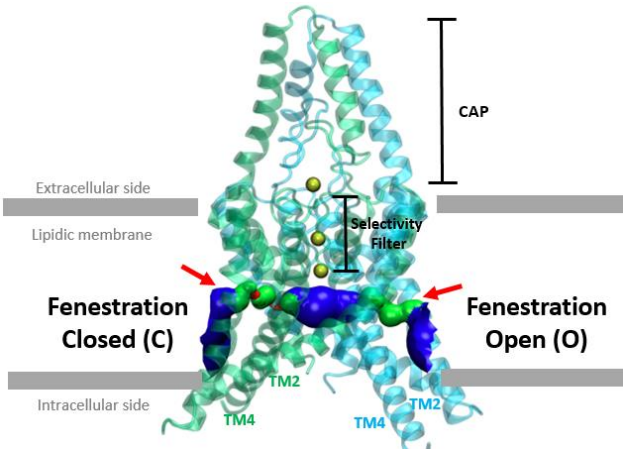
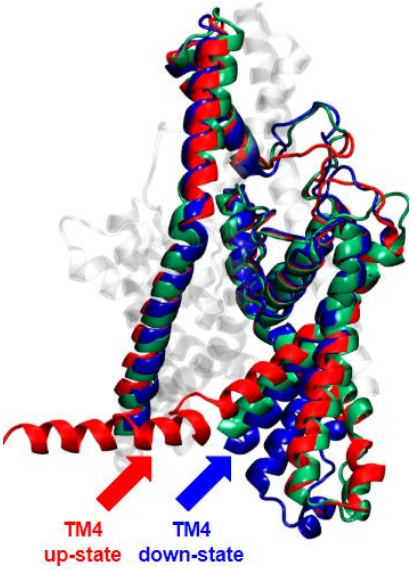
MOL2NET, International Conference Series on Multidisciplinary Sciences

Elucidating the role of the intracellular pH sensing mechanism of TASK-2 K_2P channel

Bustos Daniel[a], Sepúlveda Francisco[b], Concha Guierdy[c], Zúñiga Leandro[c], González Wendy[a].

[a]: Center for Bioinformatics and Molecular Simulations, Universidad de Talca, Chile.
[b]: Centro de Estudios Científicos, Valdivia, Chile.
[c]: Centro de Investigaciones Médicas, Escuela de Medicina, Universidad de Talca, Chile.





Abstract.

Two-pore domain potassium (K_2P) channels are responsible for maintaining the background conductance essential to the resting membrane potential[1]. K_2P channels assemble as dimers containing two pore-forming domains and four transmembrane segments per subunits. Two fenestrations connect the lipid membrane with the central conduction cavity, which can be open or closed depending of the movements of helix TM4[2]. TALK subfamily of K_2P channels is activated by alkaline extracellular pH and is formed by 3 members: TALK-1, TALK-2 and TASK-2. TASK-2 is also gated by intracellular pH (pH_i), being closed by intracellular acidification and activated by increasing pH_i. The neutralization of lysine positioned at the end of TM4 helix, and probably within the fenestrations, by a mutation to K245A abolishes pH_i-gating[3]. The molecular mechanism by which pH_i-sensing K245 exerts its gating role is unknown. A possible mechanism suggest that K245 protonated is able to open the fenestrations and therefore close the channel[4]. Through computational studies, we modeled the 3D structure of TASK-2 channel in both fenestration states, these models were used as a starting point to perform molecular dynamics simulations. The trajectories analysis reveals a good agreement between the $pK_{1/2}$ of K245 obtained experimentally and the pK_a predicted

	when the fenestrations are closed. Besides, we proved that Norfluoxetine compound is a potent blocker of TASK-2 channels and its putative binding site is within the fenestrations (data not shown).

