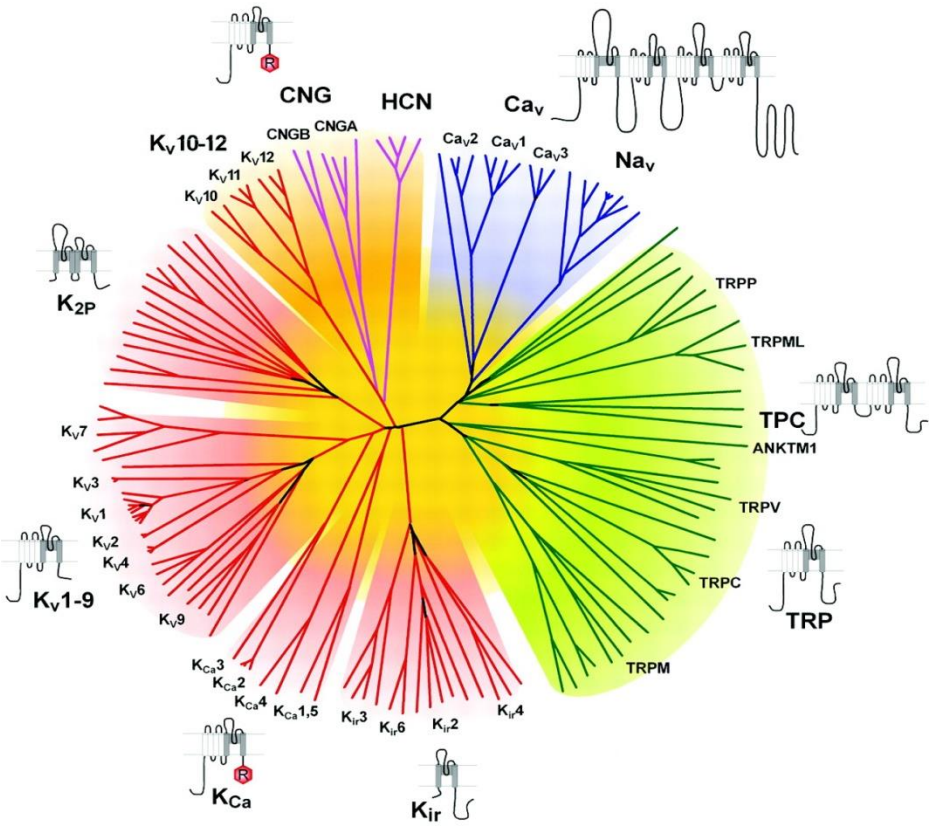


Ion Channel Structure and Function (part 1)

The most important properties of an ion channel

Intrinsic properties of the channel:
Selectivity and **Gating (activation, inactivation)**

+

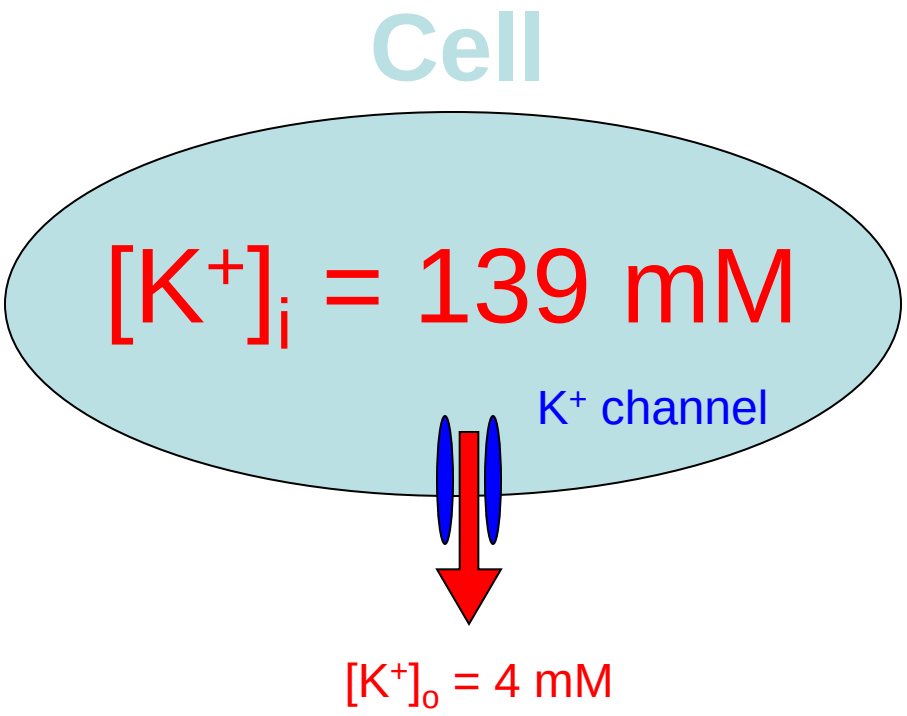
Location

Types of ion channels by selectivity

Ion channels can be:

- Potassium ($K+$)
- Sodium (Na^{+})
- Calcium (Ca^{2+})
- Nonselective Cation (Na^{+}, K^{+}, Ca^{2+})
- Proton (H^{+})
- Chloride (Cl^{-})
- Hydroxide (OH^{-})
- Nonselective Anion (Cl^{-}, P_i, ATP^{4-}, small negatively charged metabolites)
- Large-conductance nonselective (Na^{+}, K^{+}, Ca^{2+}, Cl^{-}, P_i, ATP^{4-}, small metabolites)

Potassium (K^+) channels



$V_m \rightarrow$ - 94 mV

Equilibrium (Nernst) potential for K^+ :

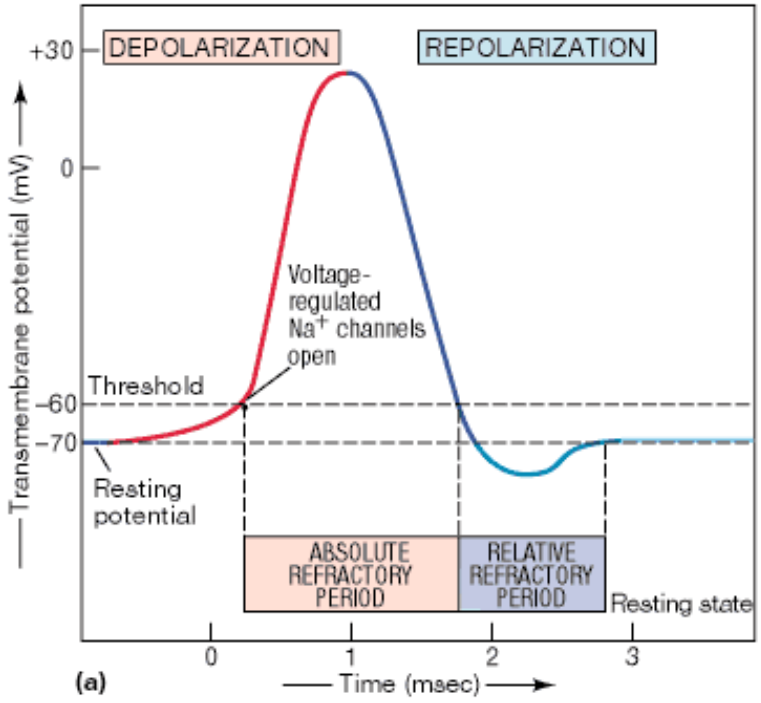
$E_K = RT/zF \{\ln[K^+]_o/[K^+]_i\} = 61 \{\log_{10}[K^+]_o/[K^+]_i\} = - 94$ mV

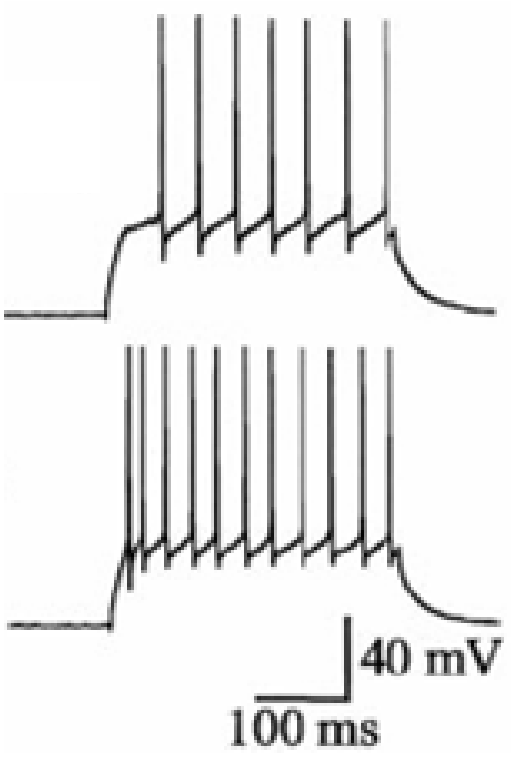
K_v, voltage-gated K^+ channels

Gating: opened by membrane depolarization; there are fast inactivating (A-type, ms) and slow inactivating (delayed-rectifier type, s) K_v channels

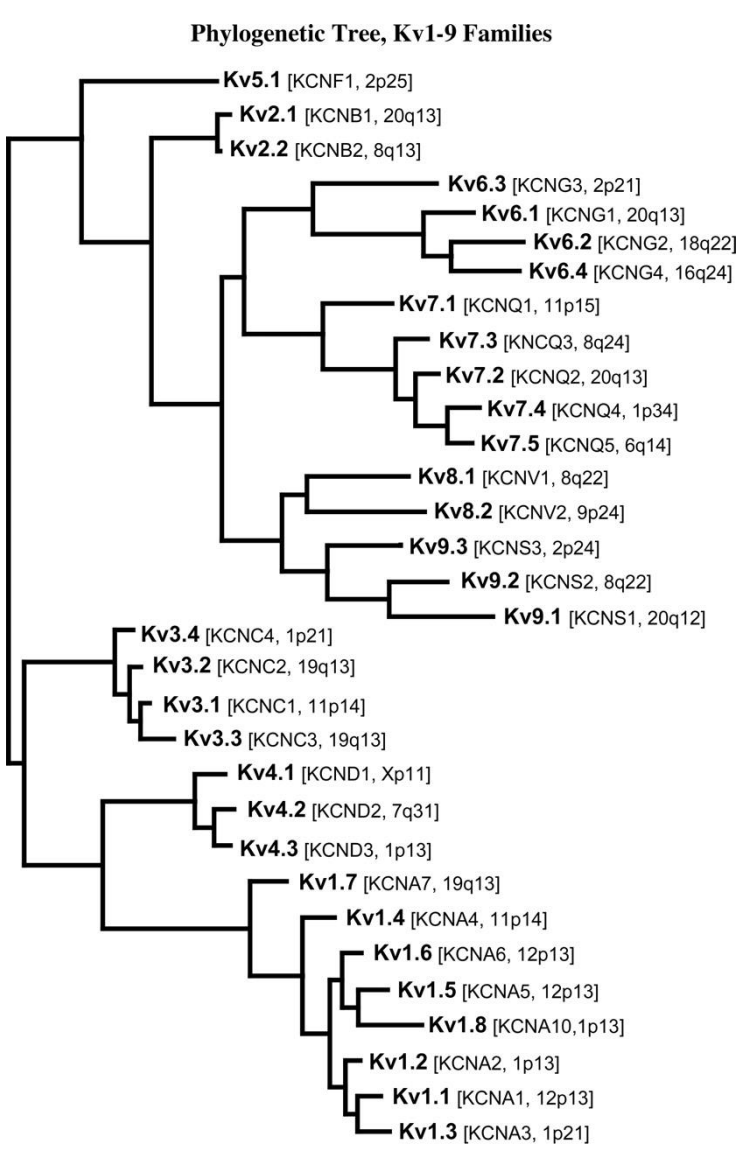
Location: plasma membrane of neurons, muscle cells, and many non-excitable cells

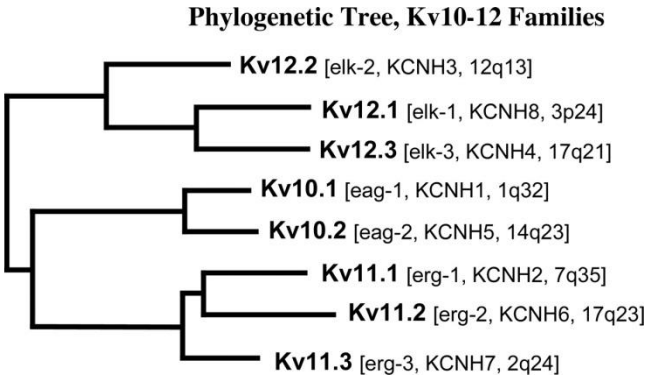
Function: maintaining membrane potential; repolarization of action potential and shaping its waveform; modulating firing pattern and electrical excitability in neurons and muscle.



