The selectivity of K^+ ion channels: Testing the hypotheses – Supplementary Information

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References

[1] Bostick, D. L., and C. L. Brooks III, 2007. Selectivity in K^+ channels is due to topological control of the permeant ion's coordinated state. *Proc. Natl. Acad. Sci. U. S. A.* 104:9260–9265.

[2] Varma, S., and S. B. Rempe, 2007. Tuning ion coordination architectures to enable selective partitioning. *Biophys. J.* 93:1093–1099.

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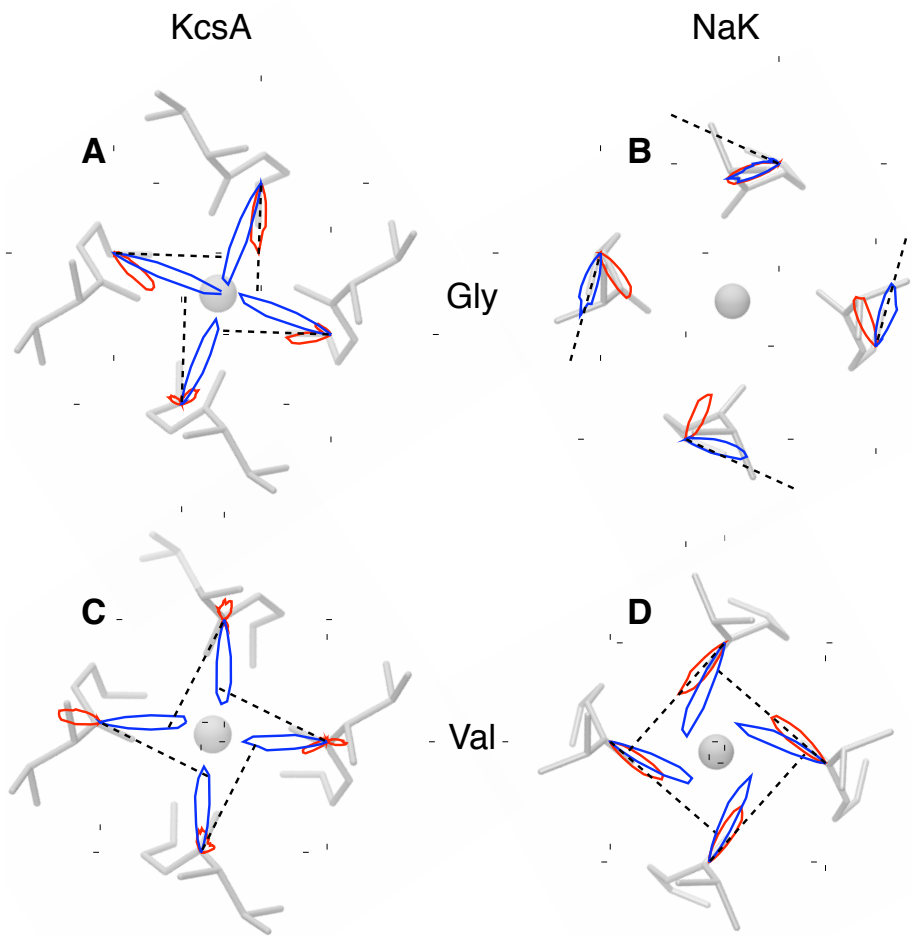

Figure S1: The polar distributions of the angles made in the plane of the lipid bilayer by the backbone carbonyl bonds from residues Val-76 (A,B) and Gly-77 (C,D) which form S2 of the selectivity filter. For clarity only 2 of the 4 K^+ bound simulations are depicted; the results for the remaining two simulations, which are similar, can be found in the main body of the paper. The dashed lines indicate the angles found in the crystallographic structures. The angular distributions from the CHARMM27 and GROMOS43a1 simulations are drawn with blue and red lines respectively. The magnitude of each curve represents the probability of that angle occurring and therefore the areas sum to unity. Note that the dynamic angular distributions have been superimposed on the static crystallographic structure.

