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COMMENTARY

Voltage-Gated Na Channels

How could simulations elucidate $Na_v1.5$ channel blockers mechanism?

Tanadet Pipatpolkai[1]

Tao and Corry used metadynamics, an enhanced sampling method to identify and classify Na_v channel blockers.

Ion channels are a class of membrane proteins that enable ion permeation across the membrane, controlling cellular and organelle excitability. Voltage-gated sodium (Na_v) channels are involved in our day-to-day physiology, including pain, propagation of nervous signals, and our heartbeat. Na_v channels are large monomeric membrane proteins of ~2,000 amino acids, organized into four distinct domains (DI, DII, DIII, and DIV) with fourfold pseudosymmetry. Each domain consists of six transmembrane helices (S1–S6), where four are voltage sensor modules (S1–S4), and S5–S6 contribute one-fourth of the pore modules (Catterall, 2014). For decades, efforts from electrophysiology, structural studies, and, very recently, computational approaches have been harnessed for the pharmacological design of pore blockers for Na_v channels, such as in anesthesia, epilepsy, or anticonvulsants (Roden, 2014).

Let me take you back to the discovery of the first Na_v channel in the Hodgkin and Huxley era in the 1940s–1950s when the word "pore blocker" was first introduced in the context of sodium and potassium channels (Catterall et al., 2012). We would imagine that a pore blocker for cation channels would have similar physiochemical features to the permeant ions, i.e., carry a positive charge so that it can block the entry of the ion to the pore—almost like a cork to the bottle of champagne. These blockers, such as tetrodotoxin, a toxin from puffer fish, or saxitoxin from the marine dinoflagellates or freshwater cyanobacteria, block the Na channel non-selectively from the extracellular side (Lipkind and Fozzard, 1994). Several pore blockers block the pore intracellularly, and these molecules are likely to be transitioned between charged and uncharged states; otherwise, they will not be able to cross the membrane (Yue et al., 2019). These intracellular blockers must be hydrophobic enough to cross the membrane and charge enough to be specific to their binding site under the selectivity filter from the cytoplasm (Starmer et al., 1984). The other mechanism is that the pore blockers enter the channel pore module through the membrane and bind at the lateral side of the ion channels via window-like tunnels appropriately dubbed "fenestrations"—preventing ion channels from opening or occluding the pore or both (Gamal El-Din et al., 2018). In this issue of *JGP*, Tao and Corry (2025), have studied nine Na_v channel blockers in multiple protonation states and revealed their blocking mechanisms using molecular dynamics simulations. This study not only highlights the importance of Na_v channel blockers but also shows the fascinating application of computational methods—particularly metadynamics—to enhance the sampling of the pore blocker and obtain the binding site of the drugs in the $Na_v1.5$ channel.

Computational approaches to the study of pore blockers

Both X-ray crystallography and cryo-EM have provided their share to the communities of Na_v channel structures—the very first of which was Na_vAb channel—the bacterial Na_v channels from *Aliarcobacter butzleri* in 2017 (Lenaeus et al., 2017), to the very recent rat $Na_v1.5$ channel with the bound pore blocker, flecainide—an antiarrhythmic drug solved in 2020 (Jiang et al., 2020). But what can we do with these structures, and how far can we extrapolate from them? Back in the early 2010s, when there were few mammalian K_v channels or Na_v channels structures, homology models based on bacterial structures were used as the initial point for docking studies. In docking studies, the protein backbone is usually kept rigid with flexibility in the side chains and the conformation of the drug molecules. The conformation of the drugs is then sampled within the docking space, usually defined as a cubic box, and the binding energy is then calculated to generate a docking score. The method is reliable when the binding site is known from electrophysiology, for example—in the alanine screening of the binding site of the pore blocker S-bupivacaine for the hERG channel (Siebrands and Friederich, 2007; Dempsey et al., 2014).

[1]Division of Physics and Applied Physics, School of Physical and Mathematical Sciences, Nanyang Technological University, Singapore, Singapore.

