Supporting Information

Ultrasensitive Detection of SARS-CoV-2 Spike Protein by Graphene Field-Effect Transistors

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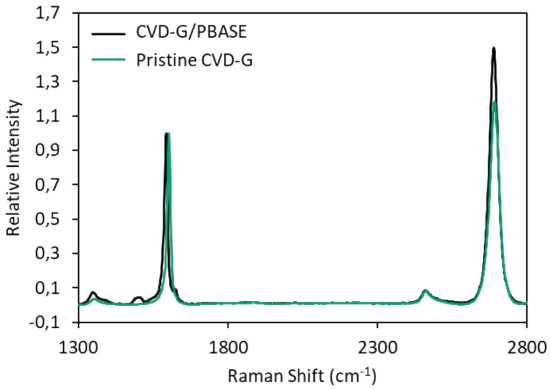


Figure S1. Raman spectra of Pristine CVD-G and CVD-G/PBASE.

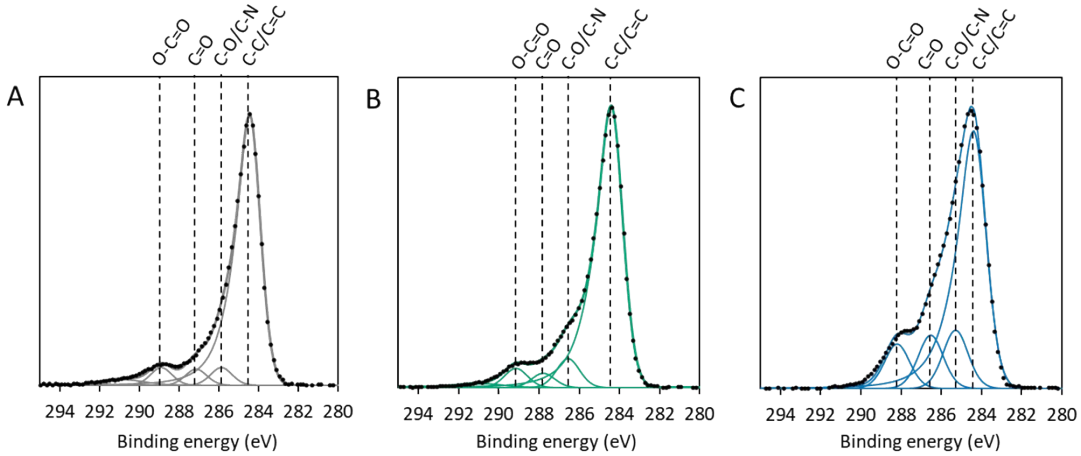
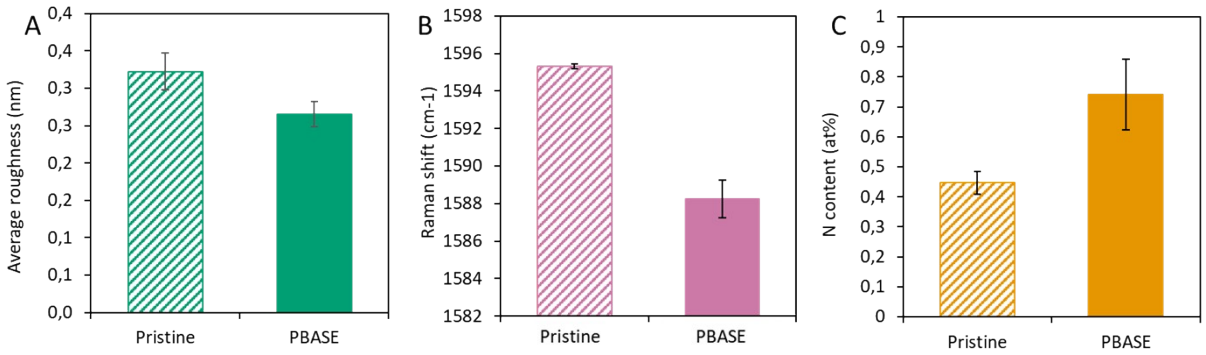


Figure S2. High resolution XPS spectra of the C 1s core of Pristine CVD-G, CVD-G/PBASE, CVD-G/PBASE/ACE2.

