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.

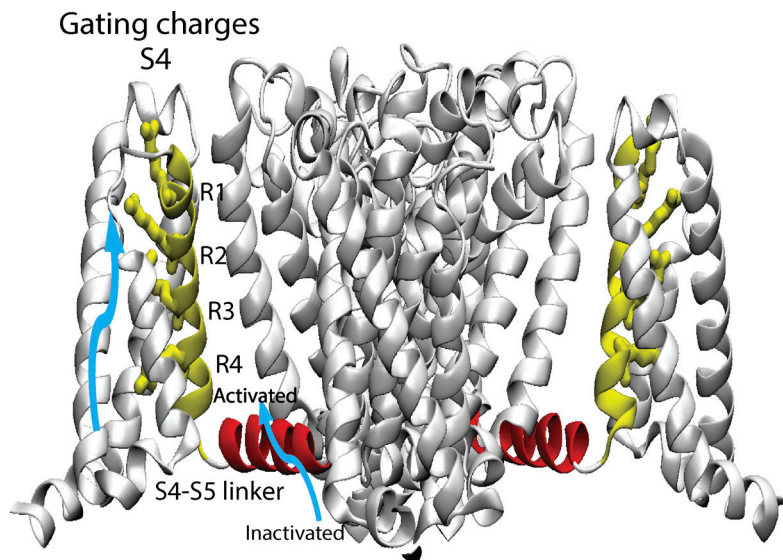Correspondence to Christian Jorgensen: christian.jorgensen@port.ac.uk

Figure 1. **The Na$_v$1.5 voltage-sensing domain and its associated S4–gating charges (shown in yellow), as well as the S4–S5-linking loop (red) as investigated by Elhanafy et al.** Depicted is the conformational change that Na$_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$_v$1.5. To do so, they utilized computer simulations with a MD engine in which each atom is explicitly modelled with a force field potential to describe the system potential energy. Briefly described, the MD engine moves the atoms forward by propagation of Newton's second law, thus generating a trajectory in time. The authors used ~120 µs of MD simulations coupled with an external electric field applied to accelerate the VSD structural transitions. This coupling to an external electric field has been well parametrized in the wider ion channel simulation community. The authors found from these simulations that they could observe spontaneous microsecond-timescale VSD conformational changes in the WT system, in which they observed the up-to-down state transition. The simulations were complemented by electrophysiological measurements of the voltage dependence of steady-state activation and mutation effects. The simulations revealed intriguing structural impacts of the mutants on the VSD. For example, the R225Q mutant showed resistance to VSD transition, remaining in the up state for the full duration of the trajectory. When studying identical mutations at an equivalent position in the VSD$_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$_v$1.5. Such emerging insights will prove useful for future work on disease-associated missense variants for cardiovascular disorders.

References

Akbari Ahangar, A., E. Elhanafy, H. Blanton, and J. Li. 2024. Mapping structural distribution and gating-property impacts of disease-associated mutations in voltage-gated sodium channels. *iScience*. 27: 110678. https://doi.org/10.1016/j.isci.2024.110678

Choudhury, K., M.A. Kasimova, S. McComas, R.J. Howard, and L. Delemotte. 2022. An open state of a voltage-gated sodium channel involving a π-helix and conserved pore-facing asparagine. *Biophys. J.* 121:11–22. https://doi.org/10.1016/j.bpj.2021.12.010

Elhanafy, E., A.A. Ahangar, R. Roth, T.M. Gamal El-Din, J.R. Bankston, and J. Li. 2025. The differential impacts of equivalent gating-charge mutations in voltage-gated sodium channels. *J. Gen. Physiol.* 157:e202413669. https://doi.org/10.1085/jgp.202413669

Fozzard, H.A., and D.A. Hanck. 1996. Structure and function of voltage-dependent sodium channels: Comparison of brain II and cardiac isoforms. *Physiol. Rev.* 76:887–926. https://doi.org/10.1152/physrev.1996.76.3.887

Gosselin-Badaroudine, P., L. Delemotte, A. Moreau, M.L. Klein, and M. Chahine. 2012. Gating pore currents and the resting state of $Na_v1.4$ voltage sensor domains. *Proc. Natl. Acad. Sci. USA*. 109:19250–19255. https://doi.org/10.1073/pnas.1217990109

Groome, J.R., and L. Bayless-Edwards. 2020. Roles for counter-charge in the voltage sensor domain of ion channels. *Front. Pharmacol.* 11:160. https://doi.org/10.3389/fphar.2020.00160

