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: Agnese Roscioni

Title of PhD research: Computational Study of Gain of Function Point Mutations in a Potassium Channel

Name of PhD supervisor: Prof. Luca Maragliano
Research lab name: Modeling and Physiology Laboratory

Cycle:
[ ] XXXVI
[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
[ ] MaSBiC
[x] Simulation/informatics lab
[ ] Other. Please, indicate: .....

ABSTRACT (1000 characters, including spaces):

Kv7.2/3 channels are responsible for the generation of the M-current, which plays a critical role in neuronal excitability. While most mutations in the Kv7.2 pore reduce the M-current, a new class of Gain-of-Function (GoF) variants, affecting residues near the intracellular side of the inner gate (IG) of the pore, is being currently described. In this project, the impact of three IG mutations on the structure of the wild-type (WT) Kv7.2 protein is investigated using all-atom molecular dynamics (MD) simulations on the microsecond timescale, analyzing both closed and open channel configurations. All the examined mutations induced a widening of the gate of the closed configuration with respect to the WT protein, consistent with a GoF effect revealed by electrophysiological experiments, while they showed no effect on the open conformation. In conclusion, the structural characterization paves the way to understand the molecular mechanisms underlying the different phenotypes.

Part 1. Scientific case of the PhD Research (2 to 3 pages, including figures)

BACKGROUND

Potassium channels are among the most diverse classes of ion channels, absolving a variety of key regulatory functions in the human body. In particular, the KCNQ genes encode four voltage-gated potassium channels (named Kv7.2 to Kv7.5), that are expressed in neuronal tissues [1]. Among these proteins, Kv7.2 and Kv7.3 are the main components of a slow activating, non-inactivating potassium flux named M-current, which contributes to stabilize the membrane potential of excitable cells in presence of depolarizing currents. These channels activate at subthreshold potentials through the opening of the inner gate (IG) [2], and unlike other potassium channels, such as for example Kv7.2 and Kv7.3, do not inactivate, thus maintaining a conductive configuration of the selectivity filter (SF) as long as the membrane is depolarized. The atomic structure of Kv7.2 is shown in Figure 1.

Due to their fundamental regulatory role in neuronal excitability, genetic defects in Kv7.2/3 altering the physiological function of these channels are linked to the emergence of several neurological disorders [2,3]. Remarkably, from the large number of pathological mutations documented in the context of the RIKEE project (https://www.rikee.org), a significant prevalence of genetic defects of Kv7.2 is observed, thus indicating this protein as an important target for drugs. Typically, these mutations are located at the level of the Selectivity Filter (SF) (Fig.1), where they induce an inactivation of the channel and the reduction of the M-current, causing the occurrence of epileptic seizures and neurodevelopmental disorders of various severity [4]. However, an alternative class of pathological mutations eliciting (GoF) effects has recently emerged. Some of these modifications, localized at the Inner Gate (IG) (Fig.1), have been found to increase the single-channel opening probability without affecting the membrane subunit abundance or the single-channel conductance [5]. This variable modulation of the channel activity induced by mutations suggests that the design of drugs for the treatment of the KCNQ2-related illness requires a detailed knowledge of their pathophysiological characteristics, in order to mitigate symptoms and restore healthy conditions.

In this context, the group of our collaborators at the "Università Federico II" of Naples, lead by Prof. Maurizio Taglialatela, studied experimentally three Kv7.2 point mutations correlated to GoF phenotypes.

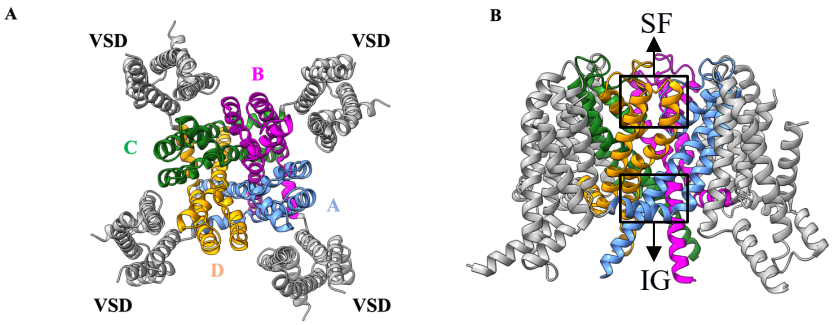

Figure 1. *Three-dimensional structure of the Kv7.2 channel (PDB ID: 7CR0). Apical (A) and lateral (B) views of the transmembrane domain. The pore helices of distinct chains are colored differently. Voltage sensors domains are colored in gray.*

SCIENTIFIC AIMS

In this PhD project, we aim to use *in silico* techniques to investigate the effect of three Kv7.2 pathogenic mutations at the channel's IG. In order to dissect the molecular basis of the experimentally observed GoF effects, we will inspect the structural and functional properties of the mutated channels and compare them to the WT one.