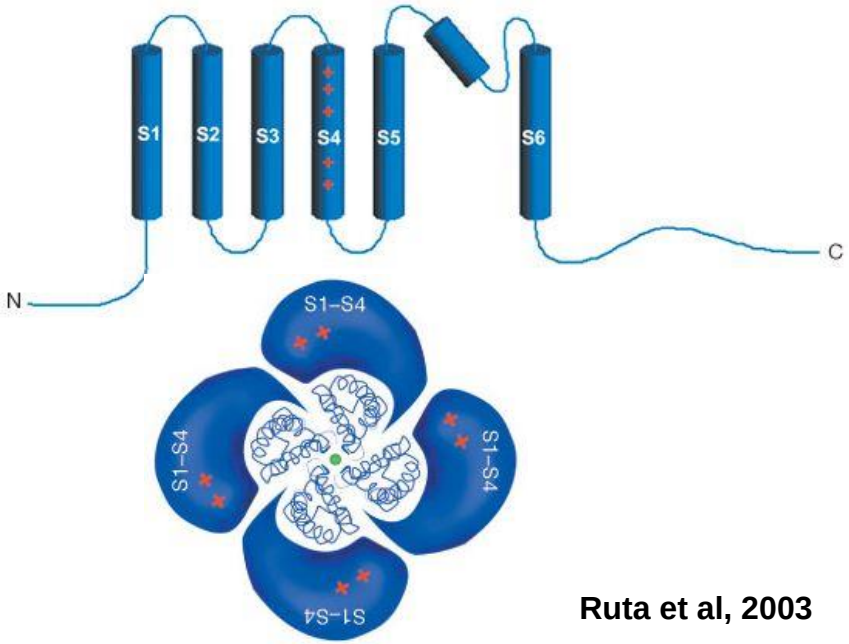


K_v, voltage-gated K^+ channels





K_v α subunits make homo- and hetero-tetrameric complexes



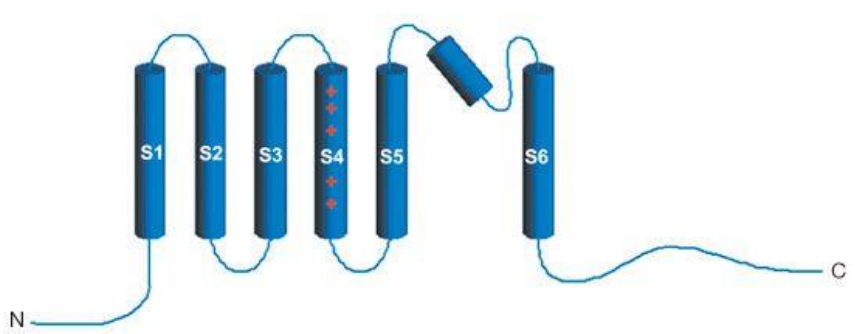
Ruta et al, 2003

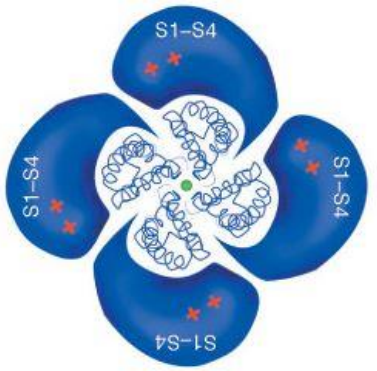
Important questions about molecular architecture of K_v channels

1. How high permeability and amazing selectivity for K^+ are simultaneously achieved?

   - K_v channels enable extremely fast ion flow, ~ 10^8 K^+ per second
   - K^+ is at least 10,000 times more permeant than Na^+, a feature that is essential to the function of K^+ channels

