Perspectives on: Ion selectivity

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The purpose of the Perspectives in General Physiology is to provide a forum where scientific uncertainties or controversies are discussed in an authoritative, yet open manner. The Perspectives are solicited by the editors—often based on recommendations by members of the editorial advisory board. To frame the issue, two or more experts are invited to present brief points of view on the problem; these are published consecutively in the Journal. One or more experts and the organizer review the contributions, but the comments and opinions expressed in the Perspectives are those of the authors and not necessarily those of the editors or the editorial advisory board. The Perspectives are 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 are published in a single, predetermined issue (usually three months after publication of the Perspective). After the letters to the editor have been published, further responses are limited to full manuscripts.

In this issue of the Journal, Youxing Jiang (University of Texas Southwestern Medical Center) together with Amer Alam; Crina M. Nimigean (Weill Cornell Medical College) and Toby W. Allen (University of California at Davis); Benoît Roux (University of Chicago) together with Simon Bernèche, Bernhard Egwolf, Bogdan Lev, Sergei Y. Noskov, Christopher N. Rowley, and Haibo Yu; Dilip Asthagiri (Johns Hopkins University) together with Purushottam D. Dixit; and Susan B. Rempe (Sandia National Laboratory) together with Sameer Varma, David L. Bostick, David Rogers, Lawrence R. Pratt, and Charles L. Brooks III provide different perspectives on the ion selectivity of cation-selective channels and transporters.

Current thinking about the mechanisms underlying ion channel selectivity are rooted in concepts dating back to the BC (before crystals) era. Mullins (1959) proposed that the selectivity of excitable membranes arose from the existence of membrane-spanning channels with diameters that were able to accommodate the preferred ion (e.g., Na^+ or K^+); he further noted that ions preferentially would move through pores that fit them rather well, in order for the pore lining to solvate the permeating ions. Eisenman (1961) developed the equilibrium theory of ion selectivity, in which the Na^+/K^+ selectivity of an ion-binding site is expressed in terms of the free energy difference ($\Delta\Delta G_{K^+\rightarrow Na^+}$) for the reaction

$$Na^+(\text{aqueous}) + K^+(\text{site}) \rightarrow K^+(\text{aqueous}) + Na^+(\text{site}),$$

where

$$\Delta\Delta G_{K^+\rightarrow Na^+} = \left(G^{site}_{Na^+} - G^{aqueous}_{Na^+}\right) - \left(G^{site}_{K^+} - G^{aqueous}_{K^+}\right) = \left(G^{site}_{Na^+} - G^{site}_{K^+}\right) - \left(G^{aqueous}_{Na^+} - G^{aqueous}_{K^+}\right).$$

Ion selectivity thus arises when the difference in the ions' interaction energy with the site, $\Delta G^{site}_{K^+\rightarrow Na^+} = G^{site}_{Na^+} - G^{site}_{K^+}$, differs from the difference in their hydration energies, $\Delta G^{aqueous}_{K^+\rightarrow Na^+} = G^{aqueous}_{Na^+} - G^{aqueous}_{K^+}$. Approximating the ion–site interactions as simple electrostatic interactions, Eisenman further introduced the concept of "electrostatic field strength" to characterize the strength of the interactions between the ions and the ligands that constitute the site. In the simplest case, the ion–ligand interactions are described by

$$G^{site}_{ion} = \frac{q_s \cdot q_i}{4\pi \cdot \varepsilon_0 \cdot \varepsilon_r \cdot (r_s + r_i)},$$

where q_s and q_i denote the charges on the ligand and ion, respectively, r_s and r_i are the radii of the site and ion, and ε_0 and ε_r are the permittivity of free space and the relative dielectric constant. In sites with high field–strength ligands (meaning that the magnitude of q_s/r_s is large), the variation in $G^{site}_{ion} - G^{aqueous}_{ion}$ is dominated by changes in G^{site}_{ion}, and the site would be expected to select for small ions; in sites with low field–strength ligands (meaning that the magnitude of q_s/r_s is small), the variation in $G^{site}_{ion} - G^{aqueous}_{ion}$ is dominated by changes in $G^{aqueous}_{ion}$, and the site would be expected to select for large ions.

