Perspectives on: Ion selectivity

Thermodynamics of ion selectivity in the KcsA K^+ channel

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I. Introduction

In the voltage-gated K^+ channels, measurements of ion channel permeability show that the protein selectively allows the flow of K^+ over Na^+ with a fidelity of better than 1 part in 1,000 (Latorre and Miller, 1983). Recent measurements (Lockless et al., 2007) of equilibrium selectivity also suggest that, relative to K^+, Na^+ hardly binds to the selectivity filter. These observations are striking given that the Pauling radius of K^+ is only ~0.4 Å greater than that of Na^+ (Pauling, 1988). Here, we provide our perspective on the thermodynamics of this selectivity.

The elucidation of the crystal structure (Doyle et al., 1998) has allowed for detailed molecular scale investigations of the selectivity filter (Allen et al., 2000; Åqvist and Luzhkov, 2000; Bernéche and Roux, 2000, 2001; Luzhkov and Åqvist, 2001; Shrivastava et al., 2002; Miloshevsky and Jordan, 2008). The selectivity filter contains five ion-binding sites, S_0–S_4. S_4 is the site on the intracellular side and S_0 is near the extracellular mouth of the protein (Noskov et al., 2004). The S_2 site forms the narrowest part of the pore. In the S_2 site, for example, the K^+ ion snugly fits (Doyle et al., 1998) in a pocket comprising eight backbone carbonyl ligands, where each monomer of the homotetramer contributes two carbonyl ligands from adjacent residues (Fig. 1). Each site can accommodate either an ion or a water molecule, and the filter as a whole has two or three bound ions with the remaining sites filled by water molecules.

In computer simulations of equilibrium selectivity of the S_2 site (Noskov et al., 2004; Dixit et al., 2009), it is common to consider an ion in each of the S_0, S_2, and S_4 sites, with a water molecule each in S_1 and S_3. It is expected that configurations of the selectivity filter where adjacent binding sites are occupied by ions will be disfavored, and configurations where adjacent sites are occupied by water molecules are likely to be unstable as well (Bernéche and Roux, 2000). In our studies of selectivity, we consider the entire protein–lipid bilayer complex with this canonical occupancy of ions and water.

The background literature (Allen et al., 2000; Luzhkov and Åqvist, 2001; Zhou et al., 2001; Shrivastava et al., 2002; MacKinnon, 2003; Noskov et al., 2004; Asthagiri et al., 2006, 2010; Noskov and Roux, 2006, 2007; Bostick and Brooks, 2007, 2009; Lockless et al., 2007; Thomas et al., 2007; Varma and Rempe, 2007, 2008; Miloshevsky and Jordan, 2008; Varma et al., 2008; Dixit et al., 2009; Roux, 2010; Dixit and Asthagiri, 2011) and the other articles in this series can be consulted to gain some appreciation for the vibrant discussions on selectivity. We aim for a pedagogical approach here. We first present the thermodynamic framework defining selectivity. Our aim is to emphasize the quantities that go into quantifying selectivity. It is our thesis that this is necessary to help focus the discussion on physically realizable systems that are of first interest here. Then we discuss the statistical mechanical framework that, together with the known structure and computer simulations, can address each factor that goes into the metric defining selectivity. It is hoped that the insights obtained from these studies can helpfully advance our understanding of the molecular determinants of selectivity.

II. Thermodynamics of selectivity

The reversible work of transferring a Na^+ ion from the aqueous phase to the ion-binding site (S) in the selectivity filter relative to the case for a K^+ ion provides a thermodynamic measure of selectivity. Thus,

$$\begin{aligned}\Delta\mu^{ex} &= \left[\mu^{ex}_{Na^+}(S) - \mu^{ex}_{Na^+}(aq)\right] - \left[\mu^{ex}_{K^+}(S) - \mu^{ex}_{K^+}(aq)\right] \\ &= \left[\mu^{ex}_{Na^+}(S) - \mu^{ex}_{K^+}(S)\right] - \left[\mu^{ex}_{Na^+}(aq) - \mu^{ex}_{K^+}(aq)\right] \\ &= \Delta\mu^{ex}(S) - \Delta\mu^{ex}(aq),\end{aligned} \tag{1}$$

is the measure of equilibrium selectivity, where μ^{ex}_x is the excess chemical potential of the ion (x) in the site (S) or in bulk water (aq). The excess chemical potential, μ^{ex}_x, is the contribution to the Gibbs free energy per

