How membrane proteins sense voltage

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Abstract | The ionic gradients across cell membranes generate a transmembrane voltage that regulates the function of numerous membrane proteins such as ion channels, transporters, pumps and enzymes. The mechanisms by which proteins sense voltage is diverse: ion channels have a conserved, positively charged transmembrane region that moves in response to changes in membrane potential, some G-protein coupled receptors possess a specific voltage-sensing motif and some membrane pumps and transporters use the ions that they transport across membranes to sense membrane voltage. Characterizing the general features of voltage sensors might lead to the discovery of further membrane proteins that are voltage regulated.

Electrochemical gradient
The electrical and chemical driving force that moves ions.

Membrane potential
The difference between the internal minus the external potential in a membrane.

Dielectric
An insulator or substance of very low electrical conductivity.

Capacitor
A device that can store electrical charge.

Electric field
The space that surrounds an electric charge. For a stationary charge, the electric field E at the position where a particle of charge q is located is defined by the vector E = F/q, where F is the force exerted on the particle.

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Living cells are delimited by the lipid bilayer membrane, which separates an internal environment from a drastically different external medium. Although the total ionic concentration is similar on both sides of the bilayer, the concentration of specific ion species (for example, potassium ion (K^+)) is different. The electrodiffusion of the ions down their electrochemical gradient generates a charge separation across the membrane, which translates into a membrane potential that is on the order of −100 mV (negative inside the cell). The ionic gradients would eventually dissipate if it were not for the presence of ATP-driven ionic pumps that maintain the gradients. In most cases, these pumps do not have a significant role in the actual generation of the membrane potential.

The hydrophobic part of the lipid bilayer acts as the dielectric of the capacitor, which separates the charge difference and so generates the membrane potential. As this region is extremely thin (around 27 Å), the membrane potential translates into an intense electric field reaching values of ~10^7 V/m. Several membrane proteins use this electric field to regulate the function of the cell and to harness the membrane electric field, so the embedded membrane proteins must sense changes in this field. It is easy to envision how an electric charge or an electric dipole can be reorientated within a protein when the field is changed and therefore can produce a conformational change in the protein that may regulate its function. The movement of the charge or the dipole induces a transient current (gating current) that can be measured experimentally and provides direct information about such conformational changes.

Recent progress has provided insights into the mechanisms of voltage sensing and has identified new voltage-dependent proteins. In this Review, I will address how the membrane electric field is used by some membrane proteins to generate specific functions. First, I discuss the basic principles of voltage sensing, and the origin and detection of the gating charge. I then consider the different ways by which voltage is sensed and transmitted into cellular responses for different types of proteins. Voltage-sensing proteins have fundamental roles in many cell functions, such as setting the resting membrane potential in most cells, generating the nerve impulse and mediating the voltage regulation of phosphorylation. These proteins are also involved in synaptic transmission and regulating homeostasis in most cells.

What constitutes a voltage sensor?

The electric field is sensed through the translocation of charges or the movement of dipoles within the membrane field. The charged groups, arrangements, local field strength, disposition and movements of the charges or dipoles can be variable; however, the final result is that changes in the electric field are tranduced into a conformational change that accomplishes the function of the protein.

There are several ways in which proteins can sense voltage (FIG. 1). Charged residues such as Asp, Glu, Arg, Lys and His are likely candidates because they can reorientate in the field; this mechanism of voltage sensing has been found in voltage-gated ion channels (FIG. 1a). Side chains that have intrinsic dipole moment such as Tyr (FIG. 1b) might also orientate in the electric field. Although not yet identified as a voltage sensor, the α-helix with its intrinsic dipole moment represents a potential voltage-sensing structure by reorientating in the field (FIG. 1c). Proteins also contain cavities in which free ions can become associated. Changes in an electric field might move the free ion, which may initiate or are the result of a conformational change. This mechanism has been found to operate in

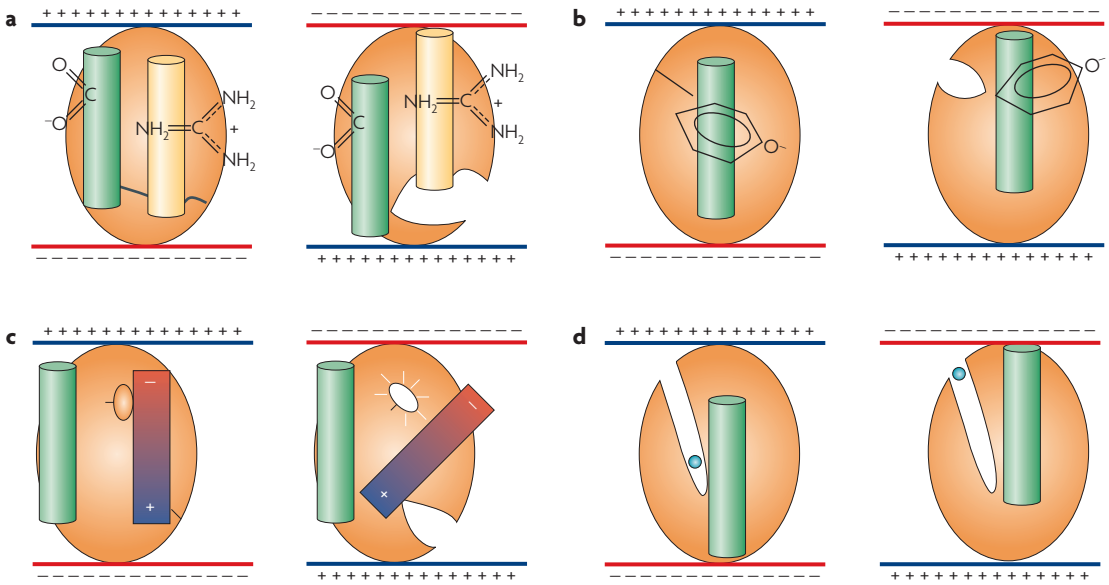


Figure 1 | **The possible structures of voltage sensors.** The diagrams show a hypothetical protein (orange oval) and the formation of an active site, which is due to a voltage-induced conformational change that is mediated by the defined regions of the protein (green and yellow cylinders). **a** | Charged amino acids may move within membranes in response to changes in voltage. The side groups of Asp and Arg are shown. **b** | Reorientation of an intrinsic residue dipole, such as Tyr, through changes in the field. **c** | An α-helix that is the length of the membrane (red to blue gradient) has a dipole moment that is equivalent to the length of the helix that separates ±0.5 electronic charges (e_0); therefore, it can also reorientate when the field is changed. The oval that is attached to the α-helix corresponds to a fluorophore that is quenched on the left and unquenched on the right, which indicates a conformational change. **d** | A channel within the protein can redistribute ions (light blue circle) according to the direction of the field and initiate a conformational change. Alternatively, a conformational change may form a channel that confers voltage dependence to the process as the ions move in the channel.

