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K^+/Na^+ Selectivity in K Channels and Valinomycin: Over-coordination *Versus* Cavity-size constraints

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Potassium channels and valinomycin molecules share the exquisite ability to select K^+ over Na^+. Highly selective K channels maintain a special local environment around their binding sites devoid of competing hydrogen bond donor groups, which enables spontaneous transfer of K^+ from states of low coordinations in water into states of over-coordination by eight carbonyl ligands. In such a phase-activated state, electrostatic interactions from these 8-fold binding sites, constrained to maintain high coordinations, result in K^+/Na^+ selectivity with no need for a specific cavity size. Under such conditions, however, direct coordination from five or six carbonyl ligands does not result in selectivity. Yet, valinomycin molecules achieve selectivity by providing only six carbonyl ligands. Does valinomycin use additional coordinating ligands from the solvent or does it have special structural features not present in K channels? Quantum chemical investigations undertaken here demonstrate that valinomycin selectivity is due to cavity size constraints that physically prevent it from collapsing onto the smaller sodium ion. Valinomycin enforces these constraints by using a combination of intramolecular hydrogen bonds and other structural features, including its specific ring size and the spacing between its connected ligands. Results of these investigations provide a consistent explanation for the experimental data available for the ion-complexation properties of valinomycin in solvents of varying polarity. Together, investigations of these two systems reveal how nature, despite being popular for its parsimony in recycling functional motifs, can use different combinations of phase, coordination number, cavity size, and rigidity (constraints) to achieve K^+/Na^+ selectivity.

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Natural systems possess the remarkable ability to distinguish between two equivalently charged ions, Na^+ and K^+, which differ in size by less than 0.4 Å. As a consequence of this ability, virtually all cells can transport these ions selectively and regulate their concentration gradients across membranes.[1] Preferential binding of one of these ions affects the activity of several other globular protein[2] and RNA[3] enzymes. Selective ion permeation and binding ultimately enable a wide variety of high-level physiological tasks to be accomplished; from nutrient uptake and volume control in cells, to generation and propagation of nerve signals, maintenance of rhythmic heart-rates, vision and dialysis in eukaryotes.

Among biomolecules that differentiate between these two ions, some selectively bind K^+, while others bind Na^+. What drives selectivity in favor of a particular ion? Equilibrium thermodynamics dictates that transfer of a particular ion A from one solvation phase m, say water, to another solvation phase p, say protein, is favorable when the solvation free energy of the ion in phase p is lower than its value in phase m:

$$\Delta\Delta G_A(m \rightarrow p) = \Delta G_A(p) - \Delta G_A(m) < 0 \qquad (1)$$

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Abbreviations used: QC, quantum chemical; MD, molecular dynamics.

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where ΔG_A (m) is the free energy change for ion A in phase m relative to the gas phase. Selective partitioning of ion A over another ion B into phase p is favorable when the free energy change for transfer from phase m to p is lower for A relative to B:

$$\Delta\Delta\Delta G_{B\to A}(m\to p) = \Delta\Delta G_A(m\to p) - \Delta\Delta G_B(m\to p)$$

$$= \Delta\Delta G_{B\to A}(p) - \Delta\Delta G_{B\to A}(m)<0 \qquad (2)$$

While these equations describe the thermodynamic driving forces required for selectivity, they do not indicate the determining factors. The determinants of A/B (K^+/Na^+ or Na^+/K^+) selectivity are the chemical and structural features present in phase p that alter the free energy difference between these ions with respect to their difference in phase m.

The pursuit to understand the determinants of K^+ over Na^+ selectivity began several decades ago, and now with the advent of new initiatives to engineer biomimetic nano-devices for addressing critical issues concerning dialysis, desalination and power generation,[4] such efforts are being undertaken with an added impetus. Early investigations led to the formulation of two main concepts: one built around host-guest steric relationships,[5,6] where the host molecule maintains a specific cavity size that fits one ion better than the other, and the other built around host–guest chemistry,[7,8] where specific electric field strengths of the host molecule ligands solvate one ion better than the other. These concepts revolutionized the field of ion channel selectivity, especially concerning equivalently charged ions. Until a few years ago, however, lack of theoretical and experimental data limited testing of these mechanisms to determine how well they explain selectivity in biomolecules.

Here, we first briefly review recent investigations of potassium (K) channels to determine how current ideas regarding K^+/Na^+ selectivity relate to the two older mechanisms. Next, we describe new conclusions regarding the K channel mechanism raise critical questions regarding the mechanism of K^+/Na^+ selectivity in valinomycin, a tiny ionophore (169 atoms) in comparison to K channels. Finally, we present our investigations of valinomycin ion selectivity using quantum chemical (QC) methods.

When the seminal work of Mackinnon and coworkers led to the determination of the first crystal structure of a strongly selective K channel,[9] K^+/Na^+ selectivity was attributed to a host–guest steric mechanism.[6,9–12] As illustrated in Figure 1(a), the crystal structure showed that the channel selectivity filter coordinates K^+ using eight carbonyl ligands. Potassium ions were thought[10,11] to partition spontaneously into such highly coordinated 8-fold binding sites because these sites mimicked coordination of K^+ in aqueous phase.[13,14] This notion of ion partitioning, however, no longer holds in light of new experimental[15,16] and theoretical[17–20] data, which show that the coordination number of K^+ in water is much lower than 8, and the probability that a K^+ coordinates simultaneously with eight water molecules in aqueous phase is negligible. At the same time, highly selective ion discrimination was attributed to binding sites that formed rigid cavities matching K^+, but not the smaller Na^+, size. Several subsequent molecular dynamics (MD) simulations,[21–23] however, demonstrated that, in accord with experimental B-factors from the crystallographic data, fluctuations of the coordinating carbonyl oxygen atoms were larger than the size difference between Na^+ and K^+ ions, thus challenging the validity of the host–guest steric mechanism of selectivity. In light of this realization, recent theoretical work based on free energy perturbation calculations demonstrated how the host–guest chemistry

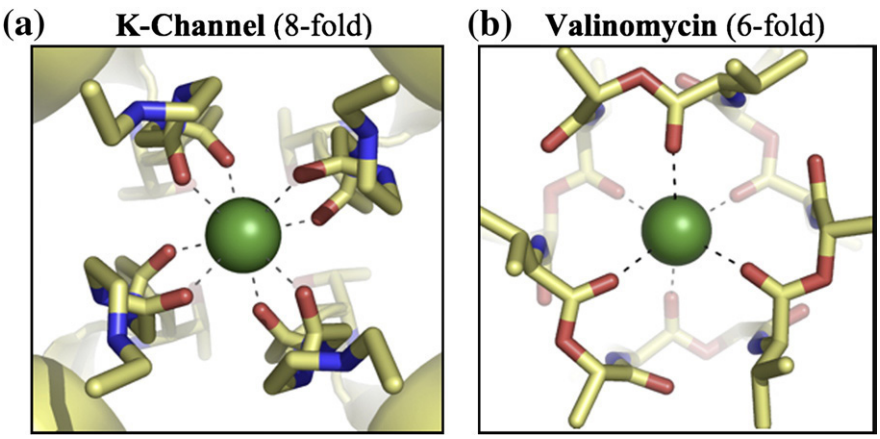

Figure 1. Structural differences between K^+-binding sites in strongly selective K channels and valinomycin. (a) Partial view of the X-ray structure[9] of a representative K channel, KcsA, illustrating a K^+ occupying the S2 site of the selectivity filter in a state of high coordination by eight carbonyl oxygen atoms. (b) X-ray structure[36] of valinomycin illustrating a K^+ coordinated by six carbonyl oxygen atoms. Potassium ions are drawn as green spheres and all other atoms as sticks with oxygen in red, carbon in yellow and nitrogen in blue. Broken lines connecting the ion and the oxygen atoms represent coordination with K^+.

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model could instead be invoked to explain K^+/Na^+ selectivity in K channels.[24]

Certain critical experimental and theoretical observations, however, still remained unexplained.[20,25] For instance, how does K^+ partition between low coordination states in bulk water and a high 8-fold coordination state in the filter? Why should the chemically identical binding sites S1, S2 and S3 in strongly selective KcsA channels have strikingly different degrees of computed selectivities?[26,27] Why should specific mutations in the region adjacent to these binding sites render the filter non-selective?[28,29] Why should NaK channels have reduced selectivity despite sharing two structurally and chemically identical binding sites with strongly selective K channels?[30]

Recent QC-based studies have helped reconcile these seemingly disparate observations by identifying the roles of the chemical and structural properties of the solvation phase in modulating ion coordination architectures, and the importance of maintaining specific high numbers of coordinating ligands in the binding sites for selectivity.[20] Because the spatial region proximal to the binding sites in strongly selective K channels is devoid of competing hydrogen bond donors, the protein solvation environment cannot interact directly and favorably with the coordinating carbonyl oxygen atoms. Within such a local "quasi-liquid" pocket, electrostatically equivalent to a low dielectric phase ($\varepsilon<3$), the thermodynamic probability for formation of an 8-fold K^+ coordination complex with carbonyl ligands increases to a value that no longer obstructs K^+ partitioning between water and the binding sites. At the same time, the free energy associated with transferring a Na^+ from liquid water to the same 8-fold carbonyl binding site is unfavorable, resulting in K^+/Na^+ selectivity. Furthermore, as also inferred from previous MD simulations,[24] such an 8-coordinate K^+-selective binding site does not require restrictions on its cavity size. Nevertheless, if the binding site were fully flexible, occupation by Na^+ could reduce the number of coordinating ligands to five or six and consequently eliminate selectivity.[20,31] Therefore, although a specific cavity size is not required, some form of rigidity[20] or topological constraint,[31] which could be supplied by structural and chemical properties of the solvation phase or the binding site itself, is necessary to prevent distortion of the binding sites upon Na^+ occupation.[32] In particular, rigidity is needed in strongly selective K channels to enforce direct ion coordination by high numbers (>6) of carbonyl ligands.

