

# Ionic Channels in Biological Membranes: Natural Nanotubes

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## Introduction: "Holes in the Wall"

Ionic channels are protein molecules that conduct ions (like  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Cl}^-$ ) through a narrow tunnel of fixed charge formed by the amino acid residues of the protein. Channels are ideally placed (in membranes in series with the cell's interior) to control biological function.<sup>1</sup> Membranes are otherwise quite impermeable to natural substances, and so channels are gatekeepers for cells.

Channels open and close stochastically, allowing rectangular pulses of current to flow, forming a nearly one-dimensional "reaction" path for solute movement (Figure 2). Ionic channels are natural nanotubes that link the solutions in and outside cells to the electric field in the cell's membrane.<sup>2,3</sup> Only electrodiffusion moves ions through channels, and so this biological system is "a hole in the wall" (Abe Nitzan's choice name for a channel in a membrane) that we should be able to understand physically.

Channels are responsible for signaling in the nervous system, coordination of muscle contraction, and transport in all tissues. Each of these functions has been so important for so long that evolution has probably produced a nearly optimal adaptation (within historical and physical constraints) and conserved it, using the same design principle again and again. Channels are obvious targets for drugs and disease:<sup>4</sup> many of the drugs used in clinical medicine act directly or indirectly through channels.

## The Biological Tradition

Channel currents are manipulated in many ways in the biological tradition, yielding an avalanche of experimental results. Solutions and electrical potentials are changed, and the structure of the channel protein itself is modified

using the dazzling techniques of molecular biology. Channelologists measure currents flowing through just one channel molecule. Molecular geneticists determine the sequence of amino acids of many channels. A successful but simple physical model of electrostatics of enzymes (the nonlinear Poisson–Boltzmann equation) has been developed.<sup>5</sup> Three-dimensional structures are known, however, for only a few channels, most notably the porins<sup>6</sup> (Figure 2). The lack of structures is a serious limitation to investigation.

Channel studies are mostly descriptive,<sup>7</sup> as is certainly necessary and appropriate, given the enormous diversity in channel type and behavior that takes several volumes even to summarize.<sup>8–10</sup> Each membrane of each cell in the body contains different types of channels, and each channel type is likely to differ in different types of cells. For example, biologists study the many "isoforms" of sodium channels present in each region of the brain, or present in each subtype of skeletal muscle, because each isoform has a distinct biological role, controlling current flow in its own way.

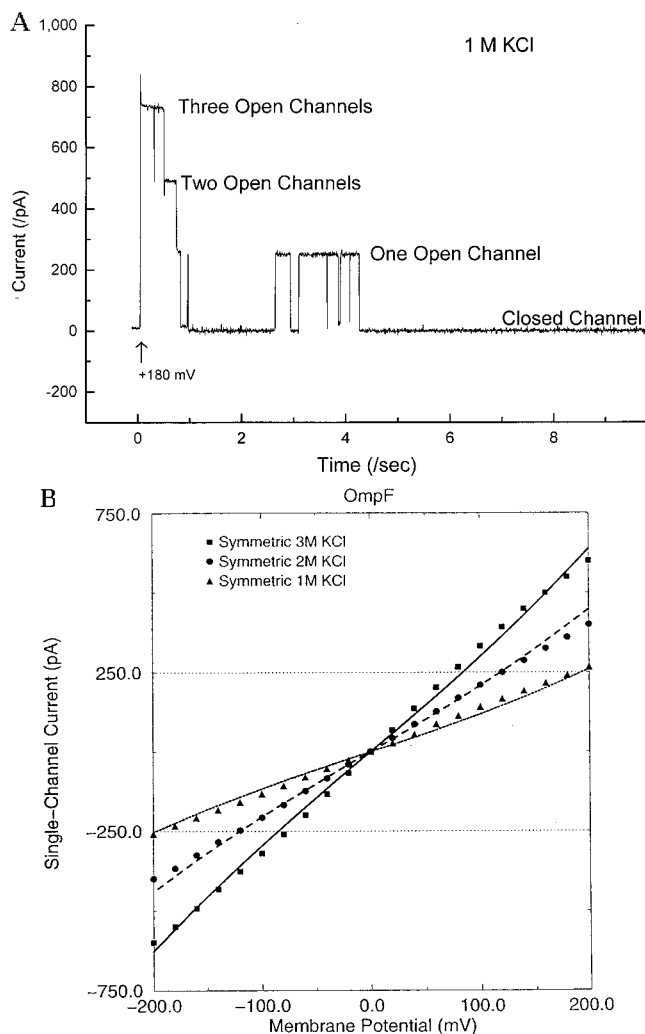
A single ionic channel controls current by opening and closing ("gating"), thereby making a random telegraph signal (Figure 1A). The properties of gating are complex, and the structure(s) and mechanism(s) that produce gating are not known (see ref 7, p 479). Physical analysis is difficult until the underlying mechanism is known, in my view.

