APPLICATIONS OF PHYSICAL CHEMISTRY: A BIOLOGICAL EXAMPLE

A few pages for the second edition of

Berry, Rice and Ross: Physical Chemistry

by

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Physical Chemistry Applied to Biology. Biologists try to understand how complicated structures (built by evolution) use physical laws to produce their natural function. The primitive model of electrolyte solutions, which we have previously studied at length **[p. 997, Section 26.5 of BRR1],** provides the many of the physical laws of life because much of life occurs in salt solution.

Here, we consider proteins called ionic channels embedded in the membranes that form the 'walls' of cells. Channel proteins form 'holes in the wall' lined by fixed charge that controls many of the properties of channels. The physics that governs ionic channels is important in all proteins. Proteins are the main components of most biological systems and so understanding the physical laws governing proteins is in a very real sense understanding the physical basis of life.

Ionic Channels. Channels are probably the simplest protein structures of general biological importance. Channels are responsible for signaling in the nervous system, for co-ordination of muscle contraction, including the co-ordination that allows heart muscle to function as a pump. Channels are intimately involved in the secretion of urine and hormones and most other transport processes in cells; they are natural targets which viruses attack and use to enter cells.

Fig 1
Channel in a membrane

Each of the functions of channels has been important for so long in the history of life that evolution has probably produced a nearly optimal adaptation (within historical and physical constraints) and conserved it, i.e., used the same fundamental design principle again and again. The transport and gatekeeper functions of channels are obvious targets for drugs and disease: many, perhaps most, of the drugs used in clinical medicine act directly or indirectly through channels.

Ultimately for these reasons—because of their medical significance—hundreds of types of channels are studied by thousands of biologists, who discover and describe new properties of channels every day. This work must be described (and understood) in the language of physical chemistry because the words of channels are the words of physical chemistry. What is measured and controlled are ionic currents, concentrations, and electrical potentials in aqueous solutions.

Channels as conductors of current. Channels conduct a definite amount of current, once they are open, and this single channel current can be easily recorded by the patch clamp method introduced by Sakmann and Neher [Sakmann, 1995 #135]. When the solutions on either side of the channel are kept at definite concentrations, and the electrical potential between those two solutions is maintained at a fixed value, the ***mean*** current carried by permeant ions as they

move through the open channel is remarkably constant and reproducible. Once the channel is open, the instantaneous current recorded shows substantial variance, not all of which is instrumentation noise, but the ***mean*** current does not drift at all, on the time scale relevant to biology, longer than say 10 μsec. The mean current is the same from opening to opening, from channel to channel, from day to day, from animal to animal, and from laboratory to laboratory, within the error of measurement, with a precision more commonly found in measurements of physical than biological systems. This reproducibility—along with their simplicity and great clinical and biological importance—make open channels an inviting target for physical study.

Of course, it is not trivial to maintain the concentration of ions fixed, or the electrical potential fixed near a channel, when a large current flows through it. Complex experimental apparatus and procedures have been developed over the years for this purpose, and their developers of have won several Nobel Prizes. The 'voltage clamp' or 'patch clamp' were designed to mimic properties of the biological cells that sustain channels, while allowing precise experimental measurement of current flow.

The large currents that flow through channels guarantee that they are not isolated systems in any sense of the phrase; rather, the biological cell must use elaborate machines to supply matter and charge to control the environment around channels, e.g., to maintain boundary conditions of constant concentration. Indeed, in a sedentary human being—who is reading this textbook (for example)—a substantial fraction of all metabolism is used (in the nerve cells of the brain) to maintain these boundary conditions. If channels are to be studied as biological systems, they cannot be isolated from their environment.

Channels are a nonequilibrium system. Channels only function when they are coupled to their environment and so currents and fluxes must be present in a theory (or simulation). A model of a channel as an isolated system is not likely to be useful. Channels carrying current must be studied in the spirit of the nonequilibrium systems described earlier in this book **[Ch. 27-31, for example, of BRR1]**.

Steady-state flux can flow across boundaries of the system only if boundary conditions of the system are (spatially) nonuniform. If the boundary conditions surrounding the system are everywhere the same, then clearly no steady flux can flow. These simple statements have profound consequences because they rule out many of the traditional models of channels and biochemical systems, which use spatially uniform boundary conditions and so do not allow steady flux.

Channels can also be viewed as proteins that modify and control the flow of current, like devices of our electronic technology. Current is driven through channels by external sources; it is driven through transistors and other semiconductor devices by power supplies. The equations we use to describe channels are the same as those used to describe semiconductor devices, like transistors and integrated circuits[Lundstrom, 1992 #88]. These apparently dissimilar systems would then be expected to have some similar behaviors.