Factors that disturb the quasi-liquid environment,[20] via structural or chemical modifications, can lead to distortions in binding site coordination architectures, which can in turn result in a complete loss of K^+/Na^+ selectivity. For example, hydrogen bond donor side-chains introduced next to the selectivity filter binding sites can compete with the permeating ion for direct favorable interactions with carbonyl oxygen atoms, and thus reduce the probability for formation of binding sites with high numbers of coordinating ligands.[20] Fewer ligands coordinating the ions in such a binding site result in significantly reduced selectivity, as was demonstrated recently using thermodynamic calculations.[20] Experimental observations of reduced selectivity with mutations that introduce hydrogen bond donor groups near K channel binding sites also lend support to such a mechanism. For example, when tryptophan, a hydrogen bond donor, replaces serine at the S177 position in GIRK2, selectivity is reduced substantially.[28,29] Note that while serine is also a hydrogen bond donor, its distant location on the transmembrane helix and shorter length in comparison to tryptophan may provide it with fewer opportunities to physically approach the carbonyl oxygen atoms of the binding sites. In a different example from the comprehensive sequence alignment of K channels,[33] weakly selective K channels carry hydrogen bond donors in the form of arginine residues in proximity to their selectivity filters, and these side-chains are completely absent from all strongly selective K channels. Note that arginine side-chains are normally implicated in salt-bridge formation. In the absence of proximal negative charges, however, they can also engage in hydrogen bond formation.

Since water is also a hydrogen bond donor that can disrupt the quasi-liquid environment, exposure of K channel binding sites to different amounts of intra- or extracellular water can naturally be expected to affect the degrees of their selectivity. For example, binding site S2 in the highly selective KcsA channel is the site least exposed to water.[9] Even though it is chemically identical with sites S1 and S3, all-atom MD simulations[24,27,34] predict a higher selectivity of −5 kcal/mol for this site, as compared to a −2 kcal/mol selectivity for the other sites. On the same note, water penetration into the protein matrix behind the selectivity filter can be expected to reduce selectivity. Such water penetration has been reported in several MD simulations, and has been seen in X-ray studies. This is perhaps one reason why the computed selectivity of approximately −10 kcal/mol for an isolated binding site is higher than the measured selectivity of K channels.[20] In another example, the NaK channel shows weak selectivity and, in contrast to highly selective K channels, all its ion-binding sites are exposed to water.[30] Note that increased flexibility[20] or reduced topological constraints[31] in the NaK binding sites, compared with KcsA, can contribute to its reduced selectivity. Increased flexibility is evident from the recent MD simulation data[34] on the NaK channel, where its binding sites are seen to adopt substantially different coordination structures around the two ions relative to KcsA.

Exposure of ion binding sites to water can reduce their selectivity *via* two different mechanisms. In a host–guest chemistry mechanism, water molecules on average replace some of the carbonyl ligands during ion complexation and thus reduce the electric field density to values that exhibit lowered K^+/Na^+

selectivity, as explored recently using reduced models.[34] The reason weaker-field water molecules would preferentially replace some of the stronger-field carbonyl ligands in a channel binding site is not simply because more water molecules are present near those binding sites, but because water molecules are energetically easier to extract from aqueous phase in comparison with carbonyl ligands.[20] In an alternative mechanism, the exposure to water could result in an overall decrease in binding site coordination numbers, without ligand substitution, which also reduces selectivity.[20,31] Regardless of the mechanistic details, the degree of exposure of binding sites to water indeed determines the absolute potency of a given carbonyl binding site to carry out K^+/Na^+ discrimination.

In summary, recent work suggests strongly that K channels use a "phase-activated" mechanism[20] where the local environment around their binding sites is tuned to sustain high coordination numbers (>6) around potassium ions, which otherwise are rarely observed in liquid water. When such high coordinations are enforced, using some form of rigidity[20] or topological constraint[31] provided by properties of the protein solvation phase or binding site, and combined with the field strength of carbonyl ligands, they create the electrical scenario necessary for rapid and selective K^+ partitioning,[20] with no need to maintain specific cavity sizes.[20,24,31]

Note that in these and other simulation studies,[20,31,34,35] the general strategy to investigate the effects of structure on ion selectivity was to utilize representative models of ion-binding sites, without an explicit description of the rest of the protein matrix. Such representative models were constructed only after a systematic analysis[20] of the effects of both ligand numbers and chemistries on ion partitioning and selectivity. The results of these simulations were then analyzed in the context of known experimental and theoretical data, which consequently led to an improved understanding of the role of the protein matrix[20,31] in driving ion selectivity. This is not a shortcoming of the approach. Instead, it is part of a strategy essential for understanding the individual and collective roles of the physiologically relevant degrees of freedom available to ion binding sites, a seemingly impossible task if the remaining protein matrix is explicitly incorporated, as done in standard MD simulations or in wet-lab experiments. In addition, such a strategy provides an opportunity to utilize QC methods that excel at describing complex interactions, such as those occurring between an ion and its coordinating ligands.

In view of this K channel mechanism, which depends on the electrostatic and structural properties of the local environment along with constraints that enforce high ion coordination (>6), how does valinomycin achieve K^+/Na^+ selectivity by providing only six carbonyl ligands for ion coordination?

Valinomycin is a tiny ionophore that has, over the past decades, helped provide a wealth of information on biomechanisms at the molecular level. Chemically, it is a cyclic depsipeptide built from a threefold repetition of the alternating amino acid and hydroxy acid residues L-valine, D-α-hydroxyvaleric acid, D-valine, and L-lactic acid [D-Val-D-HyV-L-Val-L-Lac]$_3$. Its structure, in the absence of bound cations, is solvent-dependent. When bound to K^+, however, it adopts a configuration in which its amino acid and hydroxy acid side-chains always point outward, creating a hydrophobic exterior, while the six carbonyl oxygen atoms from the hydroxy acid residues point inward, forming a cavity for ion complexation,[36] as illustrated in Figure 1(b). Such a structural configuration allows valinomycin to transport the bound potassium ions across cellular membranes. Because of this selective ion transport property in combination with its small size, valinomycin has found important applications in numerous areas,[36] including investigations of oxidative phosphorylation reactions in biosystems, as ion-selective components in liquid–membrane electrodes, and in synthesis of antilipolytic agents, insecticides and nematodicides.

Several independent experiments[36–42] have demonstrated that valinomycin selects between K^+ and Na^+ in low as well as high dielectric solvents. For example, salt extraction equilibrium measurements show[41] that its selectivity $\Delta\Delta\Delta G_{Na^+\rightarrow K^+}(\varepsilon \rightarrow Val,\varepsilon)$ of −7.6 kcal/mol is almost independent of solvent dielectric coefficients ε in the range 2 (hexane) through 9 (dichloromethane). In addition, permeability ratio measurements in lipid membranes result in a selectivity of about −6 kcal/mol.[41,42] In methanol, which has a higher dielectric coefficient of 33, complexation constant experiments[40] find that valinomycin prefers binding K^+ to Na^+ by −5.4 kcal/mol. In water, which has an even higher dielectric coefficient value of 80, valinomycin still selects for K^+, but its degree of selectivity has not been quantified in this medium.[36,39] This ability of valinomycin to select K^+ over Na^+ in solvents of varying polarity indicates that the core mechanism underlying the selectivity of valinomycin does not rely on interaction with solvent and should be amenable to evaluation in the gas phase. Nevertheless, solvent can be expected to modify its degree of selectivity.

To gain insight into the mechanism of selectivity in valinomycin, we first investigate how additional ligation from solvent molecules, which raise the coordination numbers of K^+ or Na^+ to the high values found in K channels, can alter its degree of selectivity. We choose water as a representative additional ligand because water extraction from aqueous solution and addition to the ion-complexed valinomycin molecule simulates the physiologically most relevant scenario that occurs in lipid membranes. We compute the free energy change $\Delta\Delta g_n(\varepsilon)$ associated with extracting n water molecules from aqueous phase (*aq.*) and adding them as extra

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ligands to K^+ when it is already complexed with valinomycin $[K^+.Val]$ in a dielectric phase ε,

$$[K^+.Val](\varepsilon) + n.H_2O(aq.) \xrightarrow{\Delta\Delta g_n(\varepsilon)} [K^+.Val.(H_2O)_n](\varepsilon) \quad (3)$$

We use a molecular association statistical theory[43,44] to determine the values of $\Delta\Delta g_n(\varepsilon)$, an approach identical to the one used previously for investigating selective ion partitioning in K channels.[20] The ion, along with its directly coordinating ligands, which in this case consist of the valinomycin molecule and the n ligating water molecules, is treated quantum chemically using density functional theory (DFT) and the B3LYP[45,46] functional implemented in the Gaussian03 package.[47] The only difference in parameters, as compared to those used previously,[20] is with respect to the choice of basis set functions. Here, all optimization and frequency calculations were done with a 6-311+G(d) basis set for K^+, and a 6-31G(d) basis set for the remaining atoms, which reproduces the experimental vibrational spectra of valinomycin.[48] For computational feasibility, the hydrophobic side-chains of the valinomycin molecule are clipped and replaced with methyl groups. Note, however, that the resulting decrease in the number of atoms in valinomycin (from 169 to 114) does not affect the high-frequency vibrational spectra of the molecule,[48] implying that the computed reaction free energies will be affected only minimally.

