Conformational changes upon gating of KirBac1.1 into an open-activated state revealed by solid-state NMR

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Introduction

KirBac1.1 is a prokaryotic inward rectifier potassium channel (148.53 KDa) that performs the same function as eukaryotic inward-rectifier K^+ channels found in heart, kidney, liver, neural, and other cell types[1]. In order to study the structure-activity relationships underlying channel function using solid-state NMR (SSNMR), site-specific chemical shifts must be assigned. These chemical shifts report directly upon channel structure and conformational changes.

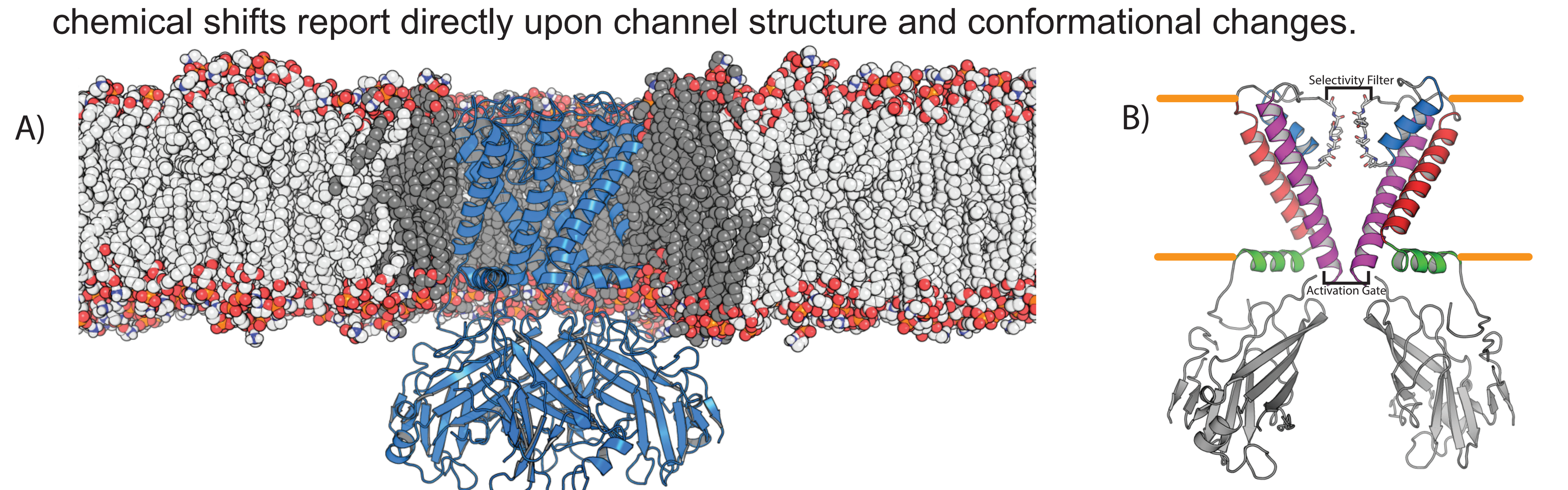


Figure 1. A) KirBac1.1 in a phospholipid bilayer B) Anatomy of kirBac1.1. Red is TM1, purple is TM2, blue is pore helix, green is slide helix

Chemical shift measurements revealed two different conformations of the transmembrane (TM) region of KirBac1.1 in 3:2 POPC:POPG proteoliposomes. The opening of the activation gate causes the extracellular turret and selectivity filter loop to pivot and introduces small kinks at glycines along the inner TM2. As TM2 twists open, it contacts multiple sites along TM1, which allows sites further along TM1 to contact the pore helix. In contrast, only the conductive state of the selectivity filter is observed in POPC bilayers.

