The Predominant Role of Coordination Number in Potassium Channel Selectivity

Michael Thomas, Dylan Jayatilaka, and Ben Corry
School of Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia, Crawley WA 6009, Australia

ABSTRACT Potassium channels are exquisitely selective, allowing K^+ to pass across cell membranes while blocking other ion types. Here we demonstrate that the number of carbonyl oxygen atoms that surround permeating ions is the most important factor in determining ion selectivity rather than the size of the pore or the strength of the coordinating dipoles. Although the electrostatic properties of the coordinating ligands can lead to Na^+ or K^+ selectivity at some values of the dipole moment, no significant selectivity arises at the specific value of the dipole moment for carbonyl groups found in potassium channels when the ligands have complete freedom. Rather, we show that the main contribution to selectivity arises from slight constraints on the conformational freedom of the channel protein that limit the number of carbonyl oxygen atoms to a value better suited to K^+ than Na^+, despite the pore being flexible. This mechanism provides an example of a general framework for explaining ion discrimination in a range of natural and synthetic macromolecules in which selectivity is controlled by the number of coordinating ligands in addition to their dipole moment.

INTRODUCTION

Potassium channels control ionic concentration gradients and electric potentials across cell membranes. To do this they are highly selective, being up to 1000 times more permeable to K^+ than Na^+, and yet they pass ions at near diffusion-limited rates (1–4). As inferred from crystal structures, ion discrimination takes place in a narrow portion of the pore, known as the selectivity filter, in which permeating ions interact directly with a series of backbone carbonyl oxygen atoms (5,6). However, the exact origins of this selectivity remain a topic of much debate. The discrimination within potassium channels can largely be explained in thermodynamic terms, quantified by the difference in free energy of two ion types in the bulk solvent and in the pore. The degree of selectivity estimated by the measurement of competitive fluxes of ions through the pore suggests a free energy difference of 5–6 kcal/mol ($\sim$10 kT, 4×10^{-20} J) for K^+ relative to Na^+ (1–4). Here we describe ion selectivity in potassium channels as the competition between the solvent and the selectivity filter for these ions and, using computational approaches, demonstrate that selectivity is determined by the ability of the protein to more favorably coordinate only some ion species as they pass through the pore. This explanation does not rely on the pore having a snug fit for only K^+, as had been suggested (5,7,8), but rather highlights the effect played by constraints on the way the carbonyl oxygen atoms can surround ions in the pore. Furthermore, we present evidence that indicates that the magnitude of the dipole moment of the carbonyl ligands is not the predominant factor creating K^+ selectivity in potassium channels, as has also been suggested (9–12). Rather, we find that selectivity arises primarily from limitations on the way in which these dipoles can orient around an ion, in particular restrictions on the number of dipoles surrounding the ion, a result of their being bound together in chains and surrounded by the remainder of the protein.

An explanation of the free energy difference between K^+ and Na^+ was suggested when the first crystal structure was determined, noting that the selectivity filter is too narrow to fit an ion with a shell of water molecules, and thus, ions must be dehydrated to enter this region of the channel. The size of the crystallized filter is such that when a K^+ is within the pore, the interactions with the carbonyl oxygens can compensate for the dehydration energy, whereas they are too far apart to adequately do so for the smaller Na^+, and it is effectively excluded from the pore (5). But, as pointed out previously (11–13), there is a problem with an explanation of ion selectivity that relies on the precise dimensions of the pore. There is evidence that the KcsA filter is a relatively flexible structure that undergoes rapid thermal fluctuations of magnitude much greater than the 0.38 Å radius difference between Na^+ and K^+ as evidenced from the crystallographic thermal parameters (11), simulations (14–19), and structures obtained at low concentration (6), suggesting that the protein could easily adapt to the smaller ion.

An alternative explanation of selectivity has also been proposed in which the physical characteristics of the coordinating ligands, most notably the dipole moment of the carbonyl groups, are suggested to be such that the channel naturally favors binding of K^+ (9–12). This would arise through the competition between the strong electrostatic attraction of the ion with the oxygen atoms that determines the structure of the filter around the ion (and allows the pore to close about small ions), and the weaker repulsion between the oxygen atoms that would regulate selectivity. Initial calculations by

Submitted March 7, 2007, and accepted for publication June 11, 2007.

Address reprint requests to Ben Corry, School of Biomedical, Biomolecular and Chemical Sciences, The University of Western Australia, Crawley WA 6009, Australia. E-mail: ben@theochem.uwa.edu.au.

Editor: Peter C. Jordan.

0006-3495/07/10/2635/09 $2.00

doi: 10.1529/biophysj.107.108167

Eisenman suggested that ligands of differing field strength could selectively bind differently sized ions (9). This was further supported by pioneering free energy calculations from MD simulations that highlighted the key role that the electrostatic attraction between ions and their coordinating ligands plays in determining the selectivity of the cyclic antibiotic valinomycin (10). Although this study noted that steric factors that influence the ability of ligands to pack around the ion (and presumably influence the number of ligands contacting the ion) also contribute to the selectivity of the host molecule, this effect has been given less importance than the field strength of the ligands in many discussions of potassium channels. In recent MD free energy calculations on the KcsA channel, the importance of having eight coordinating ligands for achieving K^+ selectivity has been noted, but the influence of the ligand dipole moment on creating selectivity was highlighted, albeit to a lesser extent in the most recent study (11,12,20). A difficulty with explaining potassium channel selectivity predominantly in terms of the field strength or dipole moment of the coordinating carbonyl ligands, however, is that it does not easily account for the conflicting fact that smaller ions tend to coordinate with ligands with the stronger electric field better than larger ions (9). This is simply a consequence of the fact that there can be larger electrostatic interactions when the ion can move closer to the ligand (notwithstanding any repulsion between the oxygen atoms). In the case of the potassium channels, the dipole moment of the carbonyl ligands is slightly larger than that of the surrounding water, and thus, using a rationale of dipole strength alone, one might expect that the smaller Na^+ ions would be favored in the pore over K^+.

Here we wish to show that the number of coordinating ligands, limited by the Angstrom-level constraints on the protein, is the most important factor creating K^+ selectivity in potassium channels. We present evidence that neither the specific size of the pore nor the intrinsic dipole moment of the carbonyl ligands is the main discriminating factor that leads to K^+ selectivity in potassium channels. Using ab initio calculations and MD simulations we show that ion selectivity is primarily a consequence of limitations on how the carbonyl ligands can coordinate permeating ions, in particular constraints on the number of ligands that surround the ion.