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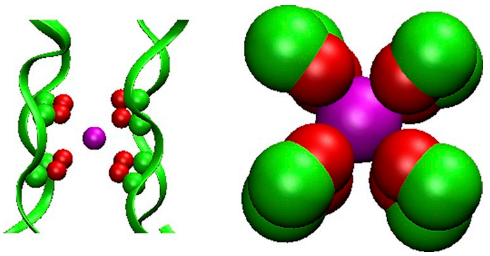

Figure 1. (Left) K^+ ion (magenta sphere) is shown bound in the S_2 site of the selectivity filter (green ribbon). The backbone carbonyl groups of the S_2 site are shown as spheres. (Right) View looking down the pore axis. Atoms are shown in space-filling format.

mole beyond the ideal gas contributions at the same temperature and density, and is also the quantity sought in computer simulations of ion selectivity.

Eq. 1 shows that two factors determine selectivity: (1) the hydration thermodynamics of the ion in water, and (2) the thermodynamics of the ion in the binding site. Each of these quantities is itself a large number on the thermal energy scale. For example, $\mu^{ex}_{Na^+}(aq) \approx -88.7$ kcal/mol (Asthagiri et al., 2003), $\mu^{ex}_{K^+}(aq) \approx -70.5$ kcal/mol (Rempe et al., 2004), and thus $\Delta\mu^{ex}(aq) \approx -18$ kcal/mol. (For the classical model used here [Dixit et al., 2009], $\Delta\mu^{ex}(aq) \approx -20.7$ kcal/mol.) But the net selectivity, $\Delta\mu^{ex} \approx 6$ kcal/mol (Noskov et al., 2004; Dixit et al., 2009), is substantially smaller than the individual differences.

Neglecting small corrections as a result of the changes in the isothermal compressibility and thermal expansivity of the system upon replacing K^+ with Na^+, the free energy change $\Delta\mu^{ex}(S)$ is given by Dixit and Asthagiri (2011) and Dixit et al. (2009):

$$\Delta\mu^{ex}(S) = \Delta\left\langle U_{sys}\right\rangle - T\Delta s^{ex} \tag{2}$$

In Eq. 2, T is the temperature, $\Delta\left\langle U_{sys}\right\rangle$ is the change in the average potential energy of the ion–protein system, and Δs^{ex} is the change in the excess entropy. (The angle brackets denote averaging with the appropriate ion in the binding site.) Eq. 2 can be taken as the definition of entropic effects in selectivity.

Formally, U_{sys} can be written as (Dixit and Asthagiri, 2011),

$$U_{sys} = \varepsilon_{local} + \varepsilon_m + U_s + U_m + U_{s\text{-}m}, \tag{3}$$

where ε_{local} is the interaction energy of the ion with protein groups in some defined neighborhood, and ε_m is the remaining long-range contribution to the ion–protein interaction energy. (Thus, the total ion–protein interaction energy is $\varepsilon = \varepsilon_{local} + \varepsilon_m$.) We will call the local neighborhood of the ion the "site" and the remaining part of the protein as the "medium"; for example, the eight carbonyl groups complexing the ion can be termed the site. In the presence of the ion, the potential energy of the site and the medium are given by the site–site (U_s), medium–medium (U_m), and site–medium ($U_{s\text{-}m}$) contributions to the potential energy.

