Structural basis of voltage-gated ion channel function

Introduction to ion channels
Subunits and their assembly
Activation gate
Ion selectivity
Voltage sensor
Inactivation gates

Ion channels: general properties

membrane bound proteins that conduct ions
at a rate near the diffusion limit across the plasma membrane, or intracellular membrane of organelles (e.g., mitochondria).

ions move faster through ion channels than via carriers
throughput rates for selective ion channels: 10^{6} – 10^{8} ions/s
current equivalent: 10^{-12} – 10^{-10} Ampere (1 – 100 pA)

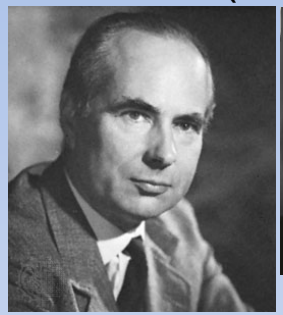
transfer rate for carriers (Na-K exchanger): 300 Na^{+}, 200 K^{+}/sec
current equivalent: 1.5 x 10^{-17} A

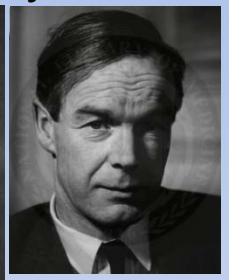
frame of reference:

electronic circuits:	*10^{-2} A*
light bulb:	*10^{-1} A*

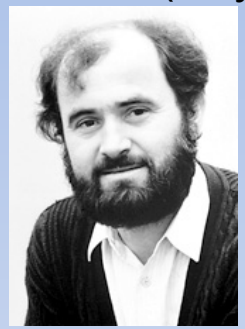
Four major breakthroughs in ion channel biology

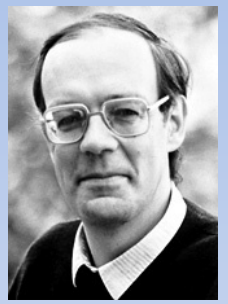
1 **Ionic conductances**
Nobel 1963 (Physiol/Medicine)


Andrew F. Huxley


Alan L. Hodgkin

2 **Patch clamp methodology**
Nobel 1991 (Physiol/Medicine)


Erwin Neher


Bert Sakmann

3 **ACh receptor channel cloning/sequencing**

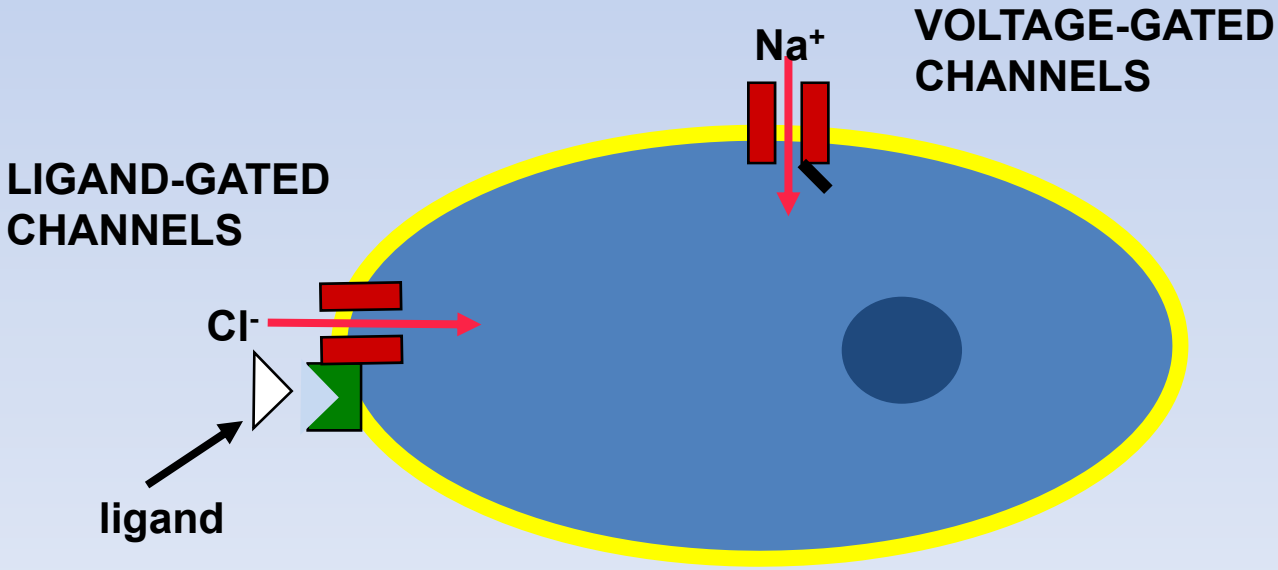
Shosaku Numa
(Kyoto)

4 **K channel structure**
Nobel 2003 (Chemistry)


Rod MacKinnon

Classification of ion channels

1) **Voltage-gated**: based on ion selectivity
   (K, Na, Ca, Cl channels)
2) **Ligand-gated**
   (ligands: glutamate, GABA, ACh, ATP, cAMP)
3) **Specialized channels**
   (connexins - gap junctions, mechanosens. channels)



Physiological functions of ion channels

Maintain cell resting potential: *inward rectifier K and Cl channels*

Conduction of electrical signals: *Na and K channels of nerve axon*

Synaptic transmission at nerve terminals: *glutamate, glycine, acetylcholine receptor channels*

