Supplemental Information

Electron spin echo envelope modulation (ESEEM) reveals water and phosphate interactions with the KcsA potassium channel

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Table S1. Data collection and refinement statistics for KcsA V48R1.

	KcsA V48R1
Data collection	
Wavelength	1.03 Å
Space group	C2
Cell dimensions *a*, b, *c* (Å), β	130.97, 76.63, 112.97, 125.83
Resolution (Å)	50.0-4.10 (4.21-4.10)
R_{merge} (%)	8.6 (42.1)
$I / \sigma I$	4.0 (1.7)
Completeness (%)	99.4 (99.4)
Redundancy	3.5 (3.6)
Refinement	
Effective Resolution (Å)	25.0-3.56
No. reflections	7898
R_{work} / R_{free}	27.1/30.2 (35.4/46.2)
No. atoms	2841
Protein	2818
B-factors	
Protein	66.4
R.m.s. deviations	
Bond lengths (Å)	0.055
Bond angles (°)	1.779

Figure S1

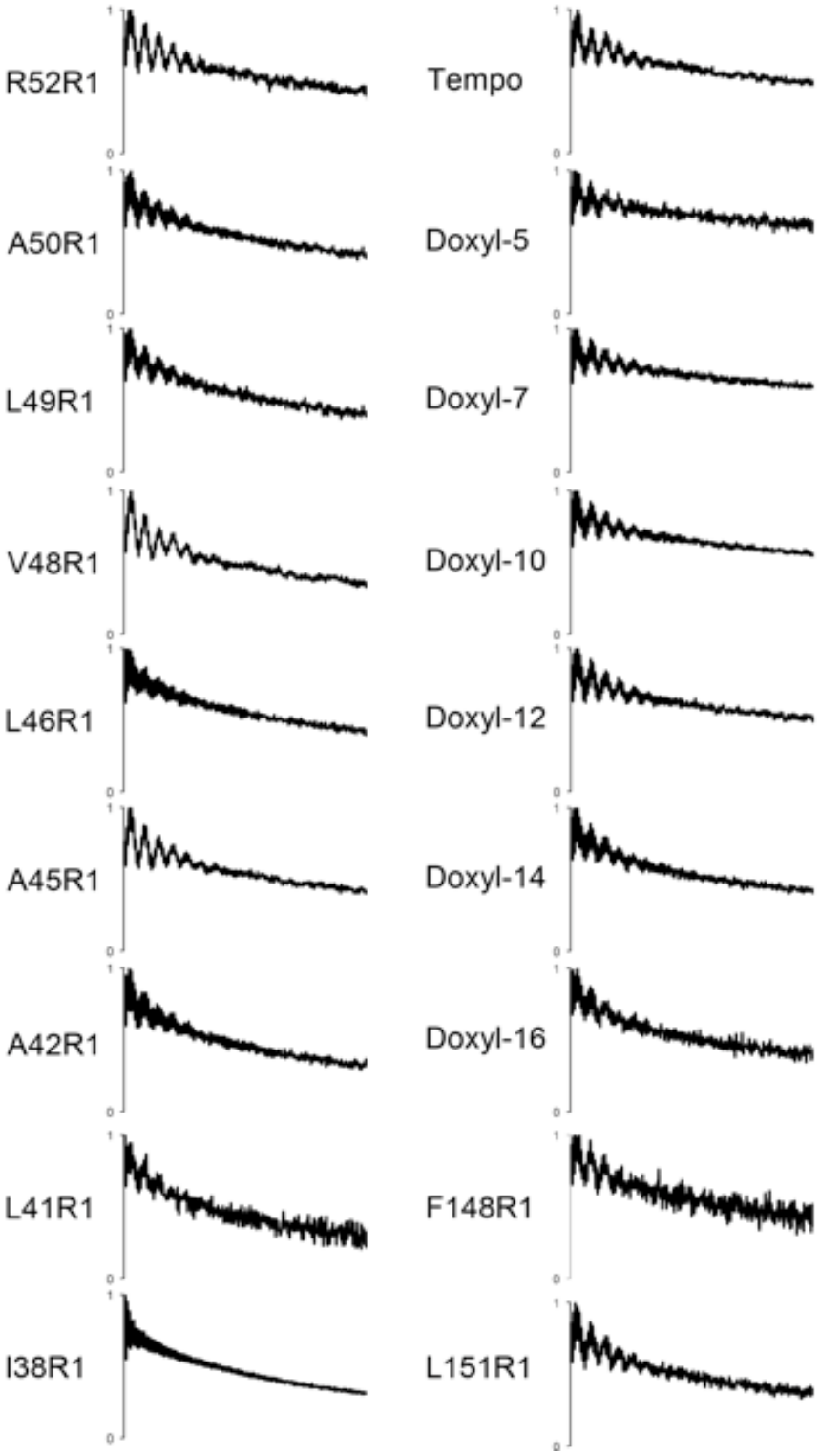

Figure S1. Normalized raw ESEEM data.

