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Computer Simulations of Voltage-Gated Cation Channels

Werner Treptow[†] and **Michael L. Klein**[‡]

[†]Universidade de Brasília, Laboratório de Biologia Teórica e Computacional, Departamento Biologia Celular, BR-70910-900 Brasilia, DF, Brazil

[‡]Institute for Computational Molecular Science, Temple University, 1900 N. 12th Street, Philadelphia, Pennsylvania, 19122-6078 United States

Abstract

The relentless growth in computational power has seen increasing applications of molecular dynamics (MD) simulation to the study of membrane proteins in realistic membrane environments, which include explicit membrane lipids, water and ions. The concomitant increasing availability of membrane protein structures for ion channels, and transporters -- to name just two examples -- has stimulated many of these MD studies. In the case of voltage-gated cation channels (VGCCs) recent computational works have focused on ion-conduction and gating mechanisms, along with their regulation by agonist/antagonist ligands. The information garnered from these computational studies is largely inaccessible to experiment and is crucial for understanding the interplay between the structure and function as well as providing new directions for experiments. This article highlights recent advances in probing the structure and function of potassium channels and offers a perspective on the challenges likely to arise in making analogous progress in characterizing sodium channels.

Keywords

Ion Channels; Ionic Conduction; Gating; S4 helix; Regulation; Molecular Dynamics Simulations; Structure and Function

Background and Significance

Voltage-gated cation channels (VGCCs) are sophisticated membrane proteins controlling the flow of ions across membranes. This remarkable property is of paramount importance for electric signaling involved in cellular secretion, muscle contraction, and nerve transmission.[1] VGCCs control ionic conduction by cycling through three main states states in response to variations of the transmembrane (TM) voltage (ΔV), *viz*. *resting-closed*, *activated-open* and *inactivated*. The *resting-closed* occurs at hyperpolarized membrane potentials, activation (opening) is induced by membrane depolarization, while inactivation leaves the channel non-conductive.[1] Transitions between these states are usually treated as a general gating mechanism.

The superfamily of VGCCs includes four major classes: Na^+, Ca^{2+}, K^+ and non-selective cationic channels, corresponding to Nav, Cav, Kv, and HCN channels, respectively. As revealed by decades of research and more recently by crystallography (see below), VGCCs are either tetrameric or pseudo-tetrameric (e.g., a single protein comprising four homologous

mlklein@temple.edu (M.L.K.) Phone: +1-215-204-1927 Fax: +1-215-204-2257. Address: Institute for Computational Molecular Science Temple University 1900 N. 12th Street Philadelphia, Pennsylvania, 19122-6078 United States. treptow@unb.br (W.T.); Laboratório de Biologia Teórica e Computacional Departamento Biologia Celular, CEP: 70910-900 Brasilia, DF, Brazli.

repeats); each subunit is built from six TM helices, labeled as S1 through S6, respectively (Figure 1). Segments S5 through S6 constitute the central pore domain (PD) and delineate the hydrophilic pathway for the ionic current and a conserved motif: the so-called selectivity filter (SF) responsible for ion selectivity. The bundle of segments S1 through S4, identified experimentally as the voltage-sensor (VS) domain, is connected to the PD via the S4–S5 linker. The VS senses variations in the TM voltage and triggers the conformational changes leading to activation (pore opening) of the channel. Channel inactivation proceeds by molecular rearrangements resulting from two mechanisms: fast and slow (C-type) inactivation. The first involves blocking the inner mouth of the pore with the intracellular N-terminus of the TM domain. After long membrane depolarization, the pore domain becomes non-conductive through slow inactivation involving the structural collapse and blocking of the SF.[2] Excellent reviews describe in detail the behavior of such channels.[3,4]

Apart from intellectual curiosity concerning the fundamental design of nature's nanomachine, an understanding of the structure and function of VGCCs offers the prospect of ultimately designing therapies for diseases associated with channel disorders, the so-called channelopathies.[5]

VGCC Structures

For many years, the scarce atomic-level structural and dynamical data available for VGCCs and other related channels severely limited knowledge on the interplay between the structure and the function of this protein family. This limitation was due primarily to the difficulty of obtaining crystals suitable for determining high-resolution structures. In 2005, the MacKinnon group reported the first and so far only x-ray crystal structure for a mammalian voltage-gated K^+ (Kv) channel,[6] the Kv1.2 channel, which was crystallized in the open-activated state, thereby enabling new possibilities in deciphering key aspects of VGCC function.