2. How changes in membrane voltage are coupled to channel opening?

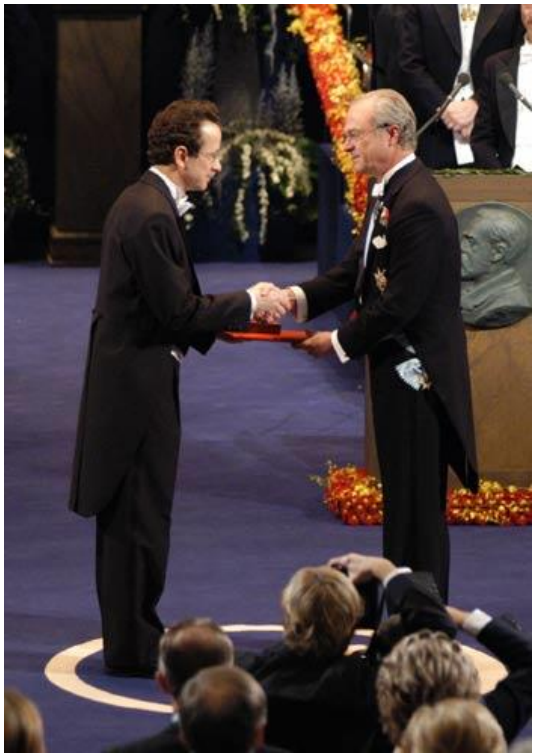


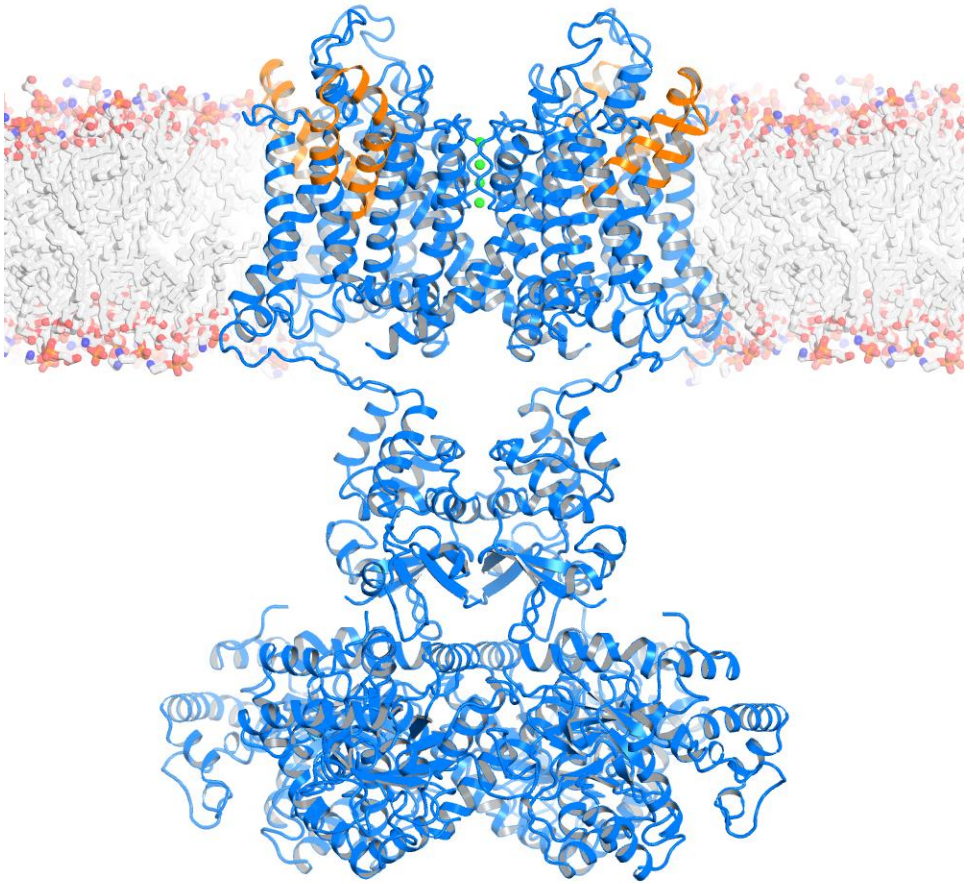


Ruta et al, 2003

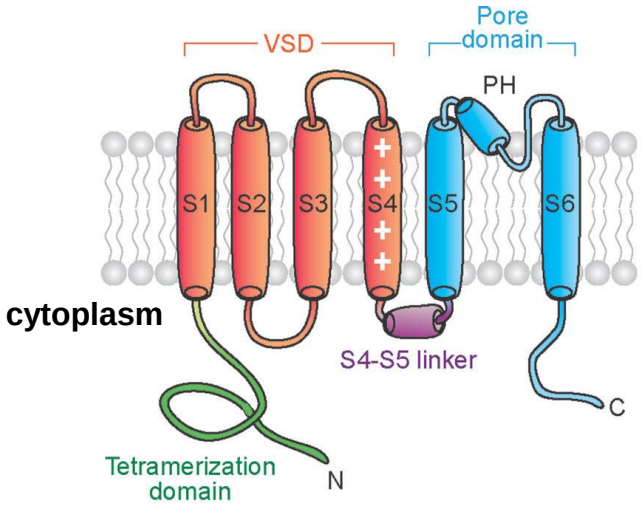
Roderick MacKinnon

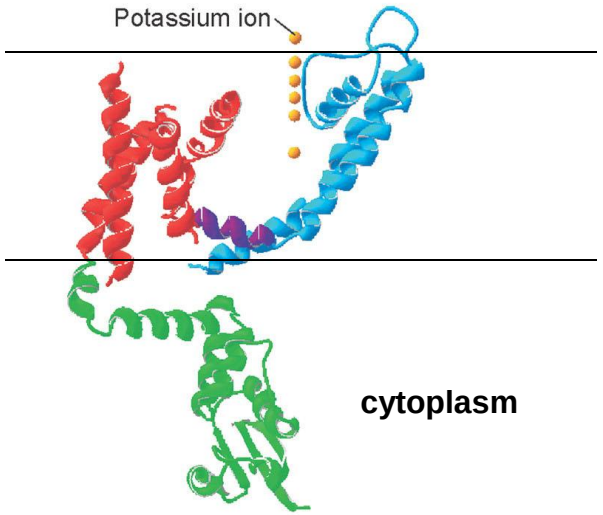
Nobel Price in Chemistry 2003



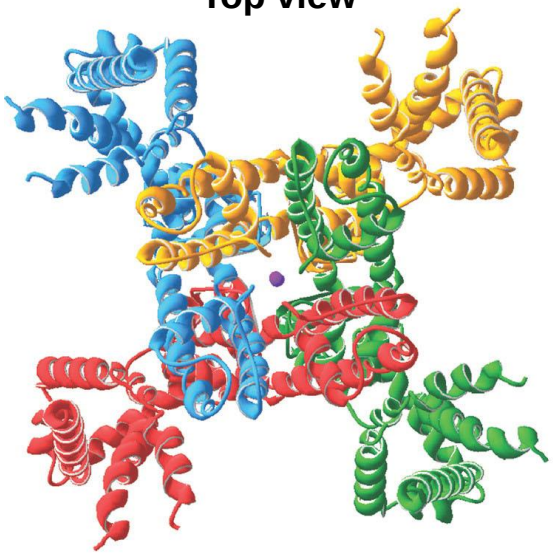


Overview of the K_v channel structure

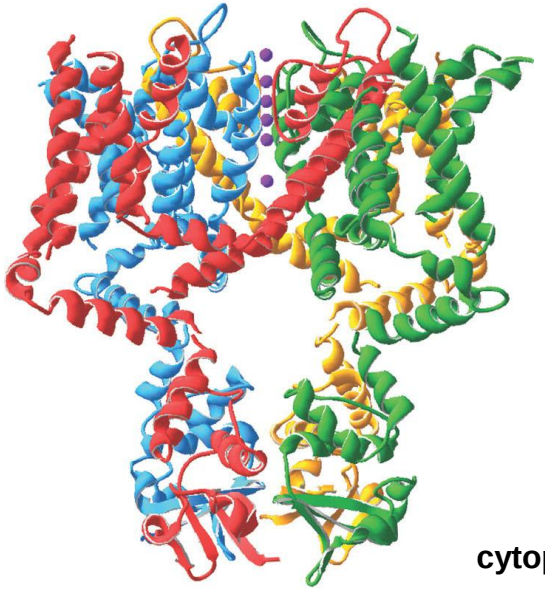




Top view

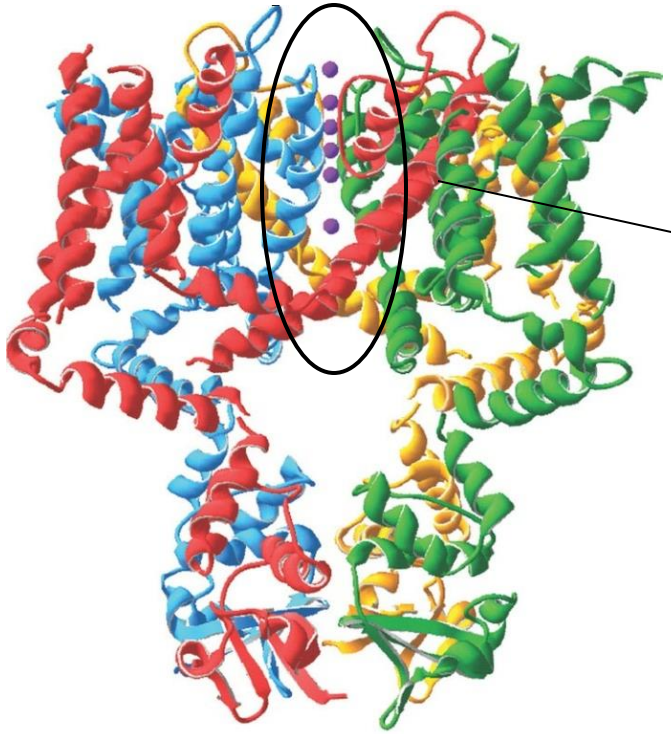


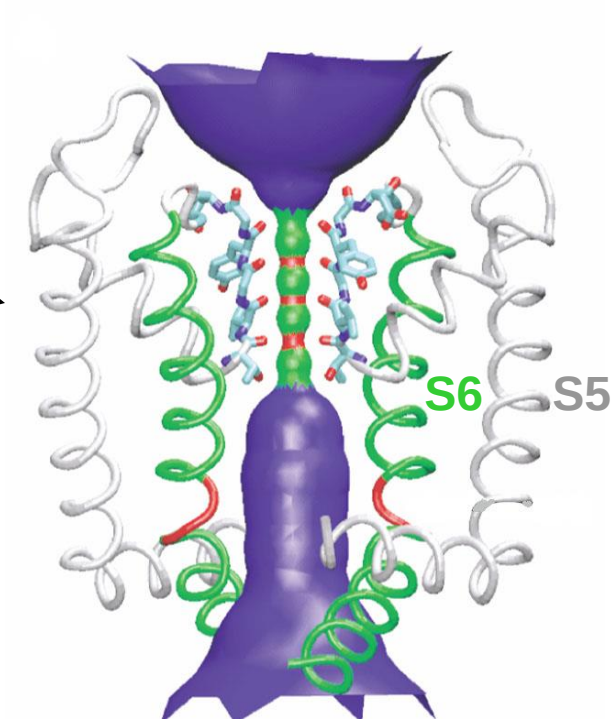
Side view



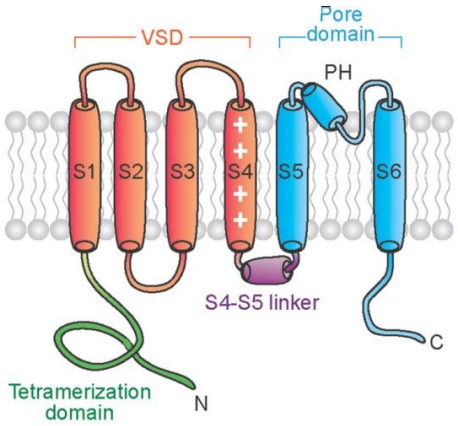
Tombola et al. 2006

Pore Region





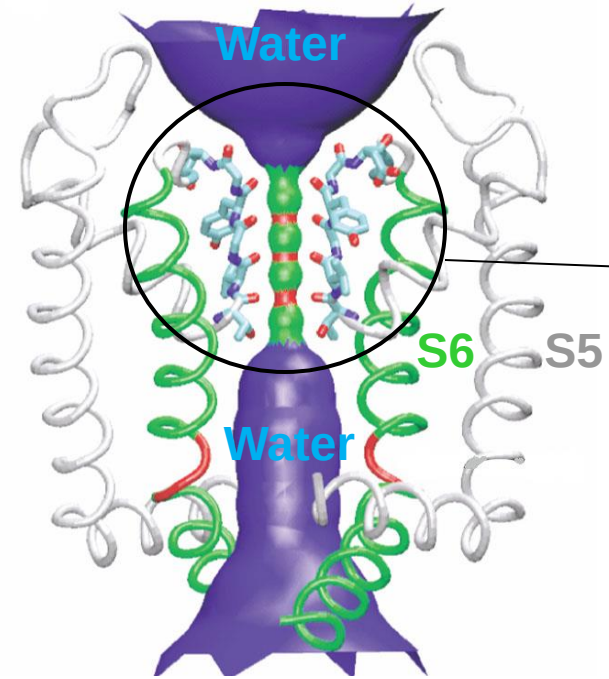
cytoplasm

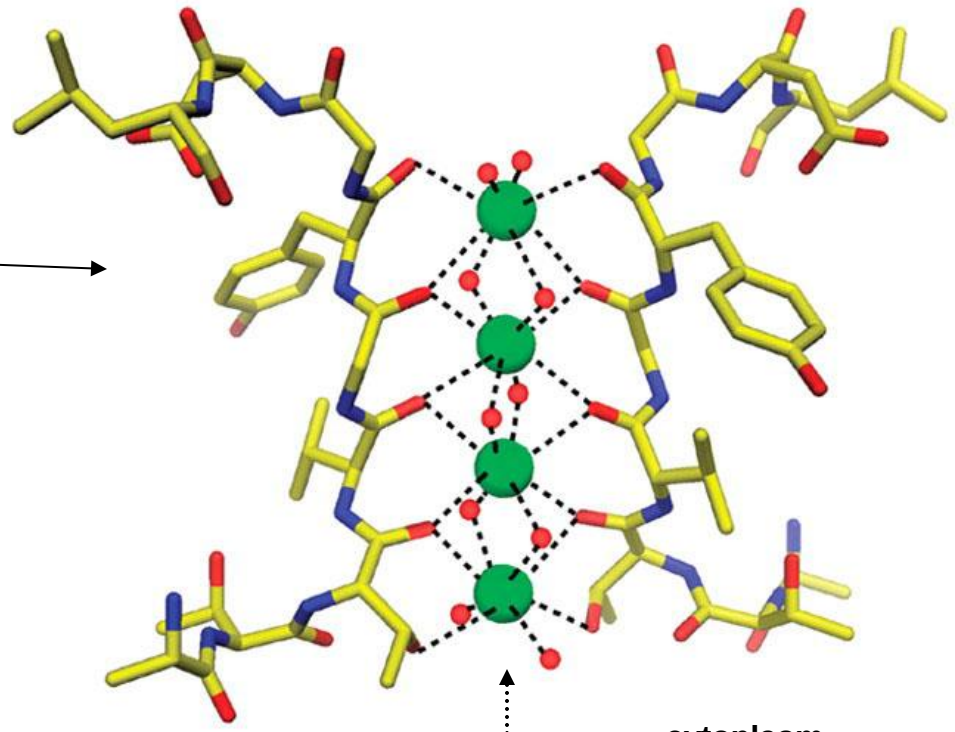


Tombola et al. 2006

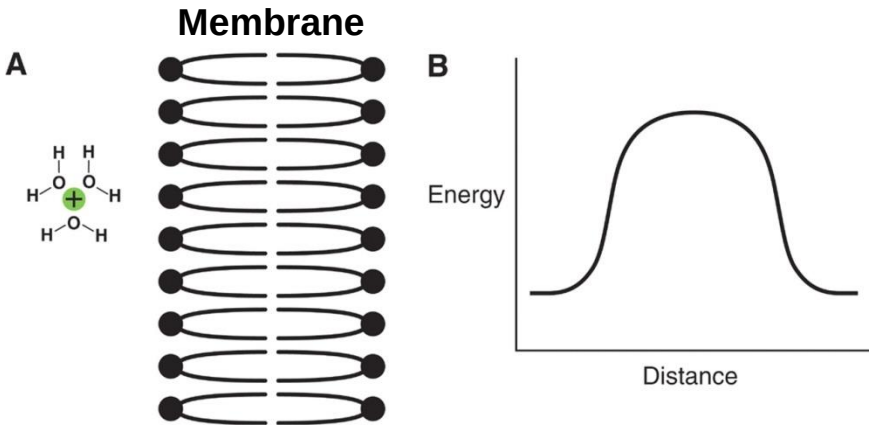
Grottesi et al. 2005

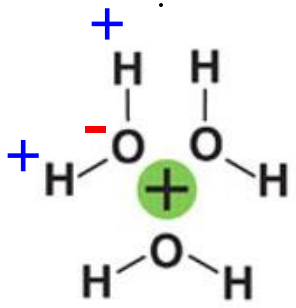
Pore Region - Selectivity Filter





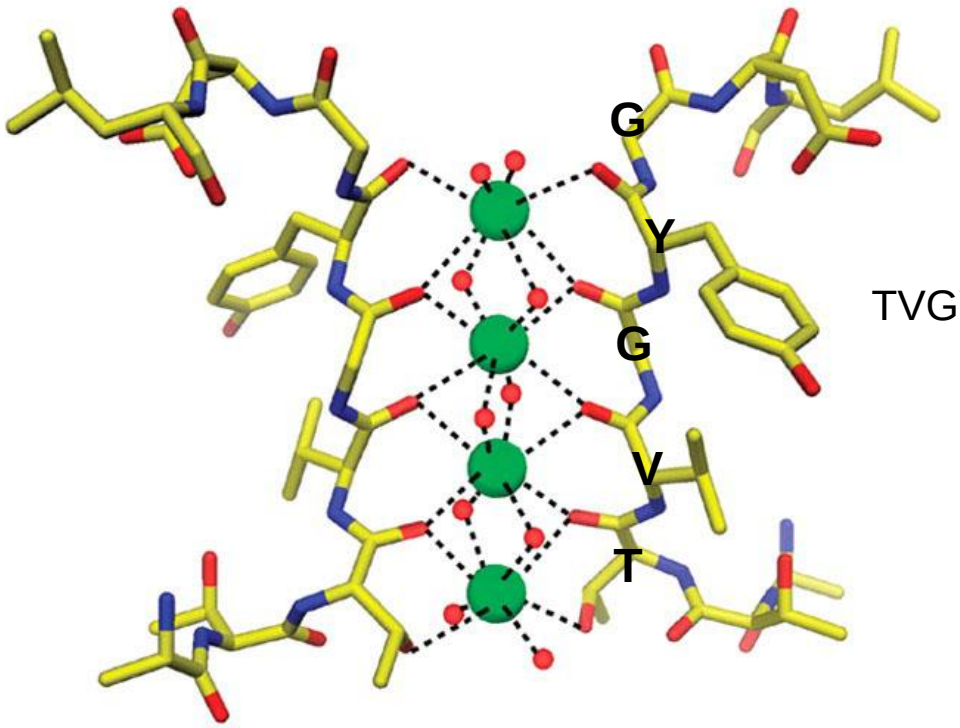
cytoplasm



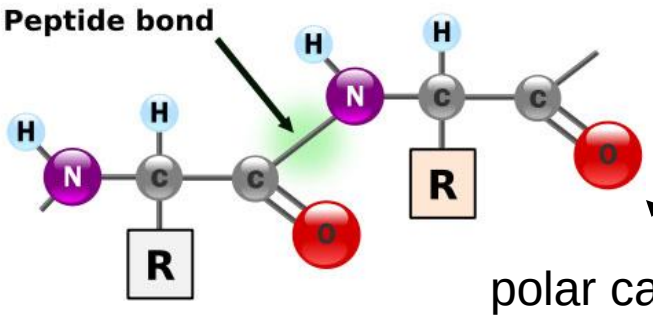


Grottesi et al. 2005
Gouaux & MacKinnon, 2005

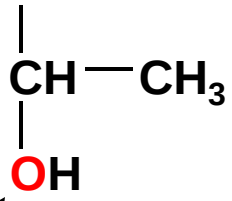
Pore Region - Selectivity Filter



TVGYG (threonine-valine-glycine-tyrosine-glycine)
– signature motif of K^+ channels



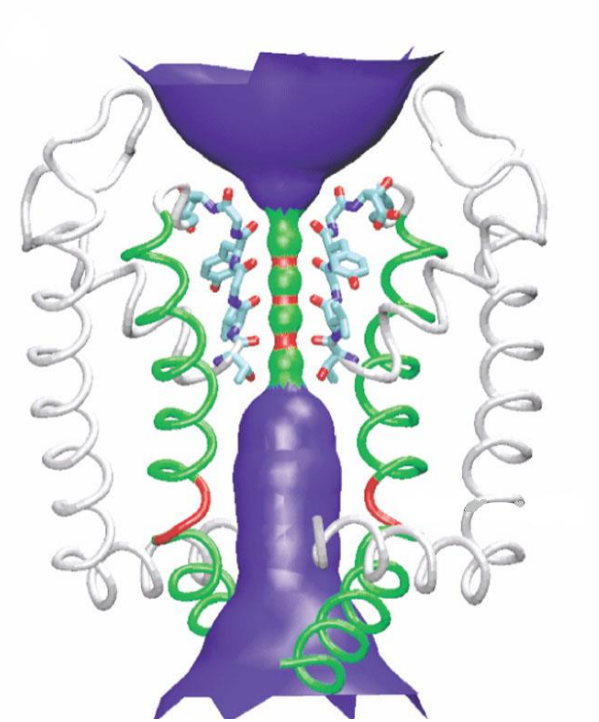
polar carbonyl oxygen
(main chain)

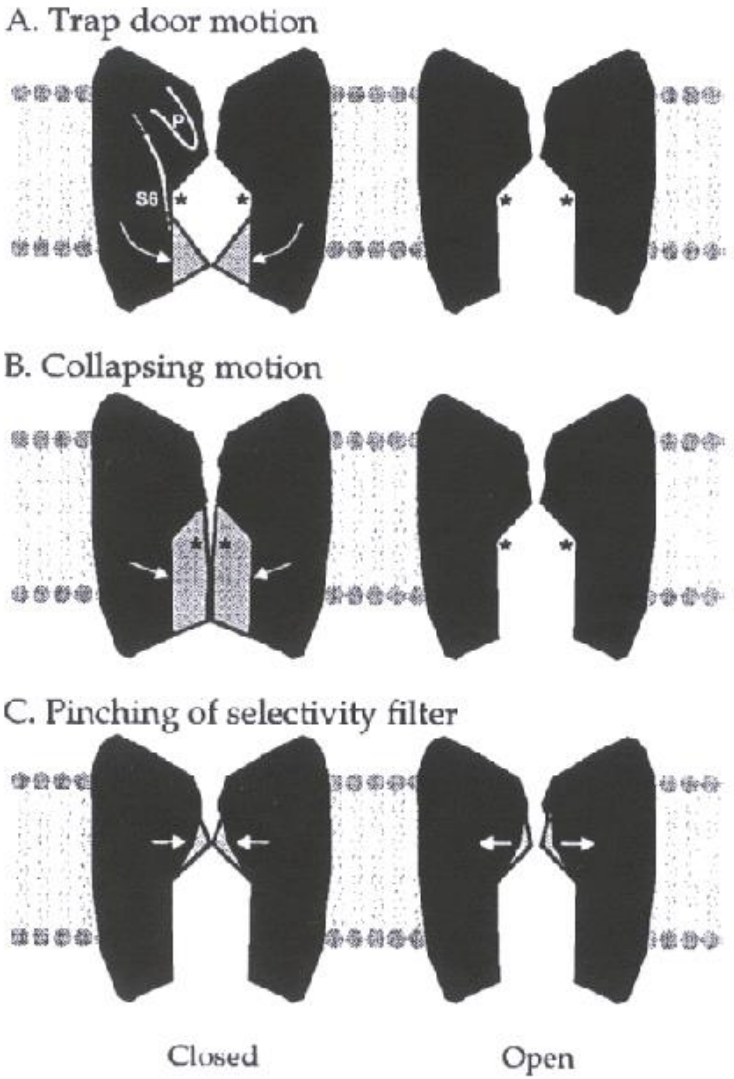


polar hydroxyl oxygen of threonine
(side-chain)

