Exploring the activation mechanism of TRPM8 channel by targeted MD simulations

Matteo Lo Monte [a], Alessandro Pedretti [a], Alessandra Labozzetta [a], Andrea R. Beccari [b], Alessio Moriconi [b], Giulio Vistoli [a]

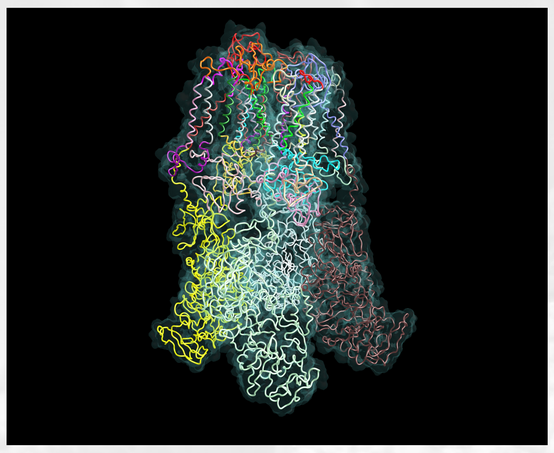
[a] Dipartimento di Scienze Farmaceutiche ‘‘Pietro Pratesi’’, Facoltà di Farmacia, Università degli Studi di Milano, Via Mangiagalli, 25, I-20133 Milano, Italy
[b] Research & Development Centre, Dompé S.p.A., Via Campo di Pile, 67100 L’Aquila, Italy

- The TRPM8 cation channel belongs to the superfamily of transient receptor potential (TRP) channels. It is involved in non-painful cool sensation and triggered by diverse chemical and physical stimuli (mild cold, voltage, compounds evoking cooling sensations such as menthol and icilin), whose precise activation mechanism is still unknown.
- It is mostly expressed in somatosensory neurons and acts as a non-painful cooling sensor. TRPM8 is found also in prostate, bladder and male genital tract, thus suggesting additional physiological roles. Lastly, TRPM8 expression markedly increases in several tumor cells[1], although the mechanism by which influences the cellular differentiation is still unclear.
- The TRPM8 transmembrane portion is formed by six helices (S1–S6). The first four helices (S1–S4) constitute the voltage sensor module and include the binding sites for menthol and icilin, the last two TM helices (S5–S6) constitute the pore module,
- Mutational and structural studies emphasize the key role of S4 helix whose downstream elongation, induced by agonists and membrane potential, can promote the pore opening acting on S4-S5 loop.

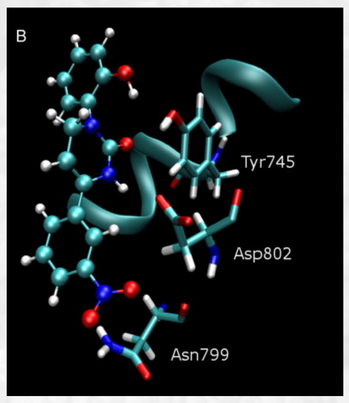
Aim of the study

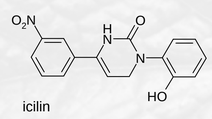
- ***This study exploits the TRPM homology model as previously generated by us to carry out a set of targeted MD simulations involving the TRPM8 channel alone and in complex with selected agonists and antagonists with a view to studying the mechanisms underlying the pore opening. Such MD runs could be also useful to predict the activity of novel ligands[3].***

Homology model for TRPM8



The model was built by fragments using the experimental structures of the Kv1.2 Shaker channel and HCN2 pacemaker channel as the final templates. The tetramer was generated by protein-protein docking simulations[2].

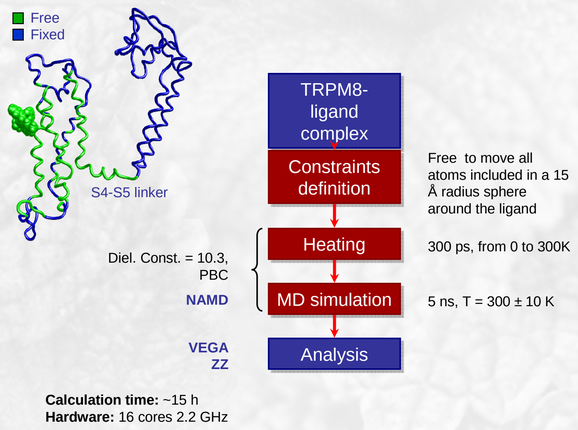




The putative complex for icilin evidences:

- the key H-bond with Tyr745 that should trigger the activation mechanism;
- the hydrophobic contacts elicited by apolar residues in S2;
- the additional interaction with Asn799 (S3) thus explaining the remarkable activity of Icilin;
- in the resting state Tyr745 interacts with Asp802.

