Exploring conformational states and helical packings in the P2X receptor transmembrane domain by molecular dynamics simulation

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Received: 18 August 2017 / Accepted: 19 March 2018 / Published online: 2 April 2018
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Abstract The P2X receptor is a trimeric transmembrane protein that acts as an ATP-gated ion channel. Its transmembrane domain (TMD) contains only six helices and three of them, the M2 helices, line the ion conduction pathway. Here, using molecular dynamics simulation, I identify four conformational states of the TMD that are associated with four types of packing between M2 helices. Packing in the extracellular half of the M2 helix produces closed conformations, while packing in the intracellular half produces both open and closed conformations. State transition is observed and supports a mechanism where iris-like twisting of the M2 helices switches the location of helical packing between the extracellular and the intracellular halves of the helices. In addition, this twisting motion alters the position and orientation of residue side-chains relative to the pore and therefore influences the pore geometry and possibly ion permeation. Helical packing, on the other hand, may restrict the twisting motion and generate discrete conformational states.

Keywords Helical packing · P2X · Ion channel · Molecular dynamics · Transmembrane helix

1 Introduction

The folds of helical proteins or protein domains are largely determined by interhelical packings. This is especially true when the helices are relatively straight and the protein structures can be described in terms of simple geometric parameters such as interhelical distances and angles [1–3]. Detailed analysis of high-resolution membrane proteins shows

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10867-018-9493-8>) contains supplementary material, which is available to authorized users.

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that most interacting helical pairs cluster into five major groups (helical packing motifs) according to their three-dimensional similarity [4]. Another study [5] used bundles of straight helices with rotational symmetry to represent the pore lining helices (PLHs) of ion channels and found that the accessible conformational space for close packing is narrow. These studies have strong implications regarding the organization of helical proteins. First, the packing motifs are largely determined locally by the surfaces of interacting residues; second, it is possible to build a helical protein model from straight helices if proximity relationships between residues are known, for example, from cysteine scanning mutagenesis and thiol cross-linking experiments; third, helical packing is expected to associate with the dynamic nature of proteins, namely, when helical proteins undergo global conformation changes, the helical packing patterns are expected to change accordingly.

The P2X receptor is a trimeric transmembrane protein that acts as an ATP-gated ion channel. Each subunit contains only two transmembrane helices, M1 and M2. The M2 helices line the ion conduction pathway. The crystal structures of the zebrafish (zf) P2X4 receptor [6–8], the giant panda P2X7 receptor [9], the human P2X3 (hP2X3) receptor [10] and an invertebrate (Gulf Coast tick) P2X receptor [11] have been solved. The closed states of the P2X receptors share similar architectures in both the extracellular and the transmembrane domain. The open state models, however, are quite different, notably in the transmembrane region. The TMD for the hP2X3 structure is longer and more complete than that for the zfP2X4 receptor and both the M1 and M2 helices deviate less from a straight helix than those in the zfP2X4 models.

The question I ask here is how different packing patterns between M2 helices associate with different conformational states of the P2X receptor. In a previous study [12], I investigated the packing patterns of the M2 helices in different lipid environments, including POPC, 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC) and 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) bilayers. Changes in the pore geometry were interpreted as a result of knob into pocket packing, a concept discovered more than 50 years ago [13]. The study, however, failed to observe continuous open pores from the extracellular to the intracellular sides. Here I extended the previous study and found four major conformational states based on how the M2 helices pack together and geometric properties such as the orientations of the M2 helices. Packing in the extracellular half (from I335 to A347) of the M2 helix produces two closed conformations (C1 and C2), while packing in the intracellular half (from L348 to I359) produces an open (O1) and closed conformation (D1). These four conformational states are associated with four types of helix packing and these distinct packing patterns are suggested as the basis for the discrete nature of channel gating.