Figure S2

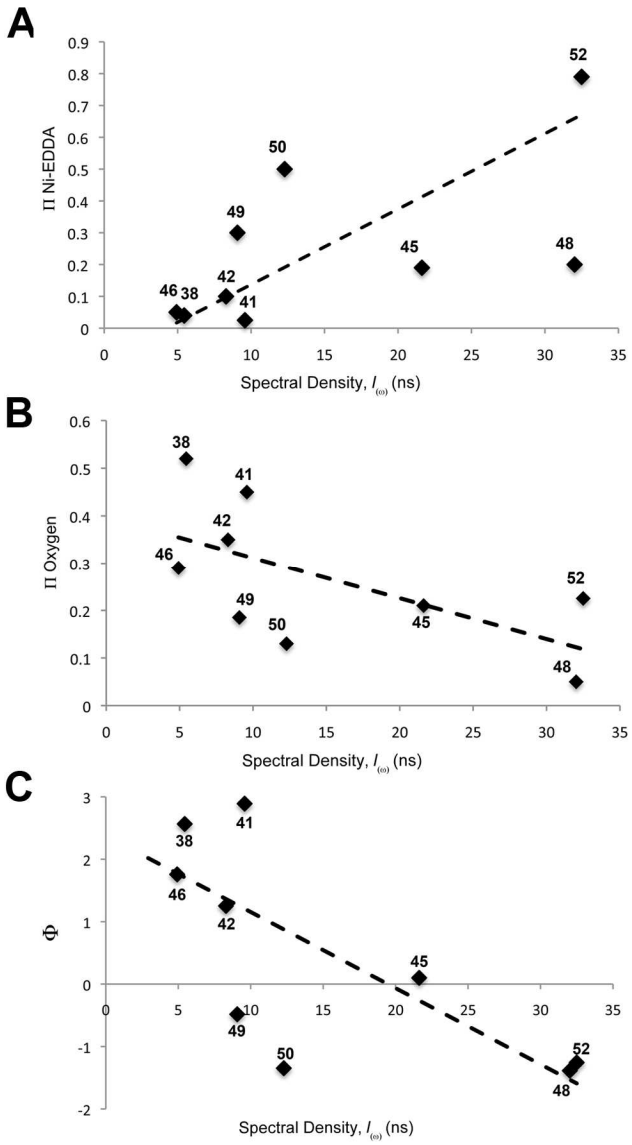

Figure S2. Correlation between collision (*J*) and deuterium ESEEM data. (**A**) NiEDDA vs deuterium ESEEM data. The dashed line is a linear regression to the data (R^2=0.81). (**B**) Oxygen vs deuterium ESEEM data. The dashed line is a linear regression to the data (R^2=0.38). (**C**) Immersion depth parameter vs deuterium ESEEM data. $\Phi = \ln(\Pi(O_2) / \Pi(\text{NiEDDA}))$. The dashed line is a linear regression to the data (R^2=0.45).

Supplemental Methods

Structure determination. The high-resolution crystallization method utilizing KcsA complexed with Fab fragments (*2*) could not be used because the KcsA-V48R1-Fab complex was not sufficiently stable. Instead, the original crystallization method was followed (*3*). This method yields crystals of lower diffraction quality compared to the Fab method. Briefly, carboxy-terminal residues were removed by chymotrypsin treatment and the truncated channel was purified by gel filtration and dialyzed into the crystallization buffer (150 mM KCl, 50 mM Tris pH 7.5, 5 mM LDAO). The protein was concentrated to 5 to 10 mg/mL and crystals were grown by vapor diffusion using the sitting drop method by mixing equal volumes of protein and reservoir solution (200 mM $CaCl_2$, 150 mM KCl, 100 mM HEPES pH 7.5) with PEG 400 (19-49%) as the precipitant. Crystals were flash cooled and data were measured under a stream of nitrogen at beamline 23-ID-B (GM/CA) at the Advanced Photon Source at Argonne National Laboratory.

Diffraction data were integrated using MOSFLM and scaled using SCALA. The dataset was anisotropic, with diffraction limits of 3.9 Å, 4.3 Å, and 3.5 Å, along the a*, b*, and c* directions respectively, and initially the data were processed to 4.1 Å. Phases were obtained by molecular replacement using Phaser with the KcsA structure (PDB entry 1BL8) as the search model. The electron density maps obtained were contiguous but lacking in detail. Anisotropy correction to an effective resolution of 3.5 Å was performed through ellipsoidal truncation and anisotropic scaling of the data using the Diffraction Anisotropy Server of UCLA (*4*). The anisotropically corrected data were then used to refine the structure at 3.56 Å resolution. The resulting electron density maps contained sufficient detail to model side chain features. The model was refined by several cycles of manual rebuilding in COOT, followed by minimization and grouped B-factor refinement using REFMAC. Data collection and refinement statistics are given in Table S1.

Supplemental References

1. Perozo, E., Cortes, D. M., and Cuello, L. G. (1998) Three-dimensional architecture and gating mechanism of a K^+ channel studied by EPR spectroscopy, *Nat Struct Biol 5*, 459-469.
2. Zhou, Y., Morais-Cabral, J. H., Kaufman, A., and MacKinnon, R. (2001) Chemistry of ion coordination and hydration revealed by a K^+ channel-Fab complex at 2.0 Å resolution, *Nature 414*, 43-48.
3. Doyle, D. A., Cabral, J. M., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., and MacKinnon, R. (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity, *Science 280*, 69-77.
4. Strong, M., Sawaya, M. R., Wang, S., Phillips, M., Cascio, D., and Eisenberg, D. (2006) Toward the structural genomics of complexes: crystal structure of a PE/PPE protein complex from Mycobacterium tuberculosis, *Proc Natl Acad Sci U S A 103*, 8060-8065.