Intracellular transfer of ions, metabolites: *gap junctions*

Cell volume regulation: *Cl channels (+ aquaporins)*

Sensory perception: *cyclic nucleotide gated channels of rods, cones*

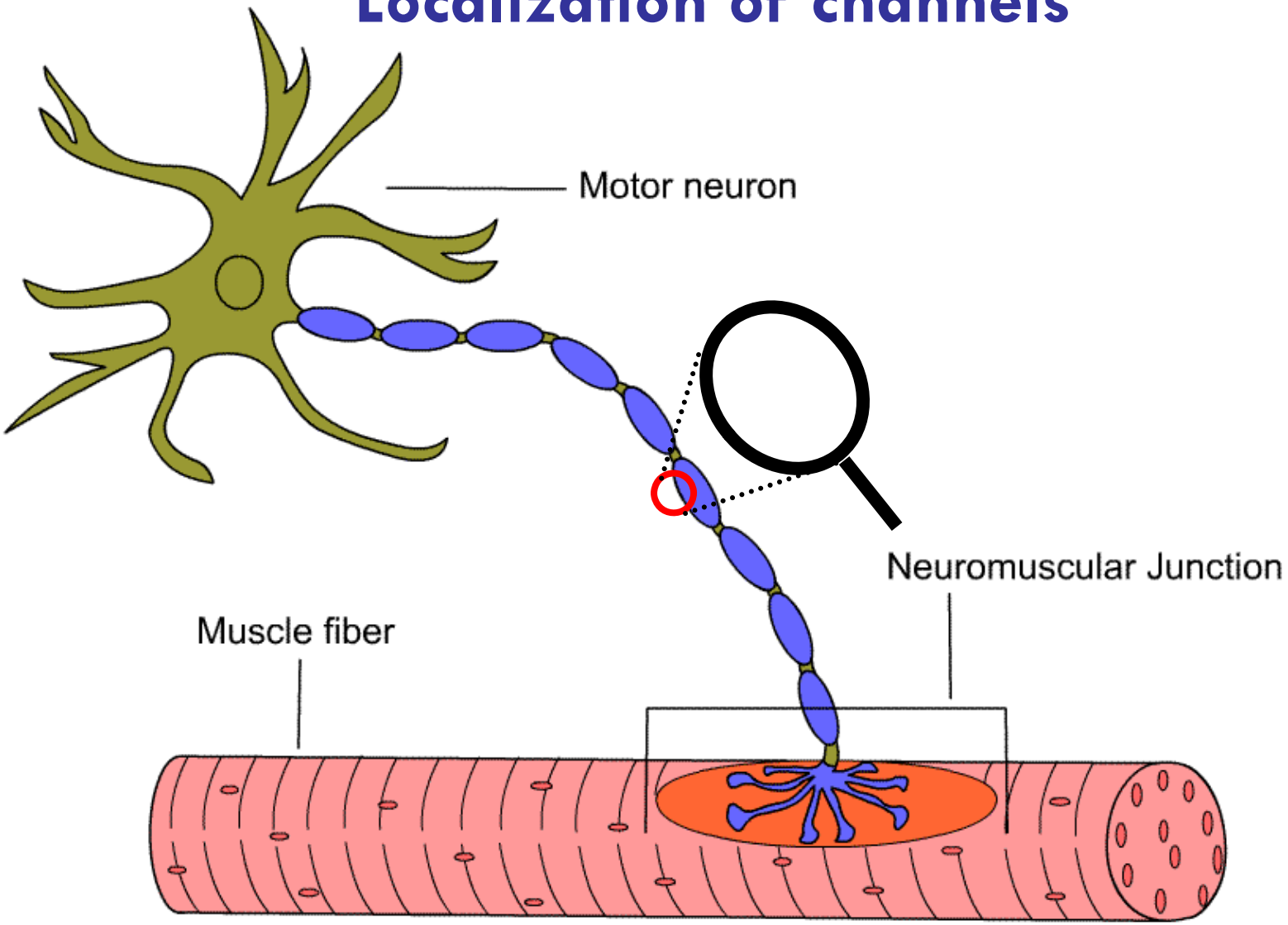
Oscillators: *pacemaker channels of the heart and central neurons*

