Numerical Modelling of Ion Transport in 5-HT3 Serotonin Receptor Using Molecular Dynamics

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Abstract. Cation selective ligand-gated ion channels are pore-forming membrane proteins. They are responsible for generating of transmembrane voltage and action potential, playing an important role in functioning of nervous systems. Mathematical modelling of transmembrane transport in membrane and membrane/protein structures using molecular dynamics (MD) method is often associated with difficulties, because it is nearly impossible to observe spontaneous diffusion in MD experiments. In this work Molecular Dynamics (MD) and Umbrella Sampling (US) methods are used to study ion transport through 5-HT3 Serotonin receptor.

Keywords: Biophysics · Molecular dynamics · Biomembranes · Ion channels · Transmembrane transport · Serotonin receptor

1 Introduction

Cation selective ligand-gated ion channels form a large class of functional membrane proteins and are involved in regulating a variety of cellular processes. Pore-forming membrane proteins responsible, in particular, for generating of transmembrane voltage and action potential, playing an important role in functioning of nervous systems [1,2]. Dysfunctions of ion channels can lead to severe neurological diseases. At the same time studying of such objects is of considerable interest from fundamental and therapeutic points of view. Ion channels are the third largest group of targets in drugs development [3,4]. All this makes studying of the structure and the function of ion channels of considerable interest.

At the same time, these complex objects are very difficult for experimental study. Only a limited number of precise 3D atomic structures are available, while molecular mechanism of functioning is not completely clear [5,6]. Computer simulation methods have shown to be effective instrument in many different areas of science, and using computer modelling techniques is widely used for studying biological objects [7,8].

One of the popular methods here is molecular dynamics (MD) method, widely used for mathematical modelling of biological systems. Unfortunately modelling of dynamics and transmembrane transport in big systems, such as membrane and

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membrane/protein structures, is often associated with computational difficulties, therefore, of interest are those approaches and methods that we can use to get adequate results in reasonable time.

In this work molecular dynamics simulations and umbrella sampling methods are used to study of mouse serotonin 5HT-3 receptor. In 2014 high resolution (0.35 nm) structure of the 5-HT3 receptor in complex with stabilizing nanobodies was determined. Homology modelling revealed, that thansmembrane domain of the structure is highly possibly in the open state, which makes studying of the structure using modern computational methods of high interest [6].

In this study the model of the 5-HT3 receptor was built with explicit membrane and solvent. The simulations show good agreement with experimentally known macroscopic parameters, such as area per lipid, thickness and compressibility of the membrane, acyl chain order parameters and crystallographic protein structure.

2 Materials and Methods

In essence, the molecular dynamics method is based on representing the system under study as a set of material points whose interaction is described by the laws of classical mechanics. Each point is an atom or a group of atoms. The total potential energy of the system is described as a sum of partial potentials:

$$U(r) = \sum_{i,j} U_{ij}^{v}(b_{i,j}) + \sum_{i,j,k} U_{ijk}^{\theta}(\theta_{i,j,k}) + \sum_{i,j,k,l} U_{ijkl}^{\phi}(\phi_{i,j,k,l}) + \sum_{i \neq j} \left[U_{ij}^{c}(r_{i,j}) + U_{ij}^{vdw}(r_{i,j}) \right] \quad (1)$$

where the first three terms represent interaction between chemically bonded atoms (energies of valence bonds, valence angles and torsion angles), and the last two terms represent the electrostatic and Van der Waals forces between the pairs of atoms which are not bonded. Partial potential functions are represented with a differentiable function of atomic coordinates. The motion of atoms in the potential field is described by the system

$$\left\{ m_i \ddot{\vec{r}_i} = -\left(\frac{\partial U}{\partial x_i}, \frac{\partial U}{\partial y_i}, \frac{\partial U}{\partial z_i} \right) \right. \quad (2)$$

of second order ordinary differential equations, which is to be solved numerically. The applicable numerical methods differ in accuracy and computational complexity. The Verlet method and its variants are widely used in molecular dynamics, as a compromise between the accuracy of the procedure and the calculation speed. The coordinates of atoms in the new temporal layer are calculated as

$$r_i(t + \triangle t) = 2r_i(t) - r_i(t - \triangle t) + \frac{F_i(t)}{m_i} \triangle t^2 \quad (3)$$

$$v_i(t) = \frac{1}{2\triangle t} \left(r_i(t + \triangle t) - r_i(t - \triangle t) \right) \quad (4)$$