Correspondence to Tanadet Pipatpolkai: tanadet.pipatpolkai@ntu.edu.sg

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A major advancement in the 2010s was the increase in computational power due to GPU acceleration and MPI parallelization. This opened novel avenues for molecular dynamics (MD) simulations to gain more insight into the binding modes and mechanisms of pore blockers, as we can then probe both where they might bind but also how they get there. In addition, MD simulations are usually conducted in the explicit solvent and lipids environment, which allows the role of lipids near the binding site to be studied. Thus, MD simulations have been used to gain insights into pore blocker binding on an ion channel structure. With MD simulation, the drug is initially docked near the binding site; then, an unbiased simulation is conducted to equilibrate it within the site. This approach was used to highlight two possible binding sites of lidocaine on the generated model of the human $Na_v1.5$ channel (Nguyen et al., 2019). However, the approach requires several microseconds of simulation time. Another common approach is to flood the simulation box with the drug and conduct multiple equilibrium simulations to obtain the binding site, which has successfully obtained the allosteric site for Yoda1 on the Piezo1 channel (Botello-Smith et al., 2019).

The bottleneck of MD simulation is the timescale due to the time cost of the long simulation. Thus, enhanced sampling methods, such as metadynamics, have been used to study pore blocker affinity and binding mode. In this study, Tao and Corry (2025) employed metadynamics to increase the exploration of the blocker within the pore module of the rat $Na_v1.5$ channel. Metadynamics gradually increases the sample by slowly titrating external forces along the collective variable—i.e., an expected parameter that defined the slowest mode of motion during the sampling process. In this case, Tao and Corry used the x, y, and z positions of the drug's center of mass along the pore's central axis. This approach allows the drug molecule to sample the pore cavity and its fenestration site thoroughly. The common binding poses were then clustered and ranked based on the size of the population within the cluster. Thus, the most explored conformation is the most common pose, which should reflect the observed structural and experimental data. By placing the drug in the cavity, their simulations have reliably captured the binding configuration similar to those observed in the cryo-EM structure of flecainide in rat $Na_v1.5$ channel (Jiang et al., 2020). By comparing the binding pose from the protonated state and the deprotonated state, they observed that the protonated flecainide adheres more closely to the experimental structure, thus highlighting that the structure of the drug observed by cryo-EM is likely to be in the protonated state.

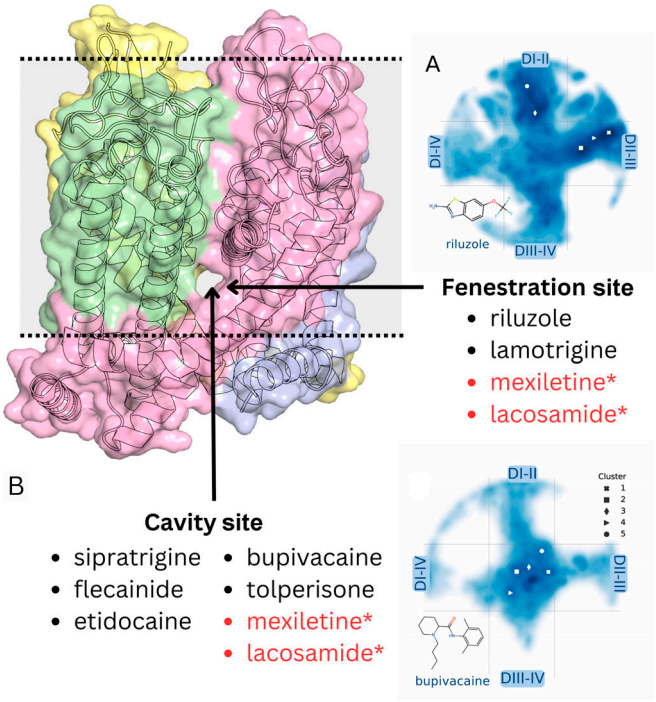

Figure 1. **Location of the binding sites of rat $Na_v1.5$ channel pore blockers through the cavity site and/or the fenestration site.** The structure of the pore module of rat $Na_v1.5$ (left) is shown as a surface. **(A and B)** Free-energy surface extract from Tao and Corry (2025) from metadynamics sampling using riluzole (A) or bupivacaine (B). Drugs that bind solely from the fenestration site or cavity site are listed in black, whereas the drugs that can bind from both sides are denoted in red.

