Perspectives on: Ion selectivity

Origins of ion selectivity in potassium channels from the perspective of channel block

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Potassium channels play crucial roles in physiology, and one of their more important roles is to repolarize the membrane after an action potential in excitable cells (Hille, 2001). During an action potential, Na^+ channels open first and depolarize the cell membrane, which is followed closely by their inactivation and subsequent opening of K^+ channels that repolarize the cell membrane by allowing K^+ to flow out of the cell. If Na^+ were allowed to move through K^+ channels, the influx of Na^+ would compete with the outflow of K^+, and the sharp membrane repolarization would no longer occur. It is then paramount that K^+ channels select keenly against Na^+ ions.

The mechanism by which K^+ channels select for K^+ ions against the smaller Na^+ ions has fascinated scientists for over 50 years. Here is why: K^+ ions, with an ionic radius of ~1.33 Å, are able to flow through K^+ channels at very high rates, close to diffusion limited, as measured with current recordings; on the other hand, Na^+ ions, with a similar ionic radius of ~0.95 Å (just ~0.4 Å smaller), are not able to generate measurable ionic current. In this Perspective, we review the existing hypotheses of thermodynamic and kinetic-based selective permeation through K^+ channel pores, and discuss recent evidence emerging from channel blocking and simulation studies that may help resolve the uncertainties for this important family of ion channels.

Kinetic and thermodynamic views of K^+ channel selectivity

Before any structural or even amino acid sequence data were available for K^+ channels, Bezanilla and Armstrong (1972) proposed, based on electrophysiological recordings on squid giant axons, that the binding sites in the selectivity filter are made out of oxygens from the backbone carbonyls of the amino acids, similar to what Hille (1971) had proposed for Na^+ channels. It was put forward that the disposition of these oxygens is such that the sites mimic the arrangement of water dipoles in solution, thus perfectly coordinating a K^+ ion, but that they may be too far apart to properly coordinate a Na^+ ion (see also Mullins, 1959, 1960). Despite this statement suggesting a difference in thermodynamic stability of the two ions inside the channel pore, they deduced that selectivity between Na^+ and K^+ ions must be given by the difference between the entry rates into the pore, rather than the depths of the wells once inside the pore. This calculation was made with the assumption that the selectivity filter of these channels has just one binding site for ions, which was already known to be an oversimplification (Hodgkin and Keynes, 1955), with ionic selectivity likely being a multistage process (Hille, 1973). Thus, the distinction between a thermodynamic and a kinetic mechanism for K^+ over Na^+ selectivity (Bezanilla and Armstrong, 1972) was not possible at that time, and all models were highly speculative.

Using barium block experiments performed on BK K^+ channels, Neyton and Miller (1988a,b) proposed the existence of at least four ion-binding sites with different affinities for different cations in the pores of K^+ channels. They suggested that the cation selectivity sequence in these channels was strongly correlated with the affinities of these sites for different cations. These experiments, however, provide measures of the thermodynamics of binding to a site located very close to at least one site already occupied by Ba^{2+}, and do not offer any information about barrier heights for these ions inside or upon entry into the K^+ channel pore.

The solution of the crystal structure of KcsA, a prokaryotic K^+ channel from *Streptomyces lividans* (Doyle et al., 1998; Morais-Cabral et al., 2001; Zhou et al., 2001) (Fig. 1), revealed, close to the prediction by Neyton and Miller (1988a,b), that the selectivity filter consists of four sites S1–S4 formed by oxygens from the protein backbone carbonyl and side-chain hydroxyl groups, adjacent to an aqueous-like cavity region. Two additional

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Abbreviation used in this paper: MD, molecular dynamics.

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sites were identified in the structure: site S0, located at the extracellular mouth of the selectivity filter, partly hydrated and partly coordinated by carbonyl oxygens from the protein; and site S_{ext}, located above S0 (predicted computationally; Bernèche and Roux, 2001) further up from the mouth and not directly coordinated by the protein (not depicted and not to be discussed further here). The protein sequence making up the selectivity filter includes the GYG signature sequence for K^+ channels (Heginbotham et al., 1992, 1994). It is evident that all sites in this selectivity filter are not equivalent, yet they share a common cage of tightly packed ligands for the K^+ ions, which is able to match the bulk water hydration number for K^+ ions of 5–7 (Neilson and Skipper, 1985) and stabilize the nearly completely dehydrated ions.

This crystallographic evidence for K^+-binding sites, where the K^+ ions appear perfectly coordinated by protein-derived ligands, which, according to the authors, could not properly coordinate a Na^+ ion, appeared entirely consistent with the picture of selectivity depicted by Neyton and Miller (1988a,b); although, as we discuss below, a "snug-fit" hypothesis may not be the true origin of such a thermodynamic preference within the crystallographic K^+ sites. As a result, the current view in the field has favored the hypothesis of selective permeation via selective binding, where K^+ channels prevent Na^+ from permeating because accommodating a Na^+ inside the selectivity filter is a thermodynamically unfavorable process.

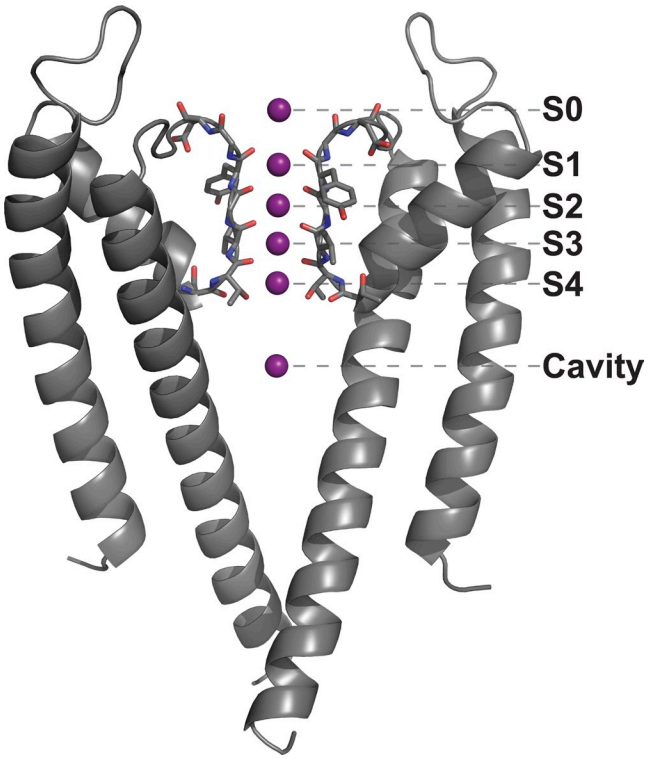

Figure 1. Structure of KcsA (Zhou et al., 2001) (deposited in the Protein Data Bank under accession no. 1K4C) with only two opposing subunits shown for clarity: the carbonyl oxygens forming the K^+-binding sites in the selectivity filter (S0–S4) in red and K^+ ions in purple.

