Published in final edited form as:

Nature. 2008 December 18; 456(7224): 891–897. doi:10.1038/nature07620.

Sensing voltage across lipid membranes

Kenton J. Swartz¹

¹ Porter Neuroscience Research Center, Molecular Physiology and Biophysics Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892 USA

Abstract

The detection of electrical potentials across lipid bilayers by specialized membrane proteins is required for many fundamental cellular processes such as the generation and propagation of nerve impulses. These membrane proteins possess modular voltage-sensing domains, a notable example being the S1-S4 domains of voltage-activated ion channels. Ground-breaking structural studies on these domains explain how voltage sensors are designed and reveal important interactions with the surrounding lipid membrane. Although further structures are needed to fully understand the conformational changes that occur during voltage sensing, the available data help to frame several key concepts that are fundamental to the mechanism of voltage sensing.

Most of us appreciate the importance of electricity at a young age. It powers many useful and entertaining man-made devices; a light for seeing the world, a computer for searching the internet, or an omnipresent iPod for listening to music. The concept that living organisms use electricity as a fundamental mechanism for signaling across membranes and between cells is less widely appreciated, even though scientists have been thinking about the biological roles of electricity since the late 18th century. Indeed, Galvani's experiments showing that spark generators can elicit contraction of frog muscles were contemporary with key developments in understanding the phenomenon of electricity itself. Today we know a great deal about the biological roles of electrical signals and it would be impossible to overstate their profound importance. In humans, electrical signals are used to complete a computation within the cerebral cortex, to secrete insulin after a meal, or to signal that a sperm has entered an egg and for embryogenesis to commence.

In cells, electrical forces arise from the separation of Na⁺, K⁺, Ca²⁺ and Cl[−] across a lipid membrane that is intrinsically highly impermeable to these ions. ATP is consumed by pumps to produce concentration gradients across the membrane for these ions, creating a chemical potential that can generate an electrical potential when ion-selective channel proteins open and provide pathways for these ions to move down their concentration gradients. In a quiescent neuron, for example, K⁺ channels most commonly establish an electrical potential, or voltage, that is negative inside relative to outside because the concentration of K⁺ is high inside the cell and low outside. A cell membrane can be depolarized (less negative inside) when neurotransmitters open ion channels that are permeable to Na⁺ or Ca²⁺ because the concentrations of these ions are high outside and low inside. For such electrical potentials to generate action potentials¹, to trigger neurotransmitter secretion or initiate muscle contraction, voltage-activated ion channels must sense the voltage across the cell membrane and react by opening or closing an ion conduction pore. In these instances, changes in membrane voltage are detected by voltage sensors that are intrinsic to the ion channel; in most instances the voltage sensors move between 'resting' and 'activated' states to open the pore when membrane voltage becomes depolarized, but there are varieties that trigger pore opening when the membrane

becomes hyperpolarized (more negative inside). Investigation into the mechanism of voltage sensing has progressed rapidly since the classical studies of Hodgkin and Huxley on the Squid giant axon¹, with the cloning of the voltage-activated Na⁺ (Nav), K⁺ (Kv) and Ca²⁺ (Cav) channels in the late 1980's²⁻⁴ and the first X-ray crystal structure of a Kv channel in 2003⁵ representing historical landmarks. Although many mechanistic aspects of voltage sensing are debated, this review focuses on the emerging mechanistic concepts for which there is growing consensus. I will begin by discussing the architecture and structure of voltage-sensing domains in Kv channels, where our understanding is most advanced, and outline four key concepts that emerge from both functional and structural studies. I will end by discussing how voltage sensors might move within the membrane and highlight exciting directions for future research.

Voltage-sensing domains in membrane proteins

The best understood voltage sensors are found in the voltage-activated ion channels that are so crucial for electrical signaling in the nervous system. When these channels were first cloned, the fourth putative membrane-spanning segment, termed S4, stood out because it contains many basic Arg or Lys residues that are capable of carrying a positive charge^{2-4, 6, 7}. The S4 segment is always found together with three other transmembrane segments (S1-S3) that contain negatively charged acidic residues, and the four segments together collectively form an S1-S4 voltage-sensing domain (Fig 1a). The concept that S1-S4 is a domain in the formal sense grew out of comparisons of Kv channels and other simpler K⁺ channels that lack the S1-S4 region⁸, experiments identifying important acidic residues in S2 and S3⁹, and the discovery that tarantula toxins can bind to S1-S4 domains in different types of voltage-activated ion channels¹⁰. That S1-S4 voltage sensors are functionally independent protein domains was demonstrated by Lu and colleagues when they found that the S1-S4 region of a Kv channel could endow the KcsA K⁺ channel with strong voltage-sensitivity^{11, 12}. Bacteria can synthesize, properly fold and insert isolated S1-S4 voltage sensing domains into their membranes^{5, 13}, further supporting the idea that S1-S4 is an independent domain.

For S1-S4 voltage sensing domains to sense voltage across membranes, they must change conformation between states where charged residues or dipoles have different relative free energies. In principle, this conformational change could result from charges moving through a stationary electric field or the electric field reshaping around fixed charges. An important advance in studying this conformational change was the discovery that the movement of charges relative to the field could be detected electrically as a non-linear capacitive current, or gating current¹⁴⁻¹⁶. Measurements of this sort led to two key conclusions about the conformational change occurring when voltage sensors activate. First, the conformational change involves the movement of 3 to 3.5 elementary charges relative to the full membrane electric field for each S1-S4 voltage sensor¹⁷⁻²¹. Second, the external four Arg residues in S4 (Fig1b) carry most of the measured gating charge^{18, 19, 21}. Although reshaping of the electric field may play a role, it is clear that S4 moves outward in channels that activate with membrane depolarization²²⁻³⁷ (see Voltage sensor motions).

