Supplementary Information

A point-of-care immunosensor based on quartz crystal microbalance with graphene biointerface for antibody assay

Piramon Hampitak¹, Thomas A Jowitt², Daniel Melendrez¹, Maryline Fresquet², Patrick Hamilton³,⁴, Maria Iliut², Kaiwen Nie¹, Ben Spencer¹, Rachel Lennon³,⁵, and Aravind Vijayaraghavan¹,*

¹ Department of Materials and National Graphene Institute, Faculty of Science and Engineering, The University of Manchester, Oxford Road, Manchester, UK.
² School of Biological Sciences, Faculty of Biology Medicine and Health, The University of Manchester, Oxford Road, Manchester, UK.
³ Wellcome Centre for Cell-Matrix Research, Division of Cell-Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology Medicine and Health, The University of Manchester, Manchester Academic Health Science Centre, Manchester, UK.
⁴ Manchester Academic Health Science Centre (MAHSC), The University of Manchester, Manchester, UK.
⁵ Department of Paediatric Nephrology, Royal Manchester Children’s Hospital, Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK.

*Corresponding author: aravind@manchester.ac.uk

Here, we provide supporting information including the production of the receptor NC3, the protocol for QCM measurement using the QSense and our customised OpenQCM systems as well as supplementary results and analysis.

1. Production of NC3

PLA2R NC3 was produced by the modification of human extracellular PLA2R (NC8) previously described by Kanigicherla et al.[1]. To clone PLA2R NC3, the following primers were first used to amplify an N-terminal portion of the NC8 by PCR using the following oligonucleotides:

5‘CCCGCTAGCCGAGGGAGTGGCCGCTGCCC3’ (Forward) and
5‘CCCGCGGCCGCTCAATGGTGATGGTGATGGTGGTGATGATGGTGTCCGGACCCGGG GTGAAATGGCCATTTTTCGTACTCAGC3’ (Reverse)[2].

Further details about protein expression and characterisations can be found in ref. 2. The cloned NC3 presented high binding affinity for human anti-PLA2R IgG [2], [3].

![Figure S1](image)

Figure S1 (a) 3D PLA2R NC3 model structure adapted from Ref. [3] (b) tertiary structure of immunoglobulin G (IgG) antibody, image from the RCSB PDB (rcsb.org).
2. The customized OpenQCM instrument development

The customised OpenQCM (C-QCM) instrument was developed based on an open-source QCM (OpenQCM, Novaetech Srl). To set up the QCM system, the temp-control chamber will be powered on and the QCM device will be connected to the laptop via USB (Figure S2(a)). A custom-made graphic user interface (GUI) was developed in Matlab R2018a (Figure S2(b)). It is used to perform QCM data acquisition, temperature, baseline calibration, the change of frequency and data post-processing.

3. QCM-D principle and procedure

3.1 Principle of QCM-D

In 1959, Günter Sauerbrey [5] demonstrated that upon adding mass to a QCM sensor surface a frequency decrease proportional to the added mass occurs and given that the mass is small compared to the total weight of the crystal the frequency change is directly proportional to the mass of the crystal [5], therefore their use as microbalances is based on the linear relationship between changes in the resonator mass and in the resonance frequency [5]. Under vacuum, the rigid attachment of a
film of mass $\Delta m$ to the crystal surface causes a decrease $\Delta f$ in the resonant frequency $f_0$. Sauerbrey was the first who derived that the relationship between $\Delta f$ and $\Delta m$ is linear in the limit of small $\Delta m$ and is expressed by a simple relationship:

$$\frac{\Delta f}{f_0} = -\frac{\Delta m}{m_Q} \quad (Eq. \, S1)$$

where $m_Q$ is the mass of the unloaded resonator.

From Eq. S1 one can introduce the mass sensitivity factor $C_r$, and depends only on the fundamental resonant frequency $f_0$ and the material properties of the crystal used [6].