Computational methods: MD simulations



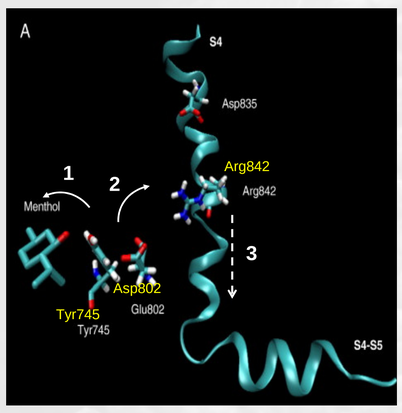
Calculation time: ~15 h
Hardware: 16 cores 2.2 GHz

The simulations involved a single monomer and their constraints and conditions were purposely optimized to render them as fast as possible so as to allow future analyses of large datasets.

Considering the proposed activation mechanism, the analysis of the performed simulations was focused on:

- the moving away between Tyr745 and Asp802;
- the approaching of Asp802 to Arg842;
- the resulting conformational changes in the S4 segment as assessed by RMSD analysis.

Activation mechanism



Based on TRPM8 structure and docking results, a three-step activation mechanism was proposed:

1. the agonist, strongly interacting with Tyr745, breaks the intramolecular interaction between Tyr745 and Asp802;

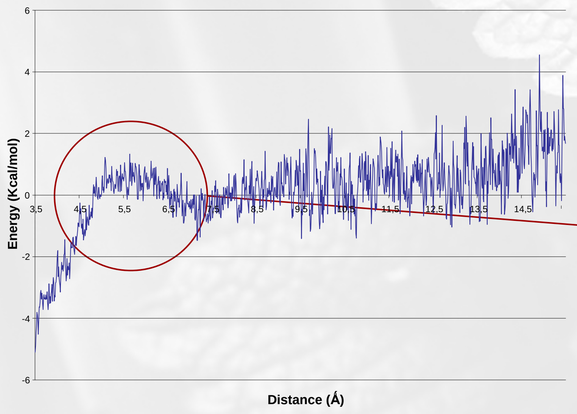
2. Asp802, now free, approaches Arg842;

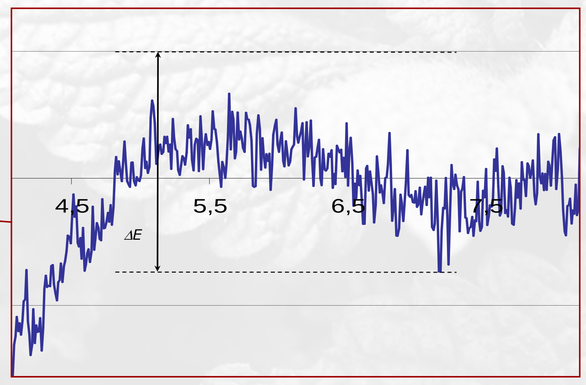
3. this new salt bridge induces the extension of the S4 domain, which shifts from a canonical α-helix to a more elongated 3_{10} motif, and consequently the pore opening.

The comparison between modeled close state (red) and open state (white) as generated by extending S4 confirms that this transition induces a downstream S4 elongation of about 4–5 Å with resulting variation of the angle between S4 and S4–S5 linker which ranges from 89° to 80°. Consequently, the pore helices vary their slope of about 16°, as measured for S5. Such a change induce s a widening of pore entrance of about 9 Å per monomer. The movement of the pore helices reflects on the large EL3 extracellular loop, which approaches the other two extracellular loops. In particular, the open state is characterized by a clear ion-pair that Glu906 realizes with Lys719 and which can contribute to the open state stabilization.

ABF MD simulations

The promising results obtained by targeted MD runs prompted us to exploit Adaptive Biasing Force[4] MD simulations to derive quantitative measures of the propensity of a given ligand to trigger or block the monitored activation mechanism. The (ABF) method is indeed able to calculate the free energy along a reaction coordinate which was chosen to correspond to the Asp802 and Arg842 distance.





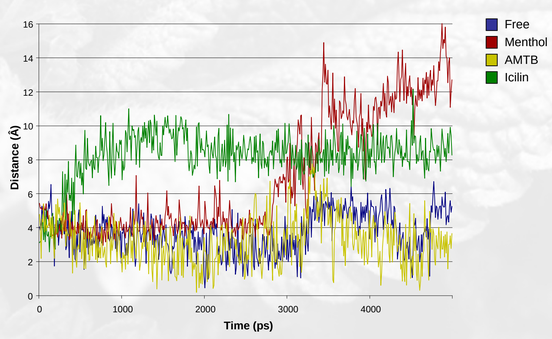
- Evaluation of free energy between Asp802 and Arg842, considering their distance as reaction coordinate.
- Same operative conditions of the previous MD, increasing heating (1.3 ns) and simulation (10 ns) times.
- Considered agonists: menthol, icilin, CA10, WS3, WS12, WS14, WS23.
- Considered antagonists: AMTB, BCTC, capsazepine.

