Supplementary Text

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

Authors: Sameer Varma, Dubravko Sabo, and Susan B. Rempe*
Sandia National Laboratories, Albuquerque, NM-87185, USA
****Corresponding Author:*** MS 0310, PO Box 5800, Albuquerque, NM 87185.
Email: slrempe@sandia.gov, Phone: (505) 845-0253, Fax: (505) 844-5670.

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.*

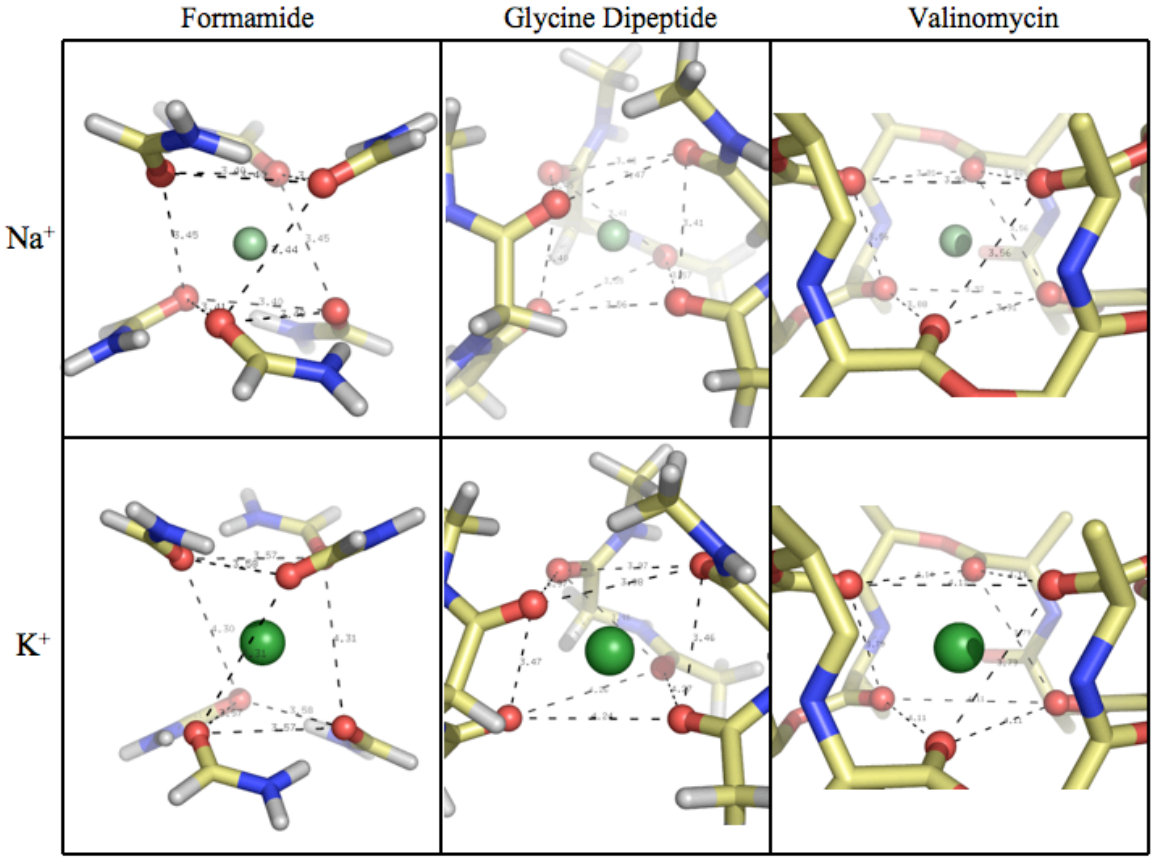
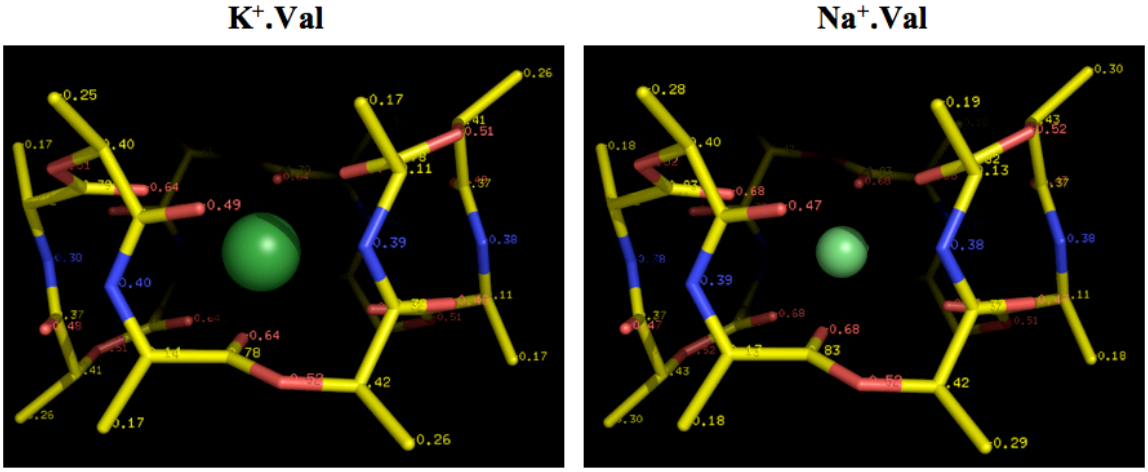


