THE PHYSICAL BASIS OF ION CHANNEL KINETICS: THE IMPORTANCE OF DYNAMICS

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Abstract. Ions cross the cell membrane through ion channel proteins. The traditional approaches have analyzed channel proteins as if they existed in a few stable conformations and switched rapidly between them. However, we show here the importance of dynamical properties in these proteins. These properties include continuous

internal motions and continuous changes of state, the interactions of the ions with themselves and with the channel protein, deterministic forces, and time dependent molecular memory. This new dynamical perspective changes how we interpret experimental data from patch clamp experiments that record the ionic currents through individual channels. It also helps us to understand how ion channel proteins function as molecular machines and how their molecular properties are related to their physiological function.

1. Introduction. Proteins are linear chains of heterogeneous units called amino acid residues. The forces among these units and between them and the external salt-water environment folds proteins into three dimensional shapes called conformational states. Protein function depends on these conformational states. In enzymes, they determine the activity of the binding sites for ligands. In ion channels, they determine the ability of those membrane bound proteins to pass ions, such as potassium, sodium, chloride, and calcium, across the cell membrane. Until recently, it had been implicitly assumed that channel proteins existed in a few stable conformations and switched rapidly between them. Thus these conformational changes were represented by: 1) kinetic diagrams consisting of a few states and the transition probabilities between those states and 2) by energy level models consisting of a few energy minima and the pathways between those states defined by fixed energy barriers. Data from biochemical experiments were used to compute the switching probabilities and their associated energy barriers between these different conformational states. However, over the last two decades theoretical and experimental evidence has shown that the dynamics of proteins have continuous internal motions and continuous changes of state that play a crucial role in protein function. We present here the evidence in support of the importance of dynamics in ion channel proteins by reviewing previous studies and by presenting new calculations on the relative importance of deterministic and stochastic motions in proteins. These results put into question the relevance of the traditional kinetic diagrams and energy models that have been used to represent ion channel proteins. This new, dynamical perspective may help us understand how proteins function as miniature molecular machines and how their molecular properties at the microscopic level determine their physiological function at the macroscopic level.

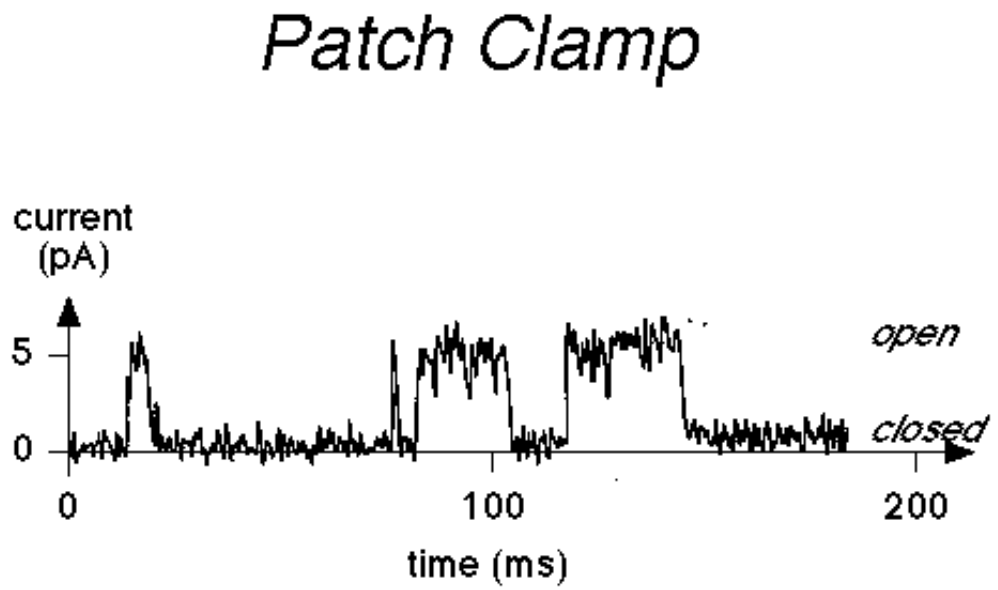
2. Ion channels. Cells use energy from ATP to pump ions across the cell membrane to establish a concentration difference of ions, such as potassium and sodium, between the inside of the cell and the surrounding media. This concentration difference is used to drive biochemical reactions and maintain an electrical voltage across the cell membrane. Ions which are soluble in the hydrophilic solutions inside and outsdie the cell cannot easily cross the hydrophobic lipid cell membrane. An important pathway for the passage of ions across the membrane is through the core of ion channel proteins in the cell membrane [11]. The core of these proteins mimics a hydrophilic environment which therefore allows the passage of ions across the cell membrane. There are many different such channels with relatively different permeabilities to sodium, potassium, chloride, and calcium. In nerve cells, these channels generate and propagate the action potential, the wave of electrical depolarization that transmits information down the axon. In muscle cells, ion channels determine calcium levels that initiate contraction. In the photoreceptors in the retina, channels transduce light into electrical signals. In intracellular organelles, such as as mitochondria, channels control the ionic concentrations of the internal fluid.

The ability of the channel to allow the flow of ions depends on its conformational state. The conformational state of a channel changes from those shapes that are open to those that are closed to the passage of ions. These changes of current can be measured across an individual ion channel protein. They can be used to infer information about the changes in state of the ion channel protein. A small *patch* of cell membrane, containing only one ion channel, can be sealed in the mouth of a glass micropipette. When the voltage is *clamped* across this patch the ionic current flowing through it can be measured. This *patch clamp* technique can resolve the picoampere currents through an individual ion channel protein [28]. Thus, unlike most biochemical or biophysical

techniques that measure the combined signal from a very large number of molecules at once, this technique can measure the properties of a single molecule at a time. Neher and Sakmann were awarded the Nobel prize in Physiology or Medicine for this achievement.

As illustrated in Figure 1, the patch clamp technique shows that an individual ion channel fluctuates between states that are closed and open to the flow of ions. These current recordings have time resolution of about 0.1 ms which is set by the electrical resistance and capacitance of the patch, micropipette, and electronic amplifiers. In these recordings the channel appears to switch instantaneously between the closed and open states. The intermediate states, if they exist, are not detected at this resolution. These data are analyzed by measuring the durations of the closed and open times and determining their statistical properties, such as the probability density function (PDF) of the closed and open durations, and the statistical correlations between those durations.

Fig. 1 Ion channel proteins fluctuate between conformational states that are closed and open to the flow of ions. The patch clamp technique can resolve the picoampere currents through an individual ion channel. The current is low when the channel is closed and high when it is open. Thus, it is possible to measure the sequence of durations of conformational states in an individual protein molecule. The data shown here were recorded by Liebovitch and coworkers from a potassium channel.