Properties of open channel currents. Channel currents are constant in the sense that the mean current through a particular type of open channel, under a particular set of conditions, does not vary with time (on the biological time scale). The average amount of current that flows through a channel varies according to the concentration, electrical potential, and type of permeating ion, as well as the type of channel. Channels are characterized by their *I-V* curves, that display the dependence of current *I* on electrical potential *V*, usually in the range of ± 150 mV. Concentrations are usually changed in the physiological range around 300 mM (corresponding very roughly to the concentration of ions in the blood of a variety of animals, or in sea water); the overall concentration range is from 20 mM to 2 M. The change in potential and concentration are usually substantial, RT/F or larger.

Different types of channels are different proteins, with different blueprints (genes), made of different sequences of amino acids, folded in different ways, with different primary and tertiary structures. They have different functions, and (usually) different locations in different cells and organs of the body. The amplitude of the current through a channel depends on the type of channel through which the ions flow. In some channel proteins, currents are carried by just one type of ion, say by potassium, sodium, or calcium ions. In other types of channels, any cation can carry the current; in still others, any anion will do. Channels are selectively permeable: some types of ions can carry current and some cannot.

The selectivity of channels is of such great biological importance that many channels are named that way: Na–channels are types of proteins that conduct mostly Na^+ ions; K–channels are types of proteins that conduct mostly K^+ ions, and channels are called by those common names (to the confusion of students) even if they are quite different in other respects, and are in fact different proteins, with quite different structures and functions.

Most investigators are rightly fascinated by the chemical basis of selectivity and the role of ‘binding sites’ (i.e., specific parts of the channel’s pore) in determining that chemistry. The selectivity of channels is immensely interesting but a little beyond what we can discuss here because it involves quite complex,

non-ideal behavior in mixed solutions of different electrolytes. Mixtures have non-ideal behavior, difficult to understand, as we have already seen **[BRR1: section 25.4]**.

Selectivity is usually studied indirectly by measuring current flow in different solutions. Chemical fluxes (e.g., as carried by radioactive tracers) are difficult to measure but even the picoamps of current that flow through one channel molecule can easily be captured and recorded using the techniques that have made channology a molecular science, patch clamp and reconstitution [Sakmann, 1995 #135].

Imagine then that we have a complete set of measurements of current through a potassium channel, in a wide range of solutions and over a wide range of potentials. How do we interpret these results? How can we analyze and then predict these *I-V* curves in terms of the structure and properties of the ionic channel itself? Can we develop a theory to predict the properties of the hole in the wall, from the structure of the protein that forms the hole?

Theory of the Open Channel. A mature and proven theory of an open channel would start with the three dimensional structure of the channel protein, with the coordinates of all the atoms. It would combine that information with the concentrations of ions and the electrical potential maintained experimentally in the baths, and predict the current that flows through the channel perhaps using only the friction (i.e., diffusion coefficients) of the permeant ions as parameters.

A theory of this sort is not available yet. The main impediments are technical, namely the problems involved in solving the three dimensional field and transport equations for given macroscopic boundary conditions. Thus, we resort to a common tactic of science: we average away some of the three dimensional detail, hoping that a one dimensional theory will retain enough of the essential physics to be able to predict the currents observed. We expect biology will use a simple one dimensional mechanism, however hard that mechanism is to discover.

We are optimistic about the likely success of this simplifying approach because of the simple structure of channels and the (relative) simplicity of electrodiffusion compared to other physical processes—like hydro- or quantum dynamics. It should be possible to understand the physical chemistry of a tiny highly charged hole in the wall filled with water and ions.

A typical channel ***protein*** might be 40 Å long and perhaps even 40 Å in diameter. The protein is embedded in a lipid bilayer some 30Å thick. The pore of the channel protein is much smaller than the protein, often not more than 7 Å in

diameter. The most important part of the pore is the narrow portion (more or less a cylinder 10 Å long and 7 Å in diameter)—the 'selectivity filter' that controls current flow. The total electrical charge of the charged and polar residues of the protein that line the walls of the selectivity filter is of the order of 1 ***e***. Because the system is approximately (but ***not*** exactly, as discussed later) electrically neutral, the number of mobile ions of opposite charge in the pore (averaged over a time of microseconds, for example) should be roughly 1 as well, giving a concentration of some 5 M, much higher indeed than biological electrolyte solutions surrounding the channel.

