The Potassium Ion Channel: 1952-1998

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Ions:

- Cell volume regulation
- Electrical impulse formation (e.g. sodium, potassium)

Lipid membrane: the dielectric barrier

- Pro: compartmentalization
- Con: Dielectric barrier
- Solution: water filled pores

The Pore Theory

- Traced as far back as the 1840s in attempt to explain osmosis
- Pores would pass water and small particles

Ions in the squid axon

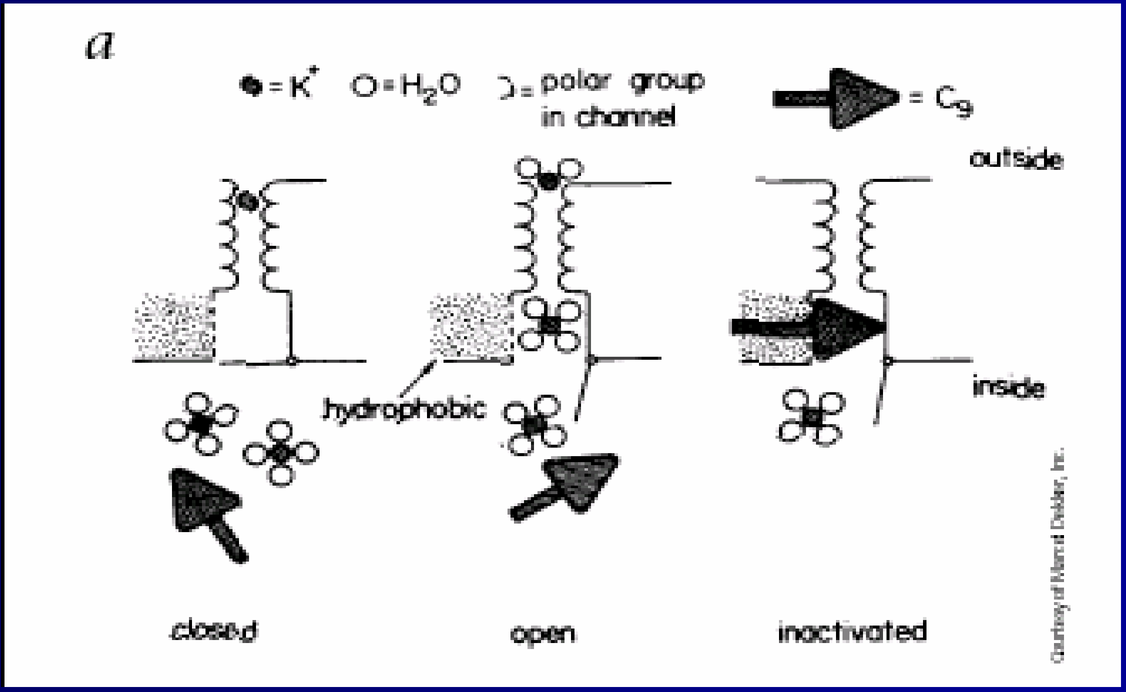
- 1952: Hodgkin and Huxley describe action potential propagation
- Describe changes in Na+ and K+ permeability
- Hodgkin and Keynes' isotopic K+ flux-ratio experiments:
  - "ions cross the membrane along a chain of negative charges or through narrow tubes or channels…in which they are constrained to move in single file [with] several ions in the channel at any moment."

The debates of 1965 - 1973

- Ions pass through aqueous pores called channels
- Ion channels are proteins
- The channels for Na+ and K+ are different
- They have gates that open and close them in response to changing membrane voltage

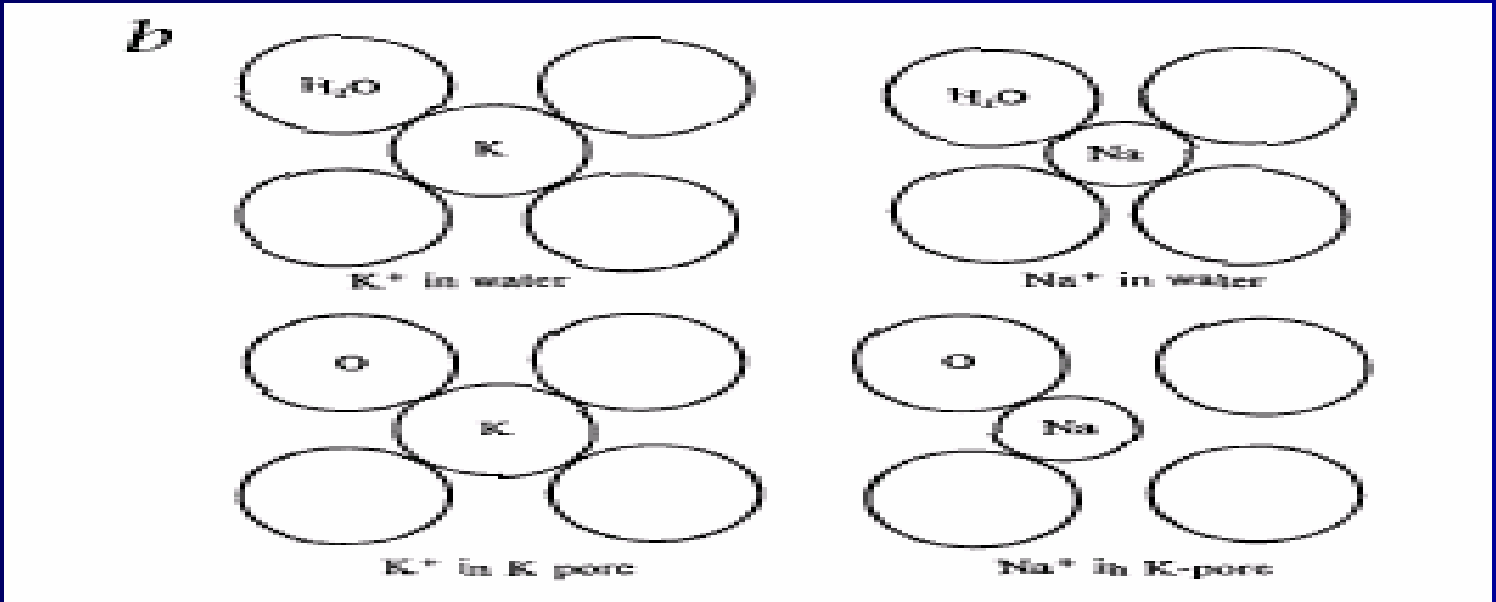
Early ideas of channel architecture and selectivity: 1971-1972