Very recently, the x-ray structure of the *prokaryotic* voltage-gated Na^+ channel from *Arcobacter butzleri* (NavAb) was determined at 2.7Å resolution[7] in a closed-pore conformation with the four VS domains activated, a conformation that was interpreted as representative of a “pre-open” state. As one member of the Nav subfamily, NavAb provides the structural template required to extend our knowledge on the structure and function of Na^+ channels and the related Cav channels for which much less is known.

The following offers an overview of the recent advances emanating from the many recent MD simulations of Kv1.2, which serve as a template for an important perspective on the challenges facing future MD characterization of other members of the VGC family in light of the very recent NavAb crystal structure.

Molecular Dynamics Simulations

Following publication of the landmark structure for Kv1.2, a number of groups immediately used MD simulation and an arsenal of cutting-edge MD-related methodologies on this new structure in a series of studies aimed at characterizing the structure and function of this channel. It is a testament to MD simulation capability that with the latest force fields proved to be a powerful tool predicting, with surprising accuracy, the channel gating motions and conduction properties, as well as channel modulation by ligands.[8]

Pioneering MD simulations of ion channels focused on the ion conduction properties of the prokaryote potassium channel KcsA;[9–12] the structural archetype for the pore domain of all K^+ channels, including Kv, which had its atomic x-ray structure[13] solved before that of Kv1.2. The KcsA structure unveiled the SF in K^+ channels as a narrow pore ~12 Å long,

formed by a tetrameric arrangement of the carbonyl groups of a highly conserved sequence TVGYG, which promotes high throughput and selection of K^+ over other ionic species (Figure 2). On passing through the SF, which is lined by carbonyl oxygens, each K^+ coordinates only two waters, both lying on the channel symmetry axis. As such, binding of multiple ions to the SF is constrained to single-file geometry, exactly as envisage by Hodgkin and Huxley in the 1950's. Indeed, in the preferred “knock on” conduction mechanism, two K^+ ions and waters alternate occupancy of 4 possible SF binding sites (s1–s4) and move coherently when nudged by a K^+ that occupies a site external to the SF.[14–17] Although the SF structure is highly conserved in the K^+ channel family, the latter displays a wide range of absolute rates of ion permeation, suggesting that ion transport likely involves subtly different mechanisms. Indeed, free-energy calculations on KcsA and Kv1.2, found that the electrostatic environment of the SF affects the energetics of ion conduction and therefore conductivity.[18]

The diffusion of ions through a channel requires a hydrophilic environment provided by transient hydration of the ionic conduction pathway, such as that encountered in the open gate of Kv1.2. Independent MD investigations have demonstrated that diffusion is energetically restrained by narrowing of hydrophobic regions of the gate that excludes water molecules from the permeation pathway.[19] In Kv1.2, Val^{478} was identified to form a major hydrophobic gate along the permeation pathway.[20] Interestingly, μs-timescale MD simulations of (rat) Kv1.2, starting from the open channel, revealed further that a dewetting transition of this pore region closes the channel by means of an intrinsic hydrophobic gating mechanism.[21] Taken together, these MD studies demonstrated that hydrophobic gates are efficient devices designed to gate the pore and to control ion transport. Indeed, hydrophobic gates are present in a variety of ion channels, including not only K^+ channels, but also the nicotinic receptors[22,23] and the bacterial mechanosensitive channel MscS.[24] MD studies have also contributed to identifying another physical gate of K^+ channels located in the SF,[25,26] namely intermittent or long-lived flips of SF carbonyls were demonstrated to lead to a constricted non-conducting structure. Moreover, as first reveal in metadynamics MD simulations[26] and later shown experimentally, C-type inactivation in K^+ channels involves simultaneous formation of multiple conformational defects in which the peptide chains that comprise the filter adopt an unusual structure with their dihedrals alternating between left- and right-handed Ramachandran angles.[26] Future multi-μs MD simulations are likely to be very useful in clarifying the many subtle features of VGCC structure and function.