Hence:

$$C_r = \frac{m_Q}{f_0} \quad (Eq. \, S2)$$

where the mass of the resonator can be expressed as the product of the average density of quartz crystal, $\rho_Q$ and its thickness $h_Q$. Thus, $C_r$ can be expressed in terms of an appropriate dimensional analysis:

$$C_r = \frac{m_Q}{f_0} = \frac{\rho_Q h_Q}{f_0} = \left[ \frac{ng \cdot cm}{Hz} \right] = [ngcm^{-2}Hz^{-1}] \quad (Eq. \, S3)$$

For 10 MHz quartz crystals, $C_r$ equals $4.5 \, ngcm^{-2}Hz^{-1}$ if only one side is covered [7]. For crystals with $f_0 = 5 \, MHz$, the density is $\rho_Q = 2.648 \, g/cm^3$ and the average thickness is $h_Q \approx 300 \, \mu m$, thus $C_r \approx 18 \, ngcm^{-2}Hz^{-1}$.

Assuming rigid adsorption to the substrate enables the use of Sauerbrey modelling to approximate the mass adsorbed on Au electrodes. To obtain the aerial mass density, the values captured for $\Delta f$ can be plugged in the Sauerbrey equation (Eq. S1). Rearranging terms, we obtain.

$$\frac{\Delta m}{A} = -\sqrt{\frac{\rho_Q \mu_Q}{2 f_0^2}} \Delta f \quad [g/cm^2] \quad (Eq. \, S4)$$

where $A$ is the area of the working electrode ($0.2043 \, cm^2$), $\mu_Q$ is the shear modulus of AT-cut quartz crystals ($2.947 \times 10^{11} \, g cm^{-1}s^{-2}$) and $f_0 = 10 \, MHz$ for the crystals used in all C-QCM instrument experiments.

Therefore, the amount of adsorbed mass can be determined by using Equation S1, and combined with viscoelastic modelling [8] one can obtain a physical description of the adlayers. Higher mass adsorption will respond in large frequency down-shifts. Results obtained from Sauerbrey computation will be more accurate if the adsorbed film is rigid, otherwise, dissipation parameter could play a key role for the viscous adsorbed film.

The QCM-D monitoring technique provides a real-time measurement of changes in both frequency ($\Delta f$) and dissipation ($\Delta D$) as molecular adsorption on surfaces. The dissipation value is the sum of total energy losses in the system in an oscillation cycle defined as the lost energy divided by the total energy stored in the system, $E_{loss}/E_{total}$[7], [9]. High dissipation values will result from viscoelastic adsorption of molecules generating a soft film on the surface. On the other hand, a rigid film usually gives low dissipation values. The slope of the plot of $\Delta D$ vs $\Delta f$ could reveal regions of different adsorption behaviour in terms of the viscoelastic structure of the adsorbed material.
3.2 Initial preparation of quartz crystals

QCM chips with gold surface electrode for both QSense (QSX-301, Biolin Scientific) and our C-QCM system (QCM10140CrAu051-051-C1, Quartz Pro) were used in this study. We used the same protocol for cleaning and surface coating.

To prepare the sensors for studying the adsorption of biomolecules on graphene derivatives surfaces, the QCM chips were cleaned following a modification of the protocol that in Ref 6, then coated with GO using a spin coater to get the GO chips. To get rGO chips, the GO pre-coated chips were thermally reduced to rGO at 180 °C for 6 hours.

3.3 QCM-D monitoring procedure for binding studies

The QCM-D measurements were carried out using the QSense Omega Auto system (Biolin Scientific). The system consists of eight sensing ports that can be controlled to be fed through customized scripts. Before every experimental session, the ports, tubing and sensors holders were cleaned using clean maintenance chips following the protocol described below. The temperature of the system was set at room temperature (25 °C). Once the system was cleaned and primed, the sensors for experimentation were loaded on the sensors holder.