Excitation-contraction coupling: *Ca channels of skeletal & heart muscle*

Stimulation-secretion coupling: *release of insulin from pancreas*

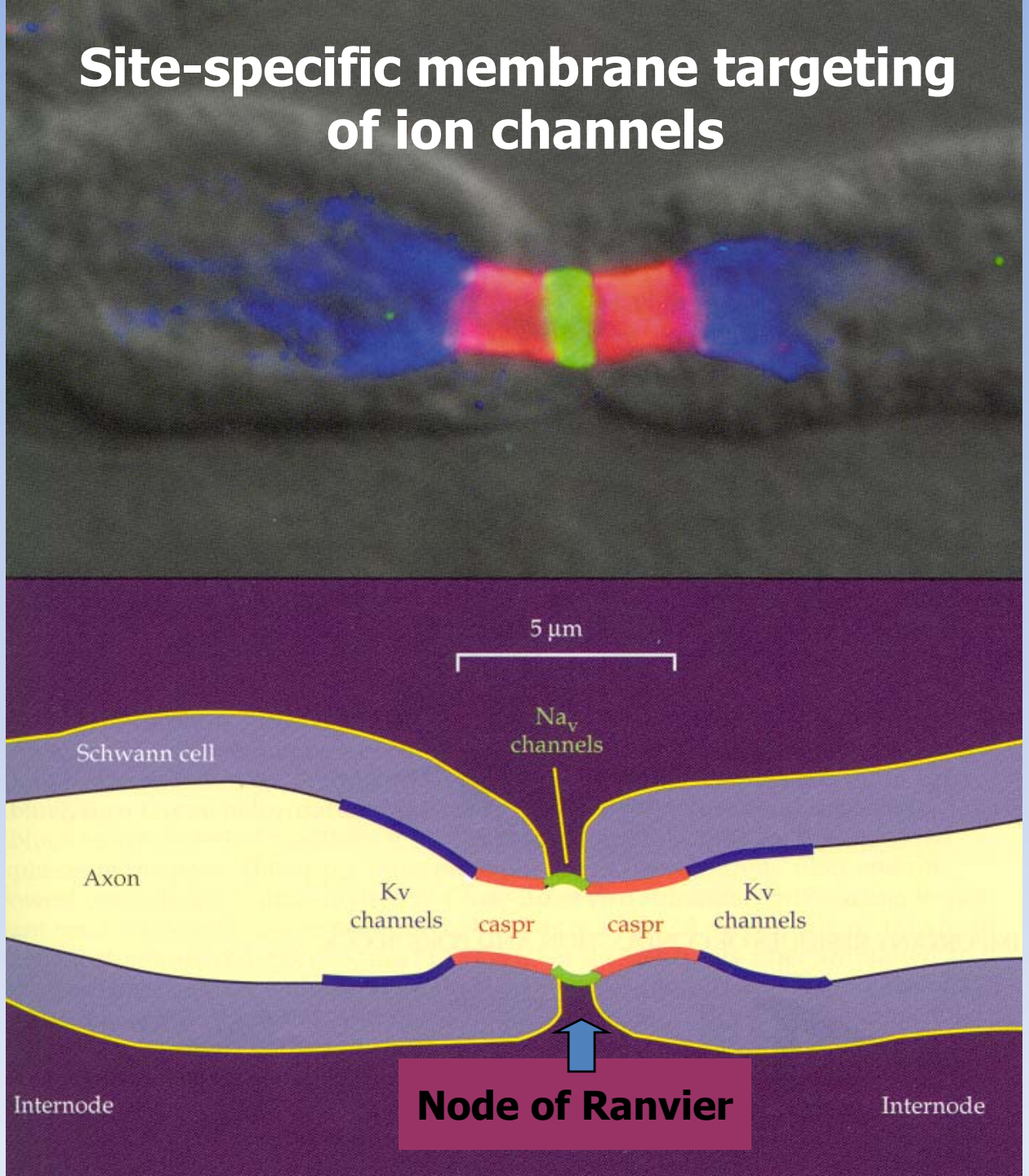
Ion channels can be highly localized

Localization of channels



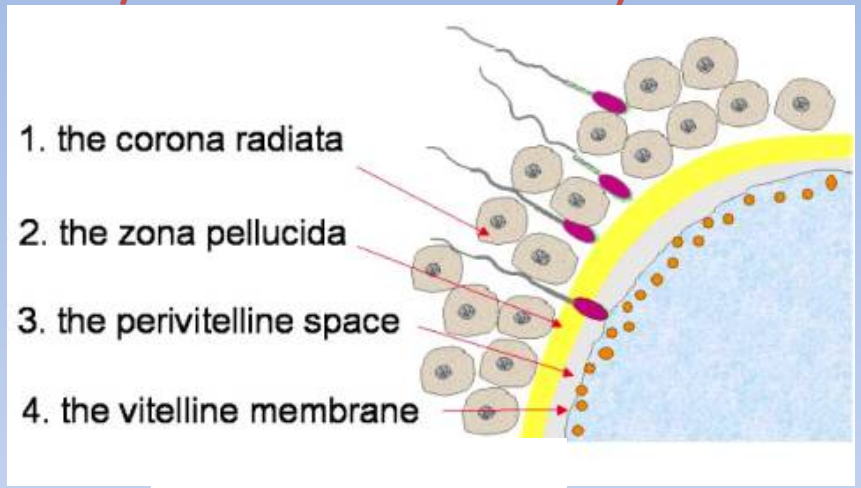
Adapted from: Kandel, Schwartz, and Jessell. *Principles of Neural Science*

Site-specific membrane targeting of ion channels

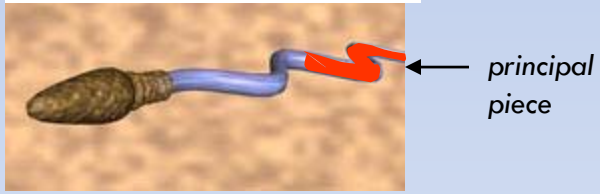


CatSper Ca channels

(6TM domains/subunit like Kv channels)