When the density of electrical charge is very large, like this, one might naively expect electrical interactions with the mean field to dominate, and theoretical work on the electrochemistry of highly charged surfaces supports this view. Thus, we will try a simple mean field theory, very much in the spirit of the Debye-Hückel theory of ionic solutions, or the Gouy-Chapman theory of interfaces, or the Poisson-Boltzmann theory of proteins **[p. 997, Section 26.5 of BRR1]**. But these theories must be generalized to allow current flow. Mean field theories depend on approximations that are hard to evaluate *a priori*, and that cannot be true for all conditions and all systems and so it is necessary to check them with real data.

The theory begins with Poisson's equation **[eq. 26.61, p. 998 BRR1]** which describes how the average charge produces the average electrical potential φ (units: volts), where x is the location along the channel axis, or more precisely, along its reaction coordinate. Here, we assume that all quantities are averaged over time for the duration of the shortest current we can measure, namely a few μsecs. We consider only the dominant charges, namely the ions of species j and charge z_jF (units: coulombs mole^{-1}) and the permanent fixed structural charges of the protein $P(x)$ (units: coulombs cm^{-1}) that line the wall of the channel. The mobile species j are Na^+, K^+, Cl^-, and/or Ca^{++} in most biological problems. $\varepsilon_{pore}\varepsilon_0$ is the product of the dielectric constant of the channel's pore and the permittivity of free space (units: farads cm^{-1} or cou volt^{-1}cm^{-1} or amp sec volt^{-1}cm^{-1})

$$-\varepsilon_{pore}\varepsilon_0 \frac{d^2\varphi}{dx^2} = \overbrace{\boldsymbol{e}P(x)}^{\text{Fixed Charge}} + \overbrace{\boldsymbol{e}\sum_j z_j C_j(x)}^{\text{Channel Contents}} \qquad (1)$$

We assume that the Poisson equation is true on all scales, that is to say, the Poisson equation can be used on any length or time scale provided we average the potential (on the left hand side) and the charge (on the right hand side) the same

way. This assumption provides a good way to start an analysis of a novel physical system, like an open ionic channel. How reasonable the assumption is theoretically can be seen by considering the alternative: what would happen if the average potential did not correspond to the average charge: what would sustain the extra forces, where would the extra energy come from to do that?

Mobile ions in the channel (that contribute so importantly to the charge on the right hand side of eq. (1)) move and carry current and so we need an equation to describe how their mean flux J_j (units: concentration×$cm^{-2}sec^{-1}$) varies with potential and concentration. The simplest relation between mean flux and potential and concentration (units: cm^{-3}) is the diffusion equation (see eq. 20.15-20.16, Table 28.1: p. 1069, and p. 1099 of **BRR1**), which is written here in its form as the Nernst-Planck equation, using the Einstein relation (**BRR1 p. 1162, eq. 30.94)** between mobility and diffusion coefficient D_j (units: cm^2sec^{-1}). The Nernst-Planck equation is simply the diffusion equation [**BRR1 p. 1099, 719]** for charged particles.

$$J_j = -D_j\left[\frac{dC_j(x)}{dx} + \frac{z_jF}{RT}C_j\frac{d\varphi}{dx}\right] \equiv \frac{-D_j}{RT}C_j(x)\frac{d\mu_j(x)}{dx} \qquad (2)$$

The electrochemical potential $\mu_j(x)$ of ion species j is discussed in Ch. 26 **[BRR1]**, $\mu_j(x) \equiv z_jF\varphi(x) + RT\ln C_j(x)$. The current I (amps) through a channel of radius r is simply $I = \pi r^2 \cdot \sum_j z_jFJ_j$, see Section 29.6 **[BRR1]**. Note that in this simple first treatment ions behave ideally, with no excess chemical potential, and thus have the same activity coefficient and standard chemical potential in the bulk solution and in the channel (see Ch. 25, e.g., p. 906–909, of **[BRR1]**, and Ch. 26, e.g., eq. 26.7-12 of **[BRR2])**. It is extraordinary that a theory with such an unlikely assumption fits so much data.

Simplified boundary conditions specify both (1) the concentrations of each species $C_j(L)$ and $C_j(R)$ in the solutions outside the channel and also (2) the potential difference $V_{applied}$ (inside – outside) maintained by the voltage clamp apparatus. The original publications [Eisenberg, 1996 #24] describe the more realistic (and complex) boundary condition that are needed to fit experimental data.

