Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Large Area Epitaxial Graphene Nanomesh: an Artificial Platform for Edge-Electrochemical Biosensing at sub-Attomolar Level.

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SI.1 RAMAN spectra analyses

Figure SI.1 (a) shows one typical Raman spectrum recorded with the SiC substrate before growth of graphene. This spectrum exhibits more peaks that are related to SiC response (between 1300 cm^{-1} and 2000 cm^{-1}). Figure SI.1 (b) shows the RAMAN spectra recorded after the first step of the electrochemical grafting of the amine which validates this first functionalization. The downward shift of 2D peak positions can be attributed to N-doping of graphene from the formation of the C-N bond.

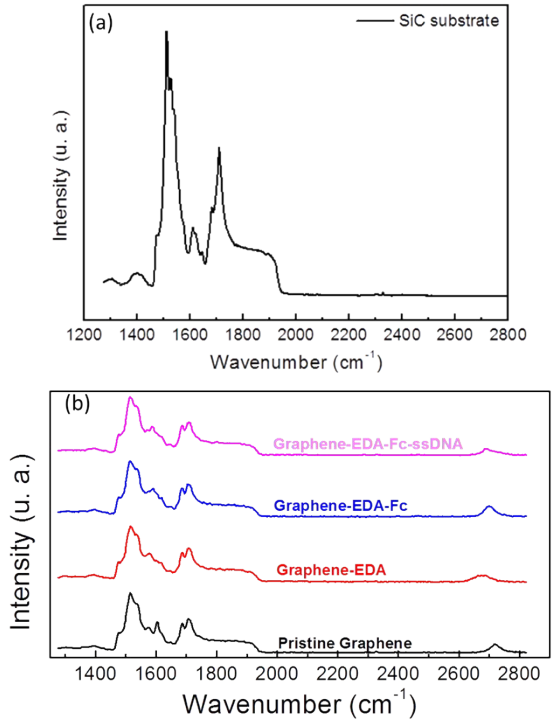

Figure SI.1 (a) RAMAN spectra recorded after the various steps dedicated to the construction of the DNA biosensor on the epitaxial graphene electrode and **(b)** Raman spectra of SiC recorded before growth of graphene.

SI. 2. XPS spectra analysis

All the different chemical steps have been characterized by XPS that shows the appearance of 5 peaks that are related to silicon, carbon, nitrogen, iron, and phosphate (Figure S2). After the grafting of the amine, the nitrogen peak (N1s) shows clearly the presence of amine functions at 399.9eV. After ferrocene and ssDNA grafting, the width at half maximum of this

nitrogen peak increases as observed in Figure SI.2 (b). Intensity of N1s peak also increases due to the presence of ssDNA nitrogenous bases. XPS spectra at C1s and N1s peaks (Figure (d)) recorded after the first step of amine electrografting evidence the presence of C-N bond on the surface of graphene. As explained in SI.1, the Raman spectrum after this EDA functionalization exhibits a shift towards lower frequency as the signature of N-doping. This modification induced a disorder in the graphene structure by conversation of carbon atoms from sp2 to sp3 hybridization. Indeed, electronic properties of graphene such as conductivity have been altered by electrochemical and chemical functionalization (doping).