- Binstock and Armstrong: TEA+
  - Derived K+ flux to be about one K+ ion per microsecond
- Armstrong and Hille using C9+, a TEA variant, proposed idea of 'gates':



Objections

- How can a channel be selective? If the ion stuck too tightly it would block rather than permeate
- Armstrong, 1971:



Enter Rod MacKinnon: 1986-1998

- How does a small scorpion toxin inhibit a potassium channel?
  - Scorpion toxin occludes the potassium channel's ion pathway
- How does the potassium channel select potassium over sodium while still conducting it near the diffusion limit?

1987: *Shaker* is cloned

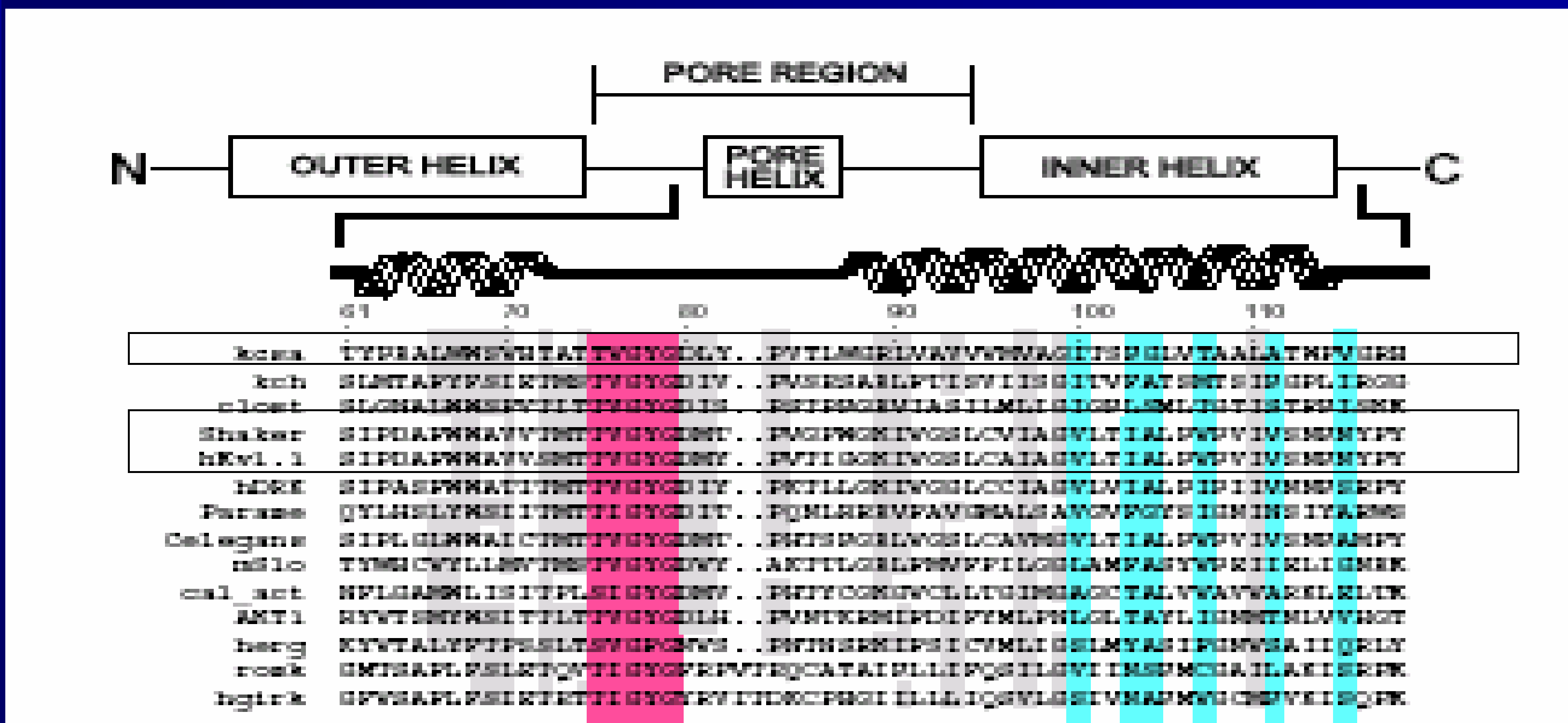
- First potassium channel gene cloned
- Little information about the channel structure
- Used scorpion toxin to identify which of the amino acids form the ion pathway
- Using methods such as site directed mutagenesis, they reached a number of important conclusions about the potassium channel architecture

K+ channel before crystallography

- The channel contains four identical subunits arranged in a symmetric ring around a central pore
- Pore loop (P-Loop): short amino acid segment between two transmembrane helices that dips into the membrane without crossing it
  - P-Loop has the "signature sequence": Thr-Val-Gly-Tyr-Gly
- This signature sequence is responsible for potassium selectivity
- The four pore loops, one from each subunit, meet near the channel's central axis to form the narrowest point along the ion pathway

