*Perspective*

# Ionic Hopping Defended

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In the whirlwind of cloning, mutagenesis, and, suddenly, structure that the ion channel field has been riding for the past 15 yr, it is easy to forget that we still don't have a satisfactory view of that most basic task carried out by these proteins: ion permeation. The diffusion of ions through the aqueous pores of ion channels (a process much simpler than gating) is being treated in two different ways by two increasingly polarized schools of thought. For want of terms that are both precise and concise, I refer to these as the "chemical-kinetic" and "continuum" descriptions of channel-mediated electrodiffusion—both of which treat ions as stumbling through one-dimensional random walks along the pore. In chemical-kinetic descriptions, ions hop along a small number of binding sites (Hille, 1991); in continuum theories, they diffuse in continuous space along the pore under the influence of local electrochemical gradients (Sten-Knudsen, 1978). This distinction may seem the stuff of academic hairsplitting, but it is not—it is fundamental, and the vituperation spent on these models in recent years attests to their current irreconcilability.

For the present discussion, I assume a qualitative understanding of the basic ideas behind the two competing views of ion conduction (but not necessarily the details of their implementation) and will offer some reasons why I consider the chemical-kinetic approach to be of greater practical utility. Rather than making general arguments about electrodiffusion to defend this position, I will illustrate the current clash of these theories by examining one particular case.

### *An Experimental Example*

The subject we will look upon is calcium channel permeation, models of which have been described in lucid detail (Almers and McCleskey, 1984; Hess and Tsien, 1984; McCleskey, 1997; Nonner and Eisenberg, 1998). This case serves to bring out essential differences in a palpable and physiologically important context. I deal with just one small corner of this subject—the key observation that launched calcium channel permeation as a rich area of investigation. This is the remarkable fact that under physiological conditions the channel is strongly selective for $Ca^{2+}$, but when bath $[Ca^{2+}]$ is reduced below 1 μM this selectivity is lost and monovalent cations easily permeate. In the well-known classical experiment, inward current through calcium channels is measured as a function of external $Ca^{2+}$ concentration in a conventional physiological bath medium at a fixed holding voltage of, say, −30 mV. At very low $Ca^{2+}$ ($<0.1$ μM) there is a large inward current carried by $Na^+$. As $[Ca^{2+}]$ is raised up to ∼100 μM, the current decreases to near zero. But then, as $[Ca^{2+}]$ increases farther into the 1–10 mM range, inward current rises again, with $Ca^{2+}$ as the charge carrier, and the reversal potential shifts positive towards $E_{Ca}$. This nonmonotonic variation in current with external $[Ca^{2+}]$, sometimes termed the "anomalous mole-fraction effect" (AMFE), is explained in vastly different ways by the two opposing viewpoints.

### *Chemical Kinetic Viewpoint: Multiple Occupancy on Discrete Sites*

According to the canonical model, the observed AMFE is a direct reflection of the binding of two $Ca^{2+}$ ions in a single-filing pore. The idea is simple, proceeding from the postulate that the channel is designed to coordinate $Ca^{2+}$ at specific anionic sites. In the absence of $Ca^{2+}$, when these are electrostatically hungry, the pore is merely charge selective, allowing virtually any monovalent cation to permeate as long as it is physically small enough to squeak through. Thus, at low $[Ca^{2+}]$, the $Na^+$ conductance is high. In the presence of micromolar $Ca^{2+}$ concentrations, the pore's selectivity region now becomes occupied by a single $Ca^{2+}$ a significant fraction of the time (which varies according to the bath concentration). Because of its intimate coordination by protein groups, this bound ion's dissociation rate from the channel is low, $\sim 10^3\ s^{-1}$, some three to four orders of magnitude slower than the throughput of $Na^+$ ions. Under these conditions, $Na^+$ roars through the pore when $Ca^{2+}$ is absent; but whenever a $Ca^{2+}$ binds, the flow of $Na^+$ current is fully blocked. This block lasts on the order of 0.1–1 ms, and it is due directly to the single-filing property: the impossibility of a $Na^+$ ion diffusing "around" a bound $Ca^{2+}$. Only after the $Ca^{2+}$ vacates the binding site can the flow of $Na^+$ through the channel resume. This effect, averaged over many channels