the sodium–potassium (Na^+–K^+) pump (FIG. 1d). In all cases, the movement of the charge or the reorientation of a region of the protein may represent an initial step that is coupled to further conformational changes or the final conformational change that regulates protein function. It is of course possible that in some cases several mechanisms operate simultaneously, as has been proposed for the Na^+–K^+ pump.

The electric field interacts with sensing charges. It is important to realize that the extent of the charge movement, as measured experimentally, depends on the magnitude of the charge and the strength of the electric field in the region where the charge moves. Owing to its complex molecular structure, the field strength near the voltage sensor can be different to the field strength near the lipid bilayer, which has a homogeneous molecular structure. So, even knowing the total charge of the sensor, an electrical measurement of the charge movement cannot be used to infer the distance travelled unless the field strength is known (BOX 1).

Gating currents. Any movement of charges or dipoles within an electric field produces an electric current. In the case of the protein voltage sensors, these charges move within the protein and are often confined to the intramembrane region. However, to maintain continuity, charges in the external and internal solution move in proportion to the charge moving in the membrane, generating a current that is detectable in the external circuit (BOX 1). When current was first measured in Na^+ channels[1] it was correlated with the opening of the channel; it was subsequently named the gating current. Since then, similar sensing currents have been measured from many other proteins. As the movement of the gating charge is an electrical marker of conformational changes within a protein, it is an extremely useful experimental measurement of intramolecular changes that might be otherwise undetectable.

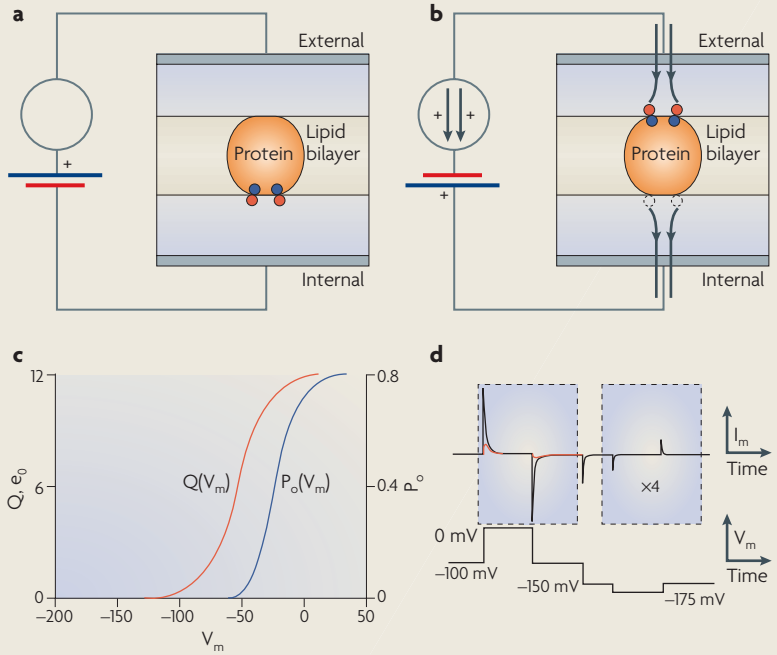
Establishing the energetic details of the charge movement at the single-molecule level will be crucial for constructing a detailed atomic model of the movement of the voltage sensor. A sudden change in the membrane potential reorientates the charges of the sensor and the kinetics of this movement depend on the energy landscape that those charges must traverse to their new position. At the single-molecule level, a decaying gating current indicates that the gating charge moves along a shallow energy profile. By contrast, if the charge encounters a high-energy barrier, a jump-like mechanism across this barrier will generate a very fast, single shot of charge. The single-molecule gating current is too small against the background noise of the current equipment to be directly measured but using noise analysis of a large ensemble of molecules it has been inferred that there is an elementary shot of 2.4 electronic charges (e_0) in both voltage-gated Na^+ and K^+ channels[2,3]. In the case of the K^+ channel the 2.4 e_0 elementary shot is preceded by a smaller elementary shot

Electric dipole
Two opposite charges of the same magnitude that are separated by a finite distance.

Gating current
The transient electric current that is produced by the movement of the gating charges.

Sensing currents
A more general term for gating currents.

Box 1 | **The origin and detection of gating currents**



Gating charges can be measured when a cell membrane is placed under a voltage clamp. In this set-up the membrane is immersed in internal and external solutions, which are connected to the electrodes that are connected to the voltage clamp (the battery). In the example, the membrane protein contains two positive charges (blue in panel **a**; negative countercharges are red). When the membrane voltage is reversed (panel **b**), these charges move from inside to outside of the membrane, crossing the entire electric field. In order to keep the membrane potential constant, the voltage-clamp circuit therefore has to remove two negative charges from inside the membrane capacitor (dotted circles) and provide two negative charges to the external side. The current recorded will therefore reflect the outwards movement of two positive charges. If the two charges of the sensor traverse only half the field, then only one external charge will be required to move. Therefore, in experimental terms, the charge transferred is the product of the magnitude of the moving charge times the fraction of the field it traverses.