Umbrella Sampling Method. Umbrella sampling method is used to improve sampling of a system where ergodicity is hindered by the form of the system's energy landscape. In this approach, we apply to the system an additional potential capable of holding the ions in a potentially unfavourable region inside of the ion channel [9,10]. The modification of the original potential energy function amounts to the addition of an external potential (Fig. 1a):

$$\dot{U}(r) = U(r) - W(r),\ W(r) = k_w(\xi(r) - \xi_0)^2 \tag{5}$$

Since the form of W(r) is known, we evaluate the free energy G of the unperturbed system as

$$G(\xi) = -k_b T \cdot ln(\dot{p}(\xi)) - W(\xi) + const \tag{6}$$

where $\dot{p}(\xi)$ is the density of the probability of finding the system in a state with this value of ξ in the perturbed system, T is temperature and k_b is Boltzmann constant.

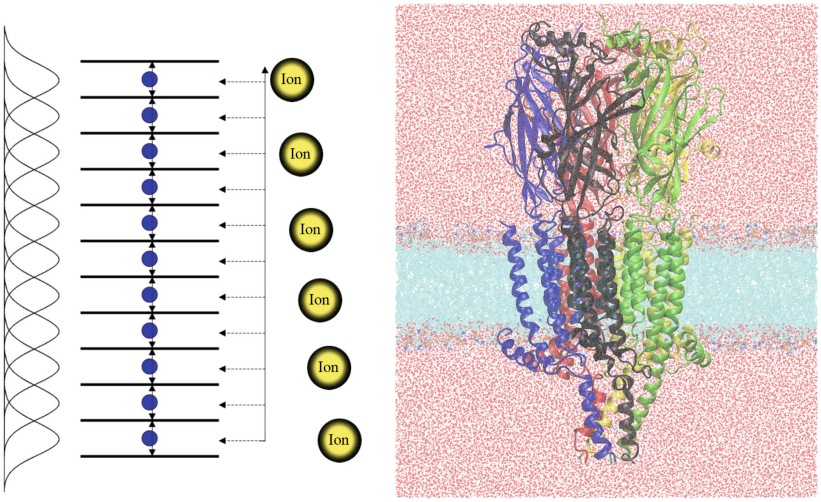
Using this approach, we can reconstruct the profile of free energy only in a small neighbourhood of ξ_0 hence, we choose a set of initial points along the reaction coordinate so that the distribution functions of the system near each initial point overlap with the distribution functions associated to the neighbouring points. There are several methods for reconstructing the original profile of free energy from the set of distributions found near each initial point. In our research we use the weighted histogram analysis method (WHAM, [11]).

Molecular Dynamics Protocol. We used Gromacs 4.6.7 [12] software for MD simulations with Gromos 53a6 [13] forcefield. As a model for eukaryotic cell membrane we used bilayers of heavy-atom model of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC), lipid structure were obtained from lipid structures database of the University of Calgary [14]. Stochastic dynamics with $\tau_t = 1\ ps$ was used for temperature control. Semi-isotropic Berendsen barostat with $\tau_p = 0.5\ ps$ was used for pressure coupling. Cut-off radius of 1.8 nm was used for calculating of non-bonded interactions.

POPC bilayer consisted of 498 lipid molecules. Initial surface area per molecule of the lipid was 68–72 $\dot{A}^2$. The resulting structures were hydrated by water molecules (Model TIP4P [15]) of not less than 150 molecules of water per one molecule of the lipid.