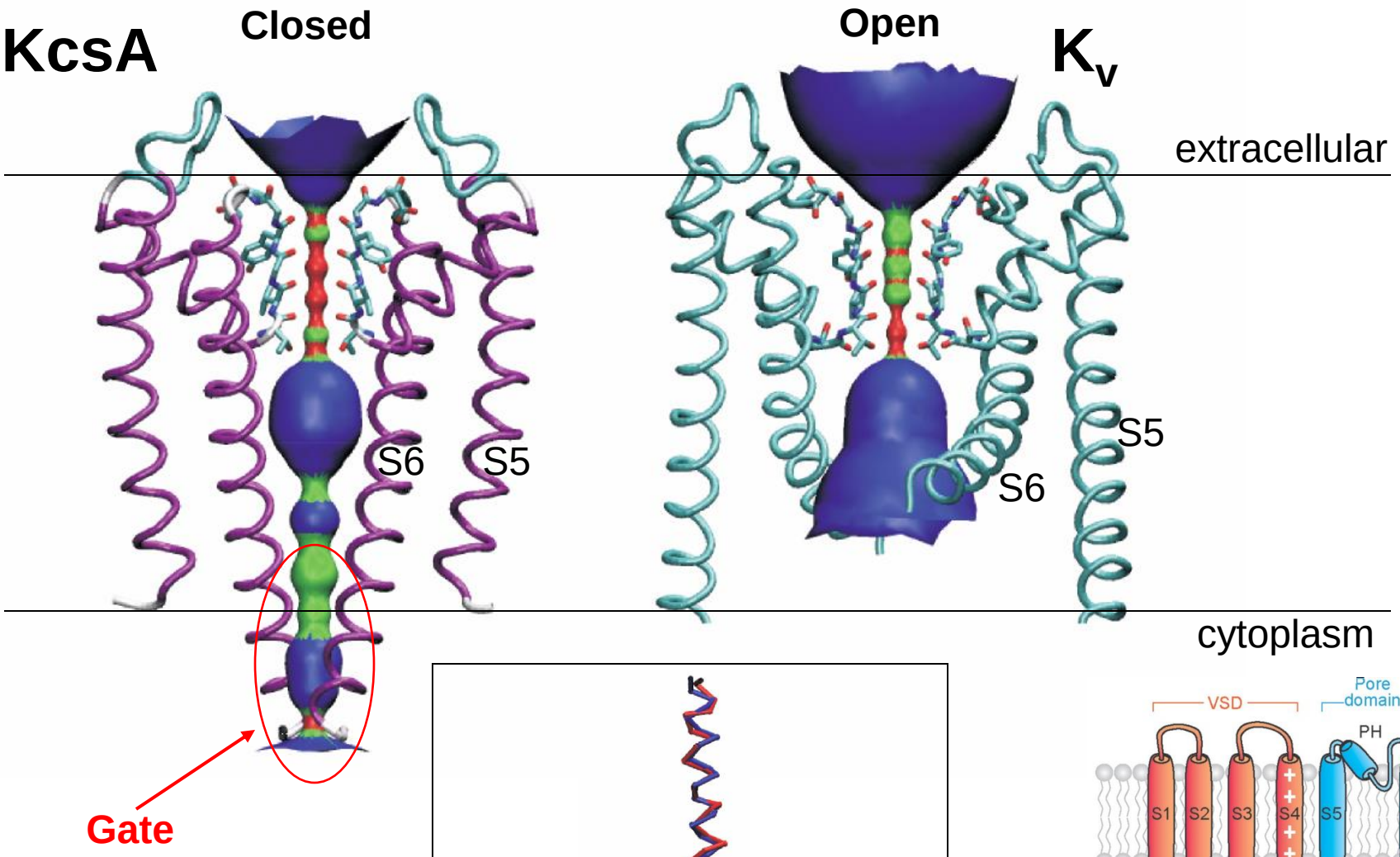
Gouaux & MacKinnon, 2005

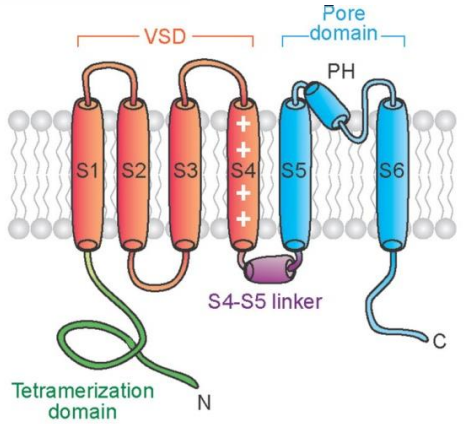
What types of gating motions might one expect?





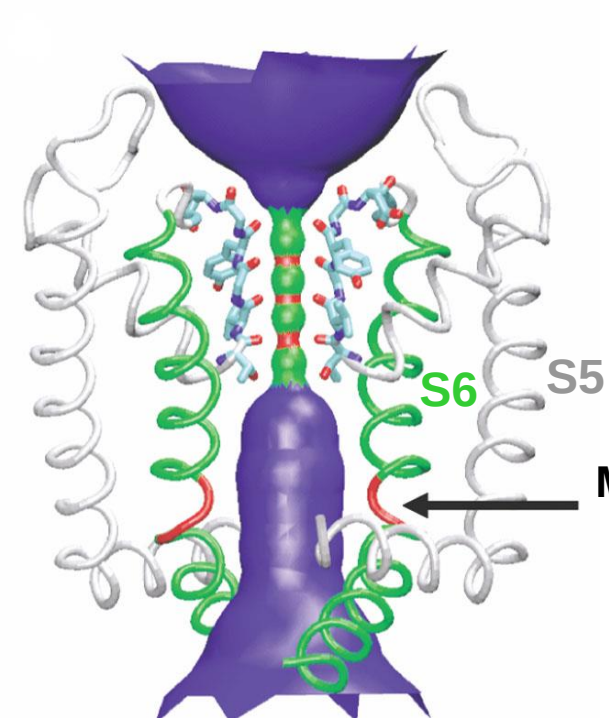
Pore Region - Gate





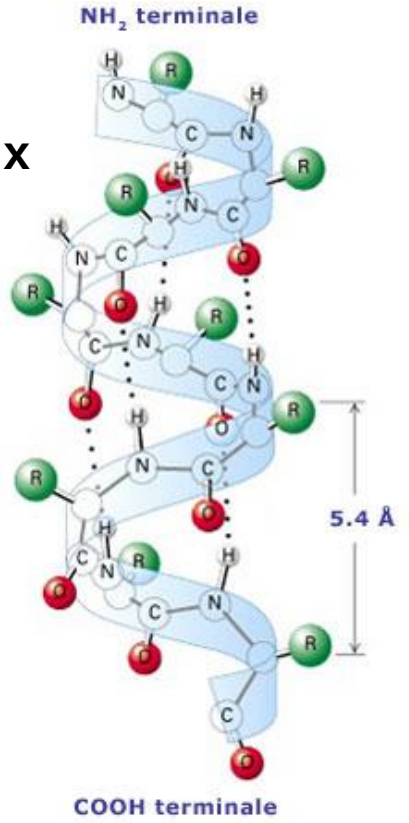
Sands el al. 2005

Pore Region - Gate

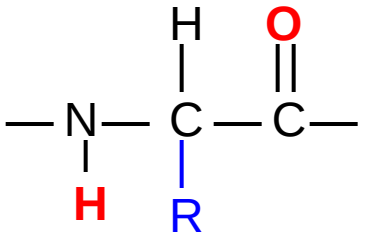


Molecular “hinge” of the “gate”, the Proline-X-Proline motif

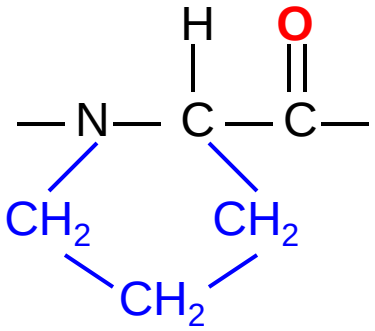
α-helix



Regular amino acid



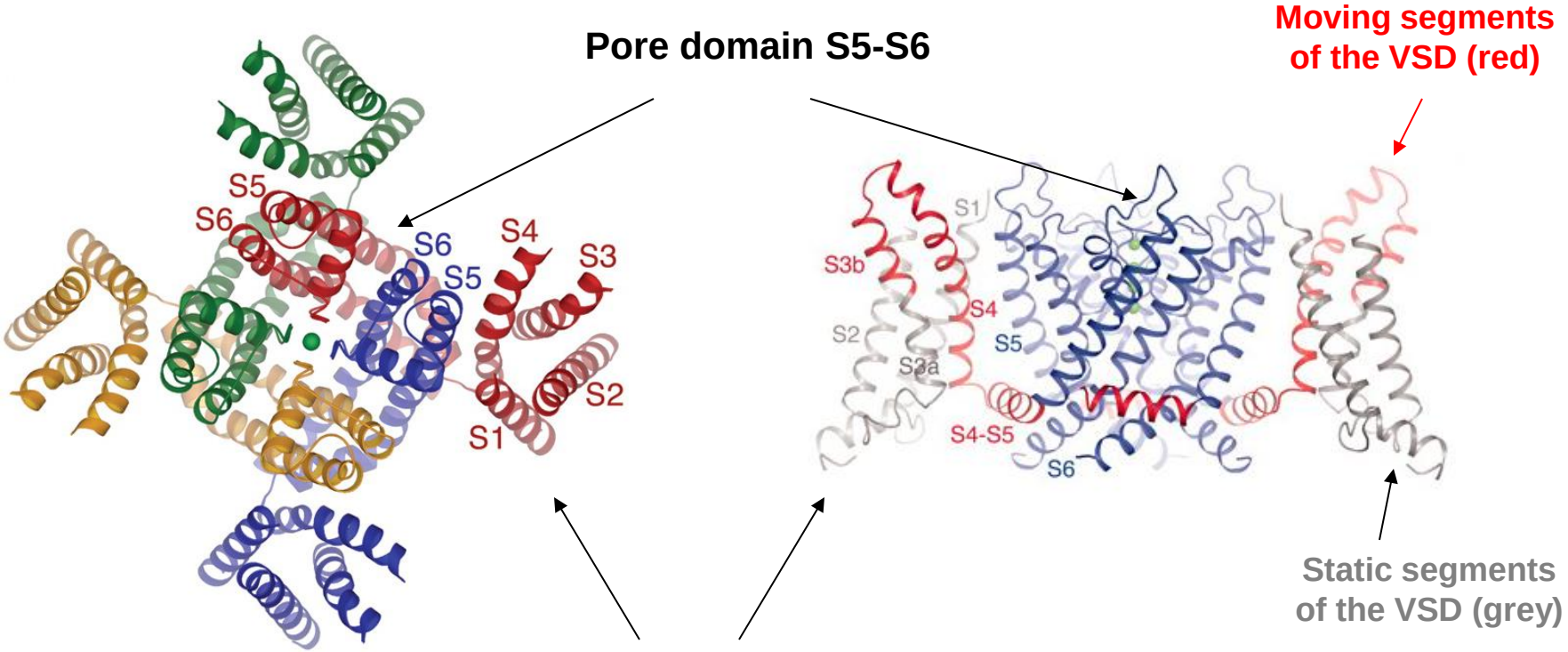
Proline



In the α-helix, N-H group donates a hydrogen bond to the backbone C=O group of the amino acid 4 residues earlier

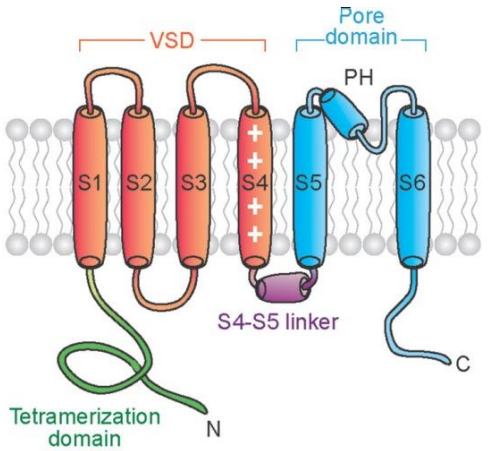
Grottesi et al. 2005

Voltage Sensor and Gating



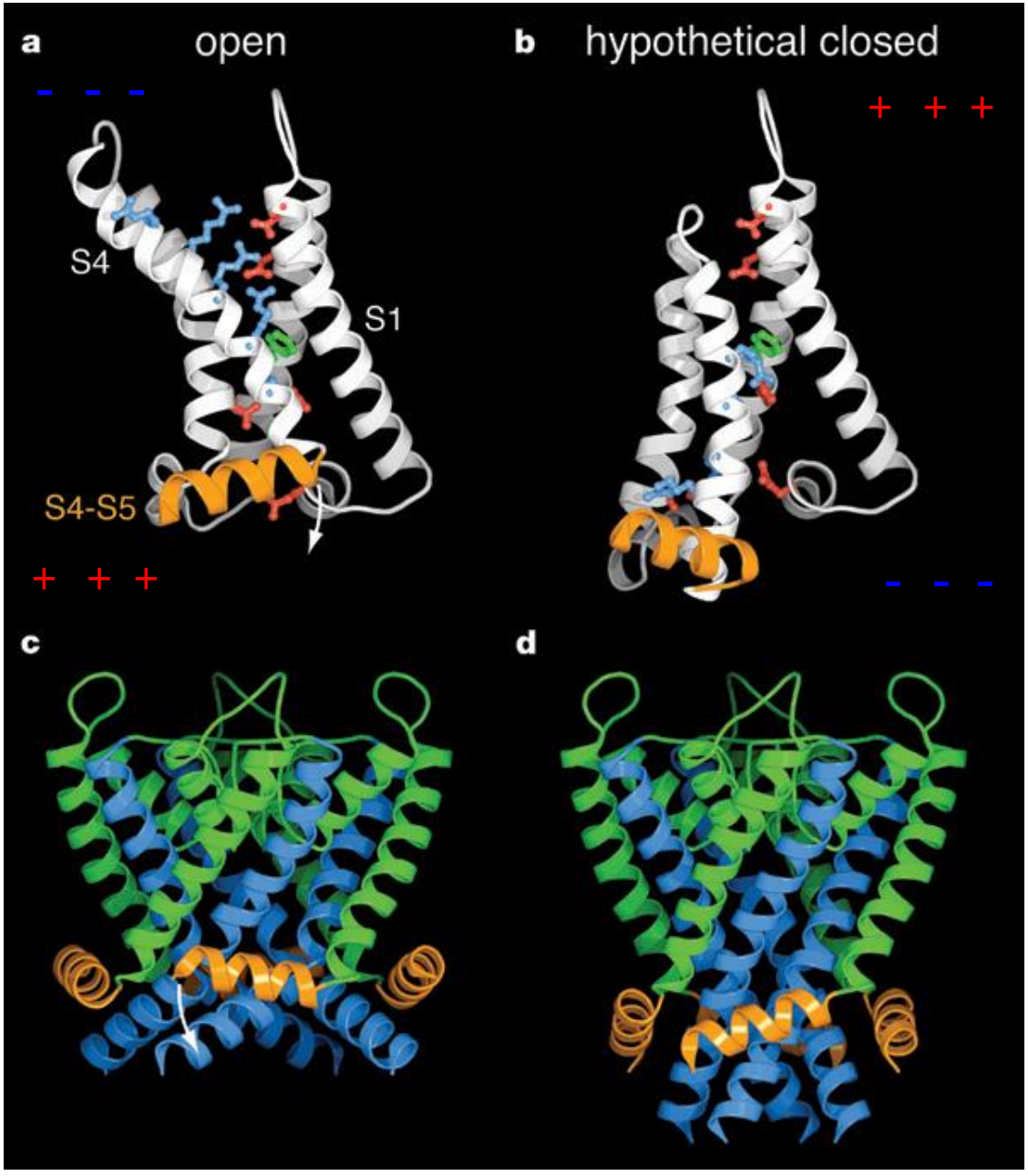
- Pore domain and VSD do not only have separate functions, but are also separated in space.

