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

Computational Study of Gain of Function Point Mutations in a Potassium Channel

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AIM OF THE PROJECT

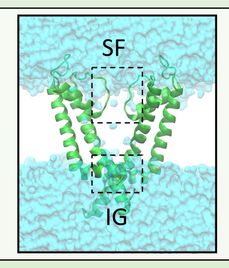
Characterization of three de novo gain-of-function mutations (GoF) of the Kv7.2 potassium channel to develop strategies to treat them.

OUTCOMES

In silico analysis underlines the channel's enhanced hydratation and the widening of the two characteristic constrictions of the Kv7.2 Inner Gate (IG, i.e. S314 and L318). Finally, a mechanicistic explanation of the phenomenon is suggested.

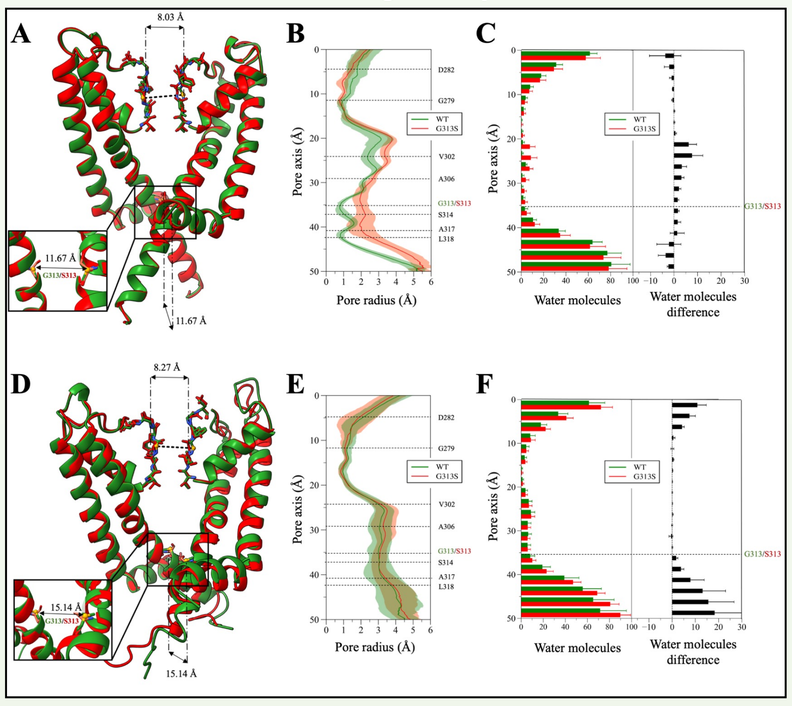
STATE OF THE ART

Kv7.2/3 channels, encoded by members of the KCNQ gene family, are responsible for the generation of the M-current, a slowly activating and deactivating potassium conductance that plays a critical role in neuronal excitability[1]. Variants in the KCNQ2 gene have been linked to phenotypically heterogeneous neurological diseases, making this protein a prime candidate for drug development[2]. A new class of GoF variants, affecting residues near the intracellular side of the inner gate (IG) of the pore, have been recently identified. Here, we investigate the impact of three IG mutations (G313S, A317T, L318V) on the structure of the wild-type (WT) Kv7.2 protein using all-atom molecular dynamics (MD) simulations on the microsecond timescale.



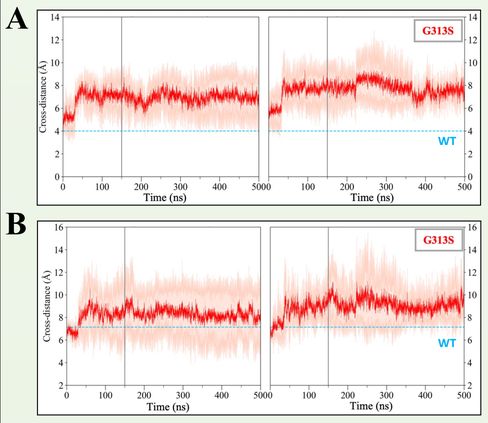
G313S

Pore widening and hydration



(A) Superposition of WT (green) and G313S (red) representative Kv7.2 closed structures after equilibration and before MD production simulations. **(B)** Channel radius profiles along the pore axis of the two proteins, averaged over all simulated replicas. Shaded regions indicate standard deviations. **(C)** Distribution of water molecules along the channel axis. Average and standard deviations are calculated over all replicas. (**D-F)** Same as **(A-C),** but for the open structures.