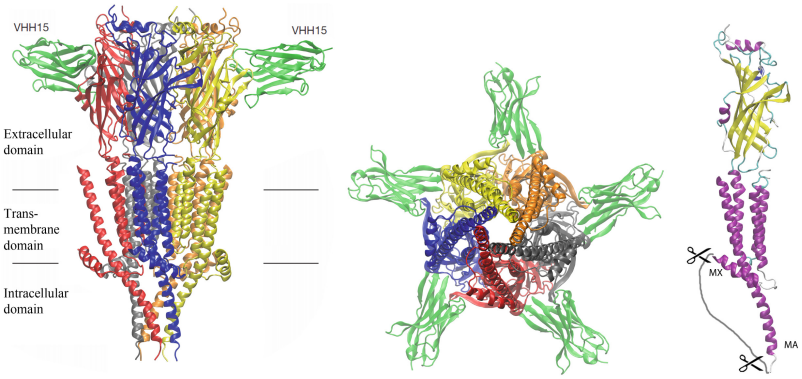
5-HT3 receptor structure was obtained from the RCSB Protein Data Bank [16] (PDB code 4PIR [6]). 3-Dimensional 5-HT3 serotonin receptor structure, obtained by X-ray analysis method is shown on Fig. 2. Because the structure is not fully determined, the ends of the missing chains were capped with ACE/NH2 residues.

For immersing the ion channel structure into the membrane we used technique described in [17]. ACE/NH2 residues spatial position was fixed with parabolic potential with the stiffness constant of $1000 \cdot kJ/(mol \cdot nm^2)$. The model system with the complete structure of the receptor, solvent and POPC bilayer contained 274,814 atoms, and is shown in Fig. 1b.



(a) (b)

Fig. 1. Harmonic potential along reaction coordinate for restraining the ion in energetically unfavourable region(1a), The system under study (1b)



(a) (b) (c)

Fig. 2. X-Ray structure of 5-HT3 receptor [6]. Parallel to the membrane plane (2a). Perpendicular to the membrane plane (2b). Structure of the subunit. Chain structure between MX-MA is not determined (2c)

Molecular dynamics simulations were performed for 13 ns at 310 K with lateral pressure of −50 bar. Pressure in a perpendicular to the membrane was 1 bar in all calculations. Structure of the ion channel was fixed, which allowed the bilayer and solvent enter into interaction with the channel structure. In the last 3 ns of trajectory we applied temperature coupling of 490 K to the water molecules, which allowed water molecules to penetrate into the pore of the ion

channel. Then we performed 30 ns MD simulation without restrictions on the ion channel structure. We used last 15 ns of trajectory for processing.

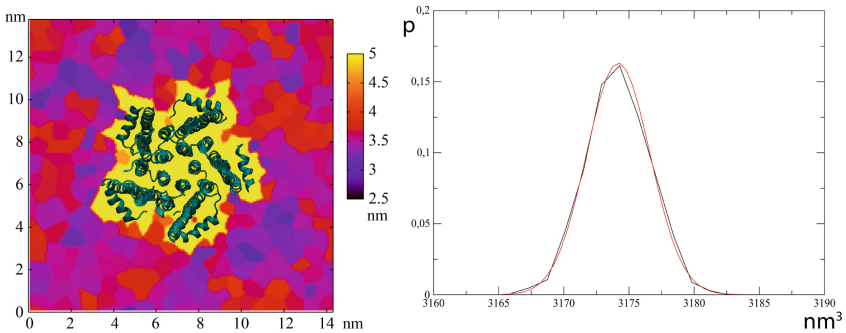
For umbrella sampling procedure we chose 129 starting configurations for the Na+ ion inside of the pore of the channel with the spatial step of 0.1 nm along the pore of the channel (z-axis, reaction coordinate). We used the harmonic potential with the force constant of $1000 \cdot kJ/(mol \cdot nm^2)$. The trajectories to gather statistics were 3 ns long. The total simulation time was 430 ns.

3 Results

After simulation fully hydrated model of the 5-HT3 serotonin receptor ion channel in the POPC membrane was obtained. The thickness of the membrane was 3.5 ± 0.2 nm, and the average area per lipid was 0.63 nm^2, which generally corresponds to the experimental estimates of 0.62–0.68 nm^2 for POPC bilayers [18,19]. The thickness of the membrane was determined as the average distance between atoms of phosphorus in monolayers.