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in a macroscopic experiment (or over time in a single-channel experiment), leads to the "falling phase" of the AMFE; i.e., the decrease of inward current as $Ca^{2+}$ increases through the micromolar range. If $Ca^{2+}$ concentration is pushed up into the millimolar range, a new phenomenon appears. Now a second $Ca^{2+}$ can bind and, as a result of this double occupancy, the exit rate of $Ca^{2+}$ from the pore increases ~1,000-fold. This huge increase in $Ca^{2+}$ off rate is usually explained by invoking electrostatic repulsion between the two ions, but other mechanisms could be involved (McCleskey, 1997). In any case, as a result of this double occupancy, $Ca^{2+}$ now flows through the channel at rates high enough to show up as current, which increases with $Ca^{2+}$ concentration to produce the "rising phase" of the AMFE.

To quantify these effects, the chemical-kinetic approach makes an explicit distinction between four different occupancy forms of the channel: a $Na^+$-conducting form with no bound $Ca^{2+}$ [O, O], two nonconducting forms, each with one $Ca^{2+}$ bound on either side [O, X] and [X, O], and a $Ca^{2+}$-conducting form with two $Ca^{2+}$ bound [X, X]. The average current and $Ca^{2+}/Na^+$ selectivity are given by the kinetic transitions among these various forms of the channel; i.e., by a set of rate constants between explicit chemical intermediates, exactly as in any conventional chemical kinetic problem. The values of the rate constants cannot be estimated from first principles, but must be derived by fitting experimental data to a kinetic model, a straightforward but rarely unambiguous procedure.

*Continuum Viewpoint: A Nanoscale Ion Exchanger*

Much of the present controversy centers on the use of Poisson-Nernst-Planck electrodiffusion models in biological channels. Such models have been in use for a long time, both as qualitative handles for the classical squid axon channels and as more intricate frameworks for permeation in ion-selective channels with firm structural foundations (Levitt, 1982, 1986). For this discussion, I will focus on a recent application to calcium channels termed PNP2 (Nonner and Eisenberg, 1998), which views the pore as a continuum containing several negatively charged groups smeared out over a reasonable pore volume; this represents a very high concentration of fixed charge (~10 M). Both $Ca^{2+}$ and $Na^+$ have free access to this forest of negative charge, where they act as gegenions, cations that are not chemically coordinated by the fixed charge, but rather are held nonspecifically by the demands of electroneutrality (or, more properly, the Poisson equation), as in an ion-exchange resin. The system is described by the simultaneous solutions of three equations in which the three crucial variables, ion concentration, electrical potential, and distance along the pore, are nonlinearly entwined. The solutions lead to mathematically self-consistent predictions of ionic current as a function of transmembrane voltage and bath ion concentrations.

In modeling the AMFE, PNP2 is a theory of ionic cleansing. With no $Ca^{2+}$ present, the pore conducts well because $Na^+$ ions dwell there at high concentration, this being the only cation available for electroneutrality. But when a little $Ca^{2+}$ is added to the bath, these divalent intruders, with their heavy artillery in the form of a +2 valence, take over, displacing the numerous but poorly armed $Na^+$ ions. In electrostatics, divalents always beat monovalents. Thus, as $Ca^{2+}$ is increased, the pore, initially $Na^+$ rich, becomes loaded with $Ca^{2+}$, and the conductance goes down because the $Ca^{2+}$ diffusion coefficient is assumed to be lower than that of $Na^+$. By the time bath $Ca^{2+}$ concentration reaches, say, 100 μM, all the $Na^+$ has been expelled from the pore, and the current is carried solely by $Ca^{2+}$. Thus, the falling phase of the AMFE.