Integrated Concentration Expression. The Nernst-Planck equations can be integrated (only once) analytically, using integrating factors, to give an explicit expression

for the concentrations $C_j(x)$ as functions of the entire potential profile across a channel $\Phi(x) = \varphi(x)F/RT$ of length d and the boundary concentrations and potentials.

$$C_j(x) = \frac{C_j(0) \cdot \exp z_j\left[V_{applied} - \Phi(x)\right] \cdot \int_x^d \exp z_j \Phi(\zeta) d\zeta}{\int_0^d \exp z_j \Phi(\zeta) d\zeta} + \frac{C_j(R) \cdot \exp\left[-z_j \Phi(x)\right] \cdot \int_0^x \exp z_j \Phi(\zeta) d\zeta}{\int_0^d \exp z_j \Phi(\zeta) d\zeta} \quad (3)$$

This expression is less helpful than it seems because the potential profile $\Phi(x)$ is not known. The profile of potential can only be determined by solving the Poisson equation (1). But the Poisson equation contains the concentration of mobile charges $C_j(x)$ and that concentration is not small. The concentration of counter ions (ions with charge opposite to that of the nearby fixed charge of the channel protein) is always of the same order as the fixed charge, because the combined system of channel wall and channel pore is fairly close to electrically neutral. Thus, the Poisson equation cannot be solved until the Nernst-Planck equation is solved. In other words, equations (1) and equation (2) [or equation (3)] must be solved simultaneously; the Poisson and Nernst-Planck equations form a coupled system.

The system of Poisson and Nernst-Planck equations is called the *PNP* equations in channology or the drift-diffusion equations in solid state physics (where they are universally used to describe current flow in semiconductor devices like transistors).

Note that neither the channel's pore, nor the channel plus surrounding baths, nor a transistor for that matter, is electrically neutral. The number of positive charges does not precisely equal the number of negative charges in any region. The potential profile in the channel's pore $\Phi(x)$ could not exist (i.e., Φ would be spatially uniform) and even the *trans*membrane potential $V_{applied}$ could not exist if the system were strictly electrically neutral. Nonetheless, electrical neutrality is approximately satisfied and the total fixed charge lining the channel

wall and the total mobile charge within the channel are within say 20% of each other.

Integrated Flux Expression. Another integrated form of the Nernst-Planck equations is helpful, particularly in making links to work on chemical reactions, because it can either be derived from the Nernst-Planck equations or from the stochastic theory of chemical reactions[Eisenberg, 1995 #25]. In fact, the integrated expression for flux J_j can be written as a form of the law of mass action, allowing a rigorous derivation of the forward and backwards rate constants for flux over any shape potential barrier $\Phi(x)$. The integrated flux equation is

$$J_j = \overbrace{D_j \frac{C_j(L)\exp\left(z_j V_{appl}\right)}{\int_0^d \exp z_j \Phi(\zeta)\, d\zeta}}^{\text{Unidirectional Efflux}} - \overbrace{D_j \frac{C_j(R)}{\int_0^d \exp z_j \Phi(\zeta)\, d\zeta}}^{\text{Unidirectional Influx}} \tag{4}$$

The flux is best written as the sum of two unidirectional fluxes: as we shall see, each component of flux has a much simpler physical meaning and dependence on experimental variables than the sum.

It is important to note that the flux depends on the integral of the potential profile, in the integrated Nernst-Planck equation (4) and the potential depends (speaking roughly) on the second integral of the fixed charge distribution, according to the Poisson equation (1). The fixed charge profile contains most of the information concerning the structure of the protein. The current through the channel has a highly integrated (and thus smoothed) dependence on fixed charge and so is expected to be rather independent of the details of charge distribution, at least if the charge distribution has one sign and never gets too close to zero.

Each unidirectional flux is carried by ions from a source concentration on the ***cis*** side (say on the left side of the channel) to the ***trans*** side (here the right side), when the ***trans*** side is held to zero concentration (even in the presence of flux) by experimental apparatus or by the metabolism of a biological cell. The ***trans*** side is then made into an absorbing boundary, by the apparatus or cell, if we use the words of stochastic processes and probability theory.

