Announcements

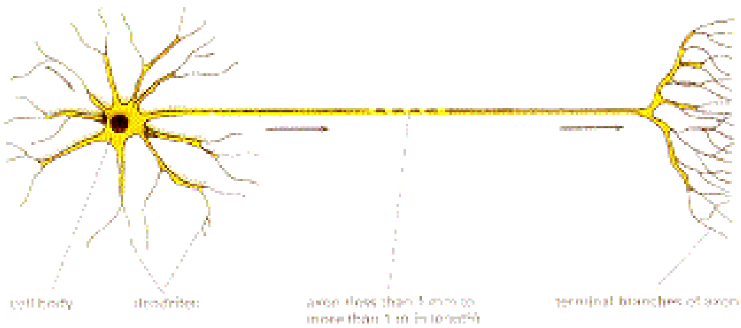
Homework due Wednesday, 4/16/08.

Also Wednesday, turn in your final paper's topic. Again, research paper and general review article. You'll give a talk and a 10 page paper– on exact same topic.

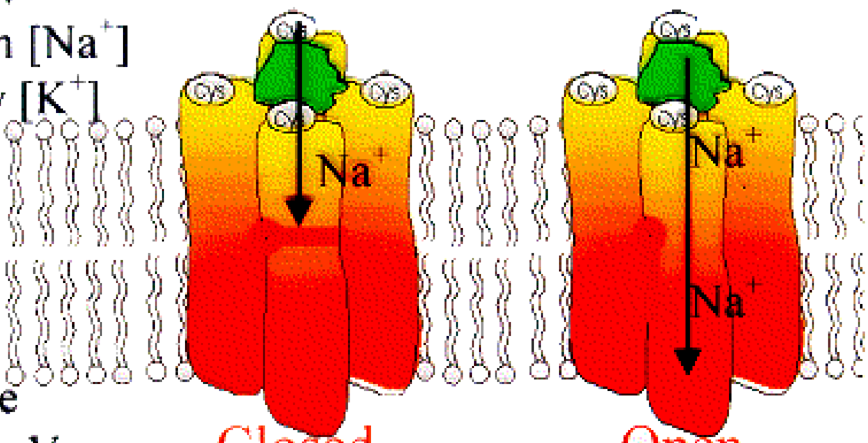
How Ion Channels Move to Create Action Potentials

Nerve cells contain ion channels

Opening/closing of (Na, K) ion channels lead to Action Potential – electrical wave.



Outside
0 mV
High [Na^+]
Low [K^+]



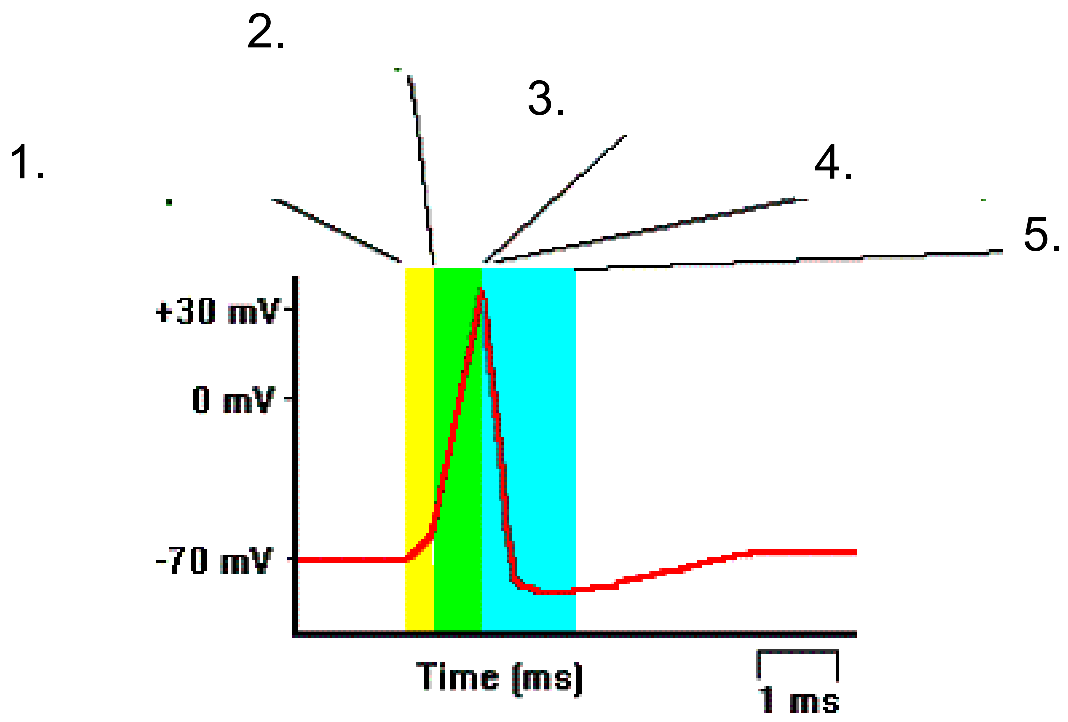
Inside
-0.1 mV
Low [Na^+]
High [K^+]

Closed
-0.1 V
"Polarized"
Resting

Open
≈ 0 V
"Depolarized"
Active

How does action potential occur?