METHODS

The energetics of ion selectivity was investigated using both ab initio and MD simulations to determine the energy of the following exchange reaction in which K^+ inside a specific binding site in the selectivity filter of the KcsA channel (K^+/S_2) is exchanged with another ion type in bulk solvent (M^+/Sol):

$$M^+/\mathrm{Sol} + K^+/S_2 \overset{\Delta G}{\rightarrow} K^+/\mathrm{Sol} + M^+/S_2.$$

Defining the thermodynamics of selectivity in this way as the difference in the free energy of the ions in the pore and in the bulk has provided valuable insight in a large number of previous studies (e.g., 10,11,19,21,22). We write this free energy difference in terms of the exchange reaction to show clearly the steps involved in the calculation and the similarity to thermodynamic calculations in chemical reactions. It was deemed computationally intractable to include the entire KcsA structure in detailed ab initio calculations. For this reason, only the S_2 binding site, which showed the greatest degree of selectivity in previous MD simulations (11), was included in our calculations. The structure of the S_2 binding site was extracted from the high-resolution crystal structure of KcsA (6). The four protein chains were terminated with hydrogen atoms, and all side chains were removed and replaced with hydrogen as illustrated in Fig. 1, *A* and *B*, for computational simplicity.

The energies of the exchange reactions were calculated differently in the ab initio and MD approaches. In the former, geometry optimized structures of each fragment (such as the ion-water complexes shown in Fig. 1, *C* and *D*) were determined first, and the energy of each was summed to produce the final value. In the molecular dynamics simulations, the free energy was determined from free energy perturbations during dynamic simulations in which an ion was slowly morphed from one type to another. Further details of two methods and the conditions employed in each case are given below. To further elucidate the origins of ion selectivity, calculations were also made in a number of hypothetical situations in which the ions were exchanged between two different bulk solvents, or a bulk solvent and a small number of specified ligands as described in the Results section.

Ab initio calculations

The geometry of each fragment of the ion-exchange reaction equation was optimized before the final energy calculations were made. The minimum energy conformations of the ion-solvent and ion-S_2 clusters were determined by geometry optimizations starting from a range of different starting configurations. Ion-water clusters for up to six water molecules were obtained using the minimum-energy geometries determined previously by Feller et al. (23,24). All geometry optimizations were made at the HF level using the 6-31+G* basis set, and final energy calculations were made using MP2/6-31+G* with counterpoise correction to minimize basis set superposition error. Standard effective core potentials were utilized for Rb^+ and Cs^+. Previous work by Feller et al. (25) has shown that this methodology gave results within 1 kcal/mol of those obtained by optimizing using MP2 or DFT with correlation-consistent basis sets for the energy of ions binding to crown ethers. Free energy calculations were not attempted in the ab initio calculations, primarily because accurate estimates of entropy require extremely accurate calculations of the vibrational modes (26), and this was decided to be too computationally demanding. In addition, our MD simulations under different temperatures suggest the change in entropy in the bound states of Na^+ and K^+ are much smaller than the enthalpic contribution (see Fig. 3, for example). Because the geometry-optimized configurations present static configurations, the number of oxygen atoms within the fist and second hydration shells can be clearly determined in the ion-solvent and ion-S_2 clusters using a cutoff of 3.6 Å for K^+ and 3.2 Å for Na^+. For example, it can be seen in the optimized ion-water complexes shown in Fig. 1, *C* and *D*, that whereas eight water molecules can directly coordinate K^+, only six do so for Na^+. All calculations were made using Gaussian03 (27).

Molecular dynamics calculations

Each ion-exchange reaction energy was determined by combining the free energy change from two alchemical free energy perturbation calculations. Free energy perturbations morphing K^+ to Na^+ and vice versa were made in 20 steps at a constant pressure of 1 atm within an initially $30 \times 30 \times 30$ Å TIP3P water box, a $30 \times 30 \times 30$ Å formaldehyde box with counterions, and within the S_2 model over a period of 20–40 ns of simulation. Unless otherwise stated, harmonic restraints of 2.2 kcal/mol/Å^2 were applied to all the carbon and nitrogen atoms in the S_2 model to replicate the root mean-square (RMS) fluctuations seen in MD simulations of the entire channel embedded in a lipid bilayer, and a weaker constraint of 0.55 kcal/mol/Å^2 was applied to keep the ions inside the site. The energy-minimized structure used

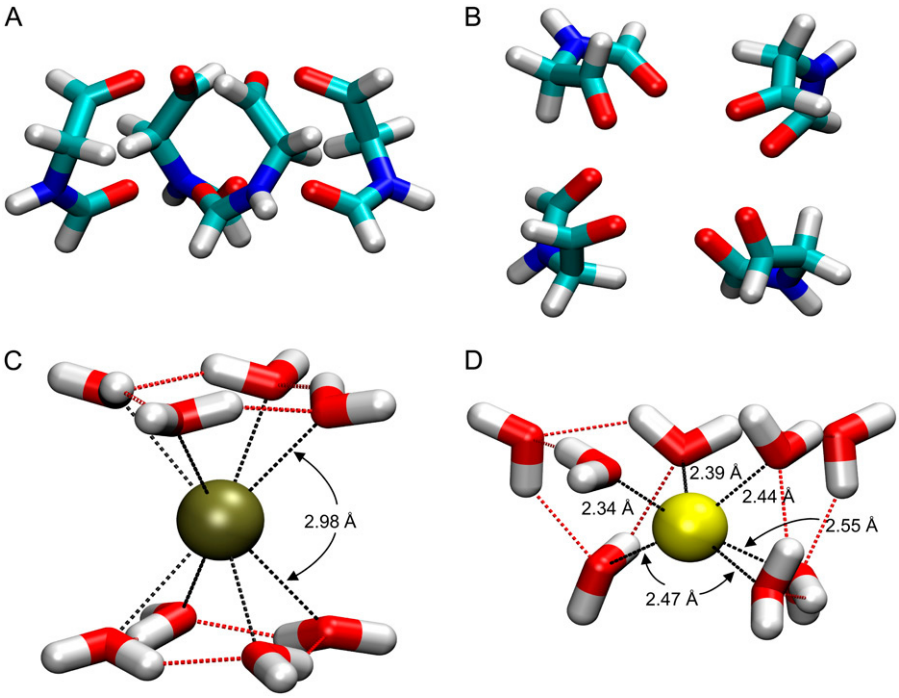

FIGURE 1 Structures used in energy calculations. (*A*) Side and (*B*) top views of the S_2 binding site model extracted from crystallographic data are shown. The ab initio geometry-optimized structure for a cluster of (*C*) K^+ and eight water molecules and (*D*) Na^+ and eight water molecules are shown with the ion-oxygen distances to the coordinating ligands noted.

