Inactivation Process in the Potassium Ion Channel KcsA

Salvatore Cosseddu
S.M.Cosseddu@warwick.ac.uk

The University of Warwick
School of Engineering
Centre for Scientific Computing

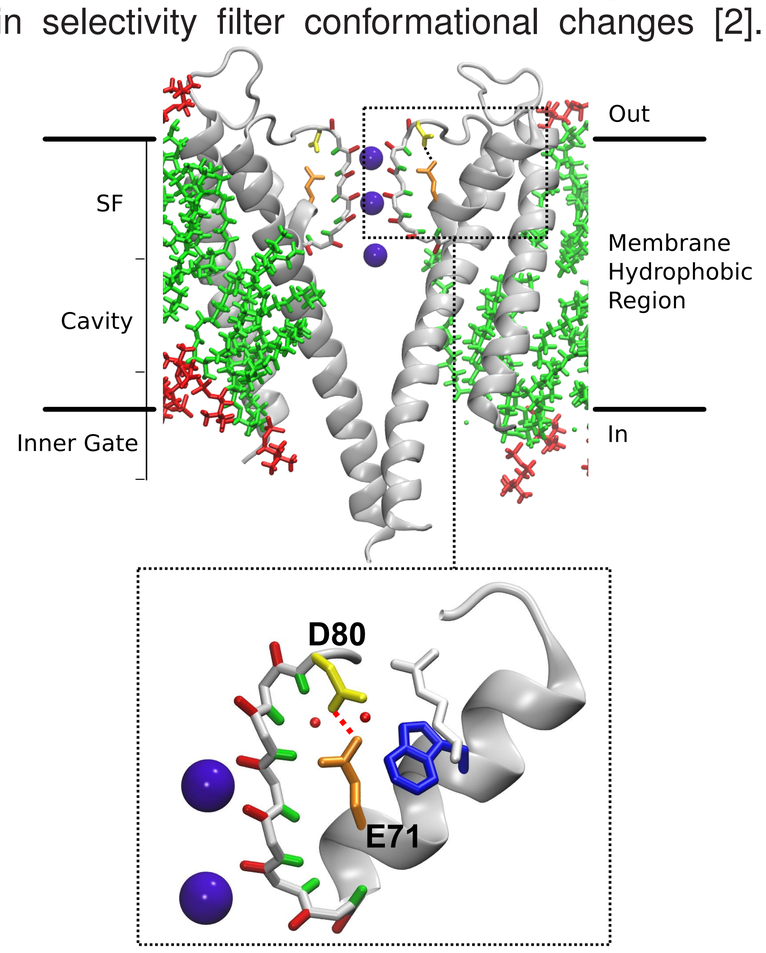
K^+ ion channel KcsA

Biological ion channels are **transmembrane proteins** that enables ions to flow rapidly according to the electrochemical gradient. They are responsible for setting membrane potential, modulation and spread of the electrical signals.

KcsA is a K^+ ion channel found in *streptomyces lividans* with a strong sequence similarity to eukaryotic K^+ channels [1] that shows a complicated time-dependence for current [2] which arises from a superposition of

1. ***Activation opening***, opening of an inner gate;
2. ***C-type inactivation***, Conformational changes in the region of the selectivity filter (SF).

Several lines of evidence have also identified a *key role* for the **E71–D80 hydrogen bond** in selectivity filter conformational changes [2].



In a recent X-ray crystallography study [3] on a mutant channel (tKcsA-OM) undergoing the opening of the inner gate, Cuello *et al.* [3] proposed a set of structures representative of the steps of a proposed mechanism for inactivation induced by the opening of the inner gate.
However a dynamical picture able to link them together is lacking.

Methods

The MD simulations on KcsA with the selectivity filter in the conductive state, show:

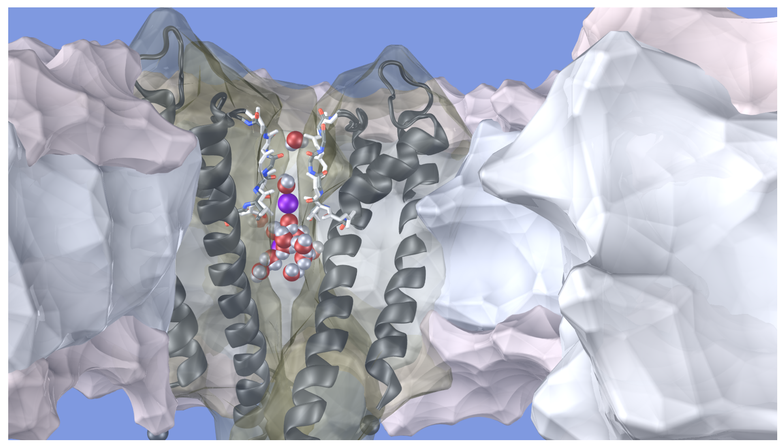
- Water molecules diffusing into the protein and breaking E71–D80 temporarily;
- A correlation between the ion mobility in the SF and the formation of inter-subunit D80–R89 H-bonds, where arginine was shown to to promote proton exchange [4].

We suggest that a deprotonation of E71–D80 pair is possible.

