

Study of the  $I$ - $V$  relations predicted by PNP in many solutions might help in developing insight into its qualitative behavior, and many such plots have been made (mostly by our collaborator and friend Wolfgang Nonner, who has generously shared them with us). But “the goal of computing is insight, not numbers,” and that goal has not yet been reached by extensive computation of PNP.

More promising is the analytical path. Barcilon et al. (1997) and Charles Peskin (personal communication) have used singular perturbation theory (cf. Kevorkian and Cole, 1996) to determine general qualitative properties of the PNP equations. Peskin has made particular progress by exploiting the large value of the permanent charge (in his *Lecture Notes on Neurobiology*). Peskin et al., (unpublished studies) are working on a related analysis of PNP itself, which should yield insight into CRC behavior, because it can be reasonably described by a uniform large  $P(x) = P_0$ .

Clearly, much more work is needed to test and then (it is hoped) exploit the PNP model of CRC. Measurements should be made in a wide variety of monovalent ions, to see if selectivity (of this type) is reasonably described. Modifications of the protein should be made (particularly of the permanent charge), and the effects on  $I$ - $V$  curves predicted and measured. Most importantly, the theory and experiments should be extended to include the divalent ions of greatest functional interest in this channel.

## APPENDIX: RATE CONSTANTS IN CHANNOLOGY

Rate models are used so widely in channology (Hille, 1975; Hille and Schwartz, 1978; Eisenman and Horn, 1983; Lauger, 1991; Andersen and Koeppe, 1992; Hille, 1992, are modern references) that we think it necessary to show explicitly how they arise in a diffusion theory applied to open channels.

Rate models of channels grew from the rate theory (sometimes called “transition state theory”) of chemical reactions developed in quantum chemistry in the 1930s. Despite its popularity then, chemists realized that rate theory must be derived (Laidler and King, 1983). It is not a fundamental physical law of either quantum or statistical mechanics, and its use must be justified by derivation, simulation, and experimentation.

Rate theory was derived in two different traditions: those of equilibrium statistical mechanics (Johnson et al., 1974; Hille, 1975; Pechukas, 1976; Chandler, 1978; Hille and Schwartz, 1978; Eisenman and Horn, 1983; Levine and Bernstein, 1987; Steinfeld et al., 1989; Lauger, 1991; Andersen and Koeppe, 1992; Hille, 1992) and diffusion theory (evidently started by Kramers, 1940; see the definitive review of Hanggi et al., 1990, citing some 700 other references; also see the textbook presentations of Berry et al., 1980, and Robinson and Holbrook, 1972, and the recent book by Fleming and Hanggi, 1993, which contains a number of articles joining the two traditions).

The tradition of statistical mechanics has difficulty accommodating flux, because flux of all types vanishes at equilibrium, where statistical mechanics is derived. Thus phenomena that occur only when macroscopic flux flows (e.g., friction or frictional heating) are not natural components of theories in statistical mechanics.

The tradition of diffusion theory has difficulty accommodating atomic detail. Frictional phenomena are natural parts of diffusion theories, but the equations of molecular dynamics used to describe molecular motion in atomic detail do not include diffusion coefficients or explicit treatments of friction.

Statistical mechanics and diffusion theory must both be extended if their relationship is to be understood. Equilibrium ideas (like free energy and its

components, energy and entropy) and atomic resolution must be present in the (extended) diffusion theories; and nonequilibrium ideas, like friction, must be present in the (extended) equilibrium theories.

The diffusion theory of channels started historically with the Nernst-Planck equations, the diffusion equations describing the concentration of charged particles, each of which following the random trajectory necessarily produced by friction (Goldman, 1943; Hodgkin and Katz, 1949; Hall et al., 1973; Levitt, 1982, 1984, 1985, 1986, 1987). Kim Cooper, then a graduate student of the biophysicist Eric Jakobsson and physical chemist Peter Wolynes, was (as far as we know) the first to use Langevin equations to describe the random trajectory of ions in a channel (Cooper et al., 1985, 1988a,b). We (and others) followed his lead. Eisenberg et al. (1995) provided a stochastic derivation of the Nernst-Planck equations, showing how those equations describe the probability density function for the location of an ion moving in a random trajectory. The stochastic derivation rationalized the analysis and demonstrated the generality of the simulations of Barcilon et al. (1993).