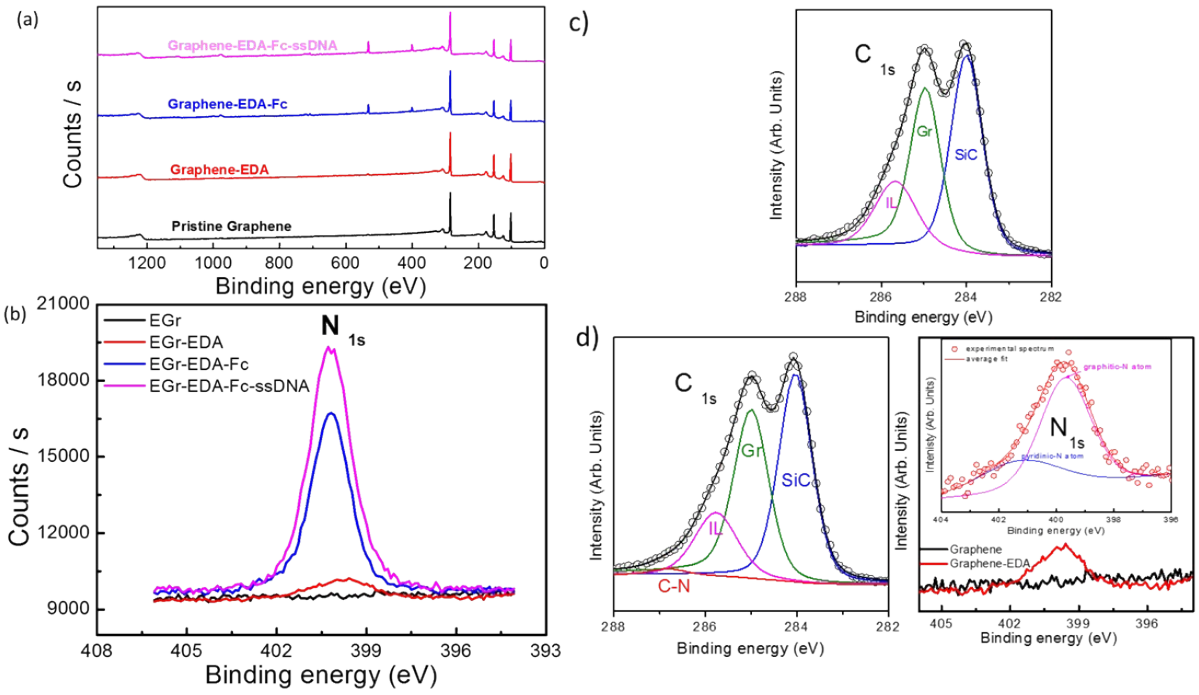

Figure SI.2 XPS spectra recorded after the various steps dedicated to the construction of the DNA biosensor on the epitaxial graphene electrode: **(a)** XPS spectra performed on a wide energy range and **(b)** High-resolution N1s spectra; XPS spectra recorded on the pristine graphene before **(c)** and after **(d)** the first functionalization step of electrografting of amine.

SI.3 Analytical performances of the biosensor

Kinetics of electrochemical DNA sensors has been studied during CV measurements through scan rate variation. Figure SI.3 presents the CVs measured during hybridization for both samples, i.e. the as grown pristine graphene and the nanomesh graphene.

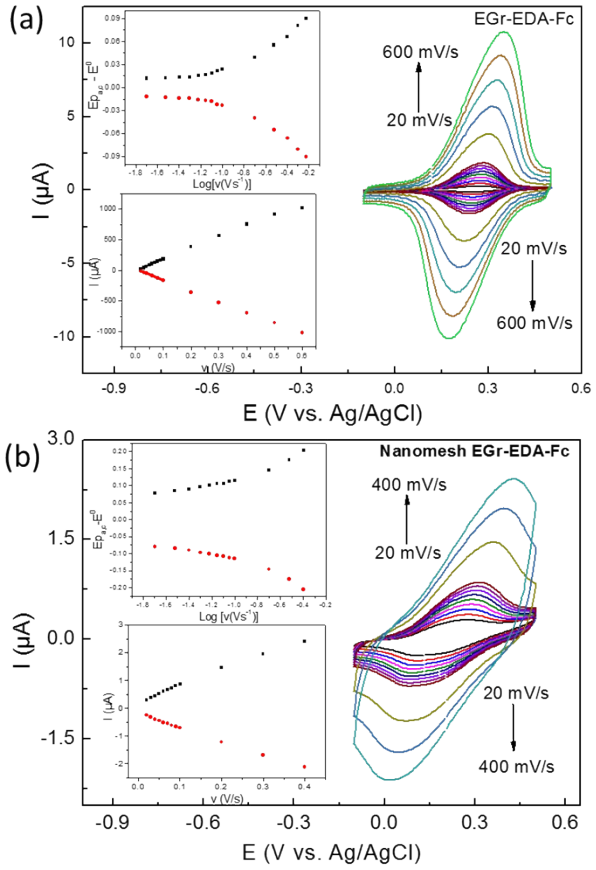

Figure SI.3 Cyclic voltammograms at various scans of a graphene electrode covered by EDA-Fc; the top inset shows the potential variation versus scan rate whereas the bottom inset shows the current variation as function of the scan rate: **(a)** with as-grown pristine graphene and **(b)** with nanomesh graphene.