KirBac1.1 is open and active in POPC:POPG Bilayers

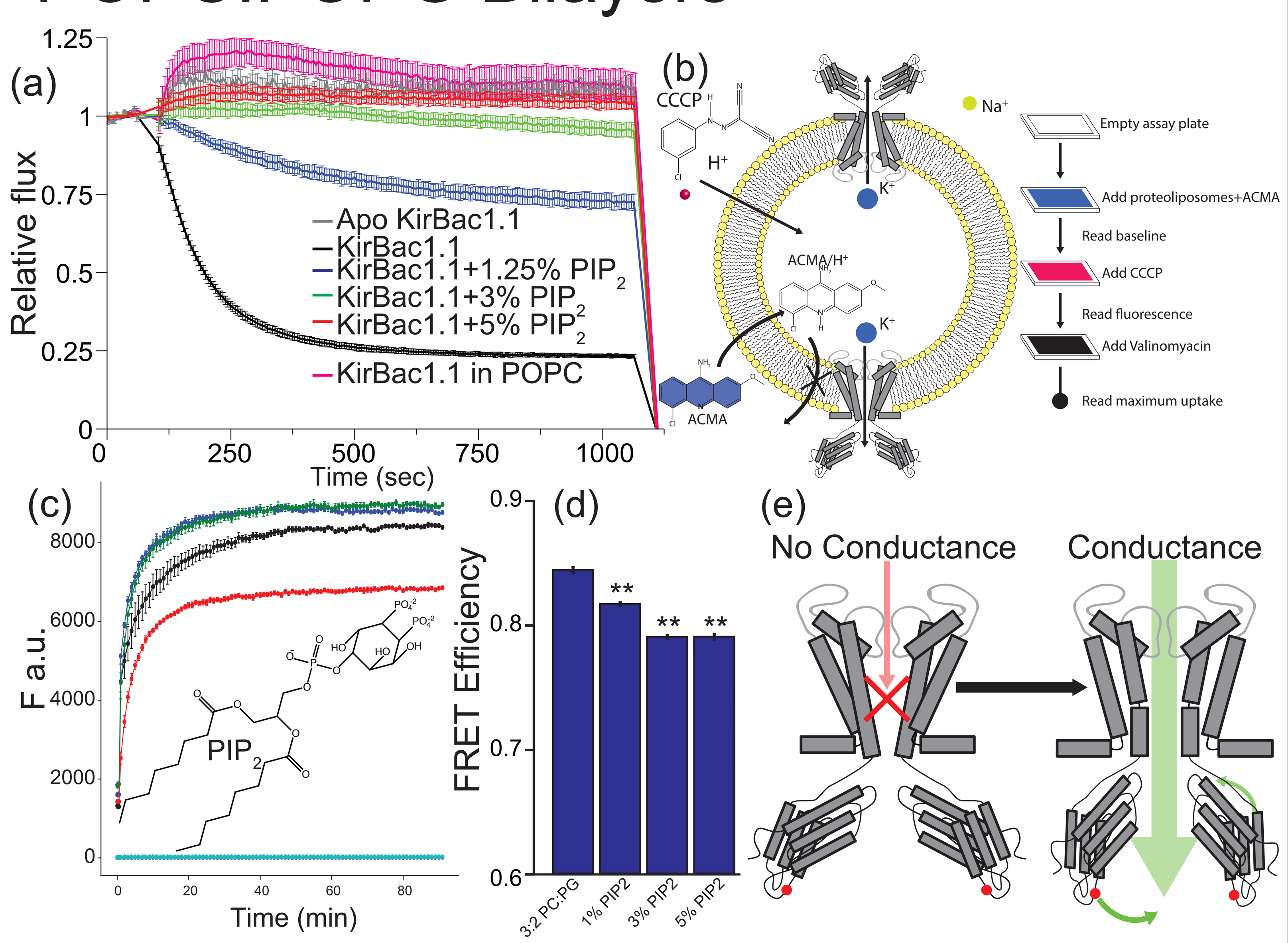


Figure 2. K^+ flux assays confirm an active channel, and FRET reveals gating motions in the C terminus upon activation. (a) Fluorescent monitoring of K^+ flux through the channel allows for real-time determination of the rate of flux. (b) Schematic outline of the K^+ fluorescence assay for K^+ flux determination. (c) Time-course FRET measurements from which F_{max} and F_0 measurements were obtained. Alexa Fluor-546 emission was monitored until we observed a maximum plateau. Samples contain A/D-labeled KirBac1.1 reconstituted into POPC:POPG in black, POPC:POPG + 1.25% phosphatidylinositol 4,5 bisphosphate (PIP_2) in blue, POPC:POPG + 3% PIP_2 in green, POPC:POPG: + 5% PIP_2 in red, POPC:POPG with nonlabeled KirBac1.1 (data are plotted behind cyan curve); POPC:POPG:PIP_2 nonlabeled in cyan. Labeled (n=6) and unlabeled (n=3) samples are reported as mean ± SEM. (Inset) chemical structure of PIP_2 (d) changes in apparent FRET (mean ± SE) upon different amounts of PIP_2 in liposome. **P < 0.05. (e) Cartoon of KirBac1.1 C-terminal domain motion previously reported to be tied to K^+ conductance, with the red mark denoting the location of G249C in the crystal structure.