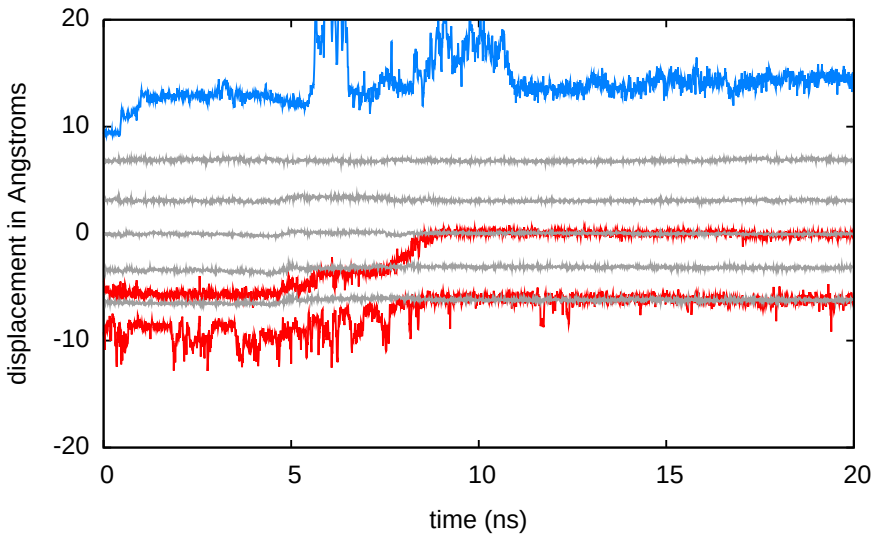

Figure S2: This is an example using the CHARMM forcefield of the motion of the ions in the selectivity filter of NaK. The displacement of the K^+ ions and the Ca^{2+} ion along the selectivity filter are drawn as red and blue lines, respectively. The positions of the centers of mass of the binding sites S0–S4 are drawn as grey lines. S4 is has the most negative displacement and S2 is centered on zero. In some K^+ bound NaK simulations this motion occurs much more quickly, sometimes during the warming, but in all cases it happens by 10 ns. Note that this motion occurs before the calcium ion unbinds from the channel.