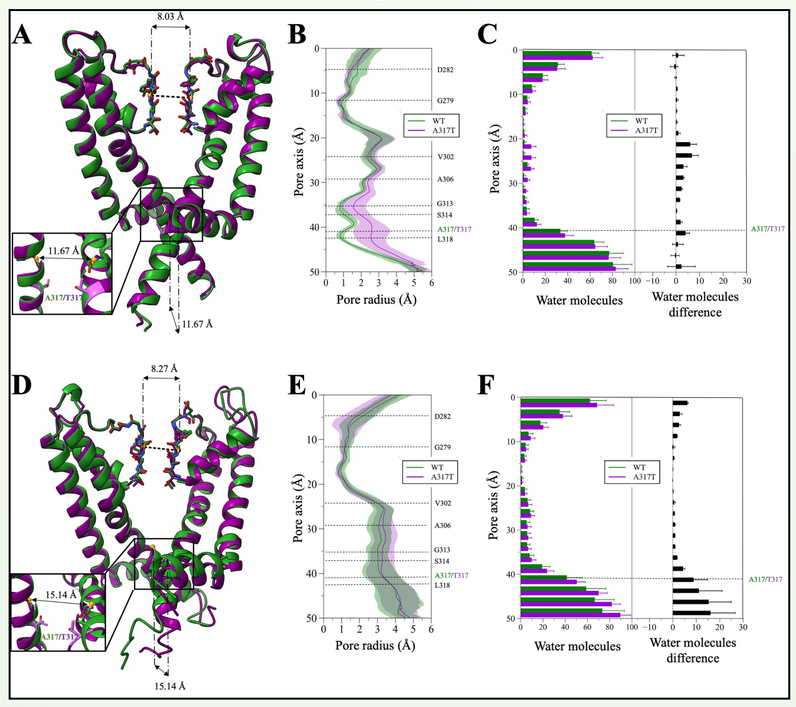
Closed IG S314 and L318 cross-distances



Time evolution (from the cumulative MD simulations) of the average S314 **(A)** and L318V **(B)** cross distances (darker solid line) and standard deviation (shaded areas). Reference value (S314=4.00 Å, L318V=7.14 Å) in dashed cyan line, measured in the starting WT structure (PDB ID: 7CR0) [4]

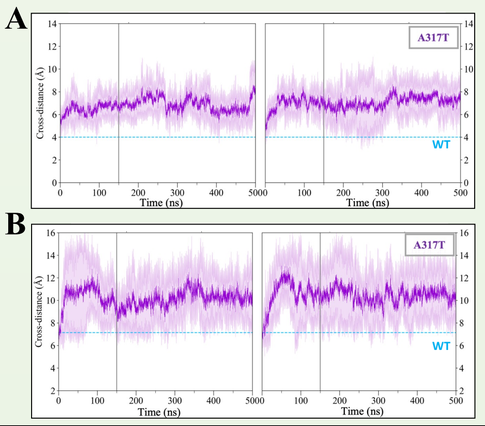
A317T

Pore widening and hydration



(A) Superposition of WT (green) and A317T (purple) representative Kv7.2 closed structures after equilibration and before MD production simulations. **(B)** Channel radius profiles along the pore axis of the two proteins, averaged over all simulated replicas. Shaded regions indicate standard deviations. **(C)** Distribution of water molecules along the channel axis. Average and standard deviations are calculated over all replicas. (**D-F)**. Same as **(A-C),** but for the open structures.