In Kv1.2, the VS forms an hourglass-like structure in which water penetrates from the extra- and the intra-cellular sides, exposing the S4 gating charges to the solvent, as demonstrated in early MD simulations targeting structural details of the channel in a membrane (Figure 3).[27,28] The hydrated environment of S4 favors a local collapse of the electrostatic potential, which shapes the TM-electric field across the domain:[27,28] a result that was further validated by simulations of the VS domain alone in a membrane and NMR experiments.[29] Specific salt-bridge interactions, between S4 basic residues with neighboring VS-acidic residues and membrane-lipid head groups, were shown to stabilize the VS structure in the activated state. In detail, the S4 top residues R^{294} and R^{297} interact with lipid-PO_4^- groups at the membrane-water interface and R^{300} is close to E^{183}; deeper within the VS, R^{303}, K^{306} and R^{309} are involved, respectively in salt bridges with E^{226}, D^{259} and E^{236}.

Given the known activated and membrane-equilibrated structure of the Kv1.2 channel, a new generation of MD studies then appeared exploring the dynamical time-resolved gating process of the channel by investigating transition events of the voltage sensor under applied TM voltage differences. In these MD simulations, ΔV was generated either by applying an external electric field[30,31] or by employing approaches in which the voltage is imposed *via* a charge imbalance protocol.[32–34] The latter based on an ionic-charge imbalance between two

disconnected aqueous baths of explicit electrolytes, is able to induce a drop in the electrostatic potential across the lipid bilayer in an almost physiologically relevant range of values, without introducing artificial external fields to the system. These studies show that in response to the applied ΔV, the S4-gating charges undergo a concerted downward motion relative to acidic residues reaching a metastable intermediate β-form. One specific study demonstrated further that the complete VS deactivation involves 5 states: the activated state α; three intermediate states, β, γ, δ; and the resting state, ε, in which the VS undergoes transitions that involve a zipper-like motion of the S4 basic residues, in a sequential ion pairing with the VS acidic residues and the membrane lipid head groups.[34]

Presently, there are several proposed molecular models for the physiologically relevant deactivated or resting-closed state of Kv1.2 (Figure 3).[4,34–37] A "consensus" model for the resting state, which is consistent with much of the experimental data, demonstrates that overall, the models are in fairly good agreement,[38] by showing the VS exhibiting specific salt bridge interactions: R^{294} with E^{226}; R^{297} lies between D^{259} and E^{236} and R^{300} is a little below E^{236}. The particular model,[34] which was obtained by considering explicitly the protein-membrane interactions under applied TM voltages, while agreeing with the "consensus" model, has R^{294} more deeply embedded within the VS domain, satisfying very recent experiments showing its proximity to F^{233}, the so-called catalytic center.[39,40] This model shows further the interaction of R^{303}, K^{306} and R^{309} with the lipid PO_4^- headgroups in the deactivated-resting state.

To validate the resting state of the channel, tests of models against gating experiments have examined the robustness of the VS geometry and gating motions associated with the open-closed transition of the channel. Here, different advanced methods have been used to quantify the gating charge, Q: (i) continuum electrostatic computations based on the Poisson-Boltzmann equation;[35, 36] (ii) energetic formulation that computes Q as the product of the channel charges times the fraction of the membrane potential each traverses and (iii) direct measurement of Q based explicitly on capacitive currents leading to TM voltage variations in a membrane-channel system.[32,34] In particular, methods (i) and (ii) allow the identification of the specific molecular components that contribute to the gating charges. From these structure-based measurements, the computed Q-values for the whole channel deactivation (12 – 14 e) were found to be in good agreement with experimental values for Shaker-like channels.[41]