Action Potential– Nerves Firing

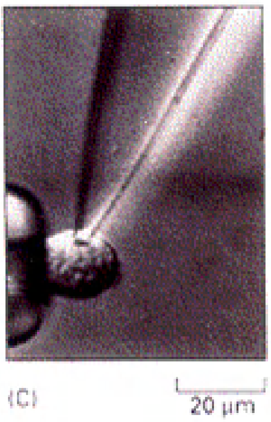


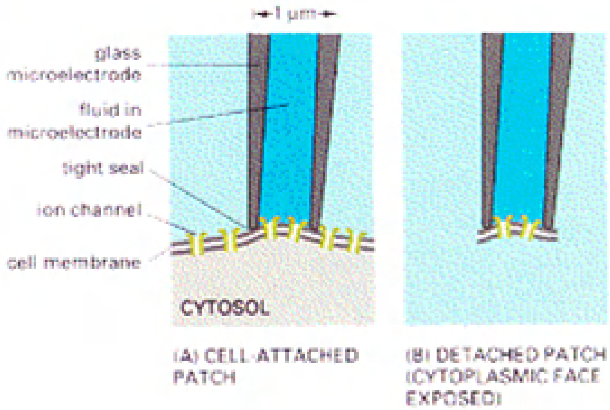
http://www.biologymad.com/NervousSystem/nerveimpulses.htm

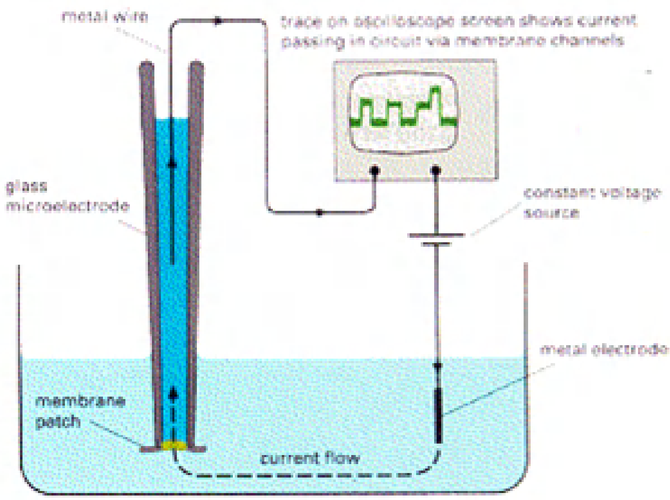
Is the Ion Channel Digital or Analog?

Current through a single channel can be recorded.

10 million charges/sec = 1pA/channel







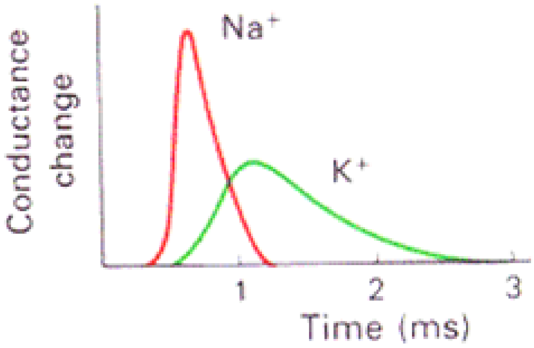
GΩ seal:
current only through channel

(microsecond resolution)

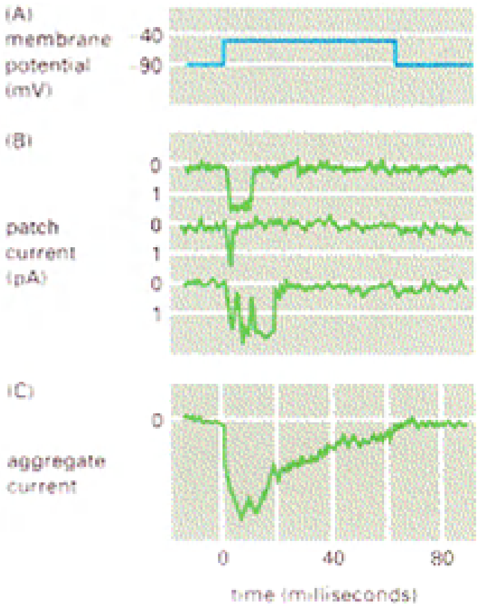
Patch-clamp technique: Nobel Prize, 1991
Erwin Neher and Bert Sakmann, invented 1976

Do ion channels open gradually or all or nothing?