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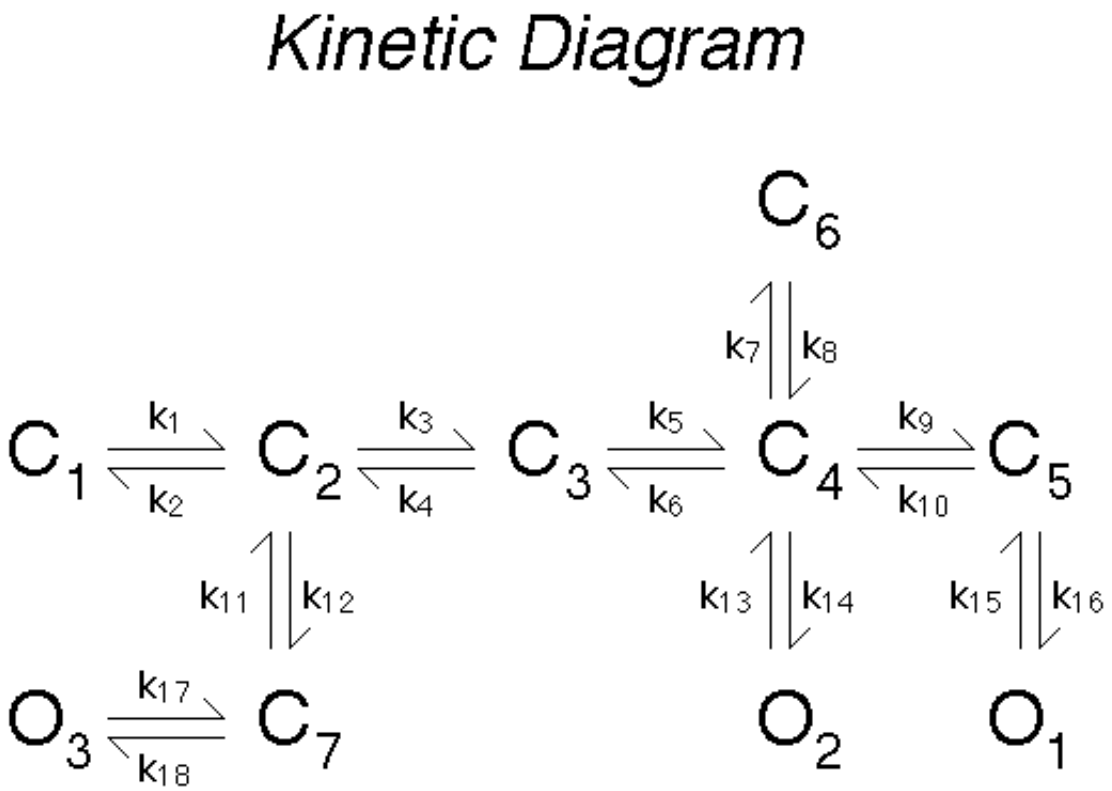
3. Traditional paradigms of ion channels. It was implicitly assumed that ion channel proteins have a few stable conformational states and that they switch rapidly between them [11,28]. It was believed that these switches are driven by fluctuations in thermal energy and the binding of ligands. The timing of these thermal or binding events was considered a stochastic process determined by chance. The probability per second of the change in conformation can be determined, but it is assumed that the exact time at which this switch occurs cannot be predicted. Two different, but related paradigms, have been used to interpret these experimental results. These paradigms, as well as some discussion of their assumptions, have been reviewed recently in the Perspectives on Ion Permeation and the subsequent letters in the Journal of General Physiology [33,34].

3.1. Kinetic diagrams. Figure 2 shows a typical kinetic diagram used to summarize the switching of an ion channel between closed and open states [3]. The PDF, the probability that a closed (or open) duration, is

greater than time t and less than time t+dt, was determined from the data. Then the PDF of the open and the PDF of the closed durations were fit by a sum of exponential terms. The data can be well represented by such functional forms. Each exponential term was identified as a unique closed or open state. The switching probabilities per second between these states, which are called the kinetic rate constants, were then determined [11,28]. This approach assumed that the switching can be represented by a Markov process. That is, it was assumed that the probability to switch states depends only on the state of the channel and not on the history of previous states and not on the duration of the time that a channel has remained in a state. Although there are many examples where Markov models have well fit the experimentally measured open and closed times, there have been very few attempts to test the complete set of mathematical assumptions that underly this assumption. Analysis of the data from some channels supported this assumption [7,9,25]. Nonetheless, although there is no memory in each pathway of this model, by having regions of the kinetic diagram connected through low probability gateway states, a kind of memory can be generated where the channel remains for long times in certain regions of the kinetic diagram.

Fig. 2 Kinetic diagrams were used to summarize the data from an ion channel protein switching between closed and open conformational states. These models assumed that the channel protein has a few stable conformational states. Here, the closed and open states are represented by C_i and O_i. It was also assumed that there is a fixed probability per second, called the kinetic rate constant k_i, to switch between the states which depends only on the present state of the channel and does not depend on the history of previous states or the time spent in a state. This kinetic diagram was used to by Blatz and Magleby to model the kinetics of a chloride channel [3].



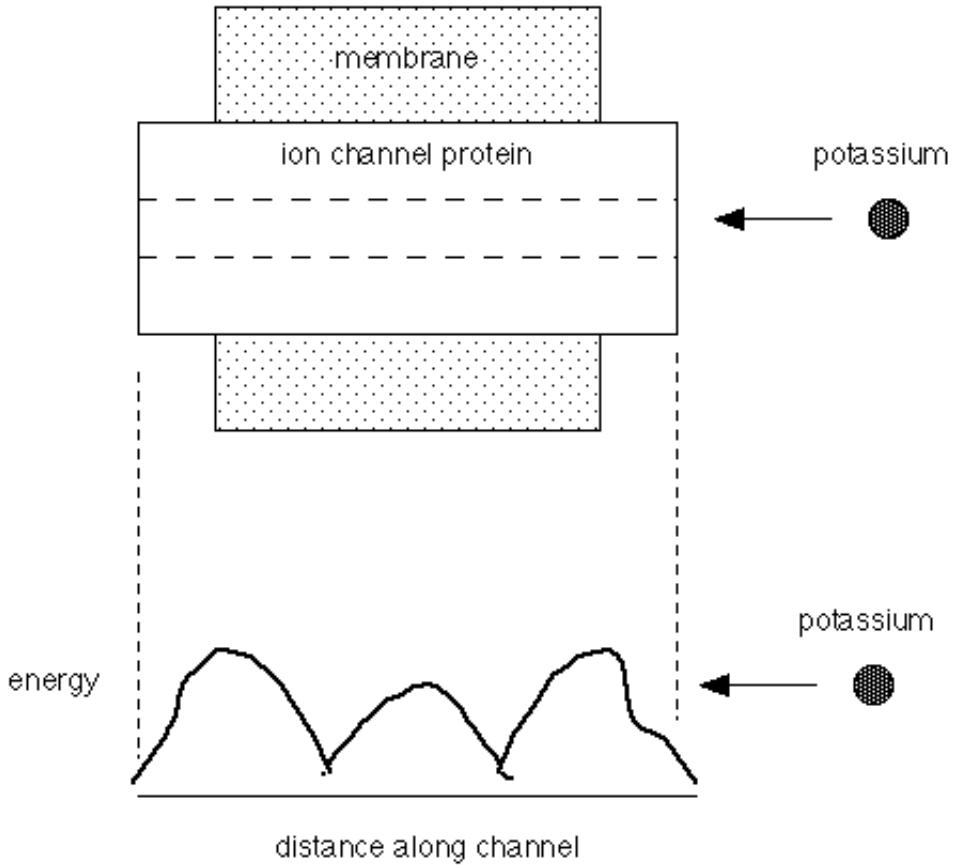
Although this approach has been widely used a number of essential technical aspects of it have been questioned recently. First, the PDFs required many exponential terms and thus a large number of adjustable parameters to fit

the data. In fact, it was shown that other functional forms with the same number of parameters are actually a better fit to the data and that the overall form of the PDF can often be fit by a much simpler power law form [17]. Second, there are far fewer parameters that can be determined from the experimental data than the number of parameters needed to define the pathways in the kinetic diagram [2,14,15]. Thus, the kinetic diagrams are not unique. Third, recent analysis of experimental data has found the presence of long term memory in the switching probabilities that are inconsistent with the assumption that the switching is a Markov process [8,32]. Fourth, the kinetic diagrams summarize the switching probabilities between states, but they do not provide a physical description of the properties of the channel protein. There have been few attempts derive a physical interpretation from this approach by using the kinetic rate constants to compute the free energy difference between conformational states. For some channels it has been shown that the kinetic rate constants and the topology of the kinetic diagrams cannot be used to generate a consistent set of energy levels [27]. This raises a question about the ability of this approach to provide insight into the physical properties of proteins.

