Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente, Ciclo XXXVII

Exploring the Potential of Graphene Field-Effect Transistors In Biosensing For Health And Environment

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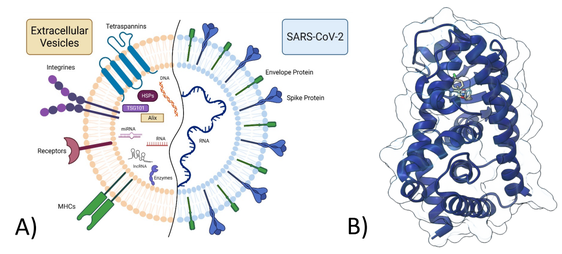
INTRODUCTION

Graphene Field Effect Transistors (GFETs) have emerged promising for biosensing due to their unique properties marked by high sensitivity and fast response times[1]. GFETs are versatile tool to detect molecules of different size, from relatively large entities like Extracellular Vesicles (150 nm) to small molecules such as environmental pollutants[2]. Extracellular Vesicles (EVs), as potential markers for various diseases, have opened a new direction for rapid and non-invasive diagnosis via liquid biopsy. This allows for early disease detection, enabling timely interventions and improving patient outcomes as well as facilitating environmental monitoring to track and address pollution concerns.

AIM OF THE STUDY

This study aims to use a GFET-based biosensor, originally designed for the detection of SARS-CoV-2[3], to track the presence of EVs from liquid biopsies and environmental pollutants in wastewater. By combining the GFET technology with the design of tailored bioreceptors, we intend to provide a valuable tool for early disease diagnosis and pollution monitoring.

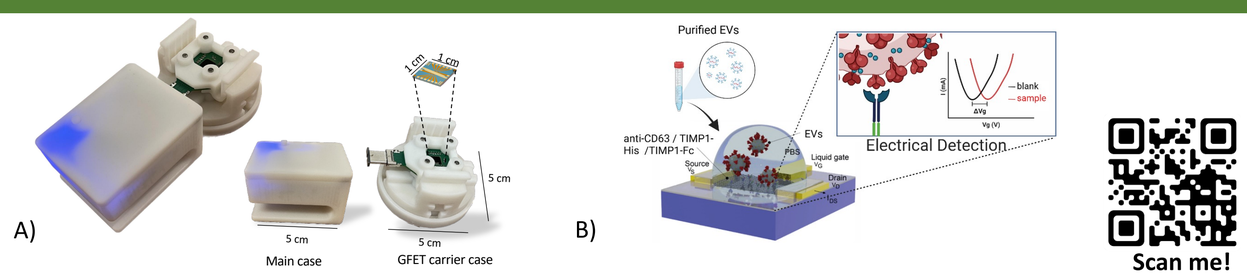


(A) Picture comparing the biological composition of SARS-CoV-2 and Extracellular Vesicles. (B) Illustration of PPAR-γ protein (PDB ID 7WGL) used for the detection of environmental pollutants

RESULTS

ELECTRICAL MEASUREMENTS

Electrical measurements were performed with our device through I_{DS}–V_{GS} curve (Transfer Curves) as electrical metric. Transfer curves were obtained while operating in liquid gating condition, maintaining fixed bias V_{ds} 0.050 V between source and drain electrodes and by sweeping the gate voltage V_g from 0 to 1.5 V. The resulting current I_{ds} were plotted as a function of the gate bias [3-4].



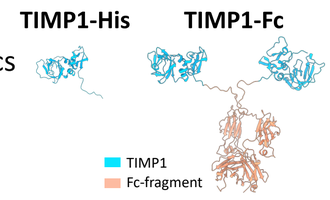
A) Picture of the device used for electrical measurements. B) Schematic illustration of the working principle of GFET

GFET IN HEALTH

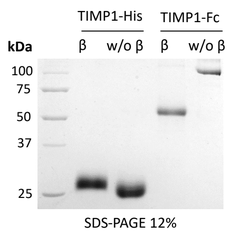
Computational design of the receptor

TIMP metalloproteinase inhibitor 1 (TIMP1) is the cell surface receptor of the EVs marker CD63.