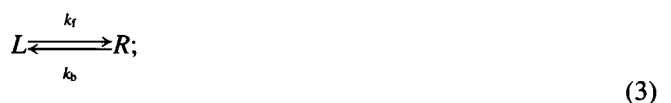
The stochastic derivation provides a pleasingly intuitive result. The flux of trajectories (and ions) is the sum of two unidirectional fluxes, each the product of a “source” concentration, “diffusion velocity” ( $D_j/d$ ) and the appropriate conditional probability:

$$J_j = d[k_f C_j(L) - k_b C_j(R)] \quad (2)$$

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

Note that the total flux cannot itself be described (in any natural way) by a (single unconditional) probability, nor can the mean first passage time or contents of an ion in a channel. All of these quantities must be replaced by the appropriate (pairs of) conditional quantities because a number of the unconditional quantities are infinite in perfectly finite and well-posed situations, as found by Barcilon et al. (1993) and Eisenberg et al. (1995).

When the unconditional quantities are replaced by the appropriate conditional quantities, the flux through the channel can be described in a simple manner, e.g., as a unimolecular chemical reaction (Robinson and Holbrook, 1972):



$$\text{where } k_f \equiv \frac{D_j}{d^2} \text{Prob}\{R|L\}; \quad \text{and } k_b \equiv \frac{D_j}{d^2} \text{Prob}\{L|R\}$$

In words: each flux can be described as a (unidirectional) chemical reaction without approximation, for any potential barrier  $\Phi(x)$  with rate constants  $k_f$  and  $k_b$  (units:  $s^{-1}$ ), determined by the conditional probabilities and diffusion velocities shown in Eq. 2, when concentration boundary conditions are in force that describe mathematically the constant-concentration/constant-potential conditions of a voltage-clamp experiment.

The conditional probabilities of Eqs. 2 and 3 require precise definition, including two boundary conditions that doubly condition the underlying trajectories, which must be described by the full (not reduced) Langevin equation, to allow the double conditioning. It was the assignment of these trajectories and boundary conditions that allowed Eisenberg et al. (1995) to specify and solve this problem, using the techniques of Schuss (1980) and Naeh et al. (1990).

The conditional probabilities of Eqs. 2 and 3 can be determined entirely numerically, by computing a random walk, or by simulating a full or reduced Langevin equation. All three numerical calculations are shown by Barcilon et al. (1993) (e.g., Figs. 4 and 5) (see also Cooper et al., 1985; Chiu and Jakobsson, 1989; Eisenberg et al., 1995). The conditional probabilities might also be determined from the simulations in atomic detail of

molecular dynamics (McCammon and Harvey, 1987; Brooks et al., 1988; Haile, 1992) or by using the Onsager-Machlup action formulation of Newton's laws, in the presence of thermal agitation (Onsager and Machlup, 1953; see modern application: Elber, 1996). The simulations fortunately require much less time than the derivation (Eisenberg et al., 1995) of their boundary conditions, which took many of us years of work (Cooper et al., 1985, 1988a,b; Chiu and Jakobsson, 1989; Barcilon et al., 1993).

Equations 2 and 3 are derived by using stochastic identities that merely assume the existence of conditional probabilities of location and so are true for a wide range of stochastic trajectories. Thus the derivation establishes the chemical reaction as a model of the open channel, and the meaning of the Nernst-Planck equations mathematically, without physical argument beyond that used in deriving the model of the open channel in the first place.

In the general case we can conclude, then, that the chemical reaction and Nernst-Planck equations are not a (perhaps vaguely derived) continuum approximation, but rather are an exact representation and description, even in atomic detail, if they use the conditional probability density functions of the location of discrete particles, as defined above and in the cited references. (See above. The meaning of the average potential profile  $\varphi(x)$  of the Nernst-Planck equations is more subtle, if not problematic, and is discussed at length in Eisenberg (1996).)

