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COMMENTARY

Voltage-gated Na channels 2026

Deep learning as a generator of sodium channel state hypotheses

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Lopez-Mateos et al. show that AlphaFold 2 can generate structural ensembles of NaV channels and that β-subunits and calmodulin reshape these ensembles, while emphasizing that these are testable structural hypotheses, not actual thermodynamic populations.

Importance

Over the past decade, cryo-electron microscopy (cryo-EM) has transformed our understanding of voltage-gated sodium (NaV) channels, revealing high-resolution structures of several conformations (Jiang et al., 2022; Noreng et al., 2021). Yet, despite these advances, the structural coverage of the full-gating cycle remains incomplete. Importantly, many antiarrhythmics, antiepileptics and local anesthetics preferentially bind to inactivated or open states of NaVs (Abdelsayed and Sokolov, 2013; Roden, 2014), that currently remain difficult to access structurally (Ullrich, 2021). As a result, the mechanistic basis of state-selective pharmacology, and thus rational design of next-generation modulators, remains only partially understood.

Physics-based MD simulations can, in principle, bridge this gap by explicitly modeling atomic motions and energetics across ion channel conformational transitions (Jensen et al. 2010; Jensen et al., 2012). However, the relevant timescales of NaV channel operation span many orders of magnitude, only a subset of which is currently accessible to routine MD simulations. Ion permeation through an open NaV channel occurs on nanosecond timescales, while local side-chain rearrangements and pore hydration fluctuate on nanosecond to microsecond timescales. Interactions with auxiliary partners, such as β-subunits or calmodulin, can further reshape these dynamics over even longer timescales. Simulating these slower, functionally critical processes in full-length human channels embedded in explicit membranes, with ions and realistic electrostatics, remains computationally demanding (Dror et al., 2012) and often calls for advanced techniques (Bergh et al., 2019).

Therefore, what is needed is a practical and computationally cheap way to generate structural hypotheses across channel states to obtain plausible conformations that can be tested experimentally, refined with physics-based methods, and which can assist the interpretation of experimental results.

In this issue of the *Journal of General Physiology*, Lopez-Mateos et al. (2026) present such an approach by deliberately exploiting the conformational diversity produced by AlphaFold 2 (AF2) and AlphaFold Multimer, applied to human NaV channels.

Ensemble generation and state definition

Previous studies showed that stochastic elements in multiple sequence alignment (MSA) subsampling and model initialization, combined with iterative refinement, allow the AF2 to converge on different locally consistent solutions to the same sequence (Kalakoti and Wallner, 2025; Riccabona et al., 2024; Wayment-Steele et al., 2024), which can be used to produce distinct conformations of the same protein.

Here, the authors used these approaches to generate large ensembles of models for all nine human NaV isoforms, both alone and in complex with auxiliary partners. Using AF2 with subsampled MSAs, multiple random seeds, and varying recycle depths, they produced hundreds of full-length models per each condition. Further, the authors defined channel "states" using simple, interpretable geometric coordinates that map onto known functional features: voltage-sensor activation inferred from S4 gating-charge displacement, activation gate (AG) openness quantified by pore-lining distances, selectivity filter (SF) geometry assessed via the signature DEKA residue spacing, and fast inactivation tracked by the distance between the IFM motif and its receptor site. Next, by correlating these coordinates across ensembles and applying clustering analyses, the authors asked two central questions:

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(1) Does MSA-subsampled AF2 sample multiple NaV-like conformations?
(2) Do auxiliary partners merely decorate the α-subunit, or do they reshape the conformational space it samples?

Key result I: AF2 ensembles sample multiple NaV-like conformations

Across isoforms, AF2 produced ensembles that spanned a broad structural range consistent with known aspects of NaV gating. Voltage-sensing domains (VSDs) exhibited shifts consistent with discrete, "click-like" gating charge movements, particularly in VSDIV, long associated with fast inactivation (Capes et al., 2013). While deeply deactivated VSDII conformations were underrepresented, partial deactivation states emerged reproducibly. The IFM inactivation motif showed a strikingly bimodal distribution, occupying either a bound position at the pore domain or an unbound, sequestered conformation.

Importantly, intermediate positions were also sampled, suggesting potential pre-inactivated or transitional states that have not yet been structurally resolved. The SF displayed modest dilation and widening in a subset of models, an intriguing observation given proposals linking SF rearrangements to slow inactivation (Ong et al., 2022; Wu et al. 2022; Wu et al., 2025; Wang et al., 2019).

