

be used to investigate distinctly non-axisymmetric shapes such as echinocytes that would be difficult to study with continuum models. In such a study one should allow for the expected partial separation between network and bilayer. Second, in this model both the bilayer and the network are still homogeneous, whereas the bilayer membrane of real cells consists of various lipids and integrated proteins and the real network has a significant number of defects. How does this inhomogeneity affect and how is it affected by strong deformations? Such a question can be addressed by decorating this model with additional particles modeling the proteins and by introducing network defects. Depending on the interactions of proteins with both the membrane and the network, enrichment or depletion will occur in a manner sensitive to local curvature and network distortion.

Finally, an outstanding challenge will be to extend this approach to study of the dynamics of shape changes induced by external hydrodynamic fields such as shear or capillary flow. This will require incorporating long-range hydrodynamic interactions into the model, a task recently achieved in the simpler model system of fluid bilayer vesicles (Kraus et al., 1996). Discher and colleagues' model and its future refinements can take us closer to a quantitative understanding of the remarkable mechanochemical properties of the red blood cell.

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## Run, Don't Hop, through the Nearest Calcium Channel

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At first glance ion permeation in calcium ( $\text{Ca}^{2+}$ ) channels presents a collection of wildly contradictory facts (Tsien et al., 1987). In the absence of divalent cations this channel behaves like a perfectly respectable cation-selective channel that discriminates poorly among alkali metal cations, much like an acetylcholine receptor channel. Monovalent current, carried for example, by  $\text{Na}^+$  ions, is blocked by submicromolar concentrations of  $\text{Ca}^{2+}$ , indicating a high affinity of the channel for  $\text{Ca}^{2+}$ . However, in physiological solutions, in which  $\text{Ca}^{2+}$  concentration is in the millimolar range, these channels select strongly ( $\sim 1000:1$ ) for  $\text{Ca}^{2+}$  over  $\text{Na}^+$ , and are capable of admitting a substantial  $\text{Ca}^{2+}$  influx despite the 100-fold higher concentration of extracellular  $\text{Na}^+$ . The high  $\text{Ca}^{2+}$  conductance indicates low free energy barriers for a  $\text{Ca}^{2+}$  ion to traverse the pore. But how is the channel capable of conducting  $\sim 10^6$   $\text{Ca}^{2+}$  ions/s if it binds  $\text{Ca}^{2+}$  so tightly? Moreover, how does raising the  $\text{Ca}^{2+}$  concentration switch off the high permeability to  $\text{Na}^+$ ?

These paradoxes have intrigued biophysicists for the past two decades. The commonly accepted explanation for the above phenomena is that the  $\text{Ca}^{2+}$  channel is a single-file pore capable of binding at least two  $\text{Ca}^{2+}$  ions at discrete sites (Tsien et al., 1987). The first  $\text{Ca}^{2+}$  ion binds with high affinity, and thus is capable of blocking the current carried by monovalent cations, which have a much lower af-

finity for the open channel. The binding of a second  $\text{Ca}^{2+}$  ion reduces the affinity of both divalent cations for the pore, perhaps by electrostatic repulsion; and now the pore is less sticky for  $\text{Ca}^{2+}$  ions, which can therefore permeate readily. The selectivity of  $\text{Ca}^{2+}$  over  $\text{Na}^+$  is a consequence of selective binding of the divalent cation. This heuristic account can be modeled quantitatively by a rate theory model of ions hopping from one binding site to the next over a small number of free energy barriers.

Enter PNP2 (Nonner and Eisenberg, 1998), and all of the sacred tenets of the rate theory description are called into question. With this Poisson-Nernst-Planck model, the successor of PNP0 and PNP1, there are no  $\text{Ca}^{2+}$  binding sites, there is no single filing, and the biophysical fingerprint of the  $\text{Ca}^{2+}$  channel is predicted under conditions in which the pore is occupied on average by less than one  $\text{Ca}^{2+}$  ion. Furthermore, the authors argue on physical chemical grounds that some assumptions underlying Eyring rate theory models of  $\text{Ca}^{2+}$  channels must be invalid.

