Different Approaches to Graphene Electrochemical Biosensors

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Abstract

Graphene biosensors have attracted growing interest in recent years due to their unique physical and chemical properties stemming from graphene's 2-dimensional, hyperconjugated carbon lattice. Although there have been many types of graphene biosensors reported or suggested, e.g., based on surface plasmon-polaritons [1], on surface acoustic waves [2], on coupling nanoparticles to graphene [3], to name just a few, most of the literature is based on liquid-gate field-effect transistors (GFETs) and other electrochemical configurations. The reason is that these architectures are relatively simple to construct, effectively exploring the graphene-electrolyte interface, allowing us to interrogate and follow the rich chemistry and other electronic processes that occur at the interface with great accuracy. Functionalizing graphene surfaces with specific molecular probes and collecting the electrical signal resulting from transducing biorecognition events makes it possible to reach untold detection limits with high specificity and sensitivity in a label-free assay [4]. Electrochemical graphene sensors can be designed and operated in different modes. This communication presents results obtained with our graphene electrochemical multi-transistor array chips fabricated using chemical vapor deposited (CVD) graphene and cleanroom technology [5], operated under DC and AC stimulation. In both cases, the sensor signal is a Dirac voltage shift upon biorecognition events that, in DC, appears as a shift in the GFET transfer curve minimum, whereas, under AC sinusoidal stimulation with a DC offset to compensate for graphene non-intentional doping, it appears as distortion in the frequency-doubled output curve. These two detection strategies are illustrated in Figure 1 by plotting simulated transfer and output curves. These curves are built with the analytical model discussed in [6], which describes the conductance of single-layer graphene as a function of the carrier resonant scattering caused by adsorbates at the graphene surface [7]. The graphene channels (20 per chip, with area $W \times L = 25 \times 81\ \mu m^2$) are functionalized using a 1-pyrene-butyric acid *N*-hydroxy-succinimide ester (PBSE) linker to which a 25-mer oligonucleotide probe sequence substituted with an amine group in the 3' position binds covalently via an amide bond. The probe will selectively hybridize with the complementary DNA strand (tDNA) containing a mutation occurring in brain tumor cells, acting as a cancer biomarker. The resulting sensor's signal, limit of detection, and sensitivity are measured and compared. Figure 2 (top) shows a set of calibration curves for tDNA detection on a GFET chip in phosphate buffer (PB) referring to binary mixtures of mutated and healthy DNA in different proportions (0%, 90%, 99%, 99.9 healthy DNA). It can be seen that the multi-transistor array chip can resolve all the mixtures for tDNA concentrations above ~ 100 aM. A critical issue that may hinder the sensor's response, particularly at minor target concentrations, is signal drift [8]. Here, it is quantified, its physical origin elucidated, and the proceedings to circumvent it are discussed. Several electrolytes (DI water, phosphate buffers of different ionic strengths, and ionic liquids) are used in measurements under different polarizations and acquisition rates to clarify the signal drift mechanisms.

The same CVD graphene devices are operated in a two-electrode configuration by shorting the source and drain contacts and using the graphene channel and the gate electrode as the working and counter-electrodes in an electrochemical impedance setup. The graphene electrode is functionalized with the same 25-mer DNA probe sequence as before, using a pyrene-derivative as the linker. It was recently demonstrated [9] that graphene electronic density of states (DOS) can be resolved using an electrochemical setup by experimentally measuring its quantum capacitance (C_q) response through electrochemical impedance spectroscopy (EIS) measurements. The latter is supported by the proportionality between C_q and DOS when plotted as an energy function: $C_q\,(eV) = e^2\,\mathrm{DOS}\,(eV)$, where e and eV correspond to the electron charge and energy, respectively. The biosensing signal is obtained by tracking the minimum graphene quantum capacitance in a series of measurements at a specific low-frequency limit and different offset bias potentials. At this frequency, there is an adiabatic coupling between Dirac electrons in graphene quantum states around the Fermi level and those of the molecular system anchored onto its surface. The displacement current resulting from the AC voltage small perturbation (10 mV RMS) reflects the dynamics of the relativistic electrons occupying states that set this electrode-molecule communication. The biosensing signal obtained in this way measures the degree of hybridization of the

