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Supplemental Data

The RCK Domain of the KtrAB K^+ Transporter: Multiple Conformations of an Octameric Ring

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Supplemental References

Krebs, W.G., and Gerstein, M. (2000). The morph server: a standardized system for analyzing and visualizing macromolecular motions in a database framework. Nucleic Acids Res *28*: 1665-75.

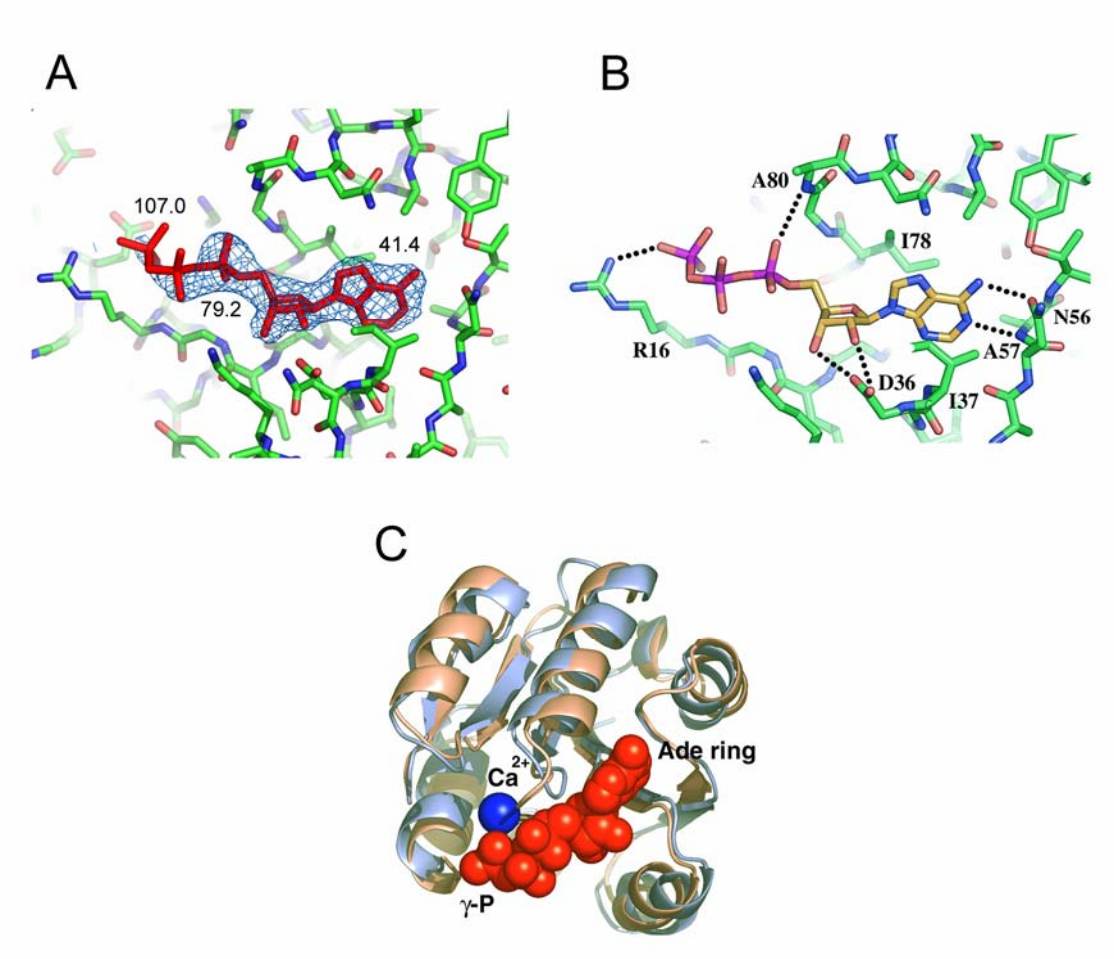

Figure S1. Ligand Binding

(A) Simulated annealing omit electron-density map of the ATP-γS molecule (in red) in the diamond octamer ring contoured at 1 sigma. As in Figure 2A of the main text, average temperature-factors for different parts of the ligand are shown.

(B) Stick representation of the KtrAB RCK domain ligand binding site with ATP-γS. Hydrogen bond interactions between ligand molecule and protein atoms are shown as dotted lines. Protein residues involved in the interactions are marked. Specificity for the adenine-end of the ligand is achieved via Watson-Crick-like interactions with the main chain nitrogen of Ala57 and the side chain oxygen of Asn56.

(C) Superimposition of RCK domains from KtrAB (pink) and MthK (blue) (Cα atom rmsd ~ 1.3 Å) viewed into the ligand binding site. The calcium ion of MthK is shown as a blue sphere and the ATP of KtrAB is shown as a red CPK molecule.

