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Permeation through the Calcium Release Channel of Cardiac Muscle

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Abstract

Current voltage (*IV*) relations were measured from the calcium release channel (*CRC*) of the sarcoplasmic reticulum of cardiac muscle in twelve KCl solutions, symmetrical and asymmetrical, from 25 mM to 2 M. *IV* curves are nearly linear, in the voltage range $\pm 150\text{mV} \approx 12kT/e$, even in asymmetrical solutions, e.g., $2\text{M} \| 100\text{mM}$. It is awkward to describe straight lines as sums of exponentials in a wide range of solutions and potentials and so traditional barrier models have difficulty fitting this data. Diffusion theories with constant fields predict curvilinear *IV* relations and so they are also unsatisfactory.

The Poisson and Nernst-Planck equations (*PNP*) form a diffusion theory with variable fields. They fit the data using adjustable parameters for the diffusion constant of each ion and for the effective density of fixed, i.e., permanent charge $P(x)$ along the channel's 'filter' (7Å diameter, 10Å long). If $P(x)$ is described by just one parameter, independent of x, (i.e., $P(x) = P_0 = -4.2\text{M}$), the fits are satisfactory (RMS error/RMS current = 6.4/67) and the estimates of diffusion coefficients are reasonable $D_K = 1.3 \times 10^{-6}$, $D_{Cl} = 3.9 \times 10^{-6}$ cm^2/sec. *CRC* seems to have a small selectivity filter with a very high density of permanent charge. This may be a design principle of channels specialized for large flux.

The Appendix derives barrier models, and their prefactor, from diffusion theories (with variable fields) and argues that barrier models are poor descriptions of *CRC* in particular and open channels in general.

Appendix

Rate Constants in Channology

Rate models are used so widely in channology (Hille, 1975; Hille and Schwartz, 1978; Eisenman and Horn, 1983; Läuger, 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 1930's. 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, the tradition of equilibrium statistical mechanics (Johnson, Eyring and Stover, 1974; Hille, 1975; Pechukas, 1976; Chandler, 1978; Hille and Schwartz, 1978; Eisenman and Horn, 1983; Levine and Bernstein, 1987; Steinfeld, Francisco and Hase, 1989; Läuger, 1991; Andersen and Koeppe, 1992; Hille, 1992), or the tradition of diffusion theory (evidently started by Kramers, 1940; see the definitive review of Hänggi *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 Hänggi, 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 difficult 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) 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 follows the random trajectory necessarily produced by friction (Goldman, 1943; Hodgkin and Katz, 1949; Hall, Mead and Szabo, 1973; Levitt, 1982; Levitt, 1984; Levitt, 1985; Levitt, 1986; Levitt, 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; Cooper *et al.*, 1988b; Cooper *et al.*, 1988a). 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.

$$J_j = d\left[k_f C_j(L) - k_b C_j(R)\right] \equiv \overbrace{\underbrace{C_j(L)}_{\substack{\text{Source}\\ \text{Concentration}}}\underbrace{\left(\frac{D_j}{d}\right)}_{\substack{\text{Diffusion}\\ \text{Velocity}}}\underbrace{\text{Prob}\{R|L\}}_{\substack{\text{Conditional}\\ \text{Probability}}}}^{\text{Unidirectional Efflux}} - \overbrace{C_j(R)\left(\frac{D_j}{d}\right)\text{Prob}\{L|R\}}^{\text{Unidirectional Infflux}} \qquad (1)$$

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 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; 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)

$$L \underset{k_b}{\overset{k_f}{\rightleftarrows}} R\ ; \qquad \text{where } k_f \equiv \frac{D_j}{d^2}\text{Prob}\{R|L\}\ ;\ \text{and } k_b \equiv \frac{D_j}{d^2}\text{Prob}\{L|R\} \qquad (2)$$

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 & k_b (units: sec^{-1}) determined by the conditional probabilities and diffusion velocities shown in eq. (1), 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 equations (1) & (2) 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, Klosek, Matkowsky and Schuss, 1990.