Whether or not well-defined sites actually existed was, however, not clear. It was generally recognized that the binding sites that appear in most kinetic models of channel-mediated ion movement (and ion selectivity) might just be figments of our imagination, as it seemed

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difficult to reconcile rapid ion movement with the existence of well-defined energy wells that would constitute such sites.

The ideas of Mullins and Eisenman laid the foundations for subsequent studies of ion selectivity in channels, with major contributions by C.M. Armstrong and B. Hille. In Bezanilla and Armstrong (1972), Armstrong noted that selective ion permeation through a pore arises from selective exclusion of the less-preferred ions, and that selectivity therefore should be considered to be, at least in part, a kinetic problem. In the simplest case, selectivity would be determined by the rate constants for ion entry into the channel. After Mullins (1959), Bezanilla and Armstrong (1972) also suggested that the permeating ions would need to fit snugly in a rigid selectivity filter, which would allow the coordinating ligands to solvate the preferred ions and provide a parsimonious explanation for the exclusion of smaller ions, such as Na^+ in potassium channels. Hille, in a series of landmark studies, explored the selectivity filters in sodium and potassium channels in myelinated nerve. Using a combination of inorganic and organic ions, he deduced that the sodium channels are aqueous pores that select among monovalent cations based on simple steric "fit" (Hille, 1971), with the inorganic ions being partly hydrated, meaning that Na^+ is coordinated by the pore-lining residues through intervening H_2O (Hille, 1972). In potassium channels, the permeant ions are coordinated directly by pore-lining (low field–strength) residues (Hille, 1973). Hille (1973) further noted that the observed selectivity (for K^+ over Na^+) was compatible both with a rigid selectivity filter, operating in a strict size-selection mode, and a flexible selectivity filter where the ion-coordinating ligands could be pulled in by smaller ions and pushed out by larger ions. The prevailing view over the next 25+ years, however, was that ion selectivity arose from size selection in a rather rigid selectivity filter.

Yet, there was ample evidence that proteins were dynamic entities. Perutz and Mathews (1966), based on x-ray crystallographic studies, concluded that there was no path for ligands to gain access to the heme group in hemoglobin unless one or more side chains would move to "open the gates," a proposal that was validated in subsequent molecular dynamics (MD) simulations (Case and Karplus, 1979). Diebler et al. (1969) pointed out that rapid dehydration/resolvation kinetics required some flexibility to allow for stepwise solvent substitution. Cooper (1976) noted that numerous spectroscopic studies provided evidence for a rather fluid, dynamic protein structure—a picture that appeared difficult to reconcile with the static structure depicted in, for example, a Protein Data Bank (PDB) coordinate file. But these two views of protein structure and dynamics are perfectly compatible once the thermodynamic fluctuations that occur in single molecules are considered. Frauenfelder et al. (1979) demonstrated that the conformational substates that had been deduced from spectroscopic measurements could also be observed by x-ray diffraction. By the early/mid-1980s, it was firmly established that proteins are dynamic, fluctuating entities (e.g., Cooper, 1984).

Studies on ion selectivity changed fundamentally with the publication of the structure of the KcsA potassium channel (Doyle et al., 1998). The structure provided immediate insight into many years of structure–function studies. It also revealed important, unexpected features including the pore-lining in the selectivity filter being formed by the peptide backbone carbonyl oxygens in the "signature sequence" Val-Gly-Tyr-Gly-Asp, and the existence of distinct K^+-binding sites formed by eight carbonyl oxygens. The "black box" era could be replaced by the modern era molecular studies! The proposed coordination of the K^+ in the selectivity filter, with K^+ fitting snugly in a cage formed by carbonyl oxygens held in place by molecular springs, was consistent with both the rigid and the flexible organization of the selectivity filter envisaged by Hille 25 years earlier.