In molecular biology, one often uses average binding strengths to rationalize binding (Alberts et al., 2002; MacKinnon, 2003). Rewriting Eq. 2 to reveal the average ion–protein binding energy explicitly, we have

$$\begin{aligned} \Delta\mu^{ex}(S) &\equiv \Delta\left\langle \varepsilon\right\rangle + \Delta\mu^{ex}_{fluc} \\ \Delta\mu^{ex}_{fluc} &= \Delta\left\langle U_s + U_m + U_{s\text{-}m}\right\rangle - T\Delta s^{ex}, \end{aligned} \tag{4}$$

where all the changes within the site and the medium contribute to the fluctuation contribution, $\Delta\mu^{ex}_{fluc}$. (The reason for this terminology is made clear below.)

Eq. 4 explicitly shows the various factors that contribute to selectivity, and, in particular, it explicitly reveals those contributions that do not directly involve ion–protein interaction. Intuitively, one normally first seeks to understand selectivity based on the local ion–site interaction, but as Eq. 4 shows, there are attendant changes in the medium that also need to be considered. For example, $\Delta\left\langle U_s\right\rangle$, the change in the average internal energy of the site upon changing the ion will sensitively depend on the composition of the binding site; thermodynamic theory makes it obvious that both number and chemical type of the ligands comprising the binding site will contribute to selectivity. Although there has been a vigorous debate about the relative importance of number versus chemistry of ligands, it appears to us that the only rigorous way to decouple the importance of either factor will be experiments where one can change the number (chemical type) without changing the chemical type (number) and keeping everything else the same. We are not aware of experimentally realizable systems that satisfy this requirement, and hence it is also not clear to us what the vigorous discussions about number versus chemistry seek to establish.

III. Statistical mechanics of selectivity

Given models of intermolecular interactions, statistical mechanics provides a way to calculate the terms in Eq. 4. In particular, the excess chemical potential of the ion is given by Widom (1982), Beck et al. (2006), Pratt and Asthagiri (2007),

$$\mu^{ex}_x = k_B T \ln \int e^{\beta\varepsilon} P_x(\varepsilon) d\varepsilon, \tag{5}$$

where $P_x(\varepsilon)$ is the distribution of binding (interaction) energies of the ion (x) with the rest of the medium, T is the temperature, and $\beta = 1/k_B T$ (k_B is the Boltzmann constant). Operationally, for the ion in the S_2 site, we construct $P_x(\varepsilon)$ by sampling configurations of the system (from a molecular dynamics simulation at constant pressure and temperature) and computing the binding (interaction) energy (ε) of the ion with all the atoms in the simulation system.

By expanding Eq. 5 into cumulants (Kubo, 1962), we can show that (Dixit et al., 2009)

$$\mu_x^{ex} = \langle \varepsilon \rangle + \mu_{fluc,x}^{ex}. \tag{6}$$

Here, $\langle \varepsilon \rangle_x$ is the mean binding energy of the ion (x) with the medium, and $\mu_{fluc,x}^{ex}$ is the remaining contribution to μ_x^{ex}. The fluctuation contribution $\mu_{fluc,x}^{ex}$ collects contributions from all cumulants of order two and greater. For example, if $P_x(\varepsilon)$ is a Gaussian distribution, cumulants of order three and greater are zero and $\mu_{fluc,x}^{ex} = \beta \sigma_x{}^2/2$, where σ_x^2 is the variance of $P_x(\varepsilon)$.

Comparing Eq. 6 with Eq. 4, we find that the spread (or dispersion) about the mean of the binding energy distribution of the ion with the protein informs us about the average potential energy of the site plus medium complex and the entropic effects in binding (Dixit et al., 2009). Thus, if the site–site interaction energy is larger in the presence of Na^+ relative to K^+, assuming entropic effects are small, we can expect the binding energy distribution of Na^+ with the protein will be broader as compared with that for K^+. As Fig. 2 shows, the binding energy distribution for Na^+ is indeed broader than that for K^+. Interestingly, the mean binding energy of Na^+ with the protein is lower than that for K^+ and $\Delta\langle \varepsilon \rangle \approx -20$ kcal/mol. As we noted above, because $\Delta\mu^{ex}(aq) \approx -20$ kcal/mol, mean binding energy alone cannot explain selectivity. Thus, we immediately infer that to understand selectivity, one must understand the changes in the medium that contribute to $\Delta\mu_{fluc}^{ex}$.