Each unidirectional flux can be written neatly, without further approximation, as the product of a 'source' concentration; 'diffusion velocity' $\left(D_j/d\right)$, sometimes called 'the permeability' in the channology literature; and the appropriate conditional probability.

$$J_j = d\left[k_f C_j(L) - k_b C_j(R)\right] \tag{5}$$

$$\equiv \overbrace{\underbrace{C_j(L)}_{\text{Source Concentration}} \underbrace{\left(\frac{D_j}{d}\right)}_{\text{Diffusion Velocity}} \underbrace{\text{Prob}\{R|L\}}_{\text{Conditional Probability}}}^{\text{Unidirectional Efflux}} - \overbrace{C_j(R)\left(\frac{D_j}{d}\right)\text{Prob}\{L|R\}}^{\text{Unidirectional Infflux}}$$

The same system can be written (*for any shape potential profile*) as a chemical reaction linking ions on the ***L***eft and on the ***R***ight side of the channel, without making any further approximations.

$$L \underset{k_b}{\overset{k_f}{\rightleftharpoons}} R$$

$$k_f \equiv k\{R|L\} = \left(D_j / d^2\right)\text{Prob}\{R|L\} \tag{6}$$
$$k_b \equiv k\{L|R\} = \left(D_j / d^2\right)\text{Prob}\{L|R\}$$

$\text{Prob}\{R|L\}$ is the conditional probability that an ion starting a trajectory on the ***L***eft side of the channel (with right-going velocity) eventually appears on the ***R***ight, when a reflecting boundary condition is imposed at the left boundary and an absorbing boundary condition is imposed on the right boundary.

The trajectories can be described by other statistics besides conditional probabilities. The time an ion takes to go from ***L*** to ***R*** is a statistic called $T\{R|L\}$, the (conditional) first passage time; the number of $\{R|L\}$ trajectories within the channel is the conditional contents of the channel $[[R|L]]$, the unidirectional flux $J\{R|L\}$ is the flux carried by the $\{R|L\}$ trajectories, and, not surprisingly

$$J\{R|L\} = \frac{[[R|L]]}{T\{R|L\}} \tag{7}$$

Equations for *un*conditional probabilities, passage times, or total fluxes are awkward to write, at best, because they often contain infinite quantities (that are difficult to compute) even in systems that are entirely finite. For example, no

simple relation exists between the (total) contents of the channel, the net flux, and the mean first passage time of all ions. Simple relations exist between these variables only if the trajectories are first separated (i.e., 'conditioned') into the subsets $\{R|L\}$ or $\{L|R\}$.

The conditional probabilities, and other statistics of equations (5)—(7) can be determined numerically [Barcilon, 1993 #13][Elber, 1993 #27] at various resolutions. For example, they can be determined by computing a random walk, or by simulating a full or reduced Langevin equation (see Section 29.3, p. 1101-1105 of **BRR1**) or from simulations of molecular and atomic dynamics.

When friction is large (as in channels on the biological time scale) and simple (characterized by a single number D_j for each ionic species j), the statistics can be determined analytically. The probabilistic analysis reduces then to studying the Langevin equation of Section 29.3 of **BRR1** and the conditional probabilities satisfy a Fokker-Planck partial differential equation. In that case, the rate constant $k\{R|L\}$ and conditional probability $\mathrm{Prob}\{R|L\}$ (of the integrated Nernst-Planck equation (4) and chemical reaction (6)) can be written exactly *for any shape potential barrier* $\varphi(x)$. [Barcilon, 1993 #13]

$$k\{R|L\} \equiv \frac{D_j}{d^2}\mathrm{Prob}\{R|L\} = \frac{D_j}{d^2}\cdot\frac{\exp\left(z_j \boldsymbol{F} V_{appl}/RT\right)}{\frac{1}{d}\int_0^d \exp\left[z_j\varphi(x)/RT\right]dx} \tag{8}$$

In this way, permeation can be described ***exactly*** both as a chemical reaction and as stochastic transport over a potential barrier of any shape. Permeation through a channel can be described as a reaction along a coordinate (as discussed on p. 1127, **BRR1**) more precisely, less metaphorically, than chemical changes can be in many more traditional situations.

John Ross was not sure the following three paragraphs should remain in the book. In view of the rigorous nature of the derivation, and the widespread use of rate theory, and the easy applicability of equation (10), I personally hope we can keep the material in the document. Of course, it should be carefully integrated with the other treatment of the Kramers/diffusion/stochastic approach to

chemical reactions, which I gather has not yet been written.