While S1-S4 voltage-sensing domains were originally thought to only exist in voltage-activated ion channels, Okamura's group made the seminal discovery that S1-S4 domains exist in proteins that lack associated ion conducting pores³⁸. The *Ciona intestinalis* voltage-sensitive phosphatase (Ci-VSP), for example, uses an S1-S4 domain to control the hydrolysis of membrane phosphoinositides by an associated phosphatase domain^{38, 39} (Fig1a). Another S1-S4 domain protein without a separate pore domain was shown to function as a voltage-activated proton channel (Hv1)⁴⁰⁻⁴² (Fig1a). In this case, the S1-S4 domain appears to provide the proton permeation pathway (see below). One of the interesting differences between voltage-activated ion channels and the other voltage-sensing proteins is the number of S1-S4 domains they contain. There are four in Kv, Nav and Cav channels,²⁻⁵ two in the proton channel⁴²,

⁴³ and only one in the phosphatase³⁹. Although much remains to be learned about these fascinating new voltage sensing proteins, key regions can be swapped with Kv channels without disrupting function or pharmacological sensitivities, suggesting that their basic voltage sensing mechanisms are related⁴⁴.

Structures of voltage-sensing domains

For many years, the lack of three dimensional structures of voltage sensors was a barrier to understanding the mechanism of voltage sensing. At present, four X-ray structures have been solved which contain S1-S4 voltage sensing domains^{5, 45, 46}. As will be discussed below, these structures are probably most relevant for thinking about the activated state of voltage sensors that exist at depolarized membrane voltages. The first two structures reported in 2003 by MacKinnon and his colleagues were of the isolated S1-S4 domain and full-length channel of KvAP⁵, an archaeobacterial Kv channel from *Aeropyrum pernix*. The structure of the isolated S1-S4 domain comprises five helices, with S1, S2 and S4 forming long uninterrupted helices and S3 containing two shorter helices (Fig1b), consistent with helix scanning studies in eukaryotic Kv channels⁴⁷⁻⁵⁰. Because this domain was crystallized without the pore domain, it wasn't obvious how it is oriented with respect to the pore or the surrounding membrane.

The structure of the full-length KvAP channel didn't answer this question because the voltage-sensing domains in this structure are distorted⁵. However, comparisons of the structures of the isolated S1-S4 domain and the full-length channel led MacKinnon and colleagues to three important conclusions^{5, 34}. First, both structures contain a conserved helix-turn-helix motif that is composed of the S3b and S4 helices, termed the voltage sensor paddle (Fig1a,b; purple helices). This motif contains the most crucial S4 Arg residues^{18, 19} (stick side chains in Fig1b) and is widely targeted by tarantula toxins^{44, 50-52} that inhibit voltage-activated ion channels by binding within the membrane⁵³⁻⁵⁵. Second, the distortions evident in the KvAP full-length structure suggest that S1-S4 domains are inherently flexible and lack tight attachments to the surface of the pore domain. In other words, the 'malleability' of voltage-sensors reveals an underlying structural flexibility that may be related to how these domains function. Third, the paddle motif moves at the periphery of the channel protein in contact with the surrounding lipid membrane. As wonderful as it was to 'see' the first structures of voltage sensors and to ponder these new ideas, many questions remained unanswered. How distorted is the structure of the S1-S4 domain in the full-length KvAP channel? How representative is the structure of the isolated S1-S4 domain of KvAP, and how is this domain oriented with respect to the central pore domain?

The X-ray structure of the eukaryotic Kv1.2 channel provided the first clear perspective of S1-S4 domains in a full-length channel and revealed how they are oriented with respect to the central pore domain and the surrounding lipid membrane^{45, 56}. Even though the quality of electron density within the S1-S4 domain is relatively weak (mean *B* factor = 162 Å²), leaving many residues unresolved, the S1 through S4 helices clearly adopt transmembrane orientations and appear loosely attached to the central pore domain. The most recent X-ray structure of the Kv1.2 channel containing the paddle motif from the Kv2.1 channel (paddle-chimera channel)⁴⁶ currently provides the clearest picture of voltage sensor structure in a full-length channel (Fig2). The close apposition of the S3b-S4 helices are similar to what is seen for the paddle motif in the KvAP structures, S1 and S4 are positioned adjacent to the pore domain and both S2 and S3 are positioned peripherally. Electron densities for many lipid molecules are also present in the maps for the paddle-chimera channel, revealing that lipids interact intimately with the channel. Lipids are present between the S1-S4 voltage-sensing domain and the pore domain, between adjacent S1-S4 domains and even intercalating between helices (Fig2a,b; teal molecules). Superposition of the structures of the paddle-chimera and the isolated S1-S4 domain of KvAP (Fig3a,b) reveals that the structures of voltage sensors are well conserved

from archaeobacteria to humans. In addition, two additional structural features can be seen in the comparison. First, both structures reveal a widespread network of electrostatic interactions between basic residues in S4 and acidic residues in S1, S2 and S3, and in many cases the side chains of these key residues virtually superimpose (Fig3a,b). In the activated conformation of the paddle-chimera there are two clusters of acidic residues, an external acidic cluster formed by Glu residues in S1 and S2, and an internal acidic cluster consisting of a Glu in S2 and an Asp in S3a. The residues in the external cluster lie within a large crevice exposed to the external aqueous environment (Fig3a; green arrow), while the internal cluster is largely buried within the protein interior. Based on their earlier mutagenic rescue experiments, Papazian and colleagues had concluded that the inner acidic residues in S2 and S3 form a stabilizing network with the fifth basic residue in S4 when the voltage sensor is in an activated state⁹, in agreement with the inner network shown in the structure of the paddle-chimera. A second structural feature evident in the comparison is that the outermost Arg residues do not interact with acidic residues, but project out from the domain towards the surrounding membrane (red arrow in Fig2a,b and Fig3a,b). Both of these structures were solved in the absence of an electric field, or 0 mV, a voltage where functional studies on KvAP and the paddle-chimera indicate that their voltage sensors are in activated conformations^{46, 57}, implying that these two structures are representative of the activated state. The internal S6 gate region within the pore of the paddle-chimera is open, consistent with the voltage sensors being activated, and there are no structural changes in the selectivity filter to suggest that the channel is inactivated⁵⁸. The paddle-chimera structure is compatible with constraints imposed by metal bridges between S4 and S5 in the activated state³⁵, further suggesting that it is representative of the activated conformation.