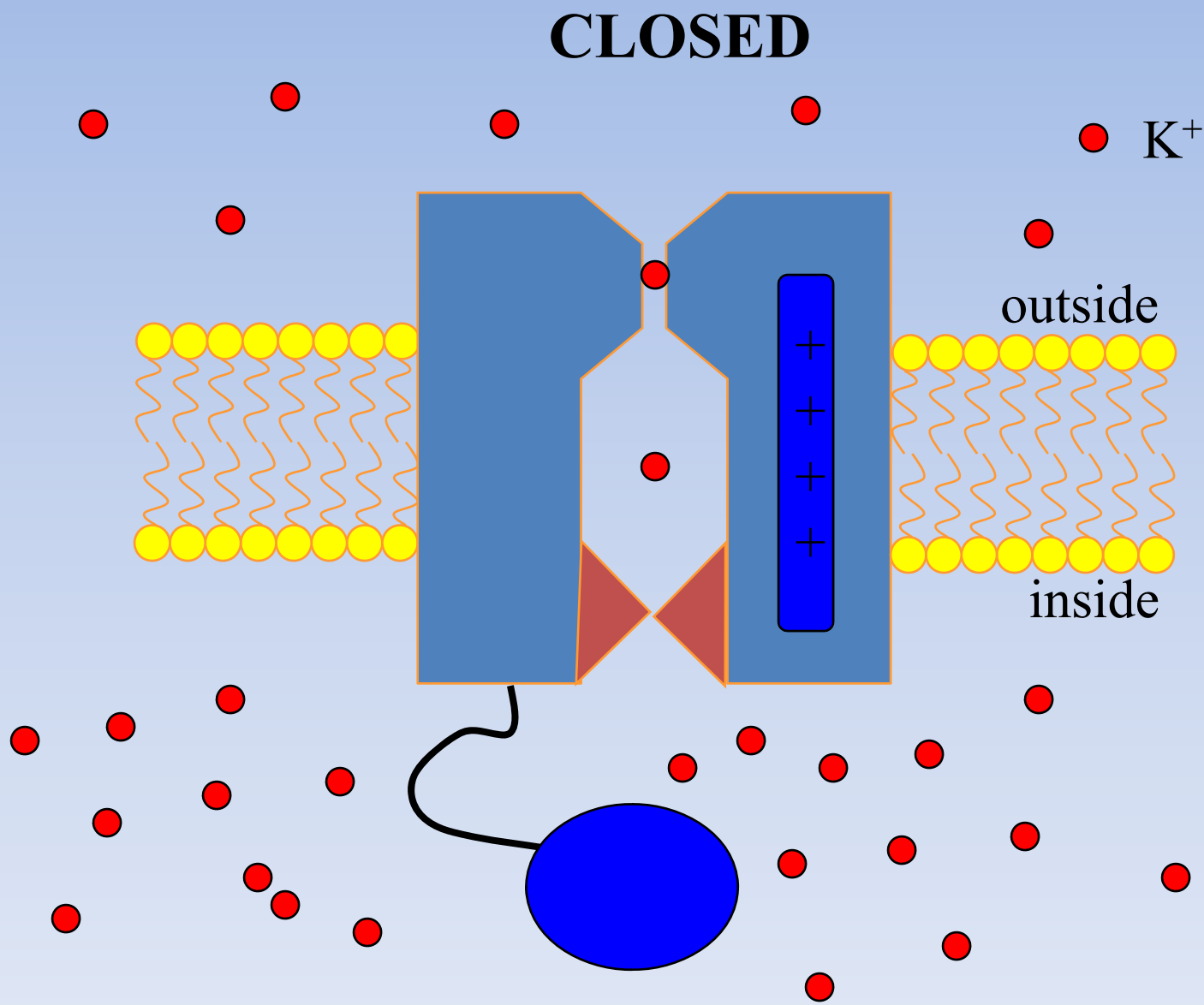
ONLY expressed in the **principal piece** of sperm (end of the tail)



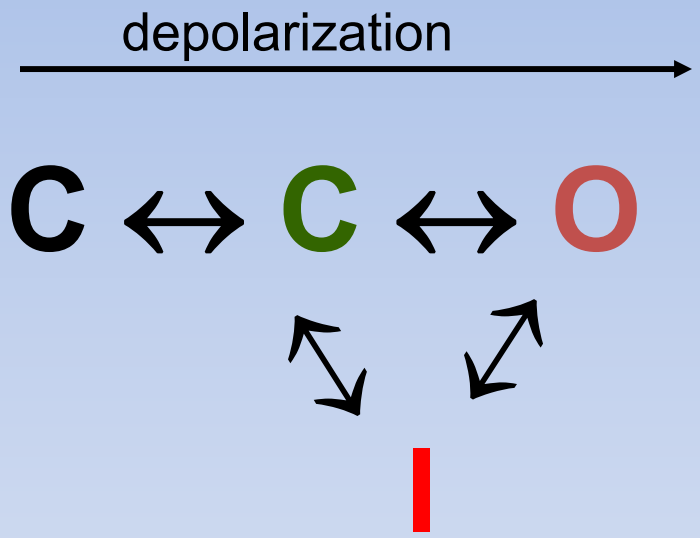
Sperm lacking CatSper are poorly motile (**no hyperactivity** during capacitation phase) and are unable penetrate zona pellucida and fertilize egg.

Genetic knock out of CatSper makes male mice infertile
- target for new contraceptive drugs?