Future Directions

The consensus picture emerging from the plethora of MD studies on Kv channels points to a voltage-dependent gating mechanism involving strong coupling between the S4 helix motion and gating of the pore, in which the S4–S5 linker, connecting these two moving parts of the channel, acts as a "cuff" surrounding the S6 bundle-crossing and directly interacts with it to control the gating of the intracellular gate.[37]

Owing to the structural similarity among members of the large family of VGCCs, the voltage-sensing process as well as the gating mechanism in the family members are expected to be similar to that unveiled for Kv1.2. The key structural elements (charged S4, helical S4–S5 linker and hydrophobic-gate containing pore) are indeed strictly preserved in the recently determined NavAb structure (Figure 1). As previously applied to Kv1.2, computer simulation approaches offer promising tools to reveal the fully atomic structure of the active and resting states for NavAb, especially clarifying whether or not the specific structural rearrangements of the channel are similar to those determined for Kv1.2. The active and resting (Rosetta) models recently proposed for NaChBac channels strongly support this conclusion.[42]

However, the intrinsic properties of the SF, including ion conduction, selectivity and gating, must be highly specific among members of the VGCC family, thus providing a key challenge to fully understand VGCC function across the whole family.[43] The structure of the NavAb SF features a pore 15 Å long,[7] formed by a tetrameric arrangement of the highly conserved sequence TLESWAS and lined with one carboxylate and two carbonyl groups from each chain (Figure 2 B). Recent MD simulations of the channel embedded in a lipid membrane demonstrate that the NavAb filter is large enough (~4.6 Å) to accommodate preferentially two hydrated bound cations, either Na^+ or K^+; the first-coordination shell of the SF-bound ion is remarkably similar to that in bulk.[44] This finding raises obvious questions concerning the mechanism of conduction in Na^+ channels. One possibility is that NavAb employs the "*knock-on*" mechanism of K^+ channels to transport ions, involving a distinct "off-register" binding mode of ions to the SF; an observation that remains to be validated by means of additional MD runs.[45]

In K^+ channels, selectivity is traditionally explained via the "snug-fit" model[46] in which the SF is finely tailored to permeant species so as to provide a rigid environment mimicking the first hydration shell of the ion in solution. By challenging this view, recent MD studies have weighted in on the role of conformational flexibility in determining selectivity in K^+ channels.[47,48] In the same direction, the selectivity basis in NavAb also appears not to follow the "snug-fit" hypothesis. In analogy with K^+ channels, the SF of NavAb features a significant degree of structural heterogeneity providing a fluctional chemical cage for ions.[44] Beside the intrinsic conformational flexibility of the SF, the self-consistent polarization of SF residues, ions, and water molecules likely also play a crucial role in selectivity.[16, 49,50] Far of being trivial, these aspects impose significant challenges in deciphering selectivity in the larger family of VGCCs; in the MD field, the computational cost of enhanced-sampling techniques and electronic-structure-based approaches has made calculation of accurate free energies an extremely challenging task. The prospect availability of peta-scale computer resources[51] and hybrid quantum-mechanics/classical-mechanics (QM/MM) MD simulations using highly scalable MD software packages[52] offer promising solutions to handle such difficulties.

Single point mutations in the SF of NavAb-related channels are able to render the channel either non-selective (LESWAD), or selective for Ca^{2+} (LEDWAS).[53] Interestingly, MD studies exploring the conduction and selectivity properties of such specific NavAb-mutant channels will contribute to building a quantitative framework to ultimately address the key long term goal of understanding the more complex and pharmacologically relevant mammalian-Nav channels and to start to glean hints on the mechanism of the yet unexplored Cav channels. Translation of MD structural studies on conduction and selectivity of NavAb to mammalian channels is likely to be challenging due to the significant sequence differences between them; instead of the four glutamates (EEEE) found in the bacterial channel, eukaryotic Nav channels feature the conserved DEKA residues.[54,55]

