

A minimalist model for ion partitioning and competition in a K⁺ channel selectivity filter

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In the recent collection of articles in Perspectives on: Ion selectivity, several authors summarized the current state of knowledge on ion channel selectivity, predominated by experimental and theoretical approaches to understanding K⁺/Na⁺ discrimination of passive ion channels. Considerable insight has been gained by complementary analyses of crystal structures as well as by molecular simulations of models ranging from fully atomistic scenarios to simple “toys” that represent essential, localizable features of the overall phenomenon. On the theoretical side, much emphasis has been put on the thermodynamic aspect of selectivity, namely modeling and understanding the free energy change associated with K⁺/Na⁺ exchange in the selectivity filter of K⁺ channels. Numerous studies have convincingly shown (e.g., Dixit and Asthagiri, 2011; Roux et al., 2011; Varma et al., 2011) that thermodynamic selectivity results from a complex interplay between the chemical nature and number of coordinating ligands and the restricted inherent local atomic flexibility. However, both experimental (kinetic and structural analyses) and computational studies (multi-ion free energy surfaces) are directed toward understanding the ionic interplay and competition in the selectivity filter of K⁺ channels that give rise to complex kinetic features that are equally important signatures of ion selectivity (Alam and Jiang, 2011; Nimigean and Allen, 2011). As emphasized in the Perspective’s editorial (Andersen, 2011), further progress relies on converging thermodynamic (single ion) and kinetic (multi-ion) approaches into a unifying picture. The success of the latter depends strongly on the adequate treatment of physiological reference states. These are (in contrast to infinite dilution states, often implicitly assumed in free energy simulations) characterized by finite ion concentrations as needed for kinetic modeling, allowing for direct ion competition as a result of partitioning.

Theoretical progress can be made by methodologies that are capable of predicting ion concentration profiles. Here, we apply the 3-D reference interaction site

model (RISM) integral equation theory (Beglov and Roux, 1997; Kovalenko and Hirata, 1998), adapted to the special case of a charged solute immersed in a salt solution (Kloss and Kast, 2008), to a truncated fragment comprising the selectivity filter of the KcsA channel in the conducting conformation (Zhou et al., 2001). The 3-D RISM approach is an approximate equilibrium solvation theory that retains the molecular detail of the solvent and that is therefore in principle capable of ion size discrimination on the basis of molecular force fields. Although it has well-known shortcomings in its established form, such as the neglect of intramolecular solute flexibility, it is nevertheless highly valuable for addressing problems that are difficult to study by molecular simulation, such as the partitioning and ion competition problem. We can turn the disadvantages into advantages by studying a sequence of progressively simpler, toy-like models that ultimately reduce the protein environment to the essential, minimal features that still exhibit one specific property, in our case the selectivity as measured by ratios of partition constants. By examining the solvent response of a simplified filter to pure monocationic K⁺ and Na⁺ solutions on one hand in comparison with the effect of a bicationic K⁺/Na⁺ mixture on the other hand, we are entitled to compare the relative trends of the reduction sequence, although the absolute numbers are of course only qualitatively meaningful. In particular, the 3-D RISM approach provides hints and answers to the following questions: (1) How different is the thermodynamic selectivity in comparison with a measure based on concentration ratios? (2) How important is the chemical composition of the residues for this measure? (3) Is the presence of more than one filter binding site relevant for the partitioning selectivity?

We apply two variants of the 3-D RISM theory, the finite concentration solute–solvent (*uv*) equations in conjunction with the hypernetted chain (HNC) closure

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and the infinite dilution HNC solute–solute (*uu*) equation between a molecule and a single ion site partner (Kloss and Kast, 2008). The resulting pair distribution functions are then used for computing *uu* thermodynamic binding constants (K) and *uv* mass action partition constants (K_c) by integration of axial concentration profiles within the pore (Kloss, 2007). We proceed by applying both theories to a truncated tetrameric selectivity filter fragment in the conducting conformation (Protein Data Bank accession no. 1K4C) comprising residues 73–81, followed by a sequence of *uv* calculations for drastically reduced filter models composed of mere carbonyl groups enclosing the binding sites S0–S4, S1–S3, and S2 only. The results are summarized in Fig. 1.

