J. Math. Study
doi: 10.4208/jms.v57n1.24.03

Vol. **57**, No. 1, pp. 53-70
March 2024

Conformations and Currents Make the Nerve Signal

Robert S. Eisenberg[1,2,*], Luigi Catacuzzeno[3] and Fabio Franciolini[3]

[1] *Department of Physiology and Biophysics, Rush University, Chicago IL 60612, USA;*
[2] *Department of Applied Mathematics, Illinois Institute of Technology, Chicago IL 60616, USA;*
[3] *Department of Chemistry, Biology and Biotechnology, University of Perugia, Via Elce di Sotto 8, Perugia 06123, Italy.*

Received July 14, 2022; Accepted September 16, 2022;
Published online March 21, 2024.

Abstract. Conformation changes control the function of many proteins and thus much of biology. But it is not always clear what conformation means: is it the distribution of mass? Is it the distribution of permanent charge, like that on acid and base side chains? Is it the distribution of dielectric polarization? Here we point out that one of the most important conformation changes in biology can be directly measured and the meaning of conformation is explored in simulations and theory. The conformation change that underlies the main signal of the nervous system produces a displacement current—NOT an ionic current—that has been measured. Macroscopic measurements of atomic scale currents are possible because total current (including displacement current) is everywhere exactly the same in a one dimensional series system like a voltage clamped nerve membrane, as implied by the mathematical properties of the Maxwell Ampere law and the Kirchhoff law it implies. We use multiscale models to show how the change of a single side chain is enough to modulate dielectric polarization and change the speed of opening of voltage dependent channels. The idea of conformation change is thus made concrete by experimental measurements, theory, and simulations.

AMS subject classifications: 92-08, 92-10

Key words: Conformation, action potential, gating current, dielectric constant.

1 Introduction

Conformation changes regulate and control an enormous range of functions, as biochemists imagined they would from the beginning of molecular biology [1,2]. Conformation changes are no longer ideas. Conformation changes now have exact coordinates of

*Corresponding author. *Email address:* bob.eisenberg@gmail.com (Eisenberg R), luigi.catacuzzeno@unipg.it (Catacuzzeno L), fabio.franciolini@unipg.it (Franciolini F)

tens of thousands of atoms in multiple conformations, thanks to the remarkable advances of structural biology [3].

Despite these wonderful advances, little is known about the physical or physiological correlates of these conformation changes. How do the atoms move in time? How do the changes in atomic locations control biological function? How do the locations reveal themselves as measurable physical properties of the protein?

One place these questions can be (mostly) answered is in the proteins that form the voltage activated sodium channel of nerve, the ion channel Na_V and the voltage activated potassium channel of nerve K_V [4]. These channels are responsible for signaling in the nervous system. Conformation changes of these channels on the atomic scale produce electrical signals that move meters and carry nearly all long range information in the nervous system. Indeed, the propagating voltage signal of nerve fibers-the action potential[†]-may have been the first binary signal to be studied in electrical detail [5], by an undergraduate, against notable opposition, at the time [6]. The binary property of the action potential is called 'all or none' in the biological literature [7, 8]. These Cambridge dons no doubt would be amused at High Table (at Trinity Cambridge) that an all-or-none, base-2 binary signal is handily misnamed as 'digital' (base-10) in popular language today.

Voltage activated channels have been studied in great detail. Indeed, their study has formed the substrate for most of our knowledge of channels, if not membranes, as recognized in a sequence of Nobel Prizes, to Hodgkin and Huxley [9-11], who worked in Adrian's department; Neher and Sakmann [12]; and MacKinnon [13]. The work of Hodgkin and Huxley has formed the keystone of membrane biophysics, as it was inherited [14] and spread [15-17] to the biological community as a whole.

Hodgkin and Huxley, following Cole [9, 18, 19], recognized even before the Second World War that the crucial mechanism generating the action potential waveform was a voltage sensitive 'conductance', as they each explained to a nineteen-year-old student many years ago (RSE personal communication). We now know that the voltage sensitivity Cole, Hodgkin, and Huxley observed in whole nerve fibers comes from voltage activated protein channels [20, 21], first proposed as voltage activated pores by Lorin Mullins [22, 23], to the best of our knowledge. Voltage acts on the sodium and potassium channels Na_V and the nerve K_V by changing the fraction of time individual channel proteins conduct current and in that sense are open. Mullins identified three key questions: the origin of selectivity [24-30] the mechanism of voltage dependence [31-37], and the difference in the time course of currents through the sodium and potassium pores, as he called them.