SSNMR identifies conformational changes upon lipid binding

We reconstituted U-^{15}N,^{13}C-KirBac1.1 into POPC and 3:2 POPC:POPG proteoliposomes with a 1:1 lipid-to-protein ratio by mass. All 2D and 3D spectra were acquired using the Rovnyak theorem[3] for nonuniform sampling (NUS) and sampling schedules were generated using NUS-tool in NMRBox. Assignment completeness has been shown in Fig3.

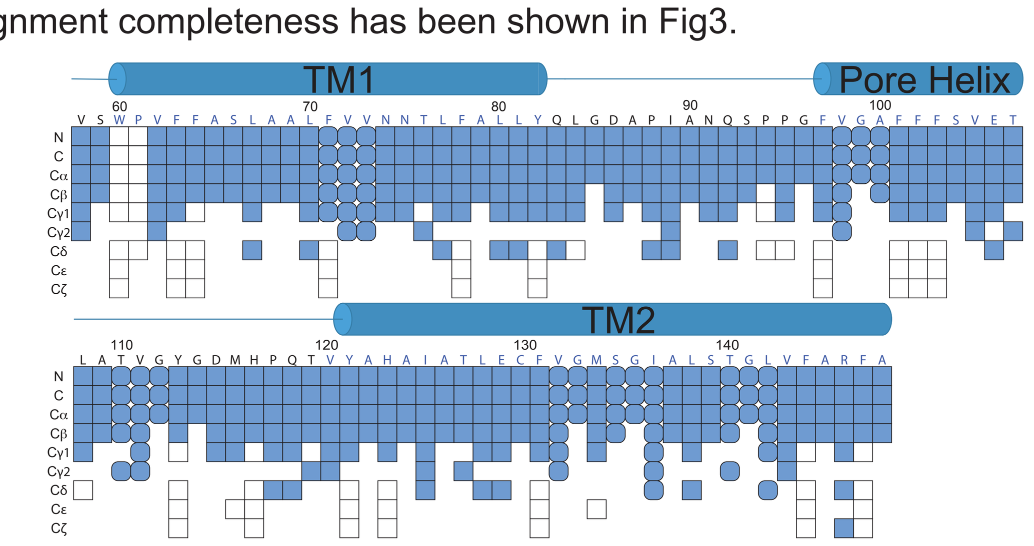


Figure 3. Assignment completeness

Chemical shift assignments of the TM region in POPC:POPG uncovered two distinct and internally consistent backbone walks. The greatest chemical shift perturbations (CSPs) were found in three VG pairs, and unique VV pair in TM1. Peaks appear as discrete states, suggesting two main conformations exchanging on a slow timescale (ms).