to produce the results in Fig. 3 was created with the nominated ion in the site and harmonic restraints on the carbon and nitrogen atoms. Direct electrostatic calculations were used for the S_2 system. Lennard-Jones parameters for the cations were chosen that reproduce the experimental free energy in bulk water. All MD simulations were conducted with NAMD (28) with the CHARMM27 force field (29), time steps of 1 ps, and a temperature of 310 K unless otherwise stated. When the ''liquid S_2'' model described in the Results section was examined, the four protein chains were cleaved between α-carbon and nitrogen atoms.

RESULTS

To demonstrate that small ions prefer to be coordinated by solvents of greater dipole moment, we determined the partitioning of Na^+ and K^+ into water and formaldehyde using MD simulations while varying the partial charge and thus the dipole moment of the carbonyl groups in formaldehyde. The thermodynamics of this partitioning was investigated by determining the free energy ΔG of the following ion-exchange reaction:

$$Na^+/H_2O + K^+/OCH_2 \overset{\Delta G}{\rightarrow} K^+/H_2O + Na^+/OCH_2,$$

in which Na^+/H_2O represents a sodium ion in water, K^+/OCH_2 represents potassium in formaldehyde, and so on. As illustrated by the diamonds in Fig. 2, Na^+ is naturally preferred in the solvent of greater dipole moment, and when formaldehyde has a dipole moment similar to that of the carbonyl ligand in the potassium channel, there is very little selectivity. This point is also illustrated using ab initio calculations in which the ion-exchange energy (ΔE) for swapping Na^+ in water with K^+ in either formaldehyde or acetonitrile (solvents with disparate dipole moments) was determined. The partitioning of Na^+ into the solvent with greater dipole moment (formaldehyde or acetonitrile) and K^+ into water is again naturally favored with an energy difference of ~3 and 5 kcal/mol, respectively. Although the size of the dipole

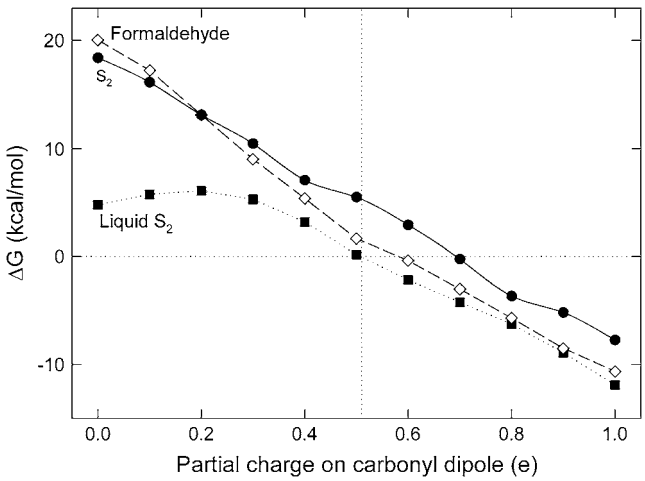

FIGURE 2 Ion-exchange free energies determined from MD calculations. The free energies are plotted for the exchange of Na^+ and K^+ between water and either formaldehyde solvent (◇), the S_2 binding site from KcsA with harmonic restraints (●), or the liquid S_2 model in which the eight carbonyl ligands forming the S_2 binding site can move independently without restraints (■). In each case the partial charge of the carbonyl dipole is altered such that the oxygen and carbon atoms carry equal and opposite charge to change the dipole moment of the coordinating ligands. The partial charge of the carbonyl group in the CHARMM27 force field is noted by the vertical doted line. The ~5 kcal/mol selectivity of the S_2 binding site is lost when the ligands have more conformational freedom.

moment of the competing ligands is important for determining ion selectivity, the carbonyl dipole moment is not such that it favors binding of K^+ over Na^+ by more than 1 kcal/mol in these situations where the ligands have complete freedom to orient about the ions. It should be noted that this study of partitioning of ions between solvents is conducted for pedagogical reasons only. Both formaldehyde and acetonitrile are miscible with water, meaning that such partitioning of ions can not be seen in practice. Also, our ab initio calculations are conducted with the ion-solvent clusters in vacuum rather than in a bulk solvent. These results do show, however, that the strength of the carbonyl dipole moment cannot itself be the cause of the 5–6 kcal/mol selectivity of potassium channels for K^+ over Na^+.

To determine the origins of selectivity in potassium channels, we conducted a series of ab initio and MD calculations to examine the selectivity filter of KcsA. Because the entire filter is too large to enable detailed ab initio calculations, we examined selectivity within just the S_2 binding site that showed the greatest degree of selectivity in previous MD simulations (11). The thermodynamics of ion selectivity was then investigated via the ion-exchange reaction described in the Methods section, an approach similar to that used previously to examine selectivity in crown ethers (26). Because we are not including all of the protein in our simulations, it is likely that the bare binding site will undergo motions that would not be possible when it is surrounded by a large number of additional protein atoms. To overcome this problem, we included harmonic restraints on the backbone carbon and nitrogen atoms to keep them close to their starting positions. A force constant of 2.2 kcal/mol/Å was used for this purpose, which reproduces the RMS fluctuations of the carbonyl oxygen atoms of ~0.75 Å seen in MD simulations of the entire protein and as estimated from the crystallographic B parameters (11).