But why does the channel conductance rise again as $Ca^{2+}$ is raised further? The surprising answer provided by the PNP2 treatment is that it doesn't! The conductance is predicted to remain essentially flat as $Ca^{2+}$ rises to high levels because electroneutrality forbids admittance to additional $Ca^{2+}$ over and above the fixed negative charge; but the current measured at a given voltage (e.g., −40 mV) does increase to produce the AMFE for a simple reason: the reversal potential keeps moving positive as external $Ca^{2+}$ is increased. It's the driving force that goes up, not the conductance. In other words, this analysis asserts, everyone in the field has been dunderheaded all these years on a most elementary point, having apparently forgotten that current equals the product of conductance and driving force! (I am oversimplifying a little here; a small rise in conductance with $[Ca^{2+}]$ is predicted by PNP2, but this is a second-order effect having to do with surface polarization.)

*Evaluation and Conclusions*

So here we have two very different ways of interpreting a fundamental set of facts about ion permeation in calcium channels. I will state my opinion bluntly. First, no theory, however mathematically sophisticated, that rejects specific ionic coordination by protein moieties, dismisses the finite size of ions, and ignores the single-filing effects necessarily arising from the small spaces in the molecular structures of ion channels can have much worthwhile to say about selective ion permeation. Second, a ubiquitous feature of continuum theory—the mean-field assumption—invalidates, or at least greatly vitiates, its application to channels in which only a small number of ions reside at any one time. Third, PNP2 is inadequate to understand the particular calcium channel problem under examination here. Fourth,

the undoubted quantitative weaknesses of the chemical-kinetic approach do not undercut its value in capturing the mechanistic essence of permeation in ion-selective channels.

*(1) The continuum approach ignores ion channel chemistry.* For many years, indirect experiments have suggested that ions permeate selective channels by binding to localized sites at which protein functional groups replace waters of hydration, and that ion selectivity mainly reflects the energetics of the switch from water solvation to protein coordination (Hille, 1991). In soluble proteins, it is hardly a radical notion that binding of dehydrated inorganic ions lies at the basis of a multitude of functions (Falke et al., 1994), and now, with the structure of KcsA (Doyle et al., 1998), this idea has been confirmed directly for a strongly selective ion channel. The KcsA structure dramatically confirms for a $K^+$ channel the multi-ion single-filing assumption, long known also to be valid for the peptide channel gramicidin A (Finkelstein and Andersen, 1981). For permeation, the qualitative consequences of localized, structured binding sites and single-filing are profound; they lead naturally and necessarily to familiar phenomena seen in many channels: strong, concentration-dependent selectivity, discrete ionic block of permeation, and anomalously high ratios of unidirectional ionic fluxes.

It is not surprising that the continuum theories presently under discussion have been unable to satisfactorily reproduce these "enzyme-like" phenomena, since they (a) disregard the close-up chemistry of ionic coordination, (b) explicitly permit ions to move through one another within the pore, and (c) treat permeation mainly in terms of electric fields acting at a distance. This approach asserts the virtue of pristine, mathematically tractable physical principles, but it commits the vice of ignoring the messy parts: the prominent, obvious structural characteristics of channel proteins. To be sure, continuum theories have traditionally endeavored to include chemistry by superimposing upon the electrical potential a position-dependent free energy profile that may differ for different ions (Levitt, 1986) or by assigning to each ion its own diffusion coefficient. These are worthy additions to otherwise "featureless" electrodiffusion theories, but they are simply not enough; nobody has yet figured out how to weld single-file arrangements of binding sites to continuum theories in a general manner, although Levitt (1982, 1986, 1991a,b) has achieved impressive success in incorporating these features in particular cases, and Nonner et al. (1998) similarly have modeled a subset of $K^+$ channel behaviors with a selective binding "region." Conquest of this analytical impediment would represent a major advance in modeling permeation; in such a case, the entire channel field would unhesitatingly embrace continuum electrodiffusion as the preferred approach to the problem.