The voltage dependence of the gating charge (the Q–V curve) has a sigmoid shape (panel **c**), which differs from the P_o–V curve (where P_o is the probability that the channel is open; panel **c**) because there are several sensors per conduction pore. In experimental conditions, gating currents (I_g) must be extracted from ionic currents — which can be blocked — and from the linear capacitive current (I_c). To separate I_g from I_c (panel **d**), the current produced by a subtracting pulse, which generates only linear current, is subtracted from the current produced by a test pulse, which generates both linear (black) and gating current (red)[62]. When the gating charge is low, there are only a few molecules or the movement is slow, I_g is undetectable. Fluorescent markers attached to residues give an indication of the charge movement (FIG. 1c).

and a purely diffusional process[4]. The gating charge may have a fractional value because the experimental measurement is in fact the magnitude of the moving charge times the fraction of the field it moves (BOX 1).

Voltage clamp
An electronic device that imposes a defined potential difference across the membrane.

Q-V curve
A plot of the voltage dependence of the gating charge.

K^+ channels: a model voltage-gated channel

Voltage-gated Na^+, K^+ and calcium (Ca^{2+}) ion channels have crucial roles in excitable cells and form the basis of the initiation and propagation of the nerve impulse. These channels contain a selective ion-conduction pore and voltage sensors. Their general structural features are similar: all three channels are made up of four independent protein subunits (K^+ channels) or one peptide that contains four homologous domains (eukaryotic Na^+ and Ca^{2+} channels). Each one of the domains or subunits contains six transmembrane segments (S1–S6) and a pore loop between segments S5 and S6. The voltage sensor is made up of the first four transmembrane segments, and the conduction pore is made up of the last two segments and the pore loop (FIG. 2). The channels are arranged symmetrically around a central conduction pore that has four voltage sensors around it.

The basic mechanism of voltage sensing in voltage-gated K^+ channels is well understood. The probability that the channel is open (P_o) is regulated by the voltage sensor, which in turn is controlled by the membrane potential. The salient feature of this superfamily of channels is their steep voltage dependence: the P_o increases by a factor of 150 with a change of only 10 mV. Many voltage-gated channels show more complex mechanisms. For example, the voltage-gated Cl^- channel is thought to derive its voltage dependence from the movement of the external Cl^- into the protein and to have protons as the gating charge[6,7].

The K^+ channel voltage sensor. The *Drosophila melanogaster* Shaker K^+ channel was named after the shaking that the fly undergoes under anaesthesia in its absence. Shaker was the first K^+ channel cloned and it has been used as a prototype for voltage-gated channels because it can be expressed at a high density in *Xenopus* oocytes. This channel forms homotetramers, so the experimental introduction of point mutations results in each channel containing four identical mutations, thereby amplifying the changes introduced by the residue replacement.

The steep voltage dependence of voltage-gated channels is a result of the sensor region moving a large amount of charge across the field. Using the Shaker K^+ channel, the number of charges per channel was determined to be 13 e_0 by measuring the gating currents in a patch where the number of channels present was known[8]. Measurements using a thermodynamic approach also gave the same number[9]. The S4 segment is believed to be a potential candidate for the voltage sensor owing to the arrangement of basic residues at every third position (Arg and Lys residues) present in this segment[10]. Indeed, it has been demonstrated that the first four most extracellular basic residues of S4 and the most intracellular acidic residue on S2 (FIG. 2) are involved in gating currents by measuring the charge per channel in mutants, in which each charged residue was neutralized[9,11]. By neutralizing one charge the total charge per channel decreased by more than the expected 4 e_0, indicating that the range of motion was modified and/or that the field seen by the other charges was modified by the neutralization. This result points to the importance of the electric field profile in the region of the sensing charges.

Charge movement during gating. Many models of voltage-sensor movement have been proposed, even before any crystal structure of a voltage-gated channel was available[12,13]. These models support either a large or a small transmembrane movement of the sensing residues. The paddle model — an example of large movement,