At the beginning of each measurement, the sensors were primed by continuously flowing PBS until a stable baseline was obtained. Once a stable baseline was observed, the corresponding measurement script was started. In the present report, the steps for monitoring the functionalisation of the assay and the binding of Ab12 were presented in Figure 3 in the main paper. The injection sequence for each sample shows as follows:

Sample (1) Au: PBS → NC3 → PBS→ antibody sample→ PBS
Sample (2) GO: PBS → NC3 → PBS→ antibody sample→ PBS
Sample (3) rGO: PBS → NC3 → PBS→ antibody sample→ PBS
Sample (4) Au-dBSA: PBS → BSA 50 µg/mL → PBS→ NC3 → PBS→ antibody sample→ PBS
Sample (5) rGO-dBSA: PBS → BSA 50 µg/mL → PBS→ NC3 → PBS→ antibody sample→ PBS
Sample (6) rGO-BSA: PBS → BSA 500 µg/mL → PBS→ NC3 → PBS→ antibody sample→ PBS
Sample (7) Au-SAM: PBS → BSA 50 µg/mL → PBS→ NC3 → PBS→ antibody sample→ PBS
Sample (8) rGO-dBSA+NHS: PBS → BSA 50 µg/mL → PBS → EDC/NHS → PBS→ NC3 → PBS→ blocking buffer → antibody sample → PBS

Once the measuring script is completed, to avoid contamination it is recommended to run a washing routine to clean the sensors, syringes and tubing using sodium dodecyl sulphate (SDS) surfactant solution to be then finally rinsed with MilliQ water and left to dry overnight. This process ensures that the system is left clean and dry for subsequent experiments.

4. Dot blot

The murine anti-PLA2R antibody (Ab12) was tested for binding affinity with NC3 using dot blotting, the measurement shown in Figure S3. Varied concentrations of NC3 (blue circle) and denatured NC3 (red circle) were blotted against the Ab12 antibody. The NC3 can be denatured by boiling it with SDS making the protein lose its secondary and tertiary structure. If the NC3 is denatured, the antibody will not bind to the NC3 resulting in no fluorescent signal. Figure S3 shows that only non-denatured BSA can present the strong fluorescent signal of antibody adsorption. The strength of the signal increases with the concentration of the protein. Thus, we confirm that the Ab12 antibody can specifically bind with the NC3.
5. Preparation of sensing surfaces for binding study

The sensing surfaces used in this study include Au, graphene oxide (GO) and reduced GO (rGO). Both physical adsorption and covalent crosslinking methods were used for functionalising the protein receptor, NC3. Therefore, eight different functional surfaces were tested as shown in the main paper. Samples 1 to 6 use the physical adsorption method for the receptor immobilisation while covalent crosslink functionalisation was used in samples 7 and 8.

GO surface coating was prepared using spin coating (speed: 3000 rpm, acceleration: 350 s⁻¹, 2 mins) to form a GO film on the entire surface of the gold surface QCM-D chip. A rGO-coated chip was performed in a vacuum oven (Townson+Mercer EV018) with temperature of 180 °C for 6 hours to reduce the GO. The full protocols were described in our previous study [10].

Au-SAM was prepared using general protocol from Sigma Aldrich following these steps 1) add 1 ml ethanol to a tube, and pipette to prepare 1 mmol/l Carboxylic acid-SAM solution, then, dilute the solution 10-fold with ethanol for Step 2) then immerse a gold substrate in the reagent solution prepared at step 1) at room temperature and leave it overnight and finally 3) wash the substrate several times with ethanol and purified water sequentially.

6. SEM imaging

The SEM imaging of GO flakes and rGO coatings was performed on a SEM Zeiss Ultra setup, using an acceleration voltage of 5 kV and 5 mm working distance. We previously reported the size distribution of GO and surface properties of GO before and after reduction [10].

Figures S4 (a) presents SEM image of GO flakes indicating that the GO is 100% monolayer. More detailed characterisation of the GO material including atomic force microscopy confirmation of layer thickness can be found in the paper in Ref. 6. As QCM crystal from QSense and Quartz Pro were used, rGO coating on crystals from both companies are presented in Figures S4 (b) and (c), respectively. There was no significant difference in the coating. Fully-coverage and uniform coating with the presence of flake overlaps display in both crystals.