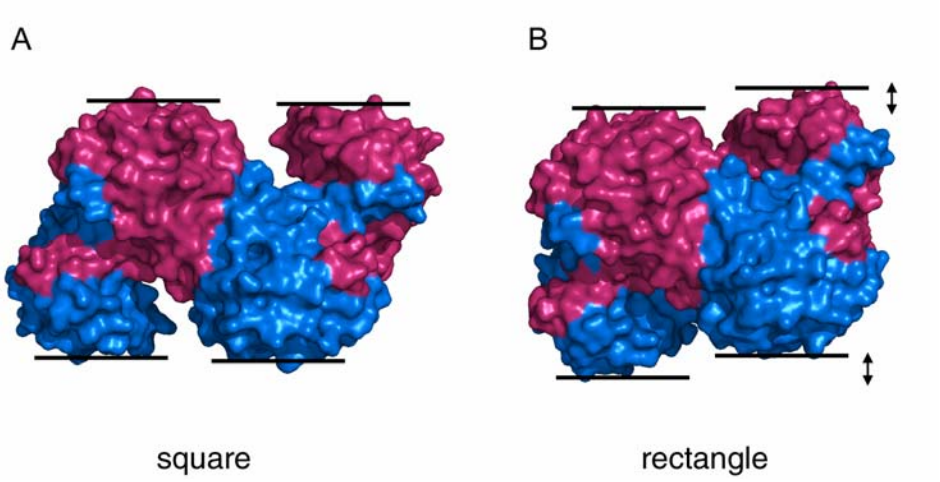

Figure S2. Topology Changes in the Ring Surface

Side views of the (A) square and (B) rectangular rings along the axis of the fixed interface. Rings are colored by chain. For clarity only the half of the ring nearest the reader is shown. In the square, the height of the four domains comprising each face of the ring are almost equal. Subunit rotations leading to the rectangular shape result in a more uneven surface along each face of the ring with some domains now protruding ~6 Å further than others.

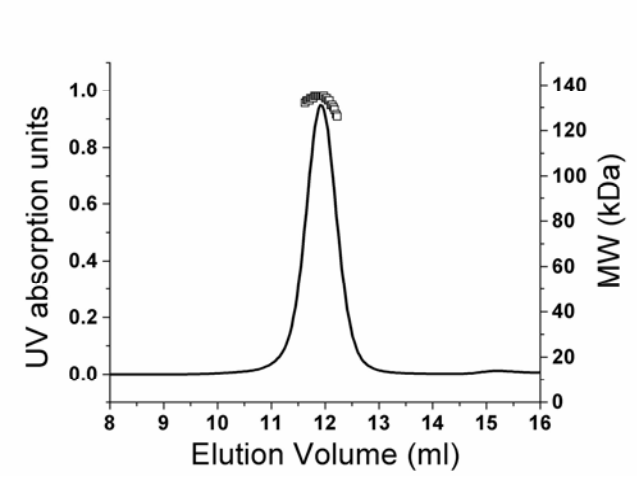

Figure S3. Molecular Weight Determination for the Truncated RCK Domain of the *M. jannaschii* TrkA Protein

Light scattering was performed in combination with size exclusion chromatography. As described in methods, the molecular weights as sampled along the protein elution peak are shown above the UV profile.