However, in the absence of explicit ions, solvent, and electrostatic forces, AF2 predictions cannot enforce the physical constraints that define a functional SF. Consequently, variations in SF geometry in these models should not be interpreted as evidence for conductive, nonconductive, or slow-inactivated states without independent validation (Naylor et al., 2016).

The AG spanned a range overlapping experimentally observed open and closed states, alongside rare "super-open" outliers. These extreme conformations might reflect model extrapolation rather than physiological states, underscoring the need for experimental validation, although large openings of AGs have been proposed before for other channels based on MD simulations (Kopec et al., 2019; Şahin and Zachariae 2025). Taken together, these results reinforce a central point: AF2 ensembles generate structural hypotheses, not verified states. Training data, internal priors, and modeling choices strongly shape what is sampled, but the diversity itself is informative and could be used, e.g., for future MD simulations as starting seeds.

Key result II: Auxiliary partners reshape the ensemble

A second major insight is that auxiliary proteins are not passive add-ons. Using AF-Multimer (Bryant et al., 2022), that more accurately models inter-subunit interfaces and context-dependent conformational changes that arise upon binding, the authors accurately reproduced known α–β subunit arrangements, validating the method's ability to capture established interfaces. More importantly, inclusion of β-subunits changed the α-subunit conformational landscape. VSD activation profiles shifted, IFM binding became more prevalent, and correlations between structural coordinates were reorganized. Calmodulin-containing models sampled Ca^{2+}-dependent states, even in the absence of explicit Ca^{2+}, with apparent consequences for IFM occupancy and inactivation-related states. These findings serve as a useful reminder that the physiologically relevant object is not the isolated α-subunit. AF2 is sensitive to molecular context, and this sensitivity can be exploited deliberately depending on how models are generated and interpreted.

Important limitations and cautions

Several caveats are worth emphasizing. First, AF2 reported scores (pLDDT and ipTM) refer to model confidence, not thermodynamic probabilities (free energies). Rare or low-confidence conformations may be incorrect, while functionally important states may be underrepresented due to biases in the training data. Second, partner-induced effects must be interpreted carefully. The finding that β-subunits and calmodulin reshape the α-subunit ensemble is biologically plausible and experimentally supported, but AF-Multimer predictions may also amplify or suppress conformations depending on how interaction interfaces are learned and weighted. The sensitivity of ensemble structure to molecular context is a strength, but it also means that incomplete or artificial modeling contexts can mislead if treated uncritically. Third, voltage, kinetics, and permeation are fundamentally outside the model's scope. AF2 has no representation of membrane potential, time-dependent gating, or ion flux. Any interpretation that links predicted structures directly to activation, inactivation, or drug-binding kinetics must therefore be indirect and hypothesis driven rather than declarative. Fourth, the choice of geometric coordinates, while pragmatic, cannot fully capture side-chain–controlled gating features, particularly at the AG or ion-stabilized SF. Finally, attempts to bias sampling using structural templates had limited success, highlighting intrinsic constraints in how strongly AF2 can be steered toward specific functional states (Kovalevskiy et al., 2024; Wayment-Steele et al., 2024; Monteiro da Silva et al., 2024). Taken together, these considerations reinforce a central message: AF2-derived ensembles should be treated as generators of candidate conformations, not as equilibrium ensembles or mechanistic proofs. Their value lies not in assigning probabilities or defining pathways, but in expanding the space of plausible structures that can be tested, falsified, or refined by experiment and physics-based simulation.

How to make it useful

Experimentally, AI-generated intermediates can be tested using state-dependent toxins, gating current measurements, voltage-clamp fluorometry, engineered disulfide cross-links, or accessibility mapping. The goal is not to confirm a model but to design experiments that discriminate between AI-proposed intermediates and known states. Computationally, AF2-derived structures can be used in physics-based refinement such as equilibration in explicit membranes and ions, assessment of structural stability, hydration or conductance proxies, and free-energy ranking using MD or enhanced sampling methods. Thus, a practical workflow emerges: starting by generating hypotheses using AF2 ensembles, filtering them by confidence and consensus, testing their stability

via MD simulations, and finally designing experiments to discriminate among them.

Outlook

Future generations of structure predictors, including AF3-like models, may improve handling of heteroatoms, ions, and molecular context, potentially refining SF interpretation and partner effects. Nonetheless, phenomena tied to voltage, ion permeation, and kinetics will remain challenging for purely data-driven models. For now, the near-term strength of deep learning in ion channel biology lies exactly where Lopez-Mateos et al. (2026) position it: as a powerful engine for hypothesis generation and prioritization, accelerating the dialog between structure, simulation, and experiment.

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