To understand the effect of the mutations on both closed and open channel structure, we will use a multi-state model of Kv7.2. Extended all-atom MD simulations at the microsecond scale will be performed using different force fields. We define two major goals: to understand the impact of the mutations on the structure of the protein, with a particular focus on the functionally relevant IG and SF regions, and to investigate how these effects are determined by the use of distinct simulation codes and Force Fields (FFs). The second goal stems from recent computational works that highlighted different structural properties of SFs depending on the FFs used [6,7].

- WORKPLAN AND RESEARCH ACTIVITIES

WP 1. Structural characterization of three Kv7.2 GoF variants.

Methods. We will simulate eight different systems: the pore region of the Wild Type (WT) protein and of the three mutants (here indicated as MUT1, MUT2 and MUT3), each in both closed and open configurations. For the closed conformations we used the cryo-EM structure of Kv7.2 (PDBID: 7CR0) [8], which shows an constricted IG, while for the open one we relied on homology modeling, using the cryo-EM open IG structure of Kv7.1 (PDB ID 6V01) [9]. For each system, we are performing five replicas of 500ns-long MD simulations using the NAMD code [10] and the Charmm36 FF [11]. Each system is composed by the protein's pore domain embedded in a POPC lipid bilayer solvated with water and a 150mM KCl^- ionic bath. The analysis performed on the simulations were based on the Samson's channel annotation approach [12] that correlates pore hydratation and channel conductance.

Expected/Obtained Results. Up to now we simulated three of the four closed channel systems, and what emerged from the analysis is that the first two mutations cause a widening of the channel pore, as can be seen from the profiles of the pore radii measured using the HOLE software (Fig2, B,E). This widening is correlated with an hydratation of the central region of the pore (Fig2, C,F), that may cause passage of ions also in the closed channel configuration. During the next months, additional simulations will be performed by applying an electric field to accelerate ion permeation and evaluate how this is affected by the mutations.

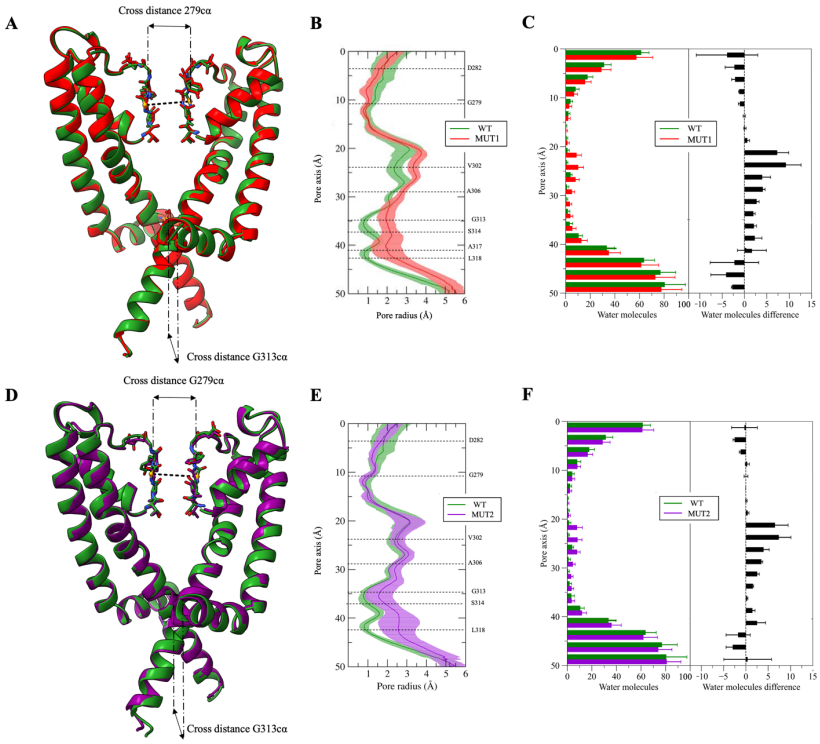

Figure 2. (A,D) Superposition of the equilibrated structures of WT (green), MUT1 (red) and MUT2 (purple) Kv7.2, all starting from a closed IG. (B,E) Channel radius profile calculated by HOLE along the pore axis of the simulated K_V7.2 WT (green) and of MUT1 (red) and MUT2 (purple). Average profiles over the five replicas are shown with solid lines, while standard deviations as bands. (C,F) Number of water molecules along the channel axis. The same color legend of the previous panels is adopted.

WP 2. Objective. Conformational heterogeneity of the SF in WT and mutant channels, and effect of different FFs.

