However, in both cases, there is the potential for multiple electrostatic interactions to sum and yield potent binding. Our data suggest that pore-block, and acceleration of inactivation, result from distinct molecular actions of the toxin.

700-Pos  Board B469
Long Molecular Dynamics Simulations of the Voltage-Gated Sodium Channel, Na\textsubscript{Ab}
Céline Boiteux\textsuperscript{1}, Igor Vorobyov\textsuperscript{2}, Toby W. Allen\textsuperscript{1,2}.
\textsuperscript{1}School of Applied Sciences and Health Innovations Research Institute, RMIT University, Melbourne, Australia, \textsuperscript{2}Department of Chemistry, University of California, Davis, Davis, CA, USA.

The solution of the Na\textsubscript{Ab} ion channel crystal structure has provided a first glimpse of the inner workings of the voltage-gated Na\textsuperscript{+} channel family, which are central to electrical signaling in the body. We have carried out a set of multi-microsecond molecular dynamics simulations aimed at shedding light on the mechanisms of permeation and selectivity for this unusual channel, with its selectivity filter EEEE locus being more reminiscent of a calcium than a sodium channel. Despite the crystal structure exhibiting a closed pore, these simulations, on the physiological timescale for permeation, have revealed exchanges between accessible multi-ion configurations (with 2-3 ions in the pore) and their coordination by water and protein groups, as well as the competition between Na\textsuperscript{+}, K\textsuperscript{+} and Ca\textsuperscript{2+} ions within the selectivity filter. We observe a critical influence of glutamate protonation on the formation of multiple ion occupancy states, as well as on protein structure and flexibility. Our long simulations have uncovered interesting conformational changes within the voltage sensors and pore lining helices, including simulations that reveal structures consistent with a putative inactivated state. These studies illustrate the valuable contribution long atomic simulations can make, through observations that can guide targeted computational and experimental studies of ion channel function.

701-Pos  Board B470
The First Crystal Structure of a Voltage-Gated Na\textsuperscript{+} Channel Predicts a New Determinant of External Access for Hydrophilic Local Anaesthetics Pètre Lukács\textsuperscript{1}, René Cervernka\textsuperscript{1}, Vaiubhavkumar Gawali\textsuperscript{1}, Xavier Koenig\textsuperscript{1}, Agnes K. Mike\textsuperscript{1}, Lena Rubi\textsuperscript{1}, Touran Zarribi\textsuperscript{1}, Karliheinz Hilber\textsuperscript{1}, Harry A. Fozzard\textsuperscript{2}, Hannes Todt\textsuperscript{1}.
\textsuperscript{1}Medical University of Vienna, Vienna, Austria, \textsuperscript{2}Cardiac Electrophysiology Laboratory, Department of Medicine, The University of Chicago, Chicago, IL, USA.

The frequently used local anaesthetic lidocaine is believed to reach its binding site in the intracellular vestibule of the voltage-gated sodium channel (Na\textsubscript{v}) via the cell membrane. QX-222 is a permanently charged, quaternary amine anaesthetic with its selectivity filter EEEE locus being more reminiscent of a calcium channel family, which drives a series of molecular dynamics (MD) simulations aimed at to study the activation process of NavAb. While identifying the reported Na\textsubscript{Ab} structure as an intermediate conformation, not fully-activated, our work has enabled us to determine channel conformations likely related to the RT and AT states of the channel. Overall, the structural results support an activation mechanism highly conserved across the entire family of VGCCs.

703-Pos  Board B472
Structure of the Ternary Complex of the C-Terminus of the Voltage-Gated Sodium Channel (Na\textsubscript{v}) with Calmodulin (CaM) and Fibroblast Growth Factor Homologous Factor (FH\textsubscript{F}) Ben C. Chung, Chao-Jian Wang, Haidun Yan, Geoffrey Pitt, Seok-Young Lee, Duke University, Durham, NC, USA.

Voltage-gated sodium channel (Na\textsubscript{v}, 1.5) is the major sodium channel expressed in heart tissue and is responsible for the initial upstroke of the action potential. Calmodulin (CaM) and fibroblast growth factor homologous factor (FH\textsubscript{F}) have been reported to regulate the inactivation of Nav1.5 by interacting with the C-terminal domain (CTD) of Na\textsubscript{v}, 1.5. Clinical studies also correlate many mutations on the C-terminus of Na\textsubscript{v}, 1.5 with arrhythmogenic heart diseases such as long QT syndrome and Brugada syndrome.

We have solved the ternary complex structure of Nav1.5.CTD/CaM/FH\textsubscript{F}2B in the absence of calcium. The calcium-free structure shows the calcium-independent binding of calmodulin to the IQ motif of Na\textsubscript{v}1.5.CTD. Strong interactions between Nav1.5.CTD and FH\textsubscript{F}2B are also observed in this structure. Several disease-causing mutations of Na\textsubscript{v}1.5.CTD are found within the regions interacting with FH\textsubscript{F}2B and calmodulin. We also identified a critical interaction between Nav1.5.CTD and FH\textsubscript{F}2B that contributes to FH\textsubscript{F}-subtype specificity. The insight provided by this structure will help us to delineate the regulatory mechanisms of CaM and FH\textsubscript{F} on the voltage-gated sodium channel.

While identifying the reported Na\textsubscript{Ab} structure as an intermediate conformation, not fully-activated, our work has enabled us to determine channel conformations likely related to the RT and AT states of the channel. Overall, the structural results support an activation mechanism highly conserved across the entire family of VGCCs.

704-Pos  Board B473
Structure of the Ternary Complex of the C-Terminus of the Voltage-Gated Sodium Channel (Na\textsubscript{v}) with Calmodulin (CaM) and Fibroblast Growth Factor Homologous Factor (FH\textsubscript{F}) Ben C. Chung, Chao-Jian Wang, Haidun Yan, Geoffrey Pitt, Seok-Young Lee, Duke University, Durham, NC, USA.

Voltage-gated sodium channel expressed in peripheral sensory neurons, play an important role in pain signaling. The human isoform hNav1.7 is a key target for the development of new analgesics for pain treatment. However, high-resolution structures of mammalian voltage-gated sodium channels that could be useful for rational drug design are not available. X-ray structures of bacterial voltage-gated sodium channels, NavAb and NavRh, have been recently solved and serve as useful templates for homology modeling of mammalian voltage-gated sodium channels. Notably, both NavAb and NavRh structures show asymmetric dimer-of-dimers configuration of the pore-forming domain. We generated homology/de novo models of the asymmetric Nav1.7 pore-forming domain using a Rosetta-membrane homology modeling method and the NavAb structure (pdb id: 4DXW) as a template. Multiple sequence alignments of the second pore helix region between hNav1.7 and NavAb have been explored and compared with experimental data concerning side-chain orientation and ligand binding. Docking of tetrodotoxin, u-conotoxin-KIIIA, and the local anesthetic lidocaine to the pore-forming domain of hNav1.7 using Rosetta-Dock and molecular dynamics simulations were performed to test the ability of structural models to fit available experimental data. Structural models of the pore-forming domain of hNav1.7 may be useful for design of analoges targeting human voltage-gated sodium channels.