Figure S3. Repeatability of functionalisation of 3 CVD-G samples with PBASE. Three parameters were monitored:



Average roughness by AFM (A), shift of the G band at Raman (B) and nitrogen content by XPS (C).

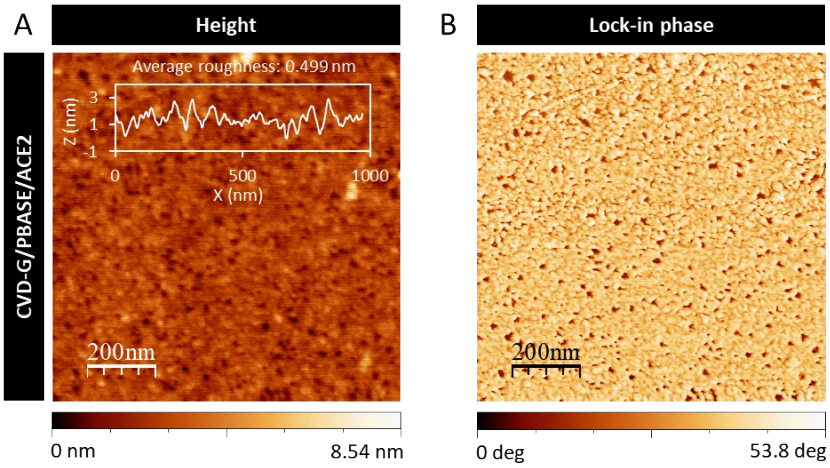


Figure S4. AFM height (A) and lock-in phase (B) micrographs of CVD-G/PBASE/ACE2.

Modelling ACE2

In order to determine the distribution of charges on ACE2 protein, we use the PDB2PQR server using AMBER forcefield at pH 7.4.[1,2] The pKa of the pH sensitive residues of the protein is calculated with the PDB2PQR web server which, at a desired pH, calculates the pKa that each HIS, ASP and GLU residue of a protein would have based on hydrogen bonding, local energetics, and model pKa values.[1] PropKa tool was also used for this purpose.[3] State of the art estimation of the protonation state of the residue uses classical approaches with remarkable accurate results. According to our simulations the total charge at pH 7.0 is -28 elementary charges or 44.86 x 10^{-19} Coulombs.[3–6]

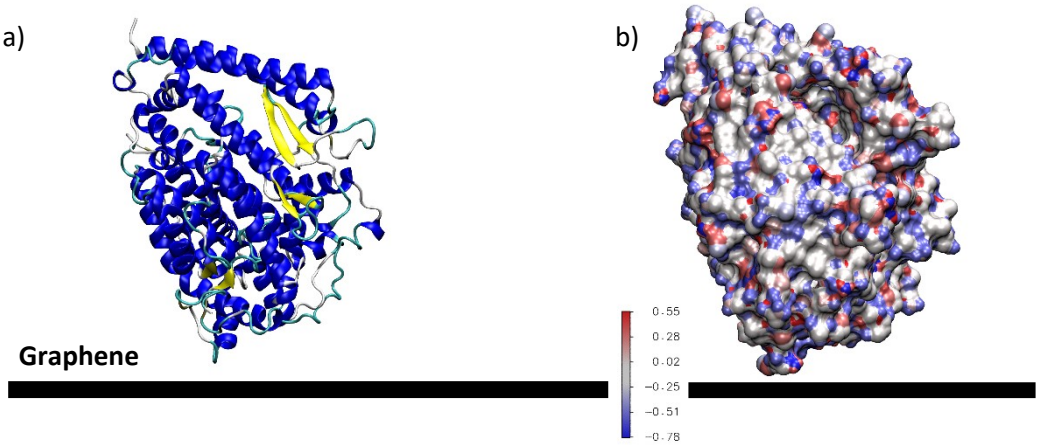


Figure S5. Extracellular section of the Angiotensin-converting enzyme 2 (ACE2) protein obtained from x-ray crystallographic data (PDB id. 1R42). a) Highlight of the secondary structure of ACE2 Alpha-Helix (Blue), Beta Sheets (yellow) and Random Loops (cyan). b) Charge distribution on the surface of the ACE2.