Ensemble



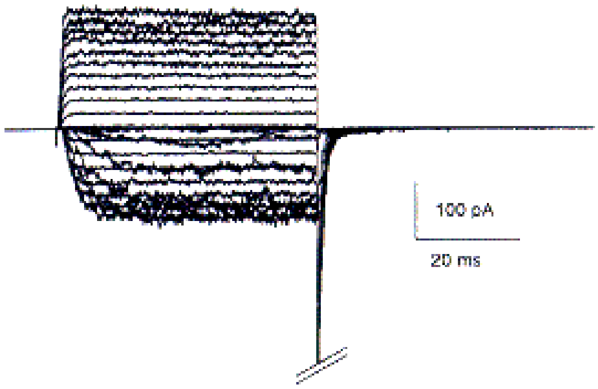
Single Ion Channel

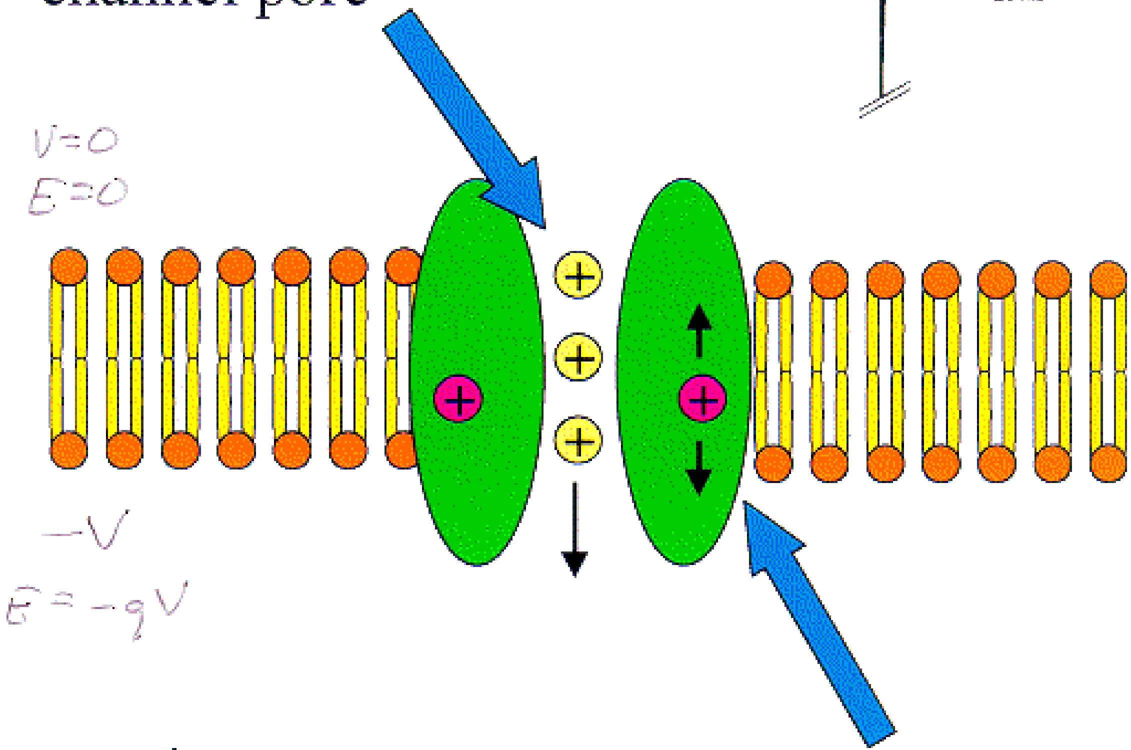


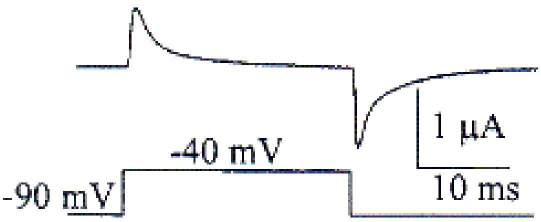
Patlack & Horn, 1982

Ionic Current and Gating Current

Ionic current results from diffusion of ions through the channel pore





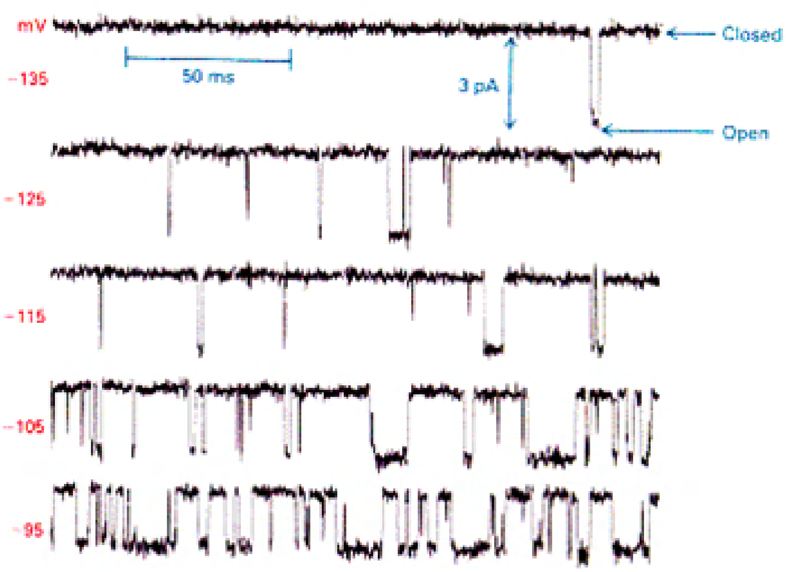


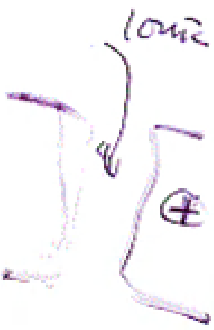
Gating current results from gating charge movement ~ 1 ms time scale