I feel this way for historical as well as logical reasons. In other situations, it has often been difficult to use the power of physical science to answer important biological questions until the underlying mechanism has been identified. If the mechanism analyzed by the physical scientist differs from the adaptation chosen by evolution, the analysis is not useful. For example, currents through membranes were analyzed for more than a century as if they flowed through one permanently open channel of fixed cross sectional area. Then, when single channels were actually recorded, we found that the complex properties of these currents were produced (chiefly) by the variation in the *number* of ionic channels that were open, and thus in the cross sectional area available for current flow, not in the properties of the open ionic channel itself.

The study of gating will have a similar history, I suspect. If the fundamental mechanism of gating is the conformation change of a protein produced by a small fixed effective charge hopping over a large barrier, then the standard analysis<sup>7</sup> using barrier models may prove to be useful, at least if it is modified to use the correct expression for rate constant in condensed phases.<sup>11</sup> But if the mechanism of gating is not that (e.g., if channel opening were analogous to the random telegraph noise of field effect transistors<sup>12</sup>), much analysis will have been wasted.

Bob Eisenberg was taught biochemical sciences at Harvard College by John Edsall, one of the patrons and founders of molecular biology, while taking courses mostly in chemistry, physics, and applied mathematics, and graduated *summa cum laude* in 1962 after three years of work. He took his Ph.D. in Biophysics at the University of London (1965), spent postdoctoral time at Duke University, and moved through the faculty ranks at UCLA from 1968 to 1976 in the Department of Physiology. Since then, Bob has been Chairman of the Department of Physiology—then Molecular Biophysics and Physiology—at Rush Medical College, Chicago, and been awarded the Bard endowed professorship.

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**FIGURE 1.** Currents measured from *ompF* porin in 1 M KCl when the electrical potential is controlled by experimental apparatus. Panel A shows current as a function of time after the electrical potential is changed from zero to +280 mV at time zero; concentrations do not change significantly even though flux is substantial. The pattern of current flow shown here is typical of porin channels, but each type of channel has its own distinctive response to voltage. In this experiment a single “molecule” of porin has inserted into a bilayer. Because the molecule is a trimer containing three pores,<sup>6</sup> we see four levels of current, one of which is the closed channel current (with added instrumentation noise), zero in the mean. The spike in current at time zero—when the voltage of +280 mV was applied—is probably an artifact, as is the following slow change in mean current. I have chosen an imperfect experiment for this illustration to bring the reader closer to the uncertainties of everyday lab work. The other currents labeled “...Open Channels” have the more characteristic and classical flat top. Other sudden changes in current are thought to be incompletely resolved openings and closings of channels. Note the sudden spontaneous stochastic opening of one trimer. Such spontaneous openings are found in nearly all ionic channels and form the “operational definition” of a single ionic channel. Panel B shows the current measured from a single channel of porin as a function of the potential across the channel. The potential was slowly swept at a constant rate and the current measured *only when a single monomer was open*. Similar recordings from different experiments in different solutions were superimposed. Families of *IV* relations such as these in a wide range of solutions characterize all known biological functions of open channels.

