Ion channels allow atomic control of macroscopic transport

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1 Introduction

Ion channels are proteins with a hole down their middle that act as nanovalves, nearly picovalves.[1–3] Ion channels — and their siblings membrane transporters — control the flow of ions and water through otherwise insulating biological membranes that surround cells, and organelles (e.g., mitochondria) inside cells. The membranes act much as SiO$_2$ does in transistors. The membrane isolates compartments allowing the flow through tiny channels (in proteins or in FETs) to control macroscopic currents and potentials.

2 Molecular biology of channels, sequence and structure

Ion channels are proteins that can be studied with the techniques of molecular biology. Thus, the genes which are the blueprints for the amino acid sequence of the protein can be isolated, grown, manipulated, mutated, and expressed much as can other genes. The astounding techniques of molecular biology can change individual amino acids with relative ease, thus allowing control of permanent (i.e., fixed) charge and local dielectric properties: acidic amino acids are permanent negative charges, basic amino acids are permanent positive charges, polar amino acids have large dielectric coefficients, nonpolar (or aromatic) amino acids have low dielectric coefficients. In this way, the channel biologist (‘channologist’) can control the physical properties of channel proteins better than the physicist can control the properties of noncrystalline physical materials.

The structure of channel proteins depends on the amino acid sequence coded by DNA but cannot be determined from the sequence. The protein folding problem remains unsolved. X-ray crystallography is usually used to determine structures of channel proteins if they can be crystallized (usually in the presence of detergents, lipids, and ions). Crystals of proteins contain large amounts of water and have structures surprisingly close to the structures of uncrystallized proteins (usually).

Only a few channel or transporter proteins have been crystallized, particularly compared to the thousands of nonmembrane proteins that have been crystallized, but a very large number of amino acid sequences of membrane proteins are known.

Mutation experiments have been surprisingly successful in finding special regions of channel proteins that control biological function. In many cases, only a handful of amino acids control a specific biological function and mu-
tations of any of the other tens or hundreds of amino acids has little effect or no specific effect on that biological function.

**3 Macroscopic function of ion channels** Ionic channels function by controlling the flow of ions, water, and electric current through the otherwise impermeable membrane of cells and organelles. Electricity in biology is almost always carried by ions: electrons are never involved in charge movement over more than 1 meter or so in biological systems. Channels were first recognized because they form the 'ionic conductances' that determine the electrical properties of nerve cells [4]. These voltage dependent channels (that carry Na⁺ or K⁺ ions but not both and so carry their names, as Na channels or K channels) open and close to control current flow across the nerve membrane. The voltage dependence of opening and closing — called 'gating' despite our lack of knowledge of what the gates are, or even if they exist in a specific structural sense — produces the propagating all or none (i.e., binary) signal called the action potential, that carries information from one end of a nerve fiber to another, e.g., from the toe of an elephant to its spinal cord.

Recordings can be made routinely (but not easily) of the current through just one channel protein molecule, using the patch clamp method of Neher and Sakmann [5] or the bilayer recording methods [6] developed by an army, or anyway a platoon of workers, led by Chris Miller. The protein can be recognized by its properties, its dependence on drugs, or in many cases it can be synthesized as a pure protein in bacterial or eukaryotic cell culture systems.

Channel proteins work independently of each other in the classical (and typical) case. If the voltage across the channels is controlled (by an amplifier or by the biological cell itself), the currents through separate channel molecules simply add. In the classical and typical case the properties of one channel do not influence the other. Of course, in many cases the function of one channel changes the voltage, and thus influences the properties of another channel indirectly, as would anything else that changed the voltage. In other cases, a channel may allow enough ions to flow that the local concentration of a 'message' species changes significantly (typically the concentration of Ca²⁺ is used inside cells as a messenger, because the background concentration of Ca²⁺ is so low some 10⁻⁶× less than K⁺ or Na⁺). Finally, some channels have nearby accessory proteins that bind ions and control channel function. Nonetheless, it is important to think first of channel molecules as independent agents, coupled by the electric field (in most cases) just as transistors in a circuit are coupled only by the electric field [7, 8].