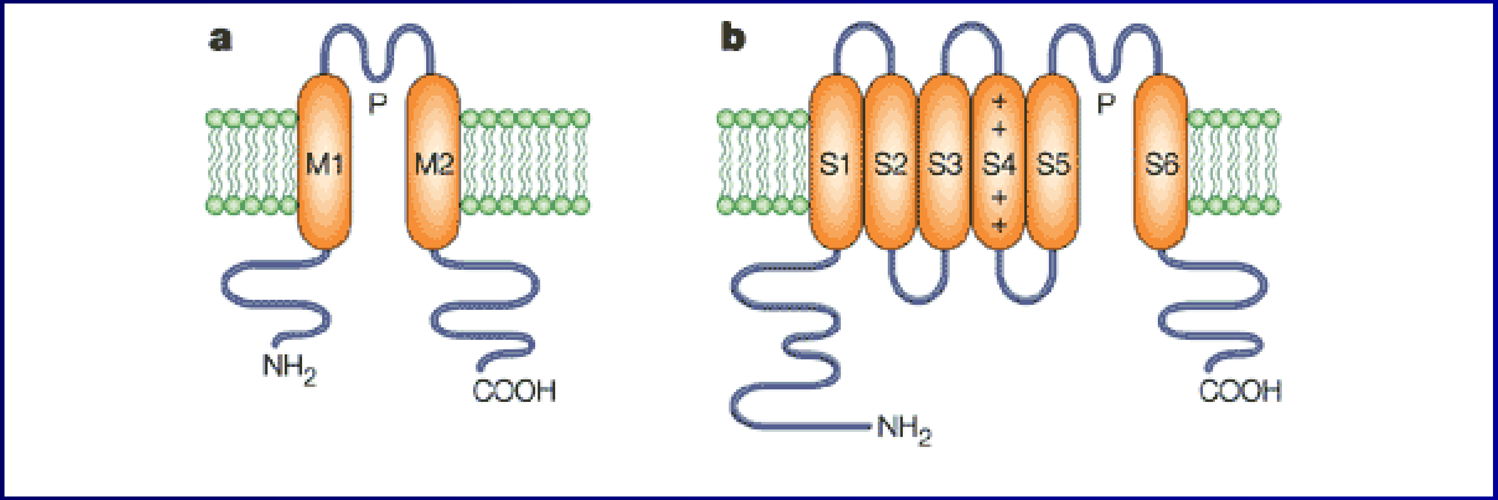
Meanwhile…more K+ channels are cloned

- Signature sequence used as a key to find potassium channels even in bacteria



Why the KscA potassium channel?

- Hyrdophobicity plot shows two closely related varieties of K+ channels



- Although KscA is a 2TM channel, its amino acid sequence is closer to those of eukaryotic 6TM channels

X-ray crystallography

- **Protein preparation**
- **Crystallization**
- **Solve the structure**
  - Measurement of native diffraction data
  - Obtaining heavy atom derivatives
  - Measurement and analysis of derivative data
  - Calculation of phases
  - Map interpretation and model building
  - Model refinement

Protein Preparation

- Obtain sufficient quantities of material
  - Overexpression of KscA in E.Coli
- Homogenous and active

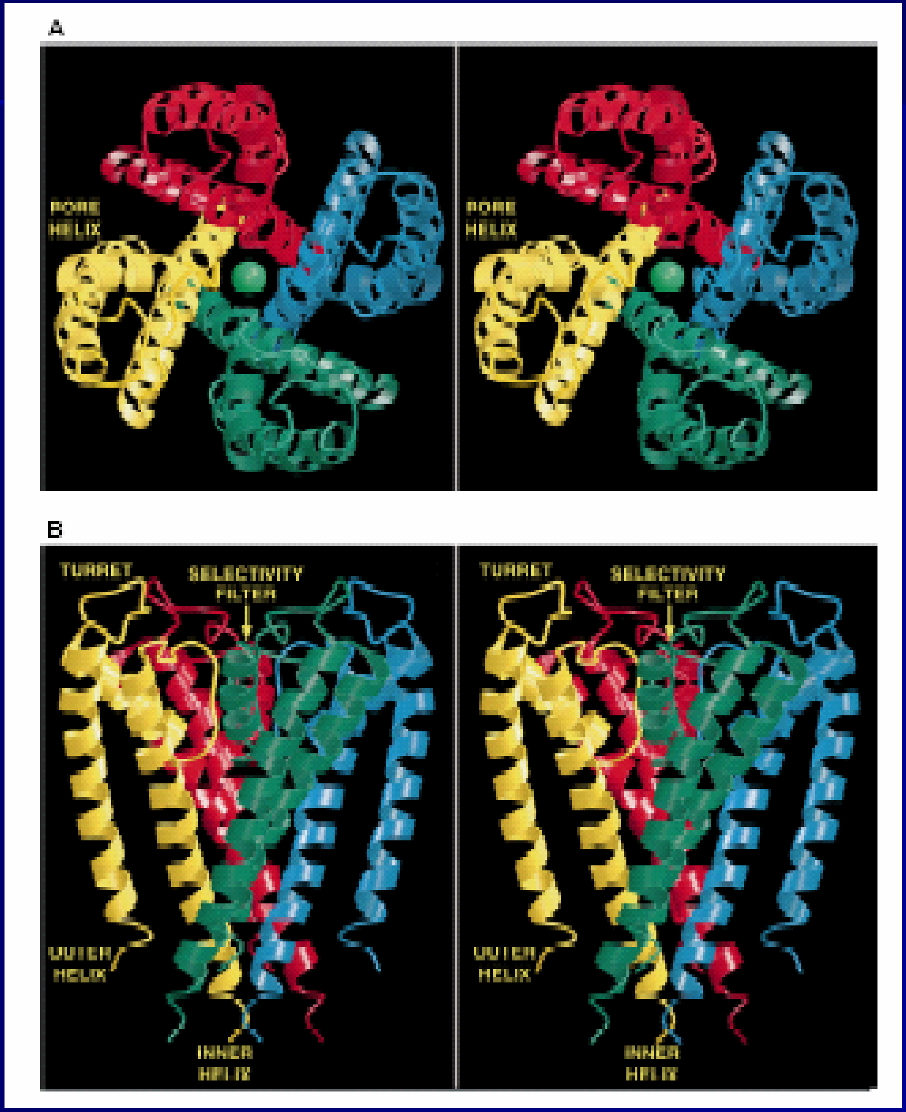
Crystallization

- Rate limiting step
- May require additives such as heavy atoms
- May require removing something

