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COMMENTARY

Voltage-Gated Na Channels

Understanding the role of mutations in voltage-gated sodium ion channels for cardiovascular disorders

Christian Jorgensen[1]

Elhanafy et al. used Molecular Dynamics simulations and electrophysiology to show how identical mutations in the volgage sending domain of sodium channels can yield differential functional effects.

In this issue of the *Journal of General Physiology*, authors from the University of Mississippi led by Eslam Elhanafy and colleagues in the group of Prof. Jing Li use a combination of electrophysiology and computational modelling to suggest that identical mutations at equivalent positions in voltage-sensing domains (VSD) of the $Na_v1.5$ channel can lead to diverse functional effects. By using multi-microsecond molecular dynamics simulations, they suggest that specific structural dynamics of the VSD, as well as gating-pore conformations, can explain these differential impacts from mutations (Elhanafy et al., 2025). Such results can have implications for the development of new cardiovascular disorder therapeutics.

The study of voltage-gated sodium ion (Na_v) channels remains a fruitful area of research dating back decades. It was Hodgkin and Huxley who discovered the sodium current that initiates the nerve action potential in their model from 1952 (Hodgkin and Huxley, 1952), and since then, significant progress has been made toward obtaining high-resolution Na_v structures to better understand the relationship between structure and function in disease. One question that persists is how mutations in the Na_v voltage-sensing apparatus play a role in excitability disorders of cardiac, muscular, and neural tissues. Solving this question carries significant implications for treating certain cardiovascular diseases.

These data are the result of the ion channel community's efforts to generate high-quality structural PDB models, which have enabled such mechanistic studies as that of Elhanafy et al. The community has solved high-resolution crystal structures of bacterial Na_v channels (Payandeh et al., 2011) as well as cryo-EM structures of eukaryotic sodium channels from nerve and skeletal muscle (Jiang et al., 2020; Pan et al., 2018; Shen et al., 2019), including the cardiac Na_V channel $Na_V1.5$ (Jiang et al., 2020).

It is the cardiac Na_v channel $Na_v1.5$ that is the subject of the work of Elhanafy et al. (2025). This channel is the predominant one in myocardial cells (Rogart et al., 1989; Fozzard and Hanck, 1996), which comprise the majority of the heart's atrial and ventricular chambers and are responsible for the contractions that pump blood through the body. For this reason, mutation-induced dysfunction of $Na_v1.5$ is known to cause dangerous cardiac arrhythmias (Liu et al., 2003). To give an example, disruption of the activation and inactivation of $Na_v1.5$ has been linked to Brugada syndrome type 1 (Kapplinger et al., 2010), long QT syndrome (Millat et al., 2006), cardiac conduction system dysfunction (Li et al., 2018), and dilated cardiomyopathy among others. The community is therefore focused on mapping out an atomic-level understanding of the role of disease-causing mutants in $Na_v1.5$ for the purposes of developing novel therapeutics. Amiodarone, for example, is an antiarrhythmic drug that is thought to target $Na_v1.5$ (de Lima Conceição et al., 2023).

To understand the pathogenicity of VSD mutations, a significant amount of work has first been put into understanding how the VSD is structured and functions. The VSD is the part of the structure responsible for sensing the changes in potential difference across the membrane, as well as initiating the process of Na_v activation and inactivation. It contains a set of highly conserved positively charged residues located at every three positions on the S4 segment (Yarov-Yarovoy et al., 2012; Choudhury et al., 2022), colloquially known as gating charges. A

[1]School of Medicine, Pharmacy and Biomedical Sciences, Faculty of Science & Health, University of Portsmouth, Portsmouth, UK.

Correspondence to Christian Jorgensen: christian.jorgensen@port.ac.uk

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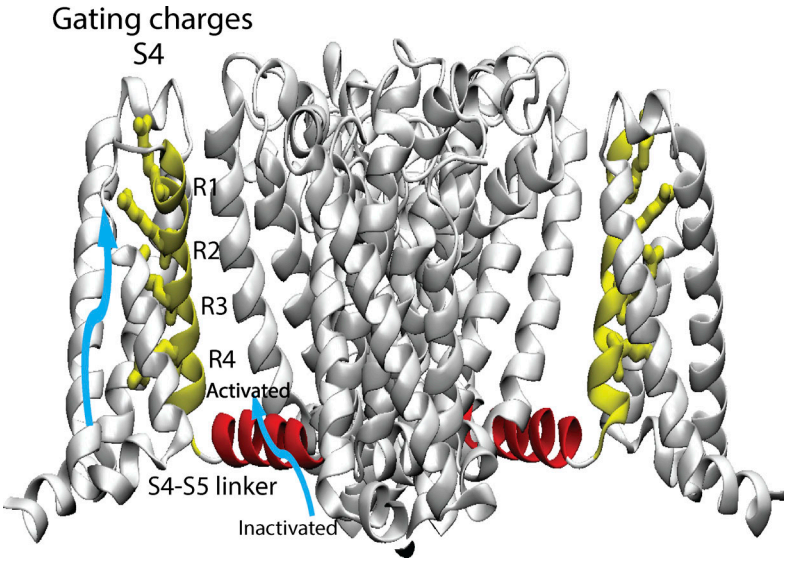