Molecular dynamics simulations using Gromacs was performed.

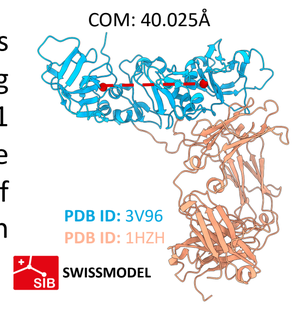


Recombinant proteins were run on SDS-PAGE gel under denaturing (β-mercaptoethanol) or non denaturing conditions (without β-mercaptoethanol).

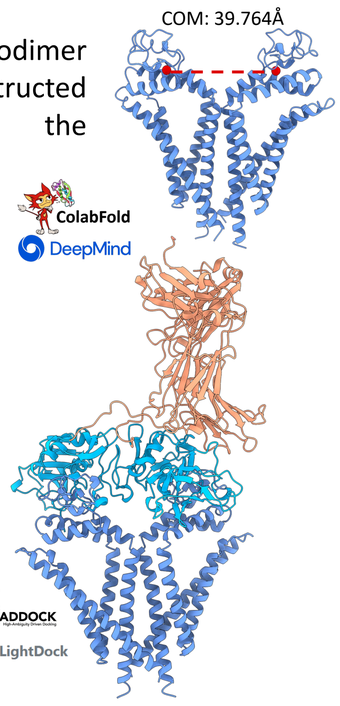


SDS-PAGE 12%

TIMP1-Fc was modelled using two TIMP1 subunits and the Fc fragment of human IgG1 with SwissModel



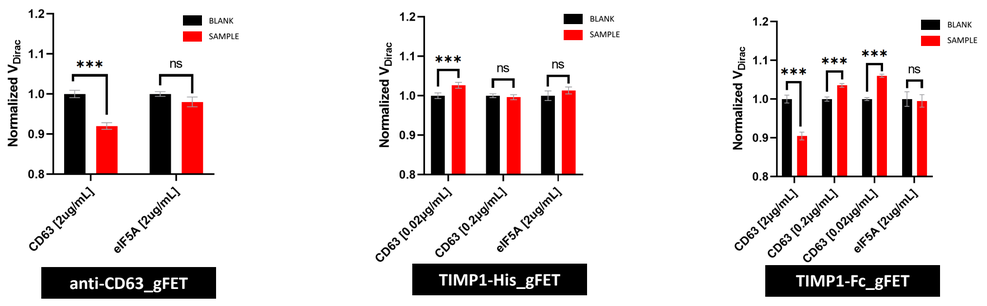
CD63 homodimer was constructed using the AlphaFold2 Multimer



Recombinant protein detection

The graphene layer was functionalized with antibody anti-CD63, TIMP1-His or TIMP1-Fc.

To assess the working of the GFET we firstly tested the device using recombinant protein CD63. eIF5A recominant protein, similar in size to CD63, was used as control.

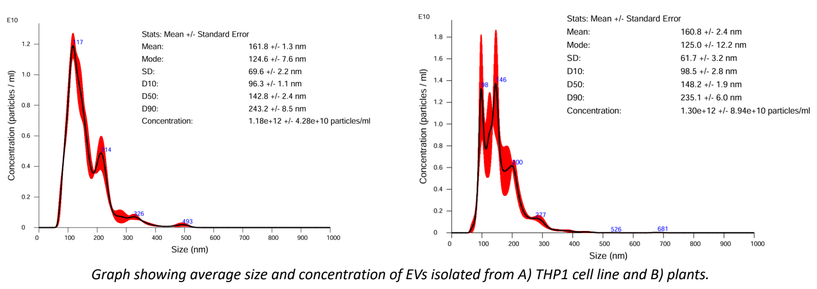


Molecular docking of TIMP1-Fc and CD63 was performed with LightDock and HADDOCK2.4

