About the painful world of cell receptors

Identification of residues involved in homotrimeric stabilization and function of the HumanP2X4R receptor channel by molecular dynamic simulations

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In Short

- combined experimental and simulative work on the structure and function of transmembrane receptors
- HP2X4R studied here is an ATP-activated cation channel and plays an important role in neuropathic pain
- atomistic insights into the structure and function of this receptor helps in the drug development
- all atom molecular dynamics studies of open and closed state

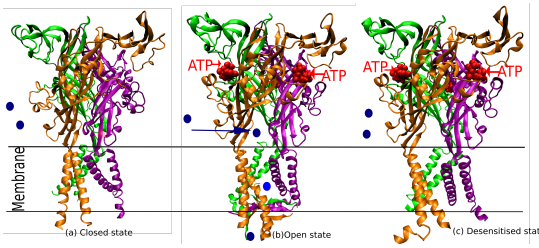

***Figure 1:** The three different conformational states of human P2X3R(HP2X3R) [2]. (a) apo-closed state (PDBID 5svj): The pore is closed and cations cannot move into the cell, (b) ATP-bound open state (PDBID 5svk): extracellular ATP binds to HP2X3R causing the opening of pore and cations can move into the cell, and (c) ATP-bound desensitised state (PDBID 5svl): In this state the pore is closed even with ATP bond.*

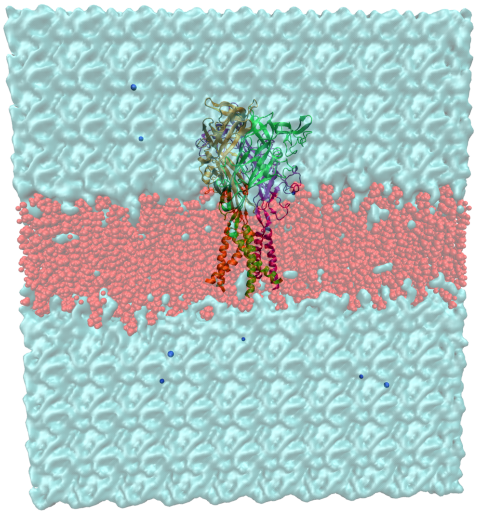

***Figure 2:** Representative simulation cell consisting of the hP2X4 receptor embedded in a membrane model (shown in red) with ionic aqueous solution (blue) on both sides of the membrane.*

Purinergic P2X receptors (P2XRs) are a family of adenosine triphosphate(ATP)-gated membrane-bound cation channels with seven members, P2X1-P2X7. P2XRs are found on almost all cells in the human body. With the existence of open and closed states of the channels, they can direct cationic transport into cells. Therefore they are capable to transport signals along cells and play important roles in numerous physiological and pathophysiological functions such as smooth muscle contraction, neuropathic pain, and inflammation. Accordingly, P2XRs are considered important drug targets [1].

Nowadays, many structures of biological macro-molecules are resolved already by Xray or NMR experiments. These structures are listed in protein data banks and available to be downloaded. For the P2X receptor family many structures are available already for many (non)human species. In figure 1 the different states of the human P2X3 receptor are illustrated. In principle, molecules of one family are very similar in structure and can be used as a template for other human P2XR family members like the one we are focused on, the HP2X4 receptor. Therefore, we get our initial structures via homology modeling server and can use it for our molecular dynamics simulations.

Anyway, for these types of simulations, the transmembrane molecule is usually embedded in a membrane model (Figure 2). The whole simulation cell is then filled with ionic aqueous solution, so we can end up with systems containing up to a million atoms. In our case it is "only" 600 000 atoms, so system of medium size. The simulation of such systems over e.g. µs would easily take several months on local computing machines. On large-scale computing centers like the HLRN, on the other hand, we can simulate up to 60 to 100 ns of one of our systems in one day, i.e. in principle 1 µs in only 10 days.

We need this demanding computational resources because the aim of the present project is the identification of the residues responsible for the non-covalent homotrimerization on the example of the human P2X4R (hP2X4R) receptor. Furthermore, the identification of individual residues being responsible for the transition of the conformational

states via molecular dynamics simulations will be combined with site-directed mutagenesis and electrophysiology experiments. Alltogether, this will provide deeper insights into the overall gating process. Such results are conceptually interesting, but also open new insights to develop alternative positive allosteric modulators (PAMs) of P2X receptors of interest for drug therapy.

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https://www.hmi.uni-bremen.de

More Information

[1] P. Illes, C. E. Müller, K. A. Jacobson, T. Grutter, N. Thomas, F. Annette , J. Samuel, C. Kennedy, G. Schmalzing, F. M. Jarvis, S. S. Stojilkovic, F. B. King, and D. V. Francesco *Br. J. Pharmacol.* **178**, 489-514 (2021). doi: 10.1111/bph.15299

[2] E. S. Mansoor, W. Lü, W. Oosterheert, M. Shekhar, E. Tajkhorshid, and E. Gouaux *Nature* **538**, 66-71 (2016). doi:10.1038/nature19367

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