The variation in anodic potential (E_{pa}) and cathodic potential (E_{pc}) versus logarithm of the scan rate (Figure SI.3, top insets) allows to calculate the rate of electron transfer k_s following Laviron model from the equation: [1]

$$\frac{1}{m} = \frac{nvF}{RT}\frac{1}{k_s}$$

where n is the number of electrons transferred in redox reaction (n=1), v is the scan rate, m is the dimensionless rate constant and R, T, F have their usual meanings.

Furthermore, the surface coverage could be calculated from the slopes of the linear plots of I versus the scan rate and according to the following Randles-Sevcik equation:[2]

$$I_p = \frac{n^2 F^2 A v \Gamma}{4RT}$$

where n represents the number of electrons involved in reaction (one electron), A is the surface area of the electrode (0.5 cm^2 with pristine graphene; 0.334 cm^2 with nanomesh graphene), T is temperature (300 K), Γ ($mol.cm^{-2}$) is the surface coverage. The average surface coverage of the modified graphene-EDA-Fc surface is estimated to be respectively 4.8×10^{-11} $mol.cm^{-2}$ with pristine graphene and 2.4×10^{-10} $mol.cm^{-2}$ with nanomesh graphene.

Two methods have been used to estimated Γ_{edge} (density on nanohole edges) and $\Gamma_{nanomesh}$.

1/ In addition to the average value of Fc molecules surface coverage on graphene nanomesh that was first estimated according to the **Randles-Sevick** equation to be around $\Gamma_{nanomesh} = 2.4\ 10^{-10}$ $mol.cm^2$, coverage density on nanohole edges Γ_{edge} has to be estimated.

Calculation is given below:

- The basal surface of graphene pristine $S_{basal\ pristine} = 0.5\ cm^2$
- With a diameter of each hole of 200 nm and a number of holes ~ $3.12\ \times10^8$ in 0.5 cm^2 the basal surface of the graphene nanomesh is estimated to be $S_{basal\ nanomesh} = 0.334\ cm^2$
- The general surface of the edges ~ $S_{edge} = 0.0013\ cm^2$

$\Gamma_{nanomesh}$ can thus be expressed by:

$$\Gamma_{nanomesh} = \Gamma_{basal} + \Gamma_{edge} = \Gamma_{Pristine\ Gr}(S_{basal\ nanomesh} / S_{basal\ Pristine}) + \Gamma_{edge}$$

$$\Rightarrow \quad \Gamma_{edge} = \Gamma_{Gr\ nanomesh} - \Gamma_{Pristine\ Gr}(S_{basal\ nanomesh} / S_{basal\ Pristine})$$

$$\Rightarrow \quad \Gamma_{edge} = 1.9\times10^{-10} - 2\times10^{-11}\ (0.334/0.5) = 1.7\ \ 10^{-10}\ mol\ .cm^{-2}.$$

$\Gamma_{edge} = 1.7\times10^{-10}$ $mol.cm^{-2}$ corresponds to 90% of $\Gamma_{nanomesh} = 2.4\times10^{-10}$ $mol.cm^{-2}$

2/ Secondly, the quantification of Fc coverage density on electrode surface $\Gamma_{nanomesh}$ can be also estimated from the charge exchanged during the redox reaction allows the following **Faraday** equation:

$$\Gamma = \frac{Q}{nFA}$$

where, Q is the charge under the cathodic or anodic waves, n is the number of electrons involved in the redox process (n=1), F is the Faraday constant (F=96500 C/mol) and A is the electrode area. $\Gamma_{nanomesh}$ is given by:

$$\Gamma_{nanomesh} = \frac{6.3 \times 10^{-6}}{1 \times 96500 \times 0.334} \sim 2.10^{-10} \text{ mol.cm}^{-2}.$$

To conclude, similar values of $\Gamma_{nanomesh}$ are obtained from either Randles-Sevcik equation or Faraday equation.

References

1 E. Laviron, *J. Electroanal. Chem.*, 1979, **101**, 19–28.

2 A. J. Bard and L. R. Faulkner, *Electrochemical methods : fundamentals and applications*, USA, 2nd edn., 2001.

SI.4 Scheme of the experimental set-up

Figure SI.4 give details on the experimental electrical set-up. All the electrochemical measurements have been performed using a small Teflon electrochemical microcell (from Metrohm). After the whole fabrication process, an additional UV lithography step was used to generate a large gold pad on the sample edge (see WE electrode in Figure SI.4 left). The sample is then placed inside the Teflon cell and the cell is closed. The two other electrodes (CE and RE) are then immerged in the central chamber. Figure SI.4 (right) shows the whole system with all electrical connections.