K^+ channels with the same GYG signature sequence display different selectivities

Early attempts to put a value on the selectivity ratio between K^+ and Na^+ ions in K^+ channels were in vain, because there was no measurable current carried by Na^+ through the K^+ channels in the squid axon, and most K^+ channels showed no detectable Na^+ currents. Estimates of lower limits of K^+ to Na^+ permeability ratios have been made for various K^+ channels by measuring the reversal potentials in mixed ionic or bi-ionic solutions by using the Goldman-Hodgkin-Katz equation (Goldman, 1943; Hodgkin and Katz, 1949). A few K^+ channels have been found in native tissues that were shown to transport Na^+, mainly in the absence of K^+ (Zhu and Ikeda, 1993; Callahan and Korn, 1994; Block and Jones, 1996), and with the advent of cloning, several types of K^+ channels were identified that allow Na^+ flux, but only in the absence of K^+ (Korn and Ikeda, 1995; Kiss et al., 1999; Wang et al., 2000, 2009). All of these K^+ channels display the same conserved canonical GYG signature sequence responsible for high K^+ selectivity, but they exhibit different degrees of selectivity. This suggests that it is not only the sequence of four carbonyl oxygen cages that determines selectivity in K^+ channels with the same GYG signature sequence, but also that there are other important factors modulating ion selectivity. For instance, in addition to the subset of K^+ channels reported to pass Na^+ ions in the total absence of K^+, some K^+ channels become more selective for Na^+ during C-type inactivation (Starkus et al., 1997, 1998; Kiss and Korn, 1998), a process believed to involve a change in the conformation of the selectivity filter prohibiting K^+ permeation (Liu et al., 1996).

Permeant K^+ ions reside predominantly in the intracellular space, with physiological concentrations of 100–150 mM, and will fill a K^+ channel pore. Although abundant in the extracellular space (~150 mM), Na^+ ions will not permeate and remain excluded from the K^+-occupied pore (the channel exhibits no known block by extracellular Na^+ [Bezanilla and Armstrong, 1972; Adelman and French, 1978; Yellen, 1984; Heginbotham et al., 1999], although for a possible exception, see Block and Jones, 1996). Intracellular Na^+ ions, on the other hand, although present at only low concentrations of 5–10 mM, also cannot pass through a K^+-occupied selectivity filter, but will reach the relatively nonselective aqueous cavity (Fig. 1) and block the K^+ flux with low affinity and fast kinetics (Bezanilla and Armstrong, 1972; French and Shoukimas, 1985; Nimigean and

Miller, 2002). Therefore, the question is, by what mechanism does the K^+ channel selectivity filter exclude the passage of Na^+ ions for this physiologically relevant situation in the presence of K^+ ions? As we shall discuss, block of permeant ions may hold the key to physiological selectivity in K^+ channels, and it is from this perspective that we focus this paper.

The prevailing view of thermodynamic-based selectivity

The snug-fit hypothesis for selectivity suggests that the crystallographically identified K^+-binding sites made by cages of carbonyl oxygen ligands, apparently of the right dimension for a K^+ ion, cannot effectively coordinate the smaller Na^+, rendering Na^+ binding thermodynamically unfavorable (Bezanilla and Armstrong, 1972; Hille, 1973; Doyle et al., 1998; Zhou et al., 2001). Such a simplified picture of size-based selectivity has existed for half a century, since the time Mullins (1959, 1960) invoked a molecular-sieving mechanism. It is a view that persisted in the following decades (e.g., Hille, 1973), and it has become the standard textbook explanation today (Hille, 2001; Alberts et al., 2007). Such an idea, although attractive because of its simplicity, is inconsistent with the natural flexibility of proteins (Allen et al., 2004; Noskov et al., 2004) and does not address the nature of microscopic ion–protein interactions and their consequences for solvation free energy, as addressed by Eisenman and colleagues over this period (Eisenman, 1961; Eisenman and Horn, 1983; Yamashita et al., 1990). Recent descriptions of the origins of a difference in thermodynamic stability in the crystallographic K^+ sites have invoked interactions among fluctuating ligand dipoles that form the binding sites (Noskov et al., 2004; Noskov and Roux, 2006), as well as the preferences of K^+ and Na^+ ions for particular coordination numbers/topologies (Bostick and Brooks, 2007; Thomas et al., 2007; Varma and Rempe, 2007).

Molecular dynamics (MD) simulations of the KcsA channel have determined that there is selective binding for K^+ over Na^+ in the four S sites inside the filter, by up to ~5 kcal/mol (Allen et al., 2000; Bernèche and Roux, 2000; Luzhkov and Aqvist, 2001; Noskov et al., 2004). It is this thermodynamic preference for K^+ in the central S1–S3 (and in particular S2) crystallographic cage sites that has provided an apparent microscopic basis for validating a thermodynamic theory for K^+ channel size selectivity. However, simulation studies (Shrivastava et al., 2002; Burykin et al., 2003; Bucher et al., 2010; Kim and Allen, 2010) and experiments (Thompson et al., 2009) have suggested the possibility that the selectivity filter consists not only of the crystallographic K^+ (S0–S4) binding sites but also sites that are selective for Na^+. Such findings illustrate the need for caution when trying to infer the mechanisms of selective permeation from the energetics within individual K^+-only binding sites, as we shall now discuss. In particular, channel blocking studies, seeking to identify the mechanism of internal block of the KcsA channel, have led to new insights into how the K^+ channel selectivity filter operates.

Insights from experimental blocking and computational studies

Using KcsA as a model K^+ channel, the interaction of the small cations Na^+, as well as Li^+ (as a sensitive probe for size-based channel blocking), in the presence of permeant K^+ ions has recently been explored (Thompson et al., 2009). KcsA is known to be blocked by Na^+ from the intracellular side during (physiological) outward flux, providing an experimentally and computationally observable phenomenon that can shed light on K^+ channel selectivity (Nimigean and Miller, 2002; Thompson et al., 2009). Electrophysiology (planar lipid bilayers), MD free energy calculations, and x-ray crystallography have been combined to probe the elusive selectivity property with some consistent observations.