It is likely that for the next few years one of the most fascinating topics of research will be the regulation of VGCCs by a large family of ligands, including divalent cations, free-sulfhydryl modifiers, toxins, and both general and local anesthetics. In a series of earlier efforts, as demonstrated by free-energy MD calculations, the tetraethylammonium (TEA) ion was found to bind with high affinity to the extracellular and intracellular entries of the SF of K^+ channels preventing ion conduction.[56] More recently, MD-docking calculations combined with alanine scanning experiments helped to identify in anesthetic-sensitive Kv channels,[57] several inhaled anesthetic sites involving the S4–S5 linker and the channel's activation gate (Figure 4). MD simulations have also explored the binding of two distinct classes of blocking toxins of Kv channels, namely the pore-blocking toxins that bind to extracellular entryway of the pore and prevent ion conduction;[58] and the gating modifier

peptides which bind to the VS domain altering the energetics of the gating process.[59] In this scenario, the appearance of the NavAb structure will motivate further investigations of ligand binding to VGCCs. The effects of the anesthetic isoflurane on NavAb-related channels, such as NaChBac[60] indicates that NavAb is likely to be a useful model, just like other specific Kv channels, for structure-function studies on the effect of volatile anesthetics on VGCCs. NavAb likely also provides a key model for investigating the binding of local anesthetics on VGCCs. Particularly relevant, given the key role of Nav channels in generating the upstroke of the action potential, the latter is targeted by a broader range of ligands compared to Kvs; for instance, the diversity of toxin action on Nav is illustrated by toxins that act at six or more distinct receptor sites on this channel compared to only two sites on Kv.[61] As previously demonstrated for K^+ channels, MD studies are likely to be a crucial ingredient in defining functional details on all of these research topics.

One notes that understanding the structural basis underlying the mechanisms by which ligands modify directly or allosterically the conducting state of the channel pore in the family of VGCCs is particularly desirable especially when searching for characterization of other sensory relevant voltage-gated cation channels that are regulated by cyclic nucleotides, such as the hyperpolarization-activated and cyclic nucleotide-gated HCN channel. Typically, these bi-regulated receptors posses the common TM domain of VGCCs physically coupled to a conserved cyclic nucleotide-binding domain (CNBD) at the C-terminus of the pore. On this desirable scenario, the availability of homologous VGCC structures for the TM region and for the CNB domain,[62] combined with comparative molecular modeling approaches,[63] offers a reliable perspective on getting insights on the mechanism by which the nucleotide-bound CNB domain modulates the pore opening on this channel subfamily.

Outlook

The nexus of fully atomistic structures, advanced computational methodologies, and powerful computational resources are likely to promote great progress in the field over the next few years. It is indeed remarkable that nowadays simulations of VGC channels are feasible to be accomplished in the timescale of hundreds of microseconds, as demonstrated in very recent simulations of Shaw and coworkers (see DE Shaw Science paper on Kv to appear in 2 weeks time – private communication). It is also remarkable that deep issues, such as, dysfunction of VGCCs, can be directly investigated with computational methods as indicated by a recent MD studies addressing the effect of mutations of S4 residues as related to certain inherited channelopathies, *e.g.*, epilepsy, long QT syndrome, and paralyses.[64,65] These MD studies, based on Kv1.2, demonstrated that S4 mutations destabilize the VS electrostatic network, giving rise to non-functional leakage of cations, the so called “omega” current, through a conduction pathway within the VS domain. Similar studies on Na^+ channels are likely to be very informative.

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Biographies

Werner Treptow is *Adjunto II* Professor of Computational Molecular Biophysics at University of Brasília. His research concerns the use of high-resolution crystal structures in combination with MD simulations and an arsenal of cutting-edge MD-related methodologies to investigate the gating and conduction properties of ion channels and their regulation by ligands.