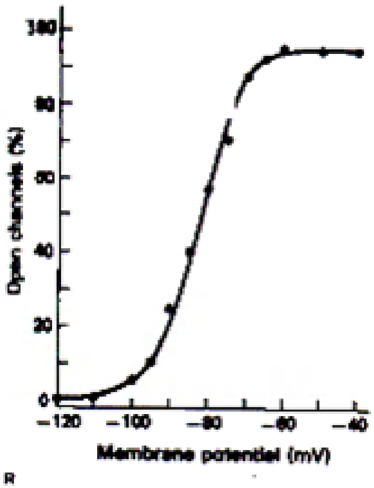
Charged amino acids (largely in the voltage sensor) move.

Voltage dependence of on/off transitions.

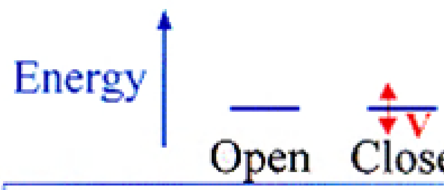
(Can measure by ensemble or single-channels)







2 state model:
Boltzman distribution



$$\{1 + \exp(-q[V-V_m]/kT)\}^{-1}$$

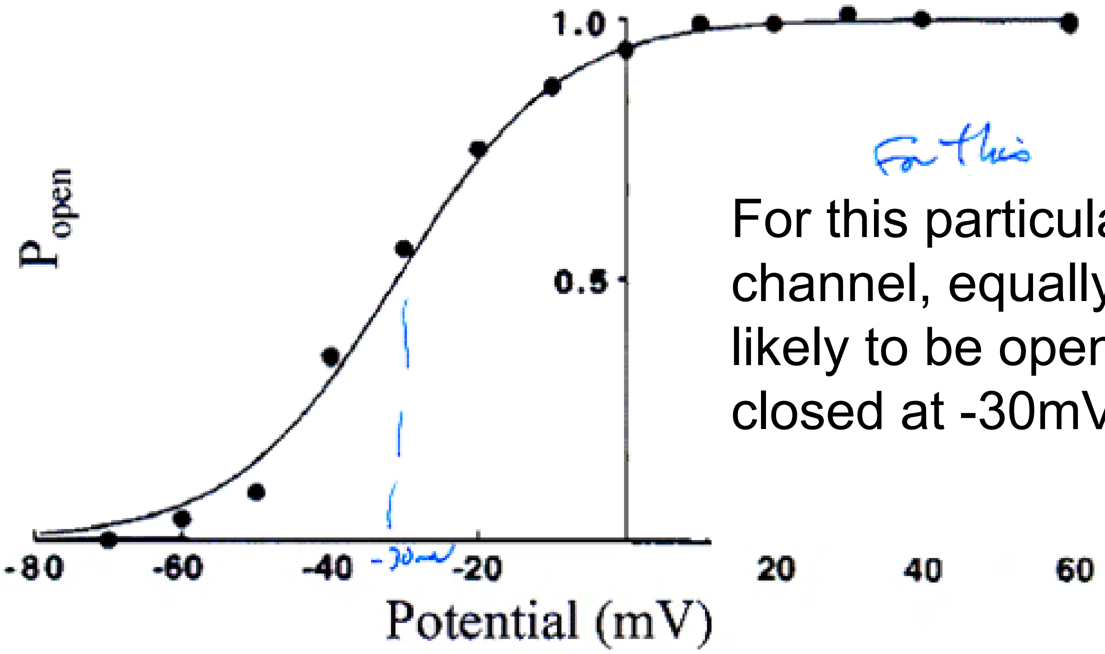
Midpoint potential: -80mV; Steepness of curve: qV