The conditional probabilities of equations (1) & (2) 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 in Barcilon *et al.*, 1993 e.g., Fig. 4 & 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; Cooper *et al.*, 1988b; Cooper *et al.*, 1988a; Chiu and Jakobsson, 1989; Barcilon *et al.*, 1993).

Eq. (1) – (2) are derived 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 location of discrete particles, as defined above and in the cited references.[1]

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 *L*eft and *R*ight 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

$$\frac{D_j}{d^2}\,\mathrm{Prob}\{R|L\} \equiv k_f = \frac{D_j}{d^2}\cdot\frac{\exp(z_jV)}{\frac{1}{d}\int_0^d \exp\left[z_j\varphi(x)/kT\right]dx} \tag{3}$$

The normalized *trans*membrane potential *V* is defined as $V \equiv \boldsymbol{e}V_{appl}/kT$.

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 & 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; Läuger, 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; Cooper *et al.*, 1988a; Cooper *et al.*, 1988b; Chiu and Jakobsson, 1989; Läuger, 1991; Roux and Karplus, 1991; Andersen and Koeppe, 1992; Barcilon *et al.*, 1993; Crouzy, Woolf and Roux, 1994; Eisenberg *et al.*, 1995). For example,

$$k_f \xrightarrow[\textbf{barrier}]{\textbf{high}} \underbrace{\frac{D_j}{d\sqrt{2\pi}}\sqrt{\left|z_j\Phi''(x_{\max})\right|}}_{\textbf{PREFACTOR}}\exp\left[z_jV - z_j\Phi_{\max}(x_{\max})\right] \tag{4}$$

There is no controversy in the chemical literature about this expression (4) or its prefactor. Exactly this expression is widely used there[2] to describe the flux over high barriers.

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 where 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. (4) 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 $\Phi_{\max}(x_{\max})$, much larger than the applied (i.e., *trans*membrane) potential *V.* Then, for example,

$$k_f \xrightarrow[\textbf{high barrier}]{\textit{PARABOLIC}} \underbrace{\frac{2D_j}{d^2\sqrt{\pi}}\sqrt{\left|z_j e\varphi_{\max}(x_{\max})/kT\right|}}_{\textit{PREFACTOR}} \exp\left[-z_j e\varphi_{\max}(x_{\max})/kT\right]; \tag{5}$$

where we use the dimensional potential $\varphi(x) \equiv \Phi(x)kT/\boldsymbol{e}$ and $x_{\max} = d/2$. 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 check which prefactor actually describes the properties of solutions and condensed phases (Fleming *et al.*, 1986; Schroeder and Troe, 1993).

Now, if the barrier is (say) 4***kT/e*** high and 1 nm long and the diffusion coefficient is some 1.3×10^{-6} cm^2/sec—as we find for 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; Chen *et al.*, 1997; Tang *et al.*, 1997)—the numerical value of the (diffusion expression for the) prefactor (for K^+) is about 2.8×10^8 sec^{-1}. The numerical value of the usual prefactor in the hopping theory is *kT/h*, which is ~2.2 × 10^4 times larger, about 6.3×10^{12} sec^{-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 the 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 K^+) is numerically equivalent to a change in the potential barrier of ln (2.2 × 10^4) ≅ 10 ***kT/e***. For example, a barrier of height 13 ***kT/e***, analyzed with the *kT/h* prefactor produces the same rate constant as a barrier of height 3 ***kT/e***, analyzed with the Kramers' prefactor. Or, in a more ominous example, a barrier of 10 ***kT/e***—which barrier is more than large enough to be described by the high barrier approximation (4) or (5)—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 3 ***kT/e*** to 12 ***kT/e***: for example, the barrier heights used to model *CRC* are 5.5 ***kT/e*** (Tinker *et al.*, 1992: Table 1, p. 498). Barrier models with such barriers cannot come close to fitting the open channel current found in most channels (Conley, 1996a; Conley, 1996b; Conley, 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; nor the diffusion coefficient D_j large enough, nor the same for different ions; nor the potential barrier $|\varphi_{\max}|$ large enough to allow *kT/h* to approximate the diffusive prefactor $\frac{2D_j}{d^2\sqrt{\pi}}\sqrt{\left|z_j e\varphi_{\max}(x_{\max})/kT\right|}$.