Emerging concepts

The available structures of activated voltage sensors represent a remarkable advance and, when considered in the context of other biophysical studies, constrain our thinking about the mechanism of voltage sensing. While it is clear that structures of voltage sensors in other conformations are needed, such as the resting state that predominates at negative voltages, the available data allow us to frame several key concepts that are fundamental to the mechanism of voltage sensing. Some of these concepts have been central to our thinking for some time, while others have their roots in the more recent X-ray structures.

Charged residues have two important roles

The external four Arg residues in S4 appear to carry the majority of gating charge, thus serving to drive conformational changes in response to changes in membrane voltage^{18, 19, 21}. Although this concept is also supported by extensive evidence that S4 actually moves²²⁻³⁷, an untested assumption in many of these studies is that neutralization of charged residues does not alter the position of the S4 helix. If this were to occur, the contribution of residues to gating charge could go undetected. The second important role of charged residues is to stabilize the basic Arg residues in the low dielectric of the membrane interior. Glu and Asp residues in S1-S3 form the external and internal clusters seen in the paddle-chimera structure and contribute to a network of electrostatic interactions that stabilize some of the S4 Arg and Lys residues within the membrane^{9, 46}(Fig3a,b). The outermost Arg residues do not appear to interact with acidic residues in the external cluster, but project towards the surrounding lipid membrane (see Fig3a,b and below).

Voltage sensors contain a water-filled permeation pathway

The first hints that water might penetrate voltage sensors and that the charged Arg residues move within an aqueous pathway came from studies by Horn and others showing extensive reactivity of water soluble MTS reagents with Cys residues introduced within S4²²⁻²⁵. In effect, the Arg residues in S4 can be viewed as tethered ions that move within S1-S4 domains.

Remarkably, untethered ions can actually permeate through voltage sensors. Bezanilla and colleagues found that His residues introduced within S4 create a proton pore^{27, 33}, and Isacoff's group made the astonishing discovery that ions as large as guanidinium can permeate S1-S4 domains when the outermost Arg in S4 is truncated^{59, 60}. The recent discoveries of voltage-activated proton channels containing only an S1-S4 domain⁴⁰⁻⁴² suggests that this permeation pathway can serve an important biological function. The paddle-chimera structure shows a large crevice between S1-S4 helices that would be contiguous with the external aqueous phase when the channel is embedded in a membrane. This region of the voltage sensor houses many of the basic and acidic residues (Fig3a; green arrow), presumably identifying the pathway for ions to permeate voltage sensors⁶¹. One important implication of water intercalation into voltage sensors is that it would be expected to focus the membrane electric field, allowing movements smaller than the full thickness of the bilayer to translocate charge across the full extent of the electric field. Indeed, focusing of the electric field has been inferred from studies with electrochromic fluorophores⁶², proton permeation mediated by introduced His residues⁶³ and charged MTS compounds with variable length tethers⁶⁴.

The paddle motif flexes within S1-S4 domains

The S3b-S4 paddle motif contains the outer four S4 Arg residues that carry most of the gating charge in Kv channels^{18, 19, 21}, and in the X-ray structures this motif is connected to other regions of the protein via flexible linkers^{5, 34}. The accessibility of biotinylated positions in the paddle motif to avidin suggests that it is a uniquely mobile motif within S1-S4 domains^{34, 36, 65}, a feature that also can be seen in electron paramagnetic resonance (EPR) measurements of spin label mobility^{13, 66}. The paddle motif can be transplanted between proteins with S1-S4 domains, whether they are found in archaeobacterial or eukaryotic Kv channels⁴⁴, Nav channels⁶⁷, or voltage-sensing proteins like Ci-VSP or Hv1⁴⁴, suggesting that this motif resides in a relatively unconstrained environment and that its function is conserved throughout voltage sensors. Another indicator that the paddle motif is a crucial flexing motif within S1-S4 voltage sensors is that it forms the receptor for tarantula toxins, such as hanatoxin^{44, 50-52}, which inhibit voltage sensor activation^{51, 54}. Many other toxins from spiders and scorpions alter the activity of voltage-activated ion channels by binding to paddle motifs⁶⁷, indicating that nature has targeted this motif throughout evolution.

Voltage sensors interact intimately with lipids

The structures of KvAP and Kv1.2 channels discussed above suggest that the paddle motif is exposure to the surrounding lipids and the paddle-chimera structure provides a glimpse of what these interactions might look like because lipid molecules actually crystallize with the channel (Fig2). EPR studies on the S1-S4 domain and full-length KvAP channel demonstrate extensive exposure of the voltage sensors to the surrounding lipid membrane^{13, 66, 68}. The interaction of tarantula toxins with paddle motifs also strongly support the idea that the paddle moves at the protein-lipid interface because these toxins bind to it within the membrane^{44, 53-55} and the toxin binding surface projects out towards the surrounding lipid membrane⁴⁴.

Although we are only beginning to learn about the nature of the interactions between lipids and voltage sensors, several recent studies suggest that these lipid-protein interactions may be fundamentally important for voltage sensor function. MacKinnon and colleagues showed that lipids with phosphate-containing headgroups are required for channel function and that they stabilize the voltage sensor in the activated state⁶⁹. One possibility is that the outer S4 Arg residues projecting out towards the surrounding membrane in the structure of the activated state of the paddle-chimera channel (red arrows in Figs2a,b,3a,b) actually interact directly with phosphate headgroups. The outer Arg residues exhibit exposure to the lipid membrane in EPR studies^{13, 66, 68} and stabilizing interactions between Arg residues and phosphate headgroups can be seen in molecular dynamic simulations⁷⁰⁻⁷².