It has been proposed that both Na^+ and Li^+ have at least one binding site within the selectivity filter, distinct from the crystallographic K^+ S sites. This site, termed the B site, is positioned between S3 and S4, in-plane with the Thr75 carbonyl oxygen atoms (Figs. 3 and 4, discussed below). The evidence for this site was the presence of a free energy minimum for both Na^+ and Li^+ at that position and a strong thermodynamic preference for both of those ions over the K^+ ion in simulation studies; a crystal structure fully consistent with the presence of a Li^+ ion in that exact planar site; and functional data consistent with these ions blocking the K^+ current in the selectivity filter with a long dwell-time, in addition to the low affinity block exerted in the aqueous cavity (Thompson et al., 2009).

As a Na^+ or Li^+ ion approaches the KcsA pore from the intracellular solution, it enters the cavity where it binds with low affinity and fast kinetics, attenuating the K^+ current by preventing permeant K^+ ions from passing. The K^+ current becomes increasingly more attenuated as the voltage is made more positive, indicating that because the binding site for Na^+/Li^+ is located in the membrane electric field, the occupancy of the cavity by Na^+ or Li^+ increases with voltage, and because these small monovalent cations do not permeate through the selectivity filter, they occlude the permeation pathway (Fig. 2, A and B). That being said, if the voltage is increased above a certain value, the K^+ current begins to increase again, suggesting that the Na^+ ion is relieving block by exiting through the selectivity filter toward the extracellular milieu, rather than back toward the intracellular solution from where it came; this phenomenon is called "punchthrough" (Fig. 2, A and B) (Nimigean and Miller, 2002). At similarly high voltages, in the punchthrough regimen, another effect of Na^+ and Li^+ becomes apparent: a decrease in the channel burst duration, as if Na^+ and Li^+ are forcefully pushed out

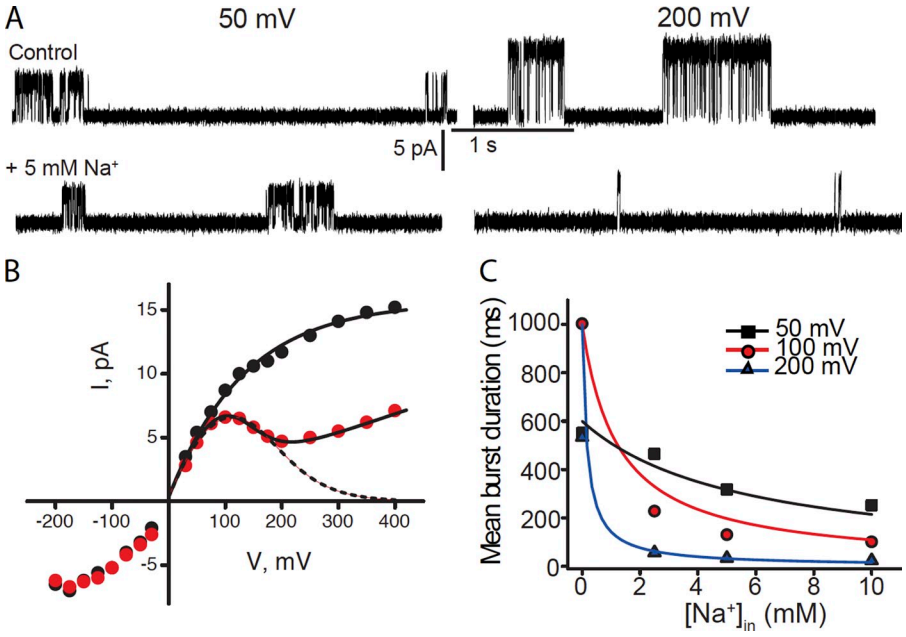

Figure 2. Na^+ blocks K^+ current through KcsA with fast and slow kinetics. (A) Single-channel current recordings adapted from Thompson et al. (2009), illustrating the decrease in K^+ current amplitude and the decrease in burst durations in the presence of intracellular Na^+. (B) I-V curves from Nimigean and Miller (2002), showing the effect of Na^+ on the K^+ single-channel current amplitude (red). Control data (black) and ideal Woodhull (1973) block (dashed line). (C) Mean burst durations decrease with increasing Na^+ and voltage. Adapted from Thompson et al. (2009).

of the cavity and into the selectivity filter by the high driving force generated by the voltage (Fig. 2, A and C). It was proposed that Na^+ (or Li^+) resides at this selectivity filter site for a long time, further blocking the K^+ current but with different, much slower kinetics than the fast block in the cavity (Thompson et al., 2009). This block with slow kinetics is consistent with Na^+/Li^+ binding in a deep well at the selectivity filter (the B site), as identified with MD simulations (Fig. 3; discussed below).

Another line of evidence that small monovalent cations can bind in the selectivity filter comes from crystals of KcsA channels grown in Li^+ solutions. If Li^+ and Na^+ bind at the proposed B site (Fig. 4), electron densities for these ions should theoretically be observed at this site. Unfortunately, Li^+ has only two electrons and it would not be seen, even at the highest resolution used today (2.0 Å; Zhou et al., 2001). It is equally difficult to identify Na^+ densities but for a different reason: Na^+, like water, has 10 electrons, so that their electron densities would be indistinguishable in an x-ray diffraction experiment. In spite of these issues, a specific location was proposed for the Li^+-binding site in the selectivity filter based on coordinating ligands (two water oxygens and four carbonyl oxygens from the Thr75 backbone; Fig. 4, inset) and the capability of Li^+ binding at this site to rescue the filter from the collapsed conformation adopted in the absence of K^+ (Thompson et al., 2009). To this end, the KcsA selectivity filter structure in Li^+ was surprisingly informative, especially after comparisons with the crystal structures of the filter in K^+ and the one in Na^+ (Fig. 4). Upon removal of K^+ from the channel and replacement with Na^+, the selectivity filter changes its conformation by drastically altering most of the K^+-binding sites and assuming a collapsed, proposed inactive, state (Fig. 4 B). The densities found in the selectivity filter in the presence of Na^+ are consistent with a mixed state where both water and Na^+ reside in the pore (Fig. 4 B), and although it is impossible to tell at this resolution between water and Na^+, there is density at the proposed B site, consistent with Na^+ binding at this location (the densities in Fig. 4 B were assigned as water or Na^+ by the authors [Lockless et al., 2007], partly based on coordinating ligands and the composition of the crystallization conditions). Unlike with Na^+, removing K^+ from KcsA and replacing it with Li^+ surprisingly does not lead to the collapsed selectivity filter state, despite the fact that all K^+ has been removed. In Li^+, the selectivity filter is still in the so-called "conductive" conformation, where the K^+-binding sites