In a special case, when friction is large (as in channels on the biological time scale) and well behaved (characterized by a single number  $D_j$  for each ionic species  $j$ ), the statistics of the conditional trajectories (e.g., mean flux, first passage times, and channel contents of left and right trajectories) can be determined analytically (Eisenberg et al., 1995), using mathematical techniques developed by Schuss. In that special case, the conditional probability and rate constant can be written as

$$\frac{D_j}{d^2} \text{Prob}\{R|L\} \equiv k_f = \frac{D_j}{d^2} \cdot \frac{\exp(z_j V)}{1/d \int_0^d \exp[z_j \varphi(x)/kT] dx} \quad (4)$$

The normalized transmembrane potential  $V$  is defined as  $V \equiv eV_{\text{app}}/kT$ .

If the potential profile  $\Phi(x)$  is dominated by a large barrier, and satisfies certain other criteria, expressions for rate constants reduce to exponential expressions (Section viii of Barcilon et al., 1993; and eq. 8.4 and 8.5 of Eisenberg et al., 1995) reminiscent of rate expressions of reaction rate theory used widely in channology (Hille, 1975; Hille and Schwartz, 1978; Eisenman and Horn, 1983; Luger, 1991; Hille, 1992). However, the prefactor (of the exponential expression derived from) diffusion theory is physically very different because it depends explicitly on friction, as noted by many biophysicists (see Cooper et al., 1985, 1988a,b; Chiu and Jakobsson, 1989; Luger, 1991; Roux and Karplus, 1991; Andersen and Koeppe, 1992; Barcilon et al., 1993; Crouzy et al., 1994; Eisenberg et al., 1995) and even more physical chemists (Hanggi et al., 1990). For example,

$$k_f \xrightarrow[\text{barrier}]{\text{high}} \frac{D_j}{d \sqrt{2\pi}} \sqrt{|z_j \Phi''(x_{\text{max}})|} \exp[z_j V - z_j \Phi_{\text{max}}(x_{\text{max}})] \quad (5)$$

PREFACTOR

There is no controversy in the chemical literature about this expression or its prefactor. Exactly this expression is widely used there to describe the flux over high barriers. (The large barrier result is derived in the equilibrium tradition in Robinson and Holbrook (1972); Johnson et al., (1974); Pechukas (1976); Berry et al. (1980); Levine and Bernstein (1987); Steinfeld et al. (1989). The prefactor in those expressions is not simply  $kT/h$ ; it includes a ratio of (factors of the grand) partition functions as well, and is in agreement with much experimental data. Fleming et al. (1986) and Schroeder and Troe (1993) both present and cite the large experimental literature.

The large barrier result is derived in the diffusion tradition by Kramers (1940), Gardiner (1985), Hynes (1985, 1986), Berne et al. (1988), Hanggi et al. (1990), and Fleming and Hanggi (1993). Many derivations (in both the equilibrium and diffusion traditions) are given by Hanggi et al. (1990), as are a detailed discussion of the prefactor and numerous ( $\sim 700$ ) references to the historical and modern literature. Fleming and Hanggi (1993)

describe the current state of knowledge: they include articles describing experimental measurement of the prefactor, a succinct reconciliation of equilibrium and rate constant traditions using variational theory, and a powerful description of the limitations of any one-dimensional theory, along with other useful articles.

It is important to note that many modern books and reviews on transport (McQuarrie, 1976; Friedman, 1985; Ma, 1985; Chandler, 1987; Mason and McDaniel, 1988; Smith and Jensen, 1989; Spohn, 1991; Balian, 1992; Mahan, 1993; Bird, 1994; Cercignani et al., 1994; Garrod, 1995) hardly mention barrier or rate models at all, preferring to deal with the general situation, in which barriers can have any shape or size, which some channologists prefer (Hall et al., 1973; Schuss, 1980; Levitt, 1982, 1984, 1985, 1986, 1987; Cooper et al., 1985, 1988a,b; Chiu and Jakobsson, 1989; Barcilon et al., 1993; Eisenberg et al., 1995; Bek and Jakobsson, 1994).