DNA probes with tDNA as those highly charged molecules locally gate the graphene, i.e., change the electrochemical potential at the electrode.
Figure 2 presents the calibration curves obtained for two biosensing assays, in which the transduction signal corresponds to the relative response of the inverse of the C_q (RR), calculated by taking as reference the response of the blank experiment - incubation of PB without tDNA. We use the inverse of the C_q as the transduction signal because it is associated with the electron energy in graphene. Thus, any biorecognition event over the graphene surface, such as a resonant coupling, changes this energy [10]. In the first assay (orange circles), the RR is recorded as a function of the logarithm of tDNA concentration, revealing a behavior roughly linear with an excellent fit parameter (R^2=0.997). Limits of detection and quantification, with values of approximately 0.08 and 0.27 aM, were calculated. The sensitivity of the biosensing assay (the line slope) unveiled a value of 117% per decade of target concentration, demonstrating the technique's performance level. In contrast, the linear fit obtained for the second assay (green circles), made in consecutive PB incubations without tDNA, presented a negligible slope of 2.4%, compared with the previous assay, demonstrating the technique's specificity.

References

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Figures

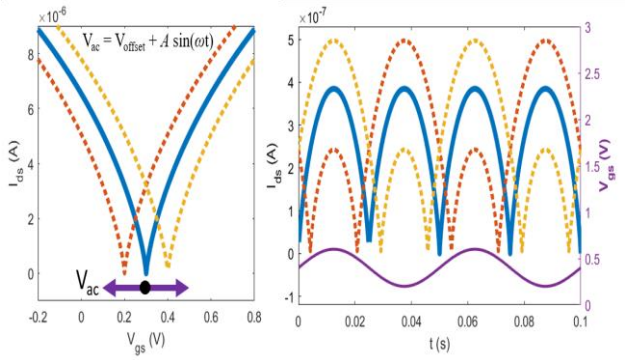

Figure 1. (left panel) A biorecognition event is detected by the shift in the GFET transfer curve minimum. Alternatively (right panel), one can use an AC sinusoidal stimulation (right axis) centered at the initial minimum (solid current line, left axis) and detect the distortion in the frequency-doubled output current upon biorecognition events in the channel (dashed lines).

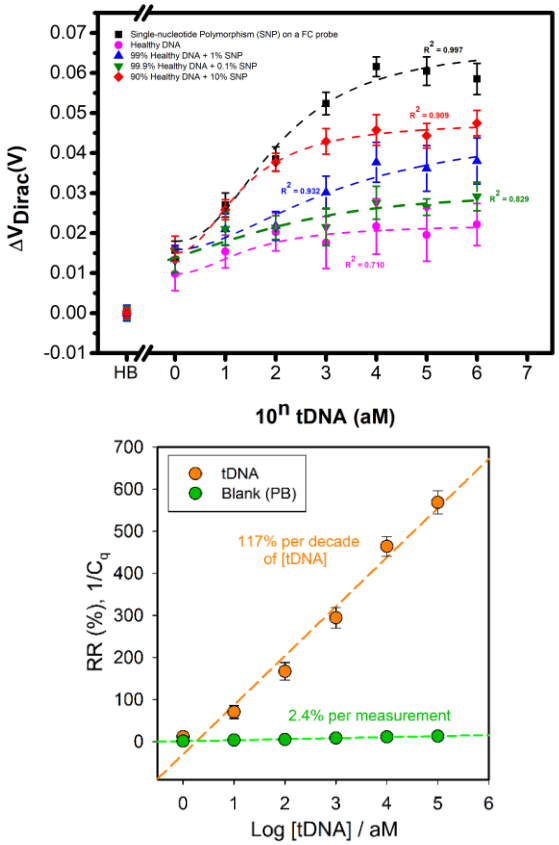

Figure 2. (top) Calibration curves for tDNA detection in PB using GFET devices. tDNA contains one SNP mutation relative to healthy DNA. The family of calibration curves refers to binary mixtures of tDNA and healthy DNA in different proportions (0%, 90%, 99%, 99.9 healthy DNA). (bottom) Calibration curves for two DNA-sensing bioassays with (orange circles) and without (green circles) tDNA. The graphene device was operated in an AC electrochemical setting. Each point of the curves corresponds to the averaged value calculated for three different graphene electrodes.