Investigating the role of conformational change of the pore in Kir channel gating

J.M. Gulbis,[1,2] K.A. Black,[1,2] D.M. Miller,[1,2] O.B. Clarke,[3] A.P. Hill,[4] J.R. Bolla,[5] C.V. Robinson,[5] B.J. Smith,[6] D. Laver,[7] R. Jin[5] and S. He,[5] [1]Structural Biology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia, [2]Department of Medical Biology, The University of Melbourne, Parkville, VIC 3052, Australia, [3]Physiology and Cellular Biophysics, Columbia University, New York, NY, 10032, USA, [4]Computational Cardiology Laboratory, Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010, Australia, [5]Physical and Theoretical Chemistry Laboratory, University of Oxford, Oxford, OX1 3QZ, UK, [6]Department of Chemistry and Physics, School of Molecular Sciences, La Trobe University, Melbourne, VIC 3086, Australia and [7]School of Biomedical Sciences and Pharmacy, The University of Newcastle, Newcastle, NSW 2300, Australia.

Potassium channels act as gates to passive K^+ diffusion across cell membranes, regulating conduction in response to cellular signals. Their exquisite selectivity for K^+ over other cations is achieved by direct interaction between K^+ and the ion selectivity filter, wherein each K^+ is coordinated by eight peptide backbone carbonyls and, at the innermost of the four binding sites, threonine hydroxyls. These amino-acid-based ligands are exchanged for water molecules at either face of the selectivity filter and it is thought that K^+ ions diffuse fully hydrated between selectivity filter and cytosol, requiring that the pore expands (relative to resting channels) to accommodate hydrated K^+ ions during activation and conduction. While the conventional model of gating rationalises this by reversible steric occlusion of the ion conduction pathway, with the pore alternating between wide ‘open’ and narrow ‘closed’ conformations at the inner helix bundle crossing, this model does not fit all available evidence. To understand channel function better we decided to test the gating model directly, embarking upon a coordinated structure-function approach utilising an inward rectifier KirBac3.1 as the subject of the study. Purified recombinant KirBac cysteine-pair mutants were crosslinked at specific sites in order to restrict the width of the pore at the helix bundle constriction (Tyr-132) in the conduction pathway. Crosslink formation between inner helices of adjacent subunits was verified by polyacrylamide gel electrophoresis, crystallographic structure analysis and native mass spectrometry. Activity of reconstituted K^+ channels was analysed by fluorimetric liposomal assays (a population method) and electrophysiological single channel analyses. The data indicate that crosslinked ‘closed’ mutants are able to conduct K^+. To substantiate our findings *in silico*, the potential of mean force as a one-dimensional function along the conduction pathway of native KirBac3.1 was calculated. The resultant energetic profiles indicate a very low free energy barrier to conduction through the narrow constriction located at the tyrosine collar, supporting the experimental results.