Three distinct mechanisms of the pore blockers

Later on, Tao and Corry (2025) extended a similar use of metadynamics to identify binding sites of eight other Na_v channel blockers—riluzole, lamotrigine, mexiletine, lacosamide, tolperisone, etidocaine, patriline, and bupivacaine, where mexiletine, etidocaine, and bupivacaine were simulated both in their protonated and deprotonated state. Their simulations have clearly shown that sipratrigine and both the protonated and deprotonated state of flecainide, etidocaine, and bupivacaine occupy the pore of the channel. The hydrophobic moiety of the blockers interacts with the hydrophobic residues at the bottom of the S6.

The smaller hydrophobic drugs, riluzole and lamotrigine, are, on the other hand, shown to occupy the fenestration site. These sites are located at the interfaces between pore helices, more commonly between DII and DIII of the Na_v channel. Interestingly, when the site is unoccupied by the blocker, the site is occupied by the tails of membrane phospholipids. Thus, we can simply explain that the blockers displace the lipid tails and occlude the pore. Interestingly, they have observed a mixed binding mode where lacosamide and mexiletine bind both at the fenestration site and at the pore. When mexiletine is protonated, the pore site is more occupied, whereas, in the deprotonated state, the binding site is more favored than the DI–II fenestration site (Fig. 1). This observation further highlighted the relationship between the protonation state of the molecules and their binding site on $Na_v1.5$ channels.

Apart from the hydrophobicity of the compound, it is important to ask questions about other chemical features of the pore blocker. The interesting observation shows that the lower molecular weight drugs (mexiletine, lamotrigine, lacosamide, and riluzole) have more flexible orientation within the pore and are also able to squeeze themselves into the DI–II and DII–III fenestration site. Tao and Corry (2025) have also observed that

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the more hydrophobic the drug, the more likely they are to be stably bound to the Na_v channel cavity.

Future perspectives

Despite having the power of computational tools as a method to reveal the binding site of ion channel blockers, this still leaves gaps as the cryo-EM structures and the simulations do not always agree. The recent structures of riluzole and lamotrigine on $Na_v1.7$ channels (Huang et al., 2023) show a binding site different from the one obtained from the metadynamics simulation. In the simulations without the drug, the site is occupied by lipids; thus, the cryo-EM structure, which has been solved in detergent, may not reflect the binding site from the simulation studies. Functional studies pairing mutagenesis and electrophysiology are needed to provide a verdict on whether the binding site obtained from simulations or structural studies is true and will be crucial to paint the complete mechanism of Na_v channel inhibition.

Multiple hydrophobic molecules—including plant alkaloids and cannabinoids—are known to be pore blockers for both potassium and sodium channels. These compounds show potent channel inhibition and are hydrophobic–suggesting they block these channels intracellularly or via channel/bilayer interfaces. The computational approach developed by Tao and Corry (2025) would greatly benefit our understanding of the binding conformation of novel pore blockers and their path of access to the pore region. Indeed, the method only provides the geometry of the binding site, which leaves us with the question of the affinity of the blockers to the channel and how we may translate the free energy from the simulations to reflect the experimental IC_{50} values.

To conclude, Tao and Corry have beautifully demonstrated the power of the metadynamics and MD simulations to gain insight into $Na_v1.5$ channel inhibition by pore blocker and correlate the effect of protonation, hydrophobicity, and the size of the blockers to three different modes of binding—"cavity block" where the drug binds in the center of the pore, "fenestration block," where it binds in the lateral fenestrations, and "versatile block" where the drug can occupy either location. These three modes of understanding will propel the development of antiarrhythmic drug screening and other pharmacological targets of the $Na_v1.5$ channel. More importantly, this well-developed tool will shed light on understanding other blockers' interaction with ion channels, leading to a greater knowledge of general pore blocker pharmacology.

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