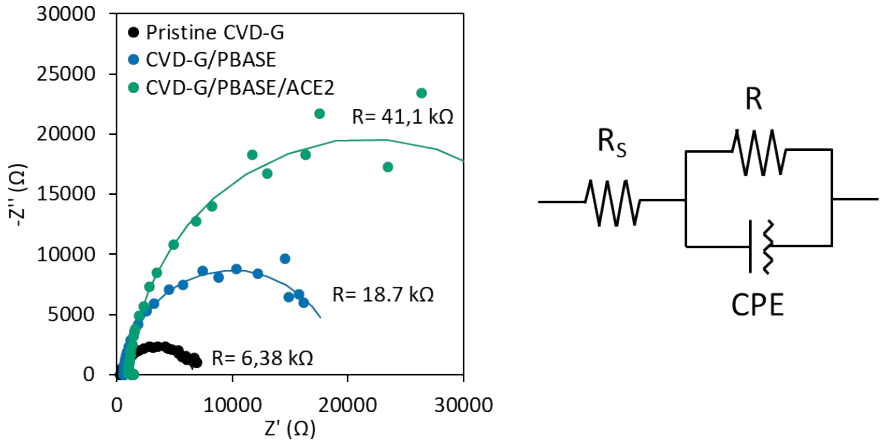


Figure S6. Electrochemical impedance spectroscopy (EIS) measurements of Pristine CVD-G (black), CVD-G/PBASE (blue) and CVD-G/PBASE/ACE2 green. The resistance of each sample has been obtained by fitting the data with the reported equivalent circuit.

Quantification of the functionalisation density by QCM

Experimental functionalisation density for PBASE

The functionalisation degree of CVD-G with PBASE was studied by QCM microbalance. A t_0 EtOH was flown over a QCM crystal on top of which was deposited a monolayer of CVD-G. At t_1 the flow was exchanged for a PBASE 1mM solution in ethanol. A sudden drop in the crystal frequency was observed due to the absorption of PBASE on top of graphene. At t_2 a stream of EtOH was flown on top of the crystal to clean the surface and remove the unabsorbed PBASE. The variation of the frequency (for n=3 Δf) after functionalisation and cleaning steps is equal to 17.97 Hz (Figure S5). Δf can be correlated to the variation in mas (Δm) applying equation S1

$$\Delta m = -\frac{A\sqrt{\rho_q \mu_q}}{2f_0^2}\Delta f$$

Eq S1.

$$\Delta m = -\frac{cte}{n}\Delta f$$

Eq S2.

$$\Delta m = \frac{17.7}{n}\Delta f$$

Eq S3.

From Δm, equal 106.04 ng cm^{-2}, the number of molecule per nm^2 (N) can be calculated by applying Eq. S4:

$$N = \frac{\Delta m}{MW_{PBASE}} NA\, 1e^{-14}$$

Eq S4.

Which it is equal to 1.62 molecules per nm^2

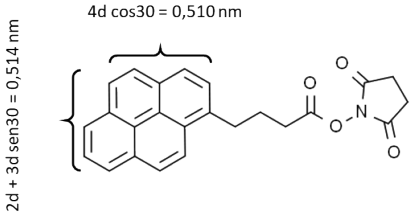
Expected functionalisation density for PBASE monolayer

The expected functionalisation density for PBASE monolayer has been calculated approximating the PBASE footprint to the area occupied by 1 pyrene moiety (Eq. S5, Figure S6).

$$A = 4d\ cos30\ (2d + 3d\ sen30) = 0.262\ nm^2$$

Eq S4.

Where d is the distance among two C atoms in an aromatic ring.