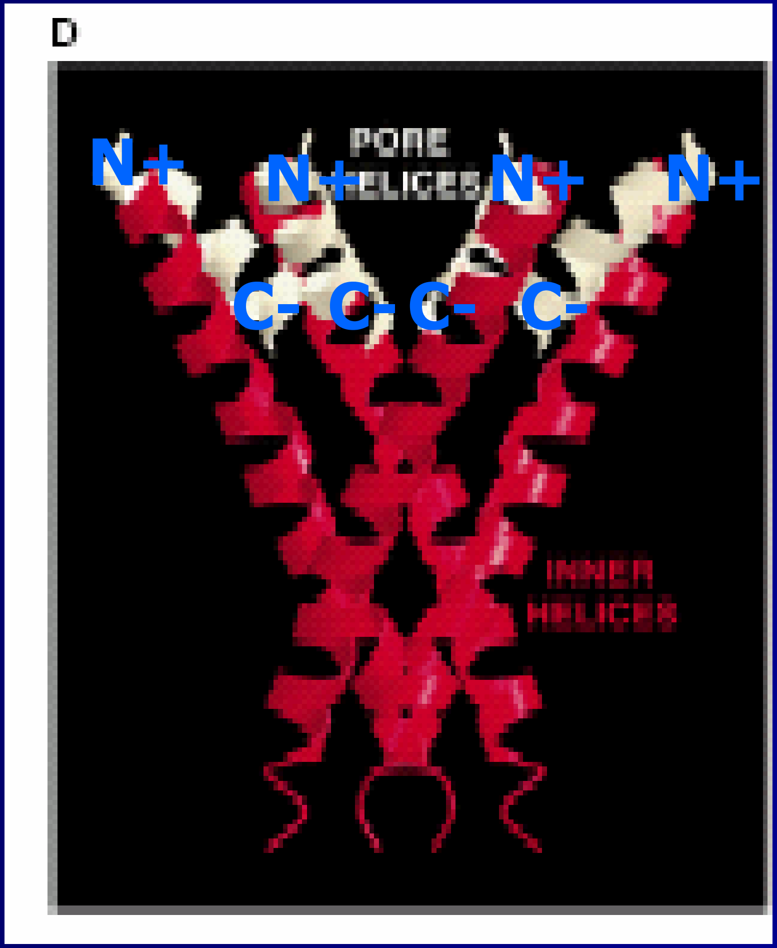
Electron density maps of KscA K+ channel

- Solved to a resolution of 3.2 Å
  - Minimum separation of two groups in the electron-density plot that can be distinguished from one another
- At this resolution the path of the polypeptide backbone can be traced