Figure S2: *Atomic partial charges for the QC optimized complexes of valinomycin. The partial charges were computed using the CHELPG method. The two complexes are approximately "iso-electric," implying that their reactions fields in a dielectric media would be similar.*



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]

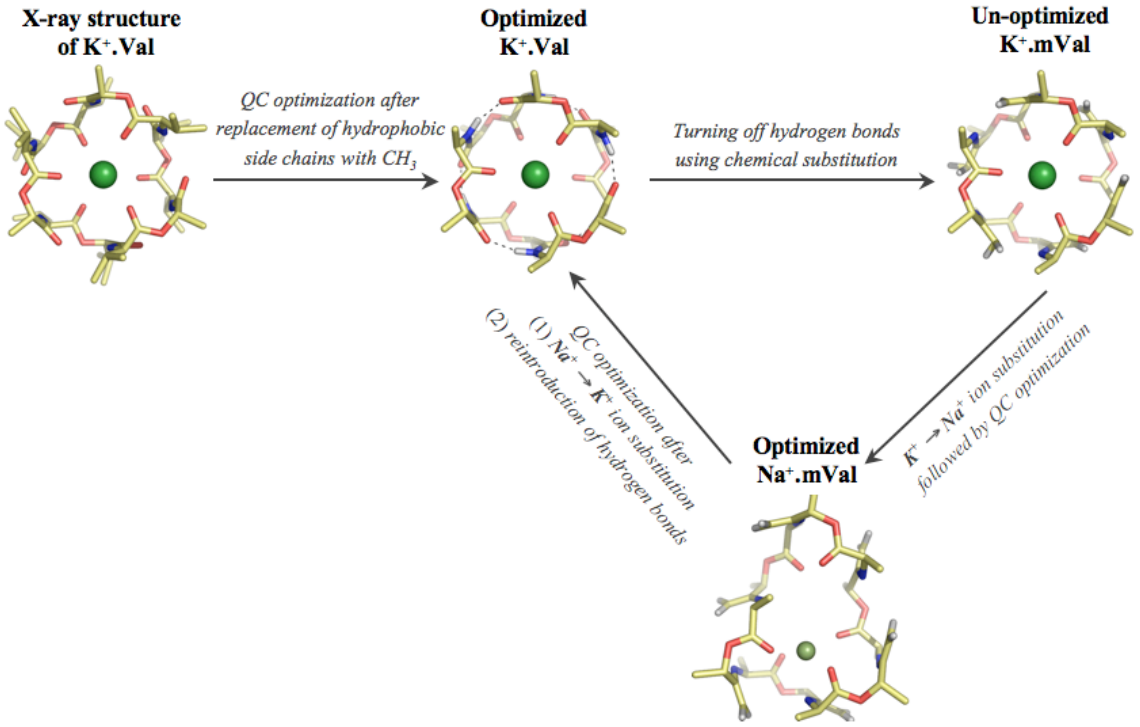
All the analyses presented in this work, and almost all of the analyses presented in our earlier work on ion hydration (see Table 5 in Ref. [1] and references therein) and K-channels[2] are based on reaction free energies and *not* differences between single point energies. Reaction free energies, in 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.

