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# Structural modeling of human cardiac sodium channel pore domain

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The pore domain of human voltage-dependent cardiac sodium channel  $Na_v 1.5$  ( $hNa_v 1.5$ ) is the crucial binding targets for anti-arrhythmics drugs and some local anesthetic drugs but its three-dimensional structure is still lacking. This has affected the detailed studies of the binding features and mechanism of these drugs. In this paper, we present a structural model for open-state pore domain of  $hNa_v 1.5$  built using single template ROSETTA-membrane homology modeling with the crystal structure of  $Na_v Ms$ . The assembled structural models are evaluated by rosettaMP energy and locations of binding sites. The modeled structures of the pore domain of  $hNa_v 1.5$  in open state will be helpful to explore molecular mechanism of a state-dependent drug binding and help designing new drugs.

Keywords: Nav1.5 pore domain; three-dimensional structure; homology modeling; binding sites; local anesthetic drugs

### 1. Introduction

Voltage-gated sodium (Nav) channels are membrane proteins for electrical signaling in excitable cells and are related with cardiovascular and neurological diseases in humans (Bezanilla, 2006; Catterall, 2012). There are nine types of Na<sub>v</sub> channels (Na<sub>v</sub>1.1–Na<sub>v</sub>1.9) in the excitable cells of the cardiovascular and nervous systems (Goldin et al., 2000). The cardiac isoform of the Na<sub>v</sub> channel, Nav1.5 is the targets of anti-arrhythmics drugs and a subset of local anesthetic (LA) drugs for treating cardiac arrhythmias (Fozzard, Sheets, & Hanck, 2011). Since its three-dimensional structure is still lacking, this has affected the detailed studies of the binding features and mechanism of these drugs. In order to solve this problem, some efforts have been made to build the structural models of Nav1.5 channels (Lipkind & Fozzard, 2000; O'Reilly et al., 2012; Pless, Galpin, Frankel, & Ahern, 2011). Nevertheless, before the first Na<sub>v</sub> channel was determined experimentally (Payandeh, Scheuer, Zheng, & Catterall, 2011), all the models were built based on potassium (K<sub>v</sub>) channels (Lipkind & Fozzard, 2000; O'Reilly et al., 2012) in which the P-loop segments are very different from those of Nav channel. It is noted that the structure of Nav1.4 was modeled recently and used to clarify the interaction between Nav1.4 and toxins (Korkosh, Zhorov, & Tikhonov, 2014; Mahdavi & Kuyucak, 2014a, 2014b). However, Nav1.4 mainly expressed in muscle, while Nav1.5 is specifically expressed in cardiac tissue. So, it is necessary to model the Na<sub>v</sub>1.5 structure for investigating cardiac drugs.

Up to now, the crystal structures of three prokaryotic Na<sub>v</sub> channels, Na<sub>v</sub>Ab (Payandeh, Gamal, Scheuer, Zheng, & Catterall, 2012; Payandeh et al., 2011), NavRh (Zhang et al., 2012), Na<sub>v</sub>Ms (Bagneris et al., 2014; McCusker et al., 2012), and a eukaryotic Nav channel Na<sub>v</sub>PaS (Shen et al., 2017) have been determined. Using the first two as templates, some structural models of mammalian Na<sub>v</sub> channel have been built. For examples, the counterpart mammalian Nav channel of NavRh was modeled by mutating the key residues at the constriction sites in order to illustrate the mechanism of Na<sup>+</sup>/K<sup>+</sup> selectivity in mammalian Nav channels (Xia, Liu, Li, Yan, & Gong, 2013). Using NavAb as the template, a closed-state Nav1.5 homology model was constructed to illustrate the interaction site of Bisphenol A and Na<sub>v</sub>1.5 (O'Reilly et al., 2012). However, no open-state structure of human  $Na_v 1.5$  (h $Na_v 1.5$ ) is available at present. It is noted that among the determined structures, only NavMs (pdb id: 4CBC) is in open conformation but it is comprised of four identical subunits, rather than four connected non-identical domains in mammalian Nav channel (Payandeh et al., 2011).

Each of  $Na_v$  channels consists of four domains (D I to D IV) and each domain has six transmembrane segments. The first four transmembrane segments (S1 to S4) of each domain compose voltage sensor domain (VSD), while other two segments (S5 and S6) and the pore loop (P-loop) between them form a central ion-conducting pore domain (PD) (Payandeh et al., 2011; Shaya et al., 2011, 2014). Similar to other voltage-gated ion channels,

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the conformations of  $Na_v$  channel alternates between open and closed states in response to the movements of VSD, which are transduced via a linker peptide located between PD and VSD to a gate located within PD.