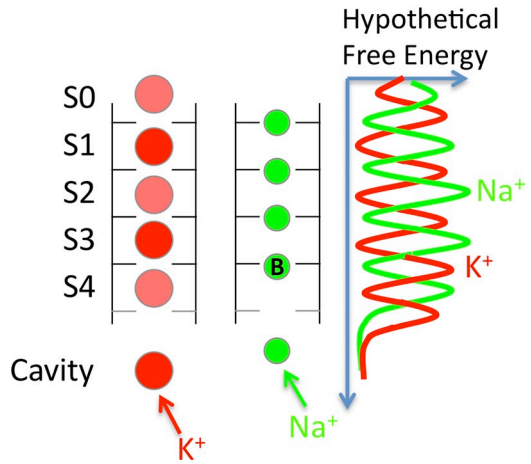

Figure 3. Selectivity filter sites suggested by MD simulations (Kim and Allen, 2010). On the right is a hypothetical free energy profile where K^+ and Na^+ would have similar thermodynamic stabilities. Precise heights of the barriers are arbitrary in this cartoon.

maintain their cage-like architecture (Fig. 4, compare A with C). It was concluded that Li^+ serendipitously keeps the filter from collapsing by binding inside the selectivity filter at the B-site location (Fig. 4, C and inset), consistent with the strong binding free energies seen computationally at this site(Thompson et al., 2009). Why would Li^+ succeed in maintaining a conductive filter conformation while Na^+ causes its collapse? Perhaps the tighter binding of Li^+ to carbonyl oxygen ligands leads to a strong inward force on the filter backbone, in addition to the presence of two tightly bound water molecules in the S4 and S3 sites that further stabilize the filter carbonyls in an inward orientation (Fig. 4, C and inset). Thus, the use of Li^+ as a probe in this case, despite the impossibility of finding electron density for it in this experiment, was particularly useful in ascertaining its binding in the selectivity filter.

Although the experimental blocking studies focused on the movement of ions into the intracellular cavity and the bottom of the selectivity filter, the presence of a planar Na^+/Li^+-selective binding site adjacent to the S3 and S4 crystallographic K^+ cage sites leads to the hypothesis that Na^+/Li^+-selective plane sites may exist between all of the crystallographic K^+ sites in the selectivity filter. Recent simulation studies (Kim and Allen, 2010), which reveal the detailed free energy profiles of ions across individual sites in the selectivity filter, have suggested that each crystallographic K^+ site is adjacent to a site selective for Na^+. It has been proposed that the most selective site, S2 (e.g., Allen et al., 2000; Noskov et al., 2004; although it has also been suggested to be S1; Luzhkov and Aqvist, 2001), actually consists of a crystallographic K^+-binding site in a cage of eight carbonyl ligands and two adjacent planar Na^+ sites made up of just four carbonyl groups (as well as water molecules above and below the ion). The cage site is selective for K^+ over Na^+ by ~5 kcal/mol, whereas each planar site is selective for Na^+ over K^+ by ~3 kcal/mol, in a similar fashion to the S4 and B site at the base of the filter. One may then ask why only one Na^+- or Li^+-binding site was suggested by the crystal structures (Fig. 4; at least for the conducting conformation presumed for Li^+), when MD simulations suggest multiple such sites. In the future we shall explore this question, including the possibility that the S3–S4 planar site is lower in free energy and is thus more prominent (Kim and Allen, 2010).

The cartoon in Fig. 3 shows a hypothesis of the free energy landscapes, with that for Na^+ "phase-shifted" by one half binding–site separation distance. A recent free energy profile for Na^+ entering from outside the channel partly supports this basic picture (Egwolf and Roux, 2010). Although it is a crude representation of the free energy profiles faced by K^+ and Na^+, and some variation in energy must be permitted (e.g., allowing for the possibility of Na^+ still having an elevated energy relative to K^+, as drawn here), the cartoon highlights a very different view of the K^+ selectivity filter. It leads us to a view that is less centered on the thermodynamic stability of K^+ and Na^+ bound in the filter. If this is the case, one must also take into consideration the kinetic description, where Na^+ might face a greater barrier for channel (or selectivity filter) entry. The question then must be asked: what is this barrier?

Possible "kinetic"-based selectivity

If the binding of Na^+ inside the filter were to be not thermodynamically unfavorable, where would the selection against Na^+ ions originate? Evidence for this barrier can come from blocking studies, which directly examine the entry of ions into the filter, at least for the case of Na^+ rejection in the presence of permeant K^+ ions. To understand this barrier, one has to recall the mechanism by which K^+ ions alone permeate this channel with such efficiency. K^+ ions follow a multi-ion permeation pathway, entailing a knock-on of ions that is almost barrier-less (of the order of a kcal/mol). This was originally proposed over half a century ago by Hodgkin and Keynes (1955), based on isotope flux coupling, and was illustrated well by Brownian dynamics simulations that revealed the principle of balancing electrostatic attraction

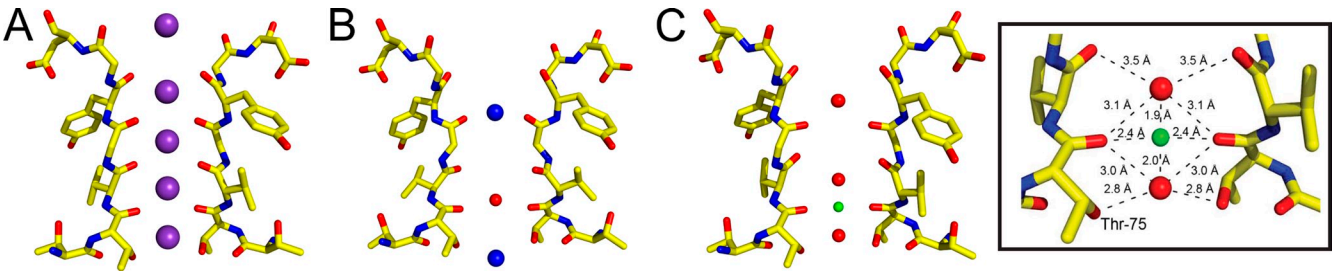

Figure 4. KcsA selectivity filter conformations from x-ray crystallography experiments in the following conditions: (A) high K^+ (Zhou et al., 2001) (deposited in the Protein Data Bank under accession no. 1K4C); (B) high Na^+ and no K^+ (Zhou et al., 2001) (Protein Data Bank accession no. 2ITC); (C) high Li^+ and no K^+ (Thompson et al., 2009) (Protein Data Bank accession no. 3GB7). (Inset) An increased representation of the S3 and S4 sites at the bottom of the selectivity filter where Li^+ is proposed to bind, with the coordination distances indicated. Purple, K^+ ions; red, waters; blue, Na^+ ions; green, Li^+ ions.

of ions to the protein and repulsion between multiple ions (Allen et al., 1999; Chung et al., 1999; Allen and Chung, 2001; Bernèche and Roux, 2003), as well as by atomistic simulations (Bernèche and Roux, 2001), that uncovered small barriers separating the low free energy configurations S0/S2/S4 and S1/S3/cavity, enabling rapid flow of ions. The low barrier to this knock-on, of only up to 2 kcal/mol (see Fig. 5, left, upper path), owes itself to the existence of a low-lying intermediate state for conduction, namely the ability of a K^+ ion to bind, partially hydrated, to S4 while K ions are residing in S1 and S3. It is the binding of this third ion in close proximity to the other two that promotes the knock-on by Coulomb repulsion. But can this occur when Na^+/Li^+ are introduced into the mix?