Of course, even if the numerical values were not too different, the meaning of the prefactors would be very different, since 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, while 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; Hille and Schwartz, 1978; Hille, 1992) shows 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 in 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 are 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 using the 'activation entropy' or 'activation free energy' (Hille, 1975; Hille and Schwartz, 1978; Hille, 1992) 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. (4), 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. Otherwise, errors some 10 $kT/\boldsymbol{e}$ will occur in estimates of the barrier height, as we have seen.

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, it must not use kT/h as the prefactor, or large errors will occur in predictions of the current. In particular, molecular models of binding sites, *whether verbal or quantitative*, must explicitly estimate both the energy and entropy term 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 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 have different prefactors and activation entropies).

We have seen that the *general* expressions (1) – (3) 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 in last paragraph of footnote 2). Perhaps, channologists should follow that practice, at least when studying open channels.

It seems worthwhile to list the difficulties (documented in this Appendix and the Results Sections) facing traditional barrier models of open channels, so 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 independent of the concentration of ions in the baths and independent of *trans*membrane 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 like to lead to qualitative errors in understanding (Eisenberg, 1996). It is important to add that the existence of these effects (and their approximate size) do not depend on details or assumptions of the PNP model.
3) Barrier models of channels assume a prefactor independent of the type of ion, in particular of its diffusion coefficient. In fact, traditional barrier models of channels use a prefactor 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 of the prefactor. For CRC the traditional prefactor is numerically too large by a large factor, 2.2×10^4 for K^+, which produces an overestimate of (a single large) barrier of ~10 kT/e.
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 ~3 kT/e. The conductance of the traditional model of CRC, predicted using the correct prefactor, using a parabolic barrier 3 kT/e high, and using the parameters of the CRC channel reported here, is some 2 psiemens, in 100 mM KCl, much less than the hundreds of picosiemens we find. Evidently barriers are low in most open channels. If that 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, equivalently, 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 *un*likely 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.

Table 1
Solutions

	Concentration (mM)											
Cis	**250**	**250**	**250**	**250**	**250**	**250**	**250**	**1000**	**1000**	**1000**	**1000**	**2000**
Trans	**250**	**2000**	**1000**	**500**	**100**	**50**	**25**	**1000**	**500**	**250**	**100**	**100**

Table 2
Parameter Estimates
Uniform Permanent Charge

Parameter Estimate			**Correlation Coefficient**		
(± Standard Deviation)			D_K	D_{Cl}	P_0
1.25×10^{-6} cm^2/sec (± 0.13)	=	D_K	**1**	– 0.88	– 0.998
3.87×10^{-6} cm^2/sec (± 0.44)	=	D_{Cl}	– 0.88	**1**	– 0.88
–4.17 M (± 0.45)	=	P_0	– 0.998	– 0.88	**1**

314 data points from 12 solutions were used to estimate the parameters and correlation coefficients

Table 3

Parameter Estimates
Non-uniform Permanent Charge P(x)

Parameter Estimate (± Standard Deviation)			**Correlation Coefficient** D_K	D_{Cl}	β_1	β_2	β_3	β_4
1.48×10^{-6} cm^2/sec (± 0.2)	=	D_K	**1**	-0.91	-0.87	-0.69	-0.01	0.29
4.12×10^{-6} cm^2/sec (± 0.9)	=	D_{Cl}	-0.91	**1**	-0.94	0.86	0.097	-0.42
– 4.82 M (± 1.1)	=	β_1	-0.87	-0.94	**1**	-0.92	-0.31	0.62
8.12 M (± 2.0)	=	β_2	-0.69	0.86	-0.92	**1**	0.15	-0.50
–4.09 M (± 8.0)	=	β_3	-0.01	0.097	-0.31	0.15	**1**	-0.93
–9.95 M (± 11.3)	=	β_4	0.29	-0.42	0.62	-0.50	-0.93	**1**