Therefore, the expected functionalisation density is equal to 3.81 molecule per nm^2

Experimental functionalisation density for ACE2

The ACE2 receptor density has been calculated from the frequency shift of the QCM balance as described previously for ACE2. At t_0 a PBS flow was stream over a QCM crystal coated with CVD-G/PBASE. At t_1 the At t_1 the flow was exchanged for a ACE2 10 µg/ml solution in PBS. At t_2 PBS solution was flown to clean the sensor surface. However, being the ACE2 covalently linked to PBASE, and the whole functionalisation highly stable, no increase in the frequency was notices upon washing. The Δf after ACE2 immobilisation was of 10.15 (Figure S7). Δm was calculated to be 18.88 ng cm^{-2}, by applying Eq S3, corresponding to a density of 0.0043 receptors per nm^2

Expected functionalisation density for ACE2 monolayer

The expected functionalisation density for ACE2 monolayer has been calculated approximating the PBASE footprint to the area occupied by a circle with a radius equal to ACE2 hydrodynamic radius (R_h=5.04nm; A=79.76 nm^2).[7] Therefore, the expected receptor density is equal to 0.012 proteins per nm^{-2}.

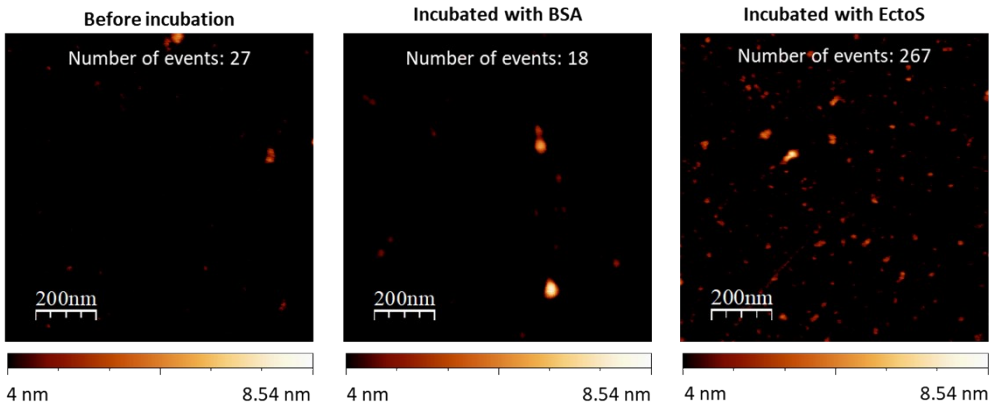


Figure S7. Height AFM images filtered with a low-pass cutoff of 4nm, to highlight the events imputable to the presence of proteins adsorbed on the surface.

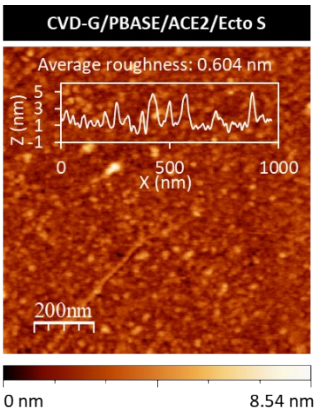


Figure S8. Height AFM micrograph of CVD-G/PBASE/ACE2 surface after incubation with Ecto S.