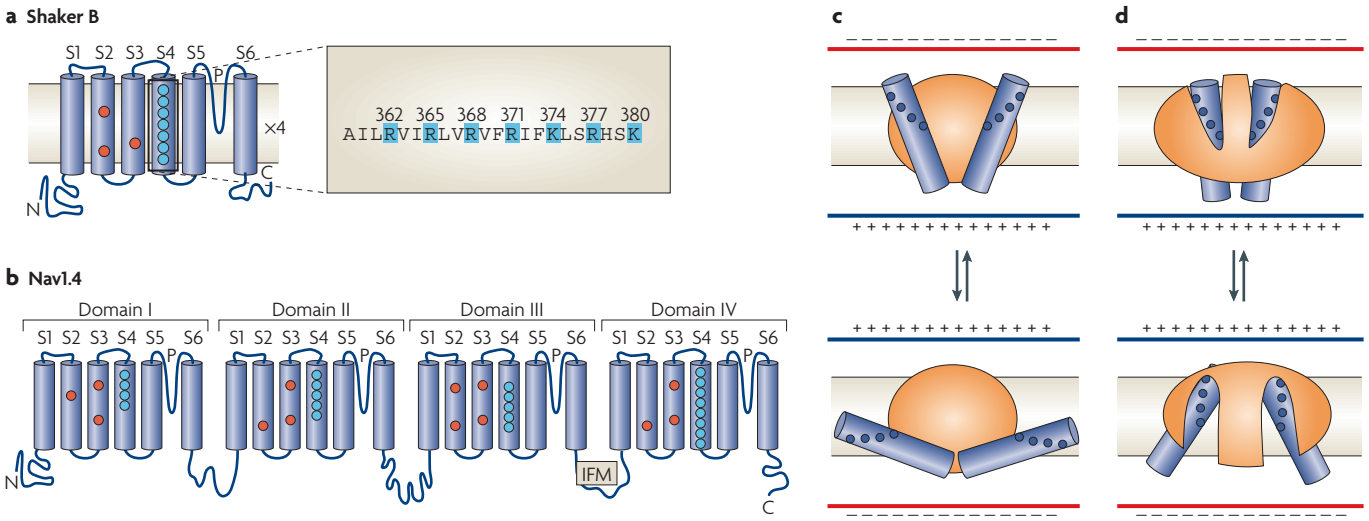


Figure 2 | **The basic architecture of voltage-gated channels. a** | Shaker B, a prototype of a voltage-gated K^+ channel, comprises four identical subunits; only one subunit is shown in this figure. Acidic residues are shown in red and basic residues in blue. The voltage sensor is made up of segments S1 to S4. Segments S2 and S3 have acidic residues, whereas S4 has a series of basic residues. The inset indicates segment S4's sequence, the main part of the voltage sensor, which contains several basic residues (Arg and Lys) separated by two hydrophobic residues. **b** | The rat mu1 (Nav1.4) is a prototype of a Na^+ channel that contains four homologous domains. Each domain has six transmembrane segments and a pore region. **c** | A gating model with large transmembrane movement of the basic residues. Changing the membrane potential (from the top to bottom panels) causes the charges to move across the field spanning the whole bilayer. **d** | A gating model with a focused field and no transmembrane movement of the basic residues. In this case large water crevices penetrate the protein, and movement of the S4, driven by the change in potential (from the top to bottom panel), rearranges the crevices, effectively translocating the charges across the entire concentrated field. IFM, isoleucine-phenylalanine-methionine; the inactivation plug in the intracellular loop linking domains III and IV.

which is based on the original crystal structure of the prokaryotic voltage-gated channel KvAP — proposes that the S3 and S4 segments translocate about 20 Å with the charges embedded in the bilayer[14]. On the other hand, a model for small movement proposes that very little transmembrane charge relocation is required for rotation and tilting of the S4 segment[15]. The results summarized below indicate that the movement is neither very large nor very small and that there is indeed tilting and rotation of the S4 segment.

Understanding the voltage sensor requires a detailed knowledge of how and where the charged residues move. This question has been addressed using multiple techniques, including Cys and His scanning, biotin–avidin reactions, fluorescence resonance energy transfer (FRET) fluorescence spectroscopy, electron paramagnetic resonance (EPR) spectroscopy and X-ray crystallography (reviewed in REFS 15,16). Here, I will summarize only the most relevant results to paint the emerging picture of how the voltage sensor operates.

Experiments using His replacement of the voltage-sensing arginines in the Shaker K^+ channel show that these residues are exposed to the inside at negative potentials and to the outside at positive potentials. A proton pore forms in the closed state when the most extracellular Arg is replaced by a His[17]. When that same residue is replaced by a small residue such as Cys, a cation pore is observed[18,19] (the omega current). In contrast to the ionic current that flows through the pore, the proton current and the omega current flow through a portion of the voltage sensor, which indicates that the internal and external solutions penetrate deep into the protein core to produce a continuous flow in the closed state of the sensor. This result implies that in the closed state the field is concentrated to a narrow region around the location of the first charge, which can also be demonstrated by experiments with different lengths of chains for the first charged residue[20].

Crystallography has been useful in determining the position of the Arg and also in computing the electric field profile. The crystal structure of Kv1.2 (the mammalian homologue of Shaker)[21] most likely corresponds to the open and slow-inactivated state of the channel because the crystal is obtained in the absence of an electric field. The central conduction pore is surrounded by four voltage sensors. The third and fourth Arg of the S4 segment are buried in the core of the protein, whereas the first two most extracellular Arg point towards the region where the bilayer would be located. A molecular dynamics simulation of the Kv1.2 structure, which included lipids and the internal and external solutions with water and ions[22], showed that the two most extracellular Arg are in contact with the phosphate groups of the lipid and water of the external solution. This simulation of the Kv1.2 structure also revealed that water penetrates the core of the protein, thereby concentrating the field in the extracellular half of the protein. A direct measurement of the field with electrochromic probes confirmed that in both the open and closed states the field is more concentrated around the voltage sensor than in the bilayer[23]. The consequence

Capacitive current
The current that flows into and out of the plates of the capacitor during its charge or discharge.

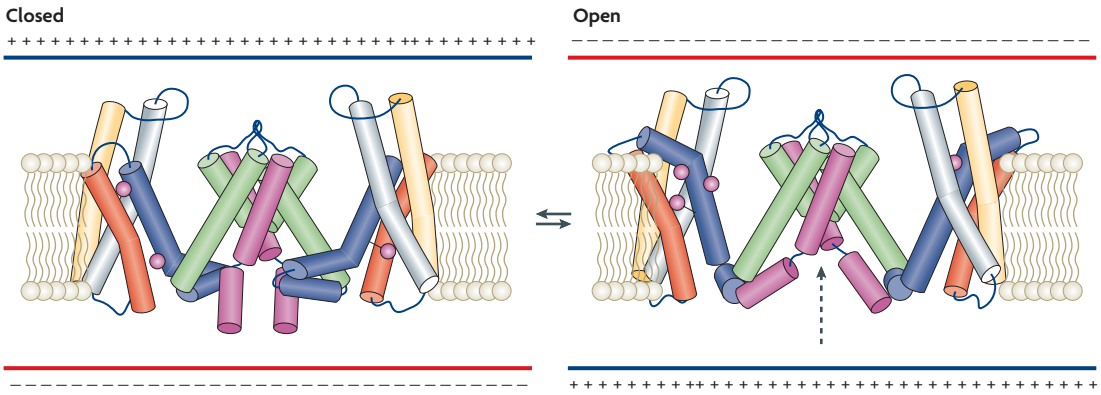


Figure 3 | **A model of the two extreme configurations of the Shaker K⁺ channel.** For clarity only two subunits are shown. When the membrane potential changes from hyperpolarized (closed; left panel) to depolarized (open; right panel), segment S4 rotates 180°, changes its tilt by about 30° and moves towards the extracellular side by about 6.5 Å. Movement of the S4 segment is transmitted through the S4–S5 linker to the intracellular part of S6 (magenta). The ion conduction pore is formed by the S5 and S6 segments and the main gate of the channel is formed by the intersection of all four S6 segments. The gate opens when the S6 segment breaks in the Pro-Val-Pro region (PVP motif) of the S6 segment, splaying apart all four segments and thereby allowing ion conduction. When the membrane is depolarized the translation rotation and tilting of the S4 segment is transmitted through the S4–S5 linker, which is in contact with the intracellular part of the S6 segment. This causes the PVP motif to bend[31], which opens the gate and initiates ion conduction. In the closed position S6 is a straight α-helix, whereas in the open position it is bent at the PVP motif, thereby opening the gate. The dashed arrow represents ion conduction through the open pore. S1, white; S2, yellow; S3, red; S4, blue; S5 and pore region, green; S6, magenta. The arginines are pictured in magenta. This model is based on data from REF 29.

of this concentrated field is that the charges do not need to traverse the whole length of the bilayer to account for the 13 e_0. The limited transmembrane movement of the S4 segment during voltage sensing has been verified through FRET measurements between fluorophores in the S4 segment and absorbing hydrophobic ions in the bilayer[24] and lanthanide-based FRET[25].