Fortunately, the current that flows through the open channel can be studied independently of the gating process that opens it.<sup>2</sup> The mean value of the open channel current is reproducible from day to day and lab to lab, within the statistical limits produced by the energy of the noise and signal during the period of measurement, typically better than 1%. Such reproducibility is not characteristic of all biological systems.

Channologists have learned to measure the currents of single channels using reconstitution and patch clamp techniques<sup>2,13</sup> and integrating picoammeters,<sup>14</sup> with (open circuit) rms noise levels (dc to 10 kHz) of less than 0.3 pA, that allow single channel currents of <1 pA to be studied (depending on the duration of channel opening and band-width of recording).

## Crossing Barriers

The current through an open channel seems to be produced by diffusion and migration of ions over an energy landscape produced by (1) fixed charge lining the wall of the channel’s pore, (2) mobile charges in the pore itself, i.e., the permeating ions, and (3) charges in the bath and electrodes that maintain the boundary conditions of (nearly) constant concentration and transmembrane potential. The current depends strongly on the ion concentration and electrical potentials in the baths (Figure 1B) as one would expect for currents carried by ions through “a hole in the wall” of fixed structure.

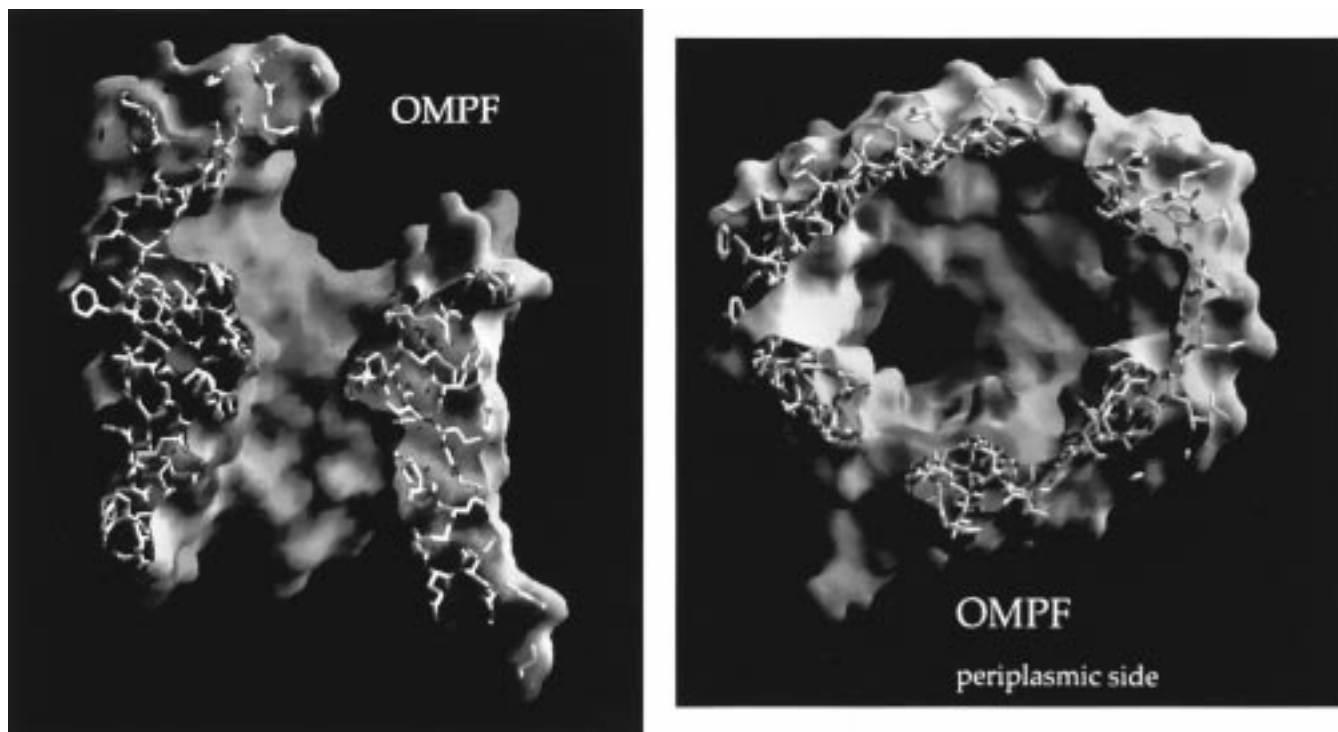
Ionic movement through channels can be viewed as a chemical reaction<sup>15</sup> involving translocation along a reaction path in real one-dimensional space. Ions actually move through channels much as reactants have been idealized to move in many theories of chemical kinetics.<sup>16</sup> Those theories usually assume movement along a one-dimensional reaction path embedded in a high-dimensional phase space. Recent work<sup>17,18</sup> suggests, however, that a one-dimensional metaphor has difficulty defining a reaction path through a multidimensional energy landscape of peaks, valleys, and cols—as anyone who tries to determine the “best” route from Denver to Salt Lake will understand.