*(2) The mean-field assumption is inapplicable in small spaces.* To obtain solutions for the ionic fluxes, continuum treatments must use concentration and electrical potential as continuous spatial variables in the coupled differential equations. The concentration at a given position determines the net charge density, which in turn influences the value of potential at that and nearby positions. Concentration is an intrinsically probabilistic quantity—the average number of ions per unit volume. For a macroscopic object such as a worm of ion-exchange gel, the number of ions present is sufficiently large that this average can be taken within each cross-sectional slab of the object at each moment in time. The concentration at each position will fluctuate with time, but, if the object is large enough, these fluctuations will be negligible and the concentration, and therefore the potential, will be a time-invariant spatial average. This is the "mean-field" assumption: this average potential may be validly used in the three crucial equations. In an object of molecular dimensions, however, a huge problem arises. For something the size of a calcium channel selectivity filter, a concentration of 10 M represents on average only one or two ions in the entire volume. "Concentration" is still defined as a statistical average, but in this case the average must be taken over time; i.e., by sitting at a given position in the pore and asking what fraction of the time an ion is present. This is a perfectly good stochastic definition of concentration, but when you try to use it to relate concentration to potential via the Poisson equation, a fundamental difficulty asserts itself. A channel containing, say, one $Ca^{2+}$ on average will be fluctuating in occupancy among 0, 1, or 2 ions (0, 5, and 10 M concentration). The mathematics represents the channel as having a time-invariant potential equivalent to the average situation: single $Ca^{2+}$ occupancy. But this is a severe misrepresentation of the potential that a $Ca^{2+}$ approaching an empty channel, or a $Ca^{2+}$ about to leave a doubly occupied channel, actually sees, and these events are often rate determining for permeation. It is as if the I.R.S. applied to every taxpayer a uniform exemption calculated for 2.6 children, the mean number of children per American family. Described another way, a $Ca^{2+}$ aspiring to enter an empty channel at a given moment is treated by the electrodiffusion equations as though it experiences the repulsive electric field that existed, say, a microsecond before this moment, when the channel had one ion in residence; since occupancy-dependent changes in field are enormous and are established instantaneously, large errors in predicted behavior will arise from using an average potential. Thus, while valid for macroscopic objects and large, wide channels, the mean-field assumption applied to physically small chan-

nels yields solutions to the electrodiffusion equations that are mathematically chaste but physically debauched.

The chemical-kinetic treatment avoids this problem by explicitly assigning distinct properties to the different occupancy forms of the channel. It asserts, for example, that the probability per unit time (i.e., the rate constant) of a $Ca^{2+}$ entering an unoccupied channel is very different from the probability of entering a singly or doubly occupied channel precisely because of the very different electric fields in the three situations. This description deliberately avoids doing what continuum theory, for mathematical reasons, must do: treating the channel as a single entity with properties averaged over the different occupancy forms.

*(3) PNP2 misrepresents calcium channel behavior.* The single example in the literature of a continuum treatment of calcium channel behavior, PNP2 (Nonner and Eisenberg, 1998), does not achieve the goal it sets for itself. The analysis is very similar to that of the classical macroscopic ion-exchange membrane, where electrodiffusion is well understood (Teorell, 1953). Emphasized in the analysis is that only the channel current at a fixed voltage, and not the conductance, is expected to show AMFE. Nonner and Eisenberg (1998) claim that published calcium channel experiments have demonstrated an AMFE only in current at fixed voltage, and that proponents of the standard view have merely assumed without evidence that the AMFE also applies to conductance, as chemical-kinetic theory says it must. This claim, if correct, would be a deadly criticism of the chemical-kinetic approach.

