies towards smaller frequencies. This could be attributed to the formation of the DNA-probes molecular layer and thus to a higher resistance at the electrolyte/electrode interface. Hybridization induces changes in capacitance from 0.1Hz to 100kHz for covalent grafting whereas for π-π stacking...
capacitance, the increase is between 0.1Hz and 20Hz. These results confirm the impedance and admittance results. Hybridization induced changes are visible for both immobilization at small frequencies (Nyquist plot, Figure 3a and b) but effect on high frequencies (admittance plot, Figure 3c and d) can only be seen for DNA-probes immobilized by covalent grafting.

Figure S3: Capacitance vs Frequency plot for DNA-probe immobilized by (a) covalent grafting and (b) π-π stacking: after anodization (blue solid square), DNA-probe immobilization (red solid triangle) and hybridization in presence of complementary DNA target (solid black curve) 1nM during 40 min at 42°C.

Equivalent circuit modeling analysis for DNA-immobilized onto anodized EG electrodes

Hybridization of grafted DNA-probes induces major changes in molecular layer parameters C1 and R2. The capacitance of the molecular double layer, C1, decreases by 70% upon hybridization. One explanation is the increased thickness of the biomolecular layers after hybridization, which arises from the rigidity of the hybridized duplex, since according to the equation: \( C = \varepsilon \varepsilon_0 \frac{A}{d} \) where \( d \) is the thickness of the film, increased thickness of biomolecular...
layers will result in a lower capacitance. The strong R2 decrease of 80% suggests that the molecular double layer is more conductive due to an increase in the density of ionic charges at the interface after DNA hybridization. From Table 1, the data reveals that in the case of covalent grafting, DNA hybridization induces significant changes in both the double layer as well the space charge layer in EG, producing changes in R3 and T by 65% and 56% respectively. Hybridization with complementary DNA brings additional negative charges near the surface, changes the surface potential and consequently shifts the flat band potential, producing changes in resistance and capacitance of the space-charge region.

For DNA immobilized by $\pi$-$\pi$ stacking, DNA-hybridization also induces significant changes. Parameters which are associated with space charge layers like R3 increases by 72% and T decreases by 52%. The molecular layer parameters C1 increases and R2 decreases by 61% and 52%, respectively. Nevertheless, DNA-hybridization presents stronger effects on anodized EG electrodes functionalized by grafted DNA-probes.

Moreover, since the capacitance for the graphene space charge layer $C_{sc}$ and the molecular layer capacitance $C_{mol}$ are in series, the total capacitance of the modified surface can be represented by $C_{tot}^{-1} = C_{sc}^{-1} + C_{mol}^{-1}$. Thus, the space-charge layer dominates if $C_{sc} < C_{mol}$, like for DNA immobilized by covalent grafting. This can explain why the admittance plot (Figure 3d) did not change upon hybridization for DNA-probes immobilized by $\pi$-$\pi$ stacking and lead to stronger change in the corresponding admittance plot for DNA immobilized by covalent grafting, enlightening the change in the space charge layer (Figure 3c).
**Equivalent circuit modeling analysis for DNA-immobilized onto anodized HOPG electrodes**

This electrical model consists of a resistance $R_{\text{sol}}$ due to the ohmic resistance of the solution, and 2 parallel combination of resistor $R$ and constant phase element CPE, reflecting the properties of the molecular layer/double layer and the anodized HOPG space charge region.

For anodized HOPG, upon hybridization, the parameters of the space charge layer and double layer increase or decrease by less than 36%. For anodized EG, hybridization induced more significant changes in the parameters associated with the molecular double layer and the space charge layer. Their values increase or decrease by more than 52%.

<table>
<thead>
<tr>
<th></th>
<th>R2 (Ω)</th>
<th>T1, $10^{-6}$</th>
<th>n1</th>
<th>R3 (kΩ)</th>
<th>T2, $10^{-6}$</th>
<th>n2</th>
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<tbody>
<tr>
<td>Probe</td>
<td>136±4</td>
<td>6.29±0.13</td>
<td>0.71±0.03</td>
<td>25±2</td>
<td>379±2</td>
<td>0.78±0.01</td>
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<td>Probe + target</td>
<td>157±3</td>
<td>6.43±0.14</td>
<td>0.71±0.02</td>
<td>16±2</td>
<td>306±3</td>
<td>0.84±0.01</td>
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<tr>
<td>% change</td>
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<td>2.2</td>
<td>0.5</td>
<td>35.6</td>
<td>19.3</td>
<td>7.3</td>
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