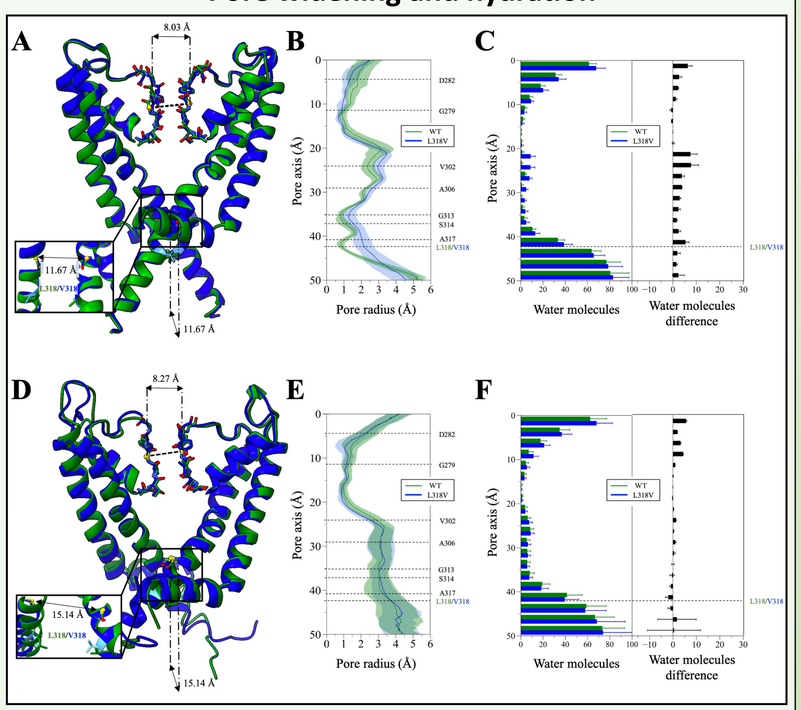
Closed IG S314 and L318 cross-distances



Time evolution (from the cumulative MD simulations) of the average S314 **(A)** and L318V **(B)** cross distances (darker solid line) and standard deviation (shaded areas). Reference value (S314=4.00 Å, L318V=7.14 Å) in dashed cyan line, measured in the starting WT structure (PDB ID: 7CR0) [4]

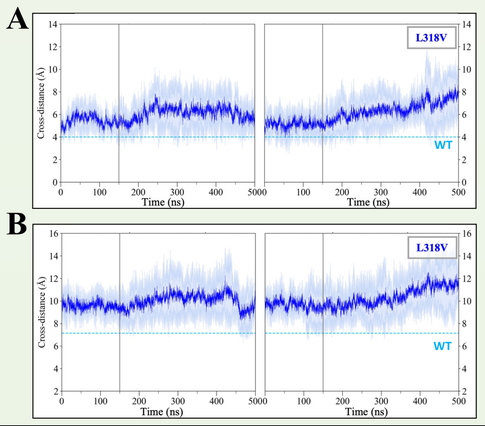
L318V

Pore widening and hydration



(A) Superposition of WT (green) and L318V (blue) representative Kv7.2 closed structures after equilibration and before MD production simulations. **(B)** Channel radius profiles along the pore axis of the two proteins, averaged over all simulated replicas. Shaded regions indicate standard deviations. **(C)** Distribution of water molecules along the channel axis. Average and standard deviations are calculated over all replicas. (**D-F)** Same as **(A-C),** but for the open structures.

Closed IG S314 and L318 cross-distances



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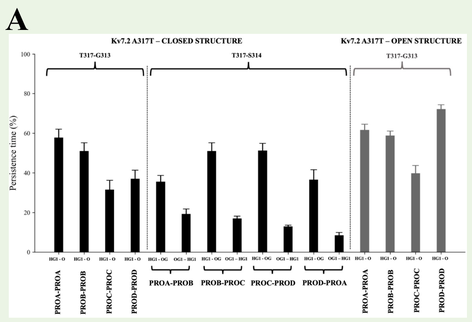
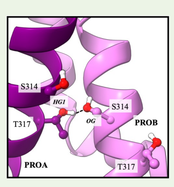
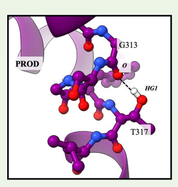
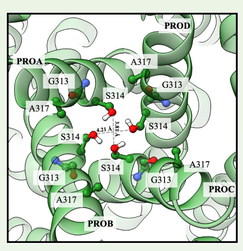
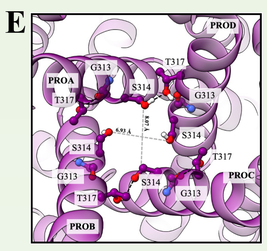
MECHANISTIC INSIGHTS