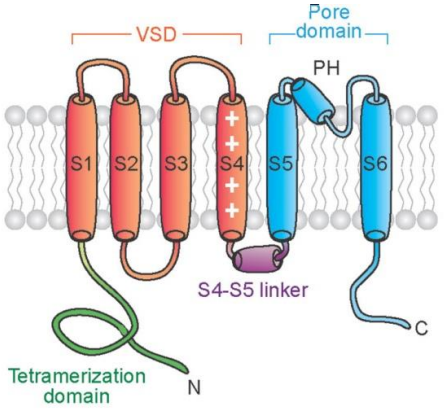
- Pore and VSD domains have only two contact points: S4-S5 linker and the top of the S1 transmembrane helix.



Long et al. 2005

Voltage Sensor and Gating



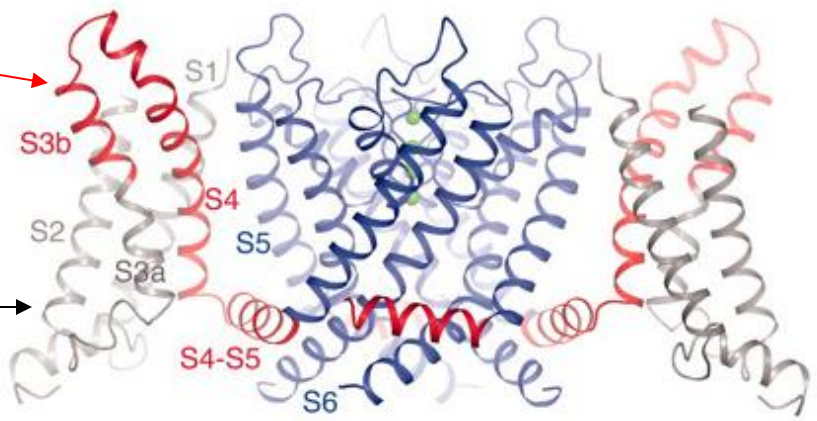


Long et al. 2007

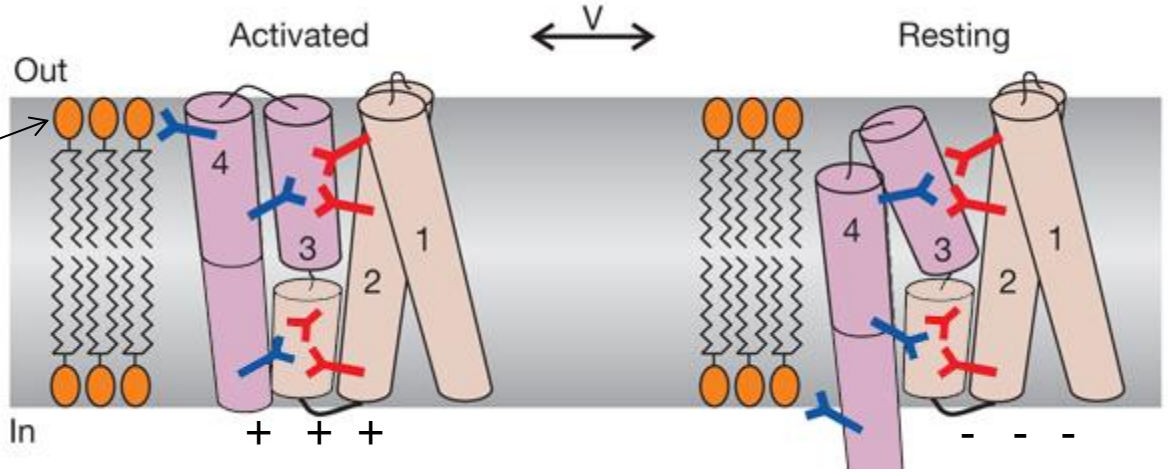
Stabilization of the voltage sensor in the lipid environment

Moving segments of the VSD (red)

Static segments of the VSD (grey)



Negatively charged polar heads of phospholipids



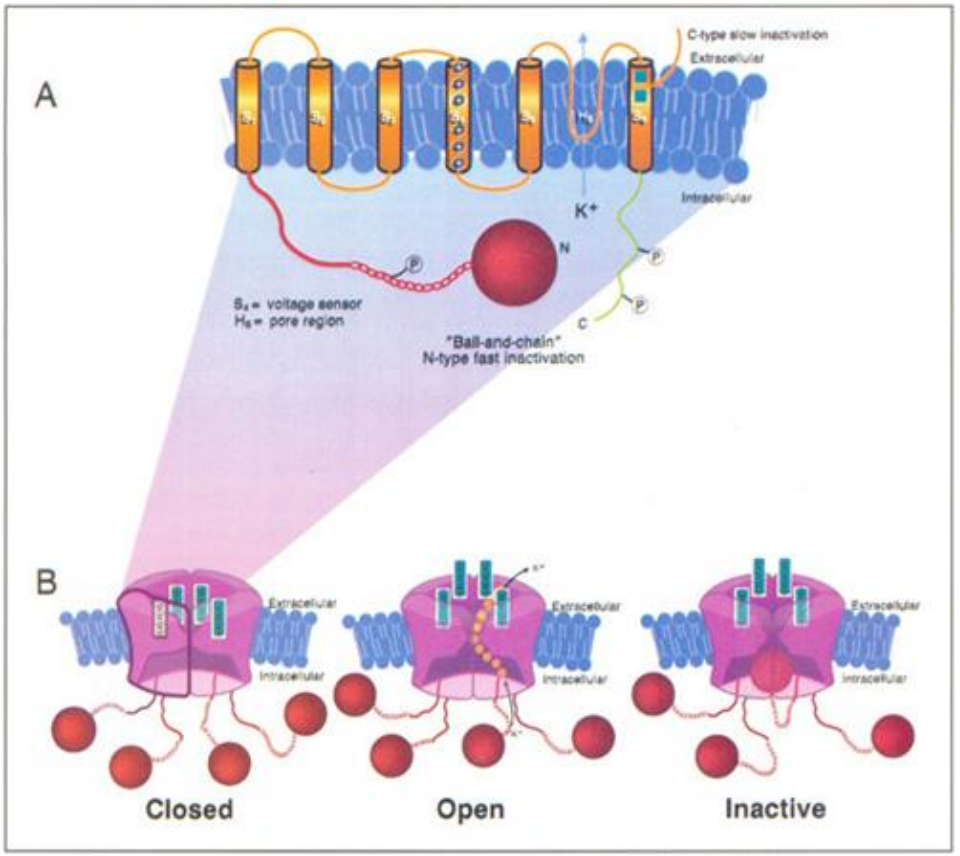
Positive charges of the S4 domain are stabilized by forming ion pairs with negative charges of the static segments of VSD and phospholipids.

Tao et al. 2010, Swartz 2008

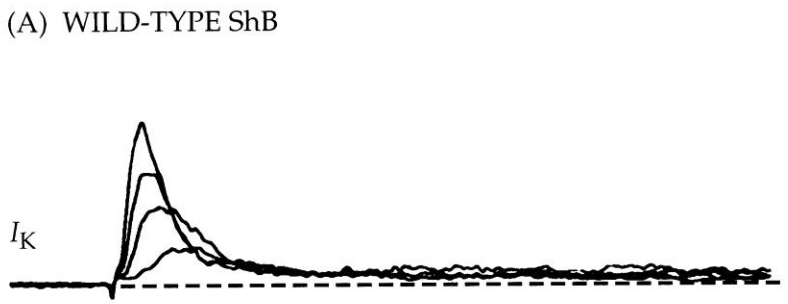
Inactivation of K_v channels

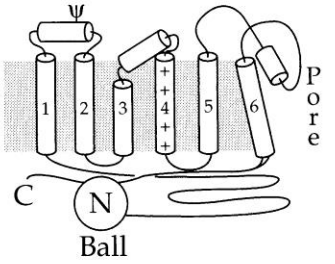
What is the difference between channel inactivation and closure?

- N-type or ball-and-chain inactivation: an N-terminal ball plugs the pore from the cytoplasmic side.
- C-type inactivation results from a localized constriction in the outer mouth of the channel pore.