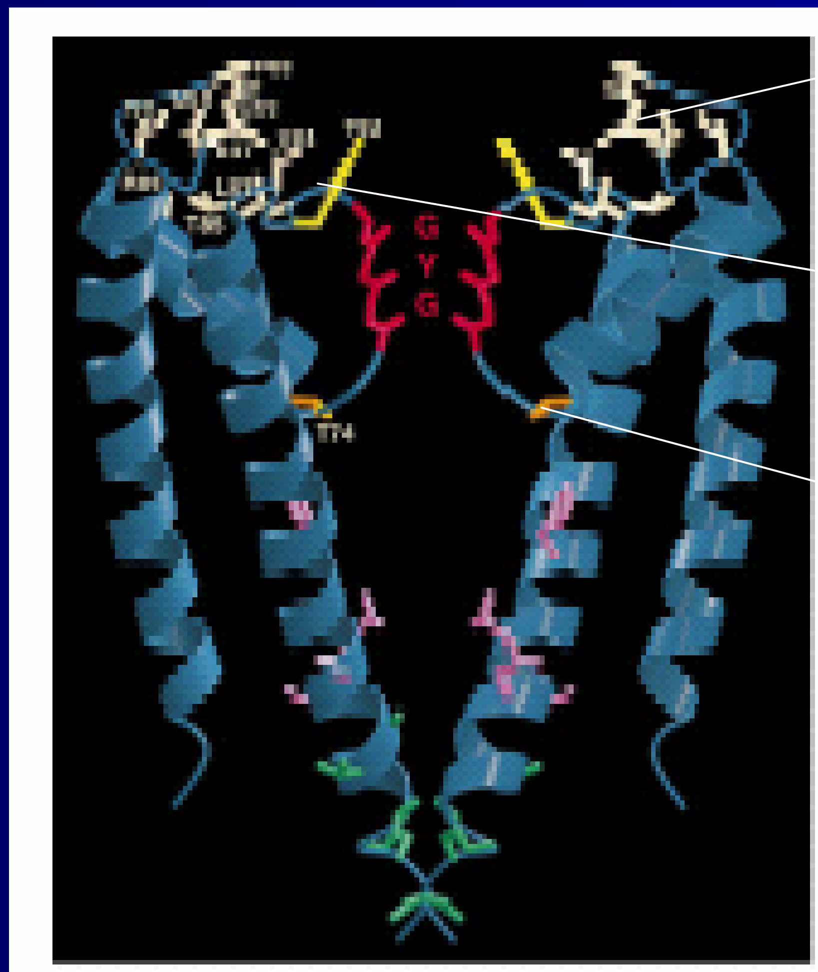
K+ channels is a tetramer



Pore helices



Mutagenesis studies on Shaker: Mapping onto the KcsA structure



Conotoxin

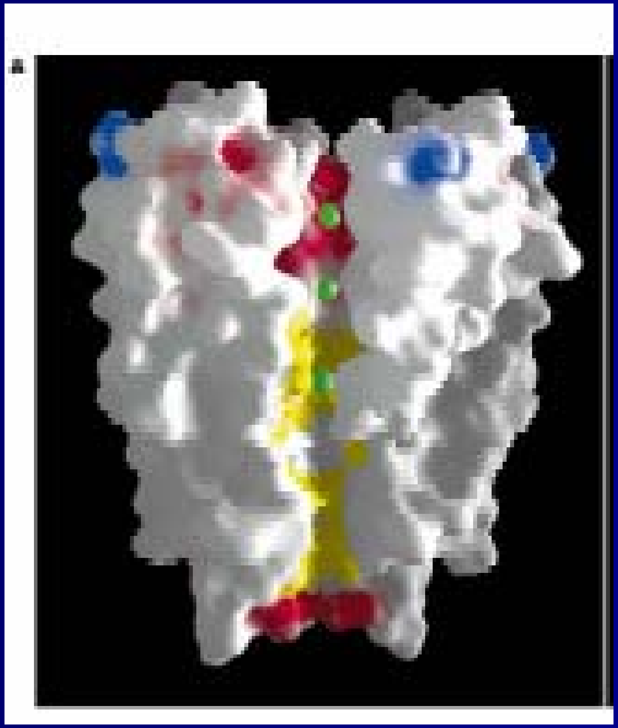
or agatoxin2

External
TEA site

Internal TEA site

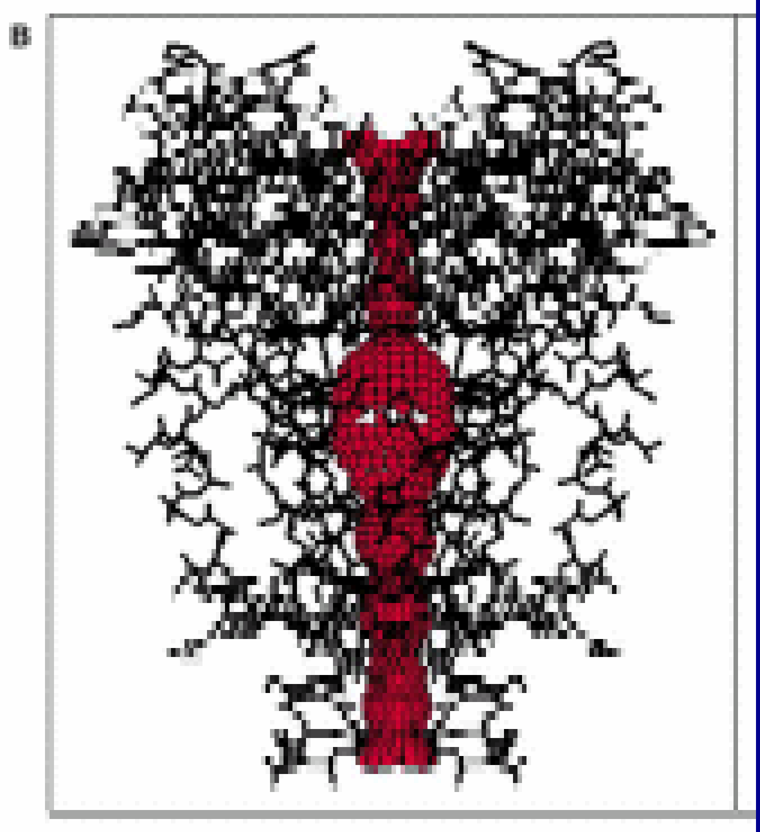
General properties of the ion conduction pore

- Both intracellular and extracellular entryways are negatively charge



General properties of the ion conduction pore

- Overall length of the pore is 45 Å



External pore:

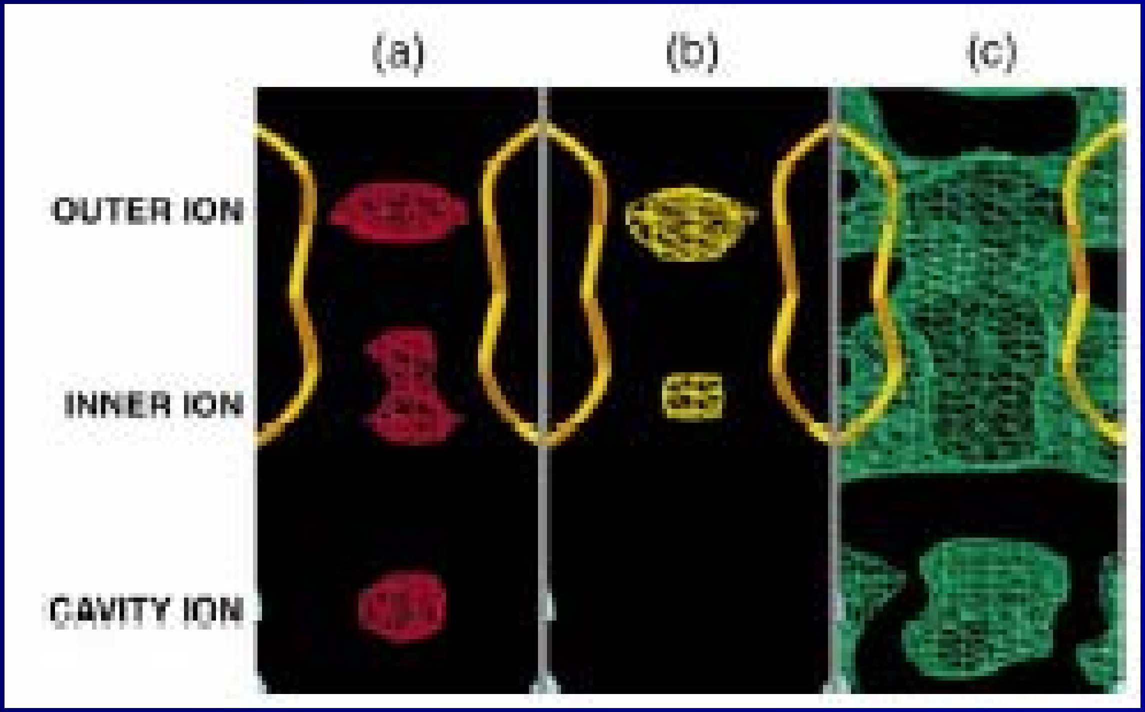
~12 Å long, ~2.5 Å across

Cavity: ~10 Å across

Internal pore: ~18 Å long

K+ ion positions in the pore

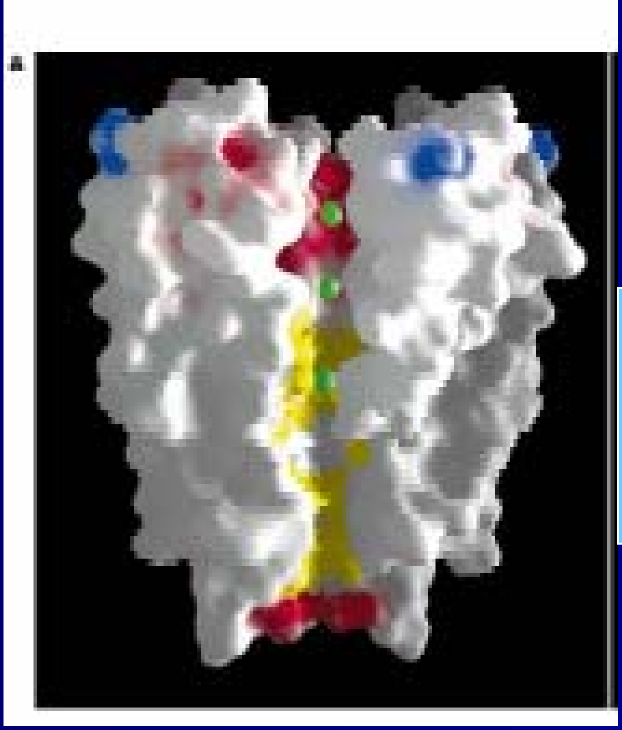
- Li^+ 0.60 Å, Na^+ 0.95 Å
- Rb^+ 1.48 Å, Cs^+ 1.69 Å, K^+ 1.33 Å



The Cavity and Internal Pore

- Why is there an ion in the 10 Å diameter cavity? Electrostatic considerations

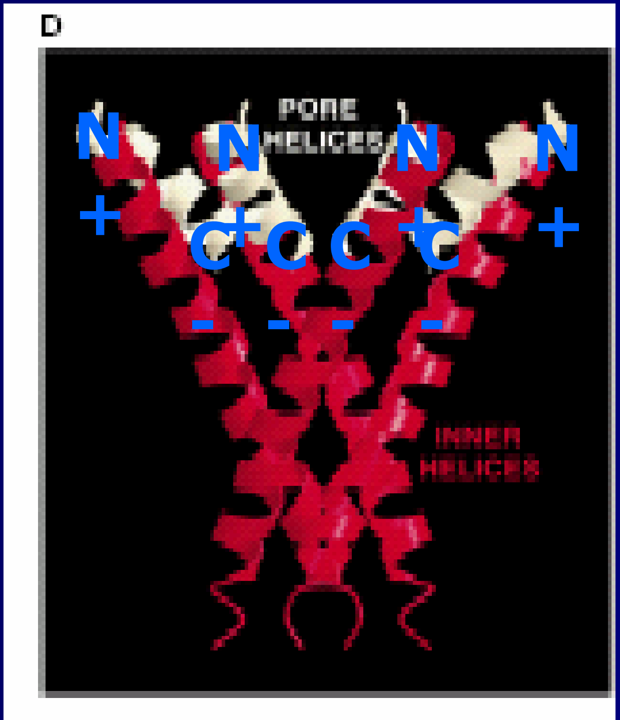
Lipid bilayer

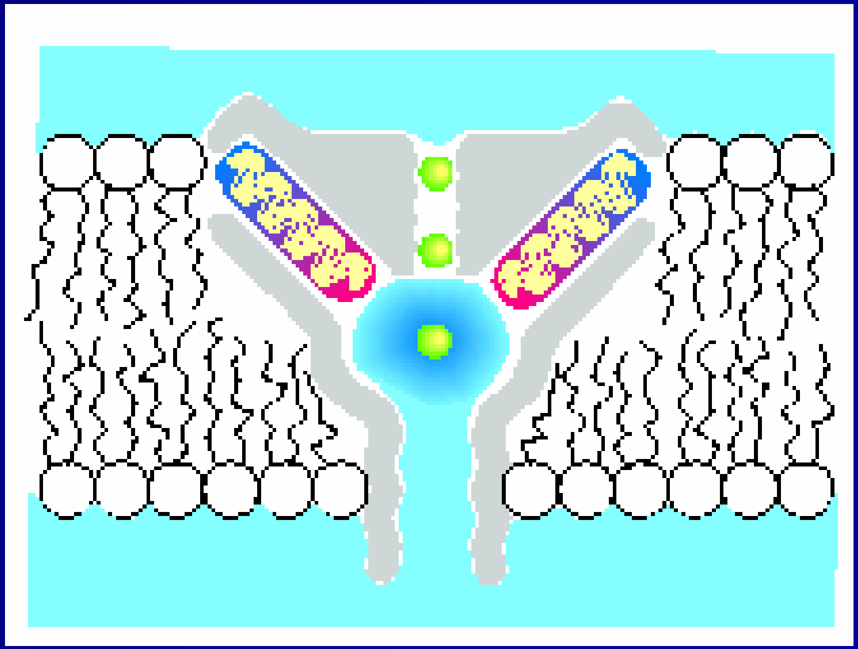


Low dielectric bilayer

The Cavity and Internal Pore

- Stabilizing the ion at the cavity center:
  - Surround the ion with polarizable water
  - Negative electrostatic potential from four pore helices