Compound	*ΔE* (Kcal/mol)	pIC50
AMTB	8.7	6.3
BCTC	5.6	6.0
Capsazepine	3.5	4.7
TRPM8 alone	2.8	-

There is an encouraging agreement between free energy and bioactivity for simulated antagonists while no correlation was found for the agonists. They may mainly influence the Tyr745-Asp802 interaction.

Computational results

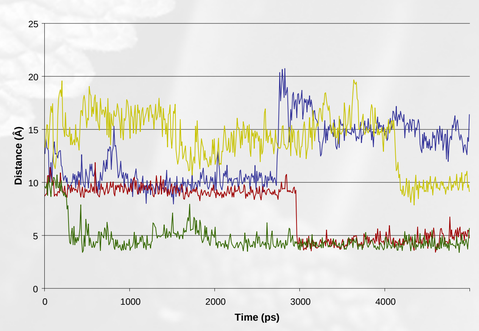
Tyr745 - Asp802 distance



The agonists (Menthol and Icilin) induce the separation.

The antagonist (AMTB) has the same behavior of TRPM8 without ligand preserving the Tyr745-Asp802 interactions.

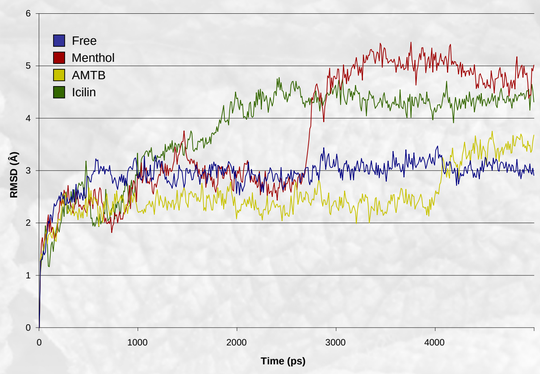
Asp802 - Arg842 distance



The agonists (Menthol and Icilin) induce the monitored approaching.

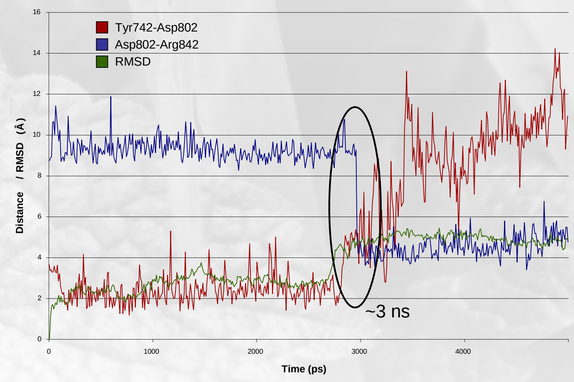
The antagonist (AMTB) and TRPM8 alone leave the residues at a distance above 10 Å.

Fluctuations in S4 domain



The agonists (Menthol and Icilin) induce more significant structure variations in the S4 domain compared to the antagonist (AMTB) and TRPM8 without ligand.

Menthol molecular dynamics



The comparison of the three plots for menthol evidences how all main transitions occur at the same time (black circle) thus suggesting that they are concatenate steps of an unique concertate mechanism.

Conclusions

- Targeted MD simulations allowed the three-step activation mechanism of the TRPM8 channel to be encouragingly confirmed.
- The simulations clearly discriminate between agonists and antagonists since only the formers are able to induce a set of conformational shifts which trigger the channel opening.
- The ABF MD runs can suitably estimate the free energy required for the second activation step and this appears to be useful to analyze the antagonists only.
- The obtained results emphasize that suitably targeted MD runs can be fast enough to be predict the bioactivity of large datasets providing it as an useful tool in rational ligand design.

References

[1]*Jordt SE, Ehrlich BE., Subcell Biochem. 2007, 45, 253-71*
[2]*Pedretti A, Marconi C, Bettinelli I, Vistoli G., Biochim Biophys Acta. 2009, 1788, 973-82*
[3]*Pedretti A, Labozzetta A, Lo Monte M, Beccari A R, Moriconi A, Vistoli G., Biochem Biophys Res. Comun. 2011, 414(1):14-9*
[4]*Darve E., Pohorille A. J. Chem. Phys. 2001, 115, 9169-9183.*