*Comparative bar charts of GFET before (black bars) and after (red bars) the addition of different concentration of samples (i.e., CD63 and eIF5A) *** p < 0.001, error bars represent standard error of the mean (s.e.m.)*

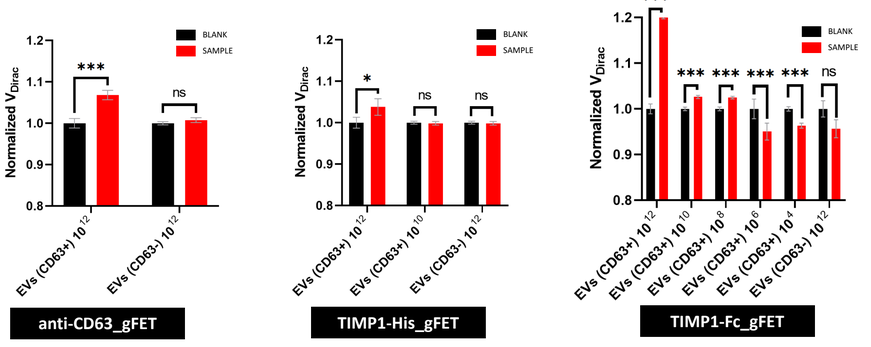
Extracellular Vesicles detection

EVs were isolated from THP1 cell line (CD63+) and plant (CD63-)[5] and quantified with Nanoparticle Tracking Analysis.



Graph showing average size and concentration of EVs isolated from A) THP1 cell line and B) plants.

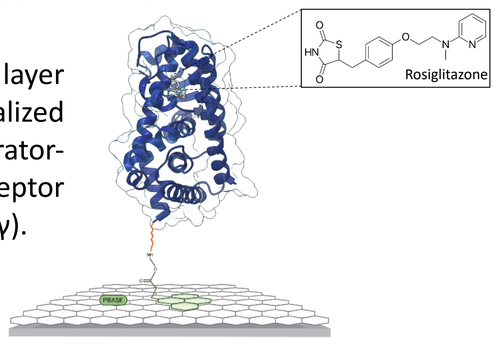
Measurements with isolated EVs were performed with antiCD63-GFET, TIMP1-His_GFET, TIMP1-Fc_GFET.



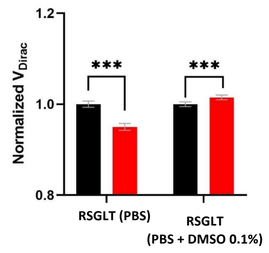
*Comparative bar charts of GFET before (black bars) and after (red bars) the addition of different concentration of samples (i.e., EVs CD63+ and EVs CD63-) *** p < 0.001, ** p < 0,01 error bars represent standard error of the mean (s.e.m.)*

GFET IN ENVIRONMENT

The graphene layer was functionalized with Proliferator-Activated Receptor gamma (PPAR-γ).



Electrical measurements with Rosiglitazone [0.1ug/mL] were performed.



*Comparative bar charts of GFET before (black bars) and after (red bars) the addition of samples. *** p < 0.001, error bars represent standard error of the mean (s.e.m.)*

CONCLUSIONS

Through our experimental evaluations, our biosensor successfully detected particles of varying size and composition, including extracellular vesicles and rosiglitazone. By targeting CD63 we were able to detect both recombinant proteins and extracellular vesicles using three different functionalized GFETs. Furthermore, the use of PPAR-γ allowed us to effectively detect its agonist. Overall, our GFET biosensor represents a significant advancement in biosensing technology, and its ability to detect extracellular vesicles and environmental pollutants highlights its potential for advancing healthcare diagnostics and environmental monitoring.

REFERENCES

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