**4 Atomic scale function of ion channels.** Individual channel molecules each have the properties of a macroscopic ensemble of ion channels, because they are independent but the current through a single ion channel does not look like the current through the macroscopic ensemble. The current through an individual ion channel is a stochastic on off signal that switches between open (a definite value of current) and closed (nearly zero current). The single channel current is a random telegraph signal. More precisely it is a random variable, a set of ‘realizations’ (individual recordings). The expected value (i.e., mean) of the set is the same as the properties of an ensemble of channels, that is to say the mean equals $1/N$ times the ensemble, where $N$ is the number of independent ion channels.

The source of variance in the single channel currents is not known, but the properties of the single channel current are remarkably simple. The average open value of the current is independent of time. More precisely, one recording of the current through a single ion channel follows a simple rectangular time course, switching from closed (i.e., zero, on the average) to a definite single value (on the average). The switching occurs stochastically, at random times, so the duration of the opening is different in each recording. The set of these recordings is a set of trajectories of a bistable stochastic process and is a random variable.

The mean current through a single channel is $\bar{I}(t) = \frac{1}{N} \sum_{k=1}^{N} I_k(t)$ where $k$ is the index of each member of the set of trajectories, i.e., of the individual time records $I_k(t)$ where $t$ measures the time after the channel opens. As long as the channel is open, the mean current through a single channel molecule $\bar{I}_{open}$ does not vary with time, on the biological time scale $>10 \mu sec$. The number of open channels $N(t)$ varies with time but the mean current $\bar{I}_{open}$ through a single open channel does not vary with time (on the biological time scale). The physical reason for this remarkable property is not known. The total current through a membrane of a cell is determined by (1) the open channel current, (2) the time the channel is open, and (3) the number of channels (that can open and close) in the membrane.

Biology evidently uses different mechanisms to control the magnitude of the bistable current and the time course of the current. The time course can be characterized by two random variables, the duration of the single channel opening and the delay (after some experimental intervention that opens the channel, e.g., applying a drug, or changing the voltage). The system that controls opening and closing is called gating, and it corresponds remarkably well to the time dependent properties of the ion conductances studied by Hodgkin and Huxley. The ionic conductances vary in time because the number of open channel changes, but the size of each open channel does not.

The size of each open channel depends on the parameters that govern current flow through a nanopore (actually a picopore) with a single structure, namely the thermodynamic ‘driving force’ (the difference of electrochemical potential across the nanopore), the number density (‘concentration’) of current carriers, and the properties of the
wall of the nanopore (e.g., its permanent charge and its polarizability, customarily described by its dielectric coefficient), along with the mobility of ions inside the nanopore. The current through the open channel is more a physical than a biological variable: it does not have a steep temperature dependence, it varies monotonically, more or less linearly with electrical potential (sometimes less than more particularly if solutions on different sides of the protein have different concentrations), it is not sensitive to biological interventions, and it has little dependence on drugs. The current through an open channel behaves the way one would expect if it represents the charge carried by (nearly) hard spheres through a structure with one geometry (on the biological time scale). The biological time scale starts around 0.01 msec, and is mostly 1 msec or slower. A single ion goes through a channel in nsec (i.e., the first passage time is often a few nsec). A channel carrying one picocamp of current, which is always occupied by one ion, neither more nor less, has one ion entering and leaving every 160 nsec. Most of the single channels we study have currents larger than 10 picoamps (because instrumentation noise prevents easy measurement of smaller channels) but there is every reason to believe that an enormous number of channels and transporters support currents very much smaller than 1 pA. We have no way of knowing what the time course of such smaller currents would look like if they could be recorded. Many important biological functions are produced by such tiny channel currents and actual measurement of them would have immediate effects on clinical medicine, e.g., on the development of calcium channel blockers which is a business involving billions of dollars/euros per year.