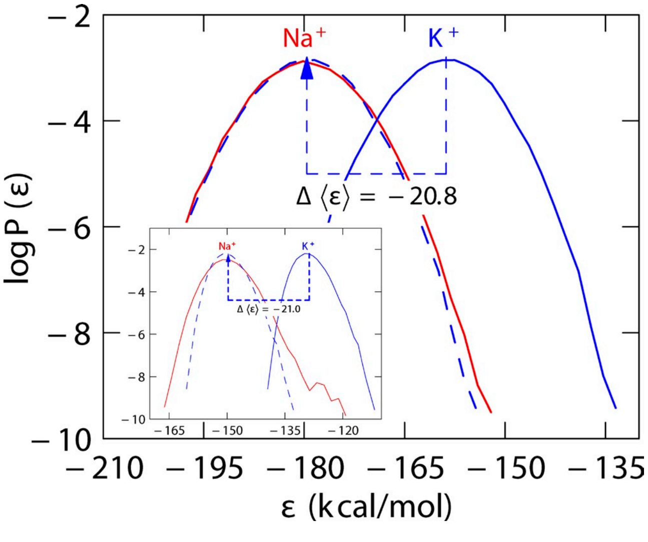

Figure 2. The logarithm of the distribution of binding energies, $P_x(\varepsilon)$, of K^+ (blue) and Na^+ (red) with the entire protein. The ions are in the S_2 site. (Inset) Binding energy distribution considering only interactions with the eight carbonyls of the S_2 site. (The eight-carbonyl site is extracted from simulations of the entire system.) Note that the local component more acutely highlights the larger range of energies sampled by Na^+. The dashed blue curve is the K^+ binding energy distribution translated along the energy axis to match the mean binding energy of the Na^+ distribution.

A. Simplified models of selectivity. Intuitively, one expects that the interaction of the ion with the protein material outside the binding site does not discriminate between Na^+ or K^+. It is thus natural to study the interactions of the ion with some defined local neighborhood to understand selectivity. (See background literature cited above.) For the potassium channels, experiments suggest that the selectivity filter can be affected by mutations outside the filter (for example, Cheng et al., 2011, and Valiyaveetil et al., 2006, and references therein), suggesting the importance of understanding the effect of the protein medium on the dynamics of the local site. To be valid models of the physical reality, a local model must factor in the effect of the environment outside the local site and satisfy some constraints. It is not always clear if this is indeed the case, yet such models have provided reasonable estimates of selectivity. In those cases, it is important to understand why such models work in the first place.

We know that $\varepsilon = \varepsilon_{local} + \varepsilon_m$. Thus, we must have

$$P_x(\varepsilon) = \int P_x(\varepsilon_{local}) P_x(\varepsilon_m \mid \varepsilon_{local}) d\varepsilon_{local}. \tag{7}$$

The condition in which the long-range contributions are nondiscriminatory requires that not only must $P_x(\varepsilon_m \mid \varepsilon_{local}) \approx P_x(\varepsilon_m)$, but also $P_{K^+}(\varepsilon_m) \approx P_{Na^+}(\varepsilon_m)$. If these conditions are satisfied, the local model is a reliable model of the entire system.

In Fig. 3 we plot ε_m versus U_{s-m}, the site–medium interaction. Here, the site comprises the carbonyl groups in the S_2 site, and for simplicity, the medium is simply the water molecules in S_1 and S_3. Treating water molecules in adjacent sites as the medium is justified because a large fraction of the effect of the entire medium is accounted for by these water molecules (Dixit et al., 2009; Dixit and Asthagiri, 2011). Furthermore, because site–medium interactions will be correlated with ion–medium interactions, if site–medium interactions are not sensitive to ion type, then ion–medium interactions will not be sensitive to ion type as well. With these observations, we find that for the canonical occupancy of binding sites in the filter, the binding energy of the K^+ or Na^+ ion in the S_2 site of KcsA does suggest that ion–medium interactions are uncorrelated with site–medium interactions, hence $P_x(\varepsilon_m \mid \varepsilon_{local}) \approx P_x(\varepsilon_m)$. They are also nearly the same for both Na^+ and K^+, hence $P_{K^+}(\varepsilon_m) \approx P_{Na^+}(\varepsilon_m)$. Observe that for a mutant analogue (Valiyaveetil et al., 2006) of the KcsA system, one with a glycine to D-alanine mutation in residue 77 ($G77A_D$) that prevents the site from collapsing in the absence of the ion, ε_m and U_{s-m} are inversely correlated: the medium is thus expected to influence selectivity.