Michael L. Klein is the Laura H. Carnell Professor of Science and Director of the Institute for Computational Molecular Science in the College of Science and Technology at Temple University. He is a pioneer in the application of computer simulation to explore the structure and dynamical behavior of molecular systems (www.temple.edu/cst/icms/index.html).

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QUOTES

"Apart from intellectual curiosity concerning the fundamental design of nature's nanomachine, an understanding of the structure and function of VGCCs offers the prospect of ultimately designing therapies for diseases associated with channel disorders, the so-called channelopathies"

"The consensus picture emerging from the plethora of MD studies on Kv channels points to a voltage-dependent gating mechanism involving strong coupling between the S4 helix motion and gating of the pore, in which the S4-S5 linker, connecting these two moving parts of the channel, acts as a "cuff" surrounding the S6 bundle-crossing and directly interacts with it to control the gating of the intracellular gate"

" It is likely that for the next few years one of the most fascinating topics of research will be the regulation of VGCCs by a large family of ligands, including divalent cations, free-sulfhydryl modifiers, toxins, and both general and local anesthetics"

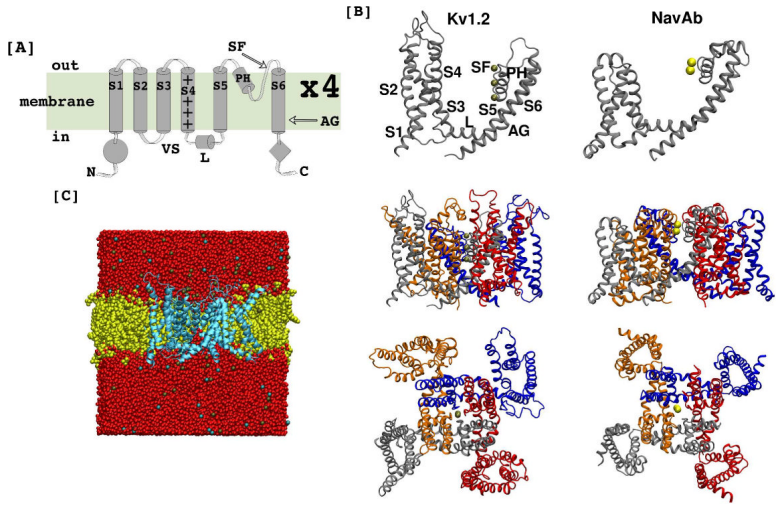

Figure 1.

Structure of voltage-gated cation channels. [A] Domain organization of tetrameric VGCCs. The TM region of the channel is built from six helices: segments S1 through S4 form the voltage-sensor domain (VSD) and segments S5 and S6 constitute the central pore domain (PD) containing the selectivity filter (SF). The N-terminal region of S6 forms the main activation gate (AG) along the permeation pathway. One notes that VSD and PD are physically coupled via the S4–S5 linker (L), a small helical segment located at the inner membrane-water interface. [B] Atomistic structures of Kv1.2 and NavAb. (Top) Structural details of a single channel subunit. (Middle, Bottom) Molecular views of the tetrameric channels from the side and from the extracellular region of the protein. Potassium (tan) or sodium (yellow) ions are depicted at the SF. [C] Typical atomistic MD-simulation system of a VGC channel embedded in a fully-hydrated phospholipid bilayer. The system contains the channel (cyan), 390 lipid molecules (yellow), 36280 solvent-water (red) molecules and salt in solution (a total of ~ 150,988 atoms).

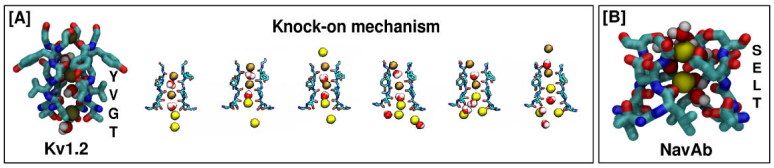

Figure 2.