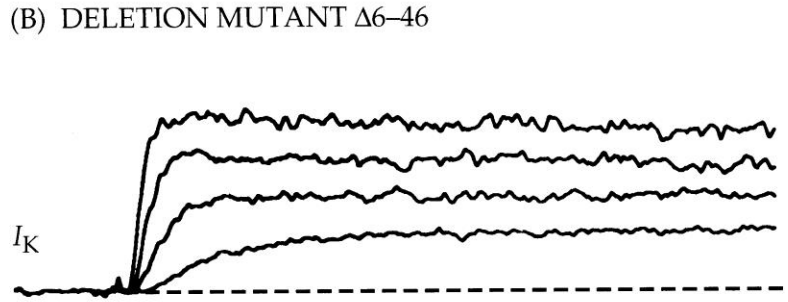


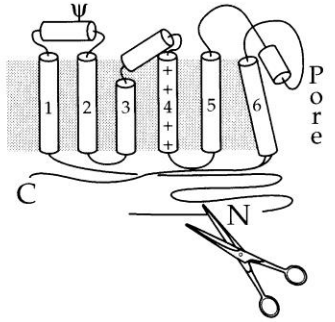
N-type inactivation: ball and chain



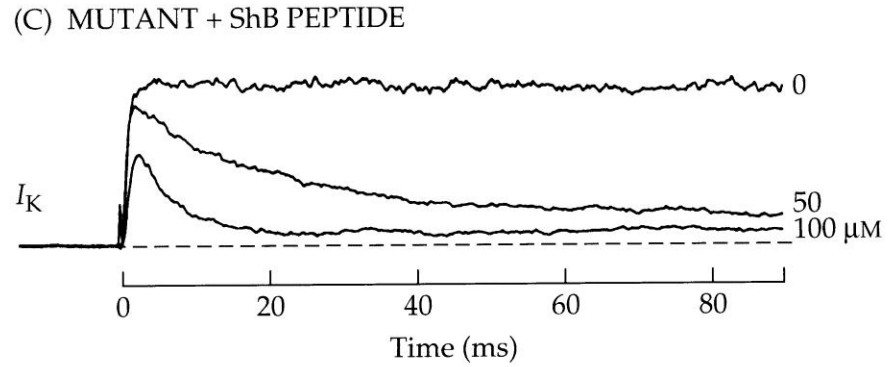


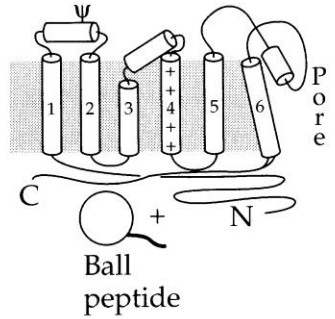
‘ball’ at N-terminus





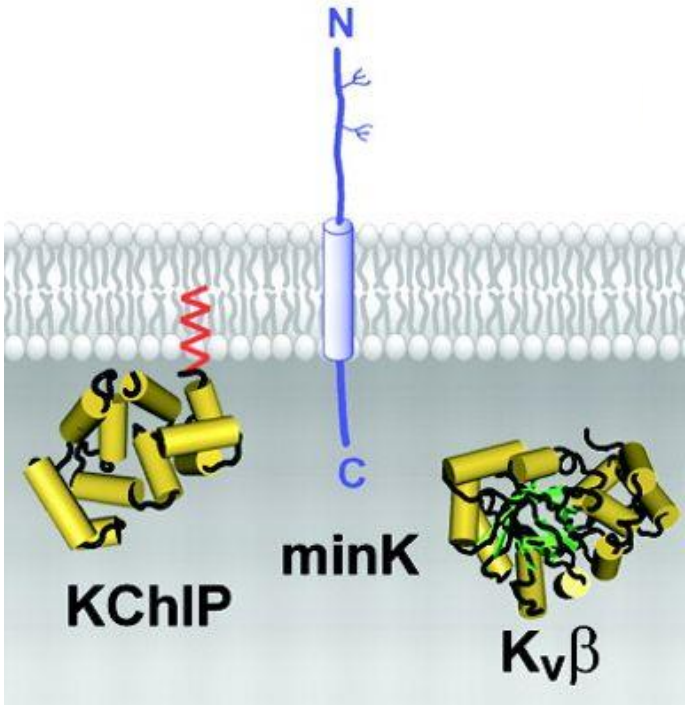
N-terminal truncated channel





N-terminal truncated channel
+
Free N-terminal peptide

Auxiliary subunits of K_v channels



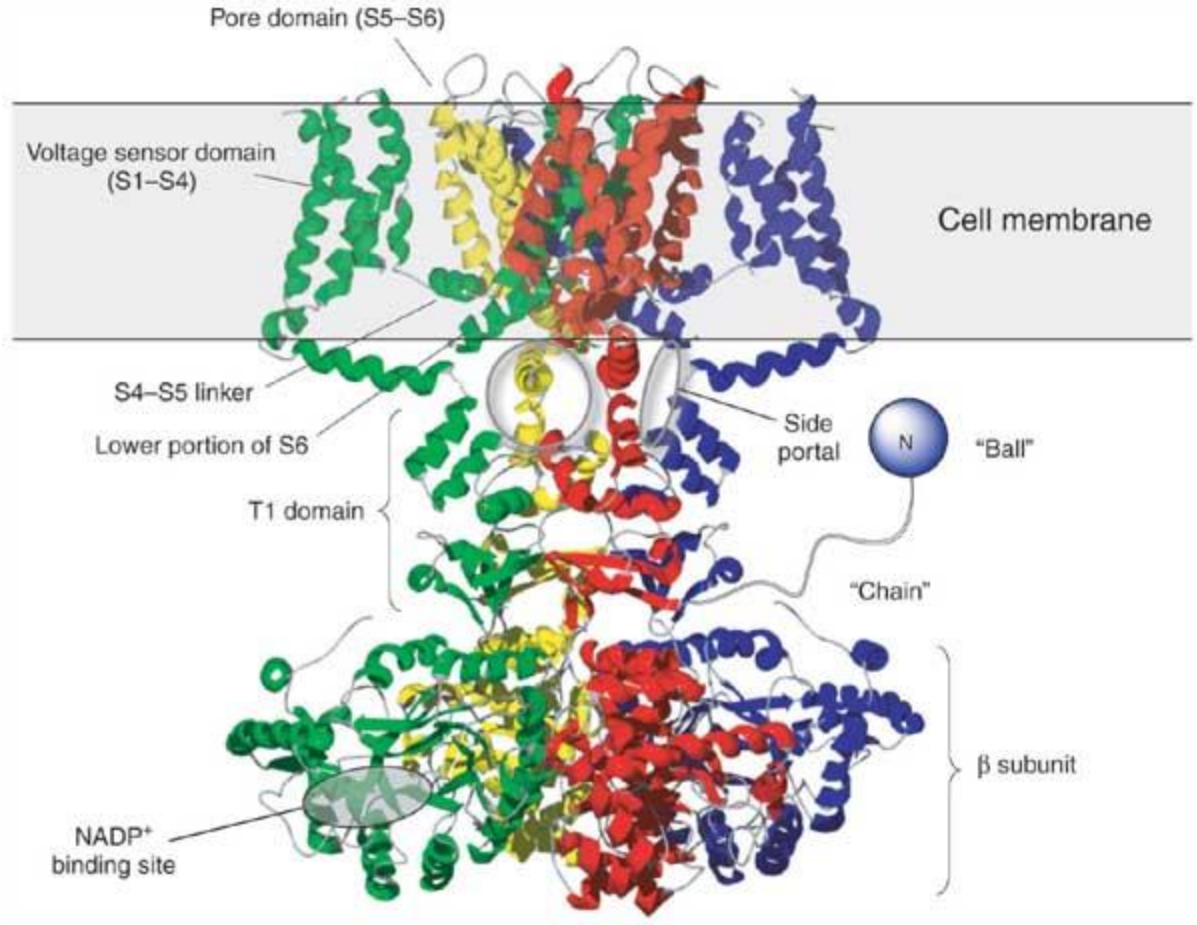
• K_V1 channels are often associated with an intracellular subunit ($K_V\beta$ 1-3). The N terminus of $K_V\beta$ subunits serves as an N-type inactivation gate for K_V1 α subunits. The β subunits bind to the N terminus of the α subunits.

• K_V4 channels interact with the K channel interacting proteins KChIP1-4. The KChIPs enhance expression of K_V4 channels and modify their functional properties.

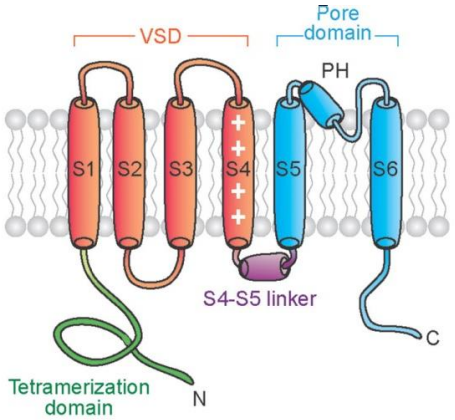
• The K_V3, K_V4, K_V7, K_V10, and K_V11 channels associate with the minK subunits (there 5 of them). The minK subunits are important regulators of K_V channel function. In particular, they significantly slow down C-type inactivation.

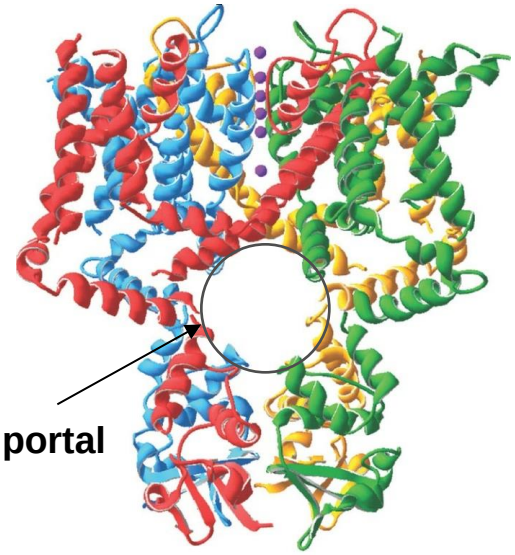
Yu et al, 2005

Crystal structure of the $K_v1.2/K_v\beta$ complex



2.9 Å





Long et al. 2005

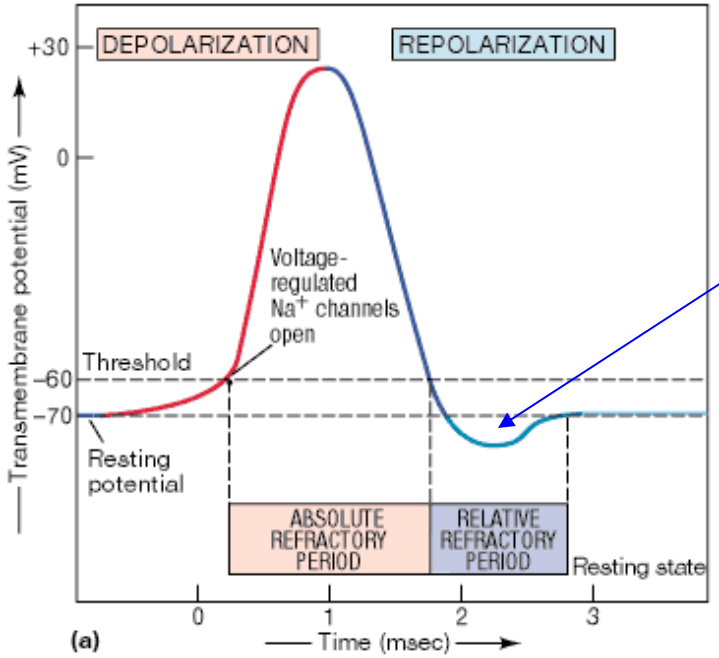
K_{Ca}, Ca^{2+}-activated K^+ channels

Single-channel patch-clamp recordings identified two types of K_{Ca} channels: small conductance (SK) and high-conductance (BK)

Gating: opened by elevation of intracellular Ca^{2+} with K_d ~ 0.5 μM (SK), opened by elevation of intracellular Ca^{2+} & depolarization (BK)

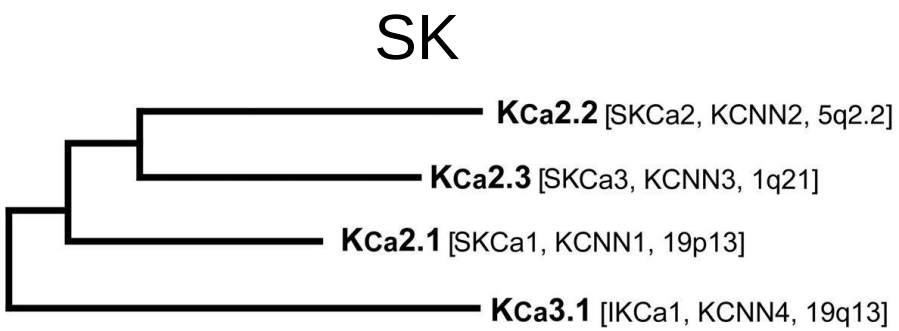
Location: Plasma membrane of neurons, muscle cells and some non-excitable cells

Function: **negative-feedback system for Ca^{2+} entry in many cell types**, slow afterhyperpolarization (up to 1 second, SK channels), fast afterhyperpolarization (several milliseconds, BK channels) and presynaptic regulation of neurotransmitter release (BK channels)

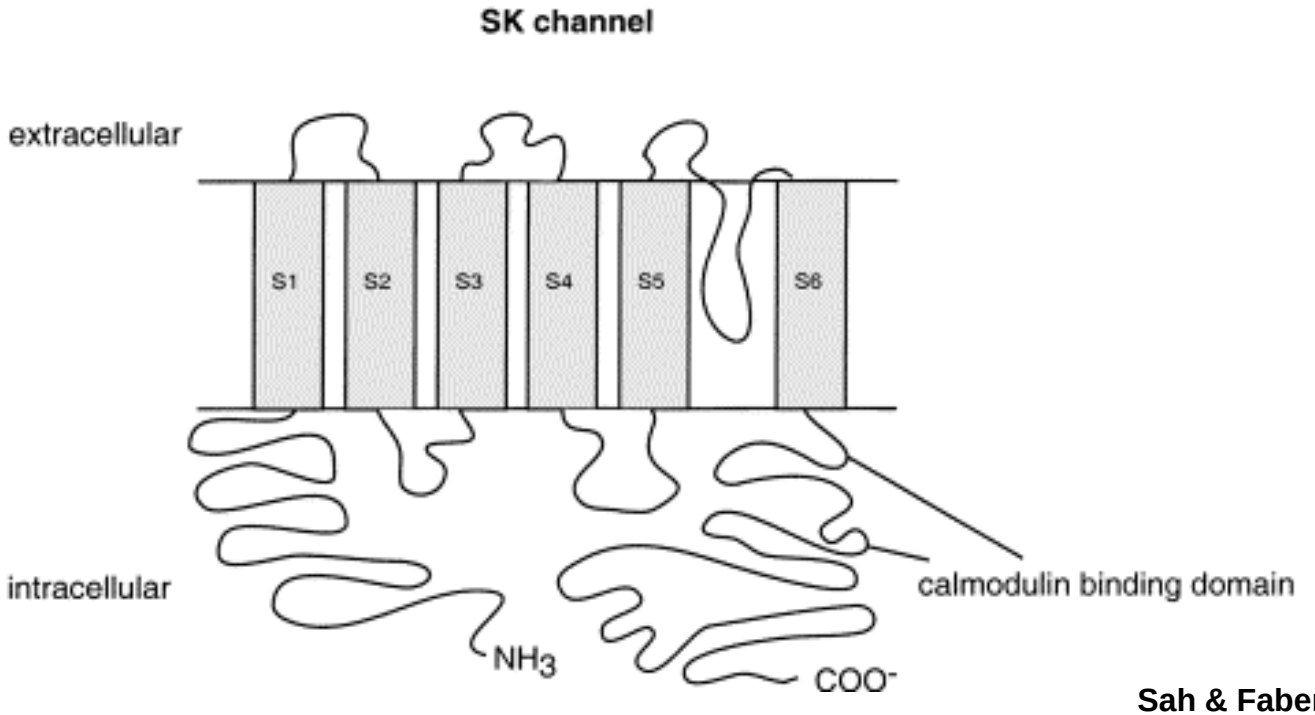


Afterhyperpolarization can be long, up to 1 second

K_{Ca}, Ca^{2+}-activated K^+ channels

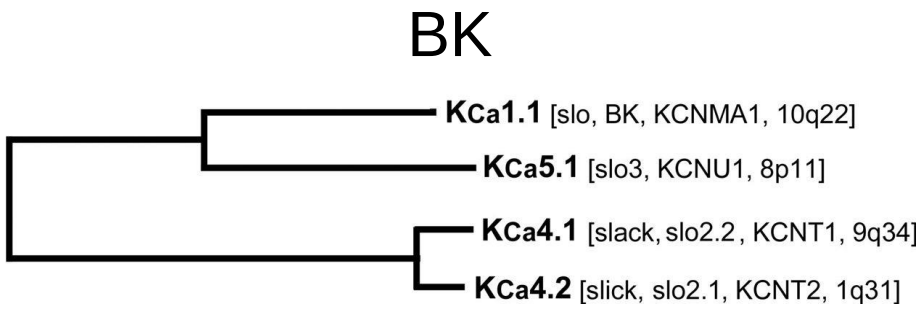


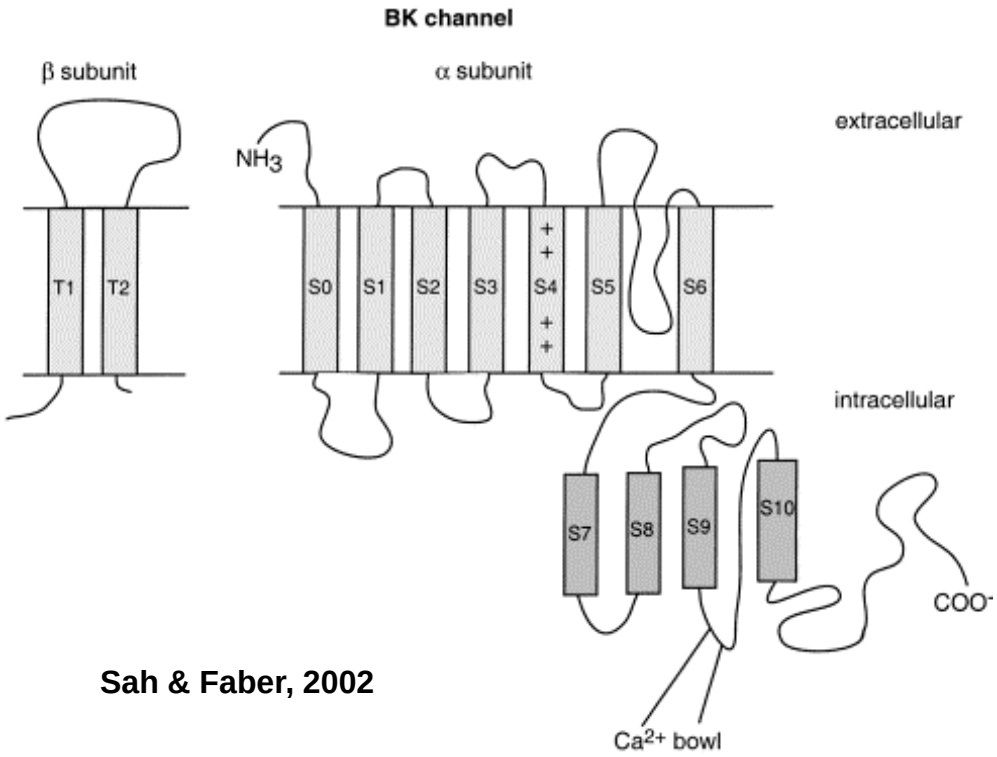
- SK α subunits make homo-tetrameric and likely hetero-tetrameric complexes.
- There is no charge in S4 domain.
- Calmodulin is constitutively bound to C-terminal domain of the channel.