At first glance, the typical system of the chemical literature seems quite different from a channel. In most chemical experiments involving flux over high barriers, concentrations change as the flux flows, in contrast to most channel experiments in which concentrations (and potentials) are kept constant (as flux flows) by the active intervention of experimental equipment (i.e., by stirring or perfusion and by the voltage/patch-clamp amplifier). However, in one special case—when barriers are high enough—these different experimental conditions produce similar fluxes (Barcilon et al., 1993; Eisenberg et al., 1995): high enough barriers are rate-limiting in both cases, even though experimental conditions are different, as are the boundary conditions that describe them mathematically. When barriers are high enough, the chemical and channel systems are nearly the same, probably because in that special case the system is nearly at equilibrium and experimental and boundary conditions do not matter very much.

The numerical value of the prefactor of Eq. 5 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  $\Phi_{\text{max}}(x_{\text{max}})$ , much larger than the applied (i.e., transmembrane) potential  $V$ . Then, for example,

$$k_f \xrightarrow[\text{high barrier}]{\text{PARABOLIC}} \frac{2D_j}{d^2 \sqrt{\pi}} \sqrt{|z_j e \varphi_{\text{max}}(x_{\text{max}})/kT|} \exp[-z_j e \varphi_{\text{max}}(x_{\text{max}})/kT] \quad (6)$$

PREFACTOR

where we use the dimensional potential  $\varphi(x) = \Phi(x)kT/e$  and  $x_{\text{max}} = d/2$ . The diffusion (i.e., Kramers) prefactor depends on the diffusion coefficient and channel length, which do not appear in the hopping prefactor  $kT/h$  at all. The diffusion prefactor varies inversely with the (square root of the) temperature, whereas the hopping prefactor depends linearly on temperature. The Kramers prefactor depends on the type of permeating particle; the hopping prefactor is independent of the type of particle(!). These different properties have made it easy for chemists to determine the prefactor that actually describes the properties of solutions and condensed phases (Fleming et al., 1986; Schroeder and Troe, 1993).

Now if the barrier is, say,  $4kT/e$  high and 1 nm long and the diffusion coefficient is some  $1.3 \times 10^{-6} \text{ cm}^2/\text{s}$ —as we find for  $\text{K}^+$  in the "filter" of the CRC channel, which is not dissimilar to the values others find for other channels (Dani and Levitt, 1981; Chen et al., 1995b, 1997; Tang et al., 1997)—the numerical value of the (diffusion expression for the) prefactor (for  $\text{K}^+$ ) is  $\sim 2.8 \times 10^8 \text{ s}^{-1}$ . The numerical value of the usual prefactor in the hopping theory is  $kT/h$ , which is  $\sim 2.2 \times 10^4$  times larger,  $\sim 6.3 \times 10^{12} \text{ s}^{-1}$  at biological temperatures. As one might expect, ions hopping over barriers experience much less friction than ions diffusing over them, and the amount of friction will depend on the identity of the ion.

The effect of friction (i.e., the ratio of the two expressions for the prefactor, one general, the other for  $\text{K}^+$ ) is numerically equivalent to a change in the potential barrier of  $\ln(2.2 \times 10^4) \approx 10kT/e$ . For example, a barrier of height  $13kT/e$ , analyzed with the  $kT/h$  prefactor, produces the same rate constant as a barrier of height  $3kT/e$ , analyzed with the Kramers prefactor. Or, in a more ominous example, a barrier of  $10kT/e$ —which is more than large enough to be described by the high barrier approximation in Eq. 5 or 6—becomes 0  $kT/e$ , which cannot be described by a high

barrier approximation, because it is no barrier at all. Indeed, almost all barrier models of open channels use  $kT/h$  as a prefactor and postulate barriers in the range of  $3kT/e$  to  $12kT/e$ ; for example, the barrier heights used to model CRC are  $5.5kT/e$  (table 1 in Tinker et al., 1992, p. 498). Barrier models with such barriers cannot come close to fitting the open-channel current found in most channels (Conley, 1996a,b, 1997) if the correct prefactor is used.