Suggests model of 2 states that differ in energy by qV
Where q is about 13e's.
(q is a part of channel; not ionic charge!)

Voltage Response of Channel

Voltage gating can be approximated by simple **two-state model**, "open" and "closed"

$$P_{\text{open}} = \frac{1}{e^{q_a (V_{50} - V)/kT} + 1}$$



For this particular channel, equally likely to be open as closed at -30mV.

V_{50} can be measured by measured experimentally

Q_e ~ 13e charges/channel for Na^+,K^+

Look for part of channel which has 13 charges!

(e.g. Lysines, Arginines, etc.)– That's voltage sensor.

Major questions

1. How does a nerve action potential begin?
Ans. Ligand gated ion channels release Ca^{2+}

2. Why is there a non-zero resting potential?
Ans: [Ion] gradient, selective ion permeability, Na-K pump.

3. What determines magnitude of action potential?
Ans: Na, K permeabilities, Nernst potentials.

4. What brings electrical potential back to resting potential? [Nerve needs to fire and then relax, ready to refire if needed.]
Ans: voltage-gated K channels.

5. Why does potential travel as opposed to simply spread?
Ans. Ion channels spontaneously close.

8. What is structure of ion channels?
Ans. Partially solved.

9. How do ion channels allow ions to flow?
Ans. Water filled tube.

10. How does voltage turn on/off channel?
Ans. Positively charged "gate" moves, opening/closing water-filled tube.

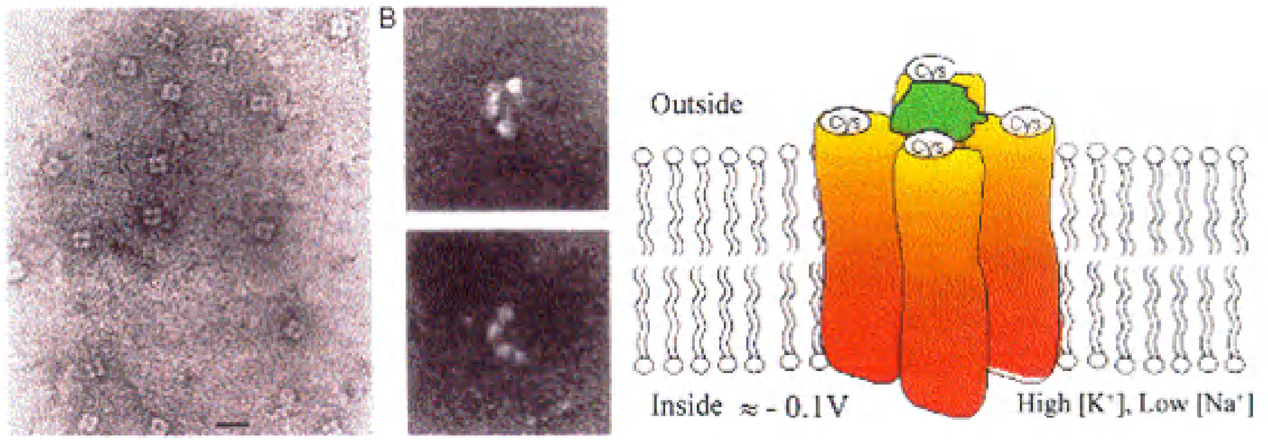
11. How does gate move, and how can we measure such small (Angstrom) changes?
Ans. My research! (Probably via a rotation, measured with FRET.)

12. How is an ion channel selective for Na or K (or other ions)?
Ans. Fine tuning of "selectivity filter" reduces energy of hydration for select ions; creates energy barrier for wrong ions.

