Modeling of Sodium Channel Inactivation - Is the Hinged-Lid Mechanism still Valid?

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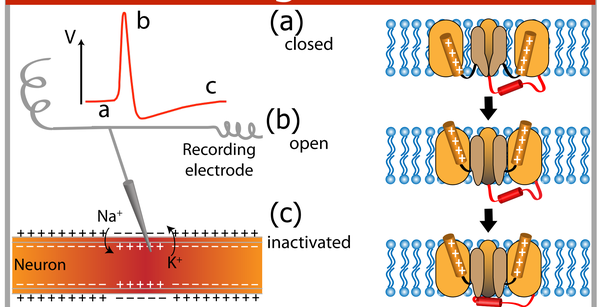
Abstract

Fast inactivation of the voltage-gated sodium (Na_V) channel is crucial in the regulation of the membrane potential in neural and muscle cells. A "hinged-lid" mechanism has been proposed to explain the rapid inactivation, in which the inactivation gate occludes the channel pore. Recent cross-link data suggest a close functional coupling between the inactivation gate and the domain IV voltage sensor (DIV VS), instead of the pore. An atomic structure of a eukaryotic Na_V channel, including both the transmembrane domain and the inactivation gate, was built by homology modeling and molecular dynamics refinement, to study the stated coupling and inactivation mechanism. The model suggests that the inactivation gate consists of a flexible linker with three short alpha-helices. Despite its high flexibility, the inactivation gate mostly interacts with the DIV VS, which agrees with previous mutagenesis studies. Novel interactions between DIV VS and the inactivation gate have been identified.

Background



Cycle of Na_V opening-inactivation regulates neural signaling. The channel is opened within 1 ms to allow Na+ ion influx into the neuron to raise its membrane potential (a→b), and then inactivated within a few milliseconds to stop the influx for the potential to relax (c).

The heterotetrameric Na_V inactivates by a "hinged-lid" mechanism, caused by the intracellular DIII-DIV linker (known as the **inactivation gate**, red cylinder).

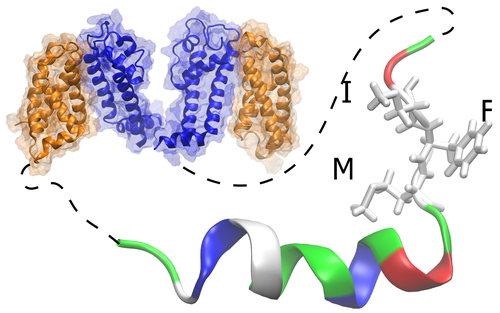
Key Questions:

Fast inactivation is known to be controlled by DIV motion, but how the inactivation gate interacts with DIV or other parts of the channel?

What are the molecular details of the inactivation mechanism?

Molecular model of Na_V channel (Extracellular view)

Modeling Na_V Fast Inactivation Gate

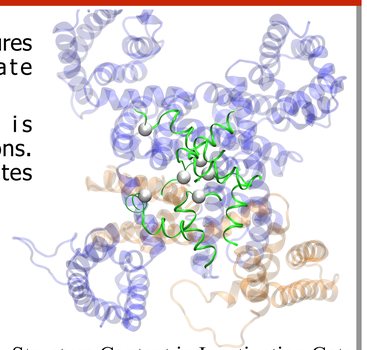


Homology model of eukaryotic Na_V channel (top) and partial NMR structural model of Na_V inactivation gate (DIII-DIV linker, bottom)

Representative structures of inactivation gate model.

The IFM helix is represented in ribbons. White sphere indicates Phe residue in IFM.

DI-DIII, **DIV**, **Inactivation gate**

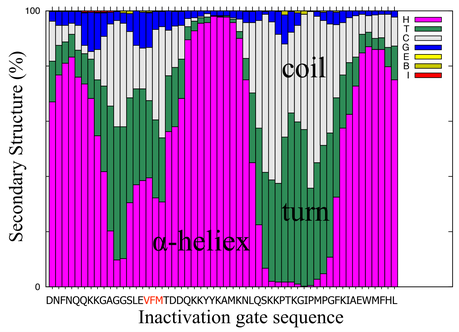


Structural models for the whole DIII-DIV linker (53 aa) with Na_V transmembrane domain were predicted by Rosetta.

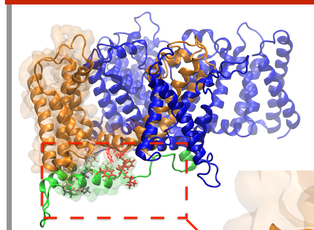
Representative structures were selected from RMSD-cluster analysis. Results shows that the linker is flexible and can possibly interact with different parts of the channel.