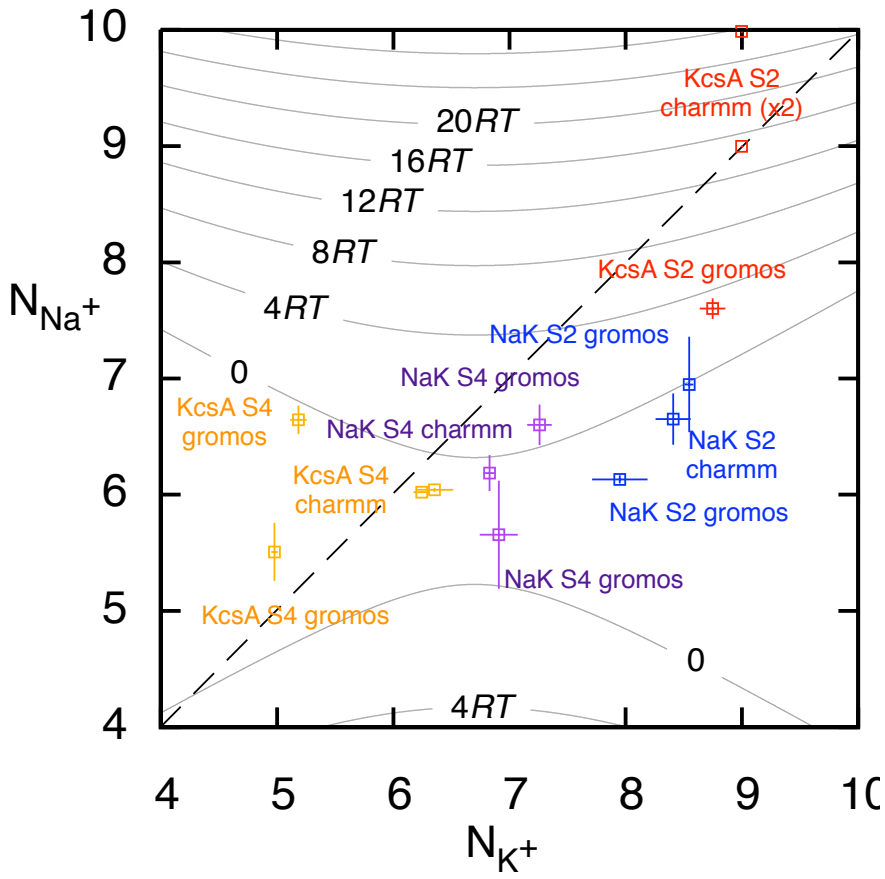

Figure S3: The average number of ligands around a K^+ ion (N_{K^+}) plotted against the number of ligands around a Na^+ ion (N_{Na^+}) in the same ion binding site. Points that do not lie on the dashed grey line therefore have different coordination numbers for each bound ion. A ligand is defined as any oxygen atom within 4.0 Å of the bound ion. Superimposed on these data are the selectivity free energy contours from Fig. 5 of the Supplementary Information of the paper by Bostick and Brooks III (1). These contours, drawn as grey lines, were derived using a non-polarizable classical forcefield. *The average number of ligands around ions at sites S2 and S4 are colored red and orange for KcsA and blue and purple for NaK, respectively*. The discrete nature of the data made estimating a correlation time difficult and so the errors were conservatively produced by dividing each simulation into only three blocks. The same analysis but where a ligand is defined as any oxygen atom within 3.5 Å of the bound ion can be found in the main body of the paper.

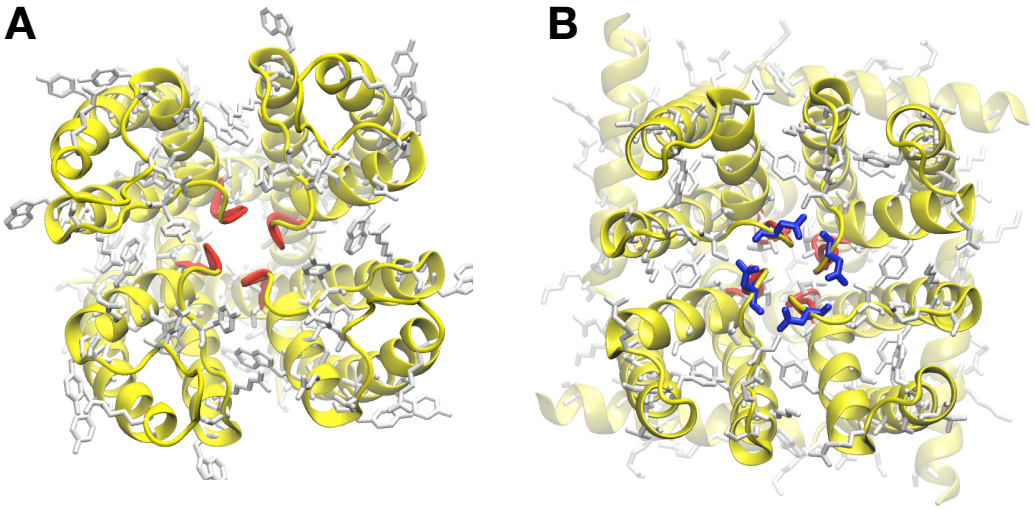

Figure S4: There are no amino acids with sidechains capable of donating a hydrogen within 5 Å of the selectivity filter carbonyl oxygens in the high-resolution structure of KcsA (PDB:1K4C, labelled A). For NaK (PDB:2AHZ, labelled B) there is a single amino acid within 5 Å(Asn-80, colored blue) but this is directly next to the last glycine of the selectivity filter so is unlikely to be able to interact directly with the carbonyl oxygens. Those with hydrogen bond donating groups greater than 5 Å from the carbonyl oxygens are colored white. For reference the backbones of the selectivity filter and the remainder of the protein are colored red and yellow respectively. For similar figures of other K^+ ion channels see the Supplementary Information of the paper by Varma and Rempe (2).

Table S-1: Selectivity filter RMSDs.