The solvent plays a critical role in determining the selectivity of the pore as evidenced from ab initio ion exchange reaction energies of the group 1 cations. In the gas phase, that is when no solvent is present when the exchange energies are calculated, the potassium channel binding site shows a very large degree of selectivity favoring smaller ions over large ones, as shown in Fig. 3, because of the greater electrostatic interactions that can arise. This property has also been seen in previous MD simulations of the KcsA selectivity filter (19). However, as also shown in Fig. 3, the selectivity sequence of the channel changes significantly when the solvent is included in the exchange reaction. In this case the final selectivity sequence of the channel is determined by the balance of the solvation and binding energies of the ions, as has been previously described (5,8–10). An ion will be favored in the channel if its binding energy minus its solvation energy is lower than that of the competing ion. With water as the solvent, K^+ is favored in the binding site, whether the protein is held fixed in its crystal structure (Fig. 3 *C*) or allowed to optimize its structure to adapt to the ions (Fig. 3 *A*). Notably, however, the model pore alters its selectivity sequence when the solvent is changed from water to acetonitrile (Fig. 3 *B*). In this case, if we extrapolate the results to larger solvent numbers, it appears that the larger Cs^+ and Rb^+ ions become favored over both K^+ and Na^+. This can be understood clearly when selectivity is considered as a competition between the channel and the solvent to coordinate the ions; the smaller ions tend to partition into acetonitrile because it has a very large dipole moment, leaving the larger ions in S_2. Because the selectivity sequence alters with a change in solvent, it is obvious that the selectivity of the pore is not an intrinsic property of the protein alone but rather a relative property that also depends on the nature of the surrounding solvent.

Although ion selectivity is dependent on the solvent, an explanation is still required to detail the factors that make the potassium channel selective for K^+ over Na^+ when immersed in water. That is, why is the difference in binding energy of the two ions less in the channel than in bulk water? In both our ab initio and MD calculations we find that K^+ is favored over Na^+ in the S_2 binding site by ~5–6 kcal/mol in agreement with the ion flux measurements of selectivity (1–4) and simulations on the entire protein (11). Altering the strength of the carbonyl dipoles in MD simulations has a significant effect on the selectivity of the pore as shown in Fig. 2 (*circles*), reversing it when the dipole moment of the carbonyl ligands is increased much above the default values in the CHARMM parameter set. Notably, this selectivity does not depend on the precise geometry of the pore: K^+ is favored over Na^+ when the site is held fixed in its crystal structure or when it is allowed to optimize its geometry (Fig. 3, *A* and *C*). A calculation of the partial charges of the carbonyl groups in the S_2 model using our ab initio calculations (using the electrostatic fitting method (30)) indicates that the dipole moment is not significantly different from that used in the CHARMM 22 force field (a partial charge of ± 0.53 for the carbon and oxygen compared to ± 0.51 in the force field).

Furthermore, the presence of thermal fluctuations of the protein does not have a great effect on selectivity. As shown in Fig. 4, whether the pore is allowed to fluctuate in a harmonic potential about the crystal structure, the structure optimized with K^+ or the structure optimized with Na^+, the degree of selectivity is relatively invariant as the size of the RMS thermal fluctuations is increased from 0 to 0.9 Å by increasing the temperature of the simulations. The degree of selectivity is notably less, however, when the structure is restrained about the energy-minimized geometry found with Na^+ in the pore. This lack of dependence of selectivity on the size of the thermal fluctuations has been noted previously (19). Another similar study noticed a slightly larger dependence of selectivity on the size of the thermal fluctuations (11); however, it should be noted that the constraints applied in that study are different from those used here. In that study, a constant temperature was used while the width of a flat-bottomed constraining potential was modified to produce

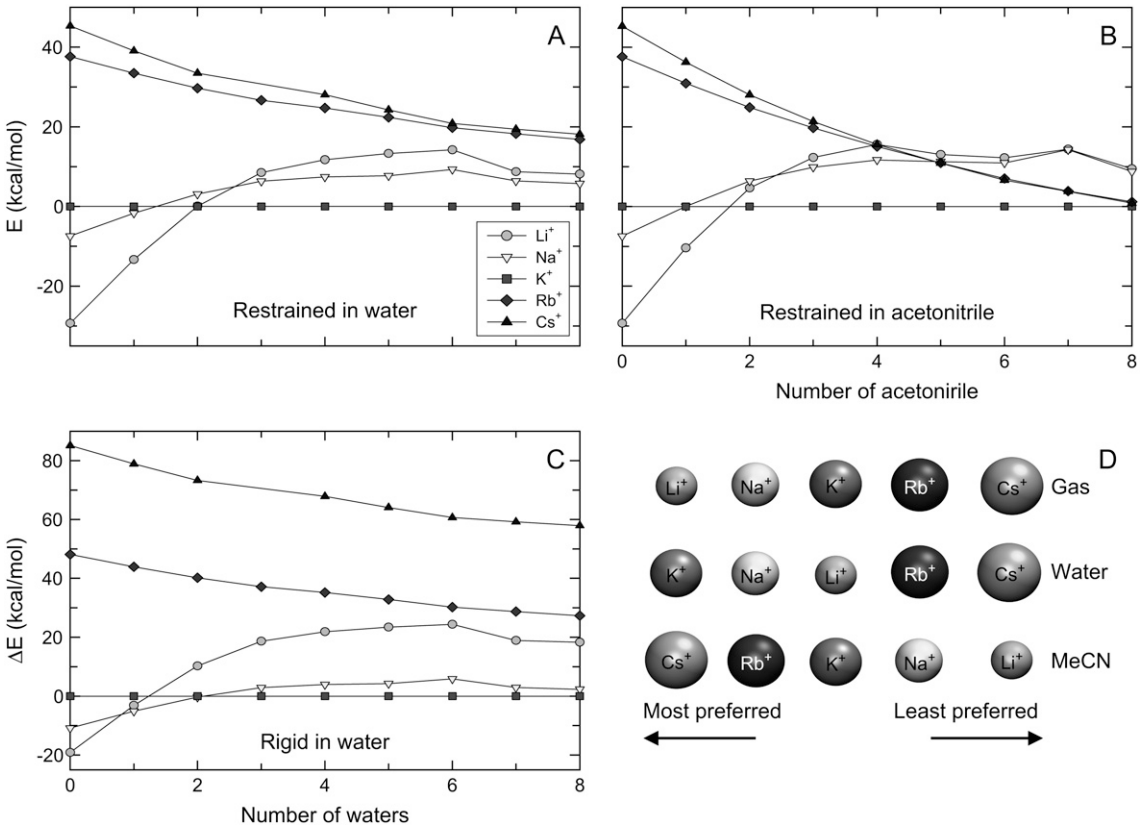

FIGURE 3 Ion-exchange energies determined from ab initio calculations. The energy of the ion-exchange reactions described in the text is plotted against the number of solvent molecules used to coordinate the ions. Exchange energies are plotted relative to K^+, which is taken as 0. Results are obtained with the protein harmonically restrained about the crystal structure using either (*A*) water or (*B*) acetonitrile as the solvent, as well as with the protein fixed in the crystal structure using water as the solvent (*C*). The ion types most favored in the channel fall at the bottom of each plot. (*D*) The selectivity sequence of the site is indicated in the gas phase, in water, and in acetonitrile (MeCN).

different RMS fluctuations of the carbonyl atoms. A complication with this protocol is that the mean positions of the atoms can alter with no strain within the width of the flat-bottomed well, and the effects of this position change on the free energy difference cannot easily be differentiated from those created by the increasing size of thermal fluctuations. Here we use a constant three-dimensional harmonic potential to constrain the backbone carbon and nitrogen atoms while altering the temperature of the simulation. This keeps the average position of the atoms similar as the size of the thermal fluctuations increases with increasing temperature.

