SUPPLEMENTARY INFORMATION

Methods

Modelling and Molecular Dynamics Simulations

Homology models of Kir1.1 (residues 39-369) were generated using Modeller v7.0 [1]. Sequence alignments were done using ClustalX [2] with an identity of 45%. A four-fold symmetry option was imposed during the modelling procedure. An ensemble of 25 models were generated and the best model selected on the basis of low energy function and root mean square deviation (r.m.s.d.) from the template structure. The Ca-Cα r.m.s.d. between the template and the final selected model was 1.3Å. The quality and stereochemical properties of the models were evaluated using ProCheck v3.4.4 [3]. Molecular Dynamics simulations were carried out using GROMACS v3.1.4 [4] (www.gromacs.org) and the GROMOS87 forcefield [5]. The protein molecule was embedded in Palmitoyl Oleoyl Phosphatidyl Choline (POPC) bilayer using protocol that has been described in detail by Faraldo-Gomez et al. [6]. High-resolution X-ray studies of K⁺ channels have revealed a vertical alignment of cationic charged ions in the filter region interspaced with solvent molecules (water). Positively charged K⁺ ions were positioned in the selectivity filter by using crystallographically determined distances (7,8). The system is then solvated with SPC waters (9) in a box of size 14nm³. Additional counterions were added such that the overall net charge on the system is zero. The final system consisted of ~100000 atoms. The protein-lipid bilayer system was subjected to molecular mechanics energy minimisation with 1000 iterations of steepest descents. This is followed by the molecular dynamics equilibration step (0.30 ns) during which the protein atoms were restrained using a force constant of 1000 kJ/mol/nm². During this equilibration process, the water molecules and the ions are free to move. The final production simulation run was carried out on the system without any restraints for 10ns.

The parameters employed to undertake simulation used Berendsen coupling (10) to maintain a constant temperature of 300K and a constant pressure of 1 bar. Van der Waal's interactions were modelled using 6-12 Lennard Jones potential with a cutoff value of 1.2nm. Particle Mesh Ewalds
method (11,12) was employed to carry out long range electrostatic interactions with a cutoff of 1.2nm. LINCS algorithm (13) was employed to constrain the covalent bonds. The time step was 2fs and the coordinates saved after every 10ps for analysis. Analysis of the simulations was carried out using various programs in the present within the GROMACS suite of molecular dynamics packages. Structural diagrams were generated using VMD (14) and PyMol (www.pymol.org).

Supplementary Figure S1:

Upper Panel: Ca root mean square fluctuation (RMSF) plotted versus residue number. Each of the four subunits are represented in different colours. High fluctuating peaks on the plot correspond to loop regions within the structure.

Lower panel: Ca root mean square deviation (RMSD) for all residues of Kir1.1 tetramer plotted versus simulation time. The overall RMSD for the 10ns simulation is 3.5Å. These plots demonstrate the structural stability of the model over the entire 10000ps simulation run.
Supplementary Figure S2:

A. Calculated distances between ‘RKR Triad’ residues. This figure illustrates the averaged distance between individual residues of the putative ‘RKR Triad’ calculated over the final 8000ps molecular dynamics simulation. The average distances are: K80-R41 (blue) = 31.5Å, R41-R311 (black) = 14.71Å, and R311-K80 (red) = 24.8Å.

B. K80 and A177 are solvent inaccessible. Solvent accessible surface area of K80-A177 interaction calculated over the 10000ps simulation run. The initial 2000ps (0-2000ps) have been treated as equilibration step and omitted. The final 8000ps have been used in the calculations using the g_sas program from the GROMACS suite of packages. K80 and A177 are located within the membrane and remain inaccessible to solvent over the entire course of the simulation.
**Supplementary Figure S3.**

**Model of Kir1.1 exposes the distance between the RKR Triad.** A) Side view of the structural model of tetrameric Kir1.1 channel to illustrate the relative position of the RKR residues (red) within the tetramer. One of the four subunits is coloured blue. The lipid (POPC) bilayer is shown as yellow spheres and tails whilst the K+ ions in the selectivity filter are shown in green. B) An expanded view of a single subunit of Kir1.1 is shown and coloured blue for clarity. The RKR residues (R41, K80, R311) are labelled red. The dotted lines and distances shown correspond to the Cα-Cα distance between them as averaged over an 8ns MD simulation (supplementary Fig. S2A).
Supplementary Figure S4.

**Calculated distances between K80/A177, R41/E318 and R311/E302.** The distance between these residues was calculated over the final 8000ps simulation. The initial 2000ps (0-2000ps) have been treated as equilibration step and omitted. The average distances are: K80-A177 (black) = 2.98Å, R41-E318 (red) = 3.72Å, R311-E302 (blue) = 3.32Å.
Supplementary Figure S5:

**K80I/A177T channels exhibit same state-dependent response to Cu-Phen.** In Kir1.1 channels the pH-inhibited state is highly sensitive to cysteine reactive agents, whereas the open state is insensitive. This sensitivity has been attributed to two cysteines; C49 in the N-terminus and C308 in the C-terminus. The regions around these cysteines are thought to move during pH-inhibition, resulting in their exposure and therefore access to modifying agents [Schulte et al. 1998]. This figure shows application of 250 µM Cu(II)-1,10, phenanthroline (Cu-Phen) at pH 8.5 and pH 5.5 to (A) wild-type Kir1.1, (B) Kir1.1-C49V/C308A, (C) Kir1.1-K80I/A177T and (D) Kir1.1-K80I/A177T-C49V/C308A channels as indicated; Cu-Phen solutions were gassed with oxygen to enhance the modification by Cu-Phen. Similar results were obtained in n>5 experiments for each mutant respectively. This demonstrates that Cu-Phen produces a state-dependent inhibition of Kir1.1 by modifying channels in the pH-inhibited state, but not in the open state, and that, as expected, the C49A/C308V mutations abolish this effect. Importantly, K80I/A177T mutant channels also exhibit similar state-dependent modification which is abolished by mutation of C49 and C308. Thus implying that the allosteric movements of the cytoplasmic domains which produce pH-inhibition are the same in both wild-type and K80I/A177T mutant channels and argue that that the pH-sensitivity is not restored via an alternative mechanism.