3.2. Fixed energy barriers. The kinetic diagrams assume that there are a few discrete states and that the probabilities to switch between them are constant. One physical interpretation of these mathematical assumptions is that the channel protein has a few, distinct conformational states, which are separated by significant activation energy barriers, that are constant in time. Physical models of the passage of ions through channel proteins have also assumed that the ion passes through a fixed, static set of energy barriers, as shown in Figure 3.

Fig. 3 Traditional models of the passage of ions through a channel protein assumed that the ions pass through static energy barriers and that their passage through the channel does not significantly change the conformational state of the channel protein.

Energy Barriers



This paradigm assumes a number of things about the physical properties of channel proteins. First, that they can be represented by static energy barriers of a fixed or average size. Second, that there is no continuous time dependent internal dynamics in the state of the channel protein. Third, that there is no interaction between the conformational state of the channel protein and the passage of the ions through it.

Although the traditional analysis of ion channels has assumed that they have these physical properties, substantial evidence over the last two decades has shown that other proteins, namely globular proteins, cannot be characterized by such physical models. For example, measurements of biochemical reactions using color change indicators, fluorescence markers, radioactive tracers, nuclear magnetic resonance, and x-ray diffraction have demonstrated that there are a wide range of molecular motions over a wide range of time scales that affect binding and enzymatic activity [21]. Moreover, the conformational changes associated with binding, such as induced fit and allosteric control, mean that ligands significantly alter the structure of the proteins when they bind. Membrane bound channel proteins share many characteristics with these globular proteins. The importance of molecular motions and ligand-protein interactions in globular proteins suggests, but does not prove, the importance of molecular motions and ion-channel interactions in channel proteins which were not taken into account in the traditional few discrete state, fixed energy models.

4. Dynamics. The kinetic diagrams and models with fixed energy barriers have been the traditional tools used to analyze and interpret the channel data from patch clamp recordings. These kinetic diagrams assume that the

switching between states is a Markov processes. The energy level models assume that the energy barriers between stable states remain constant in time. In this paper we review the existing studies and present new material that shows that these assumptions are contradicted by experimental data and theoretical analysis of channel proteins. *The central theme of this paper is that dynamical properties, continuous internal motions and continuous changes of state, play a crucial role in channel protein function.* We need to develop new approaches that take these dynamical properties into account in order to achieve an understanding of channel function.

4.1. Reaction rates depend on internal motions. One of the first indications of the importance of role of the internal motions in proteins came from theoretical studies that computed the reaction rates of the binding of ligands to proteins [13]. For example in myoglobin, the average positions of the atoms had been determined by x-ray crystallography. Thus, it was possible to determine the energy barriers that oxygen would cross in passing from the external solution to its binding site in the interior of the protein. The reaction rates predicted from these energy barriers were hundreds of times less than those experimentally measured. Molecular dynamics simulations showed that for brief times the atoms blocking the passage of the oxygen would move out of the way making it much easier than expected for the oxygen to reach its binding site. That is, for brief times the protein structure fluctuates into an appropriate structure that allows the reaction to happen. The static, average structure does not tell us how a protein really works. The reaction rate computed from the average structure, namely exp(-<deltaG(t)>/k_BT), where <deltaG(t)> is the energy barrier of the positions of the atoms averaged over time, k_B is the Boltzmann constant, and T the absolute temperature, drastically underestimated the reaction rate. The true reaction rate depends on the average of the Boltzmann factor, namely <exp(-deltaG(t)/k_B>For brief times, as the structure fluctuates, deltaG(t) decreases, and those moments contribute disproportionately to the overall reaction rate. Thus,<exp(-deltaG(t)/k_BT)> >> exp(-<deltaG(t)>/k_BT). This work clearly demonstrated that we need to take into account the motions within the protein structure if want to quantitatively predict the reaction rates and understand the process of how the ligand reaches its binding site in the protein.

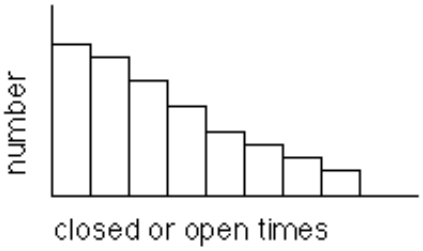
4.2. Interpretation of the PDFs of the closed and open durations. The patch clamp data can be used to determine the probability density functions (PDFs) of the durations during which the channel remains closed or open. Traditionally, it was assumed that these PDFs arise from the channel protein switching between different states. The switching probabilities between those states were then used to compute the kinetic rate constants of the kinetic diagrams or the energy barriers of the energy level models.

These PDFs need not arise at all from the channel switching between a multiplicity of closed and open states. The PDFs can also arise from a single closed and single open state, where the energy barrier between them varies in time. The variation in time of the energy barrier between the open and closed conformational states determines the PDF. Specifically, the kinetic rate constant k is the probability per second to switch to a new conformational state. This rate k is proportional to exp(-deltaG/k_B T) where detlaG is the energy barrier between the states, k_B is the Boltzmann constant, and T the absolute temperature. As a function of time t, the instantaneous k(t) proportional exp(-deltaG(t)/k_B T). From the definition of the kinetic rate constant k, it also follows that the cumulative probability of finding an open (or closed) time greater than duration t is given by P(t) = exp [-integral k(t) dt]. The probability density function PDF = - dP(t)/dt. These relationships can therefore be used to compute deltaG(t) from the PDF.

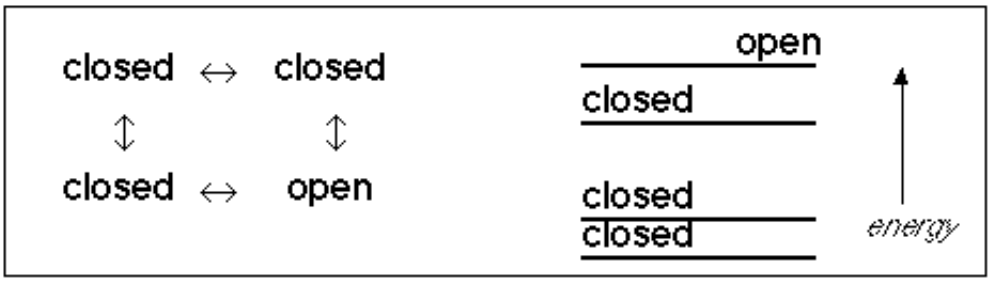
Thus, the PDFs may represent the continuous internal dynamics of the channel protein, represented by the continuous variation in time of the energy barrier between conformational states, rather than a kinetic diagram of a static set of stable states. This is illustrated in Figure 4. Liebovitch et al. illustrated how to compute the time dependency of the energy barriers form the PDFs of the channel data [17,20,21]. It will be interesting to see if the reinterpretation of other experimental data in terms of such dynamical mechanisms sheds greater light on the understanding of channel function.

Fig. 4 The PDFs of the closed and open durations have been interpreted by the traditional energy levels models in terms of the number of conformational states and the energy barriers between them. However, the PDFs can equally well be interpreted in terms of the time dependency of the energy barriers between conformational states [17,20,21].