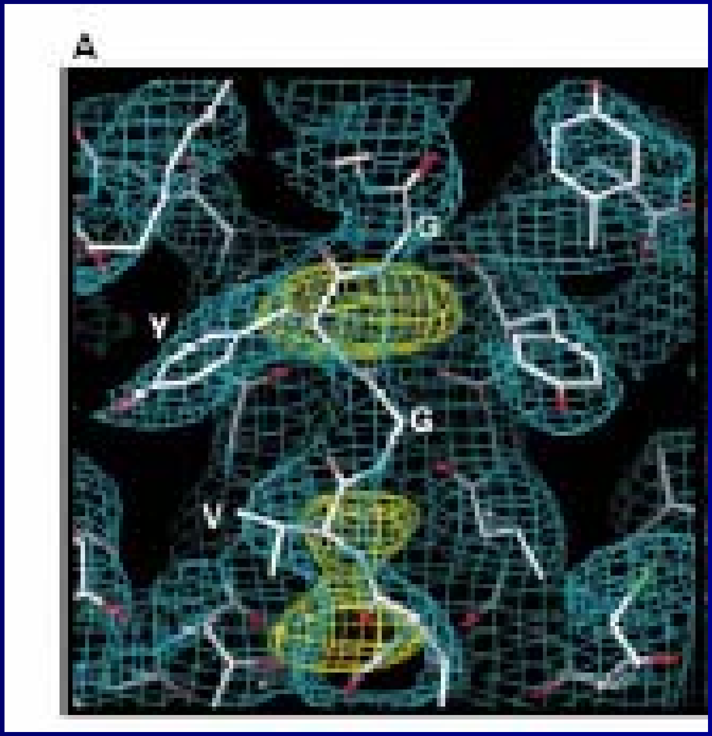


The Cavity and Internal Pore

- What is the significance of the hydrophobic lining?
  - Achieve a high throughput by having a relatively inert surface over most of the length of the pore

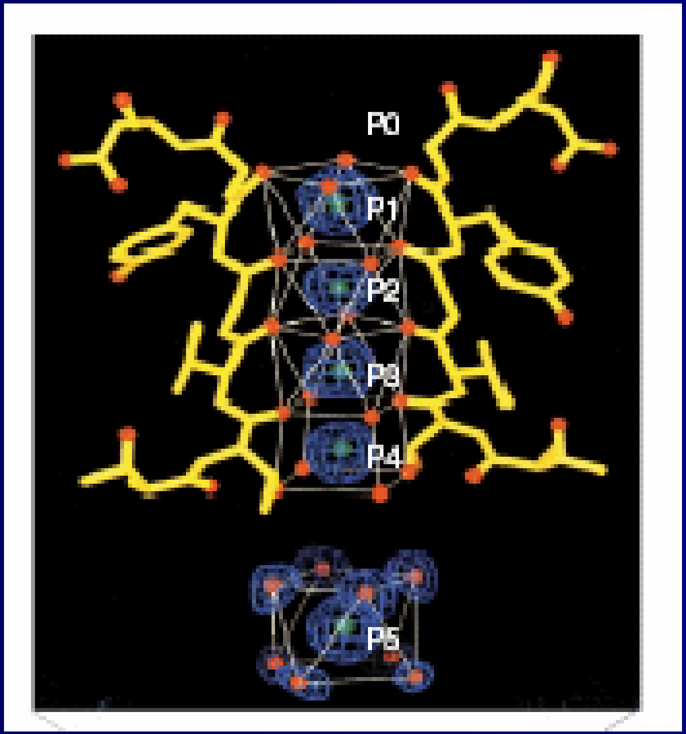
The selectivity filter

- Electron density in the selectivity filter
- Continuous ridge of electron density attributable to the main chain



The selectivity filter

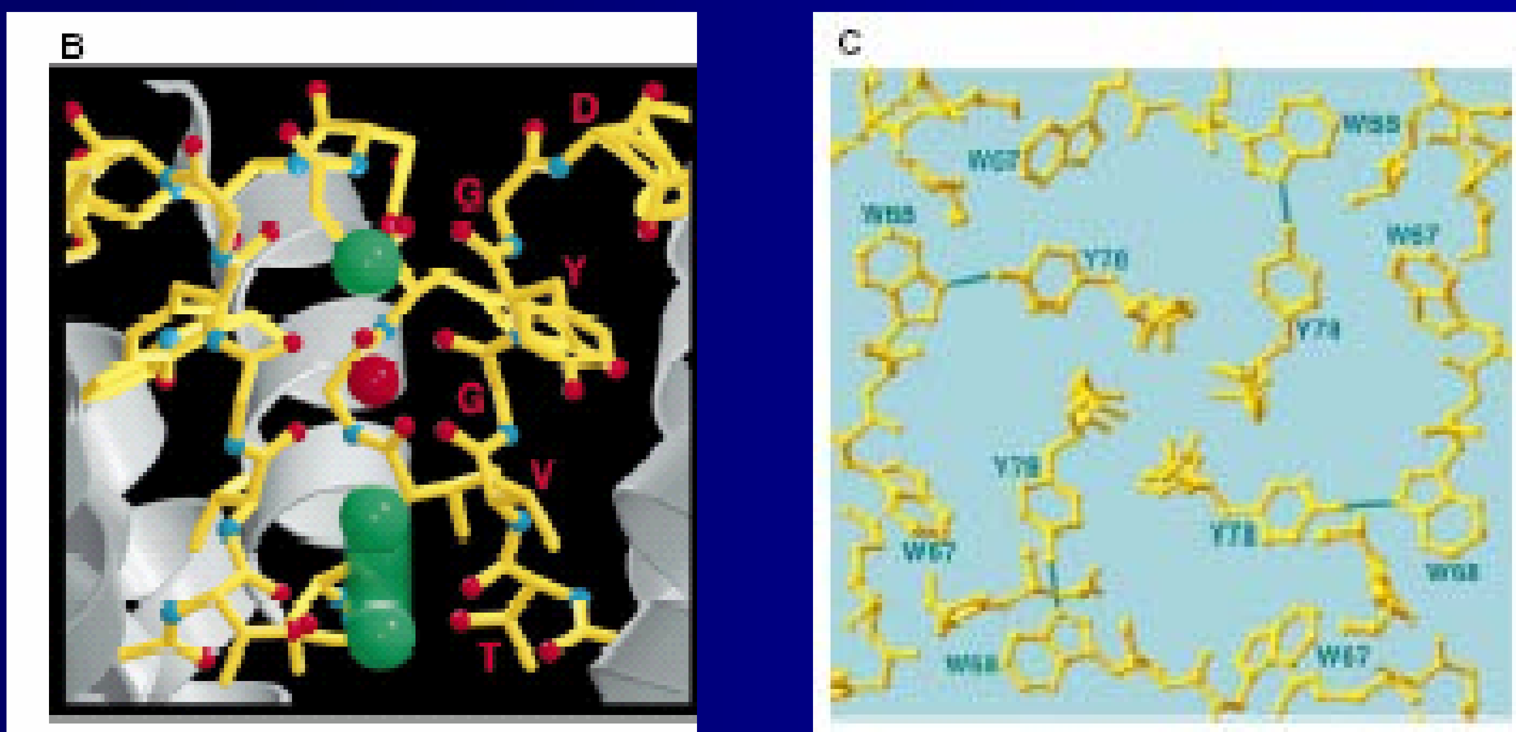
- The structure of KcsA at a resolution of 2.0 A (Zhou et al. 2001. *Nature*. 414:43-48.)