2 Methods

2.1 Model description

Currently the structural features of the modeled P2X receptor are described in terms of geometrical arrangement and inter-subunit packing of the M2 helices. The protein is oriented in such a way that the z-axis coincides with the membrane normal and the extracellular membrane side faces upward. The geometry (Fig. 1) of the M2 helices is specified by a translation parameter (r) and three orientation parameters: the tilt (θ), azimuth (γ) and axial rotation (ρ) angles. The definitions of r , θ , γ and ρ are dependent

Fig. 1 Definitions of the radial distance (r), the tilt (θ), azimuth (γ) and rotation (ρ) angles. For the definition of the tilt and azimuth angles, the M2 helix is represented by a vector $\mathbf{h}$ defined by J_{347} and J_{341} with directionality (A). The angle between z-axis and $\mathbf{h}$ is taken as the tilt angle. The projection of $\mathbf{h}$ on the xy-plane ($\mathbf{h}_{xy}$) is used for defining the azimuth angle. The center of the pore (O) is defined to be the geometric center of the triangle defined by J_{347} (B). A vector $\mathbf{r}$ is defined by joining J_{347} of an M2 helix and O with directionality. The length of $\mathbf{r}$ is defined to be the radial distance of the helix. The azimuth angle is measured as the rotation from $\mathbf{r}$ to $\mathbf{h}_{xy}$ around the membrane normal (z-axis). The rotation angle ρ of an M2 helix is defined as the axial rotation from a reference vector ($\mathbf{k}$) and a vector joining J_{347} and α_{347} (A, C), where $\mathbf{k} = \mathbf{h} \times \mathbf{z}$

on the definition of the helical axis. Several methods exist for finding the helical axis. Currently, the helical axis is simply defined by the axial projection of the C α atom:

$$J_i = \frac{\alpha_{i-1} + \alpha_i + \alpha_{i+1} + (\alpha_{i-2} + \alpha_{i+2})/2}{4}$$

where α_i represents the coordinates of the C α atom of residue i (Supplementary File 1). Based on the definition, the tilt angle of a helix is defined to be the angle made by the z-axis and the helical axis is represented by a vector ($\mathbf{h}$) from J_{347} to J_{341} (Fig. 1). The azimuth angle is defined by the vector projection of $\mathbf{h}$ in the xy-plane and a reference vector in the same plane. The reference vector is defined by J_{347} and the geometric center (O) of the triangle defined by J_{347} . The rotation angle (ρ) is measured between a reference vector and a vector defined by α_{347} and J_{347} . The radial distance (r) is simply defined to be the distance between J_{347} and O .

Global expansion or constriction of transmembrane pores is described by:

$$D\alpha(R) = \frac{L\alpha_{AB} + L\alpha_{BC} + L\alpha_{CA}}{3}$$

or

$$Dj(R) = \frac{Lj_{AB} + Lj_{BC} + Lj_{CA}}{3}$$

where R represents a residue and $L\alpha$ (or Lj) is the distance between the C α atoms (or their projections) of the residue between different subunits (namely, A, B and C). In the Results section, I chose $R = \text{L340, A344, A347 and L351}$.

To simplify the description of the positional relationship between the two subunits A and B, subunit A is said to be upstream if it is situated in the counterclockwise direction relative to subunit B when seen from the extracellular side and in this case, subunit B is said to be at a downstream position relative to subunit A.

The crossing (interhelical) angle is defined to be the angle between the axial vector of the upstream helix (**U**) and the axial vector of downstream helix (**V**). When M2 helices cross in the outer (extracellular, from I335 to A347) portion, **U** is defined from J_{343} to J_{339} and **V** is defined from J_{347} to J_{344} . When M2 helices cross in the inner (intracellular, L348 to I359) portion, **U** is defined from J_{350} to J_{347} and **V** is defined from J_{355} to J_{351} .

2.2 TMD models for MD simulations

As in the previous report [12], ideal M1 (from S28 to D59) and M2 helices (from K332 to L361) are individually relaxed in POPC bilayers and assembled into a tertiary structure based on the 4DW0 model. The residues D59 and K332 are connected to form a loop. The tertiary model is further relaxed in POPC bilayers and then assembled into a quaternary TMD model by aligning the C α atoms of L340, N341, A344, L346, A347, L348 and L351 to the correspondent atoms in the 4DW0 or the 4DW1 model (referred to as TMD-4DW0 or TMD-4DW1, respectively). The TMD-4DW0 and TMD-4DW1 are used as starting structures for MD simulation. In addition, a homology model is also built using the hP2X3 open state structure (PDB code 5SVK) as the template and the model is referred to as TMD-5SVK.