But the claim is false. The original papers on calcium channel permeation (Figure 7 in Kostyuk et al., 1983; Figure 3 in Almers et al., 1984; Figure 2 in Almers and McCleskey, 1984) reported strong AMFE in current at fixed voltage as well as in conductance, based on macroscopic I–V curves over a $[Ca^{2+}]$ range from 60 nM to 10 mM. The conductance minimum is unambiguously observed at the single-channel level as well, in both $Ca^{2+}$ and $Ba^{2+}$ (Lansman et al., 1986; Friel and Tsien, 1989; Yue and Marban, 1990; Kuo and Hess, 1993). These elementary facts, well-known to the channel community, contributed mightily to the swift and widespread acceptance of the chemical-kinetic view of permeation. The PNP2 analysis proceeds as though these facts do not exist, and it accordingly fails to explain the most basic hallmark of calcium channel permeation. This incorrect prediction of a $Ca^{2+}$-independent conductance at physiological concentrations illustrates how badly a continuum theory that uses the mean-field assumption and ignores coordination chemistry can falter.

*(4) Chemical kinetics preserves the basics.* As for the weaknesses of the chemical-kinetic view, they are certainly prominent and well-appreciated (Cooper et al., 1985; Levitt, 1986; Dani and Levitt, 1990). It is impossible to predict a priori what the absolute values of the rate constants should be or how to relate rate constants to transition-state free energies. Likewise, the use of Eyring-like exponential voltage dependence to the rate constants is theoretically unjustified and always leads to incorrect I–V curve shapes. And physical space inside of channels is in fact continuous, not a lattice of sites.

But so what? Most channel researchers don't really care about predicting absolute values of currents, just as enzymologists don't feel the need to calculate the $k_{cat}$ of an ATPase from quantum mechanics; it's the patterns of permeation behavior that count, not the absolute rates. As for the precise shapes of open-channel I–V curves, this is not a particularly compelling issue in channel physiology; the examples of unusual I–V shapes encountered in biologically meaningful contexts are invariably due not to intrinsic ionic diffusion properties, but rather to specific block (on discrete binding sites) by exogenous molecules (e.g., polyamine-induced inward rectification in $K_{ir}$ channels, or $Mg^{2+}$-induced outward rectification by NMDA-receptor channels). And chemical kineticists don't believe that ions leap over tens of angstroms of pore length in a single bound; we do posit, however, in analogy to chemical reaction mechanisms, that sojourns on binding sites represent the preponderance of time the ion spends within the pore, and thus define the important rate-determining steps of ion permeation.

Finally, there is a particularly compelling reason not to reject chemical kinetics in spite of its formal flaws: when used with an understanding of its limitations, it works. Its track record is excellent. It is primarily by chemical-kinetic analysis of ionic permeation over the past two decades that we have achieved physical pictures of ion channel proteins in the complete absence of direct structural information. It was chemical-kinetic analysis that told us that channels are built as axially symmetric structures with discrete selectivity filters and ion-binding sites at which ions are largely dehydrated, with narrow regions where ions and water lie in single file, with wide vestibules where drugs bind, and with enzymologically unprecedented regions where multiple ions bind simultaneously in close proximity. All of these features, which underpin the mechanisms by which ion channels achieve their paradoxical combination of selectivity and high transport rate, have now been observed directly in the first structure of a selective channel protein.

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## references


Almers, W., and E.W. McCleskey. 1984. A non-selective cation conductance in frog muscle membrane blocked by micromolar external calcium ions. *J. Physiol.* 353:585–608.

Almers, W., E.W. McCleskey, and P.T. Palade. 1984. Non-selective conductance in calcium channels of frog muscle: calcium selectivity in a single-file pore. *J. Physiol.* 353:565–583.

Cooper, K., E. Jakobsson, and P. Wolynes. 1985. The theory of ion transport through membrane channels. *Prog. Biophys. Mol. Biol.* 46:51–96.

Dani, J.A., and D.G. Levitt. 1990. Diffusion and kinetic approaches to describe permeation in ionic channels. *J. Theor. Biol.* 146:289–301.