The crucial step in the action potential is the flow of current inwards across the membrane, through open Na_V channels, that use a gradient of concentration to create an electrical current. The open sodium Na_V channels allow Na ions to move down their gradient of electrochemical potential changing the internal potential of the nerve from negative to positive and carrying electrical current into the nerve fiber. Note that the concentration

[†]'Potential' is usually a number in physical science. The action potential is a phenomenon, not a number, in biological science. It is best represented as a waveform V(x,t) in physical and mathematical language.

change produced by the flow of Na ions is negligible in one or even a handful of action potentials.

The action potential is propagated by current flow, not concentration changes. The change in potential is produced by electrical phenomena, following Maxwell's equations in the form of Kirchhoff's current law [38]. That channel current is then conducted macroscopic distances down the nerve axon using whatever ions are present in the axoplasm because current is conserved independent of the atomic species carrying the current, indeed independent of charge carriers altogether [39, 40]. That current is described by a version of Maxwell's equations used by Kelvin to describe the original trans-Atlantic telegraph cable [41, 42] and now usually called the telegrapher's equations in the physics and mathematics literature. It is quite helpful to use them in the modern engineering representation of two port theory [43]. In that way, matrix algebra is used to deal with otherwise awkward structures, combinations of cables, and boundary conditions as occur in the dendrites of nerve cells, for example.

The positive potential inside nerve fibers created by the inward current flow through sodium channels Na_V is restored to its normal resting level when another set of channels K_V selective to potassium, open, allowing outward movement of potassium and current.

Different Proteins have Different Roles. The two types of protein channels have distinct biological roles, as is so often the case in biology. Evolution often uses different proteins to perform different functions. The sodium channel Na_V controls the rising phase, speed, and conduction velocity of the nerve signal. The potassium channel K_V controls the duration of the signal and the time between signals (in large measure, along with a special property of sodium channels called inactivation).

The timing of the channel openings is crucial. If, for example, the sodium and potassium channels opened with the same time course, and driving forces were equal, no net current would flow across the nerve, no current would flow down the axon, the nerve signal would not exist, although of course sodium ions would enter and potassium ions would leave the nerve cell [33, 44-47].

The timing and voltage dependence of the opening of the sodium and potassium channels [48-51] is thus an essential feature that makes the nerve signal possible. It is likely that the timing and voltage dependence of channel opening and closing is a significant determinant of the total metabolism of human beings [52-54].

The voltage and time dependence of channel opening is controlled by the conformation change of a part of the sodium and potassium channels called the voltage sensor [48-51]. This voltage sensor is a distinct piece of the sodium and potassium channel proteins and is nearly identical to the proton channels found in most cells [55-57]. Proton channels are crucial parts of the systems that make chemical energy (ATP) used throughout life. They are thought to have existed from 'the beginning' of life and to have been incorporated (and slightly modified) by evolution into an ancestor sodium channel to create the voltage dependent Na_V channel studied here.

The voltage sensor. The voltage sensor is the name we give to the moving part of the

voltage sensor: think of a piston (voltage sensor) moving in a cylinder in an internal combustion automobile engine. The voltage sensor carries permanent charge on its ionized acid and base side chains. It moves in a sheath that also has permanent charge. The permanent charge of voltage sensor and sheath interact. The charges (of all types) of the sheath can move in response to the electric field, so the voltage sensor interacts with the polarization charge of the surrounding sheath as well as the polarization charge.

The conformation of charges (permanent and polarization) in sensor and its sheath is the most important conformation that determines the currents associated with the movement of the voltage sensor, usually called gating currents[‡]. That conformation is determined both by electric forces, described by a spatial distribution of potential, and mechanical forces (that has been shown to be accurately described by a steric potential in some cases [24]).

Electric forces are very much larger than diffusional forces (as made clear in the unforgettable third paragraph of Feynman's first page on electrodynamics [58]). Changes in the distribution of electrical forces are the most important conformational change determining current flow through any channel whether it is the gating pore or the main conduction channel of the protein. But the calculation of those forces is subtle because the electric field that specifies those forces is a sensitive function of the location of charges. The charges depend on the field and the field depends on the charges [59].