Methods. The analysis is performed by comparing results from the simulations described in WP1, performed using the NAMD code [10], with eight other simulations of the same systems made using the GROMACS code [13]. Eight different systems were prepared: the WT protein (PDBID: 7CR0 [8]) considering only the pore region and the three GoF mutation of the inner gate, every system both with Chamm36m [14] and Amber

FF [15]. For this other set of simulations, we are performing one replica of 2μs for each system, using GROMACS code [13] and Charmm36m [14] and Amber14SB Force Filed (FF) [15]. Each system is composed by the pore region of the protein embedded in a POPC lipid bilayer hydrated with water and KCl^- ionic bath with a concentration of 150mM. Preliminary analysis regarding the SF conformations was performed by monitoring the time evolution of the cross-distances between the Cα atoms of residues G279 from diagonally opposed chains (Fig.2 A). In addition, we also monitor the degree of opening of the IG by reporting the time evolution of the G313 Cαs cross-distances (Fig.2 D).

Expected/Obtained Results. From the preliminary data obtained from the NAMD simulations, the mutation on the IG seems to stabilize a more open, conductive state of the selectivity filter (Fig. 3), consistent with the experimentally measured GoF effect.

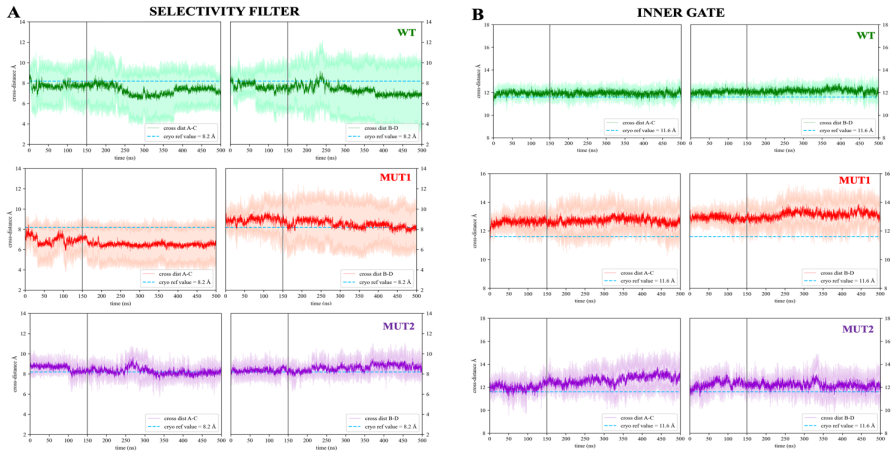

Figure 3. Average cross-distances from 5 simulated replicas, at the selectivity filter (A) and the the inner gate (B). The WT system's data are in green, while MUT1 and MUT2 in red and purple, respectively. The dashed blue line represents the value of the same distance in the cryo-EM structure.

- REFERENCES

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Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools

1. Bologna Winter school, 24th edition "Bioinformatics and Deep learning for biodata analysis" Dates: 14th - 27th February, 2023

2. Leonardo HPC course: "Introduction to Leonardo supercomputer for Eurofusion". Date: 6th June, 2023

3. Seminar of A shot of Science cicle: "Diatom frustule as a resource for innovative applications" Speaker: Alessandra Petrucciani. Date: 8th November 2023

4. Seminar of A shot of Science cicle: "Inhibiting cancer growth by targeting translational factors eIF4E and eIF5A" Speaker: Cristina Maracci. Date: 29th November 2023

5. Seminar of A shot of Science cicle: "Role of organic molecules as metal ions chelators and as antioxidants for the treatment of human diseases" Speaker: Alessandra Gilda Ritacca. Date: 9th May 2022

6. Seminar of A shot of Science cicle: "Exploring the Potential of Graphene Field-Effect Transistors in Biosensing for Health and Environment" Speaker: Jesmina Rexha. Date: 11th July 2023

List of conferences/workshops attended and of contributions eventually presented

1. Conference: "Computational Advances in Drug Discovery", Sestri Levante 2-4 May 2023 – poster presentation, title " Studying the binding of eIF4E with ligands *in silico* to obtain information for the developing of 4EGI-1 analogs"
2. Conference: "2nd Kv7 channel symposium", Napoli 13th-15th September 2023 – poster presentation, title "Structural consequences of gain-of-function mutations at the inner gate of kv7.2 subunits investigated by all-atom molecular dynamics simulations"

3. Third MaSBiC Symposium "Advances in Protein Science: Exploring Structure, Function, and Beyond" Ancona 20th – 22nd September 2023 – poster presentation, title "Structural consequences of gain-of-function mutations at the inner gate of kv7.2 subunits investigated by all-atom molecular dynamics simulations"

Part 3. PhD student information on publications

List of publications on international journals

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

13/10/2023

Student signature

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Luca Maragliano