Channel Gating: closed-open-inactivated



In response to a change in voltage, single channels can activate (Open), deactivate (Close) or Inactivate:



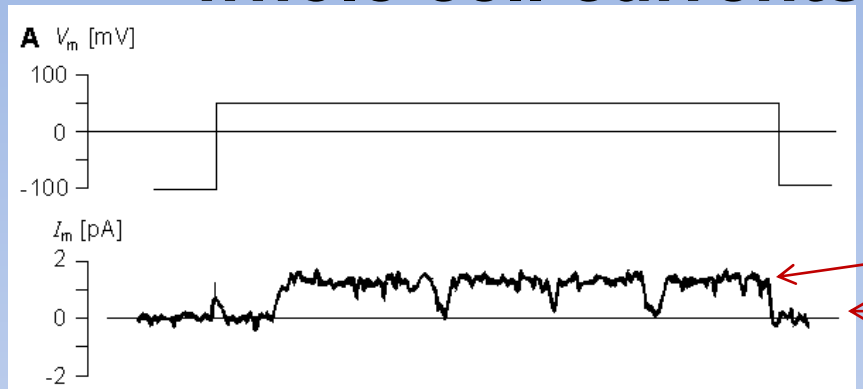
TERMINOLOGY:

Activation: C → O Deactivation: O → C

Inactivation: C → I; O → I

Recovery from inactivation: I → C; I → O

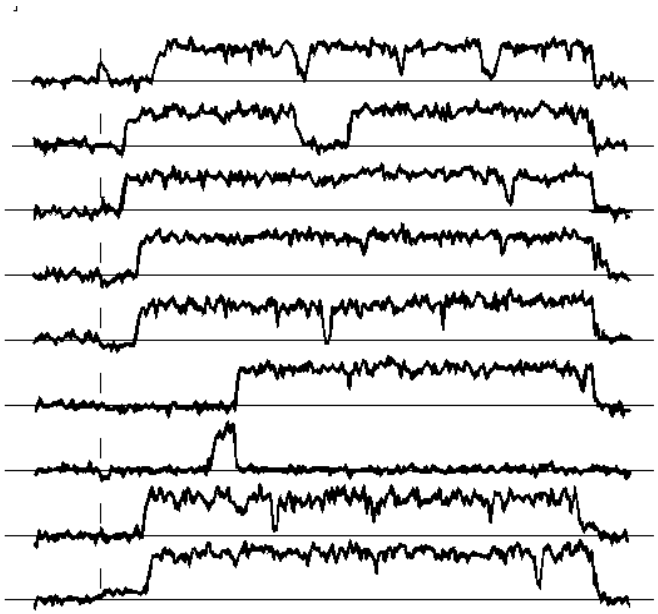
Single channel currents sum to generate whole cell currents



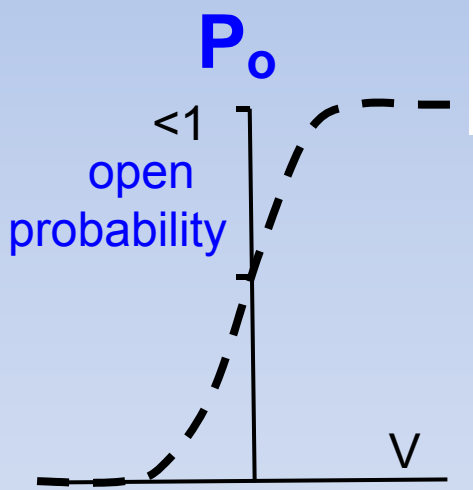
ensemble

Magnitude of whole cell current, *I* can be determined by single channel properties