7. XPS Results

The surface chemistry of three different samples including bare rGO, rGO-dBSA and rGO-dBSA+EDC/NHS were investigated using X-ray photoelectron spectroscopy (XPS) analysis to understand functionalisation mechanism of the EDC/NHS coupling on rGO-dBSA. The rGO samples were prepared by drop-casting the dispersion of GO on a clean Si/SiO2 substrate and drying at room temperature in a vacuum oven to achieve a film with a thickness of not less than 10 nm. The GO samples were thermally reduced at 180 °C for 6 hours to obtain rGO samples. The sample of rGO-dBSA

![Figure S3 Dot blot results of different concentration of NC3 binding with murine anti-PLA2R antibody (Ab12) in comparison to the denature NC3. Only non-denatured NC3 can present the response of antibody binding.](image-url)
was prepared by drop-casting BSA 50 µg/mL on rGO surface and leaving on for 10 minutes then rinsing with PBS and DI water and drying at room temperature in a vacuum oven. The rGO-dBSA+EDC/NHS samples were obtained from drop-casting EDC/NHS solution on rGO-dBSA, let it functionalised for 10 minutes and then rinsing and drying with the aforementioned conditions.

The XPS data were collected on a SPECS custom-built system composed of a Phobios 150 hemispherical electron analyser with a 1D detector. The X-ray source is a microfocus monochromated Al K-alpha (1486.6 eV) source. All spectra were collected with a pass energy of 20 eV. Combined ultimate resolution as measured from Ag 3D is 0.5 eV with the X-ray source and 20 eV pass. The XPS data processing was done using CasaXPS software (version 2.3.16 PR 1.6).

The calibration of the C1s spectrum was performed using the sp³ carbon (C=C, C-H) as a reference at 284.8 eV peak position and the background type used was Shirley. The C1s spectrum fitting was performed using synthetic components ascribed to different chemical species including hydroxyl (C-OH) and epoxy (C-O-C) groups between ~286 and 287 eV, carbonyl (C=O) and carboxyl (O-C=O) groups between ~287, 289 eV and a satellite peak, respectively. The spectrum of N1s was assigned as graphitic nitrogen at a peak of 400.5 eV. The background type Shirley was subtracted. The synthetic components including C-N and N-Ester were used for spectrum fitting [11]. The O1s spectrum is in the region of 525–580eV. The background type used was spline Tougaard. Three synthetic components including quinone, C=O and C-OH were assigned at 530.1 eV, 532.0 eV and 533.5 eV for spectrum fitting [12]. The constraints applied where the FWHM constraint (0.9-1.1) and the position constraints (between max ±1 eV to min ±0.1 eV). XPS region was fitted with the synthetic components in the manner which minimizes the total square error fit (0.91) and corresponds to the literature reports.
Figure S5 XPS results obtained from bare rGO, rGO-dBSA and rGO-dBSA+ NHS samples (a) Wide scan spectra with atomic percent. (b) detail spectra of C1s (c) O1s and (d) N1s for all samples. The sample with EDC/NHS activation clearly shows functionalisation of carbon and nitrogen on the surface.

Figure S5(a) shows the wide scan spectra for the three samples including rGO, rGO-dBSA and rGO-dBSA+EDC/NHS. The atomic percentage of carbon, nitrogen and oxygen were presented. The percentages of oxygen and nitrogen increase after the adsorption of BSA on rGO. The presence of sodium and chlorine peaks on the spectrum of rGO-dBSA+EDC/NHS could due to the salt ion in buffer solution. Figure S5(b), (c) and (d) present the XPS spectra of C1s, O1s and N1s, respectively. The sample with EDC/NHS activation clearly shows the C-N peak observed from C1s and N1s profiles. This confirms the covalent functionalisation of nitrogen functional groups on the rGO-dBSA surface.