As Table I shows, the free energy $\Delta\mu^{ex}(S)$ computed by accounting for all ion–protein interactions is nearly

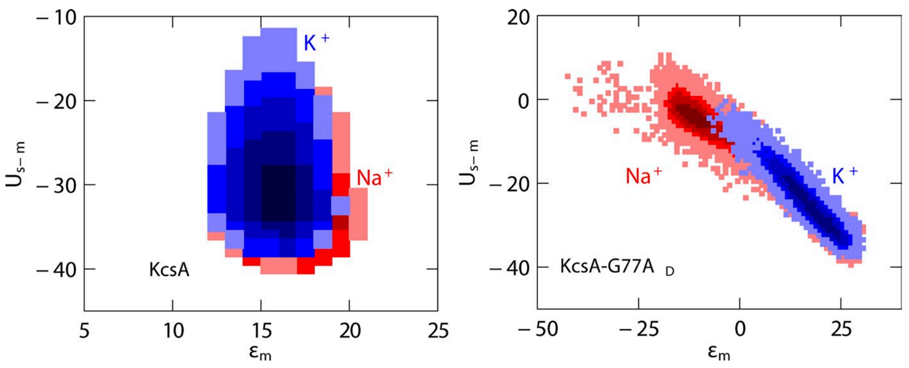

Figure 3. The joint probability distribution, $P_x(U_{s\text{-}m},\varepsilon_m)$, of the interaction of the ion with the representative protein medium ε_m versus the interaction of the site with the bulk protein medium, U_{s-m}. We regard the water molecules in S_1 and S_3 sites as the protein medium (Table I). ε_m and U_{s-m} are nearly independent of each other and similar for Na$^+$ and K$^+$ in KcsA (left), whereas they are inversely correlated in KcsA-G77A$_D$ (right). Figure reprinted from Dixit and Asthagiri (2011) with permission from Elsevier.

the same as the analogous quantity ΔW_{local} obtained by considering only ion–site interactions solely. (For calculating ΔW_{local}, we sample the local ion–site configurations from a simulation of the full ion–protein system.) The mean binding energies are, however, fairly different. As we argued recently (Dixit and Asthagiri, 2011), $\Delta\mu^{ex}(S)$ and ΔW_{local} are nearly similar because of the inverse correlation of ion–site and site–medium interactions. Thus, although the estimated selectivity free energy—a small difference of large numbers—can be insensitive to model details, the various components of the selectivity (Eq. 2) are not. Hence, a local model that reproduces the selectivity free energy may not represent the underlying physics with reasonable fidelity.

The results of Fig. 3 and Table I together suggest that, given the canonical occupancy of binding sites, a model of the binding site comprising eight carbonyl ligands (in the field imposed by the remaining protein) is acceptable for the KcsA system. Our analysis also provides a rigorous basis for the success of an isolated system of eight carbonyl ligands (Noskov et al., 2004), but in our studies, the eight-carbonyl site is always present in the field imposed by the remaining protein. Having thus established the utility of the eight-carbonyl model of the S_2 site for KcsA, we return to the question of the molecular basis of selectivity in this system.