N = *total # of channels in cell*



i single channel current amplitude



$$I = N \times P_o \times i$$

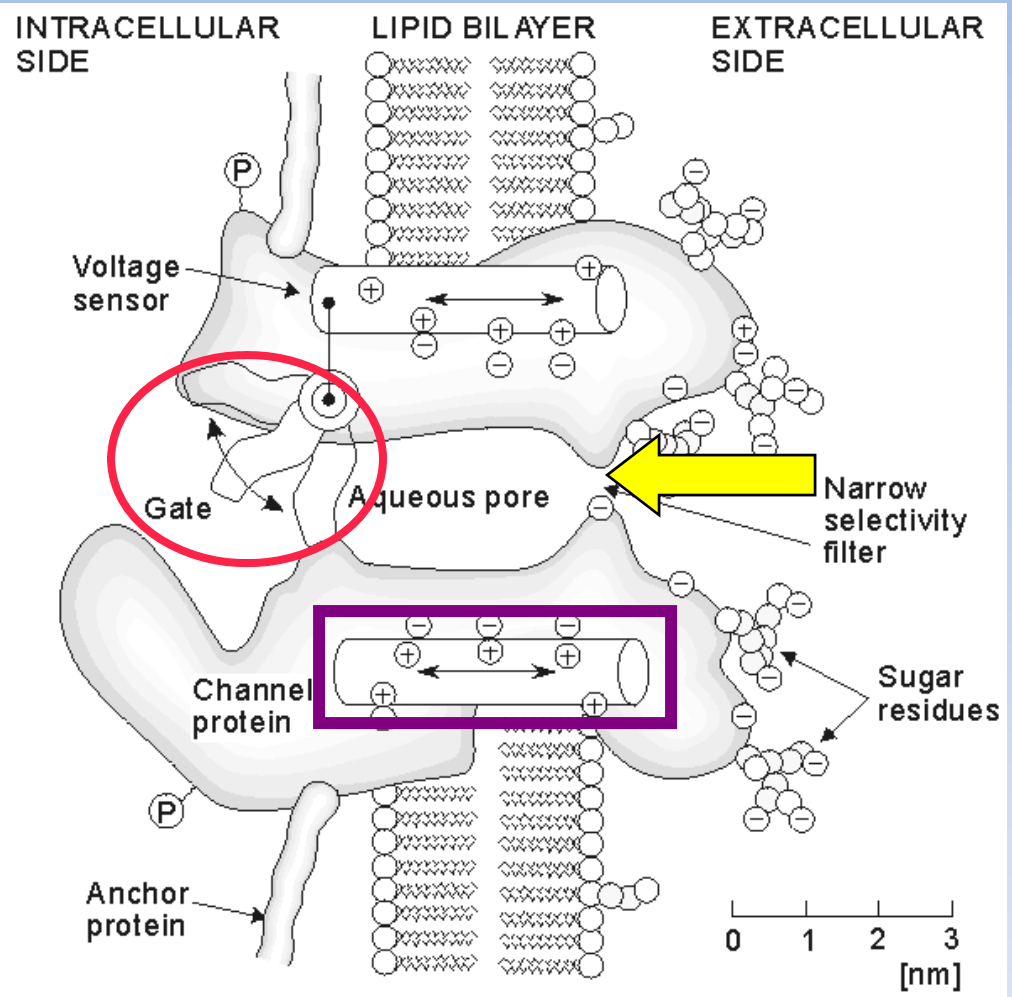
Channel structure

Transmembrane, extra- & intra-cellular domains

Gates

Pore and selectivity filter

Voltage sensor



The “Holy Grail – Part I”

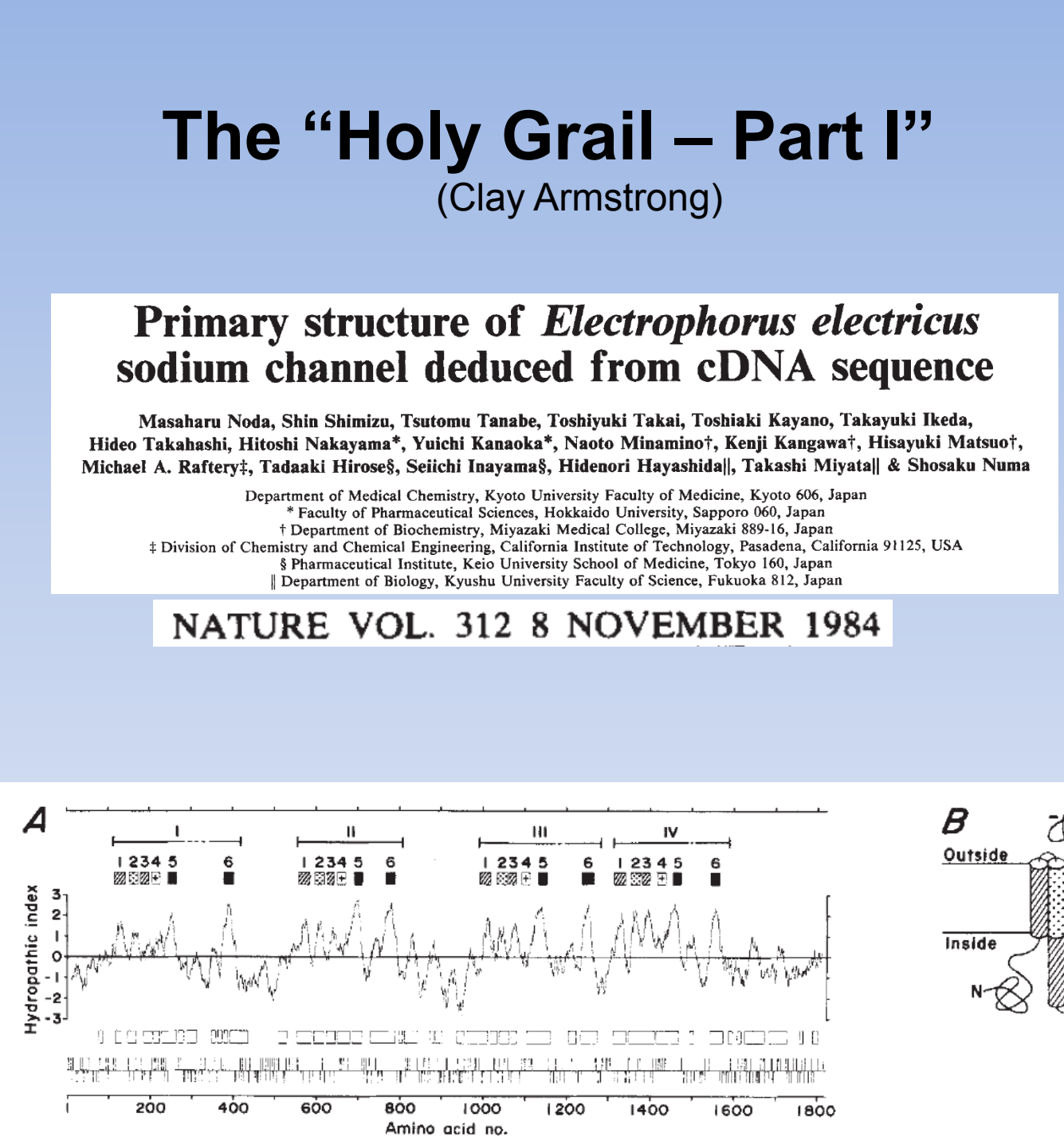
(Clay Armstrong)