Doyle, D.A., J.M. Cabral, A. Pfuetzner, J.M. Kuo, J.M. Gulbis, S.L. Cohen, B.T. Chait, and R. MacKinnon. 1998. The structure of the potassium channel: molecular basis of $K^+$ conduction and selectivity. *Science.* 280:69–76.

Falke, J.J., S.K. Drake, A.L. Hazard, and O.B. Peersen. 1994. Molecular tuning of ion binding to calcium signaling proteins. *Q. Rev. Biophys.* 27:219–290.

Finkelstein, A., and O.S. Andersen. 1981. The gramicidin A channel: a review of its permeability characteristics with special reference to the single-file aspect of transport. *J. Membr. Biol.* 59:155–171.

Friel, D.D., and R.W. Tsien. 1989. Voltage-gated calcium channels: direct observation of the anomalous mole fraction effect at the single-channel level. *Proc. Natl. Acad. Sci. USA.* 86:5207–5210.

Hess, P., and R.W. Tsien. 1984. Mechanism of ion permeation through calcium channels. *Nature.* 309:453–456.

Hille, B. 1991. Ionic Channels of Excitable Membranes. Sinauer Associates, Inc., Sunderland, MA.

Kostyuk, P.G., S.L. Mironov, and Y.M. Shuba. 1983. Two ion-selecting filters in the calcium channel of the somatic membrane of mollusc neurons. *J. Membr. Biol.* 76:83–93.

Kuo, C.C., and P. Hess. 1993. Block of the L-type $Ca^{2+}$ channel pore by external and internal $Mg^{2+}$ in rat phaeochromocytoma cells. *J. Physiol.* 466:629–655.

Lansman, J.B., P. Hess, and R.W. Tsien. 1986. Blockade of current through single calcium channels by $Cd^{2+}$, $Mg^{2+}$, and $Ca^{2+}$. Voltage and concentration dependence of calcium entry into the pore. *J. Gen. Physiol.* 88:321–347.

Levitt, D.G. 1982. Comparison of Nernst-Planck and reaction rate models for multiply occupied channels. *Biophys J.* 37:575–587.

Levitt, D.G. 1986. Interpretation of biological ion channel flux data--reaction-rate versus continuum theory. *Annu. Rev. Biophys. Biophys. Chem.* 15:29–57.

Levitt, D.G. 1991a. General continuum theory for multiion channel. I. Theory. *Biophys. J.* 59:271–277.

Levitt, D.G. 1991b. General continuum theory for multiion channel. II. Application to acetylcholine channel. *Biophys. J.* 59:278–288.

McCleskey, E.W. 1997. Biophysics of a trespasser, $Na^+$ block of $Ca^{2+}$ channels. *J. Gen. Physiol.* 109:677–680.

Nonner, W., and R.S. Eisenberg. 1998. Ion permeation and glutamate residues linked by Poisson-Nernst-Planck theory in L-type calcium channels. *Biophys. J.* 75:1287–1305.

Nonner, W., D.P. Chen, and B. Eisenberg. 1998. Anomalous mole fraction effect, electrostatics, and binding in ionic channels. *Biophys. J.* 74:2327–2334.

Sven-Knudsen, O. 1978. Passive transport processes. *In* Membrane Transport in Biology. Vol. I. G. Giebisch, D.C. Tosteson, and H.H. Ussing, editors. Springer-Verlag, Berlin. 5–114.

Teorell, T. 1953. Transport processes and electrical phenomena in ionic membranes. *Progr. Biophys. Biophys. Chem.* 3:305–369.

Yue, D.T., and E. Marban. 1990. Permeation in the dihydropyridine-sensitive calcium channel. Multi-ion occupancy but no anomalous mole-fraction effect between $Ba^{2+}$ and $Ca^{2+}$. *J. Gen. Physiol.* 95:911–939.