Secondary structure of the linker is robust and consists of three short α-helices

Secondary Structure Content in Inactivation Gate

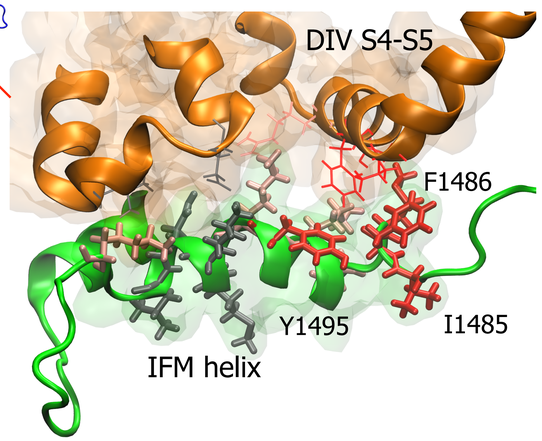


Interaction between Inactivation Gate and Na_V DIV



Squid Na_V channel with inactivation gate

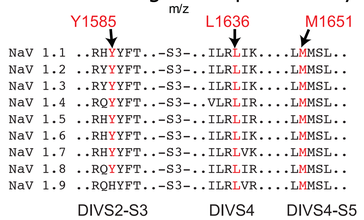
(**DI-DIII**, **DIV**, **Inactivation gate**)



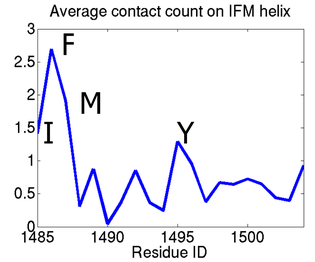
Interface residues

(**High contact count**, **Medium**, **Low**)

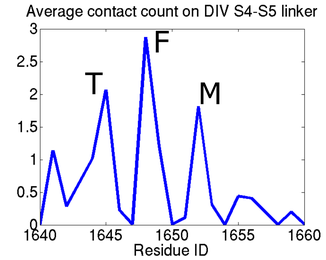
Top hits receptor sites for IFM motif in cross-linking mass spectrometry



Average contact count on IFM helix



Average contact count on DIV S4-S5 linker



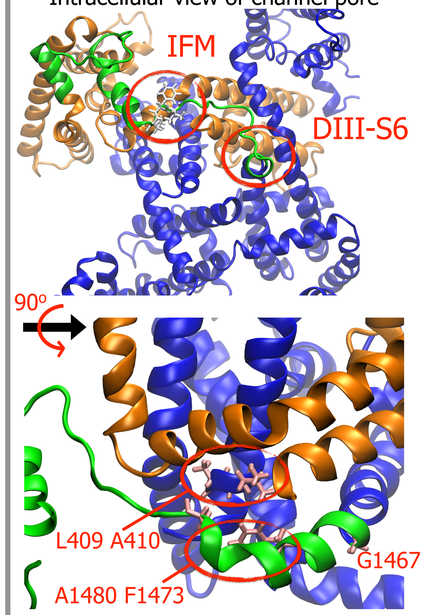
Cross-linking experiment confirms the inactivation gate is closed to DIV in inactivated state.

Molecular dynamics simulations were performed to identify the interacting interface. A few important residues (F1486, Y1495, T1645, F1648, M1652) took part in the interacting surface.

This interface is consistent with previous mutagenesis studies.

Possible Mechanism for Inactivation

Intracellular view of channel pore



IFM motif interacts constantly with DIV VSD and cannot block the pore directly as previously proposed.

Intracellular end of DIII-S6 helix could hinge around the G1467 and cover the pore. L409 and A410 located on DI-S6 are in close proximity of F1473 and A1480 on the inactivation gate.

Inactivation is completely removed by deletion of the first 10 residues of the inactivation gate [5] and L409C/A410W mutation [6], which partially support the hypothesis. However, experimental evidence to prove this interaction interface is still missing.

Conclusions

- A molecular model for the whole eukaryotic Na_V channel with inactivation gate has been built
- Based on cross-linking experimental data, the interacting interface between DIV and inactivation gate was identified
- Reclosure of the channel by DIII-S6 helix, instead of IFM motif, may lead to inactivation

Simulation Methods

The structure of a partial IFM helix was obtained from an in-solution NMR structure [1]. The structure of Squid Na_V channel with inactivation gate was built by Rosetta [2], by combining a homology model based on bacterial Na channel (Navab) [3] and the partial IFM helix.
The system was simulated for 150 ns (per trajectory) using NAMD 2.10 [4] with CHARMM36 force field for protein and lipid, and TIP3P water model. Representative structures from the three largest Rosetta model clusters were picked as initial structure. Restraint are put on the relative distance between F662 and the identified cross-link residues for first 50 ns of each simulation. All simulations were preformed using a Langevin thermostat at 300 K and pressure of 1 atm via the Langevin Nosé-Hoover method.

References

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Acknowledgment

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