A fascinating series of recent studies by Lu and coworkers points to intimate interactions between specific lipids and voltage sensors. They found that extracellular application of sphingomyelinase D, an enzyme that hydrolyzes the zwitterionic membrane phospholipid sphingomyelin to the anionic ceramide-1-phosphate, produces a dramatic stabilization of the activated state of the voltage sensors⁷³. The implication is that ionic interactions between positively charged S4 Arg residues and the negatively charged phosphate headgroup are strengthened when the positively charged choline group is removed. The effects of sphingomyelinase D can only be seen on certain types of Kv channels, suggesting that sphingomyelin may be interacting intimately with those channels that are sensitive to the lipase. In addition, sphingomyelinase D treatment dramatically alters the stability of hanatoxin bound to voltage-sensor paddles, consistent with an intimate interaction between toxins, lipids and paddle motifs⁵⁵. Treatment of membranes with sphingomyelinase C, which removes the entire headgroup of the lipid, immobilizes the voltage sensors in a resting state⁷⁴, consistent with the possibility that phosphate-containing lipids help to stabilize the voltage sensors in the activated state. Remarkably, binding of tarantula toxins to the voltage sensors appears to diminish access of sphingomyelinase C to the channel⁷⁴, suggesting that the interaction between sphingomyelin and voltage sensors are remarkably intimate. All of these studies raise the fascinating possibility that lipid interactions with voltage sensors may play crucial roles in the mechanism of voltage sensing.

Voltage sensor motions

To proceed from these concepts to a more refined understanding of the mechanisms of voltage sensing, we will need to see an actual structure of a voltage-sensing domain in the resting state. The rearrangements within the voltage sensors that occur as the protein moves from an activated state, of which we now know quite a bit about the structure, to the resting state, where we know relatively little, remains poorly constrained. If we are willing to use broad strokes, however, the emerging concepts can be put together to paint an impressionistic image of voltage sensor motions. It seems likely that the activated state of the voltage sensor is the stable conformation because this is the state found in the absence of a membrane electric field (0 mV). The domain is organized so that the S3b and S4 helices of the paddle motif reside in a relatively unconstrained and flexible environment, with much of the surface of the motif exposed to the surrounding lipid bilayer (Fig2,3). This includes the outermost Arg residues that likely interact with phosphate lipid head groups (Fig4). The deeper Arg residues are stabilized by interactions with acidic residues in S1, S2 and S3 (Fig3,4), which line a water-filled crevice projecting into the protein from the external aqueous phase. In the case of Kv channels studied thus far, the S1-S4 domains have few interactions with the pore domain within the outer half of the bilayer that are required for voltage sensing^{45, 75}. The existence of Ci-VSP and Hv1 seem to confirm this, in particular because both of these voltage sensing proteins can function as monomers^{39, 42}. When membrane voltage becomes negative (inside relative to outside), the paddle motif moves the most, with charged Arg residues in S4 moving towards the internal side of the membrane (Fig4·5). The S3b helix may move in association with S4, or the interface between the two helices may change somewhat, but in either case it seems likely that the S3b helix does not become deeply submerged in the membrane^{54, 76-78}. The outer most Arg residues probably exchange their interactions with phosphate head groups for acidic residues in the external cluster in an ion-exchange type mechanism, the middle Arg residues exchange interactions with the external cluster for those in the internal cluster (Fig4), and the inner most Arg residues might exchange interactions with the internal cluster for those with phosphate head groups of lipids within the inner leaflet of the bilayer⁷⁰. It's tempting to speculate that the prominent external facing crevice seen in the paddle-chimera structure (Fig3a; green arrow) may largely vanish when the domain adopts a resting conformation and an inward facing water-filled crevice might emerge⁷⁹.

Range of motion

While this picture captures the key concepts for which there is experimental evidence, we haven't said much about the nature of the motions within the voltage-sensing domains, and this is where things remain somewhat dicey. Viewed collectively, most of the evidence is compatible with the notion that the Arg residues in S4 move about 15 Å inward along a trajectory that is roughly perpendicular to the membrane plane as the voltage sensors move from activated to resting states. Tarantula toxins can partition into the outer leaflet of the membrane⁵³⁻⁵⁵ and bind to the paddle motif in both resting and activated states in eukaryotic Kv channels⁵⁴, a constraint suggesting that the motions of the paddle are confined to the outer half of the bilayer. Similarly, fluorescence resonance energy transfer between fluorophores attached near S4 and lipophilic acceptors that flip back and forth across the membrane suggest that motions of S4 are considerably less than the full thickness of the bilayer⁸⁰. One informative estimate of the allowed motions of S4 comes from experiments measuring the reactivity of biotinylated residues in the S1-S4 domain of KvAP with avidin applied to external or internal solutions^{34, 36}. When the accessibility data are mapped onto the structure of the S1-S4 domain of KvAP, it is evident that the S4 helix must be capable of motions of at least 15 Å (Fig5a). Because the structure represents the activated conformation of the voltage sensor, the positions towards the external side of the membrane that are accessible to internal avidin (Fig5a; yellow and blue spheres) must move to within 10 Å of the internal side of the membrane in the resting state (in the vicinity of the blue spheres towards the internal side of the structure, which are marked by a dashed line). Such a motion is consistent with MTS accessibility experiments³⁰, His-mediated proton transfer studies^{33, 63} and mutagenic rescue experiments⁸¹. Another constraint for S4 motions comes from the recent discovery by Bezannila and colleagues in the Shaker Kv channel that Cys residues introduced at the outermost Arg in S4 can form disulphide and metal bridges with Cys residues introduced into S1 or S2 when the voltage sensors are in the resting state³⁷ (Fig5b). The distance between Cβ atoms in the X-ray structure of the paddle-chimera is 19 Å for the S4 (green sphere) to S1 (light pink sphere) bridge, and 18.4 Å for the S4 to S2 (magenta sphere) bridge. Although S4 might move somewhat more or less than the 18-19 Å separating these residues (depending on the orientation of the Cys side chains), these bridges would seem to indicate that S4 motions in Shaker Kv channels are somewhat less than those proposed for KvAP based on the biotin-avidin studies. In contrast to these estimates, most fluorescence distance measurements point to motions on the order of a few angstroms^{30, 31, 82}. A recent fluorescence study on the Shaker Kv channel reporting an extensive number of distance constraints supports larger displacements of S4, on the order of about 10 Å⁸³. The question of how far particular regions within S1-S4 move will require further study and it will be important to explore the possibility that the range of motions can vary for different types of voltage-activated ion channels.