The electric field also changes dramatically with conditions, location, and time. A channel with a constant field is a degenerate system without most of the important properties of real channels [60]. The electric field also depends on the distribution of mass. The distribution of permanent and polarization charge drives and is driven by the distribution of mass. They are coupled. Theories and simulations must determine how both distributions change [24, 61]. Together the conformation change of charge and mass generate the nerve signal.

Investigation of mechanism is made possible (in large measure) because the conformation current can be measured as the permanent and polarization charges of the voltage sensor and surrounding sheath are manipulated [33, 35, 47] in mutation studies of astonishing detail (as well as difficulty) by Bezanilla's lab [33, 35, 47], more than anyone else.

These measurements involve:

(1) the not always trivial site directed manipulation of the genes that code the channel proteins,

(2) the never trivial expression of the mutation in a natural (i.e., oocyte) membrane,

(3) the measurement of the currents associated with the opening of the channel and the movement of the voltage sensor.

[‡]The conformation currents are commonly called 'gating current', despite the danger of identifying an experimental phenomenon with an unproven mechanism.

Channels are sensitive machines, easy to modify and liable to change if experimental conditions change: experiments must be made in preparations that mimic natural and resolve microsecond signals in the presence of substantial interfering admittance [11, 62, 63].

The creation of this experimental system and the melding of it with the techniques of molecular biology is a success as remarkable as the determination of protein structure, in our opinion, but one much less recognized. Perhaps electricity is harder to understand than structure for most biologists, if not physical scientists.

It is important, albeit unpopular, to realize that structural measurements of conformational changes, taken by themselves, are hard to interpret.

Structural methods at atomic resolution are rarely if ever done in systems that preserve the natural biological environment and setting. Nerve signals only exist in particular ionic conditions in channels in membranes and on a particular time scale. Natural function is changed, often destroyed altogether, if the ionic composition or concentration is perturbed significantly (particularly if the trace concentrations of the divalent calcium ion inside cells are perturbed [64]) or if the potential across the membrane is removed for times longer than a few thousandths of a second. These voltage sensitive channels are 'touchy proteins' in the laboratory (and in the natural nerve) [65-67]. They are likely to inactivate (i.e., disappear in the sense that they no longer react to stimuli, and appear dead, much like an animal ceases to move and in that sense inactivates in death [65-67]).

Structural measurements are rarely able to preserve the conditions required for natural function because of the elaborate procedures necessary to observe structures in atomic detail. Structural measurements rarely preserve the ionic composition, contents, or potentials across the channel. Thus, there is an irreducible uncertainty in the meaning of conformations observed only with structural methods: Are conformational changes observed in the structure the conformational changes that produce the actual voltage sensitivity of the natural channel?

This view of ambiguity in structural studies is an understatement. A more skeptical view would be that structures observed in systems without membrane potential or without natural calcium concentrations are likely to be different from structures in the natural state. A more hostile view would be that the structures observed are interesting artifacts, related to the natural functioning structures in unknown ways that must be evaluated by different methods with disjoint non-overlapping sets of artifacts.

Other methods of studying conformation change are fortunately possible. The study of the conformation change of these voltage sensitive channels can be done in a natural setting without the preparative procedures and separation of time scales inherent in almost all structural methods.

The conformation change can be studied by measuring the electrical currents associated with the charge movements of the voltage sensor and its surrounds. These measurements are a dielectric spectroscopy [68-70] that measures polarization currents in the time domain (although occasional crucial experiments have been done with the sinusoidal waveforms of classical impedance/dielectric spectroscopy [71-73]).

Maxwell's equations guarantee that the total current associated with the movements of these conformation charges is identical to the current flow in the surrounding baths and in the electrodes[§] connected to the baths, independent of any property of matter or polarization whatsoever [38-40, 74-78] (crystallized in [79]) because the channel is naturally a one dimensional system [38-40].

The baths can be made one dimensional conductors with little trouble [62, 63] as has been demonstrated by direct measurement [39, 40, 80].

In this way, the conformation current produced by charge movements of the voltage sensor have been made in hundreds of papers that study 'gating' current [35]. Most impressively, these measurements have been made in mutations of the sodium and potassium channels where the charge and polarization of individual side chains have been changed, for example [33, 45, 47].