The structural and energetic effects of increasing the coordination of K^+ bound to valinomycin $[K^+.Val]$ by $n=1$ and $n=2$ water molecules are illustrated in Figure 2. Additional ligation by water destabilizes the $[K^+.Val]$ complex significantly, which implies that the contribution of these higher coordination complexes to the free energy difference between the K^+- and Na^+-complexed states will be negligible. In a separate set of calculations, attempts to optimize water molecules around a $[Na^+.Val]$ complex such that they increase coordination of sodium ions to seven or eight failed, implying that the 7-fold and 8-fold Na^+ complexes are also energetically less favorable. Therefore, in a physiologically relevant scenario, additional ligation by water will not alter the selectivity of valinomycin. Thus, unlike K channels, valinomycin achieves K^+/Na^+ selectivity utilizing only six carbonyl ligands.

To understand the core mechanism behind the ability of valinomycin to select between K^+ and Na^+ using only six carbonyl ligands, we first compare the gas phase QC-optimized structures of its Na^+ and K^+ complexes. The two structures, as illustrated in Figure 3, are not in the absolute sense "isosteric,"[49] but nevertheless closely resemble each other. The root-mean-square deviation (RMSD) between the structures is only 0.14 Å, a result consistent with early circular dichroism (CD) data in low dielectric media.[39] In both cases, the ions prefer positions in the center of the cavity created by the six carbonyl oxygens, consistent with previous classical MD studies.[50] The six oxygen atoms are arranged in a skewed-triangular-prism geometry (not octahedral), with three on either side of the ions. Note that this arrangement is typical for optimum 6-fold Na^+ and K^+ interactions with carbonyl ligands (see Supplementray Data Figure S1). The average Na^+–oxygen distance in valinomycin is smaller than the average K^+–oxygen distance, but by only 0.15 Å. This is consistent with results from chromatography experiments,[50] where the Na^+ complex was found to have a cross-sectional area slightly smaller than that of the K^+ complex.

Free energies computed using these optimized ion-complexed valinomycin structures in the gas phase result in a more stable Na^+ complex relative to the K^+ complex, with $\Delta\Delta G_{Na^+\to K^+}$ $(Val,\varepsilon=1)$ of 12.3 kcal/mol. When the stability due to the reaction

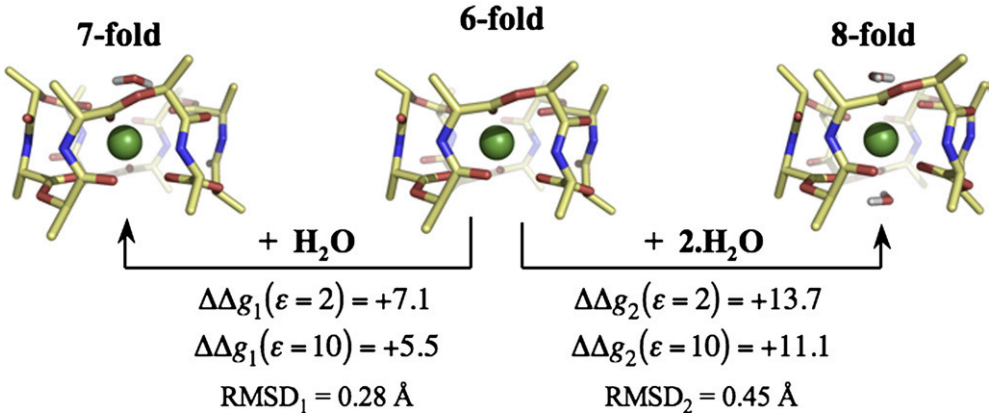

Figure 2. Structural and energetic changes associated with increasing the coordination of potassium ions bound to valinomycin in direct coordination with six carbonyl ligands. Increasing their coordination to 7 and 8 using $n=1$ and $n=2$ water molecules destabilizes the K^+ complex. $\Delta\Delta g_n(\varepsilon)$ denotes this change in complexation energy, as determined from the reaction given by equation (3). $\Delta\Delta g_n(\varepsilon)$ are provided for two different values of ε, which are representative of the dielectric constant of the lipid membranes. Note that calculations using higher values of ε, such as ε=80, also result in a greater stability of the 6-fold coordination. $RMSD_n$ reflects the change in the backbone structure of K^+-bound valinomycin due to complexation by n water molecules.

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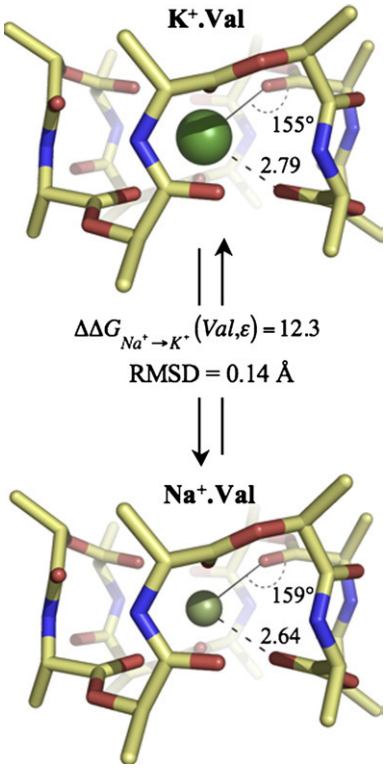

Figure 3. Optimized (DFT/B3LYP) structures of Na^+ and K^+ complexes of valinomycin. Average ion–oxygen distances are indicated in ångström units. Average angles formed between the ion and the carbonyl ligands (I^+-O-C) are also shown.

field from a dielectric medium ($\varepsilon<80$) is added (computed using the APBS package[51]), the magnitude of this relative stability $\Delta\Delta G_{Na^+\to K^+}$ (Val,ε) increases by less than 0.05 kcal/mol; a result that naturally emerges from the structural and electrical (see Supplementary Data Figure S2) similarities of the two valinomycin complexes.

This relative stability between the two valinomycin complexes is, however, much less than the relative stability of the ions $\Delta\Delta G_{Na^+\to K^+}$ (m) in relevant liquid medium m where valinomycin selectivity measurements have been conducted. In water, our computed[20] solvation energy of Na^+ is ~21 kcal/mol more favorable than K^+. In methanol, which has a smaller dielectric coefficient than water ($\varepsilon=33$), the relative solvation energy of Na^+ compared to K^+ remains close to 21 kcal/mol, as computed using methodology described previously.[20] These results are consistent with experimental data,[52] which show that $\Delta\Delta G_{Na^+\to K^+}$ (m) is invariant across these and a range of relevant solvents, including dichloromethane and other alcohols. This implies that transfer of these ions from any of these solvents m into valinomycin will result in a K^+/Na^+ selectivity $\Delta\Delta\Delta G_{Na^+\to K^+}$ $(m\to Val)$ of approximately −9 kcal/mol. This describes the core selectivity of valinomycin, independent from structural changes effected by solvent on its Na^+ and K^+ complexes. Consequences of structural changes brought upon by interaction with solvent will be considered later.

We now investigate the mechanism that gives rise to valinomycin's core selectivity, i.e. we investigate why the relative stabilities of ions in valinomycin complexes are smaller than their values in liquid media, $\Delta\Delta G_{Na^+\to K^+}$ $(Val)\ll 21$ kcal/mol. Note from Table 1 that this behavior is not typical of 6-fold coordinations in gas phase. In fact, a 6-fold coordination complex involving all carbonyl ligands results in a relative free energy difference $\Delta\Delta G_{Na^+\to K^+}$ close to 21 kcal/mol. Therefore, the specific carbonyl chemistry of the ligands in valinomycin is not responsible for its K^+/Na^+ selectivity, as postulated otherwise.[8,24,34] Note also from Table 1 that the average Na^+–oxygen distance of 2.64 Å in valinomycin is larger than the distance of ~2.4 Å that Na^+ prefers when it interacts with six fully flexible monodentate or partially flexible bidentate ligands. This increase in ion–ligand distance is seen in spite of the skewed triangular prism geometry that Na^+ prefers when coordinating with six ligands. The potassium ion, on the other hand, binds to valinomycin at an average distance similar to what it prefers when it forms 6-fold complexes with other ligands. This observation raises two questions. First, is such an increase in ligation distance sufficient to reduce the relative stability of the ions in valinomycin from ~21 kcal/mol to ~12 kcal/mol? Indeed it is, as can be verified using Coulomb's equation and the ion-ligand data given in Figure 3 (see Supplementary Data Figure S2). Second, why is the Na^+ ligation distance in valinomycin larger than its preferred length? In other words, what restrains valinomycin from collapsing onto the smaller sodium ion? There are three main interactions in proteins, or in this case a depsipeptide that define three-dimensional structure: hydrophobic forces, salt-bridges, and hydrogen bonds. Among these, hydrogen bonds are the only form of interaction relevant to such a structural feature in valinomycin, as there are six hydrogen bonds present in its optimum Na^+ and K^+ complexes. In addition, since valinomycin is a cyclic molecule, constraints that might arise from its specific ring size also matter, as explored in the past.[37,53]