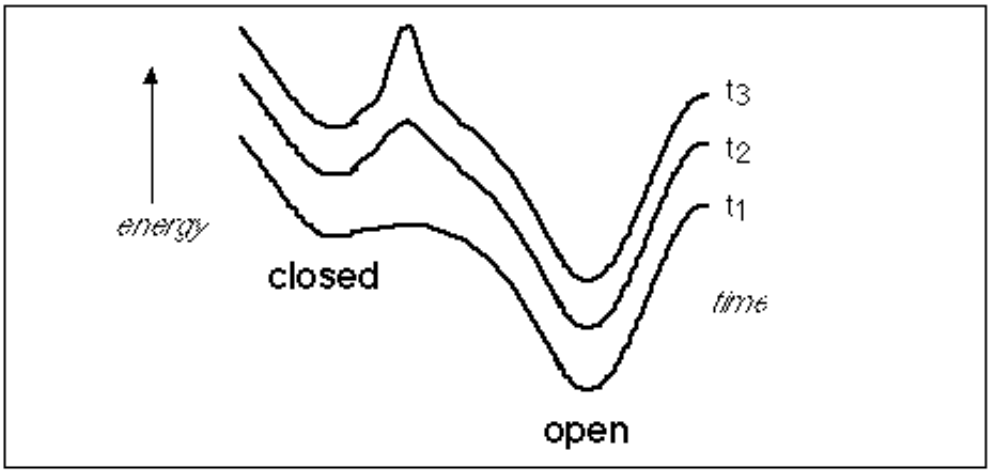
Probability Density Function (PDF) of the Closed and Open Durations



Energy Level Model:



Dynamical Interpretation:



The analysis of the patch clamp data alone cannot determine if the dynamical time dependent interpretation or the static set of stable states interpretation is the correct one. Time dependent perturbations, such as two photon experiments, where the time between the two separate stimulations is varied, may provide the way to reveal which one of these two interpretations is the correct one.

4.3. Interaction of ions with channel structure. The traditional analysis of ion channel data has assumed that there is no direct interaction between the conformation state of the channel protein and the passage of ions through it. For example, in the Hodgkin-Huxley model [12], ion channels are electrical resistance elements. The resistance of the channel does not depend on the current passing through it. More recent models assume that ions pass

through fixed energy barriers. That is, it is assumed that the passage of ions does not itself alter the conformational state of the channel protein and thus its energy barriers. Two recent theoretical models suggest that this may not be the case at all. In fact, the interactions between the channel protein and the ions passing through it may play an essential role in channel function, namely switching the channel between different conformational states. In both of these models, the dynamics of these interactions has an essential role in channel function.

4.3.1. Interaction of ions with the channel. Eisenberg et al. [4,5] modeled the interaction between the ions flowing through the channel and the channel wall. They found that a significant amount of electrical energy is exchanged between the permeating ions and the channel wall.

Chinarov et al. [6] investigated the effects of the interaction between the conformation state of the channel protein and the passage of ions through it. They represented the conformational state of the channel protein by a potential energy function. Ions passing through the channel can bind at sites inside the channel. The electrical fields of these bound ions then alters the energy function of the channel protein. They found, that for some values of the parameters of the model there was a stable regime with some fluctuations of the channel state and the current through the channel. However, for other values of the parameters, the conformational state of the channel was bistable. That is, the interaction between the ions and the channel protein caused the channel to switch between its closed and open states. This work is not a detailed model of channel function. However, the importance of this work is that it shows that the dynamical interactions between the ions and the channel, which had been ignored in previous work, can affect the most salient aspects of channel function, for example, even determining if the channel is closed or open.

4.3.2. Interaction of ions with the channel and each other. The switching of the current through the channel had always been attributed to changes in the conformational state of the channel protein. It was then quite a surprise when Lev et al. found that ionic currents passing through artificial plastic membranes with fixed pore geometry could also exhibit the same switching between discrete low and high current levels [16].

This opened the possibility that the current switching was due to a dynamical interaction between the ions and the wall of the pore. Grzywna et al. proposed that two opposing process are present. The ionic current is increased by ions drawn to binding sites in the channel. However, the ionic current is decreased when the electric field generated by too many ions reduces the ionic flow [10]. Because many ions in the channel will reduce the current, they called this the "crowding" model. The current I(t + delta t) through the channel at time t depends on the sum of these two terms, namely $I(t + detla\ t) = k_1 I(t) - k_2[I(t)]^{gamma}$, where the parameters k_1, k_2, and gamma are constants. They showed that this equation well fit the ionic current data through the pores in the artificial membrane. This equation has the generalized form of the logistic equation which is given by I(t + delta t) = A I(t) [1-I(t)] where A is a constant. The current as a function of time can exhibit periodic and chaotic behavior, depending on the values of the parameters.

This work opened the possibility that some effects, previously attributed only to the changes in conformation of the ion channel protein itself, may actual be due to the dynamical properties of the ions in their interactions with themselves and with the channel protein.

5. Deterministic dynamics. The traditional approach assumed that the switching of the channel protein between different conformational states was an inherently random event. That is, at random times the channel protein would suddenly gain energy that would shift it to another conformational state. These energy kicks could come as

thermal fluctuations from the environment or from the binding of a ligand. The changes in channel conformation then depended on these random increments of energy supplied to it.

Here we now consider another approach, and ask whether the deterministic forces within the channel itself may be partially responsible for initiating or completing these conformational changes. These forces consist of the atomic bonds between atoms, electrostatic interactions between charges, and hydrophobic and lipophilic forces between the amino acid residues in the channel protein and the lipids and water surrounding it. Perhaps these forces can drive motions that influence the conformational changes. In this approach the channel protein is more like a miniature mechanical machine, with masses, springs, gears, and levers that perform consistent stereotypical motions when excited in similar ways. The energy to run this machine could be supplied by the ambient heat bath, the voltage gradient across the channel protein, or the chemical energy of the binding of ligands.

This new approach contrasts with the traditional approach where the channel protein is thought of as a purely a passive system relentlessly kicked by random thermal or chemical fluctuations between different conformational states. The basic differences in these two approaches is the role of internal structure and forces in determining the conformational changes. In the traditional approach the energy differences alone determine the probability of reaching other conformational states. In the deterministic approach the internal structures and forces within the channel protein influence the pathways of conformational change and thus the conformation that the channel protein will attain.

In the traditional approach the probability per second of the switches between different conformational states was determined. However, the exact time of the switch, which was due to a thermal fluctuation or the binding of ligand, was considered an inherently stochastic event that could not be predicted. This assumption was based on the fact that the PDFs of the closed and open durations had the form of PDFs produced by a random timing of switching events. It was also supported by the observation that the times of the switching were not predictable.