Here we show how the change in one side chain 287 (numbered with the Shaker convention used in [47]) speeds up a potassium channel K_V so it opens at the speed of a sodium channel Na_V. The potassium channel K_V side chain isoleucine is replaced by the threonine found in sodium channels Na_V and the gating current speeds up. Threonine has a hydroxyl group and is somewhat polar and able to make low strength hydrogen bonds. Isoleucine is about as nonpolar as a side chain can be. We show that the polarization current (associated with side chain 287) is much larger with the threonine of the sodium channel Na_V (in position 287) than in the potassium K_V channel (with isoleucine in that position). The larger polarization current allows the sodium channel Na_V to open more quickly. One imagines that the larger polarization current of the threonine in the sodium channel Na_V delivers more polarization charge (in a given time) and that reduces the barrier (of electrical potential) the voltage sensor must move through. The larger polarization charge stabilizes the structure of the sheath/piston system allowing the voltage sensor piston to move more quickly into the sheath.

The importance of this mutation to 287 was shown by [33, 45, 47] and we are grateful for the wonderful afternoon in which Bezanilla explained this importance to us. That discussion motivated this hierarchical theoretical analysis establishing how the mutation might work.

The hierarchy of scales. The theoretical analysis of conformation changes is difficult because of the hierarchy of scales involved in the natural function of proteins [81]. Structural changes on the atomic scale (i.e., changing isoleucine of K_V to the threonine found in Na_V) involve less than 10^{-10} m change in structure, yet the natural function of the protein is on the scale of meters found in propagating action potentials.

The gap in time scales between the movements of side chains and natural function is at least as challenging. The natural function of channel proteins occurs on time scales starting at 100 microseconds reaching to a few milliseconds (if one excludes inactivating

[§]This property of Maxwell's equations arises immediately when Ampere's law includes the 'ethereal' current $\varepsilon_0 \partial E/\partial t$ that allows light to propagate in a vacuum devoid of charges or matter, once the law is written without mention of polarization, dielectrics or dielectric constants [38-40, 74-78], crystallized in [79].

deterioration) and action potential propagation is typically 20 milliseconds or so. The atomic motions of side chains are computed with time intervals of 10^{-14} seconds or faster.

The atomic motions of the 287 side chain are an important part of the voltage activated conformation change we wish to study and so must be resolved in computations. Indeed, metaphorical discussions of such motions have been common since the discovery of conformation currents [82, 83] and the earlier, ancestral thoughts of carrier movements and of charged molecules [19] and p. 503 of [84]. The eleven some-odd orders of magnitude difference in time scales made metaphorical discussion necessary. Direct computation is essentially impossible, as should be obvious to anyone who has numerically integrated differential equations for even thousands of time steps and compared the result to known analytical results. Such comparisons are conspicuously absent in the literature of molecular dynamics of proteins or ionic solutions[¶], despite the $\sim 10^{11}$ time steps needed to reach biological time scales [85].

Hierarchy of scales is an essential part of biology. This hierarchy of scales of distance cannot be avoided, as it is an essential part of biology. The hierarchy of scales of time cannot be avoided, as it is an essential part of thermal physics.

The hierarchy of scales in biology is needed to link the atomic scale of genes and proteins to the physiological scale of tissues, organs, and cells, even the evolutionary scale of reproducing animals [86, 87].

This hierarchy of scales makes the direct computation of function on the atomic scale forever impossible at a single scale, if electricity is involved. The number of interactions of charges involved in the conduction of the nerve signal is much more than astronomical. Every ion in a centimeter length of a nerve fiber interacts with every other ion through the electric field. Even pairwise interactions involve something like $10^{17}!$ factorial computations (!).

Electrostatic interactions, however, are not just two ions at a time. Every ion interacts with every other ion within the wavelength of action potential propagation (say 50×10^{-3} meters for a one millisecond duration action potential propagating at 50 m per second) because each ion contributes to the macroscopic electric field in that region. The macroscopic electric field controls the function of individual voltage sensors (and channel proteins) everywhere in that region as easily shown in experiments recording single channels 50×10^{-3} m away from an electrode. The function of every voltage sensor molecule is linked to the movement of every charge within an action potential wavelength. Computations of the electric field in a single atomic scale computation of a propagating action potential is clearly forever impossible.

