UNIVERSITÀ POLITECNICA DELLE MARCHE

Dipartimento di Scienze della Vita e dell'Ambiente DISVA

PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVII and XXXVIII cycles)

Name of PhD student: Nunzio Perta

Title of PhD research: Enhancing graphene field-effect transistor biosensors with DNA-mediated protein orientation

Name of PhD supervisor: Prof. Daniele Di Marino
Research lab name: Mollab

Cycle:
[ ] XXXVII
[X] XXXVIII

PhD Curriculum:
[ ] Marine biology and ecology
[X] Biomolecular Sciences
[ ] Civil and environmental protection

DISVA instrumentation labs/infrastructure eventually involved in the project:
[ ] Actea Mobile Laboratory
[ ] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[X] MaSBiC
[X] Simulation/informatics lab
[ ] Other. Please, indicate: .....

ABSTRACT:
Graphene field-effect transistor (gFET) biosensors are crucial tools for screening and quantifying biomarkers. Graphene serves as a two-dimensional semiconductor due to its superior electronic properties. In gFET biosensors, the interaction between an immobilized probe bioreceptor and the analytes is transduced as a change in electrical conductance. The most commonly used functionalization approach is to randomly immobilize the bioreceptor on graphene through linkers. Since probes usually possess defined binding sites, it is advantageous to have well-oriented biomolecules to improve biosensor performance. To address this challenge, we propose a novel approach: using DNA as a linker molecule to orient a protein on graphene. To assess the feasibility of the method and optimize the system, we used molecular dynamics simulations. The enhanced sensitivity of gFET biosensors may pave the way for a quantitative approach to studying the binding kinetics between biomarkers and proteins.

Part 1. Scientific case of the PhD Research

BACKGROUND

Biosensors play a fundamental role in diverse scientific, medical, and industrial domains, enabling the precise and sensitive detection of specific biomolecules. These adaptable devices have transformed fields such as clinical diagnostics, environmental monitoring, and biotechnology. Notably, in recent years, graphene field-effect transistor (gFET) biosensors have emerged as a pioneering technology, offering distinct advantages over traditional biosensors [1]. Central to the power of gFET biosensors is the remarkable electronic performance of graphene, a single layer of carbon atoms arranged in a hexagonal lattice. Its two-dimensional structure confers exceptional charge carrier mobility and electrical conductivity, rendering graphene an ideal platform for biosensors. The smallest fluctuations in electrical conductance can be detected with an extraordinary level of precision [2]. Molecular interactions within these biosensors are transduced into electrical changes, enabling the selective and sensitive detection of analytes. Often, linker molecules, such as 1-pyrenebutyric acid N-hydroxysuccinimide ester (PBASE), are used to facilitate these interactions [3]. Nevertheless, conventional methods involving random binding via linkers may not optimally exploit the specific binding sites of bioreceptors. Random immobilization may not harness the full binding potential of these biomolecules, leading to the need for innovative approaches to enhance biosensor performance [4]. This research is dedicated to advancing gFET biosensors, with a primary focus on the utilization of molecular dynamics (MD) simulations. These simulations serve as an indispensable tool for assessing the stability and alignment of molecular complexes, which is central to this study. The insights gained from these simulations provide a solid foundation for the subsequent experimental work [5]. Finally, advanced characterization techniques, including atomic force microscopy (AFM) and neutron reflectometry (NR), will be employed to evaluate the alignment and performance of these biosensors [6].

SCIENTIFIC AIMS

Our primary goal is to advance the potential of gFET biosensors. This effort centers on exploiting the power of MD simulations to optimize the alignment and performance of bioreceptors on graphene surfaces. By achieving this, we aim to unlock heightened sensitivity and adaptability in biosensors, widening their scope for versatile applications. Through our research, we strive to not only revolutionize the field of biosensors but also provide practical solutions that can address complex scientific challenges.

WORKPLAN AND RESEARCH ACTIVITIES

WP1. Objective. Data and Structure Preparation:

The objective of WP1 is to establish a robust foundation for our research by meticulously preparing and organizing the essential data and structures required for subsequent MD simulations and experimental investigations. This crucial phase encompasses the collection of pertinent three-dimensional (3D) structural data for biomolecules, graphene, and linkers. The gathered data will then be carefully curated and standardized into a GRO format, ensuring uniformity and accuracy. Furthermore, using advanced molecular modeling software such as ChimeraX [7] we aim to create initial molecular structures that serve as the starting point for our simulations.