The individual curve fittings of C1s, N1s and O1s spectra of all samples were presented in Figure S6. The C1s spectrum obtained from rGO displays the low intensity of oxygen functional groups but the high intensity of carbon peak. The intensity of C-C peak decreases after the adsorption of BSA, while the presence of oxygen functional groups like ether increases. After activating the surface with EDC/NHS, the graphitic nitrogen peaks (C-N and CO-NH) are obviously shown, while the carbon-carbon bond is lower than that from another two samples. The evidence from the detail scan peak of N1s in Figure S6(f) confirms the presence of covalent bonding of carbon and nitrogen in the sample of rGO-BSA with EDC/NHS. The binding energy peak at 399.7 eV could indicate the presence of the secondary amine and imine of EDC. The peak at 402.1 eV could be the characteristic of the nitrogen of the NHS ester, shifted to higher energy due to the electronegative oxygen bonded to the nitrogen [11].

Regarding the curve fittings of O1s spectra, the oxygen peak is sharper in the case of EDC/NHS activation as the C-O peak disappears. Based on these XPS results, it could be implied that certain oxygen functional groups, like carboxyl and ester, on the rGO-BSA surface were activated by amine...
8. Raman spectroscopy

Samples for Raman spectroscopy including rGO, rGO-BSA and rGO-BSA+EDC/NHS were prepared on rGO coating on QCM chips. After monitoring the adsorption of BSA on rGO and the EDC/NHS activation using QCM technique, the QCM chips were taken out from QCM equipment, rinsed with milliQ water and dried with nitrogen gas to be samples for Raman measurements. Raman spectra were taken on a Renishaw Raman system equipped with a Leica microscope and a CCD detector. Raman spectrum was recorded using 532 nm laser line (Cobolt Samba) continuous wave diode-pumped solid-state laser, 20 mW. The laser power was kept below 10 µW to avoid thermal degradation of the samples. The relative intensity ratio (I_D/I_G) was measured from the averaged acquired spectra, 10 spectra per sample. Gaussian and Lorentzian curves were used for Raman spectra fitting using Origin Pro 8.

Raman region (1000 to 1900 cm\(^{-1}\)) was fitted well to 4 peaks of Gaussian and Lorentzian curves. Two main peaks, including the graphitic (G) and defect (D), occur at ~1580 cm\(^{-1}\) and ~1350 cm\(^{-1}\),...
respectively. All samples show no difference in D and G peak positions. The $I_D/I_G$ values obtained from the sample of rGO-dBSA and rGO-dBSA+EDC/NHS show slightly higher than that of rGO samples. This indicates that more defects are presented after the physical adsorption of protein on graphene surface. The defects could originate from oxygen and nitrogen functional groups from BSA protein and the amine activation from EDC/NHS. The weak peaks labelled A and B at around 1130 and 1700 cm$^{-1}$ are the well-known peaks of defective graphite [13]. These two peaks seem to be signatures of GO thin films as well as in highly reduced GO [13]. The peak A, in this case, could be original from the edges and overlapping of the GO fakes whereas the peak B could be due to the effect of highly reduction corresponding to previous studies [13], [14].

In the Raman region between 2300 to 3500 cm$^{-1}$, three bands were observed at around 2700, 2900 and 3200 cm$^{-1}$, known as 2D, D+G and 2D' or C, respectively. The 2D peak is the overtone of the D band activated by a double-resonance process while the D–G peak is combination of overtone of the D and G bands [13], [14]. D‘iez-Betriu (2013), studied reduction mechanisms of GO using Raman, proposed that the C peak corresponds to the C–H stretching mode of aromatic C [13]. In this study, the shape and intensity ratio of these three bands looks similar in all samples, thus, no clear effect of protein doping on these bands.

Figure S7 Raman spectra of (a) three different functional surfaces including bare-rGO, BSA-rGO and and rGO-dBSA+EDC/NHS. Deconvoluted details of the spectra of (b) bare-rGO (c) BSA-rGO (d) rGO-dBSA+EDC/NHS.
Selectivity test using QCM-D measurement

The selectivity tests were performed against calf serum presented in Figure S8. The adsorption of three different samples including the negative control calf serum, 50 µg/mL Ab12 in the diluted calf serum and 50 µg/mL Ab12 in PBS, on NC3-functionalised sensing chips were measured in the same environment and time. The results are discussed in the main paper. It is confirmed that the Ab12 antibody can be selectively detected by NC3 in concentrated animal serum.