Close-up view of the selectivity filter of Kv1.2 [A] and NavAb [B] containing potassium or sodium ions, and intercalated water molecules. The primary-sequence signatures, TGVY and TLES, are indicated for each filter. In potassium channels, the conduction of ions involves a simple throughput flux in the selectivity filter, following the preferential "knock-on" mechanism. Note that, as described in ref 44, the SF of NavAb is different from that of K^+ channels in many ways: (i) the NavAb SF is wider than the K+ construct; the former can accommodate partially hydrated bound Na^+ ions in sharp contrast with the dehydrated binding of K^+ in the SF of K^+ channels; (ii) asymmetric (off-axis) Na^+-water-SF-residue configurations take place in NavAb contrasting with the single-file configuration of ions and waters in K^+ channels; and (iii) exclusive to NavAb, water can permeate the SF decoupled from ionic conduction.

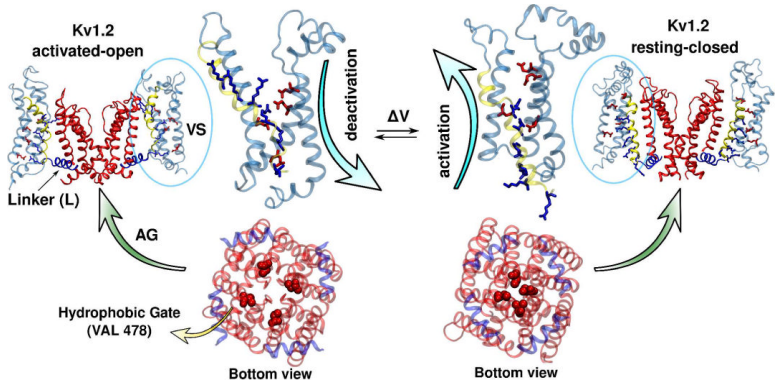

Figure 3.

Structure-function studies of Kv1.2. [Top] Membrane-equilibrated structures of Kv1.2 channel in the open-active (left) and closed-resting (right) conformations, as described in ref 34. For clarity, only the VSD (light blue; S4 in yellow) of two subunits of the channel are depicted. The close-up views of the VS domain highlights, in each state, the position of the S4-basic residues (blue sticks) and the salt bridges they form with the acidic residues (red sticks) of the other VSD segments. [Bottom] Views of the pore domain in the open (left) and closed (right) channel conformations. Note that, the VS deactivation is coupled with closure of the main pore domain; the volume accessible to K^+ is reduced at the activation gate (AG) region (intracellular entrance) of the pore upon deactivation. In this region, Val^{478} forms a major hydrophobic gate.

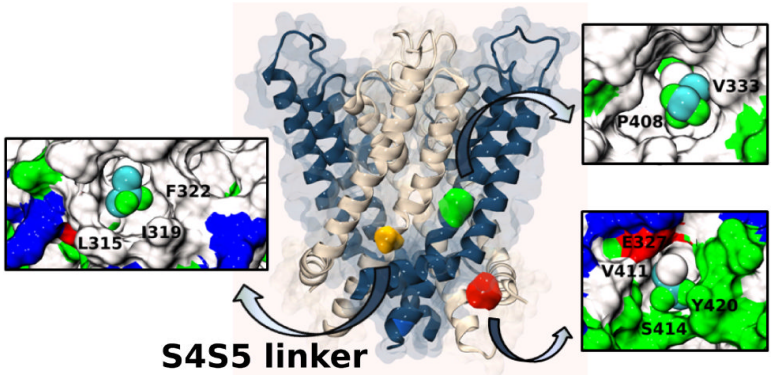

Figure 4.

MD-docking calculations for halothane binding on the K-Shaw channel, an anesthetic sensitive channel homologous to Kv1.2 (see ref 57). Side view of the channel (navy blue and beige) showing for clarity only the channel S4S5 linker and the segments S5 through S6. Colored blobs represent the density isosurface for the ligand on three affinity receptor sites. Although structurally heterogeneous, the identified sites are cavities located at the interfacial region between helical segments of the channel; they can be delineated by hydrophobic amino acids or predominantly hydrophilic as well.