Fortunately, atomic scale computation is as unnecessary as it is difficult. Macroscopic biological function follows conservation laws that are exact, on all scales, namely conservation of mass, conservation of charge, and conservation of total current (which is not quite the same thing, because of Maxwell's ethereal current $\varepsilon_0 \partial \mathbf{E}/\partial t$ that makes charge

[¶]Ref [75] is a notable exception. It shows that the trajectories of molecular dynamics are chaotic and so are not likely to uniformly sample thermodynamic phase space. They may miss some 'areas' altogether.

relativistically invariant). If these laws are exploited by accurate calculations in a hierarchical coarse graining of atomic motions, a theory and simulation can extend from atomic time and distance scales to the scales of motion in channels and nerves.

Here, we use a hierarchical coarse graining of that type, following [34, 44] who extended the scales used by [31]. We use molecular dynamics to establish the atomic scale properties and parameters. Those parameters are then used in a coarser simulation of molecular motion that uses the Langevin equations of thermal (Brownian) motion. Great care has been taken to check that the coarse grain simulations obey conservation laws. [34, 44, 88] following the practices of [89].

It is particularly important, and difficult, to show that total current (including the ethereal current) is independent of location in these one dimensional systems. Tiny numerical imprecision in the conservation of current produces large errors in results. Coarse graining procedures that do not explicitly check for conservation of total current are suspect.

We calculate the conformation current produced in Na_V channels and K_V channels. We show that changing the isoleucine of the K_V channels to the threonine found in Na_V channels speeds up the response to voltage, and thus allows the separation of opening of the Na_V and K_V channels needed to create the nerve signal.

2 Model and theory

The model is presented in Figs. 1 and 2 in outline. Detail is provided in the original papers of Catacuzzeno and Franciolini [34, 88] and its successor [44] building on the lower resolution model of Horng [89]. A representation of the structure of the voltage sensor is created in atomic detail from [90]. The rest of the channel structure was constructed using homology modeling as described on p. 2010 and in the Supplementary Material of [44].

Effective dielectric constant. Molecular dynamics was used to determine the effective dielectric constant (i.e., the polarization charge) by computing the dependence of polarization charge on the electric field, p.8 and Fig. 3 of [44], and checking that results for bulk agreed with bulk properties. This method emerges naturally from consideration of the relation of charge and electric fields in proteins [59] and has been successfully applied to estimate the dielectric constant of a protein [91, 92], as well as of water confined inside the pore of a synthetic ion channel [93]. Local polarizability was estimated from polarizability of side chains (Table 1 of Voges and Karshikoff [94], also see [95]). We avoided methods that attribute spatially homogeneous properties to proteins with internal dielectric boundaries like channels, transporters, and we suspect enzymes, that are likely to have large effects on currents [96].

The sheath around the voltage sensor was found to have a region accessible to water from the inside of the cell. The effective electronic dielectric coefficient was around 2.0 in the water accessible region and 4.0 in the water Inaccessible region. The 'effective

dielectric constant' is defined in and illustrated in detail in reference [44], particularly panel C of Fig. 3 of that reference.

The effective dielectric coefficient of dipoles was found to depend on the protein composition. It was very different (12.7) near the isoleucine 287 of the K_V channel from the effective dielectric constant of 60.1 in the threonine mutant 287T that we use to model the Na_V channel. The side chains and structure of the regions are defined precisely in Fig. 2E of [44]. We suppose that the polarization current which speeds up the Na_V channel comes from the change in dielectric properties between the isoleucine of 287 and the threonine of 287T. We propose that this atomic change in polarization is the evolutionary adaptation that produces the gap in time of activation of potassium and sodium conductance and is atomic control of action potential propagation. Biological function on the meter scale of nerve signals is controlled by side chains that differ by a handful of atoms, on 10^{-10} meter sub-molecular scale.

Isoleucine side chain is	$-CH-CH_2-CH_3$ $\vert$ CH_3	Threonine side chain is	$-CH-CH_2$ $\vert$ OH

Substituting an oxygen atom for a carbon, and removing a methylene controls the nerve action potential. This is a remarkable example of an atomic control of animal function, atoms to arm, if we may be forgiven some poetic license.