In the present paper, we report a structural model of open-state PD of  $hNa_v 1.5$  channel constructed using the crystal structure of  $Na_v Ms$  as a template through a multistep protocol. By docking open-state LAs to the modeled structure, we find that their binding sites are localized in the inner pore of the channel, where the drugs interact mainly with sites in S6 of D IV. This is consistent with the previous experimental results.

## 2. Material and methods

Open-state structures of hNav15 PD were modeled using a multi-step protocol (Figure 1).

## 2.1. Structural modeling

The ROSETTA-membrane homology modeling method (Chaudhury, Lyskov, & Gray, 2010; Subbotina et al., 2010) were used to model the PD structure of  $Na_v 1.5$  channel in open state. Homology, *de novo*, and full-atom modeling of four domains of the PD were performed using single template ROSETTA-membrane modeling

protocol (version 3.4) with the crystal structure of  $Na_vMs$  (PDB id: 4CBC). The models of each of the four domains were selected in two steps: Firstly, 10,000 models were generated using single template ROSETTA-membrane modeling protocol and clustered with a cut-off of 3.4 Å (5.1 Å for domain 1). Secondly, the models with lower free energy and score (Yang & Zhou, 2008a, 2008b) were selected from each of the largest models. Then, aligning these selected models to the corresponding domain of their templates by a structural alignment algorithm TM-align (Zhang & Skolnick, 2005). These assembled structures were clustered as above and all of the structures in the largest clusters were kept for further refinement.

### 2.2. Molecular dynamical simulation

The modeled structure was defined as the initial structure and the simulation system was prepared using Charmm-GUI (Lee et al., 2015). Default protonation states were used for all the ionizable residues. The protein was oriented using the PPM server (Lomize, Pogozheva, Joo, Mosberg, & Lomize, 2012) along Z-axis and the oriented PDB file was then embedded in a homogeneous palmitoyl-oleoylphosphatidylcholine (POPC) bilayer and solvated by 10 Å water molecules on both sides of the



Figure 1. Homology modeling scheme used for building hNav1.5 structural models. Note: Steps in the dashed box were done by ROSETTA3.4 software package.

membranes (Figure 2).  $Na^+$  and  $Cl^-$  ions were added to the solution to obtain an electro-neutral system. Finally, the system contains totally 122,949 atoms, including the 8,409 heavy atoms of protein, 801 POPC lipid molecules, 40,993 water molecules, 69  $Na^+$  ions, and 55  $Cl^$ ions.

All MD simulations were performed by the software package Amber14 using the lipid14 force field for phospholipids (Dickson et al., 2014) and the TIP3P for water molecules (Jorgensen, Chandrasekhar, Madura, Impey, & Klein, 1983). Before MD simulation, the systems were equilibrated in four steps: first of all, fixed the protein, while allowed water and lipid to equilibrate for 2 ns. Secondly, the protein alpha carbons were then restrained by a harmonic potential with force constant reducing gradually from 10 to .1 kcal/mol in four steps and each step runs 10 ns. The formal simulations were carried out for a total of 260 ns at constant temperature (310 K) and pressure (1 bar) conditions maintained using a Langevin Piston and Langevin dynamics, respectively. To avoid artifacts, the MD simulations were run twice with different starting atomic velocities. The resulting trajectories were analyzed using the cpptraj module of the AMBER14 package. Pore radius profiles were calculated with the code MOLE 2.0 (Sehnal et al., 2013).

# 2.3. Molecular docking

To determine the binding sites of open-state LA drugs, protein-ligand interactions were predicted by Autodock4.2 program (Morris et al., 2009). In this study, we downloaded the spatial structures of pilsicainide, flecainide, cocaine, and ranolazine, the open-state LA drugs, from ChemSpider (https://www.chemspider.com/), and then used these drugs as ligands to do three indepen-



Figure 2. System used for MD Simulations of  $Na_v 1.5$  PD:  $Na_v 1.5$  PD (in spectrum cartoon) embedded in a POPC lipidbilayer (in green stick). Sodium ions are purple and chloride ions are green.

dent dockings. For each independent run, Autodock Tools (ADT) was used for preparing molecules and all hydrogens were added using REDUCE (Word, Lovell, Richardson, & Richardson, 1999). All docking decoys were clustered with a cut-off of 2 Å according to root mean square deviation (rmsd) and the lowest-energy docked structures from each cluster were selected as the models of the docked ligand (MDL). The modeled structures were defined as receptors and the coordinates of AutoGrid center were determined by the center of the largest binding pocket, which was generated from receptor cavities by Discovery studio Visualizer 4.1 (Discovery Studio Visualizer 4.1, Accelrys Software Inc., San Diego, CA, USA, 2013). A grid box with the size of  $100 \times 100 \times 100$  points was built. The grid box includes almost the entire channel residues of the receptor. For drug molecules, they were defined as ligands. The procedure was carried out by considering the flexibility of the ligands, i.e. all rotational bonds were set as free.