In contrast to these results, the reaction to avidin of biotin-linked sites in different parts of the KvAP channel indicated a large movement of the S4 segment across the membrane[26]. Molecular modelling using the Rosetta method indicated that the movements in KvAP were larger than in Kv1.2 (REF. 27). However, a more recent model of Kv1.2 using the Rosetta method and based on multiple biophysical results showed a limited transmembrane movement; this model can explicitly account for the biotin–avidin results[28].

A major insight into the movement of the sensor during gating was obtained by a Cys–Cys crosslinking in the closed state between a Cys replacing the first extracellular charge of the S4 and a Cys replacing an Ile in S1 and replacing another Ile in S2 (REF. 29). These two Ile form part of the hydrophobic plug which is the main energy barrier encountered by the charged Arg when they translocate during voltage sensing. Recently a Phe that resides three residues below the critical Ile in S2 has been proposed to be such a plug[30]. The position of both Ile, as revealed by the crosslinking experiments in the closed state[29], taken together with the structure of Kv1.2 at the open conformation[21], define the movement of the voltage sensor (FIG. 3). Recent modelling using the Rosetta method has produced a similar picture of the conformational changes during gating[28].

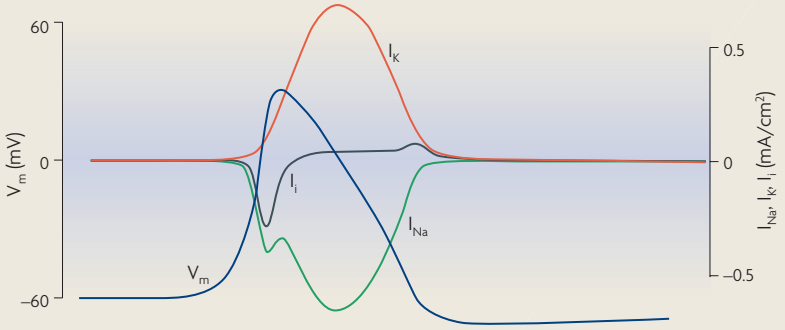
Inactivation
The process of conductance reduction during maintained depolarization.

Other voltage-gated ion channels

Although K^+ channels are excellent prototypes for voltage-gated channels, there are several other types of voltage-gated channels that differ in function, selectivity, regulation, kinetics and voltage dependence. In the following section, the discussion is restricted to Na^+ and Ca^{2+} channels, channels that close on depolarization and proton channels. Na^+ and Ca^{2+} are also voltage dependent and their S4 segments are similar to the K^+ channels (FIG. 2). Consistent with the view that their voltage dependence is similar to the Shaker K^+ channel, their gating currents show similar characteristics to K^+ channels and the charge per channel in Ca^{2+} channels was estimated as 9 e_0 (REF. 32), while in Na^+ channels it was estimated as about 12 e_0 (REF. 33). Experiments on the accessibility of S4 segment residues also confirm that the basic operation of their voltage sensors is similar to that of K^+ channels.

Specializations in the Na⁺ channel. Voltage-gated Na^+ channels have several properties that make them uniquely tuned to initiate and propagate the nerve impulse. The influx of Na^+ through Na^+ channels during the upstroke of the action potential depolarizes the membrane, which also increases the P_o, thereby opening more Na^+ channels[34]. The Na^+ influx is not maintained because the P_o spontaneously decreases while the membrane is depolarized by inactivation[34] (BOX 2). The decrease in Na^+ conductance facilitates the repolarization phase by K^+ channels and effectively shortens the duration of the action potential, which is critical for high repetitive firing, the normal way neurons communicate.

Box 2 | **Ionic currents during the nerve impulse**



The propagated action potential (V_m; blue) is a fast and transient change in the membrane potential. The normal resting potential (of around –60 mV) is normally maintained by a predominant K^+ conductance. When depolarization occurs (caused by an excitatory postsynaptic channel or by an action potential in a neighbouring region of the nerve) the Na^+ channels open; this occurs because depolarization moves the voltage sensor to the active position which increases the probability that the gates will open. The influx of Na^+ (I_{Na}; green) down the electrochemical gradient (Na^+ is more concentrated outside) depolarizes the membrane even further. This extra depolarization opens more Na^+ channels and a positive feedback ensues[34]. This positive feedback produces a rapid upstroke of the action potential in which V_m overshoots 0 mV and tends towards the equilibrium potential for Na^+ because it is the dominant form of conductance. While the membrane is being depolarized, the voltage sensors of the K^+ channels also respond by opening their gates but their kinetics are slower than the Na^+ channel sensors; therefore, the outward K^+ flow (I_K; red) down the electrochemical gradient (K^+ is more concentrated inside) is delayed. In this way, the net ionic current through the membrane is initially inwards (I_i; black), but when the sum of I_{Na}, I_K and all other forms of ionic conductance becomes zero the membrane potential reaches its peak. Beyond that point I_K dominates and the outward K^+ flow produces the falling phase of the action potential, which repolarizes the membrane. At the end of the falling phase, the K^+ conductance is very high compared with all other types of conductance, and the membrane potential tends to reach the K^+ equilibrium potential, thereby generating the undershoot of the action potential. The eventual return to the resting potential occurs through other channels that are collectively known as leakage channels. During depolarization, the inactivation gate of the Na^+ channel responds with a delay, blocking conductance and decreasing the inwards Na^+ flux, effectively allowing the K^+ conductance to take over and repolarize the membrane sooner[34]. If the inactivation gate were completely removed, the action potential would last much longer or would never repolarize.