2.3 Residue contact and helix packing

The degree of contact between two residues *A* and *B* is measured by the sum of the attractive terms of the Lennard-Jones (LJ) potentials of all possible atom pairs between *A* and *B*:

$$R = \sum_i \sum_j V_{ij}$$

where

$$V_{ij} = 4\varepsilon_{ij} \left(\frac{\sigma_{ij}}{r_{ij}} \right)^6$$

$$\sigma_{ij} = \frac{1}{2}(\sigma_i + \sigma_j)$$

$$\varepsilon_{ij} = \sqrt{\varepsilon_i \varepsilon_j}$$

and where ε is the depth of the potential well and σ is the finite distance at which the LJ potential is zero when both the attractive and the repulsive terms are present. The values of ε_i and σ_j for specific atom types are taken from the CHARMM36 force field [14].

The packing strength of a residue to a helix is defined to be:

$$PR = \sum_{i=1}^{N_A} \sum_{j=1}^{N_B} V_{ij}$$

where N_A is the number of atoms on a residue of helix *A* and N_B is the number of atoms within helix *B*. Similarly, the packing strength between two helices *A* and *B* is measured by:

$$PH = \sum_{i=1}^{N_A} \sum_{j=1}^{N_B} V_{ij}$$

where N_A and N_B represent the numbers of atoms on helices A and B , respectively.

2.4 Molecular dynamics simulation

MD simulation was performed with GROMACS 5.1 and 2016 [15] based on the CHARMM36 force field. The LINCS algorithm [16] was used to constrain hydrogen bond lengths. The protein, lipid and ion-water were coupled separately to a v-rescale thermostat at 310 K for simulations with bilayers composed of DLPC, POPC, SOPC or 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), or at 330 K for simulations with the 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayer. A constant pressure of 1 bar was applied independently in the xy-plane (the lipid-plane) and the z directions with a coupling constant of 1-ps. Periodic boundary conditions were applied and LJ interactions were truncated at 1.2 nm. Electrostatic interactions were calculated using the particle mesh Ewald method [17] with a real space cutoff of >1.2 nm on an approximately 0.16-nm grid with fourth-order spline. The simulations were terminated when the root mean square deviation (RMSD) and PH values were stable (flat plateau) for at least 30 ns (Supplementary Fig. S1). Channel pores were analyzed using the program HOLLOW [18] by filling the interior of the protein with dummy atoms (1.4 Å radius) on a grid (spacing 0.3 Å).

3 Results

Four major types of helix packing were identified in 23 independent MD simulations (60–150 ns) of TMD models (TMD-4DW0, TMD-4DW1 and TMD-5SVK) embedded in different lipid bilayers (Table 1). They are associated with four major conformational states, including one putative open state. Transitions between the open state (O1) and the closed state (D1) were also observed. The results provide insight into the mechanisms of the P2X receptor gating in the transmembrane region.

3.1 The Apo, closed state with compact organization of M2 helices (the C1 state)

In the apo state models of the P2X receptor, the M2 helices cross in the outer portion at residue G343 that packs into a downstream pocket formed by A344, A347 and L348. In most simulations of the TMD-4DW0 model ($n = 10$ out of 17), the packing of G343 remains stable in different lipid environments [12]. A344 packs into an upstream pocket formed either by L339, L340 and G343 or by L340, G343 and A344 (Fig. 2A). The use of small side-chains for

Table 1 Numbers of independent MD runs of the TMD models in different lipid environments. Simulation runs were mostly performed in the POPC lipid, the major phospholipid present in eukaryotic cell membranes. In the open state models (TMD-4DW1 and TMD-5SVK), lipid invasion of the pore region were found and these runs were excluded in the analysis

	DLPC	DMPC	POPC	SOPC	DPPC
TMD-4DW0	4	4	5	3	1
TMD-4DW1	1		3		
TMD-5SVK			2		

packing produces compact organization of M2 helices, as reflected by large PH values (Table 2). In addition, L340 and L346 also pack into their downstream pockets and A347 and L348 pack into their upstream pockets (Fig. 3A). This type of packing (referred to as type 1) produces a closed state conformation (referred to as C1) in which the residue A347 closes the ion conduction pathway (Fig. 4A) and the L340 side-chain may also block the pore (Fig. 6A), depending on the side-chain orientation [12].

3.2 Closed state with rotated M2 helices (the C2 state)

In seven simulations, the TMD-4DW0 model develops a different packing pattern (Fig. 2B) in which the M2 helices rotate about -20° on average around the helical axis (Table 2). This rotation results in loss of G343 packing, brings L339 closer to the downstream helix and reduces or eliminates L340 packing (Fig. 3B; Supplementary Fig. S2). This type of packing (referred to as type 2) also produces a closed state conformation (referred to as C2) but A347 shifts away from the pore and L340 blocks the conduction pathway (Fig. 4B). The C2 state is highly variable in the tightness of packing and the radial distance is significantly increased compared to the C1 state ($P < 0.01$, Table 2).