Introduction

Two-pore domain potassium (K2P) channels take part in stabilize the negative resting membrane potential in excitable cells. To date, 15 mammalian genes codifying K_2P channels have been identified, which are classified into 6 subfamilies[1]: TWIK, THIK, TRAAK, TRESK, TASK and TALK. Each K2P channel subunit contains two pore forming domains and four transmembrane segments (TM1-TM4) and they assembly functionally as dimers. Two unusual openings called fenestrations were discovered in crystallographic structures, which connect the lipid membrane with the central conduction cavity of K2P channels. The elucidation of TRAAK channel crystallographic structures by Brohawn[2] et al, in 2014, proves that the fenestrations can be in open or closed state by means of the movements of TM4 helix in *down* or *up* state, respectively. Likewise, Brohawn[3] et al. has postulated that the fenestrations closed corresponds to the conductive state of the channel and the fenestrations open, with lipids protruding from the fenestration[3,4] into the central cavity below to the selectivity filter, corresponds to the non-conductive state of the channel. Moreover, Dong et al. reported the structure of TREK-2 channel co-crystalized with the inhibitor Norfluoxetine (NFx, the active metabolite of Prozac), which is located inside of the fenestrations when these are in the open state. TASK-2 channel from TALK subfamily can be open by intracellular alkalinization. The mutation of a lysine residue positioned at the end of TM4 helix (K245) to K245A abolish gating by intracellular pH[5] (pH_i). Based in a comparative model of TASK-2, Niemeyer[6] et al. in 2016, has proposed an atomistic explanation about the K245 pH_i sensor due to the proximity of K245 to these hydrophobic fenestrations: "the protonated state of K245 ($K245^+$) within of the fenestration promotes their opening and therefore the closure of the TASK-2 channel". Through the Niemeyer's hypothesis is suggested the presence of an inner gate in TASK-2, which could be related with the state of the fenestrations. However, in TASK-2 channel, the inner gate has not been investigated directly, mainly due to a lack of high-affinity TASK-2 blockers that binds within the fenestrations.

Materials and Methods

Homology Modelling:

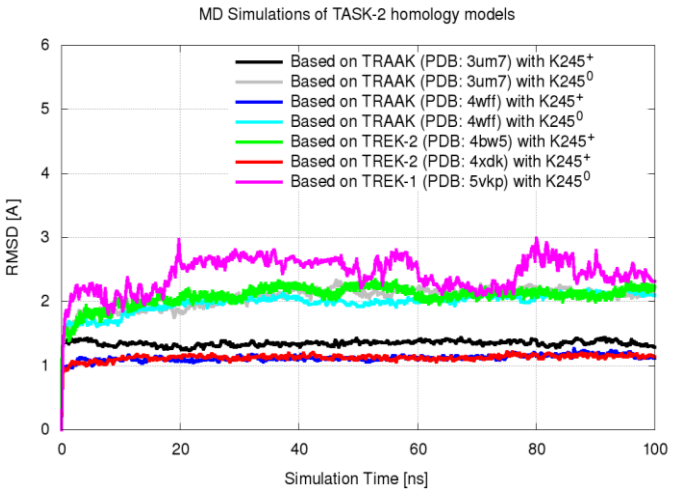
- The complete sequence of human TASK-2 was downloaded from Uniprot (ID: O95279).
- With the aim to sample both conformational states of the fenestrations in TASK-2 channel, 5 templates were selected: TREK-2 (4bw5) with both fenestrations closed (C-C) and 37% of identity, TREK-2 (4xdk) with both fenestrations open (O-O), TRAAK (3um7, O-O) with 32% of identity, TRAAK (4wff, C-O) and TREK-1 (5vkp C-C) with 32% of identity.
- The alignments between the target and each template were refined with the multiple sequence alignment of K_2P family reported by Brohawn[7] et al. The alignment was used as starting point to by I-Tasser[8] server to generate the homology models.

Molecular Dynamics simulation (MDs):

- The TASK-2 models were prepared to perform MDs with the Schrödinger[9] program. Thus, for each model two system were built: 1) with the intracellular pH sensor K245 protonated (pH = 7.5) and 2) neutral. The neutral state of K245 was predicted computationally using PropKa3.0 program[10].
- The TASK-2 systems were embedded into a pre-equilibrated POPC membrane and solvated in a periodic box of SPC water molecules, then the systems were neutralized by adding 150 mM of NaCl.
- The systems were subjected to an energy minimization and 100 ns of MDs employing OPLS[11] as force-field and thus correct the errors inherent in the modeling step. Only secondary structure restraints were applied of 0.2 kcal mol^{-1} $Å^{-2}$.

Results and Discussion

The trajectory analysis reveals that all models are thermodynamically stables under 3 Å of root mean square deviation (RMSD). Furthermore, TASK-2 based on TRAAK (3um7) with $K245^+$ (black line), TRAAK (4wff) with $K245^+$ (blue line) and TREK-2 (4xdk) with $K245^+$ (red line), are more stables than rest of the models based on TRAAK (3um7, gray line), TRAAK (4wff, cian line), and TREK-1 (5vkp, magenta line) with K245 neutral ($K245^0$). The most variable RMSD is for TASK-2 based in TREK-1 (5vkp) because is the only model including the C-terminal region of the channel, being the loops of this region the main contributors to the RMSD fluctuation.