The idea that each of the four voltage-sensor domains has a specialized function in the operation of the channel is supported by site-directed fluorescent labelling studies that can be used to track the individual time courses of the conformational changes that occur from voltage changes in each of the S4 segments[35]. The S4 segment of each of the first three domains was found to move at a rate that is compatible with the fast component of the gating current. Therefore, the S4 regions in domains I–III can be assigned as the voltage sensors for the activation of Na^+ conductance. On the other hand, the movement of the S4 segment of domain IV is delayed and also slower, following the time course of the slow component of the gating current. The Na^+ channel was partially open before this S4 segment started to move, which suggests that it does not participate in the opening of the gate. The voltage dependence of the S4 segment of domain IV correlated well with the voltage dependence of inactivation, raising the possibility that the voltage sensor of domain IV is the main sensor of the inactivation process in Na^+ channels[35].

To generate the rising phase of the action potential, Na^+ channels must open with faster kinetics than K^+ channels; indeed, the gating currents of Na^+ channels are faster than in K^+ channels, which in turn are responsible for the repolarization phase of the impulse. A partial explanation for this kinetic difference has emerged from a recent demonstration that there is positive cooperativity between domains[36,37]. Positive cooperatitivy speeds up kinetics because the probability that one sensor makes a transition is increased if another sensor has already made the transition. Introducing changes to the voltage dependence in the sensor of one domain also affected the activation of other domain sensors[36]. In addition, stabilization of the sensor of domain II in the active state by the scorpion toxin Ts1 increased the probability that the other domains would go to the open state[37]. Cooperativity in Na^+ channels occurs at hyperpolarized potentials, whereas in K^+ channels it occurs in the latest stages of activation, most likely by interactions through the opening of the pore[38,39]; it therefore does not speed up kinetics significantly.

Inactivation is another voltage-dependent process that was originally proposed to have its own voltage sensor (the *h* gate)[34]. However, gating current experiments[5] and single channel recordings[40] showed that most of the voltage dependence of inactivation is borrowed from the activation of the conductance by coupling the movement of the activation charge to the inactivation process. More recent studies have shown that some of the voltage dependence of the inactivation is associated with the voltage sensor of domain IV of the Na^+ channel[35,41]. Inactivation of conductance is a consequence of the binding of a region of the intracellular loop linking domains III and IV (the IFM motif) on the internal mouth of the channel; this interaction functions as a plug and effectively blocks ion conduction[42]. When this plug blocks the channel, it also interacts with the voltage sensors, thereby immobilizing a portion of the gating current. Only prolonged repolarization remobilizes all the charge and simultaneously removes inactivation[5]. The inactivating particle was found to interact only with domains III and IV, whereas domains I and II are free to move in the presence of the inactivating particle[43].

The proton channel. Voltage-gated proton channels open with depolarization and their voltage dependence is regulated by the internal and external pH, which results in an outward current during depolarization; thus, their function is to extrude protons from the cells. A voltage-gated proton channel has recently been cloned and heterologously expressed[44,45]. This channel has a voltage sensor that is homologous to the sensor of other voltage-gated channels, with four transmembrane segments, including the positively charged S4 segment. However, this channel does not have the two transmembrane segments and pore-loop region that is present in K^+, Na^+ and Ca^{2+} channels (FIG. 4). Although conduction might occur through an auxiliary subunit, which is co-expressed with the proton channel molecule, the lack of a canonical pore region raises the possibility that proton permeation occurs between the transmembrane

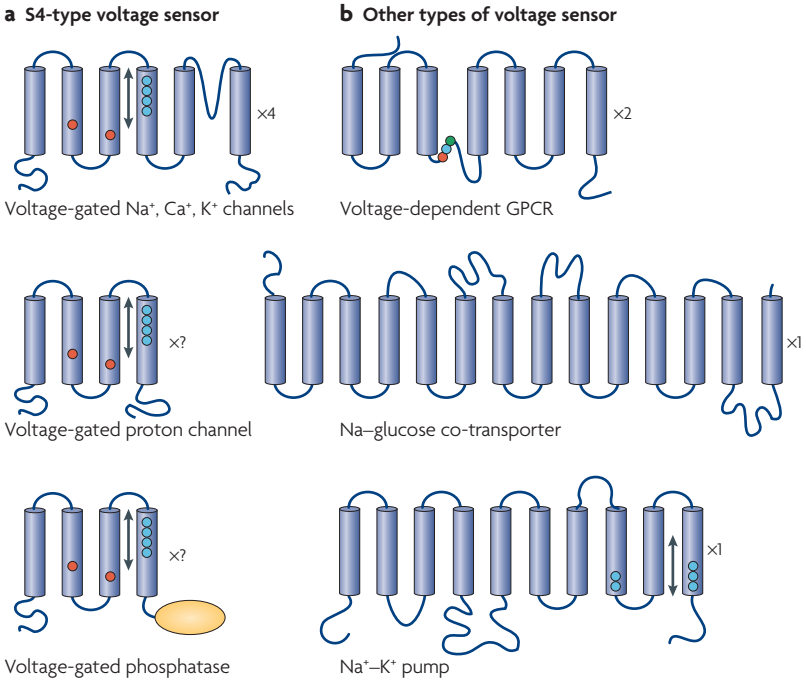


Figure 4 | **The architecture of several voltage-dependent proteins.** Panel **a** shows proteins that use the S4-type motif as their sensing unit and panel **b** shows proteins that have a different type of voltage sensor. The number of transmembrane segments and the stoichiometry (×), together with the location of the voltage sensor when known, are shown. The sensor locations in the 7-transmembrane G-protein coupled receptor (GPCR) and in the 14-transmembrane segment Na–glucose co-transporter are unknown. In the Na^+–K^+ pump the crystal structure[61] suggests that there are moving charges in segments 8 and 10, a proposal that is contrary to the view that the movement of the ions in the access channels produces the transient currents of the pump. The basic residues are represented in blue, acidic residues are represented in red and neutral residues are represented in green. The double arrow indicates the region that moves in the field. The yellow oval in voltage-gated phosphatase indicates an intracellular domain that is homologous to PTEN.

segments of the voltage sensor. Proton permeation has been demonstrated by His replacement in the S4 segment and in the S1 and S2 segments of the Shaker K^+ channel[17,29], which suggests that a similar mechanism might operate in the proton channel.