3.3 Open state with loose packing of M2 helices in the inner TMD (the O1 state)

The zfp2X4 and hP2X3 open state models (4DW1 and 5SVK, respectively) lack interhelical packing between the M2 helices. When relaxed by MD runs, the TMD-4DW1 and TMD-5SVK models develop loose packing at L351 (Fig. 3C), which packs into an upstream helix region close to G343, L346 and A347 (Fig. 2C). This type of packing (referred to as type 3) produces an open state conformation (referred to as O1, Fig. 4C) with a large azimuth angle (95.2° , Table 2). The pore size is increased significantly in the outer region and is slightly

Fig. 2 Helical net representation of the major types of packing between M2 helices. The solid lines indicate the covalent bonds between neighboring residues in sequence, the dashed lines indicate hydrogen bonds between residues i and $i + 4$ for an ideal helix, and the dotted lines indicate possible side chain ridges formed between residues i and $i + 3$. **A** shows type 1 packing from a simulation with TMD-4DW0 embedded in a POPC bilayer. The defining feature of the packing is the use of G343 as a knob residue, which packs into a downstream pocket formed by A344, A347 and L348. **B** shows type 2 packing from a simulation with TMD-4DW0 embedded in a DMPC bilayer. The M2 helices rotate about -20° , leading to collective changes in the relative positions of residues between two helices. **C** shows type 3 packing from a simulation with TMD-4DW1 embedded in a POPC bilayer. The side-chain of L351 binds to an upstream region close to G343, L346 and A347. **D** shows type 4 packing from a simulation with TMD-4DW1 embedded in a POPC bilayer. The side-chain of L351 binds to an upstream pocket formed by A347, G350 and L351. Residues I355 and W358 also act as knob residues for packing

Table 2 Geometric and packing parameters obtained for different conformations. The organization of the M2 helices are also compared with those of the hP2X3 models. The azimuth angle is the key parameter defining the major states. The C1 and C2 states have comparable values of azimuth angles ($P > 0.1$); the azimuth angle of the O1 state is significantly different from the C1 or the C2 state ($P < 0.01$); while the D1 state has a significantly larger azimuth angle than the O1 state ($P < 0.01$). The C1 and C2 states are distinguished by the rotation angles ($P < 0.01$) as well as the packing patterns (See Fig. 2)

	Cross angle (deg)	Rotation angle (deg)	PH (kJ/mol)	Tilt (deg)	Azimuth (deg)	Radial distance (Å)	RMSD (Å)
C1 ($n = 30$)	70.2 ± 7.2	18.6 ± 9.8	145.8 ± 17.2	40.3 ± 6.0	61.9 ± 6.2	5.6 ± 0.3	3.25^a
C2 ($n = 21$)	71.3 ± 11.2	2.5 ± 21.3	119.1 ± 40.9	43.0 ± 10.8	56.7 ± 18.3	6.9 ± 0.9	3.51^a
O1 ($n = 9$)	74.1 ± 7.9	19.9 ± 3.4	60.2 ± 16.6	45.5 ± 5.9	95.0 ± 3.7	7.1 ± 0.3	2.82^b
D1 ($n = 6$)	67.2 ± 6.2	24.5 ± 19.4	125.2 ± 38.4	42.2 ± 9.3	107.0 ± 10.3	7.3 ± 0.7	3.00^c

For RMSD calculation, only the C α atoms are considered.

^a Calculated against the 5SVJ model.

^b Calculated against the 5SVK.

^c Calculated against the 5SVL model

decreased in the inner region (Fig. 5), producing a continuously open pore from the extracellular to the intracellular sides (Fig. 6B). The residues A347 form a narrow constriction and may act as an ion selectivity filter.