If selectivity is not a consequence of the precise dimensions of the pore, and if it does not arise for the specific dipole moment of the carbonyl ligands in the absence of other conditions as seen in Fig. 2, then an alternative explanation must be found. One possibility is that, although the protein is not held in a rigid structure, there is nonetheless some constraint on the conformational freedom of the carbonyl ligands created by the way they are bound in chains and surrounded by other protein atoms, a constraint that limits how they can coordinate permeating ions. Even if the channel protein is relatively flexible, the coordination numbers and ion-oxygen distances may be somewhat constrained, and even a slight limitation on this will have a significant effect on the thermodynamics of ion binding. Both the geometry-optimized structures of Na^+ and K^+ in S_2 found in ab initio calculations (Fig. 5 *B*), and the average position of the ions in the MD calculations suggest that ions can be coordinated only by either four or eight oxygen atoms in the binding site (Table 1), but nothing in between. As we will show below, limiting the coordination numbers in this way has a large effect on the selectivity of the site, as it enables the difference in binding energy of the two ion types to be different in the site than in bulk solvent. One would expect from steric considerations alone that it is more difficult for eight ligands to crowd around the smaller Na^+ than for K^+, and this will favor the binding of K^+ in the channel. The results of our ab initio geometry optimization for the ions in a small cluster of eight water molecules shown in Fig. 1, *C* and *D*, support this contention, showing that eightfold coordination is a minimum energy state for K^+ but not for Na^+, for which the minimum occurs with sixfold coordination. Thus, it is reasonable to expect that forcing eightfold coordination is likely to favor K^+ over Na^+ and thus will lead to K^+ selectivity.

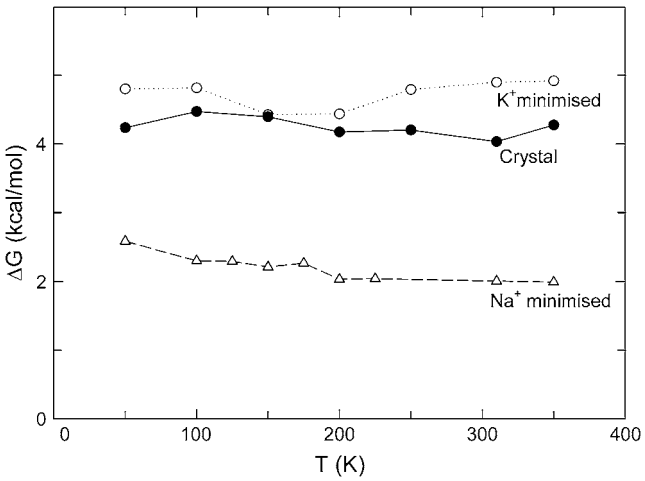

FIGURE 4 Influence of thermal fluctuations on ion-exchange energies determined from MD simulations. The free energy of the exchange reaction is plotted against the temperature of the simulations when the carbon and nitrogen atoms are held in harmonic constraints about their positions in the crystal structure (●) and structures found by minimizing the protein coordinates with either a K^+ (○) or Na^+ (△) in the pore. The size of the RMS thermal fluctuations increases linearly with temperature.

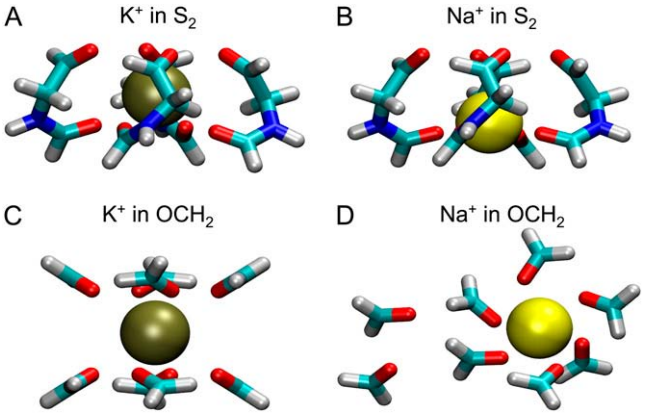

FIGURE 5 Geometry-optimized structures from ab initio calculations. The structure of the K^+/S_2 (*A*) and Na^+/S_2 (*B*) structures are shown as determined with harmonic constraints applied to the backbone carbon and nitrogen atoms. The structures determined for $K^+/(OCH_2)_8$ (*C*) and $Na^+/(OCH_2)_8$ (*D*) calculated with no constraints are also shown, demonstrating that K^+ can be coordinated by eight carbonyl ligands with little strain, as can be achieved in S_2, whereas Na^+ prefers to be coordinated by six ligands, which is not possible in S_2. (Note that eightfold coordination of Na^+ is higher in energy than sixfold coordination.)

To test whether the slight constraints on the conformational freedom of the carbonyl ligands are responsible for ion selectivity in potassium channels, we examined the effect of removing these conditions in both MD simulations and ab initio calculations. In MD this was done simply by removing the harmonic constraints on the atoms and slicing each protein chain in two such that the ion was surrounded by eight carbonyl ligands that can move independently. In the ab initio calculations, the bonds cannot be so easily cleaved; however, a similar effect can be created by replacing the eight carbonyl ligands with eight formaldehyde molecules initially arranged in the same geometry as the carbonyl ligands in S_2. As shown in Fig. 5, when the bonds between the carbonyl groups are removed, and when the restraints that mimic the effects of the surrounding protein are removed in ab initio calculations, the ligands are able to obtain new geometries that better optimize ion coordination. For K^+, the oxygen atoms remain in a position very close to that found in S_2 (Fig. 5, *A* and *C*). However, for Na^+ (Fig. 5 *D*), two of the eight carbonyl groups are pushed further away from the ion into a second solvation shell, leaving only six oxygen atoms in the inner shell (Table 1). When the coordinating ligands are free to find their optimal geometries (i.e., are not bound together, held in place by the remainder of the protein) the 5 kcal/mol selectivity of the pore for K^+ is lost (Table 2). The selectivity sequence follows that for free solvents of the given dipole moment, as illustrated by the squares in Fig. 2.

