The effect of the linker on graphene biosensing chips' performance

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Abstract

The unique physical and chemical properties of graphene make it a promising material for biosensing applications. Here, we study the effect of the linker used to immobilize single-stranded DNA probes (ssDNA) on graphene surfaces on the performance of graphene field-effect transistors (GFET)-based biosensing chips. We use quartz crystal microbalance with dissipation (QCM-D) measurements to characterize in situ and real-time the immobilization of ssDNA and the subsequent DNA hybridization on model graphene surfaces. Like those in the graphene chips [1], the surfaces were previously biofunctionalized via heterobifunctional linkers. The GFET's transfer curves were used to characterize the biosensor chip performance.

The ssDNA immobilization kinetics and thermodynamics were investigated for all the pairings between three bifunctional linkers: 1-pyrene butyric acid succinimidyl ester (PBSE), Fluorenylmethylsuccinimidyl carbonate (FSC), and Acridine Orange (AO) succinimidyl ester—and three organic solvents (DMF, DMSO, and 10% DMF/Ethanol) [2]. Spatial orientation of the linkers and effective surface modification for ssDNA attachment was evaluated based on footprints and quantification of ssDNA surface coverage. Among the investigated linker-solvent pairs, functionalization with PBSE in DMF led to the highest ssDNA surface density of up to 1.4×10^{13} cm^{-2} (Fig. 1), with a 1 aM (10^{-18} M) complementary DNA limit-of-detection (LOD), and a sensitivity of 33 mV/decade (Fig. 2). In a separate experiment, probe DNA was let adsorb onto bare graphene (no linker) via π-π interaction, and the QCM-D and GFETs' transfer curves were monitored while the complementary DNA was introduced. These experiments provide an estimation of 20% to 30% of ssDNA direct adsorption on a graphene Surface functionalized with a linker, i.e., non-specifically adsorbed.

Lastly, the GFET transfer curves using different linkers were used to simulate the output of four GFET sensors mounted in a Wheatstone bridge configuration (two graphene transistors functionalized with probe DNA, two not functionalized), showing that this configuration would result in doubling the sensitivity of the biosensor (Fig. 3). Our results demonstrate the importance of understanding the functionalization phenomena at the graphene surface to achieve high ssDNA surface density functionalization to maximize the sensor response.

References

[1] P D Cabral, T Domingues, F Cerqueira, P Alpuim, J Borme et al., Materials, 2021
[2] E Prats-Alfonso, J A Garrido, R Villa et al.. Graphene 2018 Dresden, Germany, 25-26/06/18

Figures

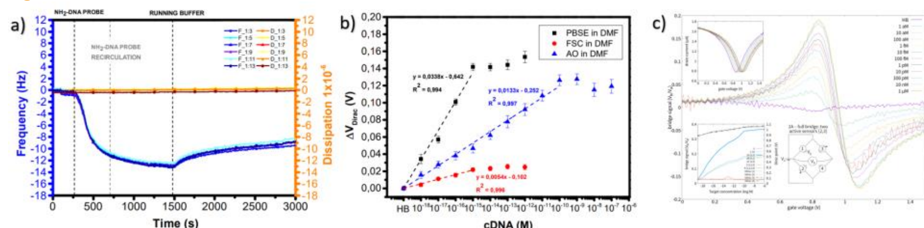

Figure 1 – (A) Measurements with quartz crystal microbalance with dissipation (QCM-D). Frequency (blue, left axis) and dissipation (orange, right axis) change vs. time response for probe immobilization with different linkers and solvents. (B) Measurements with Graphene Electrolyte-gate field effect Transistors. Calibration curve for different linkers dissolved in DMF. (C) Simulated bridge signal as a function of the applied gate voltage for different concentrations of target DNA, obtained using the measured output from 4 GFETs taken from the calibration curves of the sensor.