To investigate the contribution of hydrogen bonds to valinomycin's selectivity, we turned them off in a manner that can be emulated in experiments. As illustrated in Figure 4, we start with the K^+ optimized valinomycin configuration, and substitute all six of its hydrogen bond acceptor carbonyl oxygen atoms =O with non-hydrogen-bonding $=CH_2$ groups. A QC re-optimization in the presence of K^+ alters its backbone configuration noticeably (RMSD = 0.65 Å), but the 6-fold skewed-triangular-prism coordination geometry is retained, and the average K^+–oxygen distance increases by only 0.1 Å. A QC re-optimization in the presence of Na^+, however, results in an altogether different structure. The molecule adopts an elliptical shape and coordinates the Na^+ ion using only four carbonyl oxygen

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atoms. The four coordinating oxygen atoms are closer than in the native hydrogen bonded complex, at an average distance of 2.33 Å, while the remaining two carbonyl oxygen atoms fall away to a distance of 5.1 Å. The intramolecular hydrogen bonding in valinomycin, therefore, has a more important role in driving the structure of its Na^+ complex, as compared to its K^+ complex. These changes in coordination configurations consequently result in a significant increase in the relative stability $\Delta\Delta G_{Na^+\rightarrow K^+}$ (*mVal*) of the mutated Na^+ compared to K^+ valinomycin complex by 5.5 kcal/mol. The hydrogen bonds, therefore, contribute significantly to K^+/Na^+ selectivity because they make valinomycin less flexible and prevent its ligands from adopting an energetically more stable configuration around the smaller Na^+ ion.

The results presented above indicate that some residual K^+/Na^+ selectivity (~ -3 kcal/mol) is retained even in the complete absence of intramolecular hydrogen bonds, suggesting the presence of other structural features in valinomycin that contribute to its selectivity. Note from Figure 4 that, in the absence of hydrogen bonds, valinomycin prefers a 4-fold coordination around Na^+. Valinomycin's residual selectivity for K^+ is surprising, given previous gas phase investigations with formamide (carbonyl) ligands[20] that show 4-fold coordination of Na^+ is energetically more stable than its corresponding 6-fold coordination by 3.0 kcal/mol. In a similar calculation, we find that a 4-fold coordination from two glycine dipeptide molecules (carbonyl ligands) is also energetically more stable than its corresponding 6-fold coordination by 2.5 kcal/mol. Adding to this the observation that a 6-fold coordination from flexible carbonyl ligands leads to no K^+/Na^+ selectivity, the 4-fold coordination of Na^+ in mutated valinomycin should have led to a reversal of ion selectivity. The reason valinomycin retains K^+/Na^+ selectivity is because this 4-fold geometry, in which all the four ligands are cluttered on one side of the Na^+ (Figure 4), is energetically less stable than the preferred 4-fold geometries in gas phase, such as the tetrahedral or planar[18] geometries. These four ligands from valinomycin are unable to adopt such preferred geometries because of the combined restraints in valinomycin from structural features such as its specific ring size and the spacing between its connected ligands.

Table 1. Computed (DFT/B3LYP) structural and thermochemical properties of 6-fold ion-oxygen clusters in gas phase

Ligand	Chemistry	$\Delta\Delta G_{Na+\rightarrow K+}$ ($\varepsilon=1$) (kcal/mol)	Na^+-O	K^+-O
Valinomycin	Carbonyl	12.3	2.64	2.79
Formamide[20]	Carbonyl	20.6	2.42	2.80
Glycine Dipeptide[20]	Carbonyl	20.8	2.43	2.76
Water[20]	Hydroxyl	18.8	2.42	2.84
Methanol	Hydroxyl	18.5	2.44	2.84

Ion–oxygen distances Na^+-O and K^+-O are in ångström units and $\Delta\Delta G_{Na^+\rightarrow K^+}$ ($\varepsilon=1$) are the free energy differences between the Na^+ and K^+ complexes in gas phase.

These results on the effects of intramolecular hydrogen bonding also suggest how interaction with solvent, other than by direct coordination, can alter valinomycin's core selectivity. It is well known that thermal fluctuations cause local opening and closing of hydrogen bonds.[54–57] In the event of their exposure to solvent, as in the case of valinomycin, solvent molecules can compete for hydrogen bonding and decrease the stability of backbone hydrogen bonds. The higher the polarity of the solvent, the more disruptive its effect will be on valinomycin's intramolecular hydrogen bonding. This is also evident from valinomycin's structure in the absence of bound cations,[36] where the number of intramolecular hydrogen bonds is known to decrease with an increase in solvent polarity. Therefore, we can expect that even when valinomycin is complexed with ions, its propensity to maintain its hydrogen bonds will decrease with an increase in solvent polarity.

An increase in solvent polarity will therefore affect valinomycin in at least three ways. First, the reaction free energy associated with ion complexation to valinomycin will change. In addition to a higher energetic penalty associated with extracting valinomycin from the solvent phase, the decrease in its intramolecular hydrogen bonding will also contribute to an overall reduction (to less favorable values) in its ion complexation free energy. At the same time, however, increased flexibility will allow valinomycin to wrap itself around an ion in an energetically more favorable configuration. In addition, there will be a gain in configurational entropy. This gain, however, is expected to be less than 1 kcal/mol, as the contribution from entropy when n out of six hydrogen bonds are broken is given by $k.T.\log((6-n)!n!/6!)$. Experimental binding constant data collected as a function of increasing polarity[8,39,41,58] reveal a rough pattern wherein ion complexation energy decreases with increased solvent polarity, suggesting that the former two effects override the latter two competing effects. This specific dependence of ion complexation energy on solvent polarity could be vital for valinomycin to transport K^+ ions across lipid membranes as valinomycin would be weakly bound to K^+ at the high dielectric lipid-water interface for spontaneous ion uptake and release, and strongly bound to K^+ when inside the low dielectric lipid membrane for transport.

Second, as the solvent polarity is increased, the approximate "isostericity" observed between the Na^+ and K^+ complexes in gas phase will decrease. On the basis of the results given above, the configuration of the K^+ complex will not change as significantly as that of the Na^+ complex, which will adopt a more elliptical shape. CD and optical rotary dispersion (ORD) data[39] collected in solvents with varying polarity lend direct support to such a trend. In addition, recent MD simulations of valinomycin carried out in ethanol[24,59] show that it adopts a

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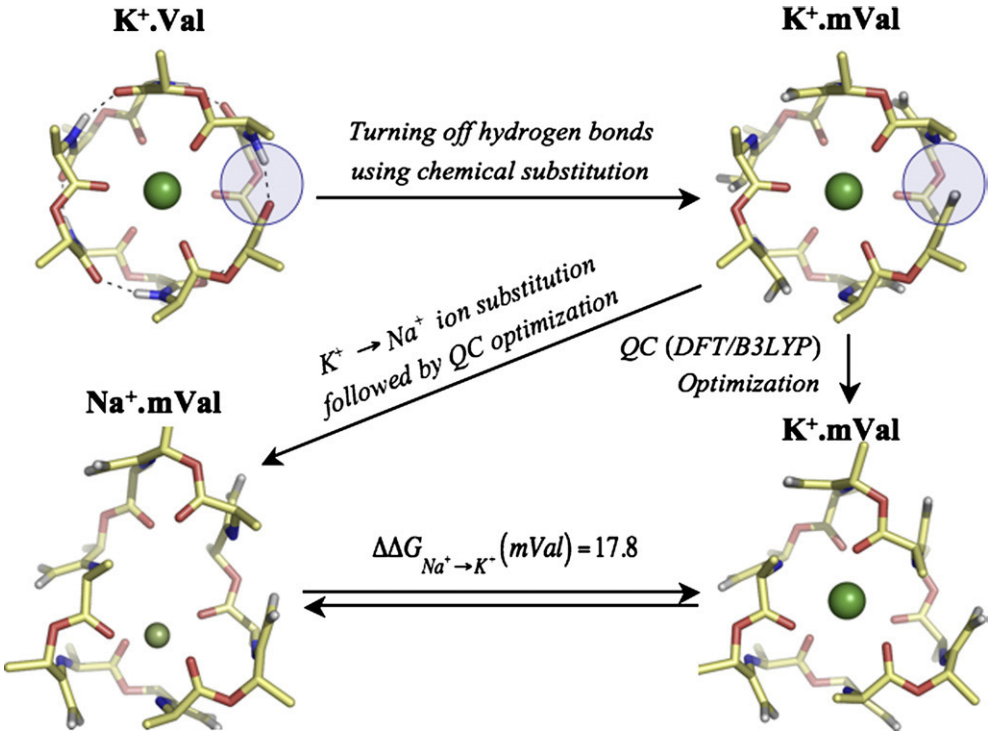

Figure 4. Structural and energetic effects of turning off the six intramolecular hydrogen bonds in valinomycin. The hydrogen bonds, represented by broken lines, were turned off using chemical substitutions involving replacement of proton acceptor atoms =O with $=CH_2$ groups. A representative substitution is highlighted inside blue circles. QC optimization following these substitutions resulted in a K^+-complexed structure K^+.mVal with a backbone atom RMSD of 0.65 Å, and a K^+–O distance of 2.88 Å. In the case of the Na^+ complex Na^+.mVal, these substitutions resulted in four of the six carbonyl oxygen atoms coordinating sodium ions at shorter distances of 2.33 Å, as compared to 2.64 Å in the native Na^+.Val structure. The remaining two carbonyl oxygen atoms are at a distance of 5.12 Å from the sodium ion. The absence of hydrogen bonds also resulted in increasing the free energy difference between the Na^+ and K^+ complexes from 12.3 kcal/mol to 17.8 kcal/mol.