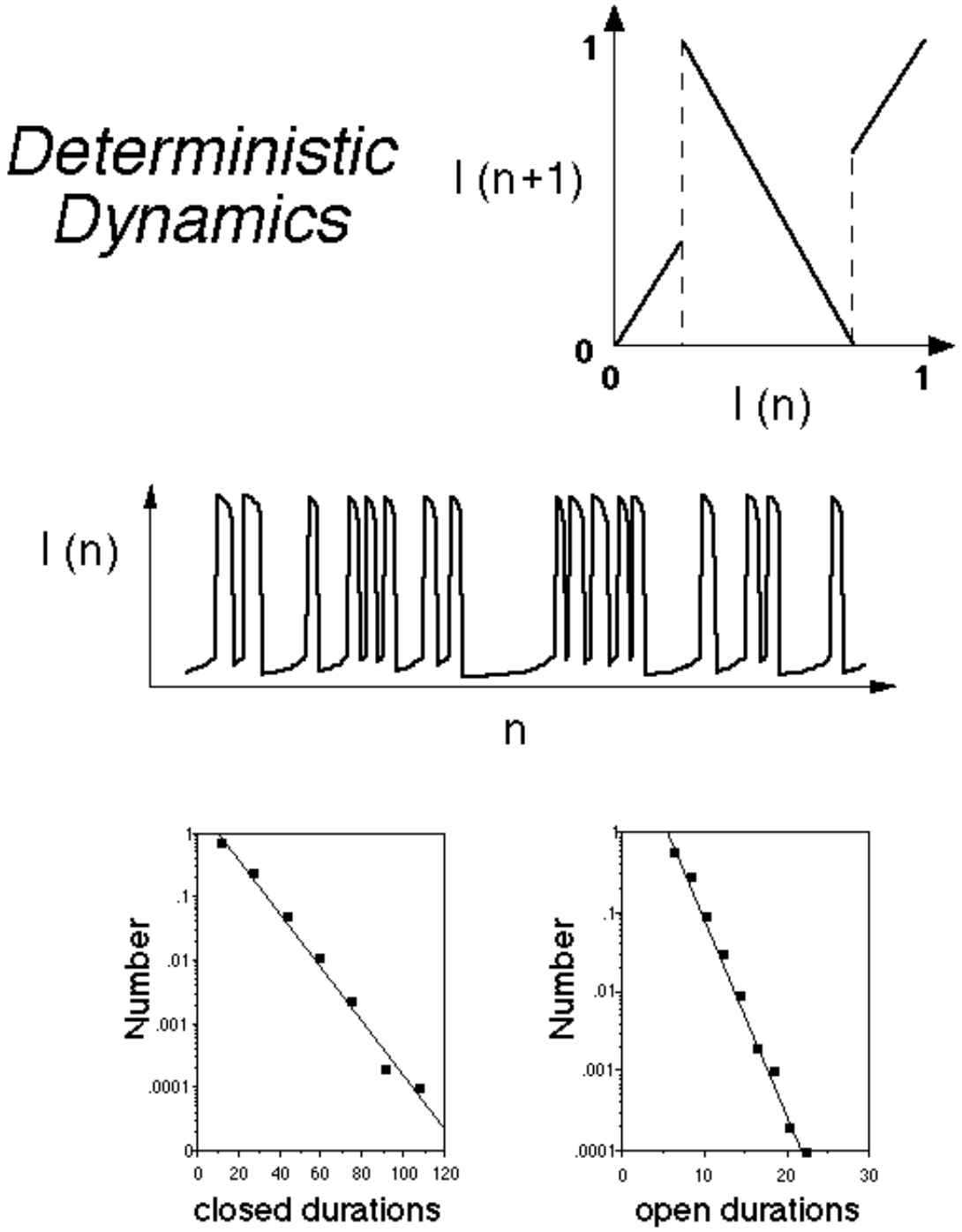
However, we now know that not everything that looks random really is random. Deterministic dynamical processes, called chaotic, can produce output so complex that it looks like that produced by stochastic processes [1,18]. In a chaotic dynamical process the variables at the next time step in time can be computed from their previous values. However, since prediction accuracy is lost at every time step, over many time steps the values of the variables are not predictable.

Deterministic chaotic processes can generate the same PDFs as those generated by stochastic processes. They can also generate switches between different conformational states of the channel protein that are not predictable. Previously it had been assumed that these properties could only be generated by stochastic processes. The fact that these experimental properties can also be generated by deterministic processes raises the possibility that the switches between conformational states of the channel may be driven by deterministic molecular forces inside the channel, rather than random thermal events. Thus, deterministic dynamical forces and motions within the channel protein could play a direct role in channel function. A search has now begun to find ways of determining the relative importance of deterministic forces compared to random thermal noise in channel function.

5.1. Deterministic, chaotic dynamics can generate the PDFs of the closed and open durations. The traditional approach assumed that the channel data were produced by stochastic processes. Liebovitch and Tóth [22] explored whether the channel data could be produced by deterministic processes. They formulated a deterministic chaotic model based on an iterated map where the current through the channel at the next time step I(n+1) is a piecewise linear continuous function of the current through the channel at the present time step I(n).

The physical interpretation of the mathematical form of this model is that the atomic, electrostatic, and hydrophobic forces in the channel amplify molecular motions until they drive the channel from one conformational state to another. In this model the next state of the channel depends on its present state. There is a "molecular memory" in the internal dynamics of the channel protein. This contrasts with the physical interpretation of the traditional Markov models of kinetic diagrams and energy level models where the channel remains in a state until a thermal fluctuation suddenly comes along and provides (or removes) enough energy for the channel to spontaneously change from one conformational state to another. In these Markov models there is no physical antecedent to the change in state, there is no memory, and the timing of the thermal fluctuation is purely stochastic. As shown in Figure 5, the current output of this deterministic chaotic model switches between closed and open states with the unpredictable timing characteristic of channel data. The chaotic model could qualitatively generate the current records and quantitatiely generate the form of the distribution of open and closed times of the experimental data. The PDFs of the closed and open durations of this deterministic chaotic model are single exponentials, a form that had previous been assumed to be an identifying characteristic of a stochastic model of ion channel function. All of the forms of the PDFs found in the experimental data could be generated by such deterministic chaotic models by adding more linear pieces to the map or making the segments nonlinear. Therefore, the channel data could be produced by either stochastic or deterministic processes.

Fig. 5 Liebovitch and Tóth showed that properties of the channel data which were thought to be characteristic of a stochastic process could also be generated by deterministic dynamics. (Top) In this deterministic chaotic model the current I(n) through the channel protein at step n determines the current I(n+1) at the next step n+1 through the function shown in the graph. (Middle) This deterministic relationship generates a current that switches between closed and open states with the unpredictable timing. (Bottom) The PDFs of this deterministic chaotic process are straight lines on a semi-logarithmic plots, that is, they have a single exponential form. The same single exponential form is also produced by a stochastic process with constant probability per second to switch between the closed and open states. Thus, exponential PDFs and unpredictable of switching between states can be generated by either deterministic or stochastic processes.



5.2. Relative importance of deterministic forces and stochastic thermal motions. The statistical properties of the channel data had been used to support the idea that the switching of the channel protein between conformational states was a stochastic process. The understanding that these same statistical properties could also be generated by deterministic, chaotic dynamical systems raised the question as to the relative importance of stochastic thermal versus deterministic atomic, electrostatic, and hydrophobic forces in channel function. This question can be addressed by using concepts from nonlinear dynamical systems.

An important property of chaotic systems in their sensitivity to initial conditions [18]. If a the computation of a chaotic system is rerun with only slightly different initial values of the variables, after many time steps the values of the variables will be markedly different between the two runs. Since it is impossible to set the values of the initial

conditions to infinite accuracy, this means that for a chaotic system repeated experiments will produce different time series of the values of the variables. This sensitivity to initial conditions is the underlying reason that a deterministic process can generate an unpredictable timing of the switching between different conformational states.

However, a time series from the same system can be transformed into a form called a phase space. The phase space set of each different time series from the same system has the same shape and dimension. The invariant of these systems is the phase space set, called the attractor. These mathematical properties have important practical consequences for the analysis and interpretation of experimental data that is not yet fully appreciated by most scientists. Experimenters have labored to reproduce experimental values in multiple experiments and theoreticians have labored to produce models that match the time series of experimental data. These are inappropriate goals that can never be achieved for a chaotic, dynamical system. What needs to be done is to transform the time series into the phase space and determine if the phase space set is reproducible in the experimental data and matched by the theoretical model.