The pK_a prediction of K245 in TASK-2 calculated with PropKa3.0 shown that the nearest values to the experimental $pK_{1/2}$ (~ 8.0) are obtained when the fenestrations are closed, and these are: TASK-2 based in TREK-1 (5vkp) in both monomers, in TREK-2 (4wff) only monomer A (with the TM4 helix in up-state and therefore the fenestration closed) and TREK-2 (4bw5) in both monomers. All the predicted pK_a values were calculated as an average over 200 ns of MD simulations evaluating 1 frame per ns (n=200).

Computational prediction of pK_a of K245			
TASK-2 Model based in:	**Monomer**	**Fenestration state**	**pK_a predicted**
TRAAK (3um7)	A	open	9.87 ± 0.12
	B	open	10.19 ± 0.04
TREK-2 (4xdk)	A	open	10.18 ± 0.03
	B	open	10.01 ± 0.05
TRAAK (4wff)	A	closed	8.98 ± 0.16
	B	open	10.03 ± 0.09
TREK-2 (4bw5)	A	closed	9.20 ± 0.19
	B	closed	9.09 ± 0.37
TREK-1 (5vkp)	A	closed	8.57 ± 0.24
	B	closed	8.59 ± 0.20
Experimental			~8.0

Conclusions

Using comparative modelling techniques and different templates, it was possible to obtain the relative position of the intracellular pH sensor of TASK-2: K245, regarding to both conformational states of the fenestrations (open & close). The computational prediction of the pK_a of K245 over a MD trajectory (n=200 structures) of all comparative models suggest that the $pK_{1/2}$ of K245 obtained experimentally was made over the channel with the fenestrations closed, in agreement with the Brohawn[3] hypothesis.

References

1. S. a Goldstein, D. Bockenhauer, I. O'Kelly, and N. Zilberberg, "Potassium leak channels and the KCNK family of two-P-domain subunits.," Nature Reviews Neuroscience, vol. 2, no. 3, pp. 175–184, 2001.
2. S. G. Brohawn, E. B. Campbell, and R. MacKinnon, "Domain-swapped chain connectivity and gated membrane access in a Fab-mediated crystal of the human TRAAK K+ channel.," Proceedings of the National Academy of Sciences. U. S. A., vol. 110, no. 6, pp. 2129–34, Feb. 2013.
3. S. G. Brohawn, E. B. Campbell, and R. MacKinnon, "Physical mechanism for gating and mechanosensitivity of the human TRAAK K+ channel," Nature, vol. 516, no. 7529, pp. 126–130, 2014.
4. A. Miller and S. Long, "Crystal Structure of the Human Two – Pore Domain Potassium Channel K2P1," Science (80-.)., vol. 432, 2012.
5. M. I. Niemeyer, L. P. Cid, G. Peña-Münzenmayer, and F. V. Sepúlveda, "Separate gating mechanisms mediate the regulation of K_2P potassium channel TASK-2 by intra- and extracellular pH," Journal of Biological Chemistry, vol. 285, no. 22, pp. 16467–16475, 2010.
6. M. I. Niemeyer, L. P. Cid, W. Gonzalez, and F. V. Sepulveda, "Gating, regulation and structure in K2P K+ channels: In varietate concordia?". Molecular Pharmacology, 2016.
7. Brohawn, S. G., del Mármol, J., & MacKinnon, R. (2012). Crystal structure of the human K2P TRAAK, a lipid- and mechano-sensitive K+ ion channel. Science (New York, N.Y.), 335(6067), 436–41.
8. Zhang, Y. (2008). I-TASSER server for protein 3D structure prediction. BMC Bioinformatics, 9, 40.J. C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kalé, and K. Schulten, "Scalable molecular dynamics with NAMD.," Journal of Computational Chemistry, 2005.
9. Schrödinger, L. (2015). Schrödinger Release 2015-1.
10. Olsson, M. H. M., SØndergaard, C. R., Rostkowski, M., & Jensen, J. H. (2011). PROPKA3: Consistent treatment of internal and surface residues in empirical pKa predictions. Journal of Chemical Theory and Computation, 7(2), 525–537.
11. Jorgensen, W. L., Maxwell, D. S., & Tirado-Rives, J. (1996). Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids. Journal of the American Chemical Society, 118(45), 11225–11236.