3.4 Closed state conformation with packing of M2 helices in the inner TMD (the D1 state)

In two simulations, a further increase in the azimuth angle (107.0° , Table 2) in the TMD-4DW1 model results in a tighter packing pattern (referred to as type 4, Fig. 2D) and a

Fig. 3 The PR values of residues 335–360 for the C1, C2, O1 and D1 states showed in (A), (B), (C), and (D), respectively. Graphs in the left column represent interactions of residues with downstream helices. Graphs in the right column represent interactions of residues with upstream helices. Values were collected at the end of the simulations. **A** The TMD-4DW0 model relaxed in a POPC bilayer for 60 ns. **B** The TMD-4DW0 model relaxed in a DMPC bilayer for 70 ns. The packing of G343 to the downstream helices is lost, and PR_{340} and PR_{346} are reduced compared to the values seen in type 1 packing. On the other hand, PR_{339} is increased. **C** The TMD-4DW1 model relaxed in a POPC bilayer for 150 ns. **D** the TMD-4DW1 model relaxed in a POPC bilayer for 150 ns

Fig. 4 Organization of key residues in different conformations. For clarity, the M1 helices are not shown. The side-chains of L340, A344, A347 and L351 are shown as sticks connecting the C α -C β atoms. L340 is in red, A344 in cyan, A347 in yellow, and L351 in blue. The C α atoms of G343 are shown as orange spheres. Images on the first and second columns are TMD snapshots viewed from the extracellular side, and the side views are presented in the third column. **A** The C1 state with type 1 packing. In this example, only A347 blocks the channel pore, while L340 points more outwards. **B** The C2 state with type 2 packing. Visual comparison of the position and orientations of side-chains shows that the M2 helices rotate clockwise in type 2 packing relative the type 1 packing. Surface representation shows that the side-chains of L340 close the pore. **C** The open conformation (O1) of the TMD-4DW1 model relaxed in POPC lipids. **D** A closed conformation (D1) with L351 and V354 occluding the pore. V354 is not shown

closed conformation (Fig. 4D), which may represent a desensitization state (referred to as D1). In this state, the conduction pathway is blocked by L351 and V354 and the L351 side-chain packs into an upstream pocket formed by A347, G350 and L351. Residues I355 and W358 also act as knob residues for packing (Fig. 3D).

Fig. 5 Pore sizes as measured by $D\alpha$ and Dj for residues L340, A344, A347 and L351. The C1 state has a narrow pore size in the outer portion of M2 helix bundles. In the O1 state, the pore size increases at the L340, A344 and A347 levels, but slightly decreases at the L351 level. In the D1 state, the pore size continues to increase at the L340, A344 and A347 levels, and shrinks at the L351 level

3.5 State transition

Conformational transition from the O1 to D1 states was observed (Fig. 7) in a simulation with a PCPC bilayer. This transition is associated with changes in azimuth angles, which involve a very fast (about 2 ns) transient increase of the azimuth angle of one M2 helix (subunit B) and a very fast transient decrease in the azimuth angle of another M2 helix

Fig. 6 Analysis of pore geometries of the C1 (A) and O1 (B) states using the program HOLLOW. The residue A340 is shown as sticks, L340, A347 and L351 shown as lines. In the C1 state L340 and A347 occlude the pore. In the O1 state, the pore size expands all the way from L340 to A347

Fig. 7 Changes in the azimuth angle (top panel) and helix packing strength (bottom panel) during transition from the O1 state to the D1 state. Letters indicate subunits

(subunit C) followed by an increase in the azimuth angle. The remaining M2 helix undergoes a slight increase in the azimuth angle. The helix packing is also increased during the state transition and the *PH* values at two interfaces reach levels (158.82 and 150.41 kJ/mol) comparable to those observed at type 1 interfaces, while the *PH* value for the third interface is 91.74 kJ/mol.

4 Discussion

In the resting state, the pore is narrow in the outer portion of the TMD and widens toward the extracellular side. This organization is very important for channel gating. The current data support a model of gating that changes primarily in azimuth angles of the M2 helices and produces an iris-like motion of the PLHs [7, 19, 20] and regulates the pore geometry (Supplementary movie 1). The increase in azimuth angles has three consequences. First, it dramatically widens the outer region of the TMD pore and slightly narrows the inner region of the pore, consistent with experimental studies [21–24] and an early normal analysis in which the constrictions at L340 and A347 are widened in a putative open state (See Fig. 2 of Du et al., 2012 [25]); second, it shifts the narrowest part of the pore to the inner portion of the TMD, altering the patterns of intersubunit interactions; third, it alters the relative position and orientation of residues, for example, the L351 side-chain splays away from the pore region in the C1 and C2 states (Supplementary Fig. S3), packs toward the upstream helices in the O1 state and lines the pore lumen in the D1 state.