Possible rotational motions

In addition to translational motions, the S4 helix has been proposed to rotate^{6, 7}, perhaps as much as 180°^{30, 31, 84}. The bridges already discussed between S4 and either S1 or S2³⁷ offer an informative perspective on the question of S4 rotations. If we consider these bridges in the context of the X-ray structure of the activated state of the paddle-chimera (Fig5c), it seems that S4 wouldn't need to rotate much for the relevant position (green sphere) to form a bridge with S1 (light pink sphere), but would need to rotate significantly (< 90°) to bridge with S2 (magenta sphere). These bridges seem to rule out large rotations of S4, but would be consistent with the possibility that S4 undergoes a modest rigid body rotation relative to S1 and S2. Another scenario put forward based on the X-ray structures of the paddle-chimera⁴⁶ and the MlotiK channel⁸⁵ is that S4 undergoes a transition from a standard α-helix with 3.6 residues per turn in the activated state to a more tightly wound 3₁₀ helix with 3 residues per turn in the resting state. Transitioning from a standard α-helix in the activated state to a 3₁₀ helix in the resting state would allow the outermost Arg residues to rotate moderately into the S1-S4 domain

without necessitating the type of rigid body motion that would require extensive adjustments in packing between S4 and the other helices in the voltage sensor. In the structure of the activated paddle-chimera channel, the internal region of S4 is also a 3_{10} helix, leading to the suggestion of a wave-like transition where the zone of 3_{10} secondary structure propagates from the internal to external regions of S4 as the voltage sensor moves from activated to resting states⁴⁶. 3_{10} helices are energetically unstable, but in the case of voltage sensors one can imagine that the energy necessary to adopt a 3_{10} helix could come from the membrane electric field. In this way, the electric field would hold the voltage sensor in an unstable or 'cocked' position at negative membrane voltages, which would readily relax to an energetically more stable conformation once the field dissipates with depolarization of the membrane towards 0 mV.

Prospects for the future

The present picture of voltage sensor structure and motions has evolved considerably since the cloning of the first membrane proteins with S1-S4 voltage sensors, yet many exciting and open questions remain. What does the structure of a voltage sensor look like in the resting conformation? How does the voltage sensor move during activation? Where exactly is the permeation pathway for ions to permeate S1-S4 domains? How do the interactions of lipids with voltage sensors influence their structure and motions? What does the structure of the lipid membrane look like around voltage sensors? The majority of structural and biophysical experiments have been done on a relatively small number of voltage-activated ion channels, mostly Shaker, Kv1.2 and KvAP, and at this point we don't know how similar the mechanisms will be for the large family of ion channels and enzymes that contain S1-S4 domains. Indeed, there is precedence from the work of Aldrich and colleagues that gating in some types of Kv channels involves only a subset of the conformational changes thought to occur in Shaker Kv channels⁸⁶. In addition, very little is known about the S1-S4 domains for ion channels such as cyclic nucleotide gated channels or transient receptor potential channels. Do the S1-S4 domains in these channels have any voltage sensing functions? Do they undergo structural changes upon binding ligands that might be related to those that occur in voltage sensors? In many respects, we have only recently developed a solid foundation for thinking about how S1-S4 voltage-sensing domains work, and there are many fascinating experiments to be done.

Acknowledgements

I thank Jeff Diamond, Joe Mindell, Shai Silberberg and the members of the Swartz laboratory for helpful discussions. I thank Rod MacKinnon for providing coordinates for the paddle-chimera and those for the superimposed structures shown in Fig 3. This work was supported by the Intramural Research Program of the NINDS, NIH.

References

1. Hodgkin AL, Huxley AF. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol* 1952;117:500–44. [PubMed: 12991237]
2. Noda M, et al. Expression of functional sodium channels from cloned cDNA. *Nature* 1986;322:826–8. [PubMed: 2427955]
3. Tanabe T, et al. Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature* 1987;328:313–8. [PubMed: 3037387]
4. Tempel BL, Papazian DM, Schwarz TL, Jan YN, Jan LY. Sequence of a probable potassium channel component encoded at Shaker locus of *Drosophila*. *Science* 1987;237:770–5. [PubMed: 2441471]
5. Jiang Y, et al. X-ray structure of a voltage-dependent K⁺ channel. *Nature* 2003;423:33–41. [PubMed: 12721618]
6. Catterall WA. Molecular properties of voltage-sensitive sodium channels. *Annu Rev Biochem* 1986;55:953–85. [PubMed: 2427018]