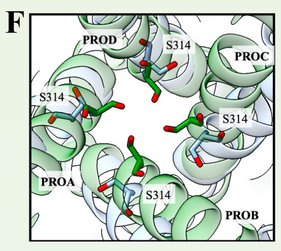


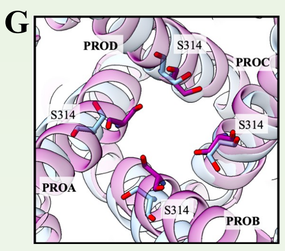






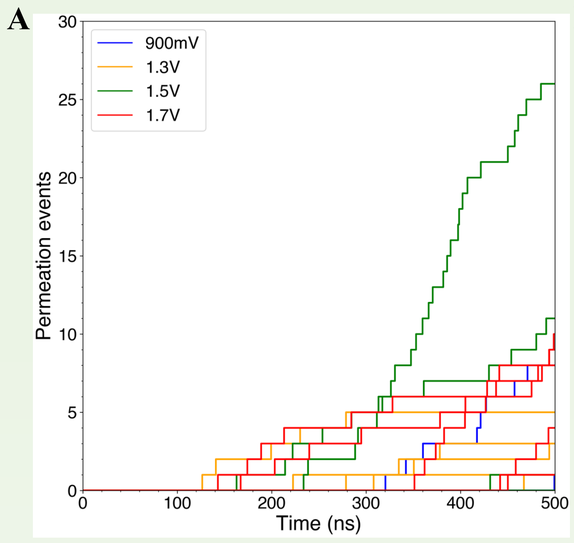






Hydrogen bond analysis performed on A317T variant **(A)** reveal the formation of a two hydrogen bonds. One between T317 and S314 (B) and the other between T317 and G313 **(C)**. The former bond move the lateral chain of the S314 from the center of the pore to the adjacent chain **(D,E)**. Similar orientation of the lateral chains can be observed in the Kv7.2 bound to the activator ebio1[5] **(E,G)**. The WT is represented in green the A317T in purple, and KV7.2 bound to ebio1 (PDB ID: 8IJK) in light blue

CURRENT EVALUATION



Voltage	Average conductance (pS)
900mV	0.65
1.3V	0.65
1.5V	2.05
1.7V	1.33

Ion permeation events were calculated for the A317T mutant for different applied voltages. The count of five 500ns indipendent replicas per voltage is shown in **(A)**, each voltage has a different colour (900mV blue, 1.3V yellow, 1.5V green, 1.7V red). In **(B)** is possibile to observe the average conductance.

METHODS

MD simulations were performed starting from the Cryo-EM structure PDB ID: 7CR0[4] for closed structure and an homology modeled structure for the open conformation (template PDBID: 6V01)[6]. MD simulations were performed using NAMD code, Charmm36m force field. 5 replicas of 500ns were perfomed for each systems (WT + three mutants, open and closed conformations). Analysis were performed using VMD with in house script.

REFERENCES

1.T. J. Jentsch, Nat. Rev. Neurosci. 2000, 1, 21.
2.Miceli, Francesco, et al. GeneReviews 2022.
3.Nappi, Mario, et al. Proceedings of the National Academy of Sciences 119.15 2022
4.Li, Xiaoxiao, et al. *Cell Research 31.3*, 2021: 52-61.
5.Zhang, Shaoying, et al. *Nature Chemical Biology*, 2024: 1-10.
6.Sun, Ji, and Roderick MacKinnon. *Cell* 180.2, 2020: 340-347.

Paper available on Biorxiv →