Channels that close on depolarization. Other channels also share the same topology to K^+ channels, which include an S4 segment with positively charged residues; however, these channels are closed at positive potentials and open when the membrane is hyperpolarized. For example, the plant channel KaT1 opens at negative membrane potentials; although the gating currents are similar to the classic channels, coupling to the pore gate is in the opposite direction. So, when the S4 segment responds to hyperpolarization, its movement must be somehow transmitted differently to the S6 segment compared with a Shaker K^+ channel because the gate opens instead of closing[46]. The hyperpolarization-activated cyclic-nucleotide (HCN)-gated channel is found in several cells and has been suggested to have a role in pacemaking. This channel also possesses an S4 segment with charged residues every third position that generate gating currents and for which the open probability increases on hyperpolarization[47]. There are other cases, such as the HERG potassium channel in which channel inactivation is faster than activation so that, upon hyperpolarization, inactivation is removed to expose the open channel[48].

S4: a modular voltage sensor?

In addition to ion channels, other membrane proteins are known to be regulated by the membrane potential. Recent findings have expanded the repertoire of membrane proteins that sense the membrane electric field and have shed light into the cellular processes that membrane voltage can regulate. In the following sections, I concentrate on a newly found voltage-dependent phosphatase, the muscarinic G-protein coupled receptors (GPCRs), the Na–glucose co-transporter, and the electrogenic $3Na^+$–$2K^+$ membrane pump.

The voltage-dependent phosphatase. The membrane protein CiVSP is abundantly expressed in the testes and is weakly expressed in the nerve tissue of *Ciona intestinalis*. CiVSP is homologous to the voltage-sensor domain of voltage-gated ion channels; it has four transmembrane segments, including the charged S4 segment, but, instead of a pore domain, it contains a large intracellular domain that is homologous to the PTEN phosphatase[49] (FIG. 4). This protein has large gating currents with similar characteristics to the Shaker K^+ channel gating currents, although its voltage dependence is shifted to more depolarized potentials. The phosphatase activity of CiVSP is increased on depolarization[50], which establishes the voltage sensor as the regulator of its phosphatase activity. Although the physiological significance of this phosphatase is not clear, CiVSP could regulate many processes in the cell, especially near the membrane, through its phosphatase activity, which is turned on by depolarization. Examples include the K^+ channel regulation of KCNQ2 and KCNQ3 (REF. 50), which are important K^+ channels in the brain.

The homology of CiVSP with voltage-gated channel voltage sensors suggests that the S1–S4 voltage sensor itself is a ubiquitous module. Thus, CiVSP can be considered an example of the voltage-sensor module coupled to an enzyme in one single polypeptide. In the case of voltage-gated channels, the sensor is also coupled as a single polypeptide through the S4–S5 linker to the pore domain. However, the action of the sensor on the pore is thought to be through hydrophobic contacts between the linker and the S6 segments, raising the possibility that the sensor is a module that is incorporated as part of the channel protein in the same way that might have happened with CiVSP. Another example is the proton channel, in which the voltage-sensor module might have adapted to have a proton pore in the sensor itself. It is tempting to speculate that this modular voltage-sensor domain may be coupled to other enzymes, thus suggesting the possibility of many, as yet undiscovered, processes that are controlled by the membrane potential.

Although the S4 sensor is found in channels and in the voltage-sensitive phosphatase, there are many other voltage-dependent membrane proteins that do not possess

HERG potassium channel
This is the human ether-a-go-go related channel, which is a potassium channel that is classified as Kv11.1.

PTEN
A phosphatase that dephosphorylates phosphatidylinositol (3,4,5)-trisphosphate to obtain phosphatidylinositol (4,5)-bisphosphate.

an S4 segment and their voltage sensor is of a different structure, such as GPCRs, carriers and pumps.

Voltage sensitivity in GPCRs

GPCRs are membrane proteins that bind to ligands and produce a cellular response through a signalling cascade[51]. Several GPCRs have been found to have a response that is affected by the membrane potential. In particular, cholinergic m2 muscarinic receptor (m2r) activity is reduced by depolarization, whereas activity of the m1 muscarinic receptor (m1r) increases with depolarization because of a change in the receptor affinity for acetylcholine[52]. It is now established that the voltage sensitivity rests in the GPCR itself[53], and gating currents were recently recorded from m1 and m2 receptors expressed in oocytes under voltage-clamped conditions[52]. Although their voltage dependence differs, both types of receptors showed similar gating currents. However, it was possible to exchange the voltage dependence of m1r to the voltage dependence of m2r by replacing the loop between transmembrane segments 5 and 6 of m2r with that of m1r. Although the voltage dependence was swapped, the gating current was not changed, indicating that this loop is only the effector of the voltage-sensor movement rather than being the sensor itself. This situation is reminiscent of the differences in voltage dependence between the Shaker channel, which opens on depolarization, and KaT1 and HCN, which close on depolarization. The structure of GPCRs is different from voltage-gated ion channels and has no region that is similar to the S4 segment of voltage-gated channels. The search for the gating charge is still on because the neutralization of the D(E)RY conserved sequence (FIG. 4), which was reported to decrease the gating charge[52], does not account for the sensing charge.

The physiological implications of the voltage dependence of the acetylcholine affinity can be assessed using the kinetics of the gating current in m2r, which has two components. The fast component is in the time domain of the duration of an action potential; therefore, the action of the receptor can be modulated at high speed by the nerve impulse. The slow component may be activated by a more prolonged depolarization that is produced by a train of action potentials, suggesting that the modulation may be further regulated by the frequency of firing of the neuron.