7. Guy HR, Seetharamulu P. Molecular model of the action potential sodium channel. *Proc Natl Acad Sci U S A* 1986;83:508–12. [PubMed: 2417247]
8. Kubo Y, Baldwin TJ, Jan YN, Jan LY. Primary structure and functional expression of a mouse inward rectifier potassium channel. *Nature* 1993;362:127–33. [PubMed: 7680768]
9. Papazian DM, et al. Electrostatic interactions of S4 voltage sensor in Shaker K⁺ channel. *Neuron* 1995;14:1293–301. [PubMed: 7605638]
10. Li-Smerin Y, Swartz KJ. Gating modifier toxins reveal a conserved structural motif in voltage-gated Ca²⁺ and K⁺ channels. *Proc Natl Acad Sci U S A* 1998;95:8585–9. [PubMed: 9671721]
11. Lu Z, Klem AM, Ramu Y. Coupling between Voltage Sensors and Activation Gate in Voltage-gated K⁺ Channels. *J Gen Physiol* 2002;120:663–76. [PubMed: 12407078]
12. Lu Z, Klem AM, Ramu Y. Ion conduction pore is conserved among potassium channels. *Nature* 2001;413:809–13. [PubMed: 11677598]
13. Chakrapani S, Cuello LG, Cortes DM, Perozo E. Structural dynamics of an isolated voltage-sensor domain in a lipid bilayer. *Structure* 2008;16:398–409. [PubMed: 18334215]
14. Armstrong CM, Bezanilla F. Currents related to movement of the gating particles of the sodium channels. *Nature* 1973;242:459–61. [PubMed: 4700900]
15. Schneider MF, Chandler WK. Voltage dependent charge movement of skeletal muscle: a possible step in excitation-contraction coupling. *Nature* 1973;242:244–6. [PubMed: 4540479]
16. Keynes RD, Rojas E. Kinetics and steady-state properties of the charged system controlling sodium conductance in the squid giant axon. *J Physiol* 1974;239:393–434. [PubMed: 4414038]
17. Schoppa NE, McCormack K, Tanouye MA, Sigworth FJ. The size of gating charge in wild-type and mutant Shaker potassium channels. *Science* 1992;255:1712–5. [PubMed: 1553560]
18. Seoh SA, Sigg D, Papazian DM, Bezanilla F. Voltage-sensing residues in the S2 and S4 segments of the Shaker K⁺ channel. *Neuron* 1996;16:1159–67. [PubMed: 8663992]
19. Aggarwal SK, MacKinnon R. Contribution of the S4 segment to gating charge in the Shaker K⁺ channel. *Neuron* 1996;16:1169–77. [PubMed: 8663993]
20. Noceti F, et al. Effective gating charges per channel in voltage-dependent K⁺ and Ca²⁺ channels. *J Gen Physiol* 1996;108:143–55. [PubMed: 8882860]
21. Ahern CA, Horn R. Specificity of charge-carrying residues in the voltage sensor of potassium channels. *J Gen Physiol* 2004;123:205–16. [PubMed: 14769847]
22. Yang N, Horn R. Evidence for voltage-dependent S4 movement in sodium channels. *Neuron* 1995;15:213–8. [PubMed: 7619524]
23. Yang N, George AL Jr, Horn R. Molecular basis of charge movement in voltage-gated sodium channels. *Neuron* 1996;16:113–22. [PubMed: 8562074]
24. Larsson HP, Baker OS, Dhillon DS, Isacoff EY. Transmembrane movement of the shaker K⁺ channel S4. *Neuron* 1996;16:387–97. [PubMed: 8789953]
25. Yusaf SP, Wray D, Sivaprasadarao A. Measurement of the movement of the S4 segment during the activation of a voltage-gated potassium channel. *Pflugers Arch* 1996;433:91–7. [PubMed: 9019737]
26. Mannuzzu LM, Moronne MM, Isacoff EY. Direct physical measure of conformational rearrangement underlying potassium channel gating. *Science* 1996;271:213–6. [PubMed: 8539623]
27. Starace DM, Stefani E, Bezanilla F. Voltage-dependent proton transport by the voltage sensor of the Shaker K⁺ channel. *Neuron* 1997;19:1319–27. [PubMed: 9427254]
28. Cha A, Bezanilla F. Characterizing voltage-dependent conformational changes in the Shaker K⁺ channel with fluorescence. *Neuron* 1997;19:1127–40. [PubMed: 9390525]
29. Cha A, Bezanilla F. Structural implications of fluorescence quenching in the Shaker K⁺ channel. *J Gen Physiol* 1998;112:391–408. [PubMed: 9758859]
30. Glauner KS, Mannuzzu LM, Gandhi CS, Isacoff EY. Spectroscopic mapping of voltage sensor movement in the Shaker potassium channel. *Nature* 1999;402:813–7. [PubMed: 10617202]
31. Cha A, Snyder GE, Selvin PR, Bezanilla F. Atomic scale movement of the voltage-sensing region in a potassium channel measured via spectroscopy. *Nature* 1999;402:809–13. [PubMed: 10617201]
32. Gandhi CS, Loots E, Isacoff EY. Reconstructing voltage sensor-pore interaction from a fluorescence scan of a voltage-gated K⁺ channel. *Neuron* 2000;27:585–95. [PubMed: 11055440]

33. Starace DM, Bezannila F. Histidine scanning mutagenesis of basic residues of the S4 segment of the shaker k⁺ channel. *J Gen Physiol* 2001;117:469–90. [PubMed: 11331357]
34. Jiang Y, Ruta V, Chen J, Lee A, MacKinnon R. The principle of gating charge movement in a voltage-dependent K⁺ channel. *Nature* 2003;423:42–8. [PubMed: 12721619]
35. Laine M, et al. Atomic proximity between S4 segment and pore domain in Shaker potassium channels. *Neuron* 2003;39:467–81. [PubMed: 12895421]
36. Ruta V, Chen J, MacKinnon R. Calibrated measurement of gating-charge arginine displacement in the KvAP voltage-dependent K⁺ channel. *Cell* 2005;123:463–75. [PubMed: 16269337]
37. Campos FV, Chanda B, Roux B, Bezannila F. Two atomic constraints unambiguously position the S4 segment relative to S1 and S2 segments in the closed state of Shaker K channel. *Proc Natl Acad Sci U S A* 2007;104:7904–9. [PubMed: 17470814]
38. Murata Y, Iwasaki H, Sasaki M, Inaba K, Okamura Y. Phosphoinositide phosphatase activity coupled to an intrinsic voltage sensor. *Nature* 2005;435:1239–43. [PubMed: 15902207]
39. Kohout SC, Ulbrich MH, Bell SC, Isacoff EY. Subunit organization and functional transitions in Ci-VSP. *Nat Struct Mol Biol* 2008;15:106–8. [PubMed: 18084307]
40. Sasaki M, Takagi M, Okamura Y. A Voltage Sensor-Domain Protein is a Voltage-Gated Proton Channel. *Science* 2006;312:589–592. [PubMed: 16556803]
41. Ramsey IS, Moran MM, Chong JA, Clapham DE. A voltage-gated proton-selective channel lacking the pore domain. *Nature* 2006;440:1213–1216. [PubMed: 16554753]
42. Tombola F, Ulbrich MH, Isacoff EY. The voltage-gated proton channel Hv1 has two pores, each controlled by one voltage sensor. *Neuron* 2008;58:546–56. [PubMed: 18498736]
43. Lee SY, Letts JA, Mackinnon R. Dimeric subunit stoichiometry of the human voltage-dependent proton channel Hv1. *Proc Natl Acad Sci U S A* 2008;105:7692–5. [PubMed: 18509058]
44. Alabi AA, Bahamonde MI, Jung HJ, Kim JI, Swartz KJ. Portability of paddle motif function and pharmacology in voltage sensors. *Nature* 2007;450:370–5. [PubMed: 18004375]
45. Long SB, Campbell EB, Mackinnon R. Crystal structure of a mammalian voltage-dependent Shaker family K⁺ channel. *Science* 2005;309:897–903. [PubMed: 16002581]
46. Long SB, Tao X, Campbell EB, MacKinnon R. Atomic structure of a voltage-dependent K⁺ channel in a lipid membrane-like environment. *Nature* 2007;450:376–82. [PubMed: 18004376]
47. Monks SA, Needleman DJ, Miller C. Helical Structure and Packing Orientation of the S2 Segment in the Shaker K⁺ Channel. *J Gen Physiol* 1999;113:415–423. [PubMed: 10051517]
48. Li-Smerin Y, Hackos DH, Swartz KJ. Alpha-helical structural elements within the voltage-sensing domains of a K⁺ channel. *J Gen Physiol* 2000a;115:33–49. [PubMed: 10613917]
49. Hong KH, Miller C. The lipid-protein interface of a Shaker K⁺ channel. *J Gen Physiol* 2000;115:51–58. [PubMed: 10613918]
50. Li-Smerin Y, Swartz KJ. Helical structure of the COOH terminus of S3 and its contribution to the gating modifier toxin receptor in voltage-gated ion channels. *J Gen Physiol* 2001;117:205–18. [PubMed: 11222625]
51. Swartz KJ, MacKinnon R. Hanatoxin modifies the gating of a voltage-dependent K⁺ channel through multiple binding sites. *Neuron* 1997a;18:665–73. [PubMed: 9136774]
52. Li-Smerin Y, Swartz KJ. Localization and molecular determinants of the hanatoxin receptors on the voltage-sensing domain of a K⁺ channel. *J Gen Physiol* 2000;115:673–684. [PubMed: 10828242]
53. Lee SY, MacKinnon R. A membrane-access mechanism of ion channel inhibition by voltage sensor toxins from spider venom. *Nature* 2004;430:232–5. [PubMed: 15241419]
54. Phillips LR, et al. Voltage-sensor activation with a tarantula toxin as cargo. *Nature* 2005;436:857–60. [PubMed: 16094370]
55. Milescu M, et al. Tarantula toxins interact with voltage sensors within lipid membranes. *J Gen Physiol* 2007;130:497–511. [PubMed: 17938232]
56. Long SB, Campbell EB, Mackinnon R. Voltage sensor of Kv1.2: structural basis of electromechanical coupling. *Science* 2005;309:903–8. [PubMed: 16002579]
57. Ruta V, Jiang Y, Lee A, Chen J, MacKinnon R. Functional analysis of an archaeobacterial voltage-dependent K⁺ channel. *Nature* 2003;422:180–5. [PubMed: 12629550]

