Exploring the interaction capacities of TRPM8 channel by docking analyses and MD simulations

Alessandro Pedretti, Alessandra Labozzetta, Andrea R. Beccari, Alessio Moriconi, Giulio Vistoli

Dipartimento di Scienze Farmaceutiche "Pietro Pratesi", Facoltà di Farmacia, Università degli Studi di Milano, Via Mangiagalli, 25, I-20133 Milano, Italy.

Dompé SpA, R&D division, Via Campo di Pile - 67100 L'Aquila (Italy).

alessandro.pedretti@unimi.it

The transient receptor potential (TRP) superfamily is a large group of ion channels that has received increased attention in recent years. TRPM8, on which this study is focused, belongs to the subfamily of thermo-TRP channels which are triggered by diverse chemical and physical stimuli and whose precise activation mechanism is still unknown. Specifically, TRPM8 is activated by cold temperature, ligands such as menthol and icilin (a synthetic derivative), positive membrane potential and the endogenous signaling lipid, PIP2. Therefore, TRPM8 could find therapeutic applications in several pathological conditions, including neurogenic inflammation, neuropathic pain, overactive bladder and prostate cancer. [1] An homology model of the TRPM8 tetramer was recently generated using a fragmental strategy by some of us. [2] Beside the global architecture of the TRPM8 channel, such a model revealed the key residues involved in ligand recognition and suggested that the agonist binding is able to induce a cascade of conformational shifts which globally may orchestrate the channel opening (at least partially). Such a mechanism was then confirmed by classic all-atoms MD simulations which evidenced how agonists are able to trigger such structural changes whereas agonists block the channel in its starting conformations. Considering the rather nonspecific nature of TRPM8 binding site and the resulting difficulty of predicting ligand bioactivity by docking calculations, adaptive biasing force (ABF) MD simulations [3] were exploited to derive the free energies involved in TRPM8 activation and the obtained energy values are in line with the activity of a representative set of TRPM8 ligands. These results emphasize that suitably targeted MD runs can be fast enough to be systematically applied to predict the bioactivity of rather large ligand datasets.

[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] Darve E., Pohorille A. *J. Chem. Phys*. **2001**, *115*, 9169-9183.