four internal binding sites (P1-P4) and two external (P0 and P5)

The selectivity filter

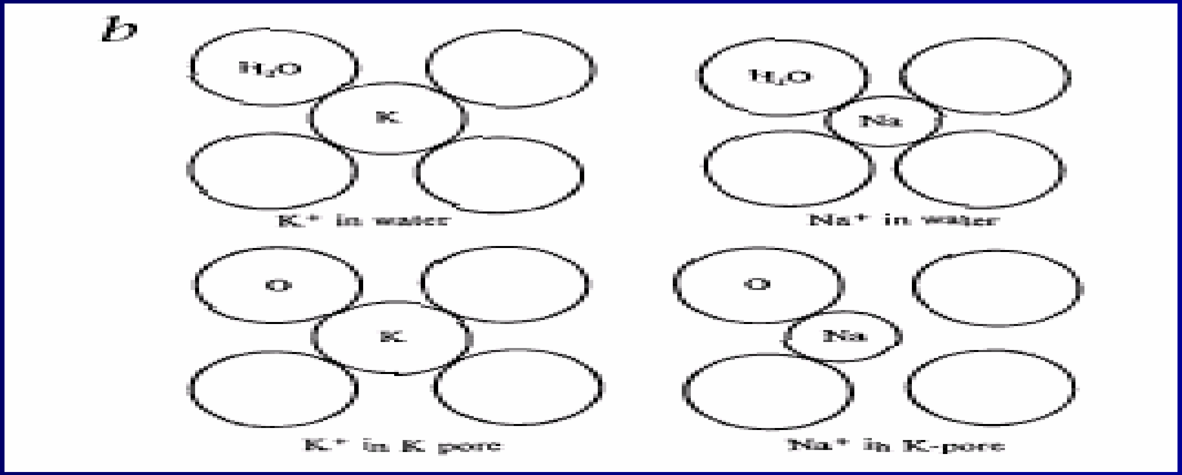
- Two essential features of the selectivity filter:
  - Main chain atoms create a stack of sequential oxygen rings
  - Side chains pointing away from the pore interact with residues from the pore helix to hold the pore open



K+ ion selectivity model: 1998

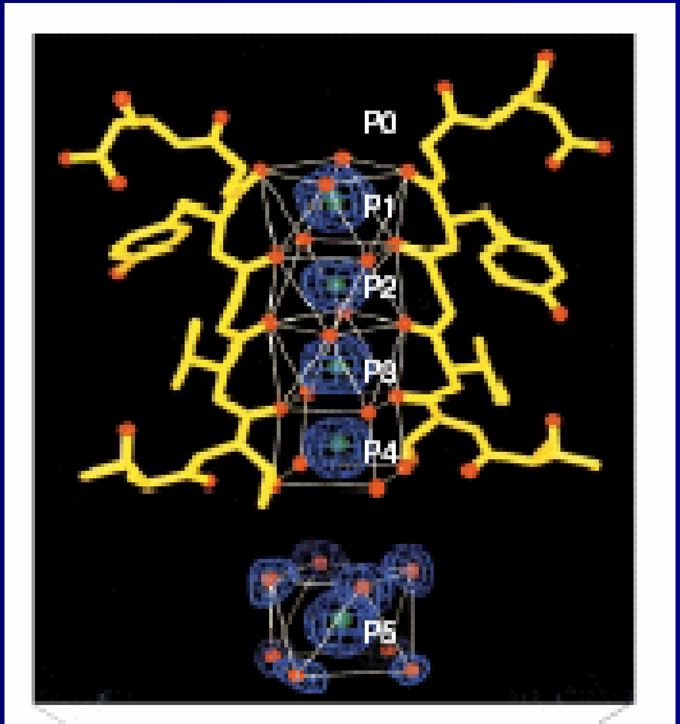
- Upon entering the selectivity filter, the ion dehydrates
- Carbonyl oxygen atoms act as surrogate water and compensate for cost of dehydration
- Selectivity filter is held open as if to prevent it from accommodating a Na+ ion

K+ ion selectivity model: 1971



K+ ion conductance model

- At 150 mM K+, selectivity filter contains two ions separated by ~7.5 Å



Summary

- The K+ channel pore is constructed of an inverted teepee with the selectivity filter held at its wide end
- The selectivity filter is ~2.5 A wide and 12 A long whereas the rest of the pore is wider with a relatively inert hydrophobic lining
- A large water filled cavity and helix dipoles help to overcome the high electrostatic energy barrier
- The K+ selectivity filter is lined by carbonyl oxygen atoms providing multiple closely spaced binding sites
- Two K+ ions at close proximity repel each other to counteract strong protein-ion interaction and allow for rapid conduction

Current and Future studies

- **What are the conformational changes that underlie pore opening in K+ channels?**
  - MthK, a Ca+ gated K+ channel crystallized in open state (Jiang et al. 2002. Nature. 417:515-522.
  - Inner helices expand its intracellular diameter in the opened state
- **What are the changes that underlie voltage sensitivity?**
  - KvAP, a voltage gated K+ channel was crystallized (Jiang et al. 2003. Nature. 423:33-41)
  - The electric field pulls on a charged helix-turn-helix structure on the channel's perimeter to bring about a conformation change