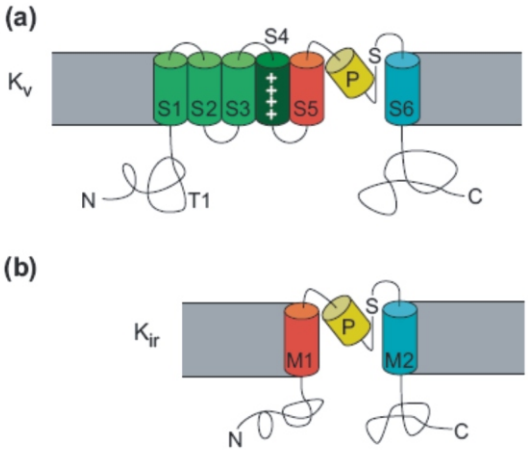
KcsA

The nobel potassium channel

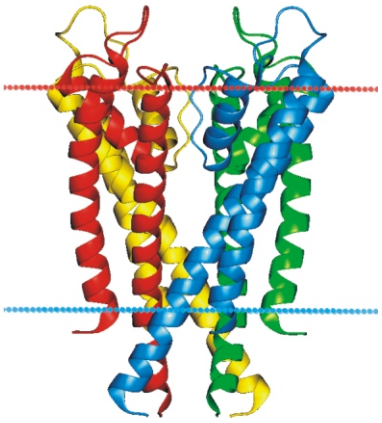
Ionovation Application Note 03

Introduction

Potassium selective ion channels play a pivotal role in many physiological processes. They have an important function in excitable and non excitable cells e.g. of the nervous system, the heart, skeletal muscle or the endothelial tissue. Besides the common structure of voltage gated ion channels with six membrane spanning domains (K_v), small potassium selective channels with only two membrane spanning domains are well-known. Prominent members of this family are the eukaryotic inwardly rectifying channels (K_{ir}), but also KcsA, a potassium channel from the gram positive bacterium *Streptomyces lividans*. It is the first prokaryotic K^+ channel analyzed on the molecular/structural level and from this studies it is convincing that KcsA is an ancestral gene in the phylogenetic tree of this ion channel family.



Schematic representation of the topologies and main features of K_v and K_{ir} potassium channels:
Transmembrane helices are numbered S1-S6 in K_v channels and M1-M2 in K_{ir} channels. The S4 segment is the positively charged putative voltage sensor. The loop between S5 and S6 (M1 and M2 in K_{ir}) represents the pore. N and C are the cytosolic amino and carboxy terminus.



Computational model of the bacterial potassium channel KcsA in the plasmamembrane.
Four identical subunits of the two-transmembrane-protein build a functional homotetramer.

Research on KcsA is of high interest for the pharmaceutical industry and the scientific community, because the results may help to understand the function and regulation of the eukaryotic potassium channels, since inwardly rectifying potassium channels are vital in many physiological processes as for example:

- Control of tone/blood pressure in arterioles
- Potassium secretion in the kidney
- Maintaining a stable resting potential in the heart
- Sensor for extracellular K^+

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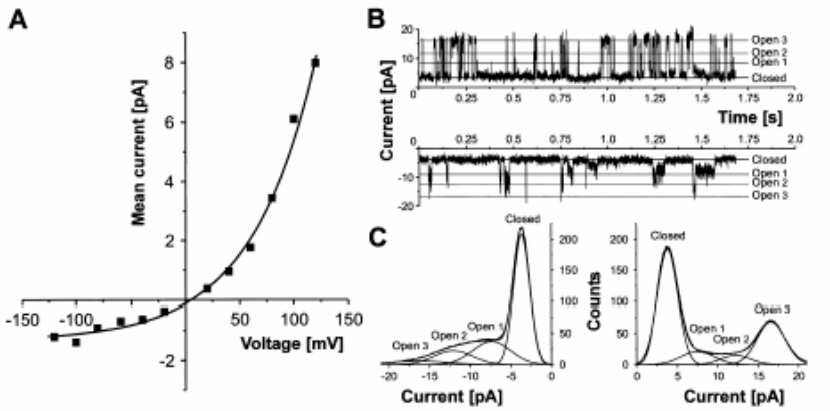
KcsA, a bacterial potassium channel from *Streptomyces lividans*

KcsA was the first discovered prokaryotic K^+-channel ion channel (Schrempf et al., *EMBO J* 1995: 14, 5170 – 78) with high resolution crystallographic structure available (Doyle et al., *Science* 1998: 280, 69 – 77). Up to date it is the best characterized ion channel and many properties have been found to be analogous to eukaryotic K^+ channels. Therefore, this data has been a starting point for detailed simulation studies on other ion channels, especially other two-transmembrane channels like the eukaryotic inward rectifier channels.
The initial work on KcsA in the labs of Hildgund Schrempf and the highly sensitive bilayer experiments conducted by Richard Wagner at the University of Osnabrück were the basis for the structural and functional characterization by Roderick MacKinnon, who was awarded in 2003 by the Nobel committee.



Rectification of potassium channels.
A: Voltage dependence of the mean current (250 mM KCl symmetric).
B: Traces at +100 mV (upper lane) and -100 mV (lower lane) showing at least three conductance levels.
C: Histograms deduced from the traces given in B. The occurrence of the closed and open levels is indicated.

Unequivocal electrophysiological characterization in planar lipid bilayers revealed unique properties:
Most strikingly, a strong pH dependence is evident. At the trans side (corresponds to the intracellular side in a conventional patch clamp experiment), a pH < 5 is essential for channel activity. It is under current debate, whether this is a physiological behavior, because bacteria normally try to keep the intracellular pH constant, similar to eukaryotic cells. Likely, the artificial lipid environment is responsible for that behavior and thus a focus in current research is the establishing of experimental conditions that maintain the physiological properties of KcsA.
Another interesting feature is the outward rectification. The reason for this behavior seems to be the different availability of subconductance states at positive and negative membrane potentials.

The available high-resolution data make KcsA the ideal candidate for structure-function analysis and drug design and is therefore invaluable for pharmaceutical purposes.

Bilayer experiments with a **Ionovation Compact** device enable highly sensitive studies concerning:

- **Drug design;** with the known structure of KcsA, highly specific drugs can be designed and tested at the channel incorporated into the bilayer.
- **Lipid-protein-interactions;** experiments on KcsA in lipid bilayers has shown an essential role of lipid-protein-interaction for proper channel function. Studies on these lipid-protein-interactions can help to understand diseases in lipid metabolism. Such experiments cannot be performed with the conventional patch clamp technique.
- **pH dependence;** the easy access to the extra- and intracellular side of the channel in one experiment make reliable experiment an ease.

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