Examining the concentration profiles, we find close correspondence with other studies. The peaks indicating a large probability of finding an ion at the respective position along the filter axis show the typical eightfold K^+ coordination (Zhou et al., 2001; Alam and Jiang, 2011) as well as preferential location of Na^+ between K^+ binding sites in the carbonyl planes (Alam and Jiang, 2011; Nimigean and Allen, 2011). The ratio of K^+ and Na^+ binding constants from *uu* calculations translates into a free energy difference for the $Na^+ \rightarrow K^+$ exchange reaction of $-3.1 \text{ kcal mol}^{-1}$ ($K = 186.4$), which is fairly in line with simulation data (Roux et al., 2011).

More interesting are the *uv* results in pure monocationic 0.1 M KCl and NaCl solution in comparison with

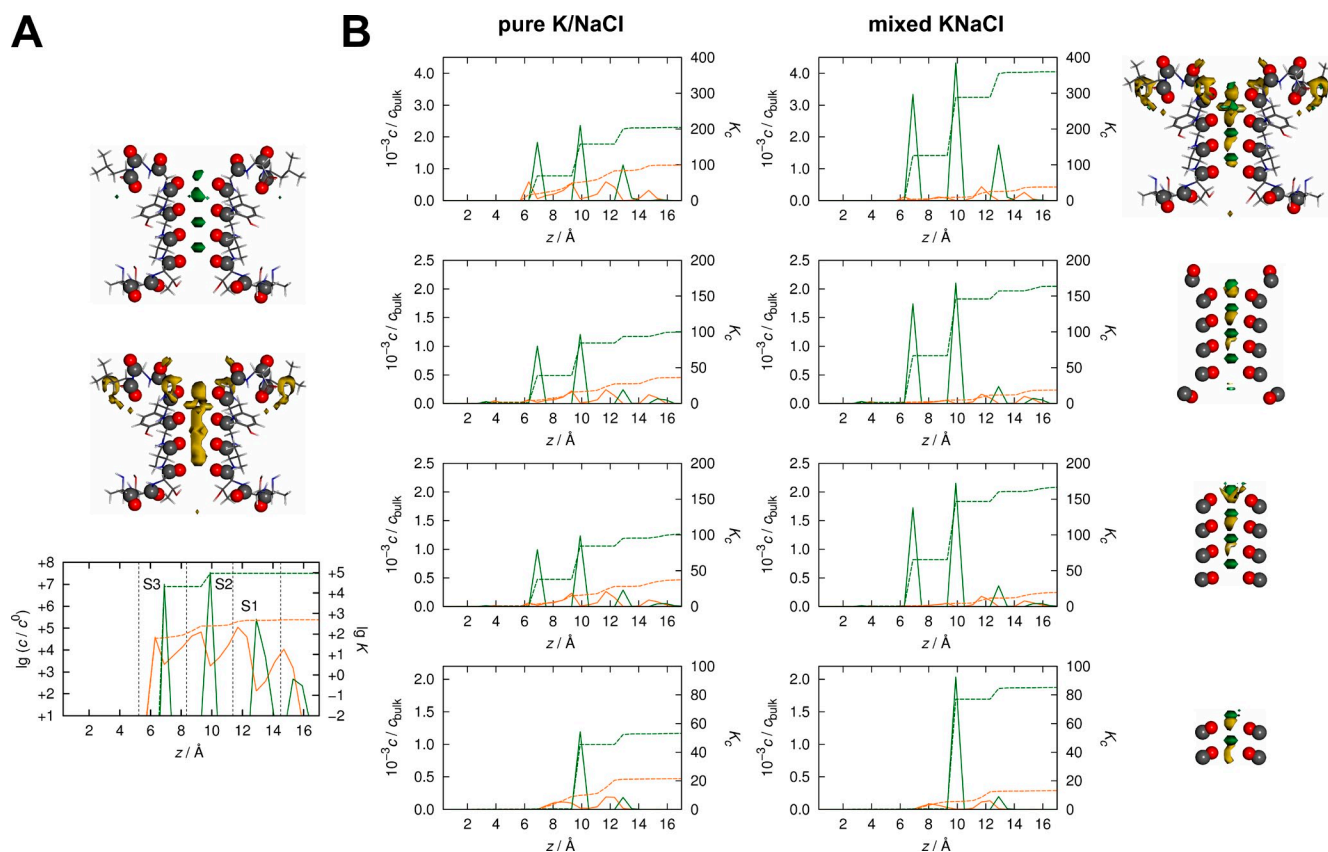


Figure 1. K^+ and Na^+ concentration profiles and isocontour surfaces of ion distributions. K^+ , green; Na^+ , yellow. (A) top, K^+ ; middle, Na^+ ; bottom, superposition of ion concentration profiles. (A and B) Results at *uu* infinite dilution conditions (A) and at *uv* finite bulk concentration (B) are depicted. (B) Profiles and their axial integral functions are shown for pure 0.1 M KCl (water density of 0.03324 \AA^{-3}) and 0.1 M NaCl (0.03327 \AA^{-3}) solutions (left) and mixed 0.05:0.05 M K/NaCl solution (middle). (right) Depictions of the corresponding structures and isocontour surfaces in mixed solution. Calculations were performed at 298.15 K on $64 \times 64 \times 64$ grids with 0.6 \AA spacing according to protocols that were described previously (Tayefeh et al., 2007). Integrals were computed over the volume generated by HOLE software (Smart et al., 1996) of 91.8 \AA^3 . The *uu* results in A and the *uv* data in the top row of B have been computed for the truncated high K^+ structure (ATTVGYGDL), which is followed in B from the second row to the bottom by results for carbonyl groups enclosing binding sites S0–S4 (TTVGYG), for carbonyl groups enclosing binding sites S1–S3 (TVGY), and for carbonyl groups enclosing binding site S2 only (VG). The largest values of the integral functions reflect the equilibrium binding constants K (A) and partitioning constants K_c (B), the ratios of which can be assigned to the equilibrium constant (A) and mass action