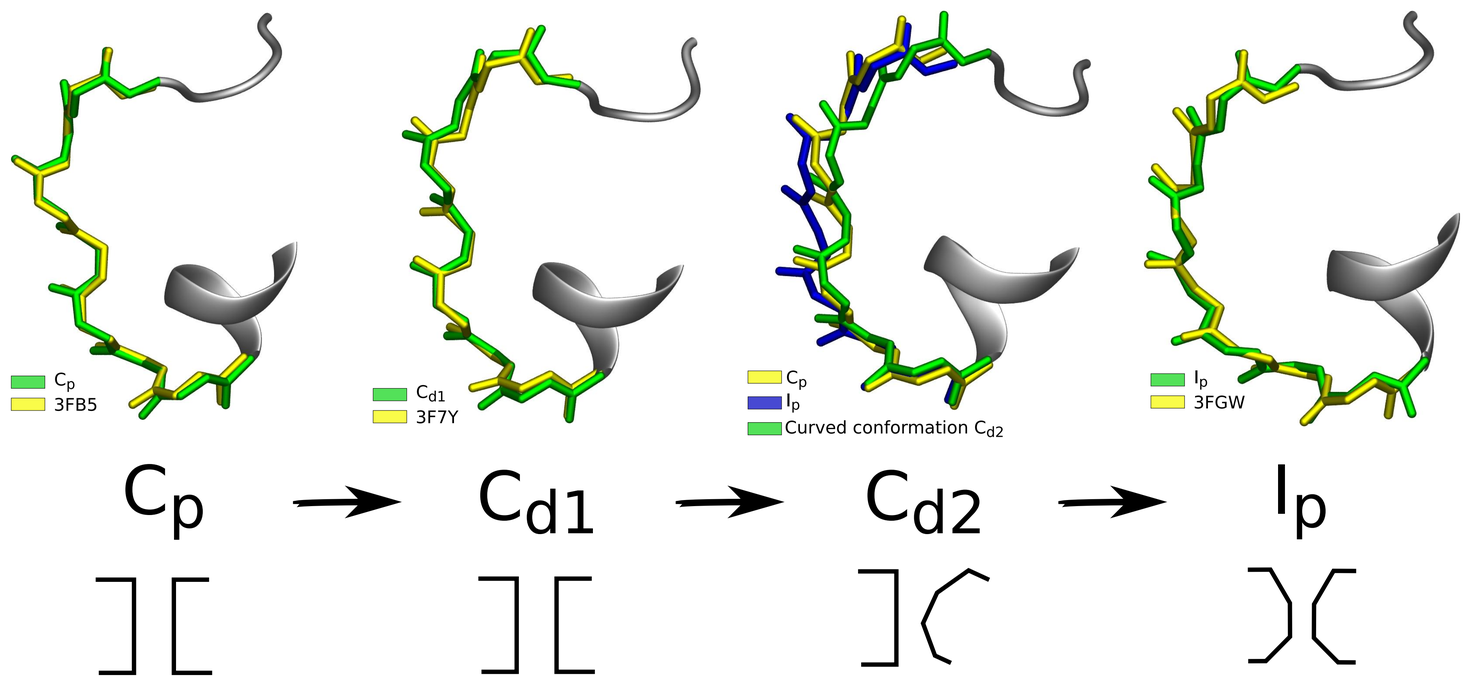
The force-fields commonly used in biomolecular simulations do not permit proton transfer events. Starting from the structure with the SF in the conductive state [1], **Various simulations of the protein in different protonation states of the E71/D80 pair have been performed**.

Inactive conformation

Starting from the X-ray structure of KcsA with the SF in conductive state (white), a conformation (coloured) that overlays with the known inactive conformation is reached.



Results



The simulations shows a series of different configurations:

1. $\mathbf{C}_p$ is considered the conductive state for the SF.
2. $\mathbf{C}_{d1}$ is obtained by the disruption of the E71–D80 bridge through the deprotonation. It overlays on a proposed intermediate structure identified by Cuello *et al.* [3].
3. $\mathbf{C}_{d2}$ is a new conformation caused by the net negative charges behind the filter, exposed as a consequence of the deprotonation, strongly interacting with the amino groups of SF.
4. $\mathbf{I}_p$ is obtained re-establishing the E71–D80 bridge in C_{d2}. It overlays on the putative inactive structure [1, 3]

Contributions

We present Molecular Dynamics (MD) simulations that provide a *mechanistic connection* among the recent studies on C-type inactivation.

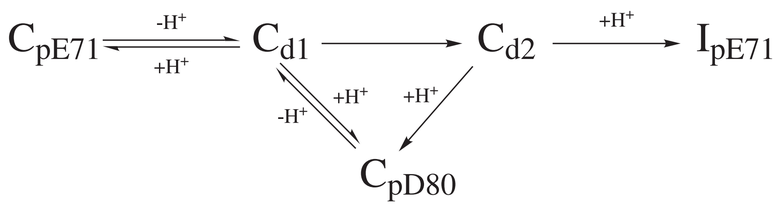
We propose:

- A possible **mechanism** for C-type inactivation;
- The importance of the disruption of the E71–D80 bridge, through a **deprotonation**, as a necessary step towards inactivation.

Furthermore we demonstrated that the stages proposed by Cuello *et al.* [3] are connected by a coherent dynamical pathway, although **the concurrent opening of the intracellular gate is not necessary to undergo an inactivation**.

Proposed mechanism

Summarizing the results of the simulations, it is possible to draw a mechanism for the C-type inactivation that relates to deprotonation of the E71–D80 H-bond:



References

[1] Zhou Y, Morais-Cabral J H, Kaufman A, MacKinnon R (2001) *Nature* 414:43-48.
[2] Cordero-Morales J F et al. (2006) *Nat Struct Mol Biol* 13:311-318.
[3] Cuello L G et al. (2010) *Nature* 466(7303):272-275.
[4] Lanyi J K (2004) *Ann Rev Phys* 66:665-688.

Project

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