On inferring the ion selectivity of the KcsA potassium channel using the distribution of coordination states of the aqueous ions

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The $S_2$ site of the KcsA $K^+$ channel has eight carbonyl ligands in the ion-binding site. A recent study suggests that the $K^+$-over-$Na^+$ selectivity of the $S_2$ site can be understood by noting the larger free energy change involved in enforcing an eight water coordination state around $Na^+$ relative to $K^+$. The free energies were obtained from the probabilities of observing eight water molecules within a coordination sphere whose radius ($\lambda_{Na^+} \approx 3.1 \, \text{Å}$ and $\lambda_{K^+} \approx 3.5 \, \text{Å}$) extends to the first minimum of the ion-water oxygen pair correlation function. Curiously, using the same coordination radius led to results that question the very idea of using coordination states in water to understand selectivity in the channel. We show that density fluctuations in neat water at the length scale of the coordination volume enter the description of the hydration thermodynamics of the ion expressed in terms of its coordination states. Density fluctuations explain the sensitivity to the choice of radius. After accounting for this effect, the results lead to the conclusion that free energy changes involved in the transition between coordination states in water are inadequate to explain selectivity in the ion-channel.

I. INTRODUCTION

Molecular simulations of the selectivity of the $S_2$ site in the KcsA $K^+$ ion channel show that \[ \Delta \mu_{\text{ex}} = [\mu_{Na^+}^{\text{ex}}(S_2) - \mu_{K^+}^{\text{ex}}(S_2)] - [\mu_{Na^+}^{\text{ex}}(aq) - \mu_{K^+}^{\text{ex}}(aq)] \]
\[ \approx 6 \, \text{kcal/mol}. \quad (1) \]

Here $\Delta \mu_{\text{ex}}$ is the free energy of transferring $Na^+$ from the bulk to the $S_2$ site relative to the same quantity for $K^+$. $\mu_{\text{ex}}$ is the excess chemical potential of the ion; $\mu_{\text{ex}}^{\text{aq}}(aq)$ ($X = K^+, Na^+$) is the hydration free energy and $\mu_{\text{ex}}^{\text{aq}}(S)$ is the analogous quantity in the $S_2$ site.

A. Inferring selectivity from the distribution of coordination states of the aqua-ions

In an effort to rationalize the selectivity of the channel, Bostick and Brooks \cite{2} suggest that the selectivity of the $S_2$ site can be understood based on the probabilities of observing specified $n$-coordinate structures ($X[H_2O]_n)$ of the aqua-ion in bulk water. (The identification of a particular $n$-cluster requires the specification of a coordination sphere around the aqua-ion.) The idea is that the lower probability of observing the eight-coordinate state of $Na^+(aq)$ relative to $K^+(aq)$ indicates a higher free energy change involved in enforcing an eight coordinate state around $Na^+(aq)$ relative to $K^+(aq)$. But the $S_2$ site also provides an eight-ligand site, albeit the ligand is a carbonyl and not water. But the behavior in water itself suggests that the “control of the permeant ion’s coordination state” \cite{3} is the basis of selectivity.

Bostick and Brooks \cite{2} seek $\Delta \mu_{\text{ex}} = \Delta \mu_{\text{ex}}(n) - \Delta \mu_{\text{ex}}(aq)$ (Fig. 1, and they express this in terms of the free energy change in restricting the coordination state of the aqua-ion to $n$. Thus
\[ \Delta \mu_{\text{ex}} = \Delta \mu_{\text{ex}}(n) - \Delta \mu_{\text{ex}}(aq) \]
\[ = \Delta \mu_{Na^+}^{\text{ex}}(n, \lambda_{Na^+}) - \Delta \mu_{K^+}^{\text{ex}}(n, \lambda_{K^+}), \quad (2) \]

where $\lambda_X$ is the coordination radius of ion $X$ and $n$ is the coordination number under consideration. ($n = 8$ is used for inferring the selectivity of the channel \cite{4}). Eq. 2 is a thermodynamic truism.
serving the $n$-coordinate state, $x_n(n)$, that the earlier analysis is deficient. In particular,