5 Analysis of channel currents. Currents through channels are usually analyzed on the scale that they are measured rather than the scale necessary to determine mechanism (or biological function, for that matter). Currents through ensembles of channels were studied for many decades using the voltage clamp method of Hodgkin, Huxley and Cole, classically applied to the giant nerve fiber of the squid [4]. The patch clamp method allowed similar measurements to be made from typical ‘round’ biological cells with the “whole cell clamp” method, although amusingly enough the measurements almost always have less time resolution than the British measurements of nearly 60 years ago! These currents are usually produced by a mixture of different types of channels (i.e., by different proteins of quite different structure and function, carrying different types of ions) and so mechanicistic study of them is foolish: there is an old saying among biochemists something like “never do clean experiments on dirty enzymes”. The cacophony of currents in typical whole cell clamp currents are hard to resolve into a coherent symphony of sounds, although specific toxins that block only one type of channel help a great deal.

Mechanistic experiments are usually done on recordings of single channels because the identity and reproducibility of the channel currents is not in question. In fact, they are reproducible to the standards of physical science, i.e., better than 1% (in the mean) from experiment to experiment and laboratory to laboratory. There is essentially no biological variance in high quality single channel recordings and most of the hundreds of scientists doing single channel recordings can do such measurements.

Single channel recording is not without its price, however. Single channels usually give results similar to those of the ensemble they are meant to represent, but not always. Channel proteins are clearly displaced and move in the lipid as a patch clamp is established, and changes in gating properties (but not the amplitude of single channel currents) are observed, presumably because the protein is damaged or its relation with its accessory proteins is disturbed. Isolated and purified channel proteins reconstituted into bilayers often ‘run down’, i.e., have slow (~ 10 minute) changes in their properties as accessory chemicals (‘cofactors’) change, because the experimenter does not know how to supply them. Properties that depend on the precise geometrical relation of channel proteins to other constituents of the cell, e.g., the cytoskeleton or neighboring proteins, or that depend on the soup of chemicals inside cells, are modified in the patch and bilayer methods. The role of these other agents must be sorted out, in any case, to understand channel function, so the vivid dependence of single channel recordings on the chemical and mechanical environment of the channel is both a blessing and a curse.

Single channels also show stochastic behaviour as we have mentioned and this too is a blessing and curse. The stochastic behaviour probably does not directly affect biological function because it is not the dominant source of variance in most currents recorded from ensembles of channels. The properties of tiny nerve endings (which fill a good fraction of our brains) and of even tinier subcellular organelles may be an exception. It is possible they fluctuate because of the variance of single channel currents. These tiny nerve endings may contain only a (single) handful of channels.

The stochastic behaviour of single channels is a blessing to those who wish to study gating because it reveals the atomic basis of gating function. The fluctuations occur in structures of atomic size, because the nanopore in ion channel proteins is from 0.4 to 1 nm in diameter, compared to typical ionic diameters of 0.2 nm. Structures of this size have enormous Brownian motion because collisions and friction dominate phenomena on this length scale. As a rule of thumb, atoms near thermal equilibrium move at the velocity of sound 1.5 km/s, which is 1.5 nm/picosecond, i.e., each atom moves 7 diameters in one picosecond. The size and nature of atomic motions is hard to comprehend, not only do atoms move at enormous speeds on the picosecond time scale, let alone on the msec time scale of biology, they also move nowhere! Atoms fill a condensed phase in liquids, proteins or ion channels and so they collide as soon (~<10^-16 sec) as they move, producing the ‘infinitely’ dense back and forth motion of Brownian particles.
that is described in a mathematical idealization as a function of unbounded variation, without a well defined velocity at any point in time!