Clearly, I want to give as little attention to transition state theory as is possible, while being fair, because it has been so badly abused in the past, but ignoring it is not the best way to deal with the past abuse, at least in my opinion. Perhaps the best way is to give a clear, trivial derivation, of an easy to use form of the equations and to ***state forcibly when they can and when not they cannot be used.***

The integrated Nernst-Planck equation (4)—(8) can also be used to derive the exponential expressions of activated-complex theory (p. 1147-1164 of **BRR1**), i.e., the transition state theory widely used to describe rate constants if the potential profile $\Phi(x)$ is dominated by a single large isolated barrier. For example, the standard expression of the Kramers' formulation of rate theory **[**reference to new section of **BRR1]** is recovered.

$$k_f \xrightarrow[\textbf{barrier}]{\textbf{high}} \underbrace{\frac{D_j}{d\sqrt{2\pi}}\sqrt{\left|z_j\Phi''(x_{\max})\right|}}_{\textbf{PREFACTOR}} \exp\left[z_j V - z_j\Phi_{\max}(x_{\max})\right] \tag{9}$$

The numerical value of the prefactor of eq. (9) can be estimated easily if the potential profile $\Phi(x)$ is a symmetrical parabolic barrier spanning the whole length d of the channel, with maximum size $\varphi_{\max}(x_{\max})$, much larger than the applied (i.e., *trans*membrane) potential $V = FV_{appl}/RT$. Then, for example,

$$k_f \xrightarrow[\textbf{high barrier}]{\textbf{PARABOLIC}} \underbrace{\frac{2D_j}{d^2\sqrt{\pi}}\sqrt{\left|z_j F\varphi_{\max}(x_{\max})/RT\right|}}_{\textbf{PREFACTOR}} \exp\left[-z_j F\varphi_{\max}(x_{\max})/RT\right] \tag{10}$$

The prefactor depends on many variables and has a numerical value very different from the expression kT/h used in simpler situations (p. 1147-1164 of **BRR1**).

Equations (9) & (10) are useful approximations and can be used when systems satisfy the conditions under which they were derived, namely, when barriers are known to be large and of a definite size that does not change in the experiments of interest. These conditions are ***not*** satisfied in most systems

involving condensed phases, for example proteins and channels. They are certainly not satisfied when *I-V* relations are measured from channels in many solutions, because changes in salt concentration do in fact change $\varphi_{\max}(x_{\max})$. No matter what the historical precedent, it is not logical to use transition state theories to describe systems with small barriers or barriers that do not have constant size in the experiments of interest.

PNP equations. The *PNP* equations are deceptively simple both in their physics and in their form. Physically, they are mean–field equations like those of other mean field theories and they depend on the same assumptions. But the *PNP* equations differ from many mean field theories because they explicitly and self-consistently allow flux. This is very different from theories that are confined to equilibrium, where no flux flows.

Systems at equilibrium have much simpler behavior than nonequilibrium systems; in particular, systems at equilibrium do not have the behaviors characteristic of (what engineers call) devices, the motors of our technology that we use every day to help us with our lives. For example, an automobile engine without gasoline is not a motor; it cannot move. A transistor at equilibrium (without current flowing into its terminals) is not a device; it cannot switch, amplify, or perform logic functions. In fact, one could measure and understand every physical property of a transistor at equilibrium, and still be unaware that away from equilibrium it can be a switch, amplifier, memory element, or indeed a part of an integrated circuit that remembers a number or name.

The *PNP* equations describe the rich behavior of semiconductor devices, such as switches, amplifiers, and memory elements, for example, even though they look like (linear) differential equations that yield only much more ordinary behavior. The equations are not linear, however, and in fact describe much richer behavior. Only the potentials at the terminals of a transistor need to be changed to convert the device from a linear amplifier to a logarithmic amplifier or even a nonlinear switch. The theory has the same properties as the physical system. Only the boundary values have to be adjusted to give this richness of behavior. ***Neither the differential equation nor its parameters have to be changed in any way.***

The *PNP* equations are deceptive in this way, giving a rich repertoire of well determined behavior from a simple pair of equations. They are deceptive in other ways as well, because they cannot be integrated by the normal numerical recipes widely available in packaged programs. Those integration schemes do not work on these equations, even approximately, for fundamental reasons that are well understood mathematically.

Solving the PNP equations: the Gummel iteration. Integration of the *PNP* equations is difficult if recipes for standard systems of equations are used, but integration is easy if a particular method called the Gummel iteration, or its equivalent, is used. . The Gummel iteration was discovered some decades ago by the semiconductor community and is a general method for producing a self-consistent solution of coupled equations closely related to the self-consistent field methods used in quantum chemistry to compute molecular orbitals (which we have discussed previously, **BRR1**, p. 176: **this is not a particularly apt reference. I hope you can find a better one.**).