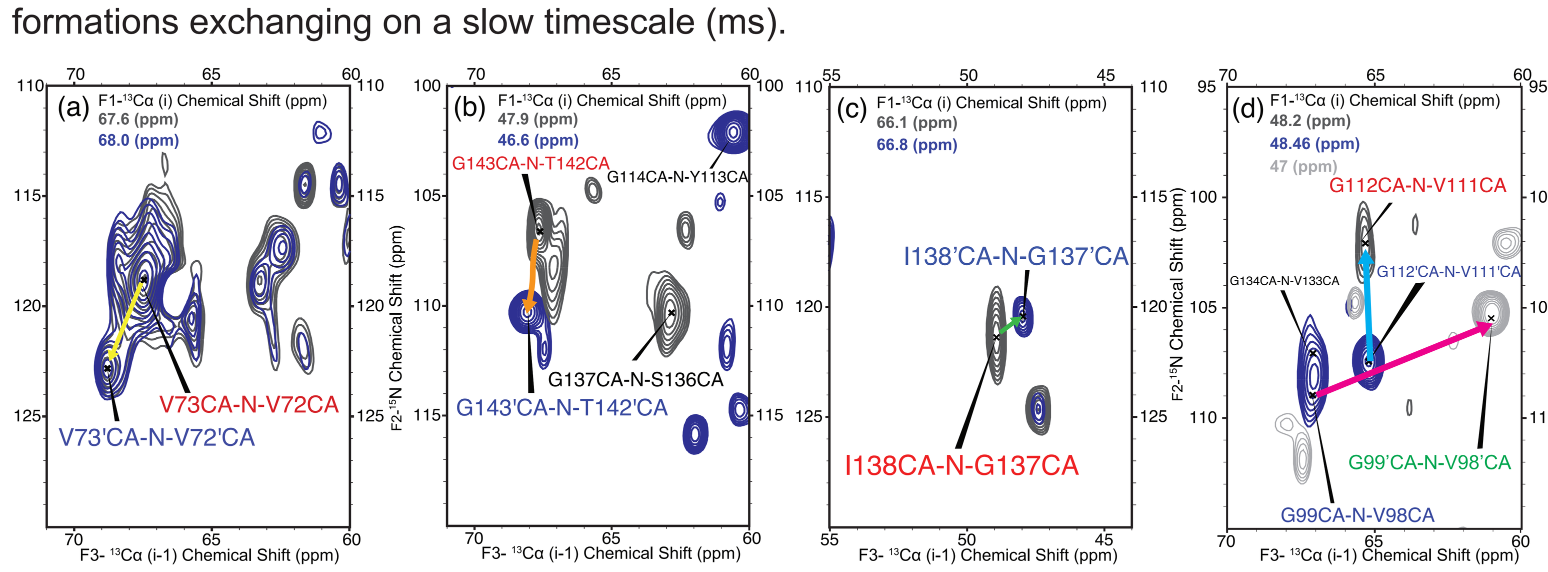
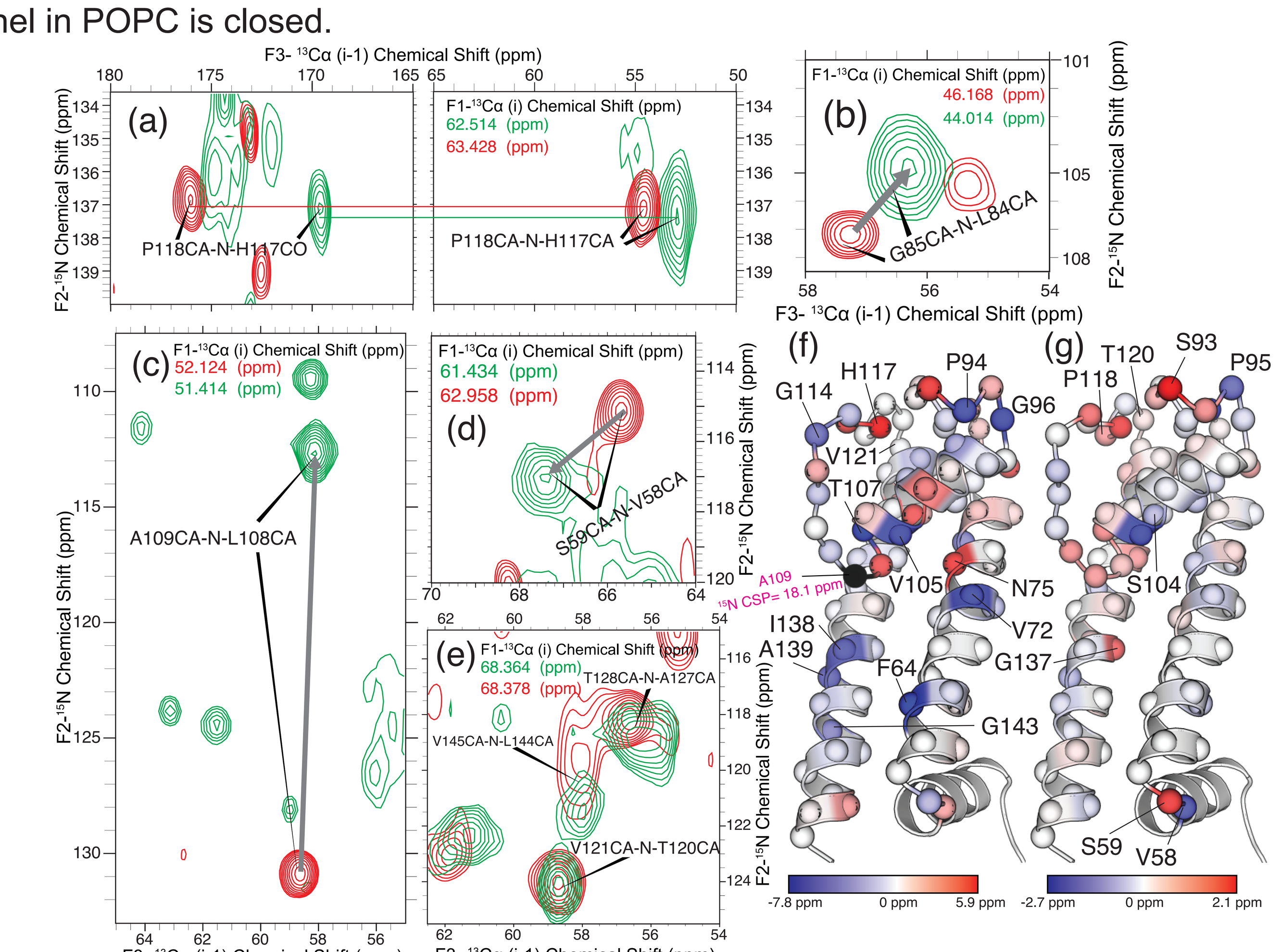