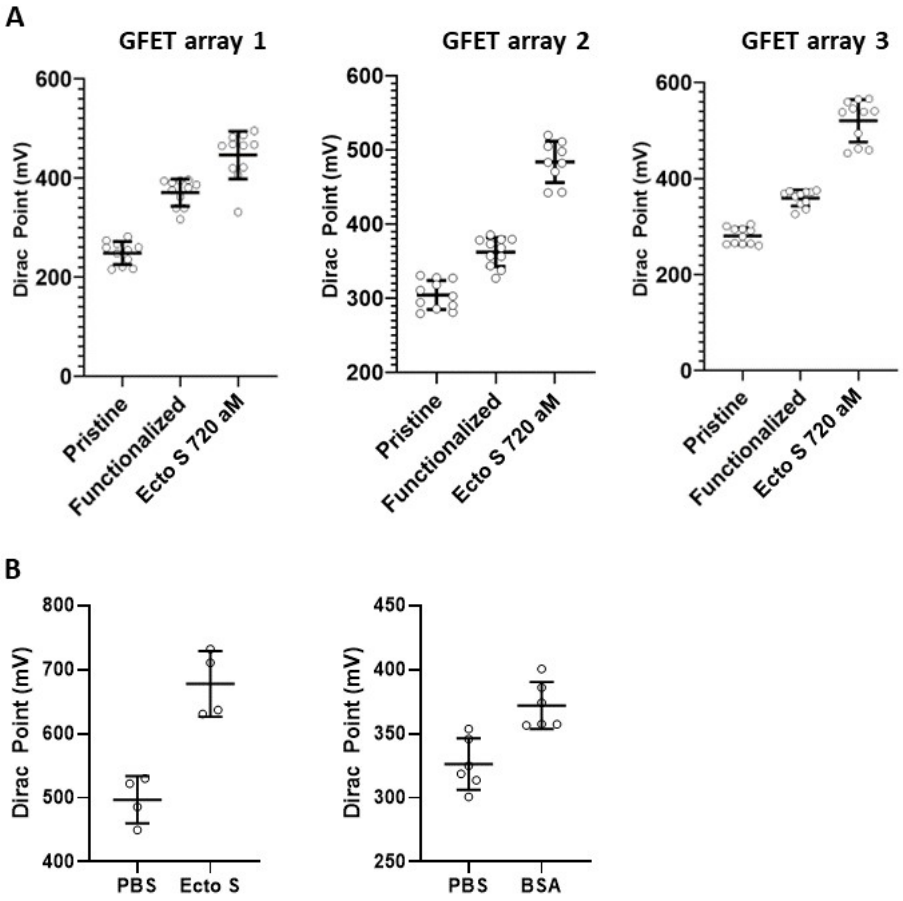


Figure S9. A) V_{CNP} for three different GFET arrays, each composed of 12 microtransistors. The V_{CNP} of each microtransistor was registered with graphene as pristine material, after its functionalization with ACE2 and then in presence of Ecto S at 720 aM. B) V_{CNP} of 4 microstransistors of a GFET array in PBS and in presence of Ecto S at 720 aM in PBS (left), and V_{CNP} of 6 microstransistors of a GFET array in PBS and in presence of BSA at 720 aM in PBS (right).

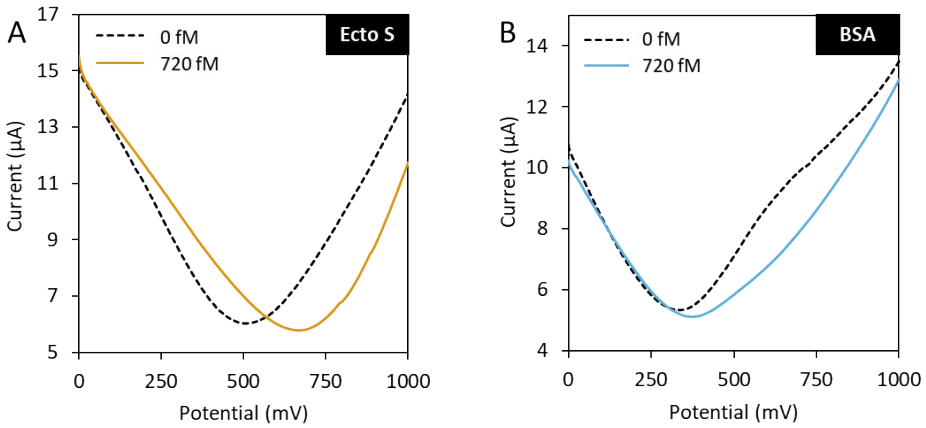


Figure S10. Transfer curves of GFET/PBASE/ACE2 devices in presence and absence of 720 fM Ecto S (A) and BSA (B).

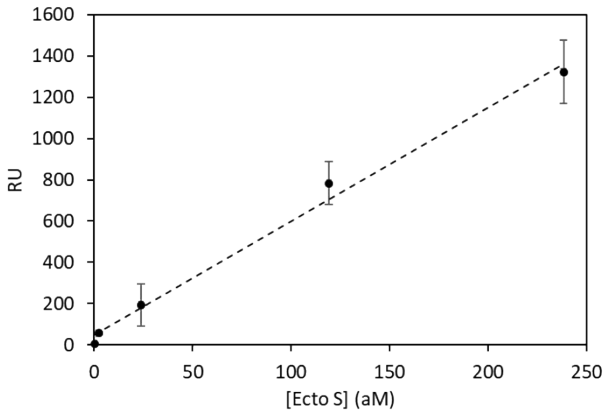


Figure S11. Change of SPR signal as function of Ecto S protein concentration.

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