Hodgkin, A.L., and A.F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* 117:500–544. https://doi.org/10.1113/jphysiol.1952.sp004764

Jiang, D., H. Shi, L. Tonggu, T.M. Gamal El-Din, M.J. Lenaeus, Y. Zhao, C. Yoshioka, N. Zheng, and W.A. Catterall. 2020. Structure of the cardiac sodium channel. *Cell*. 180:122–134.e10. https://doi.org/10.1016/j.cell.2019.11.041

Kapplinger, J.D., D.J. Tester, M. Alders, B. Benito, M. Berthet, J. Brugada, P. Brugada, V. Fressart, A. Guerchicoff, C. Harris-Kerr, et al. 2010. An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. *Heart Rhythm*. 7:33–46. https://doi.org/10.1016/j.hrthm.2009.09.069

Li, W., L. Yin, C. Shen, K. Hu, J. Ge, and A. Sun. 2018. SCN5A variants: Association with cardiac disorders. *Front. Physiol.* 9:1372. https://doi.org/10.3389/fphys.2018.01372

de Lima Conceição, M.R., J.L. Teixeira-Fonseca, L.P. Marques, D.S. Souza, and D. Roman-Campos. 2023. Interaction of the antiarrhythmic drug Amiodarone with the sodium channel $Na_v1.5$ depends on the extracellular pH. *Eur. J. Pharmacol.* 960:176127. https://doi.org/10.1016/j.ejphar.2023.176127

Liu, H., C. Clancy, J. Cormier, and R. Kass. 2003. Mutations in cardiac sodium channels: Clinical implications. *Am. J. Pharmacogenomics*. 3:173–179. https://doi.org/10.2165/00129785-200303030-00003

Millat, G., P. Chevalier, L. Restier-Miron, A. Da Costa, P. Bouvagnet, B. Kugener, L. Fayol, C. Gonzàlez Armengod, B. Oddou, V. Chanavat, et al. 2006. Spectrum of pathogenic mutations and associated polymorphisms in a cohort of 44 unrelated patients with long QT syndrome. *Clin. Genet.* 70:214–227. https://doi.org/10.1111/j.1399-0004.2006.00671.x

Moreau, A., P. Gosselin-Badaroudine, and M. Chahine. 2015a. Gating pore currents, a new pathological mechanism underlying cardiac arrhythmias associated with dilated cardiomyopathy. *Channels (Austin)*. 9: 139–144. https://doi.org/10.1080/19336950.2015.1031937

Moreau, A., P. Gosselin-Badaroudine, L. Delemotte, M.L. Klein, and M. Chahine. 2015b. Gating pore currents are defects in common with two Nav1.5 mutations in patients with mixed arrhythmias and dilated cardiomyopathy. *J. Gen. Physiol.* 145:93–106. https://doi.org/10.1085/jgp.201411304

Pan, X., Z. Li, Q. Zhou, H. Shen, K. Wu, X. Huang, J. Chen, J. Zhang, X. Zhu, J. Lei, et al. 2018. Structure of the human voltage-gated sodium channel $Na_v1.4$ in complex with β1. *Science*. 362:eaau2486. https://doi.org/10.1126/science.aau2486

Payandeh, J., T. Scheuer, N. Zheng, and W.A. Catterall. 2011. The crystal structure of a voltage-gated sodium channel. *Nature*. 475:353–358. https://doi.org/10.1038/nature10238

Rogart, R.B., L.L. Cribbs, D.D. Kephart, and M.W. Kaiser. 1989. Molecular cloning of a putative tetrodotoxin-resistant rat heart Na+ channel isoform. *Proc. Natl. Acad. Sci. USA*. 86:8170–8174. https://doi.org/10.1073/pnas.86.20.8170

Shen, H., D. Liu, K. Wu, J. Lei, and N. Yan. 2019. Structures of human $Na_v1.7$ channel in complex with auxiliary subunits and animal toxins. *Science*. 363:1303–1308. https://doi.org/10.1126/science.aaw2493

Yarov-Yarovoy, V., P.G. DeCaen, R.E. Westenbroek, C.-Y. Pan, T. Scheuer, D. Baker, and W.A. Catterall. 2012. Structural basis for gating charge movement in the voltage sensor of a sodium channel. *Proc. Natl. Acad. Sci. USA*. 109:E93–E102. https://doi.org/10.1073/pnas.1118434109