Features of ion localization in the 5-HT₃ receptor revealed by Langevin dynamics simulation

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Abstract. Localization times of Na⁺ corresponding to the pore-lining amino acid residues of the 5-HT₃ receptor are obtained using Langevin dynamics with respect to hydrodynamic and dielectric friction. The transmembrane potential is modelled by the external uniform electric field. The simulation shows that the ion localization times for T257 and E250 are similar, even though threonine has an uncharged side chain. Moreover, the time values obtained for T257 exceed more than twice the appropriate results for S253. Despite of the non-polar nature of I267 and I268, these residues are noticed to have the highest time values. This result may confirm the suggestion of the hydrophobic gating existence.

1. Introduction
Studying of proton transfer in different protein complexes such as cytochrome c oxidase and F₀F₁-ATP synthase demonstrated the possibility of proton localization at specific structural elements [1,2]. In the case of research extension to ion channels, the features of ion transport in the 5-HT₃ receptor are of great interest. The crystal structure of this protein appeared only some years ago, while the cryo-EM structure at high resolution has been just recently obtained [3,4]. Thus, the atomistic details of its functioning remain poorly understood yet. To fill up the gap, we use Langevin dynamics simulation for determination and analysis of ion trapping areas in the 5-HT₃ receptor.

2. Theoretical background
The X-ray structure of the whole homomeric 5-HT₃A receptor from Mus musculus (PDB code 4PIR) was reduced to the five transmembrane M2-helices with parts of their connecting loops making up the architecture of the ion channel pore. According to the experimental data the pore-lining amino acid residues were shown in Figure 1b. Hydrogen atoms missing in the default PDB-file were added to the each residue of the helices. The modified protein structure was further aligned along the Z-axis, and single Na⁺ was set at the entrance of the channel. Full system preparation for modeling of ion transport was made in VMD 1.9.3 [5]. The pore radius profile was obtained using CAVER Analyst 1.0 [6].

In this study the sodium cation is the only moving object, while all the protein atoms are fixed in the homogeneous implicit solvent. The Brownian motion of the ion is expressed by the Langevin equation:

\[ m \frac{dV}{dt} = -\xi V + F_e(t) + F_s(t) \]  \hspace{1cm} (1)

where \( m \) is the mass of the particle, \( \xi \) is the friction coefficient, \( F_e(t) \) is an external force field, \( F_s(t) \) is a stochastic force represented by a white noise process. In other words, the last term should...
fulfil the further conditions: \( \langle F(t) \rangle = 0 \) and \( \langle F(t_1) F(t_2) \rangle = \sigma^2 \cdot \delta(t_1 - t_2) \). External force \( F(t) \) is represented by the sum:

\[
F_e = \frac{1}{4\pi\varepsilon_0} \frac{q_i q_j}{r_{ij}^2} + \frac{48\varepsilon}{r_{ij}} \left( \left( \frac{\sigma}{r_{ij}} \right)^{12} - \frac{1}{2} \left( \frac{\sigma}{r_{ij}} \right)^{6} \right) + F_Z
\]

where \( \varepsilon \) is the absolute permittivity of the medium, \( \varepsilon_0 \) is the electric constant, \( q_i \) and \( q_j \) are charges of atoms \( i \) and \( j \) respectively, which are separated by the distance \( r_{ij} \), \( \varepsilon^* \) is the depth of the potential well, \( \sigma \) is the finite distance at which the inter-particle potential equals to zero, and \( F_Z \) is a uniform electric field which is directed along the \( Z \)-axis for simulation of the transmembrane potential. The first summand represents the electrostatic force, while the second one is Lennard-Jones type interaction. Parameters \( q \), \( \varepsilon^* \) and \( \sigma \) used for calculation of the Coulomb and Lennard-Jones forces correspond to the CHARMM22 protein force field [7]. In view of the above, velocities \( V_i \) and coordinates \( X_i \) can be obtained as follows [8]:

\[
V_i = V_{i-1} e^{-\gamma \Delta t} + F_e \frac{1 - e^{-\gamma \Delta t}}{m\gamma} + N \left( 0, \frac{\sigma^2 \left( 1 - e^{-2\gamma \Delta t} \right)}{2\gamma} \right)
\]

\[
X_i = X_{i-1} + V_{i-1} \frac{1 - e^{-\gamma \Delta t}}{\gamma} + F_e \left( \frac{\Delta t}{m\gamma} - \frac{1 - e^{-\gamma \Delta t}}{m\gamma^2} \right) + N \left( 0, \frac{\sigma^2 \left( \gamma \Delta t - \frac{3}{2} + 2e^{-\gamma \Delta t} - e^{-2\gamma \Delta t} \right)}{2\gamma^3} \right)
\]

where \( \gamma = \frac{\sigma}{m} \) means the specific friction coefficient considering both hydrodynamic and dielectric friction.

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**Figure 1.**

(a) The intrinsic electrostatic potential profile obtained for the transmembrane domain of the 5-HT\(_3\) receptor (black solid line) and the pore radius profile (red solid line). The intervals along the \( Z \)-axis corresponding to the pore-lining residues are separated by dashed lines. The category corresponding to the residues I267 and I268 is hereinafter referred to as “I267”.

(b) The reduced modeling structure of the 5-HT\(_3\) receptor (PDB code 4PIR [3]). One of five chains is omitted for illustrative purposes. The transmembrane M2-helices are represented by grey semitransparent ribbons. The pore-lining amino acid residues and the ion are shown in labeled red sticks and ball respectively.
3. Results and discussion

The Langevin dynamics simulation of ion transport in the 5-HT₃ receptor was performed with the integration time step set equal to 1 fs. The process of Na⁺ translocation was modelled at the transmembrane potential values ranging from 0 to 200 mV. Ion localization times obtained from processing of resulting trajectories correspond to the pore-lining amino acid residues of the channel.

Analysis of the simulation results represented in Figure 2 shows that potential values below 50 mV are characterized by the significant ion localization times up to 800 ps corresponding to D271. Moreover, ion translocation through the channel is possible only at the transmembrane potential above the present value (Figure 3). This point is consistent with the intrinsic potential gap observed in the region of this residue. Such external potential is probably not enough for the sodium cation to overcome the energetic barrier located after D271. However, if the transmembrane potential is higher than 50 mV, Na⁺ localizes mainly at the residues I267 and I268. Despite of the fact that the electrostatic potential profiles and pore radius demonstrate the absence of any potential gap and narrow region respectively, these hydrophobic residues trap the ion with time values up to 400 ps.

Furthermore, another curious result concerns E250 and D247, which are located at the channel exit. Both of these residues have negatively charged side chains. Nevertheless, in the case of E250 ion localization times exceed more than twice the corresponding values observed for D247. The same situation occurs with the adjacent polar residues T257 and S253, even though the pore radius corresponding to serine is significantly lower than the radius of threonine area (about 2.1 Å and 3.5 Å respectively). Moreover, ion localization times obtained for T257 are similar to the time values for E250, while the region of T257 corresponds to the hilltop of electrostatic potential and to the local maximum of the radius.

The non-polar residues V264 and L260 should be also highlighted, because at the transmembrane potential below 175 mV their ion localization times are comparable to the time values for negatively charged E250 or even higher. This fact coupled with the above-mentioned result for I267 and I268 may prove the assumption of hydrophobic gating mechanism in the ligand-gated ion channels, which is observed in recent MD simulations of the 5-HT₃ receptor and another members of the Cys-loop superfamily such as the GLIC – prokaryotic homolog of the nicotinic acetylcholine receptor [9,10].

![Figure 2](image-url)  
*Figure 2.* Ion localization time dependence on the transmembrane potential for every pore-lining amino acid residue of the 5-HT₃ receptor. Each point is the mean time over 20 model experiments consisting of 10⁶ simulation steps.
Figure 3. Relative ion localization times for the corresponding pore-lining amino acid residues at different transmembrane potential values. Each bar represents the ratio of the ion localization time value to the total time.

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