There is another important characteristic of the phase space set that can be used to determine if the mechanism that generated the time series is stochastic or deterministic [18]. The phase space set can be constructed from the time series of the experimental data. The dimension of the phase space set reveals the number of independent variables needed to generate the time series analyzed. The dimension of the phase space set of a stochastic process is infinite. That is, randomness means that so many processes are going on that we cannot write down the equations and variables for them all. The phase space set of a deterministic, chaotic process is low dimensional. Thus, in principle, the dimension of the phase space set constructed from the time series of the experimental data will reveal whether the process that generated the data is stochastic or deterministic. In practice, there are mathematical and computational difficulties in constructing the phase space and determining its dimension. These difficulties have to do with the large amount of data needed by these methods and how the parameters of the methods (such as the time lag used in the embedding) affect the results. Hence, these methods have not yet been to determine the relative contributions of stochastic and deterministic processes in channel function [18,22].

5.3. Dynamical patterns as long lived states. The traditional models assumed that long lived channel states correspond to stable states associated with local energy minima. However, another deterministic chaotic model, based on continuous differential equations, suggested that a different type of long lived channel state could also exist [19]. These were dynamical resonances. That is, the continuous dynamics of the channel protein produced states of the channel protein with almost constant current levels. These could be low or high levels of current. There were also intermediate levels of the current similar to the subconductance states found in the channel data. Nonlinear dynamical systems can behave in one manner for a long time and then switch between different types of long lived behaviors. None of the behaviors are stable in the traditional sense that they correspond to stable local energy minima. Rather, the dynamics of each behavior reinforces itself for a while, but also eventually drives the system into a different behavior. For example, in the Lorenz model of the motion of air in the atmosphere, convective rolls of air rotate and switch between rotating in the clockwise and counterclockwise directions. It is not the case that there are stable clockwise and counterclockwise states and the air switches between them. Rather, each state is inherently unstable. The dynamics of rotation in one direction is self-reinforcing for a short term and unstable in the long term. (Technically, the Lorenz system consists of saddle points in the phase space. The trajectory, the time trace of the values of the variables, is drawn towards these points, which are attracting in 2 directions, but once close to the critical point the trajectories are repelled along the third direction.)

This dynamical model suggested an alternative to the traditional concept that channel proteins switch between long lived stable conformation states corresponding to energy minima. It raised the possibility that long lived states may represent long lived dynamical patterns and that the dynamics of being in a pattern itself, rather than an external perturbation, switches the channel protein to another pattern.

6. Molecular memory. Dynamical, continuous changes of conformational state would also imply that there is long term "memory" in channel behavior. That is, in such a dynamical system the past history of the conformational states of the channel would play a direct role in determining its future conformational states. The Markov processes used to represent the kinetic diagrams and the energy level models assumed that no such long term memory was present. In those traditional models the functionality of a channel, for example its response to an applied voltage or its receptivity to bind a ligand, is entirely determined by its present state. On the other hand, new experimental results now imply that ion channels do have such long term memory. This means that the functionality of a channel depends not only on its present state, but the history of its previous states as well. That is, such states are time dependent and cannot be fully characterized as a unique, static state.

New theoretical models show that this long term memory may form a link between molecular ion channel kinetics at the microscopic scale and the transmission and processing of information in the spike trains in neurons at the macroscopic scale.

6.1. Non-Markovian behavior. In the traditional kinetic diagrams and energy level models, Markov processes are used to model the switching between conformational states. In a Markov process, the probability per second to switch states depends only on the present state of the channel. The channel data must satisfy two requirements for this assumption to be valid: 1) the conditional probability for the channel to be in state i at time t_i must depend only on the state j of the channel at time t_j and not depend on the state k of the channel at time t_k earlier than t_j, and 2) the probability of the channel to switch from state i to state j, must depend exclusively on the sum of all the probabilities of going from state i to state m, and then from state m to state j, which is called the Smoluchowski-Chapman-Kolorogorov relationship. Analysis of the data from some channels was consistent with the first requirement [25]. It was also found that for some channels the time constants of the exponential terms used to fit the PDFs were the same as those of the conditional probabilities, which is a partial test of the second requirement [9,25]. However, recently, Fulinksi et al. [8] found that both these requirements were not satisfied by the data from some channels. They showed that: 1) the conditional probabilities, over brief times (10 ms) varied with the time spent in a state and 2) the deviations from the Smoluchowski-Chapman-Kolorogorov relationship were more than one order of magnitude higher than those for a test model based on a Markov process. This means that dynamical, time dependent processes, which cannot be described by Markov processes, are present in channel function. The fact that the channel data do not have the properties assumed by the traditional kinetic diagrams and energy level models means that those models may not be able to help us fully understand channel function. We will need new approaches that better describe the dynamical properties of channel proteins in order to more fully understand channel function.

6.2. Molecular memory in inactivation. The conductance of some channels change when they are activated by a voltage pulse. However, some time must pass before the channel will respond similarly to another activating voltage pulse. During this time the channel is said to be "inactivated." Typically, the degree of inactivation is proportional to $\exp(-t/T_i)$, where t is the time since the end of the activating pulse and T_i is the inactivation time constant. In the 1950's Hodgkin and Huxley developed a model of channel function to summarize the voltage sensitive and time dependent conductances of channel experiments [12]. First a "conditioning" pulse of voltage was used to activate the channel. Then, after a delay, a test pulse was used to determine the degree of

inactivation. It's surprising (and a bit sobering) that in 40 years of extensive subsequent repetitions and variations of this protocol there were no systematic experimental studies of how the channel properties depend on broad variations in the duration of the conditioning pulse.

Recently, Toib et al. [32] used the patch clamp technique to measure the response of channels to a conditioning pulse over a large range of durations, from 1 - 100s. They found that the inactivation time constant T_i depended on the conditioning time T_c, namely, T_i was proportional to T_c^d. Such a power law relationship is typical of a fractal scaling [18]. These experiments clearly demonstrated that there is a continuous change in the state of the channel during the conditioning pulse that is later reflected in the time that it takes the channel to escape from its inactivated state. They showed that this dynamical memory was present when the patch was removed from the cell. This indicates that the memory is present in the channel protein, rather than a regulatory biochemical network in the cell. That is, the channel protein itself has a "molecular memory" of past voltage events over time scales of minutes. The scaling exponent d was different for sodium and potassium channels. They also found that changes in the scaling exponent in mutant muscle sodium channels could explain the paradoxical symptoms of hyperkalemic periodic paralysis [24].

Previous studies of the PDFs of closed and open time durations indirectly suggested that there are continuous changes of state in channel proteins [17,20]. These inactivation studies were the first direct experimental demonstration of continuous dynamical changes in state in channel proteins.

Ion channel proteins generate action potentials in nerve cells in response to voltage and chemical stimuli. The demonstration of the molecular memory in these studies implies that the history of previous action potentials determines the state of ion channels. The state of the channels will then determine if an action potential is generated in response to a particular stimulus. In this way, the information content in the spike train of action potentials is modulated by the molecular kinetic properties of channel proteins. Thus, there is a direct link between the closing and opening channel kinetics and the information content in the timing of action potentials.