constants (B) for the reaction $Na^+ - Ch_{aq} + K^+_{aq} \rightarrow K^+ - Ch_{aq} + Na^+_{aq}$. The infinite dilution result (A) is $K = 186.4$. The finite concentration results (B) are $K_c = 2.1$ (pure) and 9.5 (mixed) for the atomically detailed model (top), $K_c = 2.8$ (pure) and 8.8 (mixed) for the S0–S4 carbonyl group model (second row), $K_c = 2.7$ (pure) and 8.6 (mixed) for the S1–S3 model (third row), and $K_c = 2.5$ (pure) and 6.5 (mixed) for the S2 model (bottom row). Isocontour values were chosen as (*uu*) $g_{K^+} = g_{Na^+} = 800$ (A) and (*uv*) $g_{K^+} = g_{Na^+} = 80$ (B). Throughout, only two opposing subunits are shown. Structural images were created with MOLCAD software (MOLCAD GmbH).

0.05:0.05 M mixed K/NaCl. We find basically identical coordination geometries as before and irrespective of the level of reduction, whereas the selectivity measured by the K_e ratios in mixed solution differ drastically from the infinite dilution reference state, maximally reaching 9.5. This value compares well with early single-channel data and flux measurements on KcsA (Cuello et al., 1998; Meuser et al., 1999). Although this ratio stays basically the same throughout the multisite models (though absolute numbers change), it drops to some extent if only S2 is left. In contrast, the K_e ratios in monocationic solutions remain unaffected for all stages of reduction.

In summary, the relative trends observed suggest the following answers to the questions above: (1) Taking finite concentrations into account has massive impact on selectivity in comparison with infinite dilution. (2) The chemical composition of the environment plays a minor role in the partitioning selectivity as long as channels with similar average filter geometry and flexibility are compared. (3) Ionic competition emerging from in-plane Na^+ displacement by adjacent in-cage K^+ coordination is an important ingredient for understanding selectivity. This means in particular that only a multi (at least two)-site filter motif is fully capable of exhibiting K^+/Na^+ selectivity, which is in line with structural observations (Alam and Jiang, 2011). This emphasizes the importance of kinetics and the impact of competition effects for understanding the selectivity phenomenon fully.

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