Primary structure of *Electrophorus electricus* sodium channel deduced from cDNA sequence

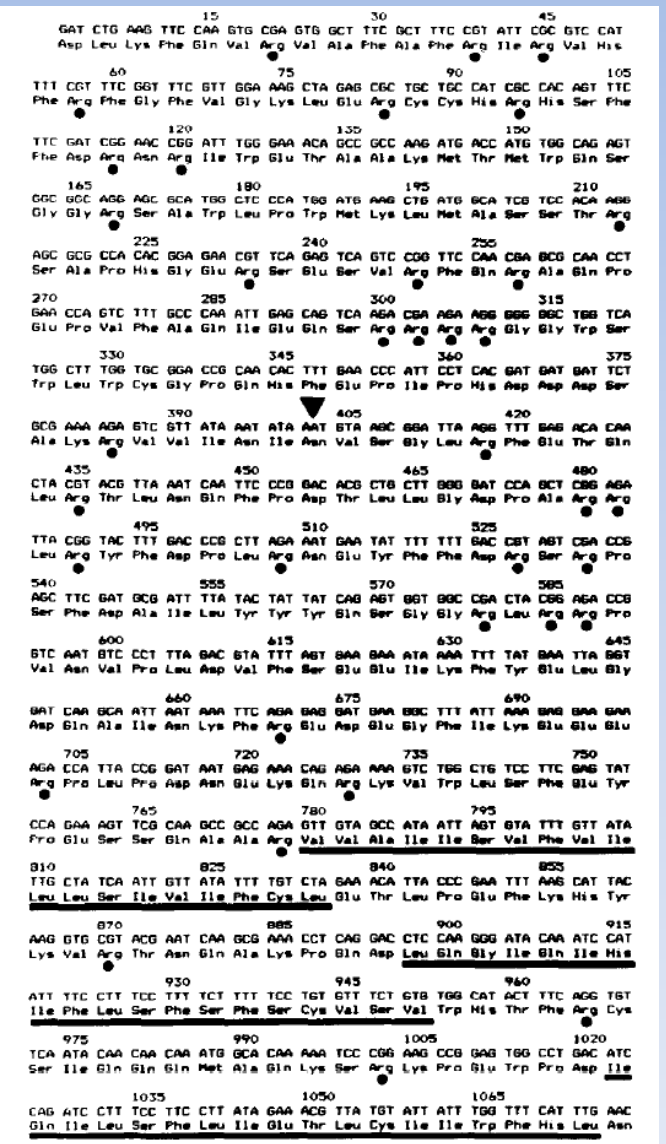
Masaharu Noda, Shin Shimizu, Tsutomu Tanabe, Toshiyuki Takai, Toshiaki Kayano, Takayuki Ikeda, Hideo Takahashi, Hitoshi Nakayama*, Yuichi Kanaoka*, Naoto Minamino†, Kenji Kangawa†, Hisayuki Matsuo†, Michael A. Raftery‡, Tadaaki Hirose§, Seiichi Inayama§, Hidenori Hayashida‖, Takashi Miyata‖ & Shosaku Numa

Department of Medical Chemistry, Kyoto University Faculty of Medicine, Kyoto 606, Japan
* Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan
† Department of Biochemistry, Miyazaki Medical College, Miyazaki 889-16, Japan
‡ Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, USA
§ Pharmaceutical Institute, Keio University School of Medicine, Tokyo 160, Japan
‖ Department of Biology, Kyushu University Faculty of Science, Fukuoka 812, Japan

NATURE VOL. 312 8 NOVEMBER 1984

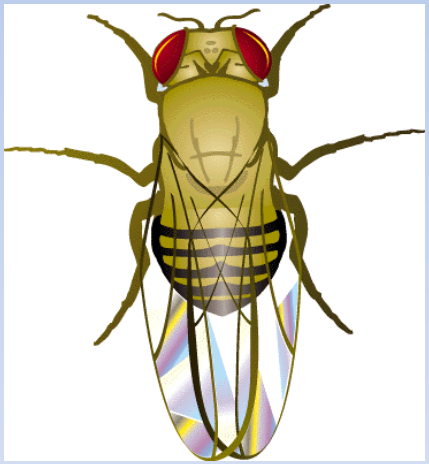


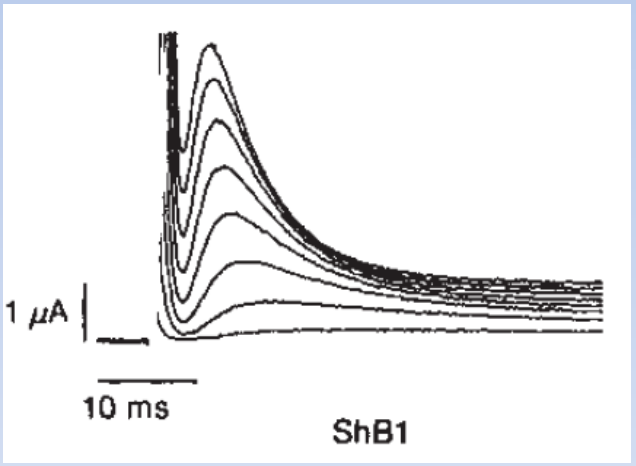
Molecular Characterization of *Shaker*, a Drosophila Gene That Encodes a Potassium Channel