Sah & Faber, 2002

K_{Ca}, Ca^{2+}-activated K^+ channels



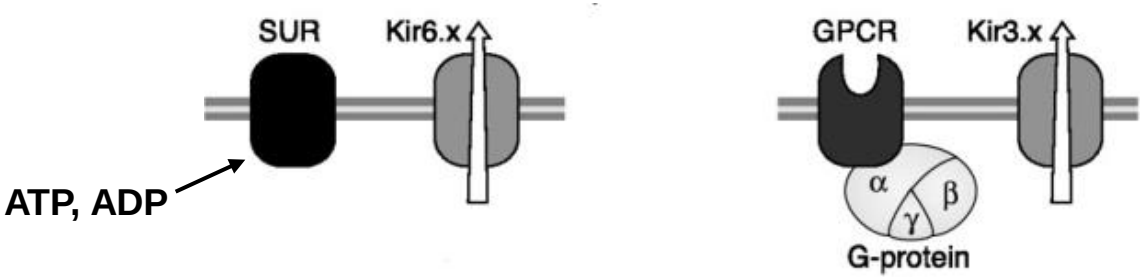


Sah & Faber, 2002

- Interestingly, $K_{Ca}4.1$, $K_{Ca}4.2$ and $K_{Ca}5.1$ are insensitive to intracellular Ca^{2+}.
- $K_{Ca}4.1$, $K_{Ca}4.2$ are gated by depolarization and synergistically by elevation of intracellular Na^+ and Cl^-.
- $K_{Ca}5.1$ is gated by voltage and elevation of intracellular pH. This channel is sperm-specific and controls sper membrane potential.
- BK α subunits make homo-tetrameric and likely hetero-tetrameric complexes.
- BK channels interact with four β subunits (β1-4) that can confer a higher Ca^{2+} sensitivity and faster inactivation on the channel.
- S0 transmembrane domain of BK channels is important for interaction with the β subunit.

K_{ir}, inwardly rectifying K^+ channels

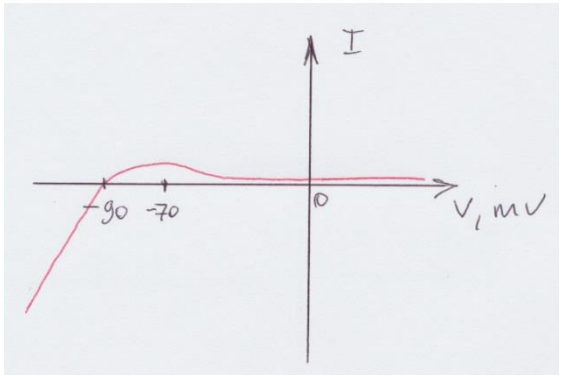
Gating: constitutively active ($K_{ir}1.1$, $K_{ir}4$ subfamily, $K_{ir}5.1$), opened at voltages negative to E_K and closed at voltages positive to E_K ($K_{ir}2$ subfamily, $K_{ir}7.1$), opened by $G_{\beta\gamma}$ subunits ($K_{ir}3$ subfamily), opened by ADP ($K_{ir}6$ subfamily), closed by intracellular ATP ($K_{ir}6.2$).



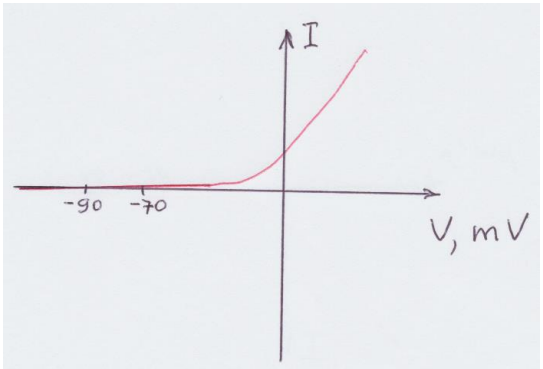
Location: plasma membrane of excitable and non-excitable cells

Function: **as a generalization, K_{ir} help maintain the resting membrane potential and control excitability of neurons and muscle cells**; transepithelial K^+ transport ($K_{ir}1.1$, $K_{ir}7.1$), G protein coupled (serotonin, metabotropic glutamate, etc.) receptor-dependent membrane hyperpolarization, regulation of insulin secretion in pancreatic β-cells ($K_{ir}6.2$), **oxygen and glucose sensor in brain ($K_{ir}6.2$)**, cytoprotection during cardiac and brain ischemia ($K_{ir}6.2$).

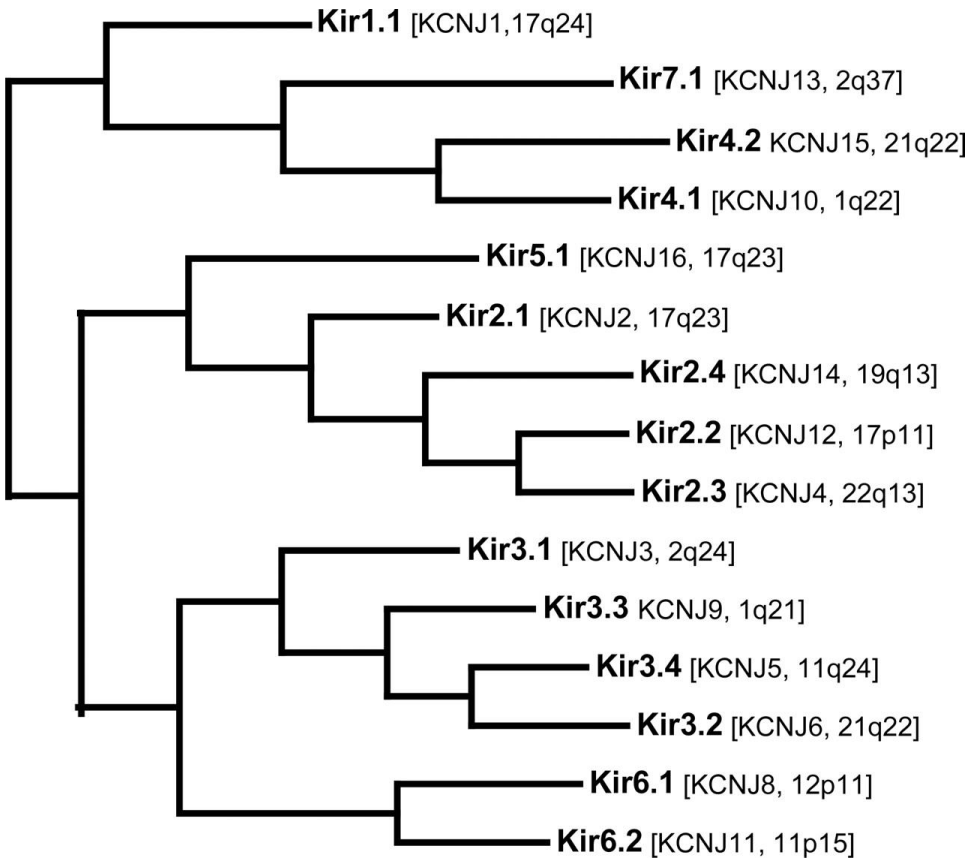
Inwardly rectifying K^+ channel



Outwardly rectifying K^+ channel

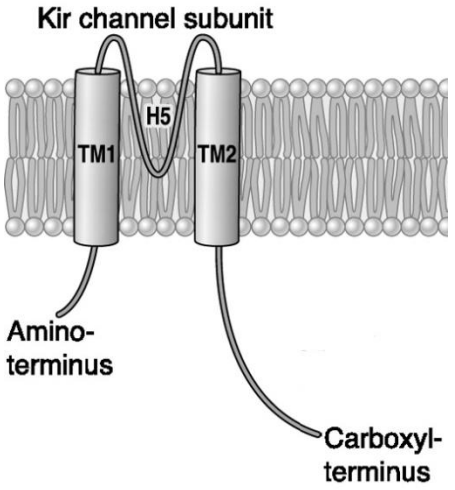


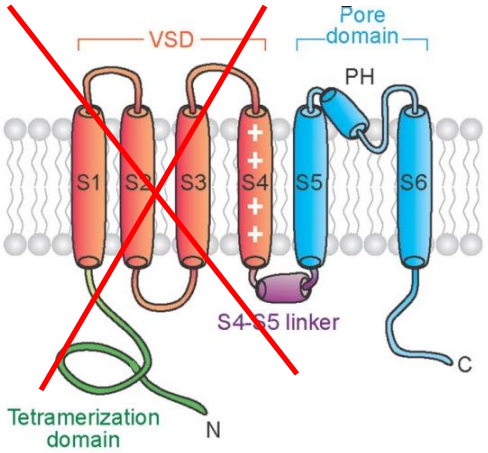
K_{ir}, inwardly rectifying K^+ channels



