

Perspectives on Ion Permeation

The purpose of the Perspectives in General Physiology is to provide a forum where scientific uncertainties or controversies can be discussed in an authoritative, yet open manner.

The Perspectives are solicited by the editors—often based on recommendations by the advisory editors or members of the editorial board, who may be asked to coordinate the process. To frame the issue, two or more experts will be invited to present a brief point of view on the problem, which will be published consecutively in *The Journal*. The comments and opinions expressed in the Perspectives are those of the authors and not necessarily those of the Editors or the Editorial Board. The Perspectives will be accompanied by a few editorial paragraphs that introduce the problem—and invite the submission of comments, in the form of letters-to-the-editor, which will be published in a single, predetermined issue (usually four months after publication of the Perspectives).

In this issue of *The Journal*, Edwin W. McCleskey (Vollum Institute), Wolfgang Nonner, Duan P. Chen, and Bob Eisenberg (University of Miami School of Medicine and Rush Medical College), Christopher Miller (Brandeis University), and David G. Levitt (University of Minnesota) provide different insights into ion permeation through membrane-spanning channels.

Following the lead of Julius Bernstein, who almost 100 yr ago used electrodiffusion theory to describe the resting membrane potential of muscle and nerve, ion movement across biological membranes has traditionally been described using the Nernst-Planck electrodiffusion equations. Beginning in the mid-1950's, however, descriptions of ion movement through membranes moved beyond the conventional implementation of the Nernst-Planck analysis. First, it was found that K^+ movement through voltage-dependent potassium channels could not be described by the Nernst-Planck electrodiffusion equations, as the flux-ratio exponent describing the K^+ tracer flux was much larger than the expected value of 1. This led to the notion of single-file flux (Hodgkin and Keynes. 1955. *J. Physiol.* 128:61–88). Second, ion–ion interactions within the membrane were introduced explicitly into the Nernst-Planck flux equations via the Poisson equation (Bruner, L.J. 1965. *Biophys. J.* 5:867–886). Third, it became apparent that ion channels were not simple aqueous pores: they could concentrate ions and exhibit saturation behavior. The latter property was not readily accounted for by the Nernst-Planck equations, which led to the development of rate-theory models of ion permeation (Läuger, P. 1973. *Biochim. Biophys. Acta.* 311:423–441).

An attraction of rate-theory models was that they not only provided a convenient way to incorporate flux saturation, they also could account for single-file flux coupling. Moreover, they might provide a way to relate the kinetics of ion movement to the underlying energetics of ion solvation by the channel (provided that the rate constant $[r]$ for the transition from an energy well across an adjacent energy barrier can be expressed by a physical model. The most commonly used model has been the Eyring Transition State Theory (TST); in its simplest form, $r = (kT/h) \cdot \exp(-E/kT)$, where k is Boltzmann's constant, T temperature in Kelvin, h Planck's constant, and E the energy difference between the barrier peak and the well trough (which includes a contribution, $\delta \cdot z \cdot e \cdot V$, from the electrical potential difference between the peak and the trough, where z is the valence, e the elementary charge, and δ the fraction of the potential that falls between the well and peak). A fundamental limitation of the rate-theory approach is that the mean free path in condensed phases is $\sim 0.1 \text{ \AA}$, much less than the distance an ion must traverse when passing through the channel. Channel-mediated ion movement thus involves many elementary steps—which can be lumped into a single transition; but in that case one can no longer use the Eyring TST to relate rate constants and barrier heights. The elementary steps occur at a frequency of $\sim 10^{13} \text{ s}^{-1}$ (based on a mean free path of 0.1 \AA and a thermal velocity of $\sim 10^4 \text{ cm/s}$; Andersen and Feldberg, 1996. *J. Phys. Chem.* 100:4622–4629), which is comparable to kT/h ($\approx 6 \cdot 10^{12} \text{ s}^{-1}$). Following standard diffusion theory, even with no energy barrier, the frequency of successful transitions over a distance of 1 \AA will be only $\sim 10^{11} \text{ s}^{-1}$ —or two orders of magnitude less than the elementary step frequency. Related to this problem, rate-theory models with only a few steps tend to predict current–voltage relations that are steeper than those observed, because the voltage dependence of the underlying rate constants vary as $\exp(-\delta \cdot z \cdot e \cdot V/kT)$. This means that δ 's need to be small—that there are many steps in the path. Läuger, in fact, used a model with 10 steps in series to describe the current–voltage relations.

Rate-theory models with sufficiently many steps become diffusion models, and there is now general agreement that channel-mediated ion movement involves an electrodiffusive transfer through the pore. As evident from the present contributions, however, there is considerable disagreement about the importance of specific ion–channel (and ion–ion) interactions—and how one should describe them. Do ions within the pore tend to be localized in a few well-defined regions? If so, are these “sites” due to specific chemical interactions between the ion and the channel protein, or do they arise from the “simple” superposition of the different contributions to the ions' energy profile?

Probably both, as suggested by the recent x-ray structure of the Kcsa potassium channel (Doyle, D.A., J.M. Cabral, R.A. Pfuetzner, A. Kuo, J.M. Gulbis, S.L. Cohen, B.T. Chait, and R. MacKinnon. 1998. *Science*. 280:69–77). Given the structural evidence for ion binding sites, how should they be introduced into the permeation model? If single-file flux coupling is a central feature of narrow ion-selective channels, how is it (most conveniently) incorporated into permeation models? Finally, notwithstanding the utility of the mean field approximation, is it appropriate for narrow channels that are occupied by only a few ions?

These are unsettled questions that are central for understanding selective ion movement in narrow pores. Their resolution is likely to be catalyzed by constructive disagreement among the proponents of different approaches to the problem. It is in this context important to keep in mind that kinetic experiments provide information about the rates of transfer (through a channel). Whatever the preferred analysis method, deductions about the height of energy barriers (and depth of energy wells) depend on an underlying physical model, which invariably involves a judicious choice of approximations and assumptions (and the assumptions we do not acknowledge are likely to cause the most problems). Moreover, even if the physical problem can be formulated to everybody's satisfaction, the mathematical development of the model and its solution may prove elusive. The challenge then becomes to balance the need for physical (and chemical) reality with that for mathematical rigor.

In this context, it is sobering to note that ion permeation is a comparatively well understood process (despite disagreements about the appropriate kinetic description, choice of prefactors, etc.). Whatever the complexities involved in developing permeation models, they pale in comparison with those involved in developing quantitative, physical models of channel gating (or any other protein conformational transition). There may be unassailable evidence for discrete states in the gating path, but the conformational transitions are likely to be so complex that deductions about barrier heights and well depths using kT/h or any other fixed prefactor will be seriously misleading because the prefactors for different steps may vary by orders of magnitude!

Letters-to-the-editor related to these Perspectives will be published in the October 1999 issue of *The Journal of General Physiology*. The letters may be no longer than two printed pages (approximately six double-spaced pages) and will be subject to editorial review. They may contain no more than one figure, and may not contain significant references to unpublished work. After the letters-to-the-editor have been published, further responses will be limited to full manuscripts. Letters-to-the-editor should be received no later than August 1, 1999 to allow for editorial review. Letters can be submitted electronically by sending a formatted text file as an attachment in an e-mail to the editorial office "jgp@rockvax.rockefeller.edu". Figures must be submitted in hard copy (they can be faxed so that they are received in the editorial office by the deadline).

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