The stochasticity of channel recordings is a curse in another context because it demands explanation before one can study the mean properties of gating, whether to understand the mechanism of gating or the function of gating. One must deal with the stochasticity even though the biology depends almost entirely on the mean value of the single channel recording. Unless one knows the physical basis of gating, it is difficult to estimate the properties of the underlying physical system or even to know how to average the stochastic records to best show the mechanism — many types of averaging are possible. It is difficult to know how ensemble properties depend on the underlying physical system without a model that defines the physical source of randomness in gating.

Almost all workers (except the author) believe that gating is produced by the motion of some part of a protein over a large potential barrier of more or less fixed size. The author does not know if this is true or not, but is fearful that the automatic acceptance of this view precludes (psychologically and socially) specific investigation of what makes the barrier and how the gate crosses it, and how the gate is coupled to sensors etc. It is also possible that quite different mechanisms, hydrophobic gates [9–11] or even bubbles (regions of gas within a channel [12]) might produce the sudden turn on and turn off of single channel currents.

6 Open channel currents. Fortunately, for those of us who seek a physical analysis of biological systems, there is one feature of single channels that is more or less immune to this uncertainty concerning the origin of stochasticity, namely the current through the open channel.

Because the single channel current is independent of time, once the channel is open, and highly reproducible (<1%), it can be studied as a physical variable. The question can be raised, what governs the flow of current as a function of voltage? What governs the flow of current as a function of concentration? How do ions of different charge and diameter go through the nanopore of the channel? How does the channel wall control the flow of ions through its nanopore?

Currents through open channels were first analyzed in the tradition of rate theory, using expressions like $I = (kT/h)\exp(-eU/kT)$ where $U$ is the height of the potential barrier, $k$ is the Boltzmann constant, $T$ is the absolute temperature, $e$ is the charge on a proton, and $h$ is Planck’s constant, but unfortunately a mistake was made, and the prefactor used was copied from theories of the gas phase [3]. Gases are (in the ideal) infinitely dilute systems of particles moving ballistic paths in straight lines without collision and without friction. Ions in solutions or channels are condensed systems with collisions and friction. Condensed phases have are phases without empty space, in which particles collide ‘infinitely’ often (in the Brownian idealization) and are dominated by friction. Their trajectories are the original fractals and mass does not appear (to first order) in their equations of motion. If the rate theory of condensed phases is used, the prefactor involves friction, and currents are very much smaller (~20,000x smaller [13]) than computed with the gas phase prefactor, and currents are then very much smaller (~20,000x) than the currents measured in typical open channel experiments [14–16].

The rate theory tradition has another more subtle fault shared by other simplified approaches, e.g., the traditional Einstein-Smoluchowski-Langevin approach to Brownian motion [17]. These simplified theories characterize the electric field by a potential $U$ without specifying what creates or maintains this potential. Electric fields and potentials are created by charge, permanent charge in the simplest case, and it is this permanent charge that is invariant in systems like ion channels and proteins (and most chemical systems in condensed phases). Only when covalent bonds change, or when charge is supplied by electrodes is the charge in a typical system changed. Both charge and potential cannot remain the same as experimental conditions change, ions move and so on. Thus, the potential that appears in rate models, and in Langevin equations, cannot be a constant. It must be computed from the configuration of charge, using Maxwell’s equations or their reduced form Poisson’s equation with boundary conditions. The assumption of a single potential $U$ cannot even serve as a useful approximation in most cases because the electric field is very very strong, and tiny changes in charge produce large changes in potentials and the resulting forces usually are very important and usually dominate small systems like ions and channels.

Thus, it is necessary to replace rate theory with something that includes both friction and charge, that computes current carried by the electrodiffusion of ions in an electric field created by the permanent charge of the ions, the channel protein, and the electrodes (or other cellular processes) that supply charge to the system [15, 16, 18, 19].