58. Yellen G. The voltage-gated potassium channels and their relatives. *Nature* 2002;419:35–42. [PubMed: 12214225]
59. Tombola F, Pathak MM, Isacoff EY. Voltage-sensing arginines in a potassium channel permeate and occlude cation-selective pores. *Neuron* 2005;45:379–88. [PubMed: 15694325]
60. Sokolov S, Scheuer T, Catterall WA. Ion permeation through a voltage-sensitive gating pore in brain sodium channels having voltage sensor mutations. *Neuron* 2005;47:183–9. [PubMed: 16039561]
61. Freites JA, Tobias DJ, White SH. A Voltage-Sensor Water Pore. *Biophys J*. 2006
62. Asamoah OK, Wuskell JP, Loew LM, Bezanilla F. A fluorometric approach to local electric field measurements in a voltage-gated ion channel. *Neuron* 2003;37:85–97. [PubMed: 12526775]
63. Starace DM, Bezanilla F. A proton pore in a potassium channel voltage sensor reveals a focused electric field. *Nature* 2004;427:548–53. [PubMed: 14765197]
64. Ahern CA, Horn R. Focused electric field across the voltage sensor of potassium channels. *Neuron* 2005;48:25–9. [PubMed: 16202706]
65. Banerjee A, MacKinnon R. Inferred motions of the S3a helix during voltage-dependent K⁺ channel gating. *J Mol Biol* 2008;381:569–80. [PubMed: 18632115]
66. Cuello LG, Cortes DM, Perozo E. Molecular architecture of the KvAP voltage-dependent K⁺ channel in a lipid bilayer. *Science* 2004;306:491–5. [PubMed: 15486302]
67. Bosmans F, Martin-Eauclaire MF, Swartz KJ. Deconstructing voltage sensor function and pharmacology in sodium channels. *Nature*. 2008in press
68. Vamvouka M, Cieslak J, Van Eps N, Hubbell W, Gross A. The structure of the lipid-embedded potassium channel voltage sensor determined by double-electron-electron resonance spectroscopy. *Protein Sci* 2008;17:506–17. [PubMed: 18287283]
69. Schmidt D, Jiang QX, MacKinnon R. Phospholipids and the origin of cationic gating charges in voltage sensors. *Nature* 2006;444:775–9. [PubMed: 17136096]
70. Jogini V, Roux B. Dynamics of the Kv1.2 voltage-gated K⁺ channel in a membrane environment. *Biophys J* 2007;93:3070–82. [PubMed: 17704179]
71. Sands ZA, Sansom MS. How does a voltage sensor interact with a lipid bilayer? Simulations of a potassium channel domain. *Structure* 2007;15:235–44. [PubMed: 17292841]
72. Freites JA, Tobias DJ, von Heijne G, White SH. Interface connections of a transmembrane voltage sensor. *Proc Natl Acad Sci U S A* 2005;102:15059–64. [PubMed: 16217012]
73. Ramu Y, Xu Y, Lu Z. Enzymatic activation of voltage-gated potassium channels. *Nature* 2006;442:696–9. [PubMed: 16799569]
74. Xu Y, Ramu Y, Lu Z. Removal of phospho-head groups of membrane lipids immobilizes voltage sensors of K⁺ channels. *Nature* 2008;451:826–9. [PubMed: 18273018]
75. Soler-Llavina GJ, Chang TH, Swartz KJ. Functional interactions at the interface between voltage-sensing and pore domains in the Shaker K(v) channel. *Neuron* 2006;52:623–34. [PubMed: 17114047]
76. Nguyen TP, Horn R. Movement and crevices around a sodium channel S3 segment. *J Gen Physiol* 2002;120:419–36. [PubMed: 12198095]
77. Gandhi CS, Clark E, Loots E, Pralle A, Isacoff EY. The orientation and molecular movement of a K⁺ channel voltage-sensing domain. *Neuron* 2003;40:515–25. [PubMed: 14642276]
78. Darman RB, Ivy AA, Ketty V, Blaustein RO. Constraints on Voltage Sensor Movement in the Shaker K⁺ Channel. *J Gen Physiol*. 2006
79. Islas LD, Sigworth FJ. Electrostatics and the gating pore of Shaker potassium channels. *J Gen Physiol* 2001;117:69–89. [PubMed: 11134232]
80. Chanda B, Asamoah OK, Blunck R, Roux B, Bezanilla F. Gating charge displacement in voltage-gated ion channels involves limited transmembrane movement. *Nature* 2005;436:852–6. [PubMed: 16094369]
81. Grabe M, Lai HC, Jain M, Nung Jan Y, Yeh Jan L. Structure prediction for the down state of a potassium channel voltage sensor. *Nature* 2007;445:550–3. [PubMed: 17187053]
82. Posson DJ, Ge P, Miller C, Bezanilla F, Selvin PR. Small vertical movement of a K⁺ channel voltage sensor measured with luminescence energy transfer. *Nature* 2005;436:848–51. [PubMed: 16094368]
83. Posson DJ, Selvin PR. Extent of voltage sensor movement during gating of shaker K⁺ channels. *Neuron* 2008;59:98–109. [PubMed: 18614032]