Certainly the successes of this paper are stunning. Nonner and Eisenberg (1998) begin with a rather featureless permeation pathway composed of a central  $6 \text{ \AA} \times 10 \text{ \AA}$  "pore proper" with conical aqueous vestibules extending into the bulk solution. Ions flow through the channel, obeying laws of bulk electrodiffusion. Using five single-valued measurements (e.g., the maximum conductances of  $\text{Ca}^{2+}$  and  $\text{Na}^+$  at high concentrations) from the  $\text{Ca}^{2+}$  channel literature, they are able to predict most of the published biophysical properties of  $\text{Ca}^{2+}$  channels under a wide variety of conditions (i.e., solution compositions and voltage). For example, the model predicts de novo 1) the "anomalous mole fraction effect" in which  $\text{Ca}^{2+}$  blocks at low concentration and permeates well at high concentration, 2) typical current-voltage relationships over a variety of ionic conditions, 3) the saturation of  $\text{Ca}^{2+}$  currents in the range of tens of mM, and 4) the voltage-dependent block of currents by protons. The ro-

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bustness of the model is shown by the insensitivity of these predictions to the few parameters of the model (e.g., the length of the pore proper, its permittivity, and the number of structural charges lining the pore).

Before accepting the demise of the widely used rate theory models of permeation, it is worth evaluating what Nonner and Eisenberg, and electrodiffusion models in general, have to offer as an alternative. It is important to note that this is not a statistical battle between mathematical models, and therefore is not a case of "model discrimination." The PNP2 model was subjected to a much stiffer test than curve fitting. After the five experimental measurements were input, there were no free parameters to adjust and no data were fit. The model was simply used to simulate channel properties under a variety of novel conditions. It is inconceivable that a rate theory model could do this with as little starting information, although such models can account for the biophysical properties of  $\text{Ca}^{2+}$  channels by ad hoc adjustment of model parameters, i.e., by curve fitting. On the other hand, it is not clear whether PNP2 has enough parametric detail to be able to fit all of the bumps and wiggles of experimental data which range from current-voltage relationships to blocking statistics in single channel currents.

The fundamental product of a model is insight, and it can be argued that rate theory models have produced this in abundance. Nonner and Eisenberg (1998) question, however, whether some of these insights (e.g., the necessity for discrete binding sites and multi-ion occupancy of the pore) are valid. A recent structural study from a related ion channel also shows a tendency for ions to be located at discrete positions along the axis of a pore with-

out obvious binding sites (Doyle et al., 1998). But what insight does this incarnation of PNP provide about  $\text{Ca}^{2+}$  channel permeation? In what sense, for example, does PNP2 provide insights fundamentally different from (or better than) an appropriately parameterized rate theory model? In some cases this is not so obvious. The PNP equivalent of an empty binding site, for example, is a "zone of depletion," due perhaps to electrostatic repulsion of nearby cations. In both models "occupancy" has a probabilistic meaning, which translates in PNP into a local concentration.

One insight from PNP that may prove critical in understanding permeation is that the selectivity filter (i.e., pore proper) may act like an ion exchange resin that buffers its ionic contents from the vagaries of the surrounding milieu. This idea is an elaboration of the Teorell-Meyer-Sievers theory of fixed-charge membranes (Teorell, 1953). Buffering of ions within the pore proper ensures a resilient balance between repulsive and attractive forces necessary to allow both high selectivity and high conductance (Doyle et al., 1998). Perhaps the most valuable insight provided by the PNP models, however, is that free energy barriers within a channel cannot be independent of ion concentrations (Eisenberg, 1996). If for no other reason, this hard-to-refute assertion is a serious challenge for standard rate theory models.

The PNP models have some serious problems to address, however, before they can be embraced wholeheartedly. Most of these problems are a consequence of the comparable physical dimensions of the pore (typically having diameters  $\leq 6 \text{ \AA}$ ) and those of individual atoms in solution. Because the selectivity filters of most ion channels are so narrow (Hille, 1992; Doyle et

al., 1998), ions must move in single file, a fact that is consistent with experimental measurements of radioactive flux coupling (Hodgkin and Keynes, 1955). Not only is single filing neglected in PNP; it is not clear how it could be added to a bulk electrodiffusion model, or how this omission biases the conclusions. Moreover, the narrow dimensions of the selectivity filter necessitate the loss of most of the hydration shell around a permeating ion, a fact also disregarded by PNP. Overall the craggy energetic landscape experienced by an ion in a restricted space is ignored by present PNP models, and may even be impossible to calculate with fine-scaled structural information. It is fair to ask, therefore, whether some of the conceptual principles generated by this relatively featureless model could produce another set of erroneous insights.

Stay tuned.

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