$$\Delta \mu^\text{ex}(\text{aq} \rightarrow n)' = -k_B T \ln \frac{x_{\text{Na}^+}(n, \lambda_{\text{Na}^+})}{x_{K^+}(n, \lambda_{K^+})} \quad (3)$$

is erroneously equated to $\Delta \mu^\text{ex}$ of Eq. 2 (cf. Eq. 10 in Supplementary Information to Ref. [4]); the prime on $\Delta \mu^\text{ex}(\text{aq} \rightarrow n)'$ is to note this error. Eq. (3) implies that (to within an additivity constant that is the same for both $K^+$ and $Na^+$ and without loss of generality taken equal to zero),

$$\Delta \mu^\text{ex}_X(n, \lambda_X) = -k_B T \ln x_n(n, \lambda_X). \quad (4)$$

It is rather surprising that the right hand side of Eq. 4 is always positive but there is no obvious physical reason that the left hand side of Eq. 4 must always be positive. Further since $\sum_n x_n(n, \lambda_X) = 1$, Eq. 4 leads to

$$e^{-\beta \mu^\text{ex}_X(n, \lambda_X)} = \sum_n e^{-\beta \mu^\text{ex}_X(n, \lambda_X)}. \quad (5)$$

If instead of the transition between the aqua-ion to the $n$-coordinate state of the ion, the transition between the most probable coordination state of the ion ($\tilde{n}$) to the $n$-coordinate state is considered, Eq. 3 becomes

$$\Delta \mu^\text{ex}(\tilde{n} \rightarrow n)' = -k_B T \ln \frac{x_{\text{Na}^+}(n, \lambda_{\text{Na}^+})}{x_{\text{Na}^+}(\tilde{n}, \lambda_{\text{Na}^+})} + k_B T \ln \frac{x_{K^+}(n, \lambda_{K^+})}{x_{K^+}(\tilde{n}, \lambda_{K^+})}, \quad (6)$$

where different values of $\tilde{n}$ are implied for $Na^+$ and $K^+$.

In the earlier study (Fig. 3B, Ref. [4]) the “most popular coordinated states” were indicated on the free energy landscape (at a free energy value near zero) on which the $n = 8$ coordinate case was also noted. Based on this, earlier we had concluded that Bostick and Brooks had used Eq. 6 when in fact they were using Eq. 3.

**Results using Eqs. 3 and 6.** Fig. 2 collects the results for estimates of the selectivity using Eqs. 3 and 6 and the corrected versions of these equations (see below). Eqs. 3 and 6 lead to an estimate of selectivity about 2 kcal/mol higher than the lower bound of selectivity ($\approx 3$ kcal/mol [4]). Observe that Eq. 6 leads to an estimate not much different from that based on Eq. 3. This is consistent with the observation [5] that in the description of the hydration free energy of the ion in terms of its various coordination states (and a correction for long-range interactions), only coordination states with $n \leq \tilde{n}$ contribute dominantly to the thermodynamics. Since for $Na^+$, $\tilde{n} = 6$ ($\lambda_{\text{Na}^+} = 3.1$ Å), and for $K^+$, $\tilde{n} = 7$ ($\lambda_{K^+} = 3.5$ Å), it is then reasonable to expect that Eqs. 3 and 6 give similar estimates.

**II. DENSITY FLUCTUATIONS AND $\Delta \mu^\text{ex}_X(n, \lambda_X)$**

We first note the following identities that readily follow from a multi-state generalization of the potential distri-

\[ \Delta \mu^\text{ex} = \frac{-k_B T}{p(n, \lambda_X)} \frac{x_{\text{Na}^+}(n, \lambda_{\text{Na}^+})}{x_{K^+}(n, \lambda_{K^+})} + k_B T \ln \frac{p(n, \lambda_{\text{Na}^+})}{p(n, \lambda_{K^+})}. \quad (9) \]
the observation volume in the ensemble of states (a). The free energy change in going from (a) to (b) is

\[
\Delta \mu^{\text{ex}}(\bar{n} \rightarrow n) = \mu^{\text{ex}}(n) - \mu^{\text{ex}}(\bar{n})
\]

