The mechanism of fast gating in ClC chloride channels

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ClC proteins are a ubiquitous family of chloride channels and transporters that perform diverse functions such as the stabilisation of membrane potentials and the regulation of cell volumes. The most widely studied member of this family is ClC-0 from the *Torpedo* electric ray that has been shown to contain two independent ion conductive pores and have two distinct voltage gating mechanisms. The 'slow' or inactivation gate operates on both pores in the dimer simultaneously, whereas the 'fast' gate acts on each pore individually and opens and closes at a much faster rate. We have investigated the hypothesis that the side chain of a single glutamate residue acts as the fast gate in these channels using molecular dynamics simulations. We find that the motion of this side chain can indeed gate the channel, and furthermore demonstrate that this mechanism explains the dependence of channel gating on extracellular Cl^- concentration, membrane potential and pH.

Using the crystal structure of a bacterial ClC protein as a template we first create a putative open state configuration of the ClC-0 channel. Using this open state model we then conduct molecular dynamics simulations to study the motion of the central glutamate side chain. We find that when the side chain extends towards the extracellular end of the channel it presents an electrostatic barrier to Cl^- conduction. However, external Cl^- can push the side chain into a more central position where, pressed against the channel wall, it does not impede the motion of Cl^- ions. Alternatively, the barrier to ion conduction can be removed by a proton from a low pH external solution binding to the side chain and neutralising its charge. Finally we use Brownian dynamics simulations to demonstrate the influence of membrane potential and external Cl^- concentration on the open probability of the channel.