It has been shown that, for intracellular Na^+ to bind at its binding site between the crystallographic S3 and S4 K^+ sites (i.e., the B site), it must follow a different pathway and suffer additional energy costs. The shift of Na^+'s binding site upward (by one half of a crystallographic site), eliminated the aforementioned intermediate state for conduction, simply because that site would correspond to overlapping Na^+ (in the S3/S4 B site) and K^+ (in the S3 site) ions (see Fig. 5 right, upper path). For a conduction event to occur, the two consecutive sites, S3 and S4, must be devoid of K^+ ions. It was determined that the outward movement of K^+ ions to free up these sites is associated with a substantial free energy barrier (of at least 4 kcal/mol). Thus, the shift of the ion-binding site by just ~1 Å (from a cage to an adjacent plane of ligands) has eliminated the high conduction pathway for the K^+ channel, effectively excluding Na^+ ions from passing the channel in the presence of K^+ ions.

The Na^+ ions therefore have affinity for the selectivity filter, but they have trouble rearranging the permeant ions to reach their different binding sites (deeper inside the filter). We suggest that the height of this K^+-induced energy barrier may underlie selectivity against small intracellular monovalent cations, at least for the case of outward flux in the presence of permeant K^+ ions. This suggestion for the initial rejection step against intracellular Na^+ from the selectivity filter of K^+ channels is consistent with the requirement for very large driving forces to push the Na^+/Li^+ from the cavity (where they block with fast kinetics) into the selectivity filter (where they block with slow kinetics) (Fig. 2). It is also consistent with the original hypothesis of Bezanilla and Armstrong (1972), who suggested that it is the rates of entry into the selectivity filter that determine the selectivity between K^+ and Na^+, despite emerging from a simpler description of the permeation process.

The future: experimental and computational tools for revealing selectivity

These recent blocking studies have unveiled a more complex picture of selectivity on K^+ channels, where Na^+ ions may not be simply rejected because of an elevated free energy in crystallographic K^+ sites. What has been shown is that even a K^+ channel selectivity filter is made up of both K^+- and Na^+-binding sites, and discrimination, at least for the case of outward flux of Na^+ in the presence of permeant K^+ ions, emerges from a multi-ion conduction mechanism that has not been optimized for K^+–Na^+ mixtures.

Obviously, the complete story of selectivity must be able to explain lack of permeability of Na^+ ions from the outside of the cell (where Na^+ is abundant). Work is underway to address the case of rejection of external Na^+ ions using a combination of electrophysiology and free energy simulations. K^+ channels must effectively exclude abundant Na^+ ions on the outside of the cell membrane. Extracellular Na^+ ions are not observed to block KcsA (and other K^+ channels; Bezanilla and Armstrong, 1972; Yellen, 1984; Heginbotham et al., 1999) with fast kinetics, possibly because of the absence of a cavity at the extracellular channel mouth (Doyle et al., 1998).

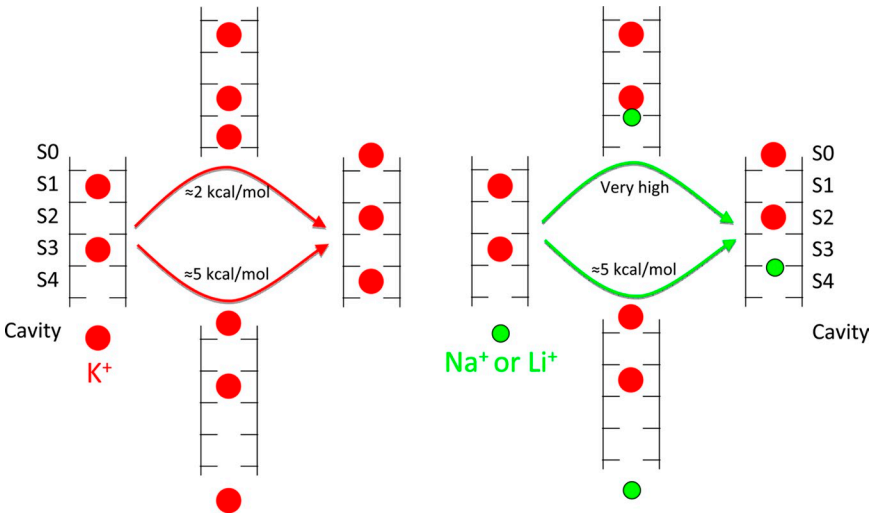

Figure 5. Origin of the multi-ion barrier for Na^+ outward permeation in the presence of K^+ ions. See text for description. Based on Thompson et al. (2009).

We are looking into the possibility of slow block of KcsA at a high concentration of external Na^+ and high negative membrane potentials, which could indicate that Na^+ may actually enter and bind inside the filter, provided a large barrier is overcome by sufficient driving voltage. MD simulations can reveal the energetics leading to selective permeation and provide an atomic-level basis for interpreting experimental observations. In the case of a long multi-ion pore, such as the K^+ channel, one must compute multi-ion free energy surfaces, using multidimensional umbrella sampling (Thompson et al., 2009), for example, as has been useful in revealing the possible barriers preventing Na^+ conduction in the presence of K^+ ions (Egwolf and Roux, 2010; Thompson et al., 2009).