Methods:

- Collect pertinent 3D structural data on the biomolecules, graphene, and the linkers.
- Organize and clean the data, ensuring it is in a standardized GRO format for further analysis.
- Utilize molecular modeling software ChimeraX to construct initial molecular structures based on available data.
- Verify the structural integrity and correctness of the generated initial structures.

Expected/Obtained Results:

- Curated and organized datasets for the biomolecules, graphene, and the linkers.
- Initial molecular structures suitable for simulations (Figure 1).

WP2. Objective. Coordinate, Topology, and Force Field Files Assembly:

The objective of WP2 is to establish a structural framework for our research, enabling precise modeling and simulations. This phase involves the generation of coordinate files that define the spatial arrangement of our molecular components, including biomolecules and graphene. Additionally, we will create topology files to precisely describe the interconnections, parameters, and interactions within the system. Finally, we will assemble the appropriate force field, specifically CHARMM36, to govern the behavior of our molecular components during simulations.

Methods:

- Generate a GRO coordinate file by assembling all the components into one system.
- Create GROMACS ITP topology files to define the system.
- Assemble the CHARMM36 force field for MD simulations.

Expected/Obtained Results:

- Coherent coordinate (Figure 2), topology, and force field files for simulations.

WP3. Objective. System Preparations and Molecular Dynamic Simulations:

The objective of WP3 is to advance our research by systematically preparing and conducting MD simulations. In this phase, we will construct the complexes comprising biomolecules, and the graphene substrate, simulating different gFET biosensor environments. The addition of an appropriate solvent will replicate physiological conditions. Employing GROMACS [8] software and the CHARMM36 force field, we will execute these MD simulations over defined time scales with specific parameters. The primary goal is to obtain comprehensive insights into the stability and alignment of the biomolecules-graphene complexes.

Methods:

- Develop multiple *in-silico* models for biosensor functionalization, varying the use of distinct DNA linkers and adjusting the number of biomolecules for placement on graphene.
- Employ the GROMACS software alongside the CHARMM36 force field to perform MD simulations.

Expected/Obtained Results:

- Comprehensive MD simulations of diverse graphene-biomolecules complexes.
- Gain valuable insights into the stability and alignment of the biomolecules on the graphene surface.

WP4. Objective. MD Analysis and gFET Biosensor Measurements:

The objective of WP4 is to bridge the gap between computational simulations and experimental measurements in our research. In this phase, we will process the data obtained from MD simulations to comprehensively analyze the behavior of the biomolecules-graphene complexes. Insights garnered from these simulations will be translated into the design and fabrication of gFET biosensors, ensuring the optimal placement of bioreceptors. We will conduct experiments using these biosensors to assess their performance and sensitivity in detecting various analytes. The data generated will be meticulously analyzed and compared with simulation findings to evaluate the effectiveness of the biosensors.

Methods:

- Analyze simulation data to evaluate graphene-biomolecules complexes behavior.
- Integrate findings into gFET biosensors.
- Perform measurements to assess biosensor performance.

Expected/Obtained Results:

- Comprehensive analysis of graphene-biomolecules complexes behavior.
- A refined and optimized DNA-linker strategy for achieving the alignment of bioreceptors on graphene.
- Integration of insights to enhance gFET biosensor performance.

WP5. Objective. Experimental System Characterization:

The objective of WP5 is to thoroughly characterize the experimental system of our gFET biosensors. In this phase, we will employ advanced characterization techniques such as AFM and Neutron Reflectometry NR to assess the alignment and performance of the biosensors. AFM will enable us to visualize the topography and alignment of the bioreceptor layer on the graphene surface with high resolution. On the other hand, NR, a diffraction technique, will be used to determine the composition of the bioreceptor layer with exceptional sensitivity. By comparing simulation results with experimental data, we aim to confirm the enhanced performance and sensitivity of the gFET biosensors, offering a tailored and efficient strategy for biosensor customization.

Methods:

- Characterize the gFET biosensor system using techniques like AFM and NR.
- Examine the alignment and performance of the biosensor.
- Correlate computational and experimental data.

Expected/Obtained Results:

- Detailed experimental characterization of gFET biosensors.
- Correlation between computational insights and experimental performance.
- Comprehensive validation data demonstrating the improved performance of gFET biosensors with the optimized DNA-linker approach.