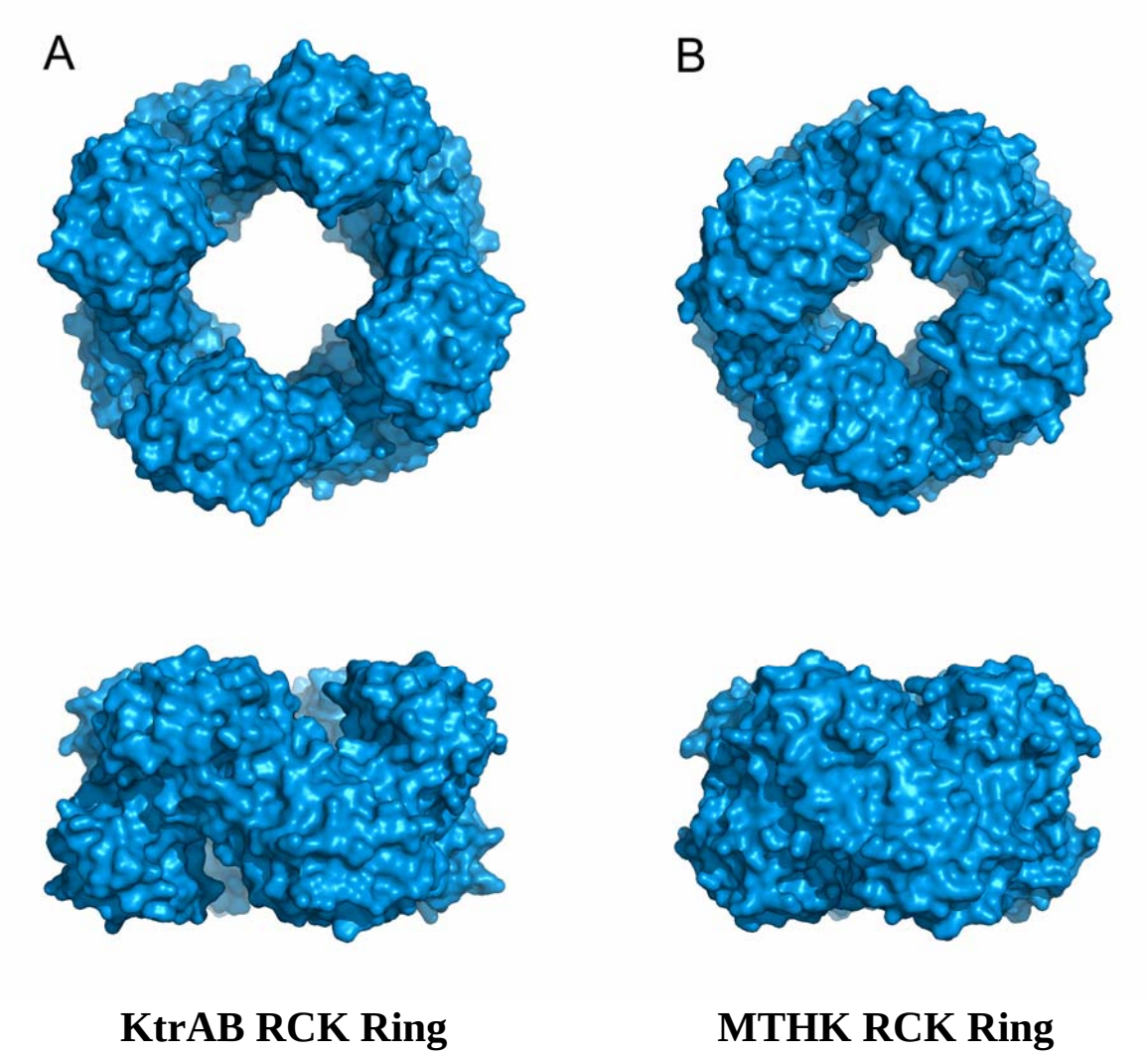

Figure S4. Comparison of the Size of the RCK Rings from KtrAB and MthK

Top and side views of the rings are shown for (A) KtrAB and (B) MthK with the peripheral C-terminal domain removed. It is noteworthy that this ligand-bound MthK ring is in its most-expanded form, shown to correlate to channel opening. Nevertheless, the KtrAB RCK ring is significantly larger in diameter with deep, wide grooves between domains. Both rings are approximately 48 Å thick (or high).

Table S1. Crystallographic Data

	Ring Structure Conformation/Bound Ligand				
	Diamond/NAD(H)	**Diamond/ATPγS**	**Diamond/ADP**	**Rectangle/NAD(H)**	**Square/ATP**
Space group	P422	P422	P422	$P3_112$	I422
Unit cell (Å)	a = 127.41 b = 127.41 c = 50.87	a = 127.67 b = 127.67 c = 51.88	a = 127.46 b = 127.46 c = 51.40	a = 164.88 b = 164.88 c = 57.24 γ = 120º	a = 126.28 b = 126.28 c = 98.61
Resolution (Å)	50 – 2.2	50 – 2.25	50 – 2.2	50 – 2.7	50 – 3.0
Rsym[a]	0.107	0.099	0.094	0.097	0.100
No. reflections observed (%completeness)	21,282 (96.5)	20,614 (97.4)	21,918 (97.6)	24,578 (99.9)	8,231 (99.7)
Redundancy	7.2	8.6	8.9	10.8	9.9
I/σI (highest bin)	12.5 (2.6)	14.8 (2.2)	17.0 (2.8)	23.5 (4.2)	19.6 (3.7)
Monomers/asu	2	2	2	4	2
Total atoms	2,407	2,325	2,333	4,455	2,191
Protein + ligand	2,233	2,207	2,199	4,428	2,191
Solvent	174	118	134	27	0
Rwork (R_{free}[b])	0.232 (0.258)	0.228 (0.245)	0.228 (0.245)	0.214 (0.249)	0.264 (0.303)
Rmsd bonds (Å)	0.007	0.007	0.007	0.007	0.010
Rmsd angles (º)	1.27	1.20	1.21	1.23	1.31
PDB ID	2HMT	2HMU	2HMV	2HMS	2HMW

[a]$\Sigma \mid I - \langle I \rangle \mid / \Sigma (I)$.
[b]Based on a random 10% of reflections.