The permanent charge is $P(x)=\beta_1+\beta_2 J_0(\pi x/d)+\beta_3 J_0(2\pi x/d)+\beta 4 J_0(3\pi x/d)$ where d is the length of the channel, and J_0 is a Bessel function of the zero order. 314 data points from 12 solutions were used to estimate the parameters and correlation coefficients

FIGURES

Figure 1. The permanent charge profile adopted as the best estimate of $P(x)$. The current voltage relations measured in several solutions, fit with a uniform spatially independent permanent charge, $P(x) = P_0 = -4.2\text{M}$, diffusion coefficients $D_{\text{K}} = 1.3 \times 10^{-6}$, $D_{\text{Cl}} = 3.9 \times 10^{-6}$ cm^2/sec, and dielectric constants of 2, 5 and 10. Curves for $\varepsilon = 2$ and $\varepsilon = 5$ are indistinguishable. Singular values were $\{9016, 306, 14\}$. The theory fits quite well, with RMS error/RMS current $= 6.4/67$ summed over all solutions.

Figure 2. Current voltage relations measured in the solutions indicated. The lines are the best fits of *PNP* with the parameters shown in Table 2 or Table 3. The fits of the two models are not distinguishable by eye. Note that small (5.7%) changes in the value of the uniform fixed (i.e., permanent charge) could account for all the deviations between theory and experiment (see text for details).

Figure 3. Panel A shows the profile of potential in symmetrical 250 mM solutions. Note the large region in which the potential in the baths differs from its bulk value (for this reason the scale of the horizontal axis is different inside the channel $0 < x < 10$Å and outside the channel in the baths, $x < 0$ & $x > 10$Å). This region is so large because the concentration of permanent charge at the ends of the channel is so large (see Fig. 1) compared to the concentration of the bathing solutions. The Debye length in the channel is thus much shorter than the Debye length in the bath. The potential profiles are clearly sensitive functions of *trans*membrane potential and bath concentration. See caption of Figure 4.

Panel B shows the profile of concentration of anions and cations in symmetrical 250 mM solutions with a logarithmic vertical axis and different horizontal scales inside and outside the channel. The concentration profiles show much less dependence on *trans*membrane potential and bath concentration than the potential profiles. This is to be expected from a channel like *CRC* with (nearly) linear *IV* relations, and is not found in channels with more complex profiles of fixed charge and thus more complex *IV* characteristics (Chen *et al.,* 1995, 1996; Tang et al., 1997).

Figure 4. Panel A shows the profile of potential in asymmetrical $250\|50$mM solutions. Note the large region in which the potential in the baths differs from its bulk value (for this reason the scale of the horizontal axis is different inside the channel $0 < x < 10$Å and outside the channel in the baths, $x < 0$ & $x > 10$Å). The potential profiles are clearly sensitive functions of *trans*membrane potential and bath concentration.

Panel B shows the profile of concentration of anions and cations in asymmetrical $250\|50$mM solutions with a logarithmic vertical axis and different horizontal scales inside and outside the channel.

Forward and backwards rate constants for permeation of each ion are determined by the potential profiles as shown in the Appendix, eq. (1)-(3). The dependence is considerable, as documented in the following table. Roughly speaking, in this channel, changing one solution from 250 mM to 50 mM, changes rate constants by a factor of 2 to 10; changing *trans*membrane potential by 100 mV changes forward and backwards rate constants by a factor of 10.