Figure 1. **The $Na_v1.5$ voltage-sensing domain and its associated S4–gating charges (shown in yellow), as well as the S4–S5-linking loop (red) as investigated by Elhanafy et al.** Depicted is the conformational change that Na_v channel S4 undergoes from the inactivated state with the S4 in a down state to the activation process in which the S4 moves to an up state (movement indicated with an arrow) during depolarization. In this work, Elhanafy et al. (2025) systematically compared the differential impacts of R-to-Q–gating charge mutations at the R1 to R3 positions.

set of negatively charged residues in the S1 to S3 segments form complements to the gating charges. Both the gating charges and complementary negative charges are highly conserved across the Na_v families (Groome and Bayless-Edwards, 2020; Choudhury et al., 2022), and as such, any study of these holds implications throughout the wider Na_v families.

The VSD plays a central role in the activation cycle of Na_v channels, in which the channel under a regular resting membrane potential is in an inactivated state with the S4-gating charges positioned closer to the cytoplasmic side in a "down" state. Then, at the onset of depolarization of the channel the S4 undergoes a conformational change and moves toward the extracellular side of the membrane (the "up" state), after which the channel is activated (Fig. 1). A lot of work has shown that this regular activation mechanism can be disrupted by mutations in the S4 segment (Moreau et al., 2015a, 2015b; Gosselin-Badaroudine et al., 2012), which creates new, alternate gating pores through which additional conduction pathways arise in otherwise nonconducting Na_v channels. Such mutations therefore lead to experimentally measurable gating-pore current.

Early work by the Li group (Akbari Ahangar et al., 2024) proposed ∼2,400 disease-associated mutations across multiple Na_v channels and sought to examine their impacts on the structure and function of the channel. They proposed that a large cluster of such mutation effects are concentrated in the VSD and that such mutations have measurable impacts on standard electrophysiological readings, including on the maximal current amplitude (I_{max}), half-activation voltage in steady-state activation ($V_{1/2,Act}$), half-inactivation voltage in steady-state fast inactivation ($V_{1/2,Inact}$), recovery rate (τ_{rec}), persistent current (I_P), and gating-pore current (I_ω). This work by the Li group underscores the need for further functional studies of VSD mutations, and perhaps serves as a call for the community to map out a broad range of standardized electrophysiological metrics when probing mutations.

In the current work of Elhanafy et al. (2025), the Li group built on this prior work to compare the differential impacts of six gating-charge mutations by focusing specifically on arginine to glutamine (Arg-to-Gln) mutations at the R1 to R3 positions in the VSD_I and VSD_{II} regions of $Na_v1.5$. To do so, they utilized computer simulations with a MD engine in which each atom is explicitly modelled with a force field potential to describe the system potential energy. Briefly described, the MD engine moves the atoms forward by propagation of Newton's second law, thus generating a trajectory in time. The authors used ∼120 µs of MD simulations coupled with an external electric field applied to accelerate the VSD structural transitions. This coupling to an external electric field has been well parametrized in the wider ion channel simulation community. The authors found from these simulations that they could observe spontaneous microsecond-timescale VSD conformational changes in the WT system, in which they observed the up-to-down state transition. The simulations were complemented by electrophysiological measurements of the voltage dependence of steady-state activation and mutation effects. The simulations revealed intriguing structural impacts of the mutants on the VSD. For example, the R225Q mutant showed resistance to VSD transition, remaining in the up state for the full duration of the trajectory. When studying identical mutations at an equivalent position in the VSD_I (R225Q) and VSD_{II} (R814Q) domains, the authors found differential effects on the channel-gating properties despite them being identical mutations, which was evidence of a differential response to disease-associated missense variants. In the VSD_I, the mutant prevents the conformational transition seen in the WT simulations, while in the VSD_{II} the mutant behaved like the WT. This is an interesting result, and the authors rationalize the salt-bridge network of the Na_v channel can explain the differential impact.

Overall, the work of Elhanafy et al. (2025) shows that a solid combination of simulation and experiment can help us probe the role of hazardous disease-causing mutants of Na_v channels, revealing that identical mutations can have differential effects on the structural properties of $Na_v1.5$. Such emerging insights will prove useful for future work on disease-associated missense variants for cardiovascular disorders.

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