If the selectivity for K^+ is largely determined by constraints on the carbonyl ligands, it is interesting to consider the kind of constraints required to yield this degree of selectivity. Is it important to place the carbonyl oxygens in a particular arrangement, or is it more important to simply limit the coordination numbers of the ligands about the permeating ions? To answer this question we calculated the selectivity of a simple model containing eight formaldehyde molecules in which the oxygen atoms are allowed to move freely within a sphere of radius 3.5 Å about a central ion as done previously (11). This constraint keeps all the carbonyl ligands close to the ions, preventing any from escaping to a second solvation shell. As illustrated in Table 2 (and Fig. 6), this system has a very similar degree of selectivity for K^+ over Na^+ as the potassium channel (5.8 kcal/mol). However, removing the constraint and allowing the formaldehyde to orient freely removes most of the selectivity. As well as reinforcing the conclusion that conformational constraints are important for creating K^+ selectivity, these results show that the specific nature of the constraints is not critical as long as the number of ligands coordinating the ion is fixed.

The simple model described above provides a system in which the effects of restricting coordination numbers on the thermodynamics of selectivity can be examined, extending a similar study presented previously (11). Constraining otherwise free ligands within a spherical boundary allows the number of coordinating ligands to be specified, such that the effect of this and the partial charge (or equivalent dipole moment) of the ligands on selectivity can be systematically studied. We have postulated earlier that K^+ selectivity arises in potassium channels primarily from having eight ligands coordinating the ions. As illustrated in Fig. 6, constraining the number of coordinating ligands to eight is likely to produce K^+ over Na^+ selectivity for almost any value of their dipole moment. A coordination number of 8 is much less favorable for Na^+ than K^+ and thus leads to K^+ selectivity. This

TABLE 1 Coordination numbers of the minimum energy structures found in ab initio calculations

Ion	H_2O/MeCN	S_2	Liquid S_2
Na^+	6	4	6
K^+	8	8	8

The ions are surrounded by either eight solvent molecules (column 1), the S_2 binding site (column 2), or the liquid S_2 model comprising eight formaldehyde molecules (column 3). The structures corresponding to these numbers are shown in Fig. 1, *C* and *D*, and Fig. 5.

selectivity is lost when the number of ligands is reduced to five or six.

Some more general properties of K^+ or Na^+ selectivity in flexible systems can also be derived from the results that highlight the role played both by the number of coordinating ligands and their dipole moment. As shown in Fig. 6, K^+ over Na^+ selectivity can be obtained either with weak-dipole-strength ligands at any coordination number (because the smaller Na^+ will have a stronger electrostatic interaction with the solvent than the ligands surrounding the binding site) or by constraining the coordination number to 8. As the strength of the dipole is increased, the effect of constraining the coordination number on selectivity also increases. In the case of the intermediate-strength carbonyl dipoles, we find that the bulk of the K^+ selectivity seen in the KcsA channel is accounted for by the difference in the energy between eightfold and sixfold coordination. Na^+ selectivity, on the other hand, will arise only for a small range of coordination numbers (from four to six ligands) and only provided the dipole strength is greater than that of carbonyl groups.

It is worth noting that the effect of coordination number on selectivity described above does not make specific reference to the bulk coordination numbers of the ions but relies only on the fact that eightfold coordination is less favorable for Na^+ than it is for K^+. Although early MD studies suggested

TABLE 2 Loss of selectivity caused by removing conformational restraints on carbonyl ligands

Ligand	ΔE restrained (kcal/mol)	ΔE free (kcal/mol)	Loss of selectivity (kcal/mol)
Formaldehyde S_2 configuration	9.99	1.26	8.73
Liquid S_2	5.24	−0.03	5.27
Formaldehyde 3.5 Å sphere	5.77	1.26	4.51

The energies involved in exchanging K^+ in the carbonyl ligands with Na^+ in water calculated from three different sets of MD simulations are shown. In the first two rows, the carbon and nitrogen atoms are held by weak harmonic constraints about the positions of the S_2 binding site in the KcsA crystal structure. These restraints are removed to produce the ''free'' data shown in the second data column. The last row of data shows results in which the oxygen atoms in the formaldehyde molecules are free to move within a 3.5 Å radius sphere centred on the ion. This constraining sphere is again removed to produce the ''free'' results. Although the carbonyl ligands provide a K^+ selective binding site when restrained in a potassium channel-like structure, this is lost when the ligands have more conformational freedom.

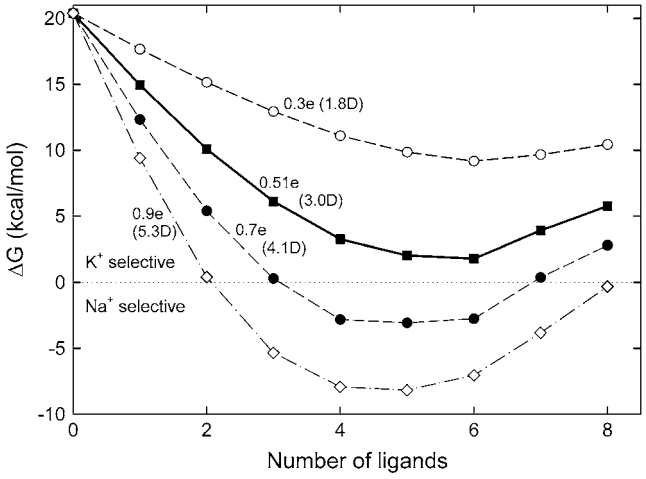

FIGURE 6 Influence of coordination number and dipole moment on Na^+/K^+ selectivity in flexible systems. The free energy determined from MD calculations in which K^+ surrounded by carbonyl ligands is exchanged with Na^+ in bulk water is plotted against the number of carbonyl ligands coordinating the ions. The coordination numbers are constrained by forcing the oxygen atoms on the carbonyl ligands to remain with in a 3.5 Å sphere. Four different calculations are made with differing partial charges on the carbonyl ligands as specified beside each curve. Equivalent dipole moments assuming an oxygen-carbon separation of 1.23 Å are also shown in brackets. Results for the default partial charge of the carbonyl groups in the CHARMM parameter set are shown by the solid line.

that the coordination number of K^+ in bulk water was above 7 (31), and for Na^+ was 5.6 (32), more recent x-ray and neutron diffraction experiments (33,34) and ab initio MD simulations (35) suggest that the number is closer to 5 or 6 for both ion types. An implication of this is that a site that replicates bulk coordination by containing six ligands with dipole moment similar to water should show little selectivity (unless changing the surrounding dielectric constant has a large influence). Our results are consistent with this expectation because the minimum selectivity for carbonyl-like dipoles arises with six ligands, as shown in Fig. 6.