Trans-membrane potential *V* *mV*	K^+ Rate Constant $k_f(K^+)$	K^+ Rate Constant $k_b(K^+)$	Cl^- Rate Constant $k_f(Cl^-)$	Cl^- Rate Constant $k_b(Cl^-)$
	μsec^{-1}	μsec^{-1}	μsec^{-1}	μsec^{-1}
KCl: $250\|250$ mM				
0 mV	148	8.92	412	6838
100 mV	585	0.737	34.0	2.70×10^4
KCl: $250\|50$ mM				
0 mV	59.7	0.722	828	6.85×10^4
100 mV	163	4.12×10^{-2}	47.4	1.87×10^5

Figure 5 shows the potential profile of *CRC* with asymmetric 250||50 salt if (a) there were no ions in the channel or bath (the solid-line), (b) if there were ions in the baths but not in the channel's pore (the dashed-line: – – –), and (c) if the ions are permeable (dot-dash line: — - —). Note that potential profiles, vary substantially with concentration and so the rate constants given in eq. (3) will also vary with concentration.

Figure 6. Comparison of *PNP* and barrier models in several solutions. Broken lines were obtained using the parameters given by Tinker *et al.*, 1992. In particular, the prefactor *kT/h* was used, although the Kramers' expression (see Appendix) would be a much better choice.

Figure 7. Two more complex profile of permanent charge. $P(x) = -4.8 + 8.1\boldsymbol{J}_0(\pi x/d) - 4.1\boldsymbol{J}_0(2\pi x/d) \; -9.9\boldsymbol{J}_0(3\pi x/d)$ (in molar, where x is the location in the channel of length d; $\boldsymbol{J}_0$ is a Bessel function of order zero) and the diffusion constants in the pore are $D_{\mathrm{K}} = 1.5 \times 10^{-6}$, $D_{\mathrm{Cl}} = 4.1 \times 10^{-6}$ cm^2/sec. This profile produces somewhat better fits than a uniform profile. Its singular values are {7621, 281, 22.7, 15.7, 1.83, 0.426} and so the parameters shown in Table 3 seem quite well determined.

When an additional term $\beta_5 \boldsymbol{J}_0(4\pi x/d)$ was added to improve the estimate of $P(x)$, the best least squares value of its coefficient $\beta_5 = -0.52$ was much smaller than the other coefficients, whose average magnitude was some 6.7M. The *SVD* showed that β_5 was reasonably well determined: the singular values were {7645, 277, 22.6, 15.7, 1.97, 0.569, 0.222} and so it seems that just the 3 Bessel functions (and constant) of Fig. 7 'exhaust the (information content of) the data'. They describe the permanent charge as well as it can be described given the limitations of theory and experiment.

The data was also fit with a profile of permanent charge made of the sum of 8 step functions. Note that it converges to give much the same estimate of diffusion coefficients and profile of permanent charge.

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Endnotes

[1] See footnote **Error! Bookmark not defined.**. 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.

[2] 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 in Kramers, 1940; Gardiner, 1985; Hynes, 1985; Hynes, 1986; Berne *et al.*, 1988; Hänggi *et al.*, 1990; Fleming and Hänggi, 1993. Many derivations (in both the equilibrium and diffusion tradition) are given in Hänggi *et al.*, 1990, as well as a detailed discussion of the prefactor, and numerous (~700) references to the historical and modern literature. Fleming and Hänggi, 1993, describes the current state of knowledge: it includes 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, Illner and Pulvirenti, 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, as some channologist prefer as well (Hall *et al.*, 1973; Schuss, 1980; Levitt, 1982; Levitt, 1984; Cooper *et al.*, 1985; Levitt, 1985; Levitt, 1986; Levitt, 1987; Cooper *et al.*, 1988b; Cooper *et al.*, 1988a; Chiu and Jakobsson, 1989; Barcilon *et al.*, 1993; Eisenberg *et al.*, 1995; Bek and Jakobsson, 1994; Eisenberg *et al.*, 1995).