6-fold coordination around K^+, but a lower 4-fold coordination around Na^+. These simulations, however, were carried out in the absence of polarization, which has been argued to be important for describing ion binding in valinomycin[8] (see also partial charges estimated from *ab initio* calculations in Table S1 of Supplementary Data).

Finally, a characteristic dependence of K^+/Na^+ selectivity on solvent polarity will emerge. In low-polarity solvents, where all hydrogen bonds are maintained and the Na^+ and K^+ structures remain approximately isosteric, the K^+/Na^+ selectivity will attain a constant high value of selectivity similar to its core selectivity. This idea was conceptualized[49] in the late 1960s and eventually proved[41] by carrying out salt extraction equilibrium experiments in low ($\varepsilon<9$) polarity solvents. In higher polarity solvents, where the proclivity to maintain all six hydrogen bonds is lower, valinomycin will be able to adopt a relatively more stable configuration around the Na^+. In such a scenario, selectivity can be expected to decrease, but further investigation is required. Nonetheless, valinomycin selectivity will not vanish even in very high-polarity solvents due to the intrinsic contribution of −3 kcal/mol from its other structural restraints that are not affected by solvent polarity.

Together, we find that valinomycin is able to use only six carbonyl ligands to achieve K^+/Na^+ selectivity because it can physically prevent all of its six ligands from collapsing simultaneously onto the smaller Na^+ ion. In other words, it creates a specific cavity size for ion binding that matches the size of K^+, but not that of the smaller Na^+ ion. Valinomycin enforces such constraints through a combination of intramolecular hydrogen bonds and other structural features, including its specific ring size and the spacing between its connected ligands.

This host–guest steric mechanism of valinomycin is, however, entirely different from K channels, which use a different variety of topological constraint. In the absence of cavity size constraints, K channels utilize constraints on over-coordination by more than six carbonyl ligands. In addition to any rigidity supplied by the binding site structure, K channels require a special local solvation phase around their binding sites that is devoid of competing hydrogen bond donors to sustain such over-coordination.[20] One of the key advantages of the K channel mechanism is that it allows the degree of selectivity to be easily tuned *via* mutations in the protein solvation environment surrounding the binding sites. In fact, nature appears to have capitalized on this feature to create strong, intermediate

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as well as weakly selective K channels without major backbone structural rearrangements.[30,33] The main advantage of the valinomycin mechanism over the K channel mechanism is that valinomycin can tolerate the presence of hydrogen bond donors in its local neighborhood, as is evident from its persistent selectivity in solvents of varying polarity. In addition, its complexation energy with K^+ is very small in liquid water.[8,39,41,58] This currently makes it the mechanism of choice for nanodevice technology. Cyclodextrins, for example, encoded with valinomycin's structural features, can be plugged into the pores of large channel proteins like α-hemolysins[60] to create artificial K^+/Na^+ selective channels for devices intended for various purposes, including power generation, drug delivery and dialysis.

In general, these and all previous investigations on K channels and valinomycin together reveal how nature has utilized different combinations of phase, coordination number, cavity size, rigidity, and chemistry to construct widely different mechanisms for K^+/Na^+ selectivity. These investigations have, however, merely uncovered what lies beneath the surface of an iceberg. Much remains to be discovered before a comprehensive picture of selective ion partitioning mechanisms in biological systems is achieved.

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Supplementary Data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jmb.2007.11.059

References

1. Hille, B. (2001). Ionic Channels of Excitable Membranes, 3rd edit. Sinauer Associates, Sunderland, MA.
2. Page, M. J. & Di Cera, E. (2006). Role of Na^+ and K^+ in enzyme function. *Physiol. Rev.* **86**, 1049–1092.
3. Draper, D. E. & Misra, V. K. (1998). RNA shows its metal. *Nature Struct. Biol.* **5**, 927–930.
4. NIH Center for Design of Biomimetic Nanoconductors URL: http://www.nanoconductor.org.
5. Pauling, L. (1948). Nature of forces between large molecules of biological interest. *Nature*, **161**, 707–709.
6. Bezanilla, F. & Armstrong, C. M. (1972). Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons. *J. Gen. Physiol.* **60**, 588–608.
7. Eisenman, G. (1961). In *Symposium on Membrane Transport and Metabolism* (Kleinzeller, A. & Kotyk, A., eds), Academic Press, New York.
8. Aqvist, J., Alvarez, O. & Eisenman, G. (1992). Ion-selective properties of a small ionophore in methanol studied by free energy perturbation simulations. *J. Phys. Chem.* **96**, 10019–10025.
9. Doyle, D. A., Cabral, J. M., Pfuetzner, R. A., Kuo, A. L., Gulbis, J. M., Cohen, S. L. *et al.* (1998). The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science*, **280**, 69–77.
10. Miller, C. (2001). See potassium run. *Nature*, **414**, 23–24.
11. Zhou, Y., Morais-Cabral, J. H., Kaufman, A. & MacKinnon, R. (2001). Chemistry of ion coordination and hydration revealed by a K^+ channel-Fab complex at 2.0 A resolution. *Nature*, **414**, 43–48.
12. Gouaux, E. & MacKinnon, R. (2005). Principles of selective ion transport in channels and pumps. *Science*, **310**, 1461–1465.
13. Ohtomo, N. & Arakawa, K. (1980). *Bull. Chem. Soc. Jpn.* **53**, 1789.
14. Tongraar, A., Liedl, K. R. & Rode, B. M. (1998). Born-Oppenheimer ab initio QM/MM dynamics simulations of Na^+ and K^+ in water: from structure making to structure breaking effects. *J. Phys. Chem. A*, **102**, 10340–10347.
15. Neilson, G. W., Mason, P. E., Ramos, S. & Sullivan, D. (2001). Neutron and X-ray scattering studies of hydration in aqueous solutions. *Phil. Trans. Roy. Soc. ser. A*, **359**, 1575–1591.
16. Soper, A. K. & Weckstrom, K. (2006). Ion solvation and water structure in potassium halide aqueous solutions. *Biophys. Chem.* **124**, 180–191.
17. Ramaniah, L., Bernasconi, M. & Parrinello, M. (1999). Ab initio molecular-dynamics simulation of K^+ solvation in water. *J. Chem. Phys.* **111**, 1587–1591.
18. Rempe, S. B., Asthagiri, D. & Pratt, L. R. (2004). Inner shell definition and absolute hydration free energy of K^+(aq) on the basis of quasi-chemical theory and ab initio molecular dynamics. *Phys. Chem. Chem. Phys.* **6**, 1966–1969.
19. Varma, S. & Rempe, S. B. (2006). Coordination numbers of alkali metal ions in aqueous solutions. *Biophys. Chem.* **124**, 192–199.
20. Varma, S. & Rempe, S. B. (2007). Tuning ion coordination architectures to enable selective partitioning. *Biophys. J.* **93**, 1093–1099.
21. Guidoni, L., Torre, V. & Carloni, P. (1999). Potassium and sodium binding in the outer mouth of the K. *Biochemistry*, **38**, 8599–8604.
22. Berneche, S. & Roux, B. (2000). Molecular dynamics of the KcsA K^+ channel in a bilayer membrane. *Biophys. J.* **78**, 2900–2917.
23. Shrivastava, I. H., Tieleman, D. P., Biggin, P. C. & Sansom, M. S. (2002). K^+ versus Na^+ ions in a K