Thickness map of the bilayer Fig. 3a shows no significant signs of clustering or other undesirable effects, which may occur during simulation. The average thickness of the membrane is within the acceptable values for membrane systems in the liquid crystalline phase [20].

The cell volume fluctuations (Fig. 3b) were 3174 ± 1.92 nm^3, which correspond to compressibility constant χ_T of $2.7 \cdot 10^{-10} Pa^{-1}$, which is in agreement with known experimental χ_T estimates for membranes from $1 \cdot 10^{-10}$ to $6 \cdot 10^{-10} Pa^{-1}$ [21]. Thus, the reasonable values of the compressibility factor χ_T, and Gaussian distribution pattern for volume fluctuations indicates local equilibrium conditions.

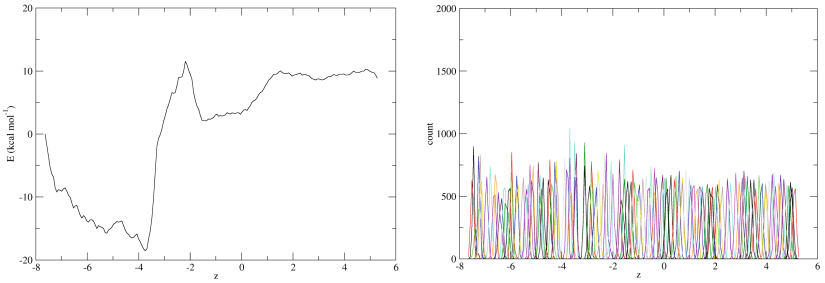


(a) (b)

Fig. 3. Thickness distribution over the bilayer (3a). Cell volume probability density function (3b).

In vivo, 5-HT3 receptors in open state are permeable to Na+ and K+ ions, and have a negligible permeability to negatively charged ions. The calculated mean force potential (Fig. 4a) indicates potential barrier of approximately

30 kCal/mol in the intracellular domain of the ion channel, where supposedly selective filter is located. Relatively high potential barrier of this magnitude can prevent Na+ cations from extracellular space from getting inside of the channel. The histograms in (Fig. 4b) characterize the statistical covering of the conformation space along the reaction coordinate in the simulations. The histogram reveals few regions with relatively low population, which suggests that using spatial step of 0.1 nm with the force constant K_{harm} of the harmonic potential equal to $1000 \cdot kJ/(mol \cdot nm^2)$ is not enough for good statistical coverage.



(a) (b)

Fig. 4. Mean force potential over the channel pore (4a). Population density histograms (4b).

4 Conclusion

Based on the simulation results we can make following conclusions:

- We have build the model of fully hydrated 5-HT3 ion channel with POPC bilayer, in agreement with known experimental macroscopic parameters such as: thickness of the membrane, area per lipid and bilayer compressibility.
- The MD protocol we used is capable for modelling of protein-membrane systems in a quasi-equilibrium state.
- Umbrella sampling analysis shows relatively high potential barrier of approximately 30 kCal/mol for penetrating Na+ cations in the region, where the selective barrier for anions is supposedly located [22]. This result indicates hindered permeability of the channel for cations and needs further investigating.