QCM-D Desorption

A series of desorption studies were conducted to confirm the covalent functionalisation of the NC3 on the functionalised-rGO surfaces. The NC3 immobilisation on rGO, rGO-dBSA and rGO-dBSA+EDC/NHS surfaces coupled with the binding with the Ab12 in serum were monitored using QCM-D, then the desorption was studied by injecting a surface active cleaning agent (Decon 14%) which was flowed over the surface for five minutes before rinsing with PBS to recover the baseline. Figure S9(a) presents baseline recovery occurring on rGO sample after rinsing the functionalisation with Decon 14%. The baseline shifts back from 53 Hz after antibody adsorption to 17 Hz after the rinsing. This means the antibody and partial amounts of NC3 were washed off. Figure S9(b) presents the desorption occurring on the rGO-dBSA surface. After the final rinsing, the signal recovers from 40 Hz to 6 Hz which is the
same level as dBSA adsorption. It could be inferred that any layers above the BSA were removed, with only the dBSA layer remaining. We suggested that the BSA molecules denatured and squeezed on the rGO surface through strong hydrophobic interaction [10]. The shift of baseline observed from the rGO-BSA activated with EDC-NHS (Figure S9(c)) recovers to the level of NC3 confirming the covalent functionalisation of NC3 on the surface. This is the only case where the NC3 cannot be washed off.

In other cases, the NC3 was mostly washed off. As the Decon 14% can remove physically adsorbed molecules but not the covalently attached ones, the shift of the baseline observed from the rGO-BSA activated with EDC-NHS confirms the covalent functionalisation of NC3 on the surface.

11. The C-QCM testing for stability

Our C-QCM system and rGO-coated chips were tested for reproducibility when cycling injection of DI water and PBS buffer on Au and rGO coated QCM chips (figure S10). The frequency shift results for seven cycles were collected presented in Table S1.

12. QCM-D patient results

Regarding QCM-D measurement for the detection of the anti-PLA2R antibody in patient sera, the collinear plot of the final ΔF the final and ΔD value for each specimen is presented with a correlation coefficient ($R^2$) is 0.98. Thus, the ΔD increases linearly with respect to the ΔF.
Table S1 Comparison of frequency shift results between Au and rGO QCM chip

| Cycle | Au (Hz) | rGO (Hz) |
|-------|---------|----------|
|       | DI      | PBS      | DI      | PBS      |
| 1     | 2209    | 2343     | 2120    | 2342     |
| 2     | 2222    | 2339     | 2076    | 2340     |
| 3     | 2232    | 2389     | 2114    | 2387     |
| 4     | 2230    | 2342     | 2088    | 2343     |
| 5     | 2253    | 2370     | 2124    | 2371     |
| 6     | 2231    | 2373     | 2114    | 2372     |
| 7     | 2252    | 2374     | 2110    | 2373     |
| Avg   | 2233    | 2361     | 2107    | 2361     |
| S.D.  | 16      | 20       | 18      | 19       |

Figure S10 QCM results for sequential injection of liquid samples by pipetting DI water and PBS buffer on (a), (b) Au and (c), (d) rGO surfaces, respectively.
References

[1] D. Kanigicherla, J. Gummadova, E. A. McKenzie, S. A. Roberts, S. Harris, M. Nikam, K. Poulton, L. McWilliam, C. D. Short, M. Venning, and P. E. Brenchley, “Anti-PLA2R antibodies measured by ELISA predict long-term outcome in a prevalent population of patients with idiopathic membranous nephropathy,” Kidney Int., vol. 83, no. 5, pp. 940–948, 2013, doi: 10.1038/ki.2012.486.

[2] M. Fresquet, T. A. Jowitt, J. Gummadova, R. Collins, R. O’Cualain, E. A. McKenzie, R. Lennon, and P. E. Brenchley, “Identification of a major epitope recognized by PLA2R autoantibodies in primary membranous nephropathy,” J. Am. Soc. Nephrol., vol. 26, no. 2, pp. 302–313, 2015, doi: 10.1681/ASN.2014050502.