The Gummel iteration starts with an initial guess of the potential profile, often as just a linear function of position connecting the boundary values of potential. That initial profile is substituted into the right hand side of the integrated concentration equation (3). This substitution determines the congruent initial guess of the concentration profile $C_j(x; \textit{initial guess})$. That guess is substituted into the right hand side of Poisson's equation (1), which is then trivially solved. The resulting estimate of potential $\varphi(x; \textit{first iterate})$ identically satisfies the boundary conditions, as do all other estimates of the potential profile. The potential profile $\varphi(x; \textit{first iterate})$ is substituted into the integrated NP equation (3) and so determines a first-iterate of concentration profiles $C_j(x; \textit{first iterate})$. These two iterates are consistent with each other and the boundary conditions. The two first-iterates $\varphi(x; \textit{first iterate})$ and $C_j(x; \textit{first iterate})$ are then substituted into the right hand side of Poisson's equation (1), which is again solved, now to determine the second-iterate $\varphi(x; \textit{second iterate})$, an updated, hopefully better approximation to the potential profile. The second-iterate of potential determines a second-iterate of concentration by equation (3); together, the two second-iterates determine the third-iterate of potential, and so on for ten iterations, (which is more than enough for good convergence in almost all cases), that take only milliseconds on a typical personal computer.

Fig. 4
I-V Relations from 4 types of channels

The *PNP* equations form a map between the structure of the channel protein, represented crudely by the function $P(x)$ and the *I-V* curves measured experimentally. Different types of channels have different pores made with linings of different charge. A useful and productive working hypothesis assumes that the only difference between different types of open channels is their different distributions of fixed charge $P_k(x)$, where the subscript k identifies the type of channel protein, e.g., a voltage activated Na–channel, a stretch–activated channel and so on. Of course, this working hypothesis cannot always be true: specific

Fig. 3
Input Output For PNP

chemical interactions, not captured in this simple mean field theory, will no doubt be important in ways we do not yet understand. Nonetheless, as we write these words, the *I-V* relations of some 7 types of channels in a wide range of solutions can be predicted by simple distributions of fixed charge $P_k(x)$. In one particular kind of channel (from cardiac muscle), a fixed charge $P_{cardiac}(x) = P_0$ independent of position, with P_0 equal to ~1***e***, predicts the currents measured in pure solutions, and most mixtures, of all the monovalent cations (i.e., Li^+, Na^+, K^+, Rb^+, Cs^+) from 20 mM to 2 M, and potentials of ±150 mV, assuming each ion has a different diffusion coefficient. The value of the diffusion coefficients are estimated by fitting theoretical predictions to the experimental data. Typically, the diffusion coefficients are some 10× less than in free solution.

The graphs below show a few *I-V* relations from four types of channels with quite different characteristics. We see the ***LS*** channel[Chen, 1997 #16] is highly rectifying, that the ***NBAC*** channel rectifies in the other direction[Chen, 1995 #87], the ***CRC*** channel is nearly linear[Chen, 1997 #128] and the porin channels [Tang, 1997 #41; Tang, 1997 #228] have still different curves. The data from the porin channels are of particular interest because the locations of the atoms of that protein are known by x-ray crystallography[Cowan, 1992 #138; Jeanteur, 1994 #140; Schirmer, 1995 #53]

Nonequilibrium effects. Nonequilibrium effects in channels are profound. These arise in at least two different ways. First, the flow of current and the flux of ions is accompanied by a significant change in electrochemical potential, a change in both the profiles of electrical potential and concentration. This is the voltage drop or change in concentration gradient given by either Ohm's or Fick's law in simple uncoupled systems (p. 1112-1115 and p. 721-725 of **BRR1**).

The other effect of moving away from equilibrium states is more subtle, but at least as important. A non-equilibrium system can exist for a very wide range of boundary conditions but equilibrium systems can exist (i.e., make sense, and satisfy the equations that define themselves) only under very special circumstances, e.g., when boundary conditions are spatially uniform so no flux flows in the system. For example, a channel and its mathematical model are nonequilibrium systems that can function (biologically) and exist (mathematically) ***no matter what the concentrations and what the electrical potentials in the baths***, or in the boundary conditions used to describe the baths. But an equilibrium theory of a channel (e.g., Poisson-Boltzmann models, or most simulations of the molecular dynamics of a channel) can only describe situations in which no flux of any species flows anywhere. If the concentrations and electrical potentials present in

the baths (and boundary conditions) do in fact produce flux, (say) because they are not spatially uniform, an equilibrium model or simulation cannot be computed, if it is programmed correctly, because the equations in fact have no solution in that case. If an equilibrium simulation or computation gives a result, that result must have zero flux everywhere, because that is what equilibrium means. If an equilibrium simulation or computation seems to give a result, when bath and boundary conditions are non-uniform, the simulation must not have converged to a solution to the equations defining the system, because no solution to the equations exists, and thus no numerical procedure can find one, in that case.