B. Selectivity: role of energetic strain. Guided by the framework developed above, for the eight-carbonyl site (in the field of the remaining protein), in Fig. 4 we plot the distribution of ion–site binding energy with the site–site interaction energy. Here, $U_s \equiv U_{CO-CO}$. Clearly, on average, relative to K$^+$, Na$^+$ is better bound to the S_2 site. Further, it is obvious that better ion–site binding causes the average internal energy of the site to increase. As we noted above, this energetic strain directly determines the fluctuation contribution, because

$$\Delta\mu^{ex}_{fluc}(S_2)=\Delta\langle U\rangle\text{-}T\Delta s^{ex}(S_2). \qquad (8)$$

For the S2 site, $T\Delta s^{ex}(S_2)$ = 2.1 kcal/mol. Relative to the observed magnitude of selectivity ($\Delta\mu^{ex}(S_2)$ = −15.8 kcal/mol), the entropic contribution to the difference in ion-binding free energy is small (Noskov and Roux, 2006; Dixit et al., 2009). As Eq. 8 shows (Dixit et al., 2009), this implies compensation between matrix strain associated with ligand–ligand repulsion and binding–energy fluctuations: $\Delta\mu^{ex}(S_2)$ = 5.2 kcal/mol is roughly the same magnitude as $\Delta\langle U_{CO\text{-}CO}\rangle$ = 7.3 kcal/mol. Thus, we see that selectivity depends on the differential strain introduced in the medium (Noskov and Roux, 2007; Dixit et al., 2009; Dixit and Asthagiri, 2011), and this strain will be sensitive to both number and chemical type of the ligands in the binding site.

TABLE I

Energetic decomposition for KcsA and KcsA-G77A$_D$

		KcsA	KcsA-G77A$_D$
	$\Delta\mu^{ex}(S)$	−15.7	−10.0
	ΔW_{local}	−15.8	−9.9
	$T\Delta s^{ex}$	1.3	−1.1
	$T\Delta s^{ex}_{local}$	2.1	4.7
Ion–site	$\Delta\langle\varepsilon_{local}\rangle$	−21.0	0.8
Ion–medium	$\Delta\langle\varepsilon_m\rangle$	2.2	−20.1
Site–site	$\Delta\langle U_s\rangle$	7.3	−6.0
Site–medium	$\Delta\langle U_{s\text{-}m}\rangle$	−2.9	14.5
Medium–medium	$\Delta\langle U_m\rangle$	0.0	−0.3
Fluctuation (total)	$\Delta\mu^{ex}_{fluc}$	3.1	9.3
Fluctuation (local)	$\Delta W^{ex}_{local,fluc}$	5.2	−10.7

The eight carbonyl ligands comprise the local site. We consider the water molecules in the S_1 and S_3 sites adjoining the S_2 site as the medium. $\Delta\mu^{ex}(S)$ and ΔW_{local} are obtained using thermodynamic integration. The selectivity in the KcsA system is 5.0 kcal/mol considering the site plus the medium, and it is 4.9 kcal/mol for the eight-carbonyl model. Fluctuation (total), the fluctuation contribution for the site plus two-water system; Fluctuation (local), the fluctuation contribution for the site alone. Note that these values are comparable to the selectivity free energy. All values are in kcal/mol.

IV. Acknowledging mistakes and correcting errors

The framework of a Perspective behooves us to review our earlier work and correct some unfortunate errors that we made in citing previous research. In discussing one model of selectivity that purported to show that enforcing an eight-coordinate structure with water as ligands can explain selectivity (Bostick and Brooks, 2007), we made two errors. First, we mistakenly noted in the introduction of our article that those authors considered water molecules within a sphere of radius 3.5 Å as the binding site. (Later in the same article, we do acknowledge that they used different radii.) Second, we mistakenly noted that those authors compare the probability of observing an eight-coordinate structure around Na^+ (or K^+) relative to the most probable coordination state of those ions, whereas those authors did not compare it with the most probable coordination state. Ironically, had those authors indeed made the first error we ascribed to them, they would have immediately recognized the problems with their model. The second mistaken attribution does not materially affect the conclusions those authors drew. We revisit those questions here, as this highlights the care needed in estimating selectivity.

The study in question (Bostick and Brooks, 2007) had suggested that the coordination structure of the ions in the bulk aqueous phase itself provides insights into the selectivity in the channel. Specifically, it was claimed that because the probability of observing the eight-coordinate structure of Na^+ is much less than that for observing the eight-coordinate structure of K^+, the free energy change in imposing an eight-coordinate structure around Na^+ is higher relative to K^+. The radius of the first coordination shell of the respective ions, different for Na^+ and K^+, was used for defining the coordination states.