But the question remained: is the selectivity filter rigid (as believed by many electrophysiologists) or flexible (reflecting the dynamic nature of proteins)? Early MD simulations (Guidoni et al., 1999) showed that the selectivity filter was flexible/fluctuating, with the RMSD of the fluctuations being less when K^+ was coordinated in the pore. Yet, how could a flexible selectivity filter, formed by relatively high field–strength carbonyl oxygens, be selective for K^+? A resolution of this seeming paradox was proposed by Noskov et al. (2004), who noted that the conventional field strength point of view, focusing on just the ion–ligand interactions, was incomplete. When ligands are packed as tightly as they are in the selectivity filter, one needs to consider not only the ion–ligand interactions but also the ligand–ligand interactions when evaluating $\Delta\Delta G_{K^+\rightarrow Na^+}$. The (attractive) ion–ligand interactions would tend to decrease $\Delta\Delta G_{K^+\rightarrow Na^+}$ and favor the smaller Na^+, and the (repulsive) ligand–ligand interactions would tend to increase $\Delta\Delta G_{K^+\rightarrow Na^+}$ and disfavor the smaller Na^+. Although this would provide an explanation for why the selectivity filter was K^+ selective, it did not account for the variation of $\Delta\Delta G_{K^+\rightarrow Na^+}$ among the different sites in the selectivity filter, as deduced from MD simulations, or for the variations in Na^+/K^+ selectivity among potassium channels with the same signature sequence (and, presumably, selectivity filter). Varma and Rempe (2007), who used quantum mechanical calculations to evaluate the ion–ligand interactions, therefore proposed that it would be necessary to also consider the environment outside the selectivity filter proper, in which the organization of the selectivity filter would be determined by ion–ligand as well as ligand–environment interactions.

A key tool in most recent studies on ion selectivity has been the so-called toy models, introduced by

Noskov et al., (2004), which emphasize the fluid-like features of the selectivity filter and allow for the isolation of key features that one would like to examine. But, although proteins may be fluid-like at small-length scales, they are not fluids—indeed, they show considerable rigidity (defined structure) at longer-length scales, as evident in, for example, a PDB coordinate file. This rigidity is important for several reasons. First, the carbonyl ligands would only be able to form a K^+-selective site if they were confined. Second, the overall organization of the selectivity filter is determined during channel synthesis and folding; thus, although there might be an energetic cost associated with organizing the selectivity filter if the ligands were in a liquid, that cost was paid during biosynthesis. Third, the short-range flexibility and long-range rigidity allow for the molecular motions necessary for rapid exchange of the coordinating ligands (or H_2O), while limiting the overall extent of the molecular transitions, allowing for rapid kinetics.

Thus, although the toy models allow for important new insights, they are toys. The goal is to transfer the knowledge that is gained into understanding the selectivity of the bilayer-spanning channels, which remains a challenge as it becomes necessary to consider not only the equilibrium situations but also the kinetics, and the competition among the permeant ions as they strive to make it through the channel. As evident from the contributions to this Perspectives series, these questions can be approached from different, complementary directions. Thus, it may be useful to note that "a model is neither right because it predicts correct answers, nor are the ideas behind a model wrong because some details do not come out exactly correct. The challenge is to deduce those features that should have enduring significance however future models are constructed" (Hille, 2001).

In this series of Perspectives, Alam and Jiang focus on what can be deduced from crystal structures. Next, Nimigean and Allen consider what can be learned from a combined electrophysiological, crystallographic, and computational approach. The last three contributions, by Roux et al., by Dixit and Asthagiri, and by Rempe and colleagues (Varma et al.), consider different theoretical and computational approaches based on MD simulations and quasi-chemical theory, including the use of simple "toy" models, to identify the mechanisms underlying ion selectivity (the contribution by Varma et al. will appear in the June 2011 issue of the Journal).

Letters to the editor related to these Perspectives will be published in the September 2011 issue of the Journal. Letters to the editor should be received no later than Friday, July 22, 2011, to allow for editorial review. 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, no more than 15 references, and no significant references to unpublished work. Letters should be prepared according to The Journal's instructions and can be submitted electronically at http://www.jgp.org, or as an e-mail attachment to jgp@mail.rockefeller.edu.

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