The current data suggest that residues (L340 and A347) in the outer portion of the M2 helices are responsible for the resting state, while residues (L351 and V354) in the inner portion are responsible for the open and possibly the desensitization states. L351 seems to play dual roles in channel gating. On the one hand, L351 stabilizes the open state by interacting with the upstream pocket. On the other hand, L351, together with V354, blocks the conduction pathway in the desensitization state.

In hP2X3, a putative desensitization state is identified that is very similar to the D1 state in that the L351 equivalent residue V334 blocks the pore. The residue V337 (equivalent to V354) does not block the pore but forms a constriction, which is absent in the open state. It is highly possible that the P2X receptor has more than one desensitization state. If I denote D0 as the desensitization state from the hP2X3 crystal structure, then a simple linear transition scheme driven by changes in the azimuth angle can be written as:

This raises the question of how the C2 state fits the scheme. Compared to other states, the M2 helices at the C2 state rotate about 20° clockwise. It is not known how this rotation occurs and if this rotation is physiologically relevant. On the helical net representation, the knob residues that bind to the downstream pockets are separated from those that bind to upstream pockets (Fig. 8A). Changes in azimuth angles switch the packing sites between the outer and the inner portion of the M2 helices. On the helical wheel representation of an ideal M2 helix, these two groups of knob residues face different directions (Fig. 8B). For example, in the C1 state ($\rho = 18.6 \pm 9.8^\circ$), A347 faces more toward the pore, while in the C2 state ($\rho = 2.5 \pm 21.3^\circ$), A347 side-chains point more toward the extracellular side. It is highly possible that axial rotation is coupled to changes in the azimuth angle and plays a role in fine tuning the

Fig. 8 Knob residues in a helical net (**A**) and a helical wheel (**B**) representation of the M2 helix. The knob residues can be classified into two groups, those in the outer portion of the M2 helices, and those in the inner portion. The knob residue in the outer portion generally packs on downstream target pockets, while those on the inner portion pack in the opposite direction

position and orientation of residue side-chains for proper packing during channel gating (Supplementary movie 2).

Currently, ideal helices are used as building blocks for TMD models. It has been shown that the structures of transmembrane helices are well reproduced in an implicit lipid membrane model by Monte Carlo (MC) simulation [26] and MD simulation [27, 28]. Packing of modeled transmembrane helices by the Metropolis MC algorithm accurately predicts the structures of bacteriorhodopsin [29] and halorhodopsin [30]. An important implication of these studies is that the two-stage folding scheme [31] can be used for structure prediction by computational assembly of helical membrane proteins. At the time when the P2X TMD models in this study were built, the known models of the P2X receptors (such as 4DW0 and 4DW1) suffered from the problem of pronounced hydrophobic mismatch, which is suggested to be inconsistent with the thickness of native lipid membranes [19]. In the hP2X3R structures, both the M1 and M2 helices are less deformed from the ideal helix than the previously solved zfp2X4 structures, justifying the use of my approach in studying the mechanisms of channel gating. Computational assembly of protein models may enhance the sampling of the protein conformational space and provide important insight into the mechanism of channel gating.

From the *PR* values for different interfaces (interfaces A/C, C/B and B/A, Fig. 3), it is obvious that the conformations at different states are asymmetric. This asymmetry may simply arise due to geometric constraints that prevent tight symmetrical packing. Asymmetric motion of the pore-lining helices was caught by plunge-freezing of the heteromeric *Torpedo* nicotinic acetylcholine receptor [32].

A key question to understanding the function of ligand-gated ion channels is how ligand binding controls the gating process. Currently only the transmembrane regions are used for MD simulation, raising issues such as the suitability and limitations of the MD systems. Kinetic analysis of ion channels shows that the transition of conformational states depends on ligand binding, while the conformational states themselves are independent of ligand binding [33, 34]. Isolated transmembrane domains of the nicotinic acetylcholine receptor are crystallized [35] with a structural organization similar to the full length structure, especially for the pore lining M2 helices [36]. Furthermore, isolated TM regions of the human glycine receptor α_1 subunits spontaneously form pentameric Cl^- -conducting channels [37], justifying the use of isolated TMD systems for understanding the structural property of channel gating.

Acknowledgments This work was supported by a grant to G.H.L. from the National Natural Science Foundation of China (Grant No. 30972861).

Compliance with ethical standards

Conflict of interest The author declares no conflict of interest.

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