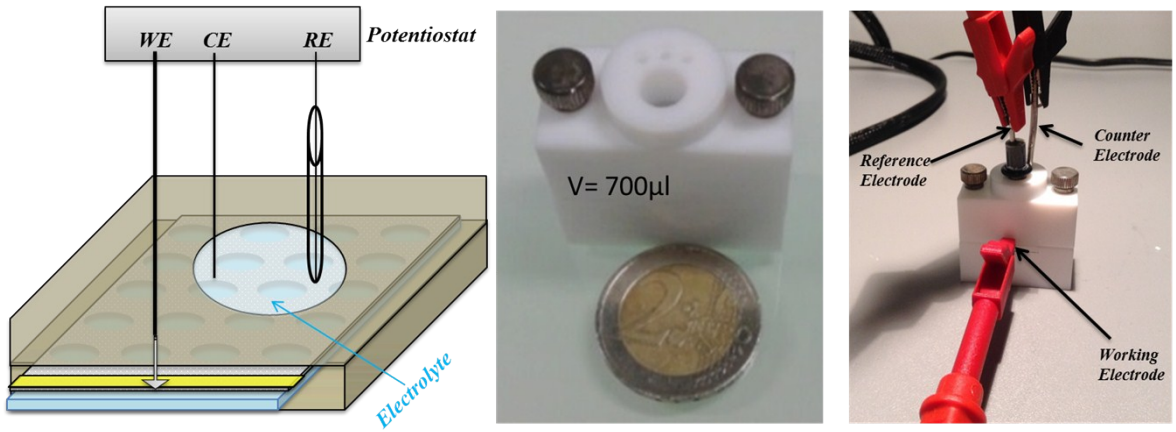

Figure SI.4 (left) scheme of the electrochemical cell and (right) an overview of the cell with the electrical connections.

Instrumentations

Raman spectroscopy was performed at room temperature with a Renishaw spectrometer, using a 532 nm laser physics argon laser focused on the sample with a DMLM Leica microscope with a 100x (NA=0.75) objective.

XPS measurements were performed on a K Alpha spectrometer from Thermofisher, using a monochromated X-ray Source (Al Kα, 1486.6 eV). For all measurements a spot size of 400 µm was employed. The hemispherical analyser was operated in CAE (Constant Analyser Energy) mode, with a pass energy of 200 eV and a step of 1 eV for the acquisition of surveys spectra, and a pass energy of 50 eV and a step of 0.1 eV for the acquisition of high resolution spectra. The spectra obtained were treated by means of the "Avantage" software provided by Thermofisher. A Shirley type background subtraction was used.

SEM images were taken with a Hitachi scanning electron microscope (SEM 4800, LPN, France).

Electrochemical measurements were performed with Autolab 30 equipped with software Nova. The macro-cell has the total volume of 1 mL and it is composed of epitaxial graphene as working electrode, and a bare platinum as counter electrode and an Ag/AgCl as reference electrode. The electrochemical measurements, in microfluidic cell, were performed by connecting one gold electrode as pseudo reference and the larger gold electrode as counter electrode. The exposed surface area of the working electrode was 0.5 cm^2 for pristine graphene and 0.334 cm^2 for nanomesh EGr.

SWV measurements were conducted based on the following parameters: 120 s accumulation time, 25 Hz frequency and 0.02 V amplitude.

Chemical reagents

Ethylene diamine and the lithium perchlorate were purchased from Sigma Aldrich. The modified ferrocene group $Fc(NHP)_2$ was synthesized following a previously described procedure [21]. The background electrolyte is 0.1 M of phosphate buffer at pH 7.4 prepared by mixing stock solutions of NaCl, KCl, Na_2HPO_4, KH_2PO_4 and ultrapure water. All other reagents are commercially available and were purchased with analytical reagent grade. The oligonucleotide containing 15 pair bases with 5' terminal amino group modification and C12 carbonyl spacer was provided by Eurogentec Company. The oligonucleotides probe with amino group at its 5' phosphate end, abbreviated NH_2-ssDNA, has the sequence as follows:N H_2–$(CH_2)_6$–5'-GATACTTCTATCACC-3'. The complementary target oligonucleotide has the following sequences: 5'- CTATGAAGATAGTGG-3'.