DISCUSSION

The discrimination of potassium channels for different ion types results from a competition between the protein and solvent to bind the ions that can be quantified by the difference in the binding and solvation energy of the species. As a consequence, the selectivity sequence is determined by properties of both the protein and the solvent, including their dipole moment and ability to coordinate the ions. Although we have examined just one binding site in the channel and not discussed how selectivity can be influenced by more distant properties, such as the presence of additional ions in nearby sites (21) or the effect of the surrounding dielectric constant, we are able to quantify some of the fundamental causes of K^+ selectivity. Here we find that the two most important factors for determining the ion selectivity of a flexible binding

site are the number of coordinating ligands and their electrostatic properties.

Our results do confirm the possibility that the electrostatic properties of the coordinating ligands can, in principle, create an ion-selective binding site. However, these results also suggest that in the case of potassium channels, the electrostatic properties of the carbonyl ligands are not the primary discriminating factor that leads to K^+ selectivity. This is evidenced by the fact that there is little or no selectivity for K^+ over Na^+ when the carbonyl ligands have complete coordinational freedom to orient about the ions (Fig. 2, *squares* and *diamonds*). Instead, we have demonstrated that by placing slight constraints on the conformational freedom of the carbonyl ligands, potassium channels limit the number of ligands that coordinate the permeating ions. Thus, they are better able to compensate the energetic cost of dehydrating K^+ than Na^+ because eightfold coordination is less favorable to Na^+ than to K^+. The Angstrom level constraints on the coordinating ligands are essential to obtain the 5–6 kcal/mol selectivity of the channel for K^+ over Na^+ because their removal leads to a complete loss of selectivity (Table 2). It has previously been noted that the number of ligands coordinating the ions influences ion selectivity (11,12,20), but here we emphasize that this appears to be the most important cause of selectivity in potassium channels. Indeed, given that there are eight ligands, K^+ selectivity will arise for virtually any value of the ligand dipole moment.

Although this theory shares some similarities with the original ''snug fit'' explanation of selectivity in that it highlights the importance of constraints on the protein, we stress that it is most important to limit the coordination numbers of the ions, not the pore size. This is evidenced by the results obtained for the carbonyl ligands constrained in a sphere in which there are no limits on how ''snugly'' the ligands can fit about the ion. Thus, selectivity can emerge in a pore that is dynamically fluctuating in size by using Angstrom scale conformational constraints that influence the number of coordinating ligands.

During final revision of this article, another quantum mechanical study came to our attention that reaches some similar conclusions on the origins of K^+ selectivity in KcsA (36). In agreement with our results, eightfold coordination is found to be necessary for selective K^+ partitioning with carbonyl ligands, and this is lost when the architectural restraints on the protein are removed to allow for Na^+ to obtain lower coordination numbers. Our ion-water clusters obtained in vacuum yield optimum coordination numbers of 8 for K^+ and 6 for Na^+, larger than the average values found in ab initio MD simulations in bulk solvent. This is consistent with the suggestion that coordination numbers are larger in low-dielectric environments (e.g., vacuum) than in higher ones (e.g., bulk water), but these numbers should not be directly compared because one represents a minimum-energy state and the other an average from a dynamic simulation that samples a region of phase space. Our results highlight that rapid transport of K^+ through the channel should not be prevented by the necessity to move into an eightfold coordinated site from bulk solution where it is on average less coordinated. However, we do not wish to directly imply that eightfold coordination in KcsA is caused by the dielectric effects of the surroundings as suggested by Varma and Rempe (36). We believe that any mechanism that enforces eightfold coordination of the permeating ions, be this from dielectric effects, steric effects, or other constraints on the protein, will suffice to produce selectivity. Conversely, any situation that enables one or both ions to obtain five- or sixfold coordination can be expected to eradicate this. For example, previous MD simulations suggest that the lack of selectivity in the NaK channel compared to KcsA can be attributed to the slight architectural differences that allow for the Na^+ ions to position themselves such that they are surrounded by six ligands rather than eight as in KcsA (20). This suggests that involving the dielectric properties of the surroundings is not necessarily required to explain selectivity.

The general rules examined here provide a framework for understanding selectivity in a range of other molecules. For example, the recently crystallized leucine transporter (37) selectively binds Na^+ with just six ligands and has some ligands of greater dipole strength than the carbonyl groups to help favor binding of Na^+, as is shown to be required in Fig. 6. Similarly, Na^+ channels use charged amino acid side chains to bind ions (38). This explanation of ion discrimination that includes strains on the protein that limit ion coordination as well as the electrostatic properties of the ligands provides a conceptual framework for understanding selectivity in a range of natural and synthetic molecules as diverse as macrocyclic ligands and biological transporters.

The authors gratefully acknowledge Graham Chandler for his helpful discussions and enthusiasm, and David Feller for supplying energy-minimized structures for various ion-water clusters.

This project was supported by funding from the Australian Research Council, the National Health and Medical Research Council, and an award under the Merit Allocation Scheme on the National Facility of the Australian Partnership for Advanced Computing, as well as computer time from the high-performance computing center iVEC.

REFERENCES

1. Yellen, G. 1984. Ionic permeation and blockade in Ca^{2+} activated K^+ channels of bovine chromaffin cells. *J. Gen. Physiol.* 84:157–186.
2. Heginbotham, L., and R. MacKinnon. 1993. Conduction properties of the cloned *Shaker* channel. *Biophys. J.* 65:2089–2096.
3. LeMasurier, M., L. Heginbotham, and C. Miller. 2001. KcsA: It's a potassium channel. *J. Gen. Physiol.* 118:303–313.
4. Nimigean, C. M., and C. Miller. 2002. Na^+ block and permeation in a K^+ channel of known structure. *J. Gen. Physiol.* 120:323–325.
5. 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.
6. Zhou, Y., J. H. Morais-Cabral, R. Kaufman, and R. MacKinnon. 2001. Chemistry of ion coordination and hydration revealed by a K^+ channel-Fab complex at 2.0 Å resolution. *Nature.* 414:43–48.