The drift diffusion equations of physics, which we call the Poisson Nernst Planck PNP equations, to emphasize the crucial role of the Poisson equation and the analogy with transistor technology, serve as a first step, only a first step, but at least a first step that includes friction and is self consistent. Self-consistency is an essential requirement in any theory including electric fields, in my view: the (electric) potentials calculated must in fact be consistent with (all the) charges and their distribution. Poisson’s equation must be satisfied. In my view, all theories and calculations including charge should be checked to be sure they satisfy Gauss’ law, the electric flux out of any closed surface must equal the charge included (with a scale factor). It is not clear that calculations of molecular dynamics, particularly those involving periodic boundary conditions, actually satisfy Gauss’ law, over arbitrarily chosen and oriented closed surfaces, even when Ewald sums etc. are used to calculate the electric potential. The various summation conventions.
do not seem likely, at least to me, to produce spatial distributions of charge and potential that identically satisfy Poisson’s equation. In my view the rearrangements of conditionally convergent series used to compute the electric field must be shown to satisfy Gauss’ law over arbitrarily chosen and oriented closed surfaces.

The PNP equations are good descriptions of the motion of point particles, such as holes and electrons, the quasi particles of semiconductor physics. But ions are spheres and occupy excluded space. Ions do not overlap and the resulting forces can be tremendous in the confined space of nanopores, ion channels, or the active sites of proteins. Indeed, correlations introduced by the finite size of ions are responsible for the nonideal behaviour of ionic solutions even as dilute as 100 mM. The concentrations of ions in channels and near active sites of proteins is enormous, often > 10 Molar (remember concentration is short hand for number density, and the ions are known experimentally to be mobile and carry current). At these concentrations, van der Waals forces (a more general and formal name for excluded volume forces) are as strong as electric fields, even those produced by say 4 Na\(^{+}\) in say the volume of an active site of a channel, some 0.4 nm long by some 0.6 nm wide.

No one knows ‘the correct’ way to include correlations in the PNP equations. The equations themselves include correlations of the means, but these hardly suffice when particles are in tight spaces; effects of individual particles dominate both in excluded volume effects and in some terms of the electrostatic interactions, like dielectric boundary force, between a particle and the polarization charge it induces at dielectric interfaces, and even in shielding effects which are different for neighboring spheres than for points or continua.

We have included correlation effects produced by finite size following the approach of equilibrium physical chemistry, assuming that nonequilibrium effects found in the theory of conductance of bulk solutions are not important when the counterions are stationary (as they are for the most part in channels, where many of the counterions are permanent charges in the side chains or backbone of the protein itself) or that the effects can be approximated by changes in the parameters of the model. We simply take the excess chemical free energy (really the excess chemical potential) computed by any of several treatments of equilibrium physical potential) (the NP part of the PNP equations can be written in terms of the gradient of electrochemical potential) [18, 20–23].

This approach has allowed us to understand the selectivity of several types of channels, the L-type Ca\(^{2+}\) channel, of such great importance in the beating of the heart, the Na\(^{+}\) channel of nerve cells, that Hodgkin and Huxley investigated, and the ryanodine receptor RyR responsible for the movement of calcium that produces contraction in skeletal and cardiac muscle (The latter is the work of Gillespie and Meissner and his laboratory [24–26]. I have only been an enthusiastic provocateur.)

In the case of the Na\(^{+}\) and L-type Ca\(^{2+}\) channel, one can account for the binding curve (the main feature) of the Ca\(^{2+}\) channel in a model with only two adjustable parameters (the dielectric coefficient and the diameter of the nanopore), treating the protein simply as 4 glutamates that move within the channel to their position of minimal free energy. Mutating the crucial side chains of the Ca\(^{2+}\) channel to DEKA produces a Na\(^{+}\) channel, just as it does in experiments, without adjusting any parameters, and, surprisingly to the authors, the resulting Na channel is K\(^{+}\) selective, without changing any parameters, for reasons not foreseen by the authors at all. In this model, the K\(^{+}\) selectivity arises from a depletion zone, and not from a binding site. Binding sites are present, as outputs of our calculation. They are the result of the forces and locations of charges and excluded volume; they do not come from preformed structures in the channel protein. Thus, the binding sites are of variable size and location. They are strong binding sites (i.e., show large local concentrations of ions) but they are not specific. Specificity comes from a depletion zone which also is the result of the forces and locations of spheres and charges. Interestingly, depletion zones are used to control the function of transistors, because they are in series, and thus allow a tiny atomic scale region to control macroscopic flows. The energy landscape of these models is variable and depends on the ionic concentrations, etc. This suggests that representations of proteins as definite unchanging (free) energy landscapes independent of ionic conditions and local electrical potentials will be misleading.