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channel selectivity filter: a simulation study. *Biophys. J.* **83**, 633–645.
24. Noskov, S. Y., Berneche, S. & Roux, B. (2004). Control of ion selectivity in potassium channels by electrostatic and dynamic properties of carbonyl ligands. *Nature*, **431**, 830–834.
25. Jordan, P. C. (2007). New and notable: tuning a potassium channel – the caress of the surroundings. *Biophys. J.* **93**, 1091–1092.
26. Aqvist, J. & Luzhkov, V. (2000). Ion permeation mechanism of the potassium channel. *Nature*, **404**, 881–884.
27. Berneche, S. & Roux, B. (2001). Energetics of ion conduction through the K^+ channel. *Nature*, **414**, 73–77.
28. Bichet, D., Lin, Y.-F., Ibarra, C. A., Huang, C. S., Yi, B. A., Jan, Y. N. & Jan, L. Y. (2004). Evolving potassium channels by means of yeast selection reveals structural elements important for selectivity. *Proc. Natl Acad. Sci. USA*, **101**, 4441–4446.
29. Bichet, D., Grabe, M., Jan, Y. N. & Jan, L. Y. (2006). Electrostatic interactions in the channel cavity as an important determinant of potassium channel selectivity. *Proc. Natl Acad. Sci. USA*, **103**, 14355–14360.
30. Shi, N., Ye, S., Alam, A., Chen, L. & Jiang, Y. (2006). Atomic structure of a Na^+- and K^+-conducting channel. *Nature*, **440**, 570–574.
31. Bostick, D. & Brooks, C. L., 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.
32. Valiyaveetil, F., Leonetti, M., Muir, T. & MacKinnon, R. (2006). Ion selectivity in a semisynthetic K^+ channel locked in the conductive conformation. *Science*, **314**, 1004–1007.
33. Shealy, R. T., Murphy, A. D., Ramarathnam, R., Jakobsson, E. & Subramaniam, S. (2003). Sequence-function analysis of the K^+-selective family of ion channels using a comprehensive alignment and the KcsA channel structure. *Biophys. J.* **84**, 2929–2942.
34. Noskov, S. Y. & Roux, B. (2007). Importance of hydration and dynamics on the selectivity of the KcsA and NaK channels. *J. Gen. Physiol.* **129**, 135–143.
35. Asthagiri, D., Pratt, L. R. & Paulaitis, M. E. (2006). Role of fluctuations in a snug-fit mechanism of KcsA channel selectivity. *J. Chem. Phys.* **125**, 24701–24706.
36. Dobler, M. (1981). *Ionophores and Their Structures*. John Wiley and Sons, Inc.
37. Shemyakin, M. M., Ovchinnikov, Y. A., Ivanov, V. T., Antonov, V. K., Vinogradova, E. I., Shkrob, A. M. *et al.* (1969). Cyclodepsipeptides as chemical tools for studying ionic transport through membranes. *J. Membr. Biol.* **1**, 402–430.
38. Lauger, P. (1972). Carrier-mediated ion transport: electrical relaxation experiments give insight into the kinetics of ion transport through artificial lipid membrane. *Science*, **178**, 24–30.
39. Rose, M. C. & Henkens, R. W. (1974). Stability of sodium and potassium complexes of valinomycin. *Biochim. Biophys. Acta*, **372**, 426–435.
40. Grell, E., Funck, T. & Eggers, F. (1975). Membranes. In *Membranes* (Eisenman, G., ed), *Membranes*, vol. 3, Dekker, New York.
41. Eisenman, G., Aqvist, J. & Alvarez, O. (1991). Free energies underlying ion binding and transport in protein channels: free energy perturbation simulations of ion binding and selectivity for valinomycin. *J. Chem. Soc. Faraday Trans.* **87**, 2099–2109.
42. Eisenman, G. & Alvarez, O. (1992). Ionic Selectivity of proteins: lessons from molecular dynamics simulations on valinomycin. In *Biomembrane Structure and Function - The State of the Art* (Gaber, B. P. & Easwaran, K. R. K., eds).
43. Widom, B. (1982). Potential-distribution theory and the statistical mechanics of fluids. *J. Phys. Chem.* **86**, 869–872.
44. Pratt, L. R. & LaViolette, R. A. (1998). Quasi-chemical theories of associated liquids. *Mol. Phys.* **95**, 909–915.
45. Lee, C., Yang, W. & Parr, R. G. (1988). Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B*, **37**, 785–789.
46. Becke, A. D. (1993). Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **98**, 5648–5652.
47. Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R. *et al.* (2004). *Gaussian 03.* Gaussian, Inc., Wallingford, CT.
48. Wang, F., Zhao, C. & Polavarapu, P. L. (2004). A study of the conformations of valinomycin in solution phase. *Biopolymers*, **75**, 85–93.
49. Eisenman, G., Ciani, S. & Szabo, G. (1969). The effects of the macrotetralide actin antibiotics on the equilibrium extraction of alkali metal salts into organic solvent. *J. Membr. Biol.* **1**, 294–345.
50. Wyttenbach, T., Batka, J. J., Jr, Gidden, J. & Bowers, M. T. (1999). Host/guest conformations of biological systems: valinomycin/alkali ions. *Intr. J. Mass Spec.* **193**, 143–152.
51. Baker, A. N., Sept, D., Joseph, S., Holst, M. & McCammon, A. J. (2001). Electrostatics of nanosystems: application to microtubules and the ribosome. *Proc. Natl Acad. Sci. USA*, **98**, 10037–10041.
52. Marcus, Y. (1983). Thermodynamic functions of transfer of single ions from water to nonaqueous and mixed solvents. Part 1 - Gibbs free energies of transfer to nonaqueous solvents. *Pure and Appl. Chem.* **55**, 977–1021.
53. Ivanov, V. T. & Ovchinnikov, Y. A. (1975). Conformational states and biological activity of cyclic peptides. *Tetra. Rep.* **31**, 2177–2209.
54. Daggett, V. & Levitt, M. (1992). Molecular dynamics simulations of helix denaturation. *J. Mol. Biol.* **223**, 1121–1138.
55. Avbelj, F., Luo, P. & Baldwin, R. L. (2000). Energetics of the interaction between water and the helical peptide group and its role in determining helix propensities. *Proc. Natl Acad. Sci. USA*, **97**, 10786–10791.
56. Garcia, A. E. & Sanbonmatsu, K. Y. (2002). α-Helical stabilization by side-chain shielding of backbone hydrogen bonds. *Proc. Natl Acad. Sci. USA*, **99**, 2782–2787.
57. Varma, S. & Jakobsson, E. (2007). The cPLA2 C2α domain in solution: structure and dynamics of its Ca^{2+}-activated and cation-free states. *Biophys. J.* **92**, 956–965.
58. Feinstein, M. B. & Felsenfeld, H. (1971). The detection of ionophorous antibiotic-cation complexes in water with fluorescent probes. *Proc. Natl Acad. Sci. USA*, **68**, 2037–2041.
59. Noskov, S. Y. & Roux, B. (2006). Ion selectivity in potassium channels. *Biophys. Chem.* **124**, 279–291.
60. Bayley, H. & Jayasinghe, L. (2004). Functional engineered channels and pores. *Mol. Membr. Biol.* **21**, 209–220.

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Supplementary Text

Manuscript Title: K^+/Na^+ selectivity in K-channels and valinomycin: Over-coordination Vs Cavity-size constraints

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Figure S1: *The skewed-triangular-prism geometry of ion coordination in valinomycin is typical of 6-fold Na^+ and K^+ complexes with carbonyl ligands. The ions and the coordinating oxygens are drawn as green and red spheres, with the remaining atoms drawn as sticks. The edges of the coordination geometry are shown using dashed lines connecting the oxygen atoms.*