7. 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.
8. Gouaux, E., and R. MacKinnon. 2005. Principles of selective ion transport in channels and pumps. *Science.* 310:1461–1465.
9. Eisenman, G. 1962. Cation selective electrodes and their mode of operation. *Biophys. J.* 2:259–323.
10. Åqvist, J., O. Alvarez, and G. Eisenman. 1992. Ion-selective properties of a small ionophore in methanol studied by free energy perturbation simulations. *J. Phys. Chem.* 96:10019–10025.
11. Noskov, S. Y., and B. Roux. 2004. Control of ion selectivity in potassium channels by electrostatics and dynamic properties of carbonyl ligands. *Nature.* 431:830–834.
12. Noskov, S. Y., and B. Roux. 2006. Ion selectivity in potassium channels. *Biophys. Chem.* 124:279–291.
13. Corry, B. 2006. Understanding ion channel selectivity and gating and their role in cellular signalling. *Mol. BioSyst.* 2:527–535.
14. Aqvist, J., and V. Luzhkov. 2000. Ion permeation mechanism of the potassium channel. *Nature.* 404:881–884.
15. Bernèche, S., and B. Roux. 2001. Energetics of ion conduction through the K^+ channel. *Nature.* 414:73–77.
16. Shrivstava, I. H., D. P. Tieleman, P. C. Biggin, and M. S. P. Sansom. 2002. K^+ versus Na^+ ions in a K channel selectivity filter: a simulation study. *Biophys. J.* 83:633–645.
17. Domene, C., and M. S. P. Sansom. 2003. Potassium channel, ions, and water: simulation studies based on the high resolution x-ray structure of KcsA. *Biophys. J.* 85:2787–2800.
18. 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.
19. Asthagiri, D., L. R. Pratt, and M. E. Paulaitis. 2006. Role of fluctuations in a snug fit mechanism of KcsA channel selectivity. *J. Chem. Phys.* 125:24701–24706.
20. Noskov, S., and B. Roux. 2007. Importance of hydration and dynamics on the selectivity of the KcsA and NaK channels. *J. Gen. Physiol.* 129:135–143.
21. Luzhkov, V. B., and J. Åqvist. 2001. K^+/Na^+ selectivity of the KcsA potassium channel from microscopic free energy perturbation calculations. *Biochim. Biophys. Acta.* 1548:194–202.
22. Luzhkov, V. B., and J. Åqvist. 2005. Ions and blockers in potassium channels: insights from free energy simulations. *Biochim. Biophys. Acta.* 1747:109–120.
23. Glendening, E., and D. Feller. 1995. Cation-water interactions: the $M^+(H_2O)_n$ clusters for alkali metals, M = Li, Na, K, Rb and Cs. *J. Phys. Chem.* 99:3060–3067.
24. Feller, D. 1997. Ab initio study of M^+:18-Crown-6 microsolvation. *J. Phys. Chem. A.* 101:2723–2731.
25. Feller, D., E. Aprà, J. Nichols, and D. Bernholdt. 1996. The structure and binding energy of K^+-ether complexes: A comparison of MP2, RI-MP2 and density functional methods. *J. Chem. Phys.* 105:1940–1950.
26. Glendening, E. D., D. Feller, and M. A. Thompson. 1994. An ab initio investigation of the structure and alkali metal cation selectivity of 18-crown-6. *J. Am. Chem. Soc.* 116:10657–10669.
27. Frisch, M. J., G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, and others. 2004. Gaussian 03, Revision C.02. Gaussian Inc., Wallingford, CT.
28. Phillips, J. C., R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kalé, and K. Schulten. 2005. Scalable molecular dynamics with NAMD. *J. Comp. Chem.* 26:1781–1802.
29. MacKerell, A. D., Jr., D. Bashford, M. Bellott, R. L. Dunbrack, Jr., J. D. Evanseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F. T. K. Lau, C. Mattos, S. Michnick, T. Ngo, D. T. Nguyen, B. Prodhom, W. E. Reiher, III, B. Roux, M. Schlenkrich, J. C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiórkiewicz-Kuczera, D. Yin, and M. Karplus. 1998. All-atom empirical potential for molecular modelling and dynamics studies of proteins. *J. Phys. Chem. B.* 102:3586–3616.
30. Besler, B. H., K. H. Merz, Jr., and P. A. Kollman. 1990. Atomic charges derived from semiempirical methods. *J. Comp. Chem.* 11:431–439.
31. Neilson, G. W., and N. Skipper. 1985. K^+ coordination in aqueous solution. *Chem. Phys. Lett.* 114:35–38.
32. Zhu, S., and G. W. Robinson. 1992. Molecular dynamics computer simulation of an aqueous NaCl solution: Structure. *J. Chem. Phys.* 97:4336–4348.
33. Ansell, S., A. C. Barnes, P. E. Mason, G. W. Nielson, and S. Ramos. 2006. X-ray and neutron scattering studies of the hydration structure of alkali ions in concentrated aqueous solution. *Biophys. Chem.* 124: 171–179.
34. Soper, A. K., and K. Weckström. 2006. Ion salvation and water structure in potassium halide aqueous solutions. *Biophys. Chem.* 124:180–191.
35. Varma, S., and S. Rempe. 2006. Coordination numbers of alkali metal ions in aqueous solution. *Biophys. Chem.* 124:192–199.
36. Varma, S., and S. B. Rempe. 2007. Tuning ion coordination architectures to enable selective partitioning. *Biophys. J.* 93:1093–1099.
37. Yamashita, A., S. K. Singh, T. Kawate, Y. Jin, and E. Gouaux. 2005. Crystal structure of a bacterial homologue of Na^+/Cl^--dependent neurotransmitter transporters. *Nature.* 437:215–223.
38. French, R. J., and G. W. Zamponi. 2005. Voltage-gated sodium, and calcium channels in nerve, muscle and heart. *IEEE Trans. Nano. BioSci.* 4:58–69.