The dashed circle around the solute denotes the coordination sphere and that in neat water is the observation volume. (a) Neat water with an observation volume that is open to the exchange of water molecules with the bulk. The arc with arrowheads on both ends represents the exchangeability of water. (b) Leaves out physical phenomena of importance in the thermodynamics of hydration, the consequences of which are apparent in Fig. 2 (the unprimed \(\Delta \mu^{\text{ex}}\) values). Including the role of density fluctuations in neat water at the length scale of the observation volume predicts a selectivity less than the suggested lower bound of selectivity. Thus control of the coordination state of the ion alone is an inadequate explanation of the selectivity of the ion channel. As suggested earlier [1, 2, 3], acknowledging the chemical difference between the coordinating ligand, water in the aqueous phase and carbonyl in the S\(_2\) site, is necessary to understand the selectivity of the ion-channel.

When \(\lambda = \lambda_{\text{Na}^+} = \lambda_{\text{K}^+}\), the second term on the right hand side of Eq. 9 is zero. In this instance Eq. 9 is the same as Eq. 3. The estimated \(\Delta \mu^{\text{ex}}\) for \(\lambda = 3.5\) Å is about 2 kcal/mol and is in fair agreement with the 1 kcal/mole estimate obtained using Monte Carlo simulations [3]. Most importantly, both these estimates are significantly different from the approximately 5 kcal/mol obtained using Eq. 3 with \(\lambda_{\text{Na}^+} = 3.1\) Å and \(\lambda_{\text{K}^+} = 3.5\) Å (Fig. 2). Thus we can understand the observation that when Eq. 3 is used with the same coordination radii, a lower selectivity is predicted. The lower selectivity arises due to the cancellation of the effects of water occupancy in an ion-free coordination volume of the same size for both \(\text{Na}^+\) and \(\text{K}^+\).

Before concluding this section, we present some thoughts on where the earlier [4] development may have faltered. The coordination sphere is an open system in contact with an external bath that provides solvent molecules at a constant chemical potential, but the coordination volume and the number of particles it can hold are nowhere close to the thermodynamic limit. Thus care is required in assigning a thermodynamic potential to the partition function of the system. Further, the small-system under consideration will be sensitive to fluctuations. Hence describing the system with different ensembles may not be equivalent. Equivalence of ensembles is strictly valid only in the thermodynamic limit when relative fluctuations in the variables conjugate to those used to describe the ensemble are small. Thus, perhaps, using an open system framework to study a system whose population is fixed (Cf. Eq. 7 in Supplementary Information to Ref. [4]) may explain the disagreement with results presented here.

The present development avoids the subtleties noted above. The theoretical framework used here [3, 5, 7, 8, 9, 10] is grounded in well-established statistical mechanics, and the results (for example, Fig. 3) agree with physical reasoning.

### III. CONCLUSIONS

The probability of observing a given number of water molecules in neat water in an ion-free coordination volume codifies density fluctuations in the liquid at the scale of the coordination volume. Including this effect suggests that free energy changes involved in modifying the coordination state of an ion in water [4] is inadequate in explaining the ion selectivity in the ion channel.

### APPENDIX A: METHODS

NVT simulations of neat water and of \(\text{Na}^+\) and \(\text{K}^+\) [12] in water were conducted using the NAMD program [13]. Liquid water was described using the TIP3P [14] model. A temperature of 298 K was maintained using a Langevin thermostat. The cubic simulation system comprises 306 water molecules for the pure water simulation; the ion-water system comprises an additional ion. The ion is always held fixed at the center of the simulation cell. The total number density (counting water molecules and the ion, if present) is 33.33 nm\(^{-3}\). For the ion water system, after over 10 ns of equilibration data was collected for 16 ns. Configurations were saved every 100 fs for further analysis. For coordination radii from 3.1 Å to 3.5 Å,
the distribution of water molecules in the coordination shell of the ion was calculated. An extensively equilibrated box of water molecules was further equilibrated for 100 ps. Configurations were saved every 250 fs during the course of a production run lasting 1.996 ns. For each configuration, observation volumes of radii between 3.1 Å to 3.5 Å were centered on 2197 points arranged in a cubic lattice within the simulation cell and the distribution of water molecules calculated.

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