Voltage sensors in carriers and pumps

The function of many carriers and pumps is modulated by the membrane potential. In electrogenic transporters there must be a voltage sensitivity in their transport rates. These transporters show charge movement that comes from conformational changes during their reaction cycle. They therefore look like gating currents, but they might not control gates. Factors such as nucleotide and substrate concentrations and the voltage-dependent transitions control the transport rate. The structure of these membrane proteins has no homology to voltage-gated channels, raising questions about how the voltage sensor is built in these proteins.

The Na–glucose co-transporter. The SGLT1 Na–glucose co-transporter found in the intestinal mucosa of the small intestine uses the energy from a downhill sodium gradient to transport glucose across the apical membrane against an uphill glucose gradient. This co-transporter, which is an example of a secondary active transporter (FIG. 4), has been studied in much detail, and the presence of a voltage-dependent conformational change has been confirmed[54]. Measurements of the gating currents have shown that the gating charge is composed of several elements. Modifying the Na^+ concentration changes the Q–V curve, suggesting that the Na^+ ions that are being transported through the transporter move in the electric field and generate the gating currents. In the absence of Na^+ the gating currents are still present — their kinetics and voltage dependence is altered and the total charge is reduced — which indicates that in addition to the movement of Na^+ there is an intrinsic charge in the transporter protein that senses voltage.

Several steps in the transport cycle of SGLT1 are voltage dependent. Kinetic modelling and fitting to the transport data[54–56] have been used to estimate that the gating charge in the absence of Na^+ is ~1.4 e_0, whereas the total gating charge has been measured to be 3.5 e_0. The difference between this 1.4 e_0 value and the 3.5 e_0 measured per molecule[57] might be a direct contribution from the Na^+ ions moving in the electric field.

A strong correlation between fluorescence changes and the kinetics of charge movement has been shown, but at this point the details of the origin of this intrinsic charge movement are unresolved. If the structure of the *lac* permease[58] may be used as a prototype for SGLT1, then a large cavity in the core of the protein might represent the site in which the electric field is focused. The large value of the gating charge of SGLT1 and the presence of different kinetic components in its gating current point to a multistep process in which charged residues move in this putative focused field.

The Na^+–K^+ pump. The Na^+–K^+ pump has a fundamental role in maintaining the ion gradients in cells because it transports K^+ and Na^+ ions against their electrochemical gradient using energy from ATP hydrolysis (FIG. 4). Its transport stoichiometry is $3Na^+$: $2K^+$ and, therefore, it is electrogenic and generates an outward current that makes the cell interior more negative. This transport may contribute to the resting membrane potential in cells, in which the ion leak is small. If the stoichiometry is constant at all potentials, then the net transport should stop if the membrane potential were made sufficiently negative to increase the work needed for active transport until it matches the energy available from the ATP hydrolysis. This means that the Na^+–K^+ pump current decreases as the membrane potential becomes more negative.

The Na^+–K^+ pump can be blocked specifically by cardiac glycosides such as ouabain. This has been an important tool in measuring the charge movements during the operation of the pump because, by subtracting the ionic currents in the presence of ouabain from the currents in its absence, it is possible to isolate the specific currents that are carried by the Na^+–K^+ pump. With this method

it has been possible to detect transient currents (gating current-like currents) that precede the steady-state transport or pump current during the pump operation cycle[59]. These transient currents have been traced to represent the release of the occluded Na^+ ions into the extracellular space. In fact, a detailed high-speed kinetic study showed that the release of the three Na^+ ions is sequential and each ion produces a different kinetic component of the charge movement[60]. The voltage dependence of the release steps can be interpreted as the effect of voltage on the ions present in a long channel, which is in the electric field within the pump protein. Therefore, this is yet another type of voltage sensor in which the ions themselves act as charge carriers and are not tethered permanently within proteins, and it has been proposed as the main voltage sensor that accounts for the pump-voltage dependence. Under saturation conditions of internal ATP and Na^+ and external K^+, the pump current has a sigmoid dependence on voltage with the current becoming negligible at negative potentials, increasing steeply over the physiological range and then plateauing at approximately 0 mV.

Morth and colleagues, who recently published the crystal structure of the Na^+–K^+ pump proposed that the cluster of Arg in helices 8 and 10 may move in response to changes in membrane potential, which act as a switch on helix 5 to affect the affinity of the third electrogenic Na^+ ion site in the pump[61]. Contrary to the view that the movement of ions in the access channels produces the transient currents of the pump, this proposal would imply a direct role for charged residues in the transient currents in a way that is similar to the known gating charges of the voltage-gated channels. A detailed study of the complex kinetics of the transient currents[60] in combination with the mutagenesis of the charged residues may provide a test to this proposal.

Conclusions and future directions

A large number of membrane proteins harness the membrane electric field to perform specific functions by means of a voltage sensor. Significant progress has been made on the molecular mechanisms of the S4-type voltage sensor that is found in voltage-gated ion channels, but more structures and biophysical analyses are still needed for a full molecular understanding of its function. The S4-type sensor has recently been found in a voltage-dependent phosphatase, suggesting that this type of sensor may be modular and might have been incorporated into other types of proteins, which are as yet unidentified, during evolution. The similarity is so high that it is tempting to believe that the S4-type sensor comes from a common ancestor; however, it could still have evolved independently in different proteins.

By contrast, other voltage-dependent proteins have evolved different types of voltage sensors such as the G-protein coupled muscarinic receptor, in which the sensor is an integral part of the structure that evolved to accomplish the specific function of coupling ligand binding to G-protein activation. It is expected that many other sensors will be discovered in the near future, thereby expanding our understanding of how the membrane potential regulates cell homeostasis. Biophysical, computational and structural methods should help to solve the dynamics of the molecular structure and to explain the operation of these sensors and their coupling to the function of the protein.

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FURTHER INFORMATION
Francisco Bezanilla's homepage: http://nerve.bsd.uchicago.edu/FB
Simulation programs for voltage-gated ion channels and nerve impulses: http://nerve.bsd.uchicago.edu
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