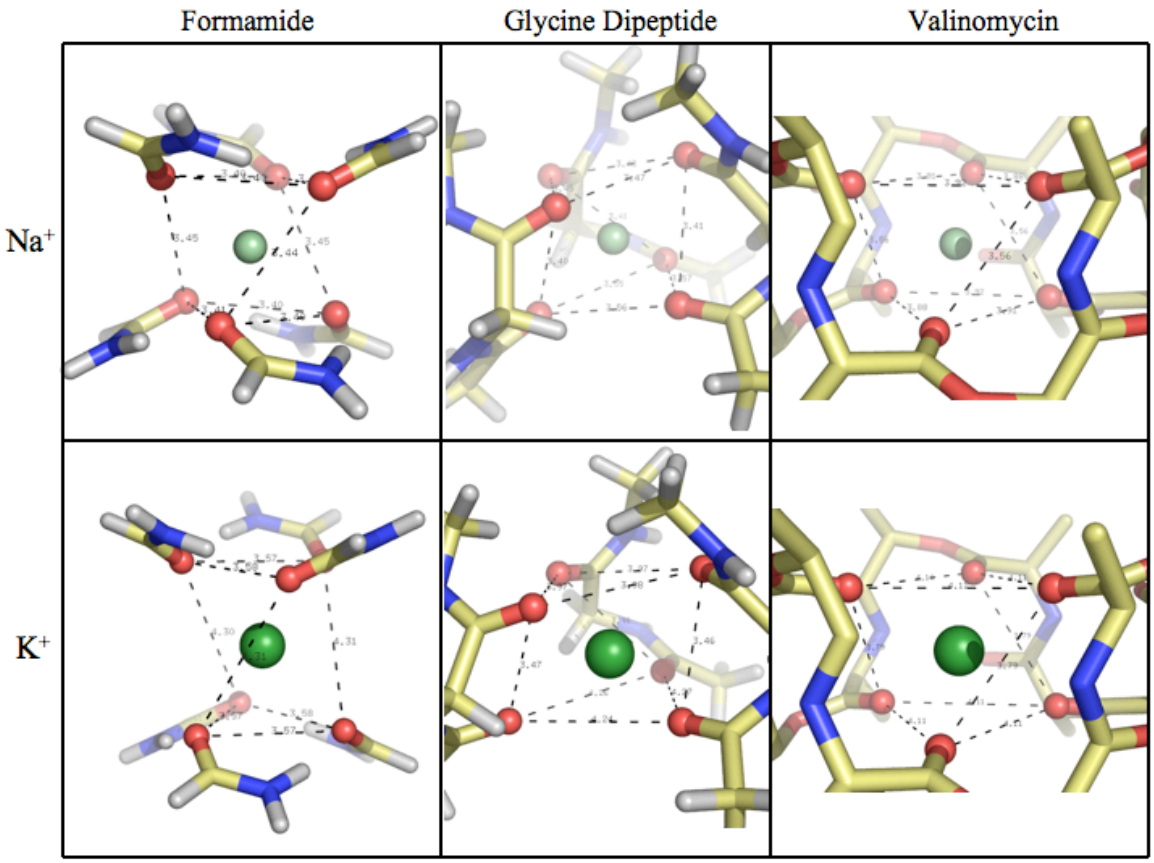
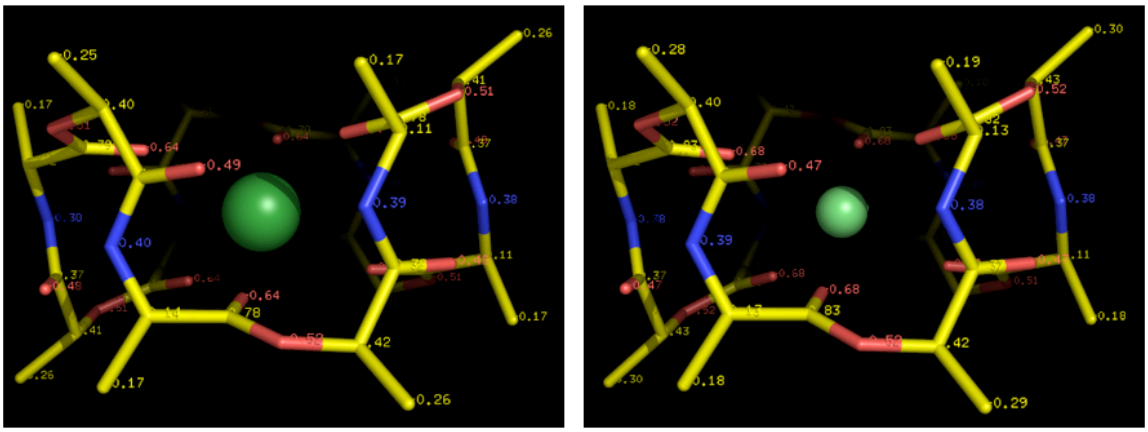


Figure S2: *Atomic partial charges for the QC optimized complexes of valinomycin. The partial charges were computed using the CHELPG method. The two complexes are approximately "iso-electric," implying that their reactions fields in a dielectric media would be similar.*

K^+.Val **Na^+.Val**



Salient features of the theoretical approach

Methodological details, including all simulation parameters, are described elsewhere.[1,2] Some simulation parameters in this work are different from those used in our earlier study on K-channels[2] and are provided in the main text. Here we focus on certain salient features of the approach left out in our previous discussions.[1,2]

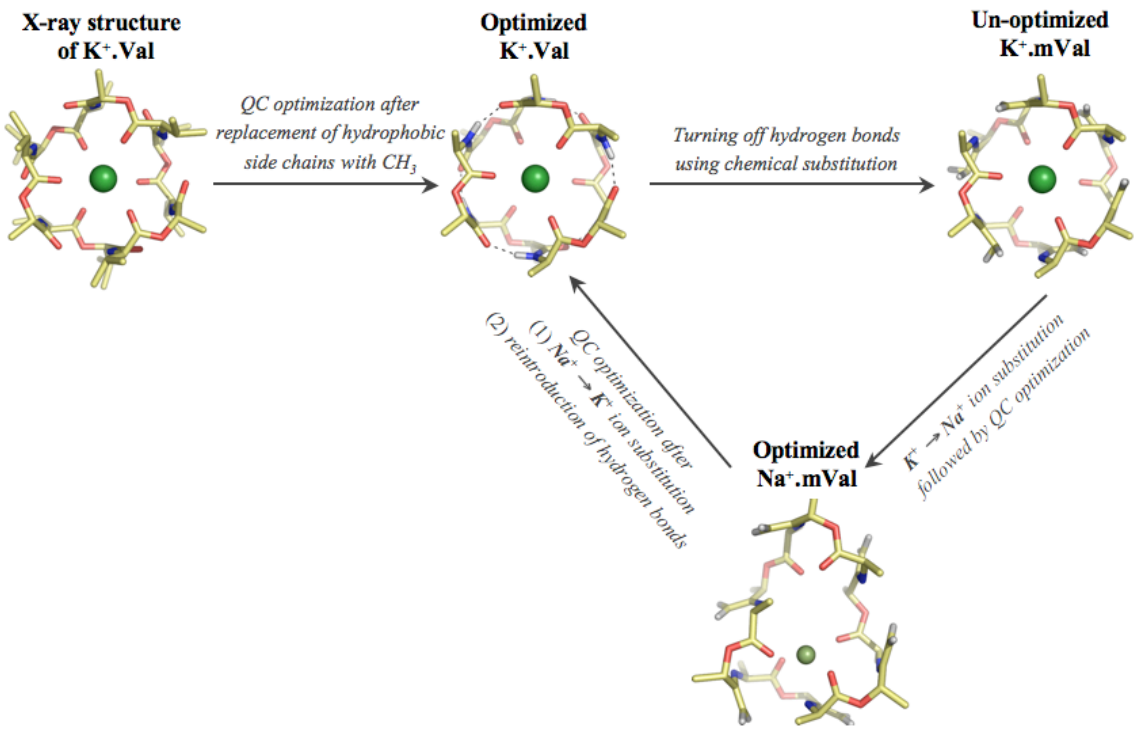
All the analyses presented in this work, and almost all of the analyses presented in our earlier work on ion hydration (see Table 5 in Ref. [1] and references therein) and K-channels[2] are based on reaction free energies and *not* differences between single point energies. Reaction free energies, in contrast to single point energy differences, include differences in zero-point energies and entropies between the reactants and products. Entropies for all reactant and product species are determined separately from partition functions[3] evaluated at their respective potential energy minima. In particular, harmonic expansions (Hessian analysis) about these potential energy minima produce estimates of the vibrational frequencies, thereby capturing the effects of structural fluctuations within each potential energy well. If optimization algorithms fail and the final geometry does not correspond to a stable minimum on the potential energy surface (PES), the computed vibrational frequencies end up imaginary. All frequencies were found to be real, confirming the existence of energetically optimized structures. Entropies estimated using such a strategy always run the risk of neglecting contributions from anharmonic vibrations. These contributions are negligible for tightly bound systems such as small ion-ligand clusters. For larger clusters, such as those that form around hydrophobic solutes,[4] they can be substantial. Their inclusion, however, only makes the reaction free energies more favorable. In addition, they can be safely neglected in certain cases, such

as during ion selectivity computations in valinomycin or in other clusters,[2] where differences in free energies between two similar ion complexes results in the cancellation of common terms, including anharmonicities arising from hydrogen bond interactions. A more detailed account on the effect of anharmonicity is included in a separate article recently accepted for publication.[4]

The PES of a system can contain multiple energy minima, all of which produce non-imaginary frequencies upon Hessian analysis. In such a scenario, which is the global potential energy minimum? For all of our optimizations, we have utilized the algorithm encoded in Gaussian03 with its default options and parameters. Gaussian03 performs optimizations via an enhanced version of an algorithm developed originally by Schlegel and coworkers,[5,6] which is described in detail on the Gaussian web site (http://www.gaussian.com/g_ur/k_opt.htm). Although no optimization algorithm can guarantee a global minimum, it is reasonable to expect, especially for strongly bound systems, that if optimizations are initiated with configurations that lie close to the global energy minimum, such as a low temperature x-ray structure, they should converge to a global energy minimum. In addition, our overall strategy has always been to carry out a more extensive exploration of the PES via utilization of multiple starting configurations. We have utilized this strategy for all our previous studies, including ion hydration[1] and selective partitioning in K-channels,[2] and also for the current study on valinomycin.

In this study, we used two very different starting configurations to search for the energy optimized configuration of the K^+ complex, one of which was the x-ray structure, while the other was the non-symmetric configuration that Na^+ adopts with the mutated form of valinomycin (Na^+.mVal). These two starting configurations yielded identical 3-fold symmetric energy optimized configurations (figure S3), suggesting that the optimum energy configuration most likely corresponds to the global minimum energy. This strategy to search the PES for energy minima is in line with an approach formalized by Stillinger and Weber in the 1980s,[7-9] where the global minimum is sought by dividing the PES into catchment basins. Formally, this is achieved by separating the configurational part of the phase space integral of the partition function into individual integrals, each representing a different minimum.