## 3. Results and discussion

# 3.1. Sequence alignment

Among the Nav channels of known crystal structures only the PD of Na<sub>v</sub>Ms channel is in open state and so is selected as the template to model the PD structure of hNa<sub>v</sub>1.5. To perform homology modeling, a target-template sequence alignment is performed using ClustalW algorithm (Thompson, Higgins, & Gibson, 1994) with Na<sub>v</sub>Ms (PDB id: 4cbc) and also with the sequences of other known crystal structures NavAb (PDB id: 3rvy) and Na<sub>v</sub>Rh (PDB id: 4dxw)). The results show that the PD of hNav1.5 shares ~20.2% sequence identity with those of NavMs, NavAb, and NavRh. The sequence differs mainly in the outer-membrane parts since the sequence identity of the inner-membrane parts is about 27%. Therefore, the modeled structures of the innermembrane part should be reliable. Previous studies showed that at the innermost bend of each P-loop there is an amino acid responding for Na<sup>+</sup> selectivity (McCusker et al., 2012; Payandeh et al., 2011; Zhang et al., 2012). The sequence alignment shows that residues D372, E898, K1539, and A1891 in hNav1.5, the purple ones in Figure 3, form the selective filter (SF) (Tikhonov & Zhorov, 2012). At the same time, residues E375, E901, M1542, and D1894 constructed the outer mouth vestibule, which is in agreement with the experimental results (Heinemann, Terlau, Stuhmer, Imoto, & Numa, 1992; Terlau et al., 1991).

# 3.2. PD models of hNa<sub>v</sub>1.5 channel in open state

The ROSETTA-membrane-homology modeling method was used to model the four domains (D I-D IV) of



Figure 3. Multiple sequence alignment for pore domains of  $hNa_v1.5$  and three  $Na_v$  channels with known structures. Note:  $hNa_v1.5$  I–IV (D I–D IV) are four domains forming the pore domain. Secondary structure elements for  $Na_vAb$  are indicated in blue. The relative accessibility of each residue is rendered as blue boxes from dark blue (accessible) to white (buried residues). S5 and S6 helix in pore domain were shown in blue helix on top. The conserved selectivity filter residues D372/E898/K1539/A1891 (D127/E235/K366/A477 in  $hNa_v1.5$  PD) are in yellow and the region of outer mouth vestibule E375/E901/M1542/D1894 (E130/E238/M369/D480 in hNav1.5 PD) are in green. The presentation of the sequence alignment was made using the online service of ESPript3.0 (Robert & Gouet, 2014).

 $hNa_v 1.5$  PD. For each of the domains, its models were constructed as follows: Firstly, the domain was aligned to the corresponding chain of  $Na_vMs$  by HHpred program (Soding, Remmert, & Biegert, 2006). Figure S1 shows that the insertion and deletion are mainly in P-segments but not in S5 and S6. Secondly, 10,000 decoys were generated based on the alignments and two models were selected through clustering and scoring (Rosetta energy and dDEFINR scores for members in the largest clusters are shown in Table S1). Then, the

selected models were refined and checked using Ramachandran plots (Figure S2). It is found that the helices S5 and S6 of the selected models are nearly the same and the main differences occur in the region of P-segments (Figure 4). The Ramachandran plots for the selected models of each domain are shown in Figure S2 and the residue dihedral angles of all selected models are located in the allowed regions. Finally, the refined models of the four domains are assembled to generate 16 PD models for further analysis.



Figure 4. The structure alignment and comparison of selected models for (A) D I, (B) II, (C) D III, and (D) D IV. Note: The two selected models of each domain were colored green and blue, respectively.