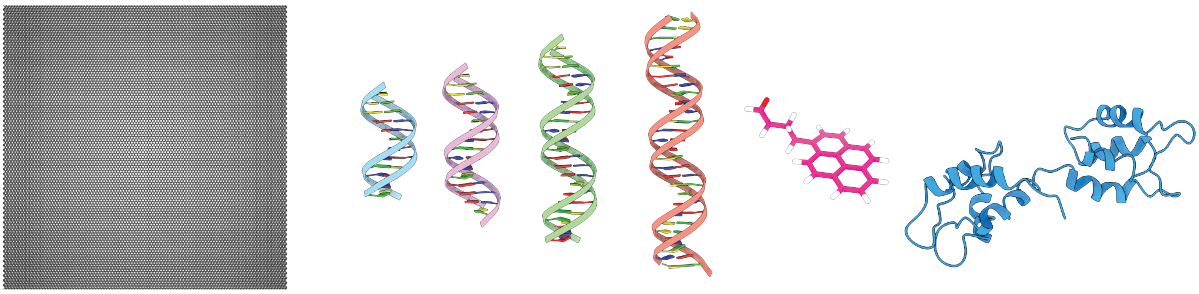

Figure 1. Initial molecular structures for initiating MD simulations. From left to right: graphene, varying sizes and compositions of DNA, the PBASE linker, and a protein (calmodulin, PDB ID: 5TP5).

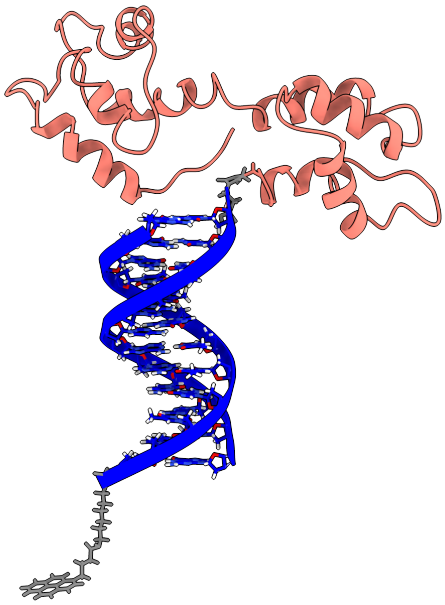

Figure 2. Individual Biomolecule Structure for Placement on Graphene to Mimic Biosensor Functionalization.

REFERENCES

[1] Béraud, A., et al. (2021). Graphene field-effect transistors as bioanalytical sensors: Design, operation and performance. Analyst, 146(2), 403-428.
[2] Rexha, J., et al. (2023). Unlocking the Potential of Field Effect Transistor (FET) Biosensors: A Perspective on Methodological Advances in Computational and Molecular Biology. Advanced Sensor Research, 2300053.
[3] Mishyn, V., et al. (2022). The holy grail of pyrene-based surface ligands on the sensitivity of graphene-based field effect transistors. Sensors & Diagnostics, 1(2), 235-244.
[4] Trilling, A. K., et al. (2013). The effect of uniform capture molecule orientation on biosensor sensitivity: Dependence on analyte properties. Biosensors and Bioelectronics, 40(1), 219-226.
[5] Romagnoli, A., et al. (2023). SARS-CoV-2 multi-variant rapid detector based on graphene transistor functionalized with an engineered dimeric ACE2 receptor. Nano Today, 48, 101729.
[6] Xu, H., et al. (2006). Orientation of a monoclonal antibody adsorbed at the solid/solution interface: a combined study using atomic force microscopy and neutron reflectivity. Langmuir, 22(14), 6313-6320.
[7] Pettersen, E. F., et al. (2021). UCSF ChimeraX: Structure visualization for researchers, educators, and developers. Protein Science, 30(1), 70-82.
[8] Abraham, M. J., et al. (2015). GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX, 1, 19-25.

Part 2. PhD student information on the overall year activity

List of attended courses

1. General courses for doctorate students: Design of research: European projects – Prof. Nicola Paone – Date: 12/01/2023-26/01/2023.
2. Swiss Institute of Bioinformatics: First Steps with R in Life Sciences – Practical course with final exam, the equivalent of 0.5 ECTS credit – Date: 02-03/02/2023.
3. ELIXIR-IIB; FABIT; SIB: 24th Bologna Winter School: Bioinformatics and Deep learning for biodata analysis – Online course – Date: 14-27/02/2023.
4. General courses for doctorate students: Technology transfer and innovation – Prof. Donato Iacobucci – Date: 01/03/2023-29/03/2023.
5. Computer Science courses for doctorate students: Introduction to the LaTeX environment for writing scientific papers – Prof. Francesco Spinozzi – Date: 26-29/06/2023.