13. Are channels analog or digital?
Ans. Digital – opened or closed. No in between.

Structural Studies

Tetramer: four-fold symmetry



Bar = 15 nm

About 8 nm x 8nm x 5nm

X-ray Crystallography

Structure of the Pore of a Bacterial Analog

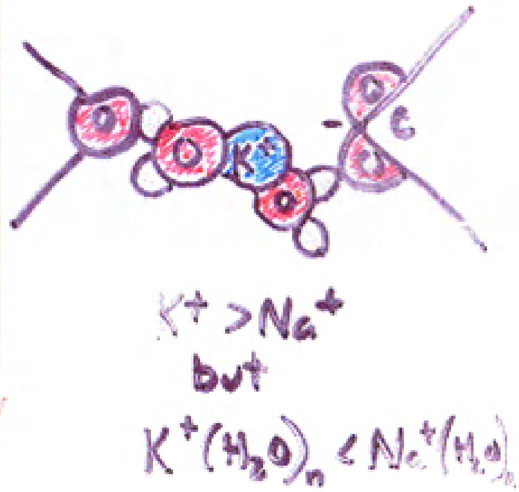
M2 in original (KcsA) structure:

S6 in K^+ Channel

M1 in original structure

S5 in K^+ Channel

Explains Ion-selectivity



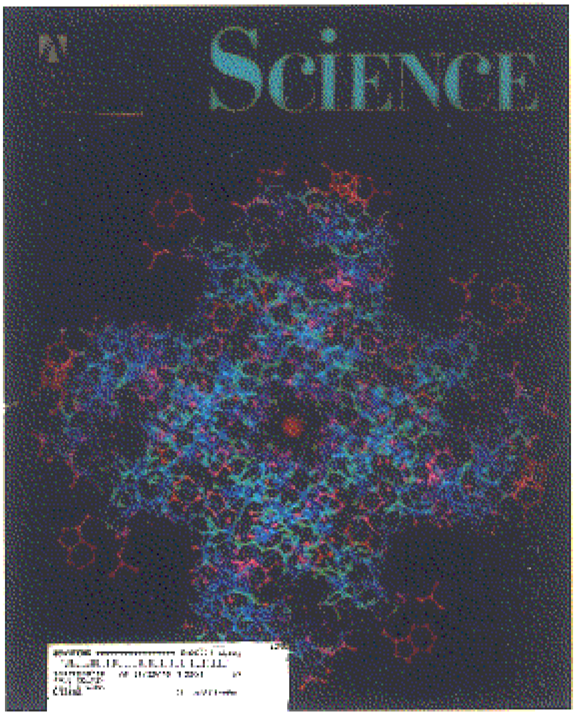
X-ray crystallography

The premier structural technique
B.C. vs. A.C.

KcsA Potassium Channel (Bacteria)

Tetramer of M1, M2

(analogous to S5, S6)



Synchrotrons have dramatically improved x-ray crystallography

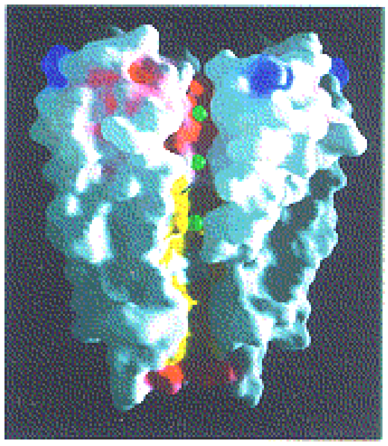
High brightness, focussed

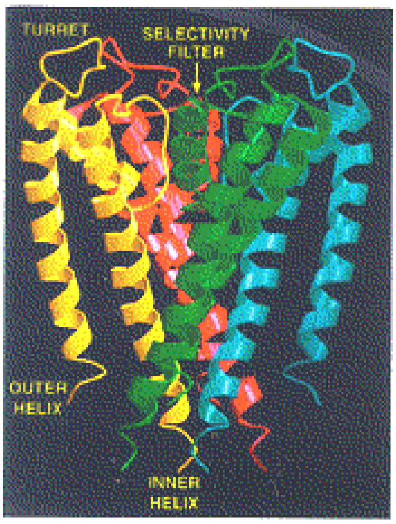
Can use small crystals/ get higher resolution