Ionic channels are then, in some ways, better realizations of classical theories of chemical kinetics than traditional chemical reactions: the reaction path is actually one-dimensional in channels and the boundary conditions allow easy computation of the flux over any shape potential barrier.<sup>19</sup>

It seems then that the hole in the wall is quite amenable to traditional chemical analysis, and so my collaborators and I have tried to describe open channels by the simplest models of electrodiffusion, in the spirit of the traditional Gouy–Chapman theory and Poisson–Boltzmann theory of protein electrostatics.

## Channels Are Nonequilibrium Devices

But we immediately face a problem with the traditional electrostatic models because they are held so tightly to equilibrium. Channels only perform their natural function



**FIGURE 2.** Structure of wild-type (*ompF*) porin as determined by X-ray crystallography<sup>6</sup> visualized by Dutzler and Schirmer using the program GRASP.<sup>41</sup> Although the representation of the structure in the figure is necessarily approximate (for psychological, psychophysical, and economic reasons), the coordinates of nearly all the atoms are known with precision.

when matter and energy and charge are supplied to them (across their boundaries) from a “power supply” (a source outside the system). Channels only work when “energized” by gradients of concentration and electrical potential, and energized systems are by definition not at, or near, equilibrium. The gradients of concentration and potential that energize channels are maintained by complex apparatus (in the lab) or by networks of metabolic pathways<sup>1</sup> in life.

### Nonuniform Boundary Conditions, *Sine Qua Non*

The concentrations and electrical potential that energize channels are necessarily nonuniform in space; otherwise, steady flux could not occur. Thus, the mathematical description of channels must be spatially nonuniform—it must include unequal sources, just as the experiment must—if it is to describe something more interesting than a dead hole in the wall. More precisely, the boundary conditions of the far field(s), away from the molecular machine that is the channel, must be described mathematically by nonuniform boundary conditions, e.g., nonuniform, inhomogeneous Dirichlet conditions.

The importance of nonuniform nonequilibrium analysis—ensured by spatially nonuniform inhomogeneous Dirichlet boundary conditions, for example—cannot be overstated. Channels with uniform Dirichlet boundary conditions are dead, but channels can live and function when they are energized by (spatially) nonuniform inhomogeneous Dirichlet conditions.

Indeed, if a system like a channel or transistor is studied just at equilibrium, its function cannot be understood,

even qualitatively, *because there is no unique trace of that function visible at equilibrium.* If a solid-state device<sup>20</sup> (e.g., a transistor) is studied with its terminals all at one potential (with its leads soldered together), the bias potentials between its terminals (that allow it to function) are not present at all. Thus, the qualitative behavior of a transistor cannot be seen at equilibrium—at all. Although the physical structure of a solid-state device exists at equilibrium, the device itself does not, at least in the functional sense. The fundamental model of the device (for example, as an amplifier with gain) is not valid at equilibrium because bias potentials (and dc current) are needed to make the physical structure function as a device, e.g., as an amplifier or switch.