Alexander Kamb, Linda E. Iverson, and Mark A. Tanouye
Division of Biology 216-76
California Institute of Technology
Pasadena, California 91125

***Cell* (1987) 50:405**

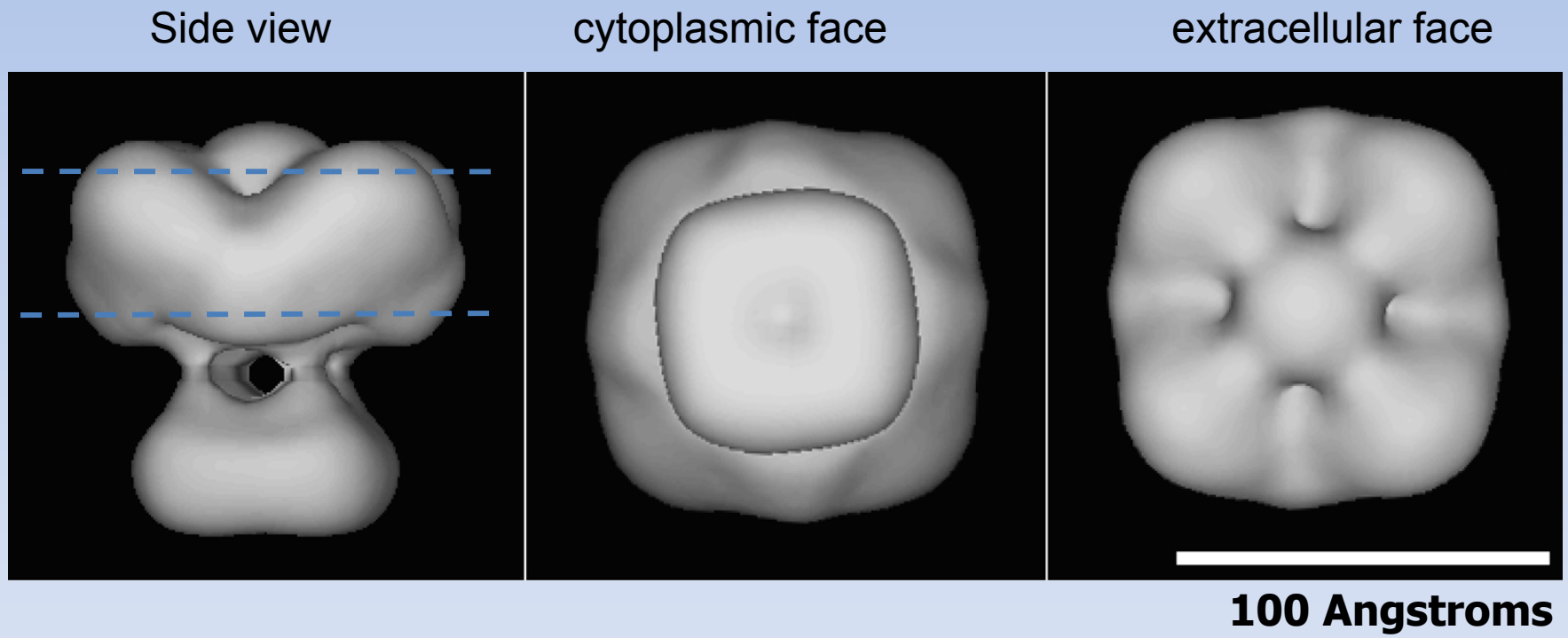




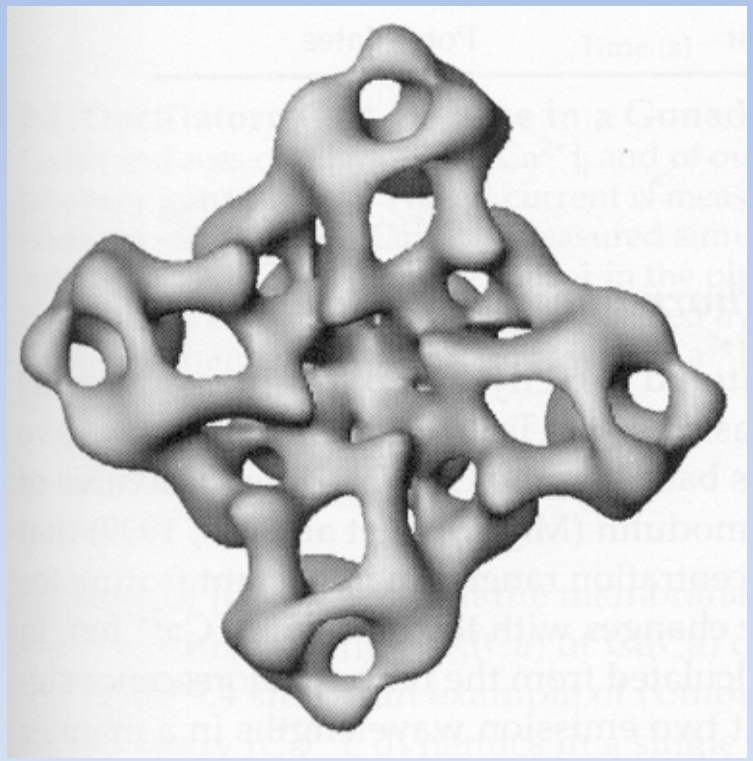
Timpe et al (1988) *Nature* 331:143

Channel Structure