### 3.3. Model evaluation

The generated 16 models of  $hNa_v 1.5$  PD structure can be evaluated using rosettaMP (Alford et al., 2015). The results are given in Table S2. It is found that open16 has the lowest energy. In fact the structures of the first four lowest energies are very similar and can be considered as belonging to the same cluster. Using this modeled structure as the initial structure, 300 ns MD simulation were taken. The backbone RMSD of the conformation showed a rapid increase to 7 Å and then fluctuated around 7 Å (Figure 5(A) black), indicating the model of open16 attained equilibration during MD simulation. In fact, the RMSD of structure without outer-membrane Ploops fluctuated around 3.8 Å after 100 ns (Figure 5(A) red). This result demonstrated that during the whole dynamics simulation, the fluctuation of RMSD is mainly due to the flexibility of P-loops in the outer membrane, especially that of the long P-loop of the domain D I (Figure S3). The equilibrated structure of open16 is shown in Figure 5. The Ramachandran plot for this model was also calculated and almost all residue dihedral angles were located in the allowed regions (Figure 5(B)). The cavity through the center of the channel is formed primarily by the C-terminal S6 transmembrane helices from the four domains (Figure 5(C) and (D)). Plots of channel radius versus distance along the channel direction (Figure 5(E) and (F)) for hNav1.5 PD structure show that in the region of the activation gate the diameter of the final simulation structure is 8.40 Å. This size is large enough for a hydrated sodium ion (roughly 7.8 Å in diameter) to pass through.

Similar to Na<sub>v</sub>PaS, the selective filter of  $hNa_v 1.5$  PD is enclosed by the side groups of the signature residues Asp127/Glu235/Lys366/Ala477 at the upper position and the carbonyl oxygen atoms of the two preceding residues in each repeat at lower level (Figure 6(A) and (B)). Above these four SF residues, Glu130/Glu238/Met369/Asp480 formed the outer negative ring that guards the entrance to the SF vestibule. Compared to Na<sub>v</sub>PaS, the differences are mainly in the orientation of residue K366 and A477 (Figure 6(C)).

The evaluation above is from the point of view of energy and structural details. To further validate the selected model open16, it's better to have experimental information of hNav1.5 PD structure but no such information is available at present. However, previous studies revealed that open-state LA drugs bound to the inner vestibule of the hNa<sub>v</sub>1.5 PD (Lipkind & Fozzard, 2005) and a series of residues in the S6 chains of D III and D IV that are important for block, including Leu1462 in S6 of D III and Phe1760 and Try1767 in S6 of D IV (Li, Galue, Meadows, & Ragsdale, 1999; Nau, Wang, & Wang, 2003; Ragsdale, McPhee, Scheuer, & Catterall, 1994). So, we see if the binding sites of the four openstate LA drugs (pilsicainide, flecainide, cocaine, and ranolazine) on open16 are consistent with the experimental results. This can validate the model open16 indirectly.

In order to answer this question, we counted the interaction sites of the model open16 with the four drugs. In all of three independent docking, there were 64, 112, 75, and 134 poses and were generated for pilsicainide, flecainide, cocaine, and ranolazine separately. We defined that LA and Na<sub>v</sub>1.5 are in contact when the distances between their closest carbon atoms are less than 4 Å. Then, we calculated the percentage of contact times for each residue with the corresponding LAs in all of the considered poses. The sites occurring larger than the average times are listed in Table 1. For the four drugs, the binding sites Phe526 (F1760 in Na<sub>v</sub>1.5 $\alpha$ ) and Tyr 533 (Y1767 in Na<sub>v</sub>1.5) in S6 chain of D IV were proved to be the critical binding sites of pilsicainide



Figure 5. Representation and structure characters of open16 after refinement (A) RMSD of C $\alpha$  atoms of structure with (black line) and without (red line) outer-membrane P-loops through 300 ns MD simulation, (B) Ramachandran plot, (C) the side view, and (D) the top view of the whole structure. All of the two figures were generated by pymol (DeLano, 2002), colored by chain (green for D I, blue for D II, purple for D III, and yellow for D IV). (E) the accessible inner surface (in blue) of the Nav1.5 pore model. Structure was shown as ribbon diagrams. (F) Plot showing the internal dimensions of the final simulation structure.



Figure 6. The selectivity filter (SF) of the model open16. (A) The SF vestibule. SF residues D/E/K/A shown in stick and colored by chain, the two preceding residues in each repeat were colored by element. The residues that constitute the negative ring above the SF vestibule were also colored by element. (B) Structures of DEKA from top view were shown. (C) Structural variations of the selectivity filter between hNav1.5 PD (green) and NavPaS (blue).

Table 1. Binding sites of the pore domain of hNav1.5 channel with four open-state drugs.