These studies have revealed an interruption of near barrier-less permeation when blocking ions, such as Na^+, bind to change the multi-ion permeation mechanism inside the K^+ channel pore. But what would happen in the case when only Na^+ is present? If the lack of Na^+ permeation in the presence of K^+ is a result of large barriers created by the mismatch of ion binding sites for K^+ and Na^+ inside the selectivity filter, one would predict that when only Na^+ is present, there could be measurable Na^+ flux with a magnitude dictated by a different multi-ion mechanism, which may vary from K^+ channel to K^+ channel. This may be implied by the wide variability of K^+ conductances for different K^+ channel types that display the same GYG signature sequence. Some K^+ channels allow measurable Na^+ flux in the absence of K^+ (Kv3 [Wang et al., 2009], Kv2.1 [Korn and Ikeda, 1995], Kv1.5 [Korn and Ikeda, 1995; Wang et al., 2000], chimeras and mutants of voltage-gated K^+ channels [Kiss et al., 1998; Ogielska and Aldrich, 1998], mutants of inward rectifier K^+ channels [Dibb et al., 2003], and KcsA mutants [Valiyaveetil et al., 2006]), and at the same time maintain high K^+ selectivity in the presence of K^+, whereas others do not allow Na^+ currents and even become defunct in the absence of K^+ (Pardo et al., 1992; Heginbotham and MacKinnon, 1993; Gómez-Lagunas, 1997; Jäger et al., 1998). Furthermore, during C-type inactivation, the selectivity filter dynamically changes its selectivity properties (Starkus et al., 1997), and changes in inactivation properties in mutants are strongly correlated to changes in selectivity (Starkus et al., 1998; Dibb et al., 2003; Sackin et al., 2009, 2010). All of these observations argue toward the existence of several modulators of selectivity in K^+ channels, in addition to the intrinsic properties of the binding sites, which are all made from the same primary sequence, GYG, and according to the same architectural principle.

One possibility, investigated by Varma and Rempe (2007), is that the dielectric constant surrounding the filter modulates selectivity by tuning ion coordination. Another possibility is that these variable residues lead to interactions behind the selectivity filter that may prevent or favor certain filter conformations, which can yield channels with different selectivities. Indeed, we recently found that a noninactivating KcsA variant, where a hydrogen bond behind the selectivity filter was disrupted through mutation of a glutamate (E71) to an alanine, displayed increased permeability to Na^+ (Cheng et al., 2011). A crystal structure of this mutant showed that in the absence of K^+, and in the presence of Na^+, the selectivity filter of this channel no longer collapses like the wild-type channel (Zhou et al., 2001) but maintains a presumably conductive conformation, encountered also in the presence of K^+ (Cordero-Morales et al., 2006). Thus, we hypothesized that there are multiple layers of selectivity in K^+ channels where the first layer, the selectivity provided by the succession of carbonyl oxygen cages from the GYG signature sequence, can be modulated by factors such as modified interactions with the variable sequence behind the filter or excursions to a different filter conformation (such as the collapsed conformation; Zhou et al., 2001).

Conclusion

Recent studies have revealed the surprising ability for a K^+ channel selectivity filter to accommodate both K^+ and Na^+ ions, challenging the prevailing view of thermodynamically driven selectivity. They have also provided a rationale for Na^+ exclusion that may be consistent with a kinetic model of selectivity. Such studies, from the perspective of channel blocking, have provided interesting new views of the selection process and have raised new questions, but they have not yet provided all of the answers. They do, however, help us begin to think about the process of channel selectivity in a way that is not restricted to simple arguments about thermodynamic stability in crystallographic K^+ sites alone. What is needed is a concerted effort between experimental structure and functional studies and all-atom simulations that can elucidate the underpinning thermodynamic and kinetic mechanisms underlying selectivity phenomena.

This Perspectives series includes articles by Andersen, Alam and Jiang, Roux et al., Dixit and Asthagiri, and Varma et al. (scheduled for the June 2011 issue).

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REFERENCES

Adelman, W.J., Jr., and R.J. French. 1978. Blocking of the squid axon potassium channel by external caesium ions. *J. Physiol.* 276:13–25.

Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. 2007. Molecular Biology of the Cell. Fifth edition. Garland Science, New York. 1392 pp.

Allen, T.W., and S.H. Chung. 2001. Brownian dynamics study of an open-state KcsA potassium channel. *Biochim. Biophys. Acta.* 1515: 83–91. doi:10.1016/S0005-2736(01)00395-9

Allen, T.W., M. Hoyles, S. Kuyucak, and S.H. Chung. 1999. Molecular and Brownian dynamics study of ion selectivity and conductivity in the potassium channel. *Chem. Phys. Lett.* 313:358–365. doi:10.1016/S0009-2614(99)01004-0

Allen, T.W., A. Bliznyuk, A.P. Rendell, S. Kuyucak, and S.H. Chung. 2000. The potassium channel: structure, selectivity and diffusion. *J. Chem. Phys.* 112:8191–8204. doi:10.1063/1.481420

Allen, T.W., O.S. Andersen, and B. Roux. 2004. On the importance of atomic fluctuations, protein flexibility, and solvent in ion permeation. *J. Gen. Physiol.* 124:679–690. doi:10.1085/jgp.200409111

Bernèche, S., and B. Roux. 2000. Molecular dynamics of the KcsA K(+) channel in a bilayer membrane. *Biophys. J.* 78:2900–2917. doi:10.1016/S0006-3495(00)76831-7

Bernèche, S., and B. Roux. 2001. Energetics of ion conduction through the K+ channel. *Nature.* 414:73–77. doi:10.1038/35102067

Bernèche, S., and B. Roux. 2003. A microscopic view of ion conduction through the K+ channel. *Proc. Natl. Acad. Sci. USA.* 100:8644–8648. doi:10.1073/pnas.1431750100

Bezanilla, F., and C.M. Armstrong. 1972. Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons. *J. Gen. Physiol.* 60:588–608. doi:10.1085/jgp.60.5.588

Block, B.M., and S.W. Jones. 1996. Ion permeation and block of M-type and delayed rectifier potassium channels. Whole-cell recordings from bullfrog sympathetic neurons. *J. Gen. Physiol.* 107:473–488. doi:10.1085/jgp.107.4.473

Bostick, D.L., and C.L. Brooks III. 2007. Selectivity in K+ channels is due to topological control of the permeant ion's coordinated state. *Proc. Natl. Acad. Sci. USA.* 104:9260–9265. doi:10.1073/pnas.0700554104

Bucher, D., L. Guidoni, P. Carloni, and U. Rothlisberger. 2010. Coordination numbers of K(+) and Na(+) ions inside the selectivity filter of the KcsA potassium channel: insights from first principles molecular dynamics. *Biophys. J.* 98:L47–L49. doi:10.1016/j.bpj.2010.01.064

Burykin, A., M. Kato, and A. Warshel. 2003. Exploring the origin of the ion selectivity of the KcsA potassium channel. *Proteins.* 52:412–426. doi:10.1002/prot.10455

Callahan, M.J., and S.J. Korn. 1994. Permeation of Na^+ through a delayed rectifier K^+ channel in chick dorsal root ganglion neurons. *J. Gen. Physiol.* 104:747–771. doi:10.1085/jgp.104.4.747

Cheng, W.W.L., J.G. McCoy, A.N. Thompson, C.G. Nichols, and C.M. Nimigean. 2011. Mechanism for selectivity-inactivation coupling in KcsA potassium channels. *Proc. Natl. Acad. Sci. USA.* 108:5272–5277. doi:10.1073/pnas.1014186108

Chung, S.H., T.W. Allen, M. Hoyles, and S. Kuyucak. 1999. Permeation of ions across the potassium channel: Brownian dynamics studies. *Biophys. J.* 77:2517–2533. doi:10.1016/S0006-3495(99)77087-6

Cordero-Morales, J.F., L.G. Cuello, Y. Zhao, V. Jogini, D.M. Cortes, B. Roux, and E. Perozo. 2006. Molecular determinants of gating at the potassium-channel selectivity filter. *Nat. Struct. Mol. Biol.* 13:311–318.

