

Computational molecular modelling of protein structure and function

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Introduction

The application of molecular modelling to study the behaviour of proteins in cells is useful in understanding the interactions they form with small molecules (ligands), and also their functions within the cell. Molecular modelling allows us to test our current knowledge of these systems and our ability to predict their behaviour *in silico*. We have been using computational techniques to study the electrostatics of conduction in potassium channels (in collaboration with Prof. M. Boyett) and in the prediction of protein binding affinity to ligands (in collaboration with Drs. R.M. Jackson and V. Gillett).

Modelling the electrostatics of conduction in a voltage-gate potassium channel and an inward-rectifier potassium channel

K^+ channels, integral membrane proteins, are universal regulators of cellular function both in excitable and non-excitable cells. Voltage-gated K^+ (Kv) channels and inward-rectifier K^+ (Kir) channels form two important families of K^+ channels. The role of these two families of K^+ channels includes the generation of the action potential and resting potential. The electrophysiological functions of key amino acids in $Kv1.4$ and $Kir3.1/Kir3.4$ channels have been studied in site-directed mutagenesis experiments. H509 and K540, located at the extracellular mouth of the $Kv1.4$ channel, for example, affect slow C-type inactivation on changing the pH of the environment. In the $Kir3.1/Kir3.4$ channel, neutralisation of R149, E139 and D173 has profound effects on, for example, the ligand (polyamine)-binding underlying inward rectification.

We have performed structure-function evaluation and electrostatics calculations for the $Kv1.4$ and $Kir3.1/Kir3.4$ channels at the atomic level using computational biology approaches. 3D models of $Kv1.4$ (S5, p-loop and S6 domains only) and $Kir3.1/Kir3.4$ channels were constructed by a standard homology modelling procedure. The electrostatic potential profiles of the channels were calculated by applying the Finite Difference Poisson-Boltzmann equation (FDPB) to the $Kv1.4$ and $Kir3.1/Kir3.4$ channels with the residues of interest in different protonation states. In addition, the electrostatic potential profile of the $Kv1.4$ and $Kir3.1/Kir3.4$ channels with different numbers of K^+ ions within the selectivity filter region was calculated.

The electrostatic potential profile along the axis of the channels shows that the electrostatic potential is generally negative along the pore of the channels, and that the electrostatic potential is lowest (i.e. most negative) in the selectivity filter region. Therefore, the selectivity filter does provide a suitable environment for K^+ ions to cross the high energy barrier of the lipid membrane. When H509 in the $Kv1.4$ channel carries a positive charge (as opposed to being neutral) the electrostatic potential is increased (i.e. made more positive) by ~38 %. On neutralising K540 in $Kv1.4$, the electrostatic potential is decreased (i.e. made more negative) by ~45 %. It is concluded that the residues of interest could be affecting the electrophysiological properties of the channels by altering the electrostatic potential of the channel pore (the electrostatic potential is expected to influence the K^+ occupancy of the channel).

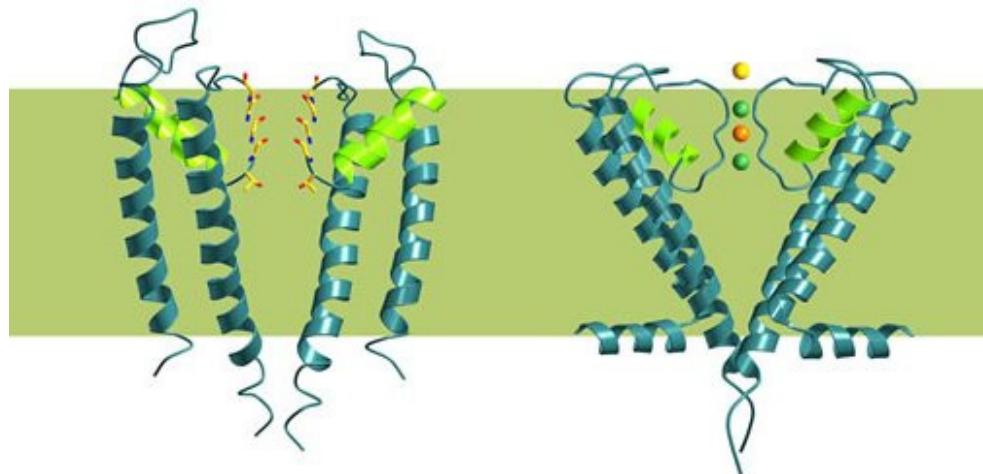


Fig. 1. Cartoon representation of transmembrane helices of potassium channel with two subunits. The selectivity filter is shown in stick (left) and four potassium ions are bound to S0 and the selectivity filter (right)

The application of multi-objective genetic algorithms to protein-ligand docking

Ligand docking is a process which computationally predicts the correct bound conformation of a given protein-ligand complex from atomic coordinates. A docking procedure employs search techniques which produce different conformations and orientations of a ligand on a target protein. A scoring function is then used to score the generated conformations. This is usually done by calculating the binding energy between ligand and protein. From the score values it is possible to infer at which confirmation the most optimal binding occurs. Docking score values are at best empirical functions showing some correction with observed binding conformations and energies. They often involve weighted combinations of possibly competing objectives, including van der Waals energy, electrostatics and desolvation. In this project we are investigating the use of multi-objective genetic algorithms in the docking problem, to investigate optimal balance between the components of the score function and its deviation between test cases, and to research the possibility of controlled consensus docking based on more than one different score function.

Collaborators

Dr. Val Gillet, University of Sheffield
Prof. Mark Boyett, University of Manchester.

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