6.3 Effects of molecular memory on the Hodgkin-Huxley model. The traditional kinetic diagrams and energy level models were based on the assumption that the probability for a channel protein to switch states depends only on the present state, but not on how long the channel protein has already remained in that state. However, previous studies had shown that the PDFs of the closed and open durations implied that the longer a channel protein remains in a state, the less the probability per second that it will exit that state [20,21]. These studies found that the typical relationship had a power law fractal scaling form where the probability per second to exit a state k(t) was proportional to t^{1-d}, where t is the time that the channel has been in a state and $d > 1$ is the fractal dimension.

Long term, power law correlations, with fractal scalings, had been found in the timing of the action potentials in the auditory and visual systems [30,31]. Lowen et al. [23] explored whether the fractal scaling of the ion channel kinetics could be one of the mechanisms responsible for these fractal correlations in the action potentials in the spike trains. They modified the Hodgkin-Huxley equations by adding a molecular memory with a power law fractal form. When there was a constant current leak across the cell membrane, the traditional Hodgkin-Huxley equations generated action potentials without long term fractal correlations. However, when the molecular memory was present, a substantial number of ion channels remained in the same state for long times and thus played a direct role over long time scales. Thus, the modified Hodgkin-Huxley equations, with a constant current leak, generated action potentials with long term fractal correlations. This demonstrated that molecular memory of a fractal form is sufficient to generate fractal correlations in the timing of the action potentials.

If the molecular properties of channel proteins determine the timing of action potentials, it raises some interesting speculations. First, memories could be stored in molecules. That is, different memories could be encoded by using ion channel proteins with different molecular properties. Second, by substituting different channels into the membrane, the nerve cell could change the algorithm that it is using to process information.

The experimental studies of channel inactivation described in the previous section and the theoretical studies described in this section both illustrate how structure and function at one level of organization is manifest in the structure and function at a higher and larger level of organization. A proper appreciation of the dynamical properties, namely the molecular memory, makes it possible to understand how the molecular kinetics of ion channels is linked to the timing of action potentials which processes and transmits information along nerves.

7. Computation of the relative importance of deterministic forces vs. stochastic thermal fluctuations. It has now been demonstrated that deterministic chaotic process can generate properties of the channel data that had previously been thought to be generated by stochastic processes. This raises the question as to the relative importance of the deterministic atomic, electrostatic, and hydrophobic forces versus random thermal fluctuations in channel function. In principle, nonlinear analysis, such as phase space plots, can determine the relative contributions of deterministic and stochastic processes in the experimental data. However, this has not yet succeeded due to technical limitations in these methods. In this section we present some elementary calculations to identify the regimes where dynamical deterministic forces will play an important role in the motions of the channel protein and its interaction with the ions passing through it.

7.1. Time scale of deterministic motions. A protein interacts with the ambient environment and by the exchange of heat these thermal fluctuations add and remove energy in a stochastic way from the protein. The energy of these fluctuations is of the order of $k_B T$, where $k_B = 1.4 \times 10^{-23}$ J/^{o}K is the Boltzmann constant and T the absolute temperature. Deterministic forces will dominate these stochastic thermal effects when the energy provided from the deterministic forces exceeds that of the thermal energy. We consider here the 2-D vibration of one amino acid residue and the rotation of one helix in the channel protein. The motion of these pieces will be controlled by deterministic forces when their kinetic energy exceeds their thermal energy. There will be a critical frequency where the energy from the deterministic forces and stochastic thermal fluctuations are equal. This critical frequency will correspond to a critical time scale. The thermal fluctuations have only enough energy to move these pieces at the critical time scale or slower than the critical time scale. Motions faster than the critical time scale must therefore be dominated by deterministic forces.

First, we consider the motion of an individual amino acid residue oscillating in a 2-D plane off the axis of its amino bonds to the channel protein. We treat its motion as that of a simple harmonic oscillator and therefore can equate its kinetic energy, $(1/2)\, m\, \omega^2\, A^2$, with the thermal energy, $2\, k_B\, T$, for such a system:

$$(7.1)\quad (1/2)\, m\, \omega^2\, A^2 = 2\, k_B\, T$$

where m is the mass of the amino acid residue, ω is the vibrational frequency, and A the amplitude of the oscillations. Solving for ω we find that

$$(7.2)\quad \omega = [\, (4\, k_B\, T) / (m\, A^2)\,]^{1/2}.$$

For m = 150 daltons, T = 300 ^{o}K, and A = 1 Å, we estimate the critical time scale as

(7.3) $(1/\omega)$ approximately 4×10^{-13} s.

At times faster than this critical time, the motions of an amino acid residue will be controlled by deterministic forces rather than thermal motions. This time scale is much less than the 10^{-4} s resolution of the patch clamp technique.

Second, we consider the rotation of one helix consisting of 20 amino acid residues of a channel protein. In this case, since we are considering twisting motions, we equate its rotational kinetic energy with the thermal energy for such a system:

(7.4) $(1/2)\ k_B\ T = (1/2)\ I\ \Omega^2$

where I is the rotational inertia and and Ω is the rotational frequency. For simplicity, we assume that $I = MR^2$, where the mass of the helix M = 20 m is uniformed distributed on the surface of a cylinder of R = 10 Å. Solving for Ω we find that

(7.5) $\Omega = [\ (k_B\ T) / I\ ]^{1/2}$

and the critical time scale is

(7.6) $(1/\Omega)$ approximately 3×10^{-11} s

At times faster than this critical time, the motions of an amino acid residue will be controlled by deterministic forces rather than thermal motions. This time scale is also much less than the 10^{-4} s resolution of the patch clamp technique.

Thus, it appears that the time scale of the deterministic motions in the channel protein is much faster than the resolution of the patch clamp technique. Hence, deterministic transitions between conformational states cannot be resolved by the patch clamp technique. However other physical measurements with better time resolution could be used to determine the time scales of motions in channel proteins. These methods include optical absorption, nuclear magnetic resonance techniques, and fast circular dichroism.

7.2. Electrostatic interaction between the channel protein and ions passing through it. The traditional models computed the passage of ions through a set of fixed energy barriers determined by the conformational state of the channel protein. It was assumed that the ions do not significantly alter the conformational state of the channel protein. Here we compare the energy from thermal fluctuations with the energy of the electrostatic interaction between ions and the channel protein.

First, we consider the electrostatic interaction of an ion moving through the channel with a charge in a the channel protein. The energy E_i iof the electrostatic interaction between two electrical point charges q_1 and q_2 is given by:

(7.7) $E_i = (\ q_1\ q_2\ ) / (\ 4\ pi\ \varepsilon_o\ d\ )$

where q_1 and q_2 are the charges, d is the distance between them, and ε_o is the permittivity constant. For $q_1 = q_2 = 1.6 \times 10^{-19}$ C, d = 10 Å, and $\varepsilon_o = 8.9 \times 10^{-12}$ C^2/nt-m^2, we find that

(7.8) ($E_i / k_B T$) approximately 55.

Thus, the electrostatic interaction between the ions and the channel protein provides much more energy into the channel than the energy from thermal fluctuations.

Second, we consider the electrostatic interaction of an ion moving through the channel with a dipole in the structure of the channel protein. The energy of the electrostatic interaction E_d at a distance d from a dipole of electrical charges q_1 and q_2 is given by:

(7.9) $E_d = (q_1 q_2 a \cos \theta) / (4 \_ \varepsilon_o d^2)$

where a is the distance between the two charges of the dipole and θ is the angle between the direction of the dipole and the direction of the ion. This formula is accurate when $d / (a \sin \theta) > 5$. The result depends on the angle θ, for a = 1 Å, then

(7.10) $| (E_d / k_B T) | <= 6.$

The electrostatic interaction, as expected, is weaker between the ion and the dipole in the channel compared to the ion and the charge in the channel. Nonetheless, even this dipole interaction can provide considerable more energy into the channel than the energy from thermal fluctuations.