It is evidently quite important to settle on the correct value of the prefactor for channel permeation before a high barrier approximation is used. The channel length  $d$  is unlikely to be short enough; the diffusion coefficient  $D_j$  to be large enough, or the same for different ions; or the potential barrier  $|\varphi_{\max}|$  to be large enough to allow  $kT/h$  to approximate the diffusive prefactor:

$$\frac{2D_j}{d^2 \sqrt{\pi}} \sqrt{|z_j e \varphi_{\max}(x_{\max})/kT|}$$

Of course, even if the numerical values were not too different, the meaning of the prefactors would be very different, because their temperature dependence is so different, and one depends on friction and the height of the potential barrier, the identity of the permeating ion, and the length of the channel, whereas the other looks more like a "constant of nature," independent as it is of the properties of the channel and ion.

Careful reading of the classical theories of barrier crossing in channology (Hille, 1975, 1992; Hille and Schwartz, 1978) shows us how to reconcile the two treatments. Those theories have defined a barrier height by its free energy (temperature times entropy plus electrical energy), not its (electrical) energy. In that case, the two treatments and prefactors can be reconciled if the frictional prefactor of diffusion theory is equated to the "activation entropy" of rate theory.

Unfortunately, the "activation entropy" is not likely to be small or have a small effect, or be the same under all conditions of biological and experimental interest, because the trajectories of the ion (that determine the entropy) are quite different qualitatively and quantitatively in the bath and in the channel. The motion is three-dimensional in the bath but (nearly) one-dimensional in the channel; and the diffusion coefficient of ions in the bath is generally much higher than in the channel's pore (Dani and Levitt, 1981).

The activation entropy of traditional barrier theories is more vaguely defined than the prefactor of diffusion theory, until the dependence of activation entropy on temperature, friction, and channel length is determined. This dependence is not derived or displayed in traditional theories of barrier crossing or in most barrier models, either, and so data measured with different permeating ions (and thus, most likely, unequal diffusion coefficients), at different temperatures, or in systems with unequal channel lengths, cannot be compared by using the "activation entropy" or "activation free energy" (Hille, 1975, 1992; Hille and Schwartz, 1978) formulation. Measurements of the value and functional dependence of the prefactor (on temperature, diffusion constant, etc.) are available in the chemical literature (e.g., Fleming et al., 1986; Hänggi et al., 1990; Fleming and Hänggi, 1993; Schroeder and Troe, 1993). They are incompatible with the expression  $kT/h$ , and in fact are close to the Kramers expression (Eq. 5) or its generalizations, under a wide range of conditions in many systems.

"Barrier heights" determined experimentally in channology (using rate theory with the  $kT/h$  prefactor) represent the free energy barrier to ion translocation. Free energies are, of course, a perfectly adequate representation of barrier heights (if barriers in open channels are in fact high), as long as the free energy is not confused with the potential energy: free energy includes entropy, and the entropy term changes current by a factor of  $\sim 2 \times 10^4$ , as we have seen. Thus a verbal model or mathematical theory (or simulation of molecular dynamics) must compute the entropy as well as the energy if it is to be compared with experimental estimates of barrier heights.

If a theory calculates just the barrier of potential energy—using Coulomb's law or Poisson's equation or a verbal version of either, to describe binding at a charged site, for example—it must not ignore the difference between potential energy and free energy, it must not ignore the entropy component of free energy, and it must not use  $kT/h$  as the prefactor, or large

errors ( $\sim 2.2 \times 10^4$ ) will occur in predictions of the current or estimates of barrier height ( $\sim 10 kT/e$ ). In particular, molecular models of binding sites, whether verbal or quantitative, must explicitly estimate both the energy and entropy terms if serious quantitative errors are to be avoided, as we have seen.

If a barrier model ignores the dependence of the entropy term on the type of permeating ion, or if it ignores the dependence on the diffusion coefficient, temperature, barrier height, and channel length, serious qualitative errors are likely to occur as well. In particular, traditional barrier models are likely to give qualitatively misleading results (because they use  $kT/h$  as a prefactor) if they are used to compare experiments involving different ions (with different diffusion coefficients and thus different prefactors and activation entropies), experiments with mutated or modified channels (which have modified potential barriers and thus modified prefactors and activation entropies), or experiments with different concentrations of ions (which are likely to have different potential barriers (Eisenberg, 1996) and thus different prefactors and activation entropies).