If $p_x(n;\lambda)$ is the probability of finding n water molecules in a coordination volume of radius λ centered on the ion x, and $p^{(0)}(n;\lambda)$ is the probability of finding n water molecules in the pure aqueous phase in an observation volume of radius λ in the absence of the ion, we have (Merchant and Asthagiri, 2009; Asthagiri et al., 2010; Merchant et al., 2011),

$$k_B T \ln p_x(n;\lambda) = k_B T \ln p^{(0)}(n;\lambda) - [\mu_x^{ex}(n;\lambda) - \mu_x^{ex}(aq)], \tag{9}$$

where $\mu_x^{ex}(n;\lambda)$ is the excess chemical potential of the ion subject to the constraint that there are only n solvent molecules within the coordination sphere. By noting that probabilities should sum to unity, it is easily confirmed that Eq. 9 leads to the correct multistate organization of the chemical potential $\mu_x^{ex}(aq)$ (Hummer et al., 1997; Merchant and Asthagiri, 2009; Asthagiri et al., 2010).

Thus, the selectivity for K^+ in the eight-water binding site, $\Delta\mu^{ex}$, is given by Asthagiri et al. (2010)

$$\begin{aligned} \Delta\mu^{ex} &\equiv \mu_{Na^+}^{ex}(n=8;\lambda_{Na^+}) - \mu_{K^+}^{ex}(n=8;\lambda_{K^+}) - \Delta\mu^{ex}(aq) \\ &= \Delta\mu^{ex}(8W) - \Delta\mu^{ex}(aq) \\ &= k_B T \ln \frac{p^{(0)}(n=8;\lambda_{Na^+})}{p^{(0)}(n=8;\lambda_{K^+})} - k_B T \ln \frac{p_{Na^+}(n=8;\lambda_{Na^+})}{p_{K^+}(n=8;\lambda_{K^+})}. \end{aligned} \tag{10}$$

λ_x (x = K^+, Na^+) is the radius of the first coordination shell. Note that $\Delta\mu^{ex}$ in the above equation depends not only on the population distribution of water ligands around the two ions but also on the population distribution of water molecules in an observation volume in the bulk aqueous phase. The latter aspect was ignored in the earlier work (Bostick and Brooks, 2007). Accounting for all the factors, as correct thermodynamic theory demands, one finds that the eight-water site is selective for K^+ (Dixit et al., 2009; Asthagiri et al., 2010) by only ~2 kcal/mol. Neglecting the $p^{(0)}(n;\lambda)$ factor can suggest apparent selectivities as high as 5 kcal/mol, comparable to those found in the S_2 site of the channel (Table I).

Eq. 9 is applicable for solutes in any medium. That equation and the above analysis emphasize that both number and chemical type of ligands in the binding site must be considered in understanding ion selectivity. Further, to describe the physics of selectivity, sufficient care is also required in designing models of the binding site.

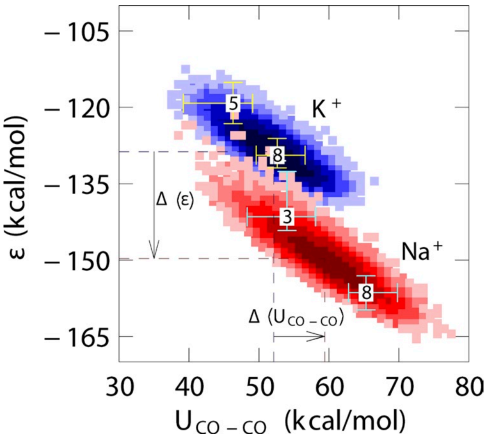

Figure 4. Density plot of the joint probability distribution, $P_x(\varepsilon, U_{CO\text{-}CO})$. ε is the binding energy between the ion and the carbonyl groups comprising the S_2 site. U_{CO-CO} is the excess internal energy of the S_2 site (but without considering the ion), which results from the repulsive interactions within the binding site ligands. The numbers indicate the ion coordination number for λ = 2.7 Å for Na^+ and λ = 3.1 Å for K^+. Observe that the low coordination states contribute to the high ε part of the distribution and vice versa. The region corresponding to ±40% probability around the mean is indicated. $\Delta\langle\varepsilon\rangle = -21.0$ kcal/mol and $\Delta\langle U_{CO\text{-}CO}\rangle = 7.3$ kcal/mol. Figure adapted from Dixit et al. (2009) with permission from Elsevier.