These order of magnitude estimates have assumed that the dielectric constants is unity. More realisitic estimates of the dielectric constant may reduce these E/k_BT rations by an order of magnitude. Nonetheless, for both cases we still find that the electrostatic interaction between the ions and the channel protein can provide significant energy into the channel compared to the energy from thermal fluctuations. This suggests that the interaction between the channel protein and the ions passing through it could be important in changing the conformational state of the protein, as was suggested by the model of Chinarov et al. [6] described in section 4.3.1.

7.3. Energy dissipation. The traditional energy level models assumed that the channel protein is in local thermodynamic equilibrium (LTE). This assumption means that there is a direct balance of the energy in the thermal fluctuations from the environment to the channel protein and from the channel protein to the environment. A system can be in a steady state but not in LTE. For example, the earth receives energy from the sun at T = 6,000 ^oK and then radiates it back into space at T = 300 ^oK. The energy the earth receives is equal to the energy that it radiates, so that it is in a steady state, but it is far from LTE. Typically, the energy flow through such steady state systems that are far from LTE generates patterns of structure in space and time. In the case of the earth, the free energy passing through the earth has driven dissipative chemical and information processes that are origin of all living things on the earth.

Channel proteins are located in the large voltage and chemical gradients across the cell membrane. These gradients represent an energy source that is much larger than the thermal fluctuations. If channel proteins dissipate this energy, then they would be in a steady state but not in LTE. The flow of energy through the channel could then generate dynamical patterns that could play an important role in channel function.

It is not clear how to determine if channel proteins are in LTE. For channels that have a cyclic kinetic diagram LTE corresponds to the chemical principle of detailed balance. In that case, the product of the kinetic rate constants clockwise around the cycle should equal the product of the kinetic rate constants counter-clockwise

around the cycle. If these products are not equal, it means that the channel is absorbing energy and is not in LTE. One study found that these products were equal and one study found that they were not equal [25,26,29].

It is not clear what are the possible mechanisms of energy dissipation in channel proteins. Channel proteins have electrical charges. The molecular motions in the channel protein mean that these charges are always changing direction and velocity, that is, they are accelerating. Since accelerated charges radiate energy, this is one possible mechanism by which proteins can dissipate energy. Here we estimate the amount of that dissipation.

We consider the amount of energy radiated during one cycle of the motion of an amino acid residue in the channel protein. The energy radiated E_r in one cycle by charge q rotating at angular frequency ω with amplitude A is given by

(7.11) $E_r = (q^2 \omega^3 A^2) / (6 \varepsilon_o c^3)$

where the speed of light $c = 3 \times 10^8$ m/s. For one electrical charge $q = 1.6 \times 10^{-19}$ C, the angular frequency of thermal motion $\omega = 3 \times 10^{12}$ s^{-1}, and amplitude A = 1 Å, we find that

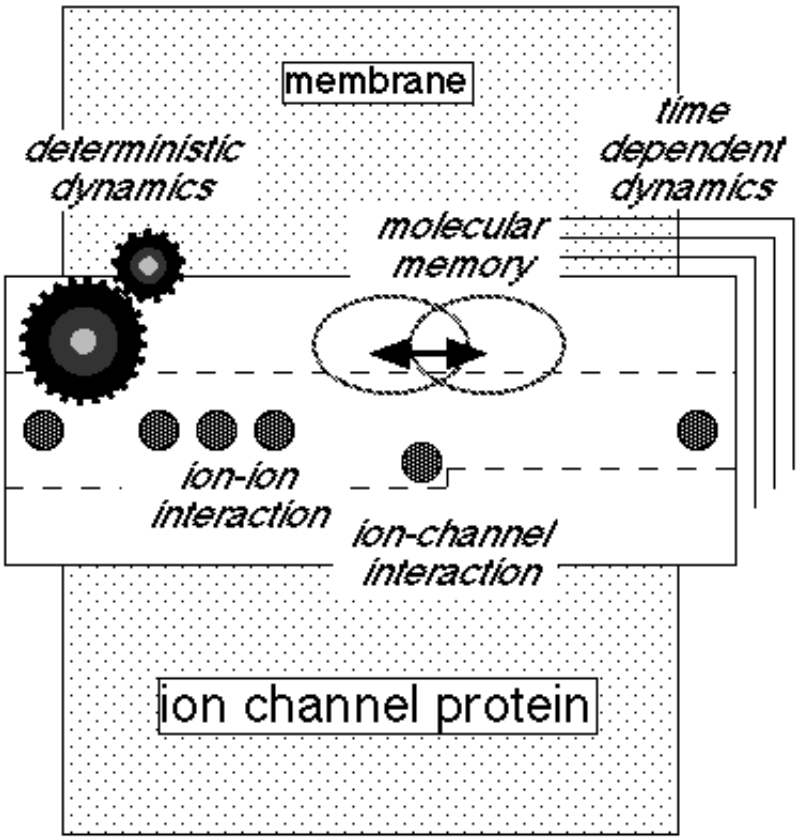
(7.12) $(E_r / k_B T)$ approximately 10^{-15}.

Thus, the amount of energy radiated is very small compared to the thermal fluctuations. The amino acid residue will complete approximately 10^5 cycles during the time it takes for an ion to pass through the channel. Even the energy radiated during that time will be still be much less than that available from the thermal fluctuations. Hence, the amount of energy dissipated by the channel protein through radiation is very small. However, note that there can be very important changes in the behavior of some physical systems when the energy dissipated changes from none to an infinitesimally small amount. In such systems, the infinitesimal dissipation breaks an inherent symmetry leading to the formation of important new patterns.

8. Conclusions. The traditional models such as kinetic diagrams and energy level models shown in Figures 2 , 3, and 4 treated ion channel proteins as if they were static structures with non-interacting pieces. We have shown here that the dynamical time dependent properties and the interactions within the channel protein and between the channel protein and the ions passing through it may play an essential role in the switching of the channel protein between states that are closed and open to the flow of ions and thus in channel function. These dynamical properties are illustrated symbolically in Figure 6.

Fig. 6 The traditional models such as kinetic diagrams and energy level models shown in Figures 2, 3, and 4 treated ion channel proteins as if they were static structures with non-interacting pieces. The point of this paper is that dynamical properties play an important role in channel protein function. These properties include continuous internal motions and continuous changes of state, the interactions of the ions with themselves and with the channel protein, deterministic forces, and time dependent molecular memory.

Dynamics



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9. Summary. The take home lesson of this paper is that dynamical properties may play an important role in channel protein function. These properties include continuous internal motions and continuous changes of state, the interactions of the ions with themselves and with the channel protein, deterministic forces, and time dependent molecular memory. These dynamical properties may be important in how the channel protein functions and how the molecular properties of channels at the microscopic level manifest themselves at the macroscopic level. These dynamical properties cannot be properly characterized by the traditional kinetic diagrams and energy level models that have been used to analyzed experimental data and model ion channel proteins. We may need to develop new approaches that take these dynamical properties into account in order to achieve a true understanding of channel function.

It is ironic that these traditional molecular models, and indeed many other present approaches in molecular biology, ignored the dynamics of time and the interactions between many elements with each other. It is as if to simplify our experiments and our thinking about them we want to dissect the living things that we study into tiny pieces and lay the cold, dead, non-interacting, non-changing pieces on our table so that we can then scrutinize them slowly and individually. We must remember that biology is not the study of dead things. Biology is the study of living things. Living things move and their pieces interact strongly with each other and with the world around them.

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