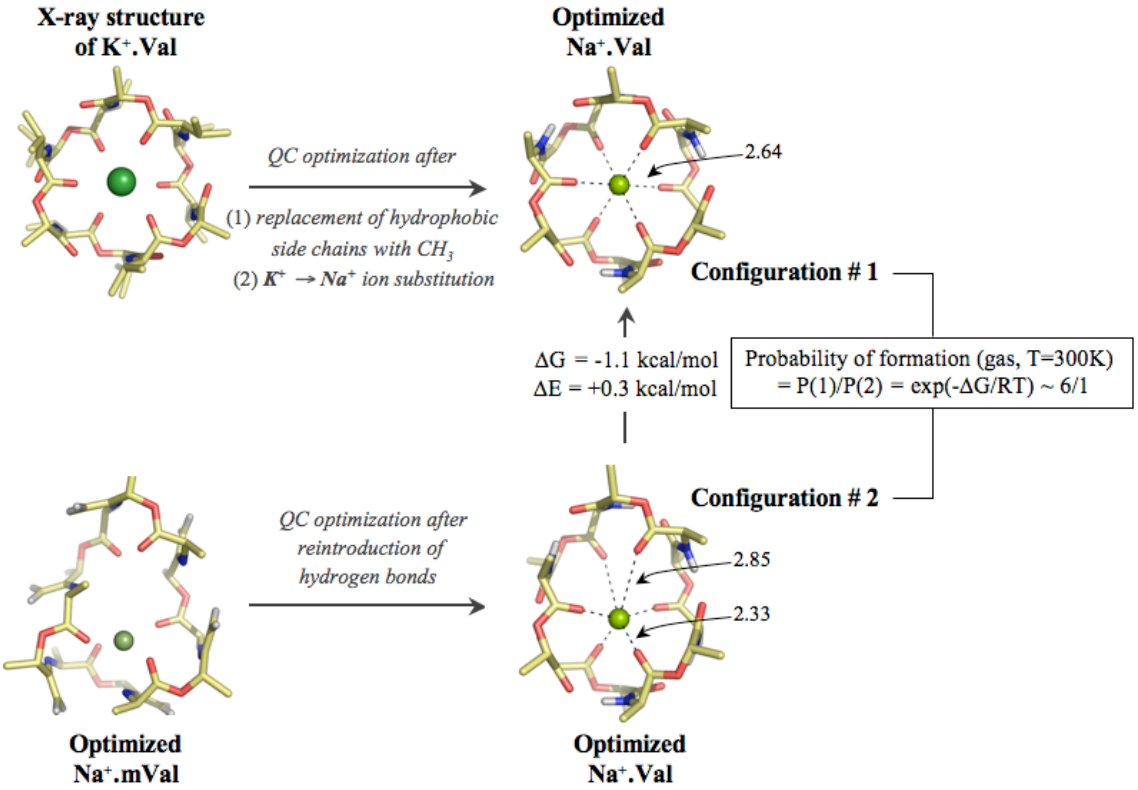
Figure S3: *Two different starting configurations were used to determine the optimized configuration of the K^+ complex with valinomycin, one of which was the x-ray structure, while the other was the non-symmetric configuration that Na^+ adopts with the mutated form of valinomycin (Na^+.mVal). These two starting configurations yielded identical 3-fold symmetric energy optimized configurations, suggesting that the optimum energy configuration most likely corresponds to the global minimum energy.*



In a scenario where there are multiple energy minima on the PES, how do they contribute to the overall reaction free energy? Boltzmann statistics dictates that the probability to form one specific configuration over another depends exponentially on the free energy difference between the two configurations. Therefore, for two configurations that differ in energy by more than a few kcal/mol, the probability to form a configuration with a higher reaction free energy is negligible, unless that configuration is favored by some kind of a constraint (i.e., potential energy barrier). In our attempt to search for the energy optimized configuration of the Na^+ complex, we also used two very different starting configurations, one of which was once again the x-ray structure of the K^+ complex, while the other was the non-symmetric configuration that Na^+ adopts with the mutated form of valinomycin (Na^+.mVal). In this case, we found two slightly different optimized configurations, with the former starting configuration yielding a 3-fold symmetric optimized configuration, while the latter produced an optimized configuration in which the position of Na^+ ion was off-center (figure S4). Nonetheless, the gas phase free energy difference between these two structures $(\Delta G_1 - \Delta G_2)$ was only 1.1 kcal/mol. In addition to establishing that the former configuration is energetically preferred, we can also invoke Boltzmann statistics to interrogate the relative probability of formation of one complex over the other. In this case, the relative probability is $e^{-\beta(\Delta G_1 - \Delta G_2)} \approx 6:1$ at a temperature of 300K.

Figure S4: *Two different starting configurations were used to determine the optimized configuration of the Na^+ complex with valinomycin, one of which was the x-ray structure of the K^+ complex, while the other was the non-symmetric configuration that Na^+ adopts with the mutated form of valinomycin (Na^+.mVal). The optimization yielded slightly*

different final configurations, with the former starting configuration yielding a 3-fold symmetric optimized configuration, while the latter yielding an optimized configuration in which the position of Na^+ ion is off-centered.



Note that in this approach, which is based on utilization of optimized configurations, the reaction free energy for complex formation is evaluated separately for each local minima of the PES. These free energies can then be utilized to estimate occupational probabilities on the PES. This differs from approaches involving MD or MC simulations where occupational probabilities on the PES are estimated before they are translated into relative free energy differences. The main advantage of the latter approaches is that no *a priori* assumption is required to describe the PES topology (such as a harmonic approximation), however, its utilization in conjunction with the *ab initio* level of theory is currently not practical due to excessively large CPU requirements. As it is, all calculations for this work on valinomycin required ~70,000 hours, while those for our previous study on K-channels[2] required more than 200,000 hours of compute time. Using the latter approaches in conjunction with classical force fields may appear to serve as an alternative. For these investigations, however, the main advantages of utilizing the former approach and employing *ab initio* descriptions of atomic interactions appears to outweigh its disadvantage of predefining the shape of the PES. First, as evident from the polarization on the carbonyl groups of valinomycin (table S1), the ion-valinomycin complex is a strongly bound system, which suggests that a harmonic approximation is a reasonable choice to describe its PES. Second, there are currently no classical force fields

that can capture the polarizability of valinomycin, which is quite substantial based on the data presented in Table S1. Switching to classical force fields therefore does not guarantee that the simulated PES would be relevant to reality, as also argued in a separate classical MD based study on valinomycin.[10] Using the former approach in conjunction with an *ab initio* level of theory provides a better representation for local interactions, which is important to understand the determinants of ion selectivity. As with any other theoretical or experimental approach, there are indeed limitations to the methods and strategy used for these investigations; however, utmost precaution has been taken to stay within those limitations, especially in choosing the issues that can be directly addressed with this approach.

Table S1: *Average CHELPG[11] charges* $\langle q_o \rangle$ *on the coordinating carbonyl oxygens in different energy optimized complexes of valinomycin. The charges are considerably more negative when valinomycin is bound to ions, implying that ion binding polarizes the carbonyl ligands. The bracelet and propeller configurations refer to the configurations of uncomplexed (apo) valinomycin in low and high dielectric media. Configurations 1 and 2 of its complex with* Na^+ *refer to the two different optimized configurations illustrated in figure S4.*

Complex	$\langle q_o \rangle$
Apo Val – *Propeller configuration*	-0.50
Apo Val – *Bracelet configuration*	-0.53
Na^+.Val – *Configuration 1*	-0.68
Na^+.Val – *Configuration 2*	-0.67
Na^+.mVal	-0.69
K^+.Val	-0.64
K^+.mVal	-0.63
K^+.Val.H_2O	-0.68
K^+.Val.$(H_2O)_2$	-0.68

References:

1. Varma, S. & Rempe, S. B. (2006). Coordination numbers of alkali metal ions in aqueous solutions. Biophys. Chem. 124, 192-199.
2. Varma, S. & Rempe, S. B. (2007). Tuning ion coordination architectures to enable selective partitioning. Biophys. J. 93, 1093-1099.

3. McQuarrie, D. A. (2000). Chapter 8 of Statistical Mechanics. 2nd Ed., University Science Books.
4. Sabo, D., Varma, S., Martin, M. & Rempe, S. B. (2007). Studies of the thermodynamic properties of hydrogen gas in bulk water. J Phys. Chem. B In press.
5. Schlegel, H. B. (1982). Optimization of equilibrium geometries and transition structures. J Comp. Chem. 3, 214-218.
6. Peng, C. Y., Ayala, P. Y., Schlegel, H. B. & Frisch, M. J. (1998). Using redundant internal coordinates to optimize equilibrium geometries and transition states. J Comp. Chem. 17, 49-56.
7. Stillinger Jr., F. H. & Weber, T. A. (1982). Hidden structure in liquids. Phys. Rev. A 25, 978-989.
8. Stillinger Jr., F. H. & Weber, T. A. (1983). Dynamics of structural transitions in liquids. Phys. Rev. A 28, 2408.
9. Wales, D. (2003). Energy landscapes: With applications to clusters, biomolecules and glasses, Cambridge University Press.
10. Eisenman, G., Aqvist, J. & Alvarez, O. (1991). Free Energies underlying Ion Binding and Transport in Protein Channels: Free Energy Perturbation Simulations of Ion Binding and Selectivity for Valinomycin. J. Chem. Soc. Faraday Trans. 87, 2099-2109.
11. Breneman, C. M. & Wiberg, K. B. (1990). Determining Atom-Centered Monopoles from Molecular Electrostatic Potentials. The Need for High Sampling Density in Formamide Conformational Analysis. J Comp. Chem. 11, 361-373.