The work on the RyR channels is quite convincing. Gillespie et al have shown that anomalous mole fraction effects once thought to occur only in single file channels occur in a PNP/DFT model of the RyR. (DFT is the density functional theory of fluids, not electron orbitals.) They fit current voltage relations in more than one hundred solutions, containing two or even three types of ions at various concentrations and over a range of some 300 mV of potential. They fit mutations which change the density of permanent charge from some 10 molar to zero! These fits are done with (two) handfuls of parameters, about 8 plus one for each ion type and these parameters have fixed values never changed in any of the calculations.

A striking feature of these models is the role of the channel protein, because that is quite different from the image impressed into the mind’s eye seen in models protein structures revealed by crystallography.

7 Role of the protein

The role of the proteins in these models of the open channel is quite specific, as it is in recent successful models of the K\(^{+}\) channel from laboratories of both Benoît Roux [27, 28] and Susan Rempe [29]. In all these models, the protein provides the environment in which selectivity occurs. In our models, we calculate selectivity properties over a wide range of conditions and mutations and we show that the protein can produce all these properties simply by maintaining a definite unchang-
ing volume, dielectric constant, and density of permanent charge, if that permanent charge is represented as spheres competing for space with mobile ions, inside the tiny confines of the nanopore of the ion channel. Evidently, more subtle and complex 'chemical' properties of the ion channel are not involved in this kind of selectivity. In all likelihood, the energies involved in crowding ions into this highly charged picovolume are much larger than these other energies.

This image of selectivity is very different from that of an ion fitting into a preformed binding site, of definite size, created by the protein, independent of the surrounding ions. That image is a natural interpretation of crystallographic structures but it has always seemed inappropriate to me (since I first saw such a crystal structure of myoglobin, literally at the knee of John Edsall in 1959 as he opened a volume of Nature to show me the first protein structure). The amount of thermal motion, and thus flexibility, the enormous electrostatic and excluded volume forces produced by ions make it impossible for the protein to have a single structure independent of conditions, in my (admittedly prejudiced) view. What is more important than my prejudice is that models with rigid structures have not allowed one to understand experimental data on selectivity over a wide range of conditions (binding curves, IV curves, mutation results) while reduced models in which the binding sites are outputs of the calculation do allow such understanding for at least three channel types, \( \text{L type Ca}^{2+} \) channel, RyR calcium channel, and the \( \text{Na}^{+} \) channel, and are being used successfully for the \( \text{K}^{+} \) channel as well.

8 Conclusion We conclude then that the open channel is a system of great biological importance, with complex biological and chemical properties that can be described accurately by a simple physical model, over a wide range of conditions, for at least three channels with quite different properties. The model includes only the charges and excluded volumes of the ions and some selected side chains of the protein. These arrange themselves in structures of minimal free energy that produce the profiles of electrical and chemical potential that in turn produce binding and IV relations. The profiles depend as much on the electrical and chemical potential that in turn produce binding sites are outputs of the calculation do allow such understanding for at least three channel types, \( \text{L type Ca}^{2+} \) channel, RyR calcium channel, and the \( \text{Na}^{+} \) channel, and are being used successfully for the \( \text{K}^{+} \) channel as well.

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References