It is possible, of course, that an equilibrium model may be a decent approximation to a nonequilibrium model, or that it may give important physical insight into the properties of the nonequilibrium system. But this must be shown to be so, it cannot be assumed, and indeed is unlikely to be the case very often in systems like channels that function nearly always away from equilibrium, with potential and concentration gradients larger than RT/F.

These abstract words have consequences for nearly all channels, because most channels carry flux under all conditions. Only a perfectly selective channel can be placed in solutions and at electrical potentials in which there is no flux of any species. Only a perfectly selective channel has gradients of potential and concentration across it that satisfy the Nernst equation of electrochemistry (p. 978 of **BRR1**; the Nernst equation is an algebraic equation defining a potential, not to be confused with the Nernst-Planck differential equation (2) that describes diffusion**)** for the permeable ion. Most channels are not so selective and allow biologically and experimentally significant flux of several types of ions at all potentials and concentration gradients. Even when the potential and concentration gradients are arranged so that one ion is at equilibrium, (i.e., the potential and concentration gradients across the channel satisfy the Nernst equation for that one ion), and so the flux of that ion is zero, other ions are away from equilibrium, and do ***not*** satisfy their own Nernst equation, and do carry flux that cannot be ignored. Thus, most channels cannot be analyzed ***under any experimental conditions*** by an equilibrium theory. They cannot be simulated by a molecular dynamics calculation that has spatially uniform boundary conditions.

The importance of nonequilibrium effects is illustrated in figures …

John and I are not sure whether the following should be included or not. We know it

is rather philosophical and self-indulgent for a book and so are inclined to cut it out.

On the other hand, I am reluctant to leave the impression that a physical analysis of this type can be easily and automatically applied to all properties of channels. I would be accused of being misleading by my colleagues if we did that.

And all too many physical scientists have analyzed biological systems prematurely, or have analyzed the wrong property of biological systems (i.e., Frauenfelder) so the warning to study the natural function of systems of known structure may be useful many of your reader.

So John and I think the authors should read, consider, and vote on whether to include the following, delete the following, or revise it.

Biological Implications. Most biological systems have much more complicated structure than channels, and often the structure is not well known, and even the physical principles involved may be in dispute. The reader must not be misled that the kind of physical analysis appropriate for open channels is immediately appropriate for other biological systems.

In particular, this kind of analysis cannot yet be applied to the processes that open and close (i.e., 'gate') channels, because the structural basis and mode of operation of those processes are not understood[p. 479–481 of Hille, 1992 #37]. Most channologists think channels open and close by changing their shape. It is also possible that they become permeable and impermeable by raising and lowering barriers of electrical or chemical potential. When studying gating, we neither understand the structures involved, nor the physical principles? Fortunately, experiments allow us to separate the properties of the open channel from the properties of gating, when currents are measured from one channel protein at a time. Those experiments, plus the simple structure and natural function of the open channel, allows our analysis of open channels as holes in the wall.

The contrast between the study of the open channel, and the gating of channels illustrates a general point. Physical analysis of a living system is not very useful until its structure and basic mode of operation has been described. Adaptations used by evolution to solve its problems are often fanciful, not obviously logical, or easy to guess, and so the structure and physical principles used to solve evolution's problem must be known ***before*** much physical analysis is useful.

The study of the open channel is relatively easy for another reason. The natural function of the channel can be directly measured and is produced by one physical mechanism that is fairly easy to describe. It is usually much easier physically, and always much more rewarding biologically, to study the natural function of a protein, than other properties of a protein, for example, the location or interactions with light of atoms not directly involved in the function of the protein. The natural function of a protein is likely to be more robust, and easier to describe by a simple model under a range of experimental conditions than the location of a specific atom having no key role in the work of the molecule.

Implications. Open channels provide a link among the communities of scientists who study electrochemical systems, who study enzymes, and who study transistors. It will be interesting to see if the physical insights of the semiconductor community—used to study charge transport in macroscopic systems with complex structure and (spatially nonuniform) boundary conditions, far from equilibrium, in atomic detail, on femtosecond time scales[Hess, 1991 #301; Hess, 1991 #302]—can be applied to the study of the atomic and molecular dynamics of electrolyte solutions, proteins, and channels.