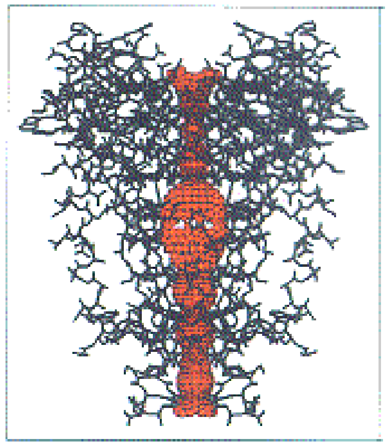
Recently → Wavelength tuneability

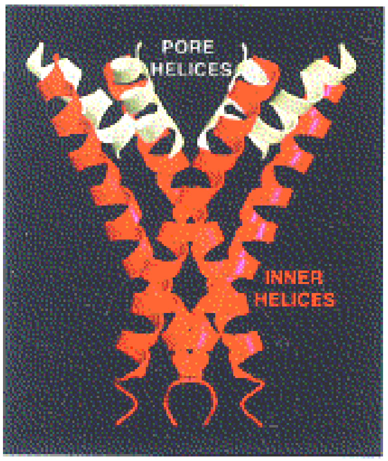
Nobel Prize- 2003, Rod MacKinnon

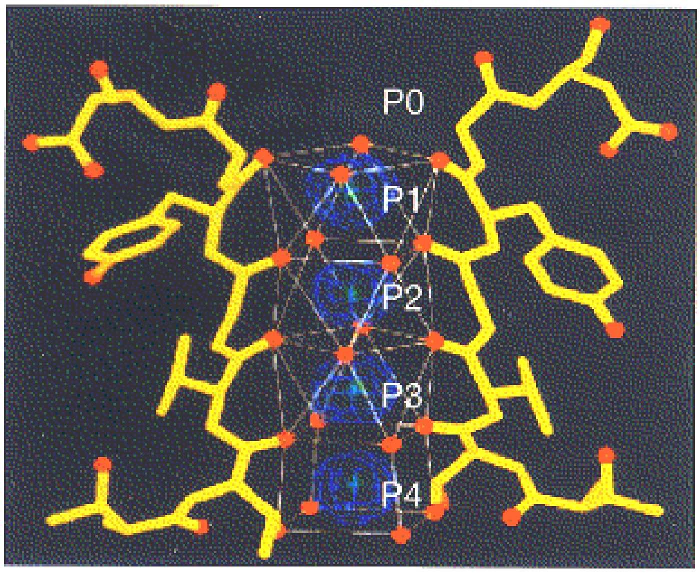
KcsA Selectivity Filter [Mackinnon, et al]











Choe, Nature Reviews, Neuroscience, 2002

Hydration Energy

To dehydrate H_2O from K^+ (or Na^+)

takes ~ 100 kT!

If passage of ion through channel requires dehydration – extremely high energy barrier

Selectivity filter is arranged so that C=O carboxylate groups of a.a. are exactly in the right position so O of H_2O is replaced by O of C=O w/o any energetic difference for K^+ in KcSA.

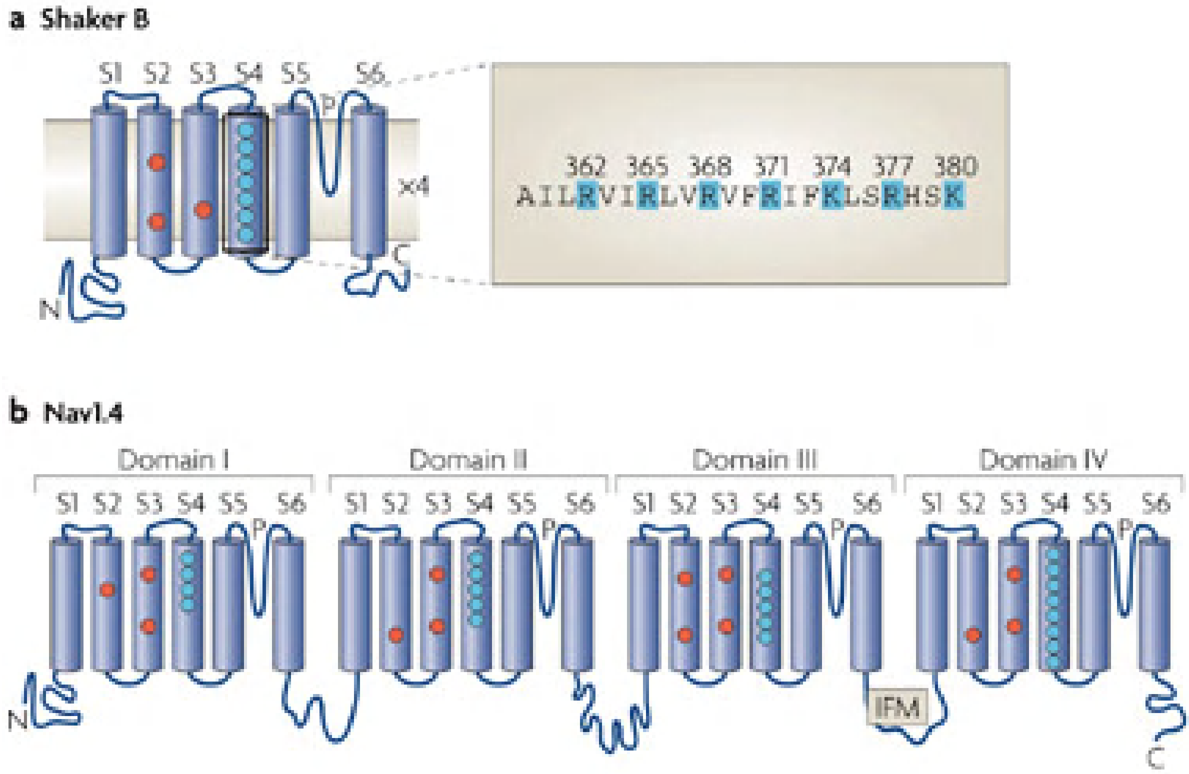
However, for smaller Na^+ ion (dehydrated), the C=O are too far apart to easily replace H_2O of Na^+-hydrated ion.