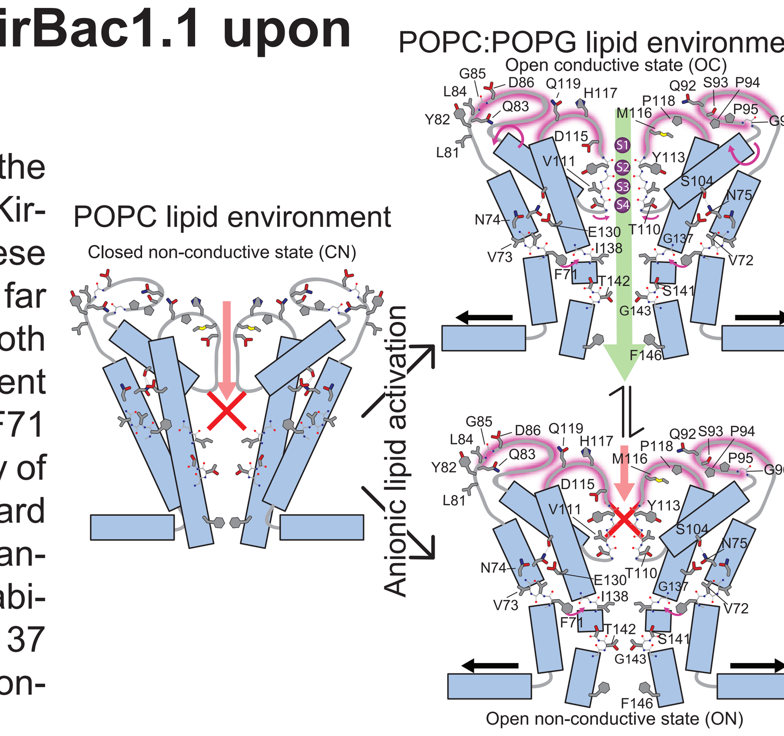
Figure 4. observed CSPs in POPC:POG sample

We also assigned the same regions of the protein in POPC liposmes. For ~80% of these residues, the chemical shifts changes are within 0.3 ppm of the assignment for POPC:POPG, confirming that the overall secondary structure of the protein is conserved. The data showed a third channel conformation in the perturbed regions. Two of the largest CSPs between the third conformer and the two purported open states are observed for A109-T110 and I138-G137. This confirmed that both observed conformers in the POPC:POPG sample correspond to an open channel, while the channel in POPC is closed.