84. Pathak MM, et al. Closing in on the resting state of the Shaker K(+) channel. *Neuron* 2007;56:124–40. [PubMed: 17920020]
85. Clayton GM, Altieri S, Heginbotham L, Unger VM, Morais-Cabral JH. Structure of the transmembrane regions of a bacterial cyclic nucleotide-regulated channel. *Proc Natl Acad Sci U S A* 2008;105:1511–5. [PubMed: 18216238]
86. Ledwell JL, Aldrich RW. Mutations in the S4 Region Isolate the Final Voltage-dependent Cooperative Step in Potassium Channel Activation. *J Gen Physiol* 1999;113:389–414. [PubMed: 10051516]

Figure 1. Types of membrane proteins that contain S1-S4 voltage-sensing domains and structure of an S1-S4 domain

a) Cartoon illustration of S1-S4 voltage-sensing domains in different types of membrane proteins. S1-S4 helices are labeled with the paddle motif colored purple. In voltage-activated ion channels the S1-S4 domains couple to an ion selective pore domain (yellow); only one of four S1-S4 domains is shown for clarity. In enzymes like the voltage-sensitive phosphates (Ci-VSP) the S1-S4 domain couples to a soluble phosphatase domain (yellow). In voltage-activated proton channels, protons are thought to permeate the S1-S4 domain directly. b) Ribbon representations of the X-ray structure of the S1-S4 domain of KvAP (PDB accession code 1ORS) with the paddle motif colored purple. The outer four Arg residues in S4 are shown as stick representations with carbon colored yellow and nitrogen colored blue. Structural representations in all figures were generated using PyMOL (DeLano Scientific).

Figure 2. Structure of the paddle-chimera Kv channel

a) Ribbon representation of the X-ray structure of the paddle-chimera channel viewed from the external side of the membrane. The paddle motif is colored purple, the pore domain is colored yellow, lipids are colored teal and basic residues in S4 are shown as stick representations with carbon atoms colored yellow, oxygen atoms colored red and nitrogen atoms colored blue. PDB accession code is 2R9R. Red arrow identifies outer S4 Arg residues projecting towards the lipid membrane. b) Side view of the paddle-chimera channel focusing on the S1-S4 voltage-sensing domain and its interface with the pore domain.

Figure 3. Charged amino acids in S1-S4 voltage-sensing domains

Superposition of the structures of the S1-S4 domains of KvAP (yellow ribbon) and the paddle-chimera channel (white ribbon) shown as a stereo pair and viewed from the side. The structures were aligned to minimize deviations between the S1 and S2 helices⁴⁶. Basic and acidic residues are shown as stick representations with carbon colored yellow or white, oxygen colored red and nitrogen colored blue. The green arrow identifies a aqueous crevice within the S1-S4 domain that would be continuous with the external solution and the red arrow identifies outer S4 Arg residues projecting towards the lipid membrane. PDB accession code for S1-S4 of KvAP is 1ORS and that for the paddle-chimera is 2R9R. b) Superimposed structures viewed from the external side of the membrane.

Figure 4. Changes in charge interactions during movements of voltage sensors

The cartoons illustrate shifting interactions between positively charged Arg residues in S4 and either negatively charged phosphate lipid headgroups (orange) or acidic residues (red) in the external and internal acidic residue clusters. Cartoon to the left is for the activated state that occurs at positive membrane voltages and that on the right is for the resting state that occurs at negative voltages. The illustrated motions of helices are not meant to imply anything about the structural changes occurring during voltage sensor movement.

Figure 5. Inferring motions from accessibility and bridging experiments

a) Ribbon representation of the structure of the S1-S4 domain of KvAP showing accessibility of biotinylated positions to avidin with 9 and 10 Å tethers³⁶. Positions marked by red spheres (α -carbon) are accessible to only external avidin, those marked by blue spheres are accessible to only internal avidin, and those marked by yellow spheres are accessible to either external or internal avidin. Black spheres mark inaccessible positions. The structure is of an activated voltage sensor, so yellow and blue positions located towards the external side of the membrane must move to within ~ 10 Å of the internal side of the membrane (dashed line) in the resting state, as indicated by the black arrow. Basic and acidic residues are shown as stick representations. b) Ribbon representation of the structure of the S1-S4 domain of the paddle-chimera channel showing positions that form disulphide or metal bridges in the resting state³⁷. The structures shown in a and b are aligned as in Fig 3. In the Shaker Kv channel, Cys substituted at R362 in S4 (equivalent to R294 in the paddle-chimera; green sphere) can bridge with either I241C in S1 (I177 in the paddle-chimera; light pink sphere) or I287C in S2 (I230 in the paddle-chimera; magenta sphere) when the voltage sensor are in a resting state. c) Bridging positions from b viewed from the external side of the membrane.