If 10,000 fold selectivity, what is E_{Na} vs. E_K ?

Ans: 9.2kT

Sodium channel been crystallized. C=O just right for Na^+.

Sodium Channel has Similar Structure



Bezanilla, 2008, Nature Reviews

Important features

1. **Aqueous cavity/channel.**
   No energetic barrier of going from aqueous (water) to low dielectric membrane.

2. **Dehydrated K^+ is bound to eight oxygen atoms** formed from carbonyl (C=O backbone) of amino acids from selectivity filter.
   No energetic barrier to dehydration for K^+.
   Large cost for ions such as Na^+ (which is dehydrated slightly smaller; hydrated slightly bigger.)

3. **Multiple ions can be in cavity at same time.**
   High throughput. (10^8 ions/sec).

Diffusion-limited passive transport of K^+ while acting like a brick wall to Na^+.

Inside

Outside

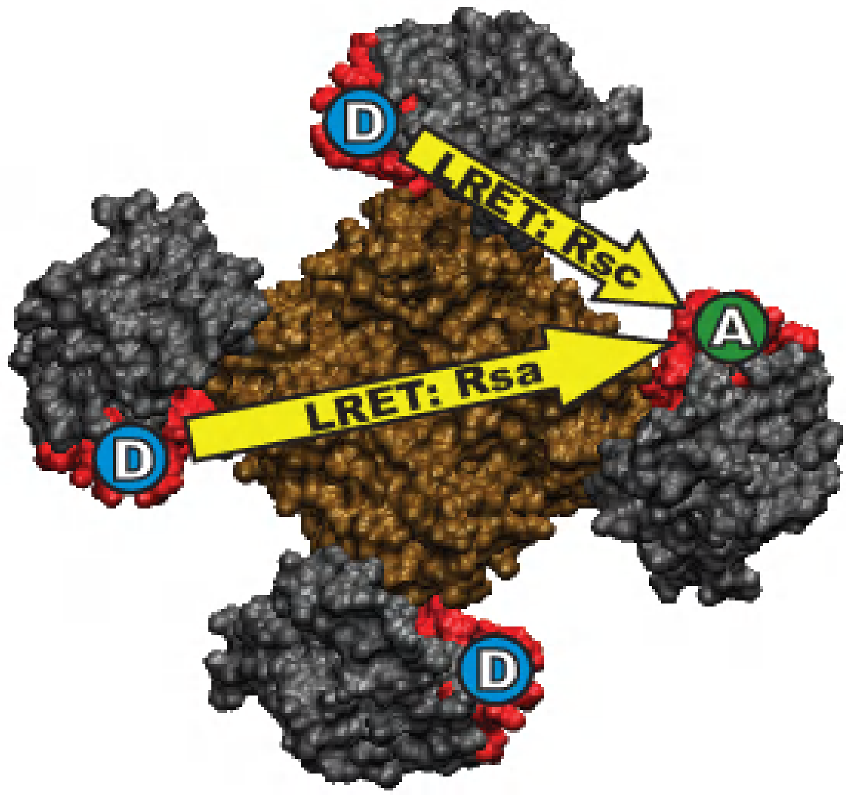
Crystal Structure of S1-S6 Ion Channel
Nobel Prize for Rod MacKinnon, 2006

Biophysics is a fast moving field!
Everything's new and open!

Nature, May 1, 2003. Crystal structure of voltage-gated ion channel.

(There were real problems with this structure.)

S1-S4 Voltage Sensor Lies on the Outside of S5/S6



Class evaluation

1. What was the most interesting thing you learned in class today?

2. What are you confused about?

3. Related to today's subject, what would you like to know more about?

4. Any helpful comments.

Answer, and turn in at the end of class.