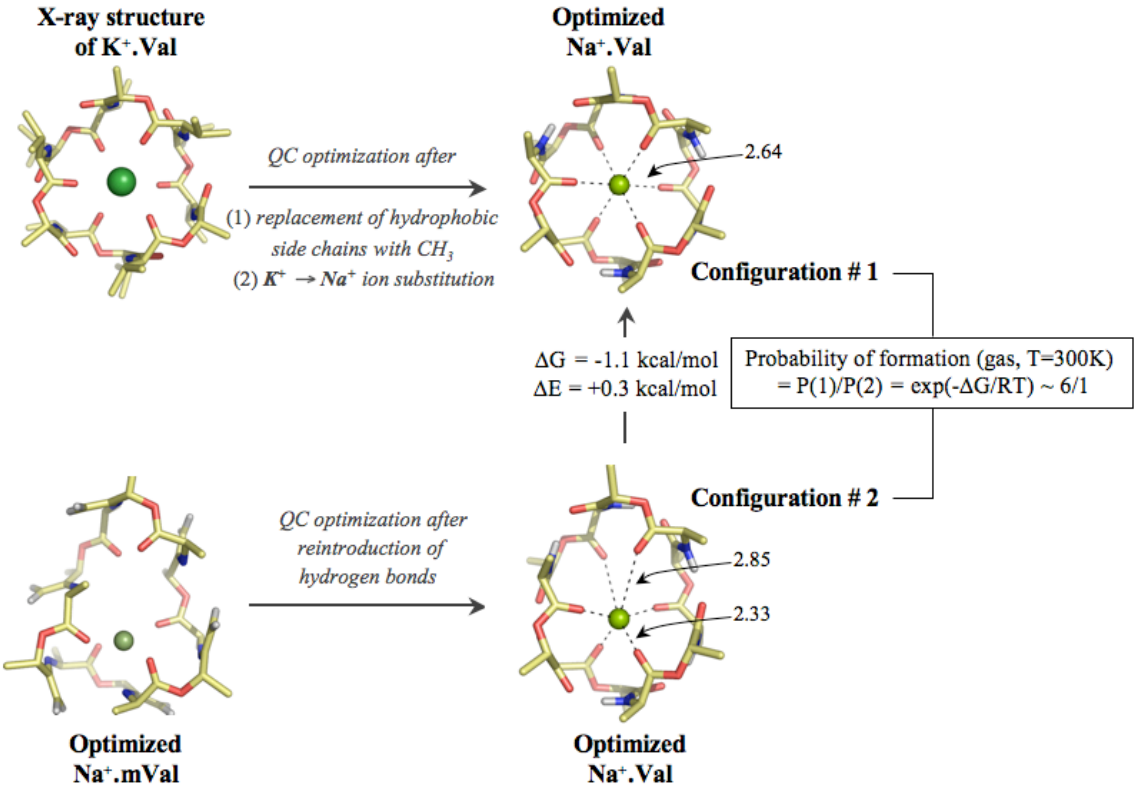
Figure S3: *Two different starting configurations were used to determine the optimized configuration of the K^+ complex with valinomycin, one of which was the x-ray structure, while the other was the non-symmetric configuration that Na^+ adopts with the mutated form of valinomycin (Na^+.mVal). These 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

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