We imagine the extra polarizability of 287T threonine that mimics the Na_V channel comes from the extra mobility and thus polarization of the electrons in the OH group of the threonine, compared to the mobility of electrons in the CH bond of isoleucine. The water that interacts with the OH probably adds more polarization as well. It is interesting that the conformation changes on a larger scale are not needed to explain this difference in gating currents between the K_V structure and the mimic of Na_V, perhaps because they occur on a much longer time scale, with more steric and frictional/entropic dissipation.

3 Results

The model and gating currents predicted are shown in Fig. 2 (and in detail in the full length papers [34, 44], particularly the supporting material for [34], that need to be consulted). Fig. 2 illustrates the main steps involved in our multi-scale hierarchical model of voltage-dependent gating.

(1) We perform all atom molecular dynamics (MD) using the atomic structure of the voltage sensor domain in order to assess the water accessibility and the polarization of the voltage sensor domain (panels A and D);

(2) The all atom structure is used to obtain the spatial distribution of mass, namely the geometrical properties of the VSD;

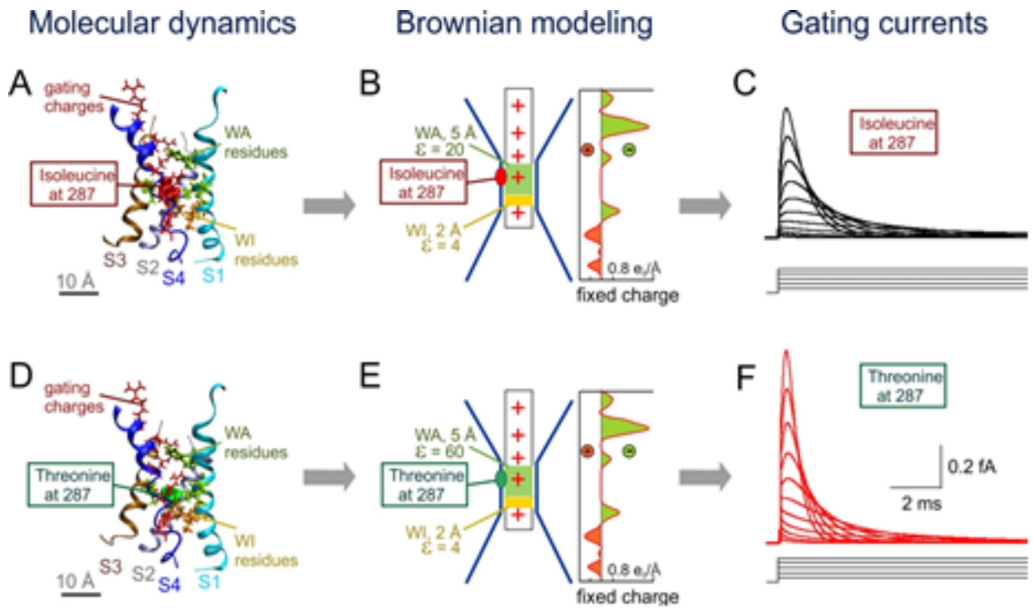

Figure 1: Multiscale hierarchical model of voltage dependent gating. See text for description.

(3) The all atom structure is also used to provide the spatial distribution of charge density. Together, the two spatial distributions provide a precise definition of the otherwise vague word 'conformation';

(4) This information is used to build a macroscopic scale, Brownian model (with thermal motion) of the voltage sensor domain (panels B and E) which, in contrast to MD, can reproduce the behavior of a population of VSDs on time scales of physiological relevance. The Langevin equation of thermal motion can be solved to give macroscopic gating currents that are close to those obtained experimentally.