Dibb, K.M., T. Rose, S.Y. Makary, T.W. Claydon, D. Enkvetchakul, R. Leach, C.G. Nichols, and M.R. Boyett. 2003. Molecular basis of ion selectivity, block, and rectification of the inward rectifier Kir3.1/Kir3.4 K(+) channel. *J. Biol. Chem.* 278:49537–49548. doi:10.1074/jbc.M307723200

Doyle, D.A., J. Morais Cabral, R.A. Pfuetzner, A. 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.* 280:69–77. doi:10.1126/science.280.5360.69

Egwolf, B., and B. Roux. 2010. Ion selectivity of the KcsA channel: a perspective from multi-ion free energy landscapes. *J. Mol. Biol.* 401:831–842. doi:10.1016/j.jmb.2010.07.006

Eisenman, G. 1961. On the elementary origin of equilibrium ion specificity. *In* Symposium on Membrane Transport and Metabolism. A. Kleinzeller and A. Kotyk, editors. Academic Press, New York. 163–179.

Eisenman, G., and R. Horn. 1983. Ionic selectivity revisited: the role of kinetic and equilibrium processes in ion permeation through channels. *J. Membr. Biol.* 76:197–225. doi:10.1007/BF01870364

French, R.J., and J.J. Shoukimas. 1985. An ion's view of the potassium channel. The structure of the permeation pathway as sensed by a variety of blocking ions. *J. Gen. Physiol.* 85:669–698. doi:10.1085/jgp.85.5.669

Goldman, D.E. 1943. Potential, impedence and rectification in membranes. *J. Gen. Physiol.* 27:37–60. doi:10.1085/jgp.27.1.37

Gómez-Lagunas, F. 1997. Shaker B K+ conductance in Na+ solutions lacking K+ ions: a remarkably stable non-conducting state produced by membrane depolarizations. *J. Physiol.* 499:3–15.

Heginbotham, L., and R. MacKinnon. 1993. Conduction properties of the cloned Shaker K+ channel. *Biophys. J.* 65:2089–2096. doi:10.1016/S0006-3495(93)81244-X

Heginbotham, L., T. Abramson, and R. MacKinnon. 1992. A functional connection between the pores of distantly related ion channels as revealed by mutant K+ channels. *Science.* 258:1152–1155. doi:10.1126/science.1279807

Heginbotham, L., Z. Lu, T. Abramson, and R. MacKinnon. 1994. Mutations in the K+ channel signature sequence. *Biophys. J.* 66:1061–1067. doi:10.1016/S0006-3495(94)80887-2

Heginbotham, L., M. LeMasurier, L. Kolmakova-Partensky, and C. Miller. 1999. Single streptomyces lividans K^+ channels: functional asymmetries and sidedness of proton activation. *J. Gen. Physiol.* 114:551–560. doi:10.1085/jgp.114.4.551

Hille, B. 1971. The permeability of the sodium channel to organic cations in myelinated nerve. *J. Gen. Physiol.* 58:599–619. doi:10.1085/jgp.58.6.599

Hille, B. 1973. Potassium channels in myelinated nerve. Selective permeability to small cations. *J. Gen. Physiol.* 61:669–686. doi:10.1085/jgp.61.6.669

Hille, B. 2001. Ion Channels of Excitable Membranes. Third edition. Sinauer Associates, Inc., Sunderland, MA. 814 pp.

Hodgkin, A.L., and B. Katz. 1949. The effect of sodium ions on the electrical activity of giant axon of the squid. *J. Physiol.* 108:37–77.

Hodgkin, A.L., and R.D. Keynes. 1955. The potassium permeability of a giant nerve fibre. *J. Physiol.* 128:61–88.

Jäger, H., H. Rauer, A.N. Nguyen, J. Aiyar, K.G. Chandy, and S. Grissmer. 1998. Regulation of mammalian Shaker-related K+ channels: evidence for non-conducting closed and non-conducting inactivated states. *J. Physiol.* 506:291–301. doi:10.1111/j.1469-7793.1998.291bw.x

Kim, I., and T.W. Allen. 2010. Multi-ion mechanism of potassium channel rejection of Na and Li ions. *Biophys. J.* 98:331a. doi:10.1016/j.bpj.2009.12.1796

Kiss, L., and S.J. Korn. 1998. Modulation of C-type inactivation by K+ at the potassium channel selectivity filter. *Biophys. J.* 74:1840–1849. doi:10.1016/S0006-3495(98)77894-4

Kiss, L., D. Immke, J. LoTurco, and S.J. Korn. 1998. The interaction of Na^+ and K^+ in voltage-gated potassium channels. Evidence for cation binding sites of different affinity. *J. Gen. Physiol.* 111:195–206. doi:10.1085/jgp.111.2.195

Kiss, L., J. LoTurco, and S.J. Korn. 1999. Contribution of the selectivity filter to inactivation in potassium channels. *Biophys. J.* 76:253–263. doi:10.1016/S0006-3495(99)77194-8

Korn, S.J., and S.R. Ikeda. 1995. Permeation selectivity by competition in a delayed rectifier potassium channel. *Science.* 269:410–412. doi:10.1126/science.7618108

Liu, Y., M.E. Jurman, and G. Yellen. 1996. Dynamic rearrangement of the outer mouth of a K+ channel during gating. *Neuron.* 16:859–867. doi:10.1016/S0896-6273(00)80106-3

Lockless, S.W., M. Zhou, and R. MacKinnon. 2007. Structural and thermodynamic properties of selective ion binding in a K+ channel. *PLoS Biol.* 5:e121. doi:10.1371/journal.pbio.0050121

Luzhkov, V.B., and J. Aqvist. 2001. K(+)/Na(+) selectivity of the KcsA potassium channel from microscopic free energy perturbation calculations. *Biochim. Biophys. Acta.* 1548:194–202. doi:10.1016/S0167-4838(01)00213-8