[3] M. Fresquet, T. A. Jowitt, E. A. McKenzie, M. D. Ball, M. J. Randles, R. Lennon, and P. E. Brenchley, “PLA2R binds to the annexin A2-S100A10 complex in human podocytes,” Sci. Rep., vol. 7, no. 1, pp. 1–11, 2017, doi: 10.1038/s41598-017-07028-8.

[4] KDE The Oxygen Team (https://kde.org), “Oxygen15.04.1-computer-laptop.svg.” Free to copy under the GNU LGPLv3 (http://www.gnu.org/copyleft/lesser.html).

[5] G. Sauerbrey, “Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mikrowägung,” Zeitschrift für Phys., vol. 155, no. 2, pp. 206–222, 1959.

[6] I. Reviakine, D. Johannsmann, and R. P. Richter, “Hearing what you cannot see and visualizing what you hear: Interpreting quartz crystal microbalance data from solvated interfaces,” Anal. Chem., vol. 83, no. 23, pp. 8838–8848, 2011, doi: 10.1021/ac201778h.

[7] M. Rodahl, F. Höök, A. Krozer, P. Brzezinski, B. Kasemo, F. H????k, A. Krozer, P. Brzezinski, and B. Kasemo, “Quartz crystal microbalance setup for frequency and Q-factor measurements in gaseous and liquid environments,” Rev. Sci. Instrum., vol. 66, no. 7, pp. 3924–3930, 1995, doi: 10.1063/1.1145396.

[8] M. V. Voinova, M. Rodahl, M. Jonson, and B. Kasemo, “Viscoelastic Acoustic Response of Layered Polymer Films at Fluid-Solid Interfaces: Continuum Mechanics Approach,” Phys. Scr., vol. 59, no. 5, pp. 391–396, 1999, doi: 10.1238/Physica.Regular.059a00391.
[9] M. Edvardsson, M. Rodahl, B. Kasemo, and F. Ho, “A Dual-Frequency QCM-D Setup Operating at Elevated Oscillation Amplitudes,” vol. 77, no. 15, pp. 4918–4926, 2005.

[10] P. Hampitak, D. Melendrez, M. Iliut, M. Fresquet, N. Parsons, B. Spencer, T. A. Jowitt, and A. Vijayaraghavan, “Protein interactions and conformations on graphene-based materials mapped using quartz-crystal microbalance with dissipation monitoring (QCM-D),” Carbon N. Y., vol. 165, pp. 317–327, 2020, doi: 10.1016/j.carbon.2020.04.093.

[11] J. E. Im, J. A. Han, B. K. Kim, J. H. Han, T. S. Park, S. Hwang, S. In Cho, W. Y. Lee, and Y. R. Kim, “Electrochemical detection of estrogen hormone by immobilized estrogen receptor on Au electrode,” Surf. Coatings Technol., vol. 205, no. SUPPL. 1, pp. S275–S278, 2010, doi: 10.1016/j.surfcoat.2010.08.006.

[12] Y. J. Oh, J. J. Yoo, Y. Il Kim, J. K. Yoon, H. N. Yoon, J. H. Kim, and S. Bin Park, “Oxygen functional groups and electrochemical capacitive behavior of incompletely reduced graphene oxides as a thin-film electrode of supercapacitor,” Electrochim. Acta, vol. 116, pp. 118–128, 2014, doi: 10.1016/j.electacta.2013.11.040.

[13] X. Díez-Betriu, S. Álvarez-García, C. Botas, P. Álvarez, J. Sánchez-Marcos, C. Prieto, R. Menéndez, and A. de Andrés, “Raman spectroscopy for the study of reduction mechanisms and optimization of conductivity in graphene oxide thin films,” J. Mater. Chem. C, vol. 1, no. 41, p. 6905, 2013, doi: 10.1039/c3tc31124d.

[14] B. Ma, R. D. Rodriguez, A. Ruban, S. Pavlov, and E. Sheremet, “The correlation between electrical conductivity and second-order Raman modes of laser-reduced graphene oxide,” Phys. Chem. Chem. Phys., vol. 21, no. 19, pp. 10125–10134, 2019, doi: 10.1039/C9CP00093C.