Panels A) and D) of Fig. 1 show the 3D structure of Shaker VSD in the active state, from (Henrion et al. 2012). A) shows the wild-type (WT) structure of Shaker VSD, and D) the structure obtained after computational mutation of the Isoleucine at position 287 to threonine. The four transmembrane segments are shown in different colors (indicated). The nonpolar hydrophobic residues forming the water accessible (WA; V236, I237, and F290) and water inaccessible (WI; S240, I241, F244, C286, I287, A319, and I320) regions of the gating pore (Lacroix et al. 2014) are shown in licorice representation, in green and orange, respectively. The gating pore residue 287, of interest here, is shown in van der Waals representation, colored in red in the WT structure (A) and green in the mutated structure (D). Gating charge side-chains are shown in licorice representation and colored in red. B) and E) Schematics showing the geometry and electrostatic properties of the VSD assumed in our Brownian model. The S4 segment containing the gating charges (red crosses) was assumed to move perpendicular to the membrane from the intracellular to the extracellular vestibules (each 31.5 Å long, and opening with a half angle of 15¡ã),

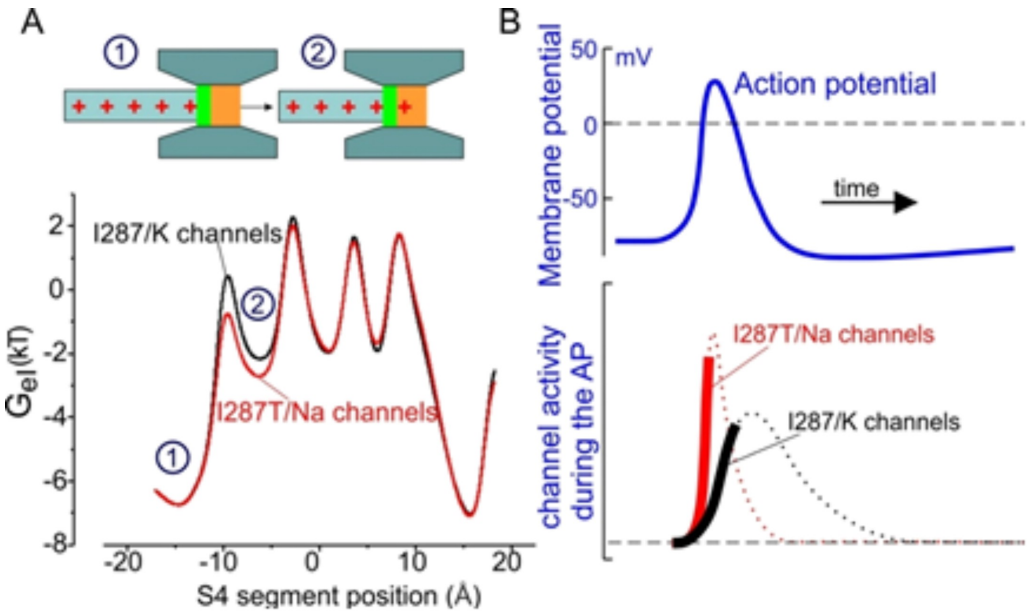

Figure 2: Physical mechanism of the different activation of Na_V and K_V channels, as computed by our multiscale hierarchical model. See text for description.

through the gating pore (7 Å long). The gating pore includes a 5 Å long water accessible region (green) and a 2 Å long water inaccessible region (orange), having relative dielectric constants of 20 and 4 in the WT structure (panel B) and 60 and 4 in the mutated structure (panel E), respectively. The drawings on the right show the fixed charge density, also obtained from all charged residues in the S1-S3 segments, with each charge contributing with a Gaussian profile having a mean determined by the charge position on the atomic structure and a standard deviation of 0.1 nm. The Panels C) and F) show simulated gating currents obtained in response to depolarizing pulses from a holding potential of $-90\ mV$, from -80 to $+20\ mV$ (delta of 10 mV) for both the WT (black, panel C) and the mutated (red, panel F) models.

Our entire model is needed to provide a specific description of our results. Only by considering the energetics of each scale of the hierarchy can the energetics and conformation change of the voltage sensor be understood. In particular, effects of the steric potential, and disorder cannot be easily summarized in conventional pictures of barriers and movements, because those effects include steric interactions, friction, and entropy production, which are more comfortably ignored.

Simplifications are nonetheless crucial to motivation and communication, if not understanding, provided they are taken as the vague approximations that they are, guides to qualitative thinking, and are not used to replace actual documented calculations.

Fig. 2 shows the classical interpretation suggested by our model and simulation of the 287T mutation from isoleucine of the K_V channel to the threonine found in Na_V channels. The difference in the activation kinetics between Na_V and K_V channels, essential for the

generation of the action potential (panel B), is produced by the different polarization charges induced by the first gating charge entering the gating pore (panel A) and the resulting differences in the electrostatic energy G_{el}.