V. Concluding perspectives

K^+ selectivity is determined by the free energy difference between Na^+ and K^+ in the binding site relative to the corresponding quantity in bulk water. Given that Na^+ is better hydrated in bulk water, there is already a large unfavorable dehydration contribution that disfavors Na^+ from the selectivity filter.

The free energy of ion binding to the protein can be decomposed into mean binding energy and fluctuation contributions (Asthagiri et al., 2006; Dixit et al., 2009; Dixit and Asthagiri, 2011). The fluctuation contribution arises because the ion in the binding site samples a range of energy values (Asthagiri et al., 2006; Dixit et al., 2009). Thermodynamics and the statistical mechanical potential distribution theorem together show that this dispersion (or spread) about the mean binding energy is related to the average potential energy of the protein (in the presence of the ion) and entropic changes incurred in ion binding.

Provided that entropic effects in binding are small, the free energy change in changing the fiducial ion to some other ion, for example changing K^+ to Na^+ in potassium channels, is determined by two competing factors. One is the change in the direct ion–protein interaction energy, and the other is the increase in the potential energy of the protein system. Relative to the fiducial ion, ions that experience better binding with the protein will also invariably increase the average potential energy of the protein; they will increase the strain in the protein matrix. Such ions can also be expected to be better hydrated in bulk water. In such cases, if the ion–protein binding energy difference is about the same as the change in the bulk hydration free energy, the selectivity free energy is dominated by the strain in the protein system. The increase in potential energy, the strain, will be dependent on the chemical type and number of ligands binding the ion, how this binding site interacts with protein material outside the site, and how that protein material itself reorganizes in the presence of the ion.

For KcsA, calculations show that entropic effects evaluated in the binding site are indeed small for changing K^+ to Na^+ in the S_2 site (Dixit et al., 2009; Dixit and Asthagiri, 2011). This statement must be qualified by noting that the remaining filter has the canonical occupancy of ions and water, that is, a K^+ ion each in S_0 and S_4 and water molecules in S_1 and S_3 binding sites. For this canonical occupancy, relative to K^+, Na^+ is better bound in the S_2 by an amount that is approximately equal to the difference in hydration free energies in bulk water; thus, mean binding energies alone cannot explain selectivity. Also, nearly the same binding energy and binding free energy differences are obtained if ion interactions with only the eight carbonyl ligands (in the presence of the field imposed by the remaining protein) are considered. Thus, thermodynamics suggests that understanding the properties of the matrix holds the key to selectivity, and, for the canonical occupancy of the filter, one can study the eight carbonyls of the S_2 site (in the presence of the protein field) to understand selectivity. Better interaction of Na^+ relative to K^+ with the eight carbonyls strains the site, and this increased strain is seen to determine the magnitude of the net selectivity (Dixit et al., 2009). (See also Varma and Rempe, 2007, for example, for discussions on the role of the protein environment around the site.)

Our analysis shows that models of selectivity that consider ion interactions with a defined local neighborhood of the ion-binding site should be carefully considered. Reproducing the free energy of ion exchange in the binding site—a small difference of large numbers—may not imply that the material outside the local environment of the ion plays no part in selectivity. This is most clearly seen in systems where the eight-carbonyl site is not an appropriate model of the binding site, as is the case for the mutant KcsA channel. This only reflects a failure in appropriately modeling the physical system under study. The ideas presented here appear to provide a safe and robust way to understand and model selectivity in such systems and in biomaterials in general.

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

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