Pilsicainide			Flecainide			Cocaine			Ranolazine		
AA	seqNo	Percent (%)	AA	seqNo	Percent (%)	AA	seqNo	Percent (%)	AA	seqNo	Percent (%)
THR	125	59.38	THR	125	66.07	THR	125	57.33	THR	125	63.43
GLN	126	43.02	GLN	126	64.29	GLN	126	41.33	GLN	126	49.25
GLY	234	36.05	ILE	163	31.25	GLY	234	32.00	VAL	160	38.81
PHE	365	56.98	GLY	234	45.54	TRP	236	29.33	ILE	163	40.30
LYS	366	27.91	TRP	236	34.82	THR	364	30.67	GLY	234	41.04
TRP	368	25.58	PHE	271	36.61	PHE	365	58.67	PHE	271	51.49
MET	369	24.42	PHE	365	82.14	LYS	366	34.67	PHE	365	90.30
SER	476	26.74	LYS	366	41.07	TRP	368	34.67	LYS	366	38.81
PHE	526	36.05	TRP	368	41.07	THR	475	30.67	TRP	368	37.31
TYR	533	24.42	THR	475	37.50	SER	476	38.67	PHE	406	38.81
			SER	476	46.43	PHE	526	29.33	SER	476	40.30
			PHE	526	69.64	TYR	533	25.89	ILE	522	19.40
									PHE	526	75.37
									VAL	530	29.10
									TYR	533	32.84

Note: "AA", "seqNo" and "Percent" denote the name of binding-site residue, its sequence number and the percentage of its occurrence in the three independent docking, respectively.



Figure 7. Interactions between LAs and  $hNa_v 1.5$  PD. All of the structures were generated using pymol. Note: The interaction diagrams (column A) were calculated and shown by Discovery Studio client (version 4.1). S6s in four domains (column B) are shown in cartoon and colored by spectrum and the binding sites are shown in sphere and colored by spectrum.

(Desaphy et al., 2010), flecainide (Carboni, Zhang, Neplioueva, Starmer, & Grant, 2005; Nau & Wang, 2004), cocaine (O'Leary & Chahine, 2002), and ranolazine (Chan et al., 2012; Fredj, Sampson, Liu, & Kass, 2006; Huang, Priori, Napolitano, O'Leary, & Chahine, 2011; Kim & Chan, 2012). These binding sites are indeed, included in our results (Table 1). Meanwhile, from the interaction diagrams (Figure 7), we can see that there are strong non-covalent Pi-Pi and Pi-sigma interactions between LAs and binding sites Phe526 and Y533. These two interactions make the LAs and hNa<sub>v</sub>1.5 interact strongly. This result suggests that Phe526 and Y533 play more important roles in the combination of local anesthetics and cardiac sodium channels hNav1.5. Previous studies have shown that F1760 (F526 in hNav1.5 PD) formed a cation-pi interaction with lidocaine and mexiletine, but not flecainide or ranolazine. However, we didn't find any cation-pi interaction with the F1760 residue. Furthermore, Y1767 (Y533 in hNav1.5 PD) did not form a cation-pi interaction with class I anti-arrhythmics (Pless, Galpin, Frankel, & Ahern, 2010). Here, we investigated the interactions between LAs and Nav1.5. The interaction diagrams (Figure 7) showed the existence of the Pi-Pi and Pi-sigma interaction between LAs and conserved aromatic side chains of Phe526 and Y533 in Na<sub>v</sub>1.5 PD. Therefore, the modeled open-state structure (open16) of hNav1.5 PD can capture some important features of its interactions with the open-state drugs and this again indicates its rationality.

# 4. Conclusion

In this paper, we constructed a structural model of the pore domain of the mammalian  $Na_v1.5$  channel in open state using bacterial sodium channel  $Na_vMs$  as a template. The mammalian  $Na_v1.5$  channel is homologous to bacterial  $Na_v$  channels but with four non-identical domains. We used a multi-step protocol to produce the models and evaluate them using the rosettaMP energy and locations of binding sites of the open-state LA drugs. The modeled structure captured certain important features of the experimental results and will be helpful to study the mechanism of interaction of local anesthetic drugs with  $Na_v1.5$ .

## Abbreviations

Na <sub>v</sub>	voltage-gated sodium
VSD	voltage sensor domain
PD	pore domain
LA	local anesthetic
K <sub>v</sub>	voltage-gated potassium
MDL	models of the docked ligand

#### **Disclosure statement**

No potential conflict of interest was reported by the authors.

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#### Supplementary material

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