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References

1. Jegla, T.J., Zmasek, C.M., Batalov, S., Nayak, S.K.: Evolution of the human ion channel set. Comb. Chem. High Throughput Screen **12**(1), 2–23 (2009)
2. Kullmann, D.M., Hanna, M.G.: Neurological disorders caused by inherited ion-channel mutations. Lancet Neurol. **1**(3), 157–166 (2002)
3. Overington, J.P., Al-Lazikani, B., Hopkins, A.L.: How many drug targets are there? Nat. Rev. Drug Discov. **5**(12), 993–996 (2006)
4. Hopkins, A.L., Groom, C.R.: The druggable genome. Nat. Rev. Drug Discov. **1**(9), 727–730 (2002)
5. Wickenden, A., Priest, B., Erdemli, G.: Ion channel drug discovery: challenges and future directions. Future Med. Chem. **4**(5), 661–679 (2012)
6. Hassaine, G., Deluz, C., Grasso, L., Wyss, R., Tol, M.B., Hovius, R., Graff, A., Stahlberg, H., Tomizaki, T., Desmyter, A., Moreau, C., Li, X.D., Poitevin, F., Vogel, H., Nury, H.: X-ray structure of the mouse serotonin 5-HT3 receptor. Nature **512**, 276–281 (2014)
7. Popinako, A.V., Levtsova, O.V., Antonov, MYu., Nikolaev, I.N., Shaitan, K.V.: The structural and dynamic model of the serotonin 5-HT3 receptor. Comparative analysis of the structure of the channel domain of pentameric ligand-gated ion channels. Biofizika **56**(6), 1111–1116 (2011)
8. Pischalnikova, A.V., Sokolova, O.S.: The domain and conformational organization in potassium voltage-gated ion channels. J. Neuroimmune Pharmacol. **4**(1), 71–82 (2009)
9. Kastner, J.: Umbrella sampling. Wiley Interdiscip. Rev. Comput. Mol. Sci. **1**(6), 932–942 (2011)
10. Torrie, G.M., Valleau, J.P.: Nonphysical sampling distributions in Monte Carlo free-energy estimation: umbrella sampling. J. Comput. Phys. **23**(2), 187–199 (1977)
11. Kumar, S., Rosenberg, J.M., Bouzida, D., Swendsen, R.H., Kollman, P.A.: The weighted histogram analysis method for free-energy calculations on biomolecules. I. The method. J. Comput. Chem. **13**(8), 1011–1021 (1992)
12. Hess, B., Kutzner, C., van der Spoel, D., Lindahl, E.: GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. J. Chem. Theory Comput. **4**(3), 435–447 (2008)
13. Oostenbrink, C., Villa, A., Mark, A.E., van Gunsteren, W.F.: A biomolecular force field based on the free enthalpy of hydration and solvation: the GROMOS force-field parameter sets 53A5 and 53A6. J. Comput. Chem. **25**(13), 1656–1676 (2004)
14. Tieleman, D.P., Forrest, L.R., Sansom, M.S., Berendsen, H.J.: Lipid properties and the orientation of aromatic residues in OmpF, inuenza M2, and alamethicin systems: molecular dynamics simulations. Biochemistry **37**(50), 17554–17561 (1998)
15. Jorgensen, W.L., Chandrasekhar, J., Madura, J.D., Impey, R.W., Klein, M.L.: Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. **79**(2), 926 (1983)
16. Berman, H.M.: The protein data bank: a historical perspective. Acta Crystallogr. A **64**(Pt 1), 88–95 (2008)
17. Schmidt, T.H., Kandt, C.: LAMBADA and InflateGRO2: efficient membrane alignment and insertion of membrane proteins for molecular dynamics simulations. J. Chem. Inf. Model. **52**(10), 2657–2669 (2012)
18. Lague, P., Zuckermann, M.J., Roux, B.: Lipid-mediated interactions between intrinsic membrane proteins: dependence on protein size and lipid composition. Biophys. J. **81**(1), 276–284 (2001)

19. Nagle, J.F.: Area/lipid of bilayers from NMR. Biophys. J. **64**(5), 1476–1481 (1993)
20. Shaitan, K.V., Antonov, M.Y., Tourleigh, Y.V., Levtsova, O.V., Tereshkina, K.B., Nikolaev, I.N., Kirpichnikov, M.P.: Comparative study of molecular dynamics, diffusion, and permeability for ligands in biomembranes of different lipid composition. Biochem. Suppl. Ser. A. Membr. Cell Biol. **2**, 73–81 (2008)
21. Braganza, L.F., Worcester, D.L.: Structural changes in lipid bilayers and biological membranes caused by hydrostatic pressure. Biochemistry **25**(23), 7484–7488 (1986)
22. Popinako, A.V., Levtsova, O.V., Antonov, M.Y., Nikolaev, I.N., Shaitan, K.V.: The structural and dynamic model of the serotonin 5-HT3 receptor. Comparative analysis of the structure of the channel domain of pentameric ligand-gated ion channels. Biophysics **56**(6), 1078–1082 (2011)