		KcsA			NaK		
		1K4C	*gromos*	*charmm*	*2AHZ*	*gromos*	*charmm*
KcsA	*1K4C*	–	1.84 ± 0.06	1.85 ± 0.04	4.26	4.21 ± 0.06	4.38 ± 0.06
	gromos	1.65 ± 0.05	–	1.52 ± 0.14	4.61 ± 0.16	4.20 ± 0.16	4.48 ± 0.18
	charmm	1.68 ± 0.01	1.27 ± 0.12	–	4.46 ± 0.05	4.14 ± 0.08	4.28 ± 0.08
NaK	*2AHZ*	1.48	2.05 ± 0.04	1.95 ± 0.01	–	1.96 ± 0.05	1.89 ± 0.04
	gromos	1.77 ± 0.04	1.37 ± 0.10	1.08 ± 0.10	1.72 ± 0.06	–	1.42 ± 0.07
	charmm	1.86 ± 0.02	1.60 ± 0.06	1.14 ± 0.07	1.70 ± 0.04	1.18 ± 0.10	–

The RMSD values in Angstroms of the monomer and tetramer selectivity filter between the K^+ bound KcsA and NaK simulations and crystallographic structures. Conformations of the selectivity filter from the simulations were fitted onto a variety of experimental structures and other simulations and the backbone RMSD (including the Cβ atoms, if present) was calculated. This was done both for the set of four monomers that make up the selectivity filter and for each monomer separately. The values for the whole tetramer and the individual monomers of the ion channels are shown above and below the diagonal, respectively. Each monomer was assumed to contribute one independent measurement of the RMSD and the errors were then calculated in the usual way. For clarity only two of the four K^+ bound simulations are compared; the results for the other two can be found in the main body paper.

Table S-2: The average number of coordinating ligands around S2 and S4.

			total		carbonyl		water	
			K^+	Na^+	K^+	Na^+	K^+	Na^+
S2	KcsA	*charmm*	8.7 ± 0.1	9.3 ± 0.1	8.0 ± 0.1	8.0 ± 0.1	0.7 ± 0.1	1.4 ± 0.1
	NaK	*gromos*	7.7 ± 0.1	6.1 ± 0.1	7.7 ± 0.1	4.2 ± 0.1	0.0 ± 0.1	1.9 ± 0.1
S4	KcsA	*gromos*	4.8 ± 0.1	6.1 ± 0.1	3.8 ± 0.1	4.1 ± 0.1	0.9 ± 0.1	2.0 ± 0.1
		charmm	6.1 ± 0.1	6.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	2.1 ± 0.1	2.0 ± 0.1
	NaK	*gromos*	6.4 ± 0.1	4.9 ± 0.3	3.9 ± 0.1	0.4 ± 0.3	2.5 ± 0.1	4.5 ± 0.4

A ligand is defined as any oxygen atom within 3.5Å of the relevant ion bound at either S2 or S4. For clarity only two of the four K^+ bound simulations are compared; the results for the other two can be found in the main body of the paper.

Table S-3: The average number of hydrogen bonds between parts of the selectivity filters of KcsA and NaK and the remainder of the protein.

	KcsA		NaK	
	donor	acceptor	donor	acceptor
All atoms	12.9 ± 1.3	2.2 ± 0.6	5.9 ± 1.1	12.3 ± 1.6
Backbone only	6.8 ± 0.9	0.7 ± 0.4	5.0 ± 1.0	0.5 ± 0.3
Tyr/Asp-78 sidechain	3.4 ± 0.5	1.4 ± 0.5	0.0 ± 0.1	11.8 ± 1.6
Thr-75 sidechain	2.6 ± 0.5	0.1 ± 0.1	0.8 ± 0.4	0.0 ± 0.1

The number of hydrogen bonds was counted for each of the seven K^+ bound simulations for KcsA and NaK for the last 5 ns of each trajectory. The data were then binned using a correlation time of 500 ps and errors calculated in the usual way. The average was then taken and the error estimated by adding the individual errors in quadrature. In all cases Thr-75 only formed hydrogen bonds with Thr-74. Note that this data includes a small number of carbonyl flips and we are therefore assuming that their presence does not significantly affect the results. A hydrogen bond is assumed to have formed if the distance between the donor and acceptor atoms is $<$ 0.35 nm and the angular deviation is $< 30\ ^\circ$.