Interestingly, one single physical structure can become several quite different devices, depending on the bias potentials that are applied to it. With some bias potentials, a transistor functions as an amplifier, with others, as a limiter or switch, and with still others, some transistors can function as a resistor, or even a multiplier. The *qualitative* behavior of a transistor depends on the value (as well as the existence) of the boundary conditions, the bias potentials between its terminals. The same physical structure can be the substrate for quite different devices. The same equations (*PNP*) can predict all these behaviors, without arbitrary adjustment of any parameter. Changing just the boundary conditions is enough to change the transistor (or its mathematical representation) from being a resistor to being an amplifier.

At equilibrium, when biases are all zero, little or no trace of this rich behavior can be seen. The physical structure of the transistor becomes a device only when it

is energized, when it is made functional by external sources. The physical structure is just the substrate of the device.

It makes no sense to study or model transistors when they cannot be the devices they are designed to be. Transistors must always be studied away from equilibrium. I believe that ionic channels must be studied similarly, away from equilibrium.

I believe that electrochemical machines and devices cannot be designed until the equilibrium or near-equilibrium treatments of traditional physical chemistry are extended to allow flux across the boundaries and, thus, everywhere else. Theories and simulations must include nonuniform far-field boundary conditions if they are to show how nanostructures can be made into useful electrochemical (nano)machines and devices.

In a way, this is just a mathematical issue. The underlying physics of the system will be the same as in traditional equilibrium systems, but the behavior will be much richer, because the nonuniform boundary conditions will produce flux, and the flux will energize the more complex behavior of the nonequilibrium system.

## Theories of the Open Channel

The simplest theory of flux through open ionic channels couples the drift-diffusion equations of traditional electrochemistry<sup>21</sup> to the Poisson equation that describes how the average charge creates the average electrical potential  $\varphi$  (volts). Here, we consider only the dominant charges, namely the fixed charges of the protein  $P(x)$  (coulombs per centimeter) that line the wall of the channel and the ions of species  $j$  and charge  $z_j e$ .  $\epsilon_{\text{pore}} \epsilon_0$  is the permittivity of the channel's pore.

$$-\epsilon_{\text{pore}} \epsilon_0 \frac{d^2 \varphi}{dx^2} = \overbrace{eP(x)}^{\text{Fixed Charge}} + \overbrace{e \sum_j z_j C_j(x)}^{\text{Channel Contents}} \quad (1)$$

The current through the channel is computed from the flux  $J_j$  over a barrier of arbitrary shape<sup>19,22</sup> using the Nernst–Planck equation

$$J_j = -D_j \left[ \frac{dC_j}{dx} + \frac{z_j F}{RT} C_j(x) \frac{d\varphi}{dx} \right] \equiv \frac{-D_j}{RT} C_j(x) \frac{d\tilde{\mu}_j(x)}{dx} \quad (2)$$

Here,  $\tilde{\mu}_j(x)$  is the electrochemical potential of ion species  $j$  and  $\tilde{\mu}_j(x) \equiv z_j F \varphi(x) + RT \ln C_j(x)$ . Of course, specific chemical interactions between ion and the channel may contribute to the energy as well, although the channels we have studied so far,<sup>23–26</sup> show little sign of them.

We call these the *PNP* equations<sup>3</sup> to emphasize the importance of Poisson's equation and the analogy with transistors. Calling them the drift-diffusion equations (as is traditional in physics, where they are widely used to describe charge transport<sup>27,28</sup>) is seriously misleading, at least in my view, no matter how well precedented, because that name ignores the Poisson equation that dominates much of the behavior of these systems.