Morais-Cabral, J.H., Y. Zhou, and R. MacKinnon. 2001. Energetic optimization of ion conduction rate by the K^+ selectivity filter. *Nature.* 414:37–42. doi:10.1038/35102000

Mullins, L.J. 1959. An analysis of conductance changes in squid axon. *J. Gen. Physiol.* 42:1013–1035. doi:10.1085/jgp.42.5.1013

Mullins, L.J. 1960. An analysis of pore size in excitable membranes. *J. Gen. Physiol.* 43:105–117. doi:10.1085/jgp.43.5.105

Neilson, G.W., and N. Skipper. 1985. K+ coordination in aqueous solution. *Chem. Phys. Lett.* 114:35–38. doi:10.1016/0009-2614(85)85050-8

Neyton, J., and C. Miller. 1988a. Discrete Ba^{2+} block as a probe of ion occupancy and pore structure in the high-conductance Ca^{2+}-activated K^+ channel. *J. Gen. Physiol.* 92:569–586. doi:10.1085/jgp.92.5.569

Neyton, J., and C. Miller. 1988b. Potassium blocks barium permeation through a calcium-activated potassium channel. *J. Gen. Physiol.* 92:549–567. doi:10.1085/jgp.92.5.549

Nimigean, C.M., and C. Miller. 2002. Na^+ block and permeation in a K^+ channel of known structure. *J. Gen. Physiol.* 120:323–335. doi:10.1085/jgp.20028614

Noskov, S.Y., and B. Roux. 2006. Ion selectivity in potassium channels. *Biophys. Chem.* 124:279–291. doi:10.1016/j.bpc.2006.05.033

Noskov, S.Y., S. Bernèche, and B. Roux. 2004. Control of ion selectivity in potassium channels by electrostatic and dynamic properties of carbonyl ligands. *Nature.* 431:830–834. doi:10.1038/nature02943

Ogielska, E.M., and R.W. Aldrich. 1998. A mutation in S6 of Shaker potassium channels decreases the K^+ affinity of an ion binding site revealing ion–ion interactions in the pore. *J. Gen. Physiol.* 112:243–257. doi:10.1085/jgp.112.2.243

Pardo, L.A., S.H. Heinemann, H. Terlau, U. Ludewig, C. Lorra, O. Pongs, and W. Stühmer. 1992. Extracellular K+ specifically modulates a rat brain K+ channel. *Proc. Natl. Acad. Sci. USA.* 89:2466–2470. doi:10.1073/pnas.89.6.2466

Sackin, H., M. Nanazashvili, H. Li, L.G. Palmer, and D.E. Walters. 2009. An intersubunit salt bridge near the selectivity filter stabilizes the active state of Kir1.1. *Biophys. J.* 97:1058–1066. doi:10.1016/j.bpj.2009.05.056

Sackin, H., M. Nanazashvili, H. Li, L.G. Palmer, and D.E. Walters. 2010. A conserved arginine near the filter of Kir1.1 controls Rb/K selectivity. *Channels (Austin).* 4.

Shrivastava, I.H., D.P. Tieleman, P.C. Biggin, and M.S. Sansom. 2002. K(+) versus Na(+) ions in a K channel selectivity filter: a simulation study. *Biophys. J.* 83:633–645. doi:10.1016/S0006-3495(02)75197-7

Starkus, J.G., L. Kuschel, M.D. Rayner, and S.H. Heinemann. 1997. Ion conduction through C-type inactivated Shaker channels. *J. Gen. Physiol.* 110:539–550. doi:10.1085/jgp.110.5.539

Starkus, J.G., L. Kuschel, M.D. Rayner, and S.H. Heinemann. 1998. Macroscopic Na^+ currents in the "Nonconducting" *Shaker* potassium channel mutant W434F. *J. Gen. Physiol.* 112:85–93. doi:10.1085/jgp.112.1.85

Thomas, M., D. Jayatilaka, and B. Corry. 2007. The predominant role of coordination number in potassium channel selectivity. *Biophys. J.* 93:2635–2643. doi:10.1529/biophysj.107.108167

Thompson, A.N., I. Kim, T.D. Panosian, T.M. Iverson, T.W. Allen, and C.M. Nimigean. 2009. Mechanism of potassium-channel selectivity revealed by Na(+) and Li(+) binding sites within the KcsA pore. *Nat. Struct. Mol. Biol.* 16:1317–1324. doi:10.1038/nsmb.1703

Valiyaveetil, F.I., M. Leonetti, T.W. Muir, and R. Mackinnon. 2006. Ion selectivity in a semisynthetic K+ channel locked in the conductive conformation. *Science.* 314:1004–1007. doi:10.1126/science.1133415

Varma, S., and S.B. Rempe. 2007. Tuning ion coordination architectures to enable selective partitioning. *Biophys. J.* 93:1093–1099. doi:10.1529/biophysj.107.107482

Wang, Z., X. Zhang, and D. Fedida. 2000. Regulation of transient Na+ conductance by intra- and extracellular K+ in the human delayed rectifier K+ channel Kv1.5. *J. Physiol.* 523:575–591. doi:10.1111/j.1469-7793.2000.00575.x

Wang, Z., N.C. Wong, Y. Cheng, S.J. Kehl, and D. Fedida. 2009. Control of voltage-gated K^+ channel permeability to $NMDG^+$ by a residue at the outer pore. *J. Gen. Physiol.* 133:361–374. doi:10.1085/jgp.200810139

Woodhull, A.M. 1973. Ionic blockage of sodium channels in nerve. *J. Gen. Physiol.* 61:687–708. doi:10.1085/jgp.61.6.687

Yamashita, M.M., L. Wesson, G. Eisenman, and D. Eisenberg. 1990. Where metal ions bind in proteins. *Proc. Natl. Acad. Sci. USA.* 87:5648–5652. doi:10.1073/pnas.87.15.5648

Yellen, G. 1984. Ionic permeation and blockade in Ca^{2+}-activated K^+ channels of bovine chromaffin cells. *J. Gen. Physiol.* 84:157–186. doi:10.1085/jgp.84.2.157

Zhou, Y., J.H. Morais-Cabral, A. Kaufman, and R. MacKinnon. 2001. Chemistry of ion coordination and hydration revealed by a K+ channel-Fab complex at 2.0 A resolution. *Nature.* 414:43–48. doi:10.1038/35102009

Zhu, Y., and S.R. Ikeda. 1993. Anomalous permeation of Na+ through a putative K+ channel in rat superior cervical ganglion neurones. *J. Physiol.* 468:441–461.