We have seen that the general expressions, Eqs. 2–4, determine the flux and (and its rate constant) exactly, for small as well as large barriers, without concern about prefactors. The general expressions have unambiguous meaning, and their functional dependence is widely accepted in the chemical literature. They are simple to compute, using generally available software that takes virtually no time to execute. Presumably for these reasons, a number of chemists do not use the high barrier theories at all (citations above). Perhaps channologists should follow this practice, at least when studying open channels.

It seems worthwhile to list the difficulties (documented in this Appendix and the Results) facing traditional barrier models of open channels, so that scientists can be aware of what they are assuming when they use them:

1. Barrier models of channels are based on a view of the trajectories of ionic motion in condensed phases which has been shown to be false, both experimentally and theoretically. Ions do not hop as they move in such systems; rather, they follow diffusive, nearly fractal paths.

2. Barrier models of channels assume potential barriers that are independent of the concentration of ions in the baths and of transmembrane potential. That is to say, they ignore the effects of the charged contents of the channel (and other mobile charges) on the potential barrier. These effects are large; indeed, these effects are what allow PNP to fit data under so many conditions from so many channels. Thus ignoring these effects is likely to lead to qualitative errors in understanding (Eisenberg, 1996). It is important to add that the existence of these effects (and their approximate size) does not depend on details or assumptions of the PNP model.

3. Barrier models of channels assume a prefactor that is independent of the type of ion, particularly of its diffusion coefficient. In fact, traditional barrier models of channels use a prefactor that is different from that derived, simulated, or measured experimentally in condensed phases. The traditional prefactor has no dependence on the type of permeating ion, its friction, or on channel length, and it has the wrong dependence on temperature. These dependencies are not just theoretical constructs; they have been measured by chemists in much experimental work on barrier crossing in condensed phases.

Thus it seems unwise to use barrier models (with the traditional prefactor) as they have often been used, namely, to compare the permeation of different ions, unless one has evidence that different ions experience the same friction and have other identical properties, as discussed previously.

4. Traditional barrier models use the wrong numerical value for the prefactor. For CRCs the traditional prefactor is numerically too large by a large factor,  $2.2 \times 10^4$  for  $K^+$ .

5. Barrier models predict much less current than flows in most open channels, if they use the correct prefactor and the barrier is higher than  $\sim 3kT/e$ . The conductance of the traditional model of CRC, predicted using the correct prefactor, using a parabolic barrier  $3kT/e$  high, and using the parameters of the CRC channel reported here, is some 2 pS, in 100 mM KCl, much less than the hundreds of picosiemens we find. Evidently barriers are low in most open channels. If this is so, traditional barrier theory makes no sense.

6. Barrier models describe the effects of mutations in channel proteins only vaguely because they do not include Poisson's equation (or, equiva-

lently, Coulomb's law applied to all charges) to show how a mutation in a protein, which often changes the fixed charge lining the wall of the protein's pore, changes the potential profile, barrier height, or rate constant for flux.

Given these difficulties, it is not surprising that barrier models of channels are unable to fit the currents measured in a number of types of channels (if measurements are made over a wide range of potentials and in a wide range of solutions) and that they are of quite limited use in understanding the general phenomena of selectivity or the specific effects of mutations in channel proteins.

## CONCLUSION

It seems to us that the time has come to abandon barrier models of the CRC channel and perhaps of other open channels as well. It seems reasonable to us to see how well PNP can serve as a replacement, by checking its predictions over a wide range potentials, in a wide range of ions and mixtures of ions.

On theoretical grounds, it seems unlikely to us that PNP in its present form will be adequate to this task. Nonetheless, an adequate replacement is likely to preserve PNP's main features, namely, the description of the channel as a distribution of permanent charge, and the calculation of the potential and concentration profiles, and flux, as the self-consistent solution of Poisson and transport equations.

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