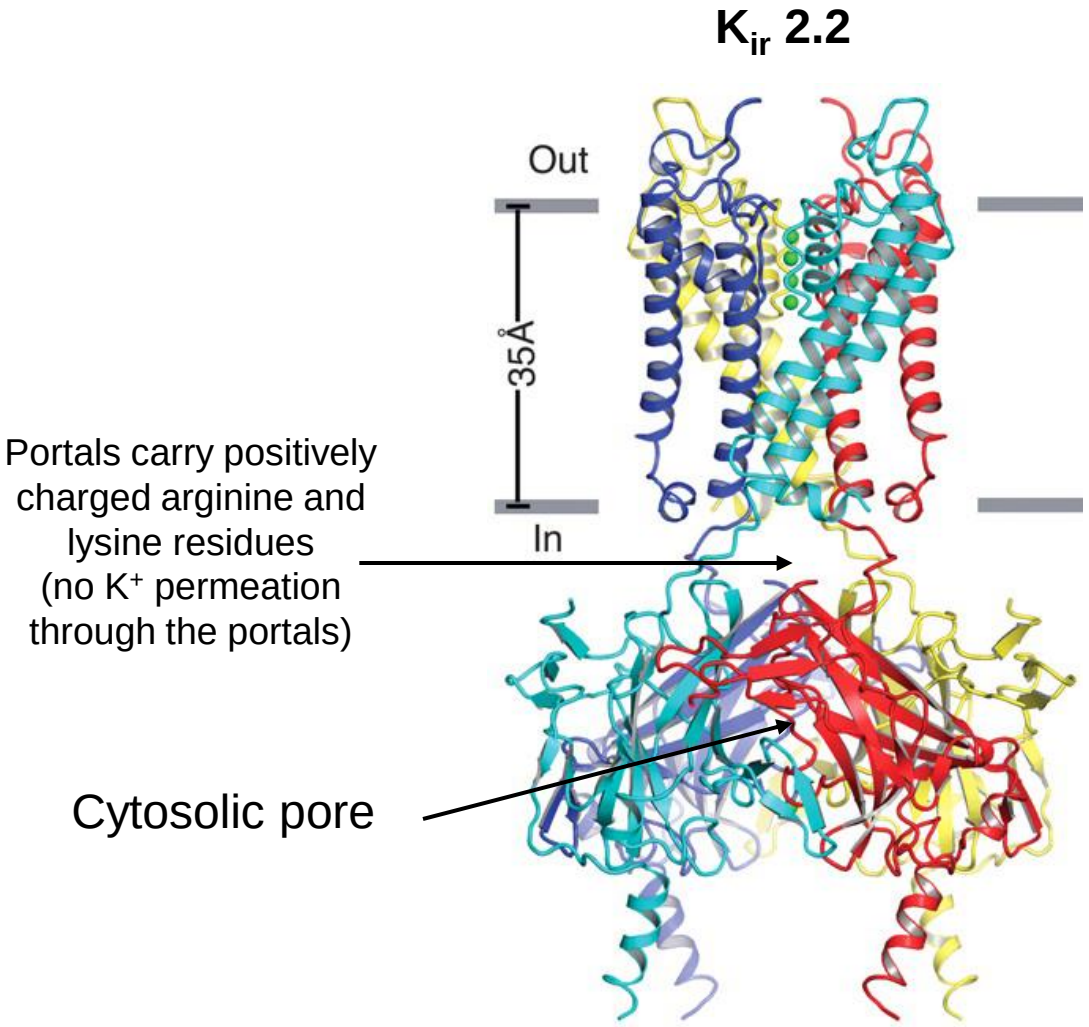
Sansom et al, 2002

- K_{ir} subunits form homo-tetrameric and hetero-tetrameric channels



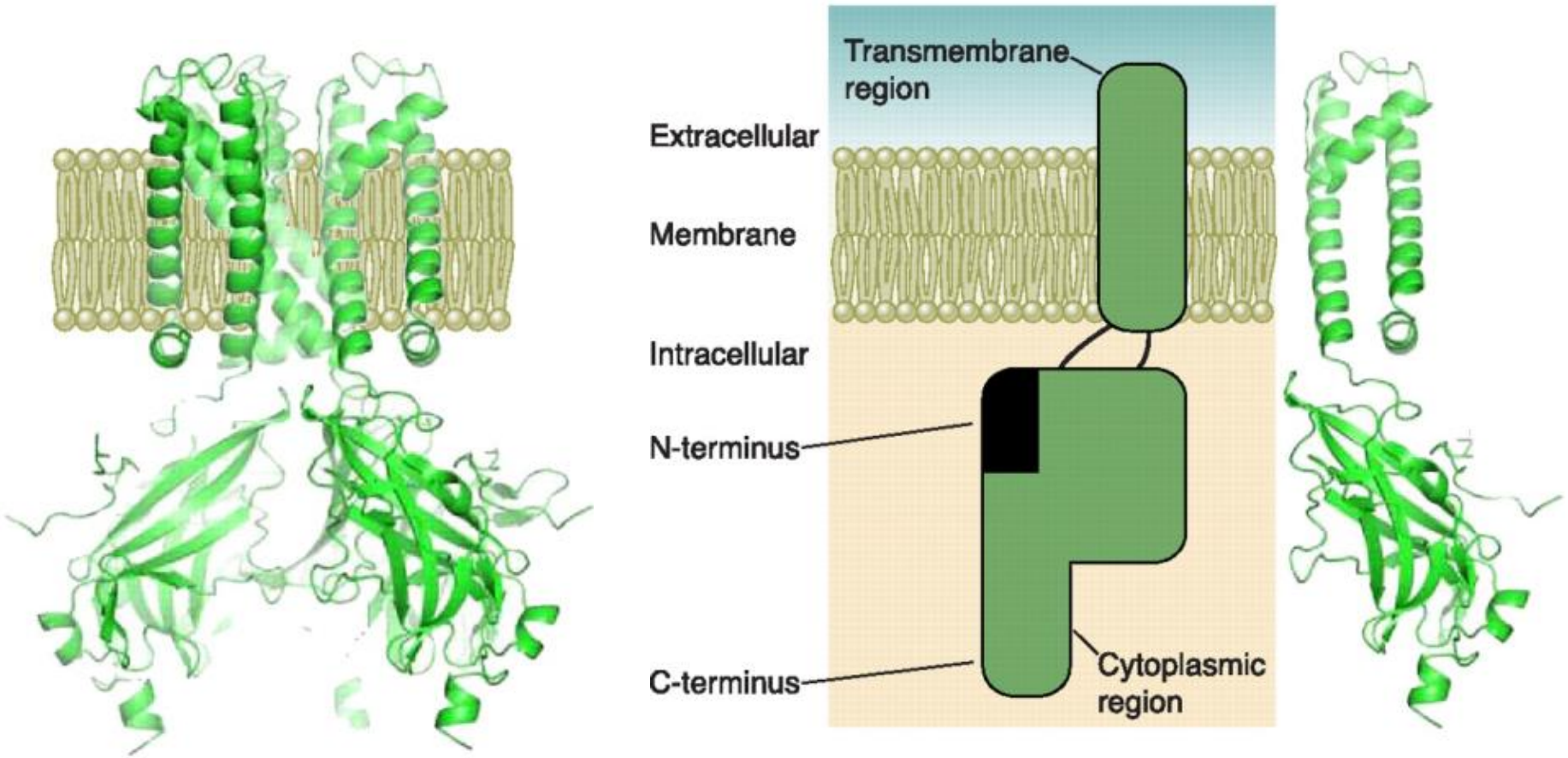


Structure of inwardly rectifying K^+ channel



Tao et al, 2009

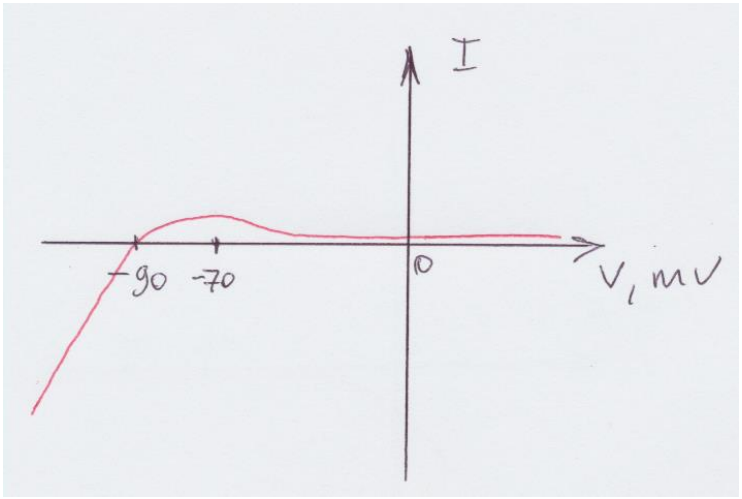
Structure of inwardly rectifying K^+ channel

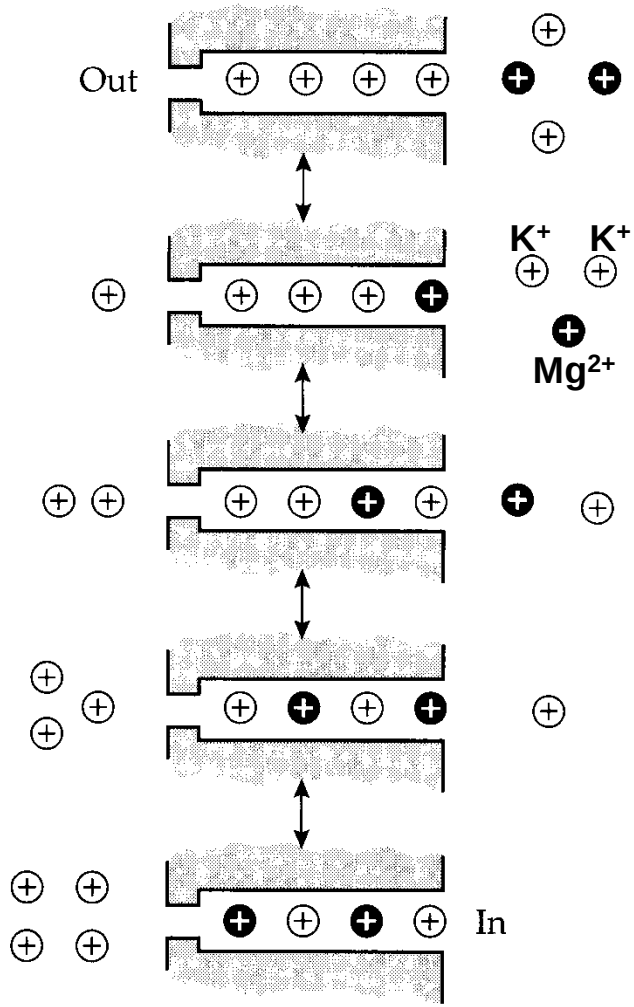


Hibino et al, 2010

Inward rectification of K_{ir} channels

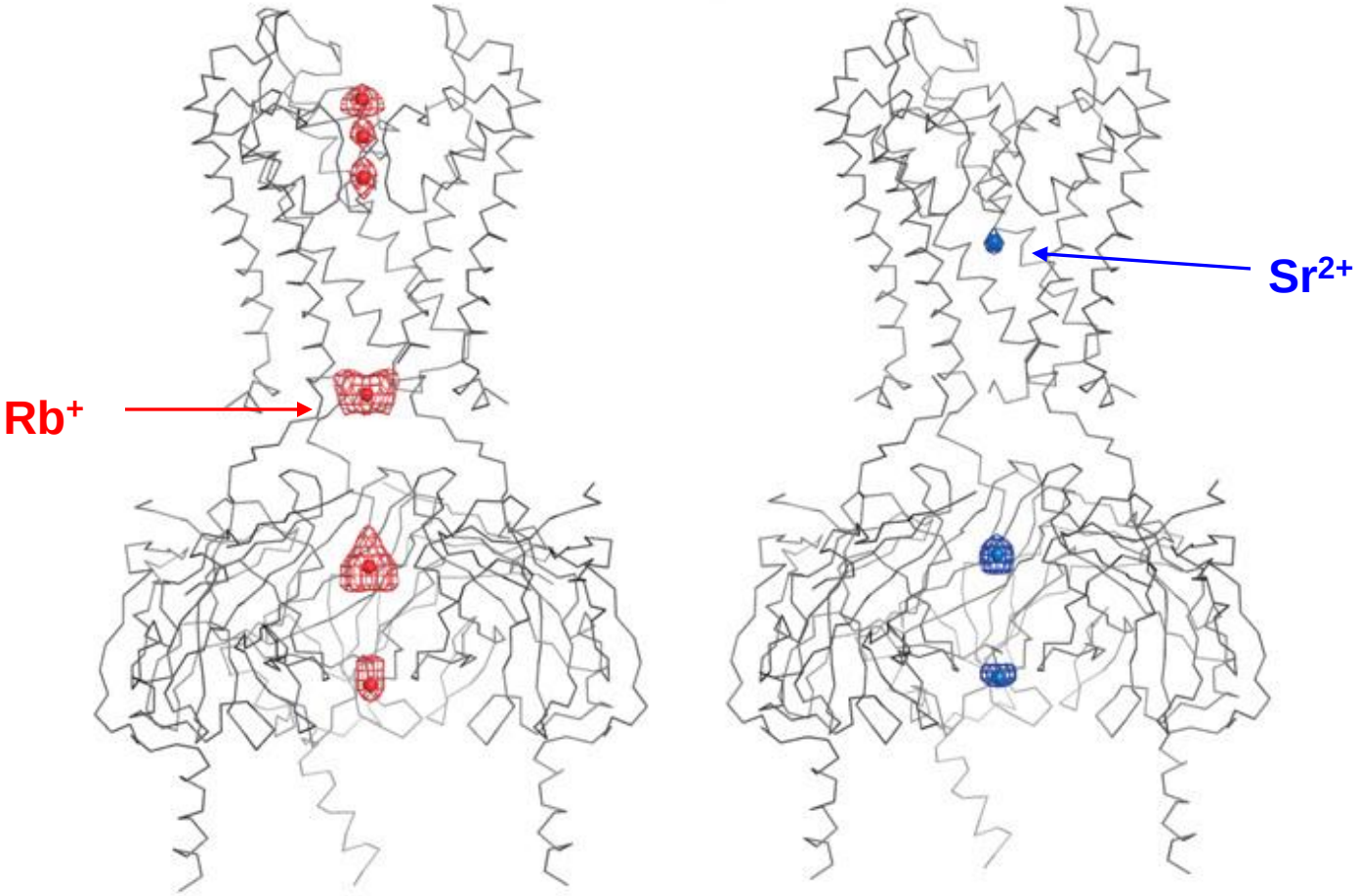
16.12 Multi-Ion Block in a Long Pore
Hypothetical occupancy states of a pore with four occupied ion binding sites. The pore is permeable to the white cation, but the selectivity filter is too narrow to pass the black ion. Current from right to left will draw the black ion from the cytoplasm into the pore, blocking further flow. Current from left to right draws permeant ions from the outside through the selectivity filter and clears the pore.





Structural basis of inward rectification of K_{ir} channels

Extracellular



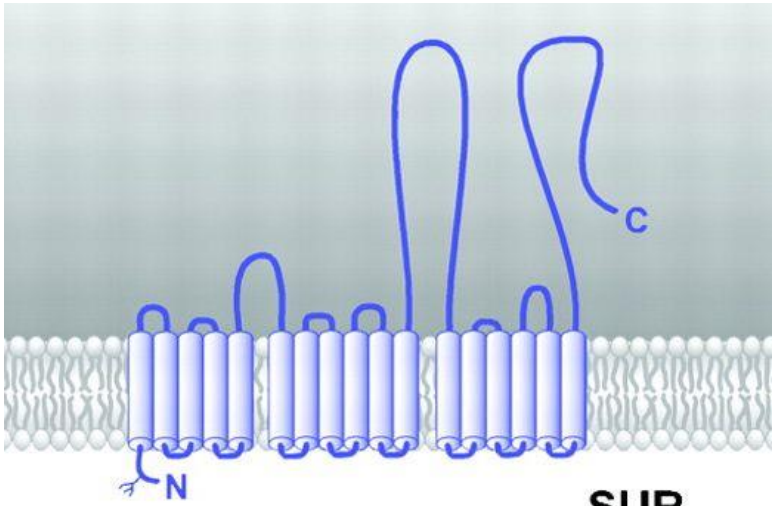
Intracellular

Tao et al, 2009

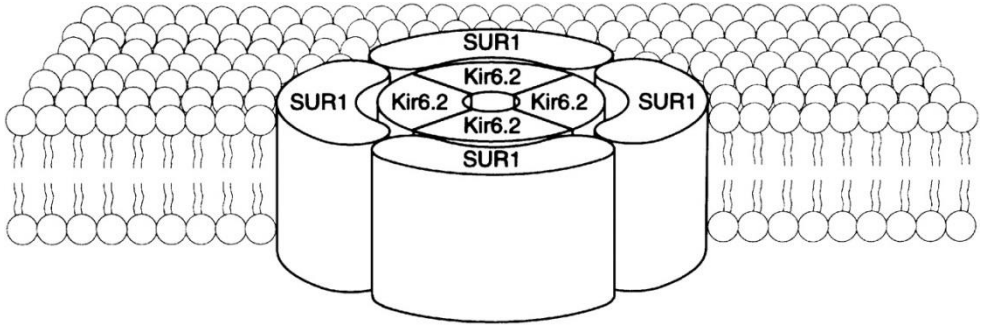
K_{ir}, inwardly rectifying K^+ channels

Sulfonylurea receptors (SUR1 and SUR2) confer ATP/ADP sensitivity on the $K_{ir}6$ ion channels.

Auxiliary subunit of $K_{ir}6$ subfamily



Modified from Yu et al, 2005



Seino, 1999