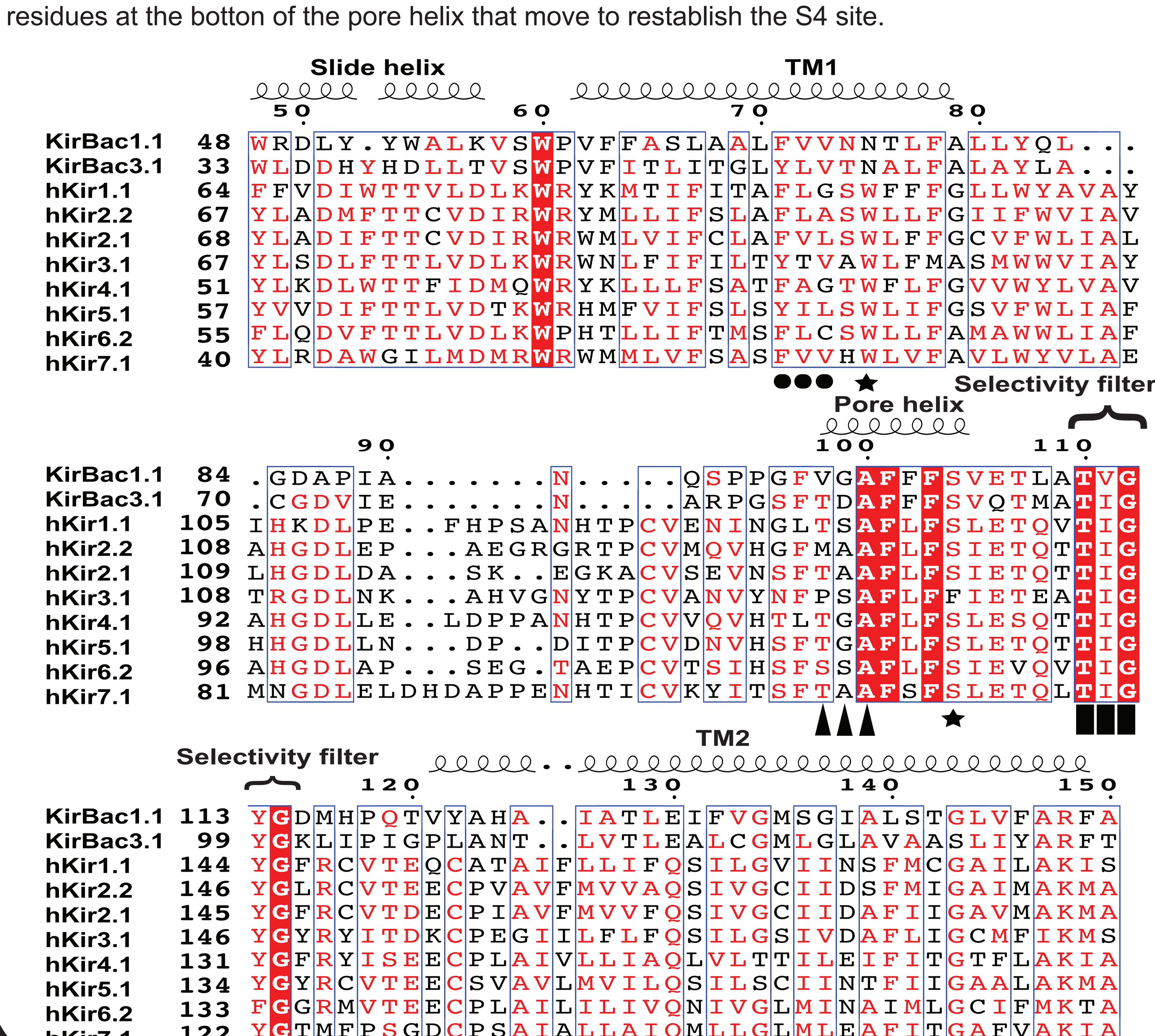


Conformation Changes of KirBac1.1 upon binding of anionic lipids

One of the largest changes is observed in the unique V72–V73 pair, the only VV pair in KirBac1.1. The phenomenological nature of these valines experiencing different conformations far from the activation gate may be a function of both conformation and lipid association. Sites adjacent to this VV pair, including F71, also shift. The F71 aromatic sidechain is oriented toward the cavity of KirBac1.1 in the crystal structure, facing toward G137 and I138. We hypothesize that, upon channel activation and gating, the F71 sidechain stabilizes the open conformation of the hinge at G137 and I138 by means of steric interaction via a concerted pivot motion from F71–V73.



Sequential alignment of KirBac1.1 shows well conserved homology between KirBac1.1 and human Kir channels. Circles denote the TM1 and TM2 hinge residues. Stars Denote the residues responsible for stabilizing rotation of the pore helix through hydrogen bonding. Triangles show residues at the start of the pore helix that are implicated in rotation of the pore helix. Rectangles are residues at the botton of the pore helix that move to restablish the S4 site.



Acknowledgments

We appreciate Colin Nichols for kindly gifting the KirBac1.1 plasmid to our lab. We would like to thank the Texas Tech Department of Chemistry and Biochemistry for material assistance and the National Institute of Health for funding support.

NIH National Institutes of Health

Funding 1R35GM124979

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