List of attended seminars

1. DiSVA seminars – Mitochondria in health and disease – Speaker: Prof. Steen Larsen – Date: 04/04/2023.
2. A shot of science: DiSVA seminars – Role of organic molecules as metal ions chelators and as antioxidants for the treatment of human diseases – Speaker: Alessandra Gilda Ritacca – Date: 09/05/2023.
3. A shot of science: DiSVA seminars – Late but good: genomic and plastic signals of adaptive mismatch in a long-lived sub-Antarctic species – Speaker: Flávia Akemi Nitta Fernandes – Date: 20/05/2023.
4. A shot of science: DiSVA seminars – Exploring the Potential of Graphene Field-Effect Transistors in Biosensing for Health and Environment – Speaker: Jesmina Rexha – Date: 11/07/2023.

List of attended schools

1. EMBL: Whole transcriptome data analysis – Flash Talk: Computational Approaches in Biology – Date: 21-26/05/2023.

List of periods spent abroad

None.

List of conferences/workshops attended and of contributions eventually presented

1. Biophysics@Rome Conference: On the path to sustainability – Date: 19-20/04/2023 – Participation with Posters and Art:
   - Poster 1 (First and Presenting Author): Enhancing gFET biosensor sensitivity with DNA linkers: a computational approach for bioreceptor orientation
   - Poster 2 (Co-Author): gFET for extracellular vesicles detection: a new rapid tool for cancer diagnosis
   - Art (First and Presenting Author): Lunar Enigma: Alien Architecture
2. Structured Based Drug Design conference (SBDD) – Date: 02-04/05/2023 – Participation with a Poster:
   - Poster (First and Presenting Author): Computational Approaches to Enhance gFET Biosensor Sensitivity
3. DiSVA PhD Week – Date: 12-16/05/2023 – Participation with a Poster:
   - Poster (First and Presenting Author): Computational Approaches to Enhance gFET Biosensor Sensitivity
4. Third DiSVA-MASBIC Annual Symposium – Date: 20-22/09/2023 – Participation with Posters:
   - Poster 1 (First and Presenting Author): Computational Approaches to Enhance gFET Biosensors Sensitivity
   - Poster 2 (Co-Author): Novel integrated approach for CoQ binding proteins identification
   - Poster 3 (Co-Author): Exploring Zebrafish Odorant Receptors: A Pilot Expression Screening in Insect Cells
   - Poster 4 (Co-Author): Exploring the Potential of Graphene Field-Effect Transistors in Biosensing for Health and Environment

Part 3. PhD student information on publications

List of publications on international journals

J1. Pirri F, Ometto L, Fuselli S, Fernandes FA, Ancona L, Perta N, Di Marino D, Le Bohec C, Zane L, Trucchi E. Selection-driven adaptation to the extreme Antarctic environment in the Emperor penguin. Heredity. 2022 Dec;129(6):317-26. https://doi.org/10.1038/s41437-022-00564-8

J2. Rexha J, Perta N, Roscioni A, Motta S, La Teana A, Maragliano L, Romagnoli A, Di Marino D. Unlocking the Potential of Field Effect Transistor (FET) Biosensors: A Perspective on Methodological Advances in Computational and Molecular Biology. Advanced Sensor Research. 2023:2300053. https://doi.org/10.1002/adsr.202300053

J3. Vitaliti A, Roccatani I, Iorio E, Perta N, Gismondi A, Chirico M, Pisanu ME, Di Marino D, Canini A, De Luca A, Rossi L. AKT-driven epithelial-mesenchymal transition is affected by copper bioavailability in HER2 negative breast cancer cells via a LOXL2-independent mechanism. Cellular Oncology. 2023 Feb;46(1):93-115. https://doi.org/10.1007/s13402-022-00738-w

J4. Romagnoli A, D'Agostino M, Pavoni E, Ardiccioni C, Motta S, Crippa P, Biagetti G, Notarstefano V, Rexha J, Perta N, Barocci S. SARS-CoV-2 multi-variant rapid detector based on graphene transistor functionalized with an engineered dimeric ACE2 receptor. Nano Today. 2023 Feb 1;48:101729. https://doi.org/10.1016/j.nantod.2022.101729

J5. Perta N, Torrieri Di Tullio L, Cugini E, Fattibene P, Borromeo I, Forni C, Rapanotti MC, Malaspina P, Cacciamani T, Di Marino D, Rossi L, De Luca A. Hydroxytyrosol counteracts triple negative breast cancer cells dissemination via its copper complexing properties (submitted).

List of publications on conference proceedings

None

List of other publications (books, book chapters, patents)

B1. Winners of the "E Se Funzionasse, edizione 2022" contest – Engaged in a dynamic 6-month incubation program at the prestigious startup incubator, BP Cube – Project name: Graphene Biosensor for health care applications.

15/10/2023

Student signature

Supervisor signature