Panel A) Plot showing the electrostatic energy associated with the S4 segment position for the two models of voltage-dependent gating differing for the dielectric constant in the WA region of the gating pore. Notice that the only difference in the electrostatic energy profiles occurs at the first peak. This can be explained by the significant difference in the charge induced and the resulting polarization energy when the first gating charge moves into the water accessible region of the gating pore (either I287 or 287T), because of their markedly different dielectric constants and thus polarization. Inset: Schematic drawings showing the first gating charge on S4 entering the WA region of the gating pore. B) Schematic plot showing the activity of Na_V and K_V channels (bottom, red, and black lines, respectively) during an action potential. Na_V channels open more rapidly than K_v channels, creating the depolarizing phase of the action potential (top).

4 Discussion

We have calculated the conformation current produced in Na_V channels and K_V channels in response to a step of voltage. We show that changing the isoleucine of the K_V channels to the threonine found in Na_V channels speeds up the response to voltage, and thus allows the separation of opening of the Na_V and K_V channels needed to create the nerve signal.

Other possibilities. Other processes could contribute to the time course of Na_V and K_V channels, as kindly pointed out to us by Bezanilla.

First, mutation of the highly conserved residue 363 in S4 from isoleucine to threonine makes the voltage sensor of K_V channels move faster, as seen in the time course of the gating currents [46]. This residue is relatively far from the gating charge, so we suspect that its contribution to the polarizability would not be significant, suggesting that a different mechanism is likely responsible for this effect.

Second, the β_1 subunit of Na_V channels speeds up the voltage sensor by an unknown mechanism [46]. Finally, the sensors in Na_V channels interact cooperatively to speed channel activation [97].

Model is sufficient and necessary. We point out that the polarization mechanism we propose is both logically sufficient and logically necessary to account for the difference in speed and the structural movements of the voltage sensor (within the limitations of our models).

It is sufficient because we reproduce the properties of gating current under a realistic range of conditions.

It is necessary because the movement of charges that we calculate must produce the currents we calculate given the universal and exact nature of the Maxwell equations that

link charge and current [39, 40]. Our conclusions arise from the universal and exact conservation of total current implied by the Maxwell equations.

Of course, evolution is not logical. It often provides redundant mechanisms that are beyond the necessary and sufficient. These mechanisms may provide properties not glimpsed in gating currents studied with step functions in the conditions they are usually measured. These mechanisms may also control the speed of gating and give the gating system biologically and evolutionarily useful properties unknown to us.

Analysis is incomplete. We do not know how the conformation change of the voltage sensor produces the opening of the channel. We can measure that conformation change quite directly by its gating current. But we do not have a model of the conformation change or of the role of gating current in the opening of the conduction channel.

It is traditional to say that the linkage between the movement of the voltage sensor and the conduction channel is mechanical but that statement is more metaphorical than physical. It does not come with a model that satisfies conservation laws of mass and electricity. Or with simulations that reach biological scales, compute electric fields with mathematics, or depend on ions (e.g., calcium) that are known to have large experimental effects.

Indeed, it is possible that the gating current itself flows in large measure through the conduction channel (as it 'completes its circuit') and triggers the opening of conduction channels. In that case, the gating current itself would be the linker between the voltage sensor and 'conductance' (i.e., number of open channels).

The traditional mechanical models ignore the reality that correlations of the motion of atoms involve electric fields [58, 98] at least as much as steric interactions or potentials [24]. Stochastic behavior that dominates the movement of charges can disappear in the movement of current [98]. Of course, a quantitative simulation and theory of channel opening is needed to establish the relative role of electrical and steric forces and potentials.

Our analysis is incomplete on the atomic scale of sensor $\Rightarrow$ channel opening, but it is complete on the larger scales of conductance (of ensembles of channels) and action potential propagation. Thanks to the work of Hodgkin [99, 100], Hodgkin and Rushton [41], Davis [42], and Hodgkin and Huxley [84], the connection is known between micrometer properties of channels in a membrane, and the nerve signal meters away. The Maxwell equations that guarantee exact conservation of current link these scales. They imply the cable equations of Kelvin (1855, presented in biological context in [101].

Acknowledgments

This research was supported in part by the United States-Israel Binational Science Foundation (Grant No. 2016106).

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