What matters ultimately is, of course, not the name of the equations, but rather the idea that the electrical potential is computed self-consistently, together with the concentrations of ions. The potential profile must be consistent with the profile of (all) charges. Neither the electric nor concentration field can be assumed; they must be computed together as self-consistent solutions of the *PNP* equations.

The charge that must be described includes the charge supplied by amplifiers and experimental apparatus to maintain the (spatially nonuniform) boundary conditions. The charge supplied by the experimental apparatus depends on experimental conditions, that is to say, it is different for different concentrations of ions, different potentials across the channel, or different structures of the channel and cannot be known a priori. We find it easier not to worry explicitly about the charge on the boundaries, supplied by the experimental apparatus, so we just solve Poisson's equation and thereby automatically supply the charge needed to satisfy the boundary conditions.

If one prefers to deal with the charge explicitly, Coulomb's law could be used in place of Poisson's equation, but *only* if the apparatus that maintains the boundary conditions is included, so the densities of *all* charges, including those on the boundaries are known. Using Coulomb's law explicitly may become a necessity, if the experimental boundary conditions are to be described realistically, including the limited frequency response and slew rate of the amplifiers that maintain them.

Little is known mathematically about the *PNP* equations and analytical results are scarce or nonexistent,<sup>27,29</sup> probably because mathematicians usually try to study the equations in their generality, and the only general properties of these equations are likely to be the conservation laws they embody.

The dearth of analytical results makes *PNP* a frustrating theory. Qualitative properties can be understood *after* an easy, nearly trivial numerical computation (see below). Then, profiles of concentration and potential along the channel (that are outputs of the theory) clearly show why currents vary the way they do, as fixed charge, transmembrane potential, or concentrations are changed. But before the computation, those profiles are hard to predict, particularly the most important, the profile of potential, and so *PNP* does not now permit the (qualitative) understanding all scientists seek, before experiments or computations are done. (We, of course, are not alone with our frustration. Semiconductor physicists have faced similar problems for nearly 50 years.)

## The Gummel Iteration

The *PNP* equations can be difficult to solve numerically, if standard methods are used. But the equations can be rapidly and accurately solved (numerically) by the Gummel iteration, used by semiconductor physicists for some 20 years.<sup>27,28</sup>

In the Gummel iteration, a profile of electrical potential is guessed (that satisfies the boundary conditions) and used to determine the concentration profile, from the Nernst–Planck equations. The concentration profiles are then substituted into Poisson's equation (and boundary conditions) to give a more refined estimate of the potential profile. That estimate is used in the Nernst–Planck equations to compute a new estimate of the concentrations. And so on, until the iteration converges (as it almost always does, in a few iterations).

The Gummel iteration is used widely—if not universally—by semiconductor physicists, but it deserves to be used even more widely, probably by everyone interested in electrodiffusion. The iteration allows the easy, fast, and accurate (numerical) solution of otherwise intractable problems, for mathematical reasons that are well understood.<sup>27</sup> Interestingly, the Gummel iteration works for high-resolution (femtoseconds and 0.1 Å) descriptions of charge movement in really quite complex semiconductor systems,<sup>28,30</sup> and so it may prove to be useful in the simulations of molecular dynamics.

## Effective Charge

Too little is known about the structure of most channels to determine the permanent charge density in one or three dimensions, and too little is known about the internal dielectric properties and dynamics of atoms within a protein on the time scale of permeation (~100 ns) to determine the (effective) charge density relevant to permeation, even in those channels where the crystal structure is known. Thus, the effective charge profile  $P(x)$  must be estimated by fitting the *PNP* theory to the experimental data itself.

Of course, the charge profile estimated this way is not as well determined as we would wish: we would like to know that charge profile in atomic detail, on the time scale of permeation. But in a certain sense that detail is not relevant to the *IV* curves measured in biological experiments, if those curves can be predicted, as we shall see, using just a few parameters.

## Experimental Applications

Five laboratories<sup>11,23–26</sup> have shown that *PNP* forms an adequate model of current voltage (*IV*) relations of six different channel proteins in ~10 pairs of solutions (20 mM to 2 M), over ±150 mV. Fits are satisfactory but not perfect, particularly at large potentials where deviations are to be expected.<sup>31</sup> The calcium release channel of cardiac muscle is particularly striking.<sup>23</sup> Only one parameter is necessary to describe its spatially uniform fixed charge.

*IV* relations are qualitatively different in different channels—some are linear, some sublinear, and some superlinear—because different channel proteins have qualitatively different profiles of fixed charge lining their pore, which arise from their different sequences of amino acids. In each type of channel, one profile of fixed charge  $P(x)$  fits nearly all the data, using just one diffusion

coefficient for each permeant ion and a single geometry. The structure is assumed entirely independent of salt concentration or electrical potential.

Traditional theories<sup>7</sup> cannot fit this range of data,<sup>32</sup> let alone with such few parameters,<sup>33</sup> which is not surprising given their use of absolute rate theory.

Electrostatic theories<sup>34–36</sup> have played an important role in the development of our understanding of channels, but they are not yet useful *in themselves* in predicting actual experimental data, i.e., current voltage curves, because they usually concentrate on dielectric properties and often ignore ions in the bath altogether and so have difficulty predicting ionic current.

Theories with higher resolution, e.g., molecular dynamics, are seductively attractive, certainly to me. We would all dearly love to understand ion channels in atomic detail just as we would love to do atomic biology in general.<sup>37</sup> Unfortunately, this desire has a certain romantic impracticality if viewed dispassionately, at least for the time being.

Existing simulations of molecular dynamics<sup>38</sup> are too brief in duration to predict flux: so far they have not observed a single ion crossing a channel, to the best of my knowledge. Nor in fact do they include the transmembrane potential that helps drive such flux. Existing calculations (in atomic detail) have also simulated small systems, which do not contain ions in the surrounding solutions at all. Such simulations of a channel in distilled water, without transmembrane potential, reveal tantalizing atomic details, but they are of limited use to the biologist, who changes ion concentration and transmembrane potential in his quest to understand channel currents. This is not to say existing simulations are useless or realistic simulations are impossible. It is just to say they have not yet been done.

The real issue is how to do realistic simulations, including all the variables known to be important, including both the atomic structure of the channel protein, and the important atomic structure of the surrounding solutions (i.e., their ions). Such simulations would actually follow a swarm of ions as they move through the channel under voltage clamp conditions.

## Testing the Theory Further

Until realistic simulations are available, we must rely on theories of lower resolution like *PNP*. Further tests of those theories are underway to discover their limitations and to focus attention on their defects, so they can be set right. It is not enough to fit data; the theory must also be tested by comparing the charge profile it estimates with known structures of channels.

Predicting *IV* data from structure requires both temporal and spatial simplifications. Temporally, the static structure measured by crystallography is assumed to be similar to the dynamic structure that accompanies permeation. However unlikely that assumption might seem to people familiar with the flexibility of proteins, the fact that a single  $P(x)$  fits such a wide range of data over a

100:1 range of ionic strength suggests that channel proteins may be quite rigid structures if a functional definition of "rigid" is used.

Spatially, simplifications are made because structures in three dimensions have a great deal more detail than the profile  $P(x)$ . A full three-dimensional theory is needed to convert the three-dimensional profile  $\bar{P}(\vec{r})$  determined by crystallography into a prediction of  $IV$  curves, or of  $P(x)$  as is done in semiconductors.<sup>28</sup>

A three-dimensional theory is not needed, however, to see how well the one-dimensional effective profile describes changes in the fixed charge of the protein, if the change in fixed charge is known independently. The structures of two channel proteins (porin and its mutant G119D) are known.<sup>6</sup> The mutant has one extra negative charge. Measurements of  $IV$  relations of porin and G119D in a range of solutions<sup>26</sup> estimate the additional charge as  $-0.97e$ , although this estimate will undoubtedly change as more work is done. (I hasten to add that no information about the proteins is used in the analysis except the length and diameter of the channel; parameters are not adjusted in any way.)

## Why does the theory work?

This success of  $PNP$  is surprising. In part, success may occur in channels because open channels have large electric fields: even a few charges in the lining of the channel's pore produce an enormous density of fixed charge.<sup>23</sup> One charge in a cylinder 6 Å diameter and 10 Å long is a concentration of  $6 \times 10^{21} \text{ cm}^{-3} \approx 10 \text{ M}$ . Interestingly, theories such as Poisson Boltzmann are thought to "become exact for large electric fields, independent of the density of hard spheres" (page 315 of ref 39) and "independent of interactions of molecules in the fluid phase" (page 972 of ref 40). And some voltage-dependent channels are thought to have as many as six charges in half that length or volume, giving  $\sim 100 \text{ M}$  fixed charge.

High densities of fixed charge also help buffer the concentration of mobile charge (of the opposite sign) in the channel's pore. The important part of the channel is not exposed to the wide changes in concentration that are used in most experiments. Concentration-independent errors in the theory can then be absorbed into its effective parameters (to some extent).

In part, the success of  $PNP$  may occur because we have stumbled on the physics chosen by evolution to control permeation. In this view, the open channel, like other biological systems, depends on fairly simple physics to do its job, evidently well described by  $PNP$ . Other physics might have been used by evolution, but were not.

In part, the success of  $PNP$  may be relative, seeming to be more than it is because of the inadequacies of other theories. The disciplines of biochemistry<sup>1</sup> and traditional channelology<sup>7</sup> usually describe their systems by networks of rate constants independent of concentration, thus not including the effects of shielding on the potential profile that determines the rate constant, even at equilibrium,

let alone away from equilibrium. Electrochemistry has not (to the best of my knowledge) used the Gummel iteration to deal with steady flux. Molecular dynamics has not used the Gummel iteration to deal with the large thermal fluctuations in electrical potential (nearly 1 V, certainly many  $kT/e$ , even in 0.01 ps) that invariably occur in the equilibrium state, on atomic time and distance scales. Potential fluctuations of this severity are likely to produce correlated movements of ions (of perhaps different species), i.e., cross diffusion and other nonlinear phenomena.

## Present and Future Directions

The apparent success of such a simple theory as  $PNP$  will probably disappear as more experiments are done. Nonetheless, it seems likely that some ideas will survive in later theories:

- (1) proteins will be described as distributions of fixed charge, not as potentials of mean force or rate constants independent of concentration;
- (2) potential profiles will be computed self-consistently from all the charges of the system, using some variant of the Gummel iteration to solve electric field and transport equations together;
- (3) nonuniform far field boundary conditions will be used to ensure steady flux.

## Hopes

Progress in membrane biology will be much faster, if more physical scientists (trained in analysis and prediction) join the molecular biologists (trained in discovery and description) who study ionic channels.

It is also possible that physical scientists will learn some useful lessons from open channels. Ionic channels may be holes in the walls that have separated molecular biology and chemical physics, natural nanotubes that provide reaction paths between these fields and so catalyze their interactions. The open ionic channel may serve as a useful paradigm for electrochemical nanomachines that only exist and function (as devices) when they are far from equilibrium.

*I thank Raimund Dutzler and Tilman Schirmer for preparing Figure 2 and helping us so much with structural issues. Duan Chen and I have shared our journey through channels from its beginning. I hope working together has been as much fun for Duan as for me. We are grateful that Mark Ratner, Ron Elber, and Abe Nitzan have tried to teach us some chemistry. I have been learning chemistry from David Eisenberg as long as I have known him, since 1959, when we discovered each other as tutees of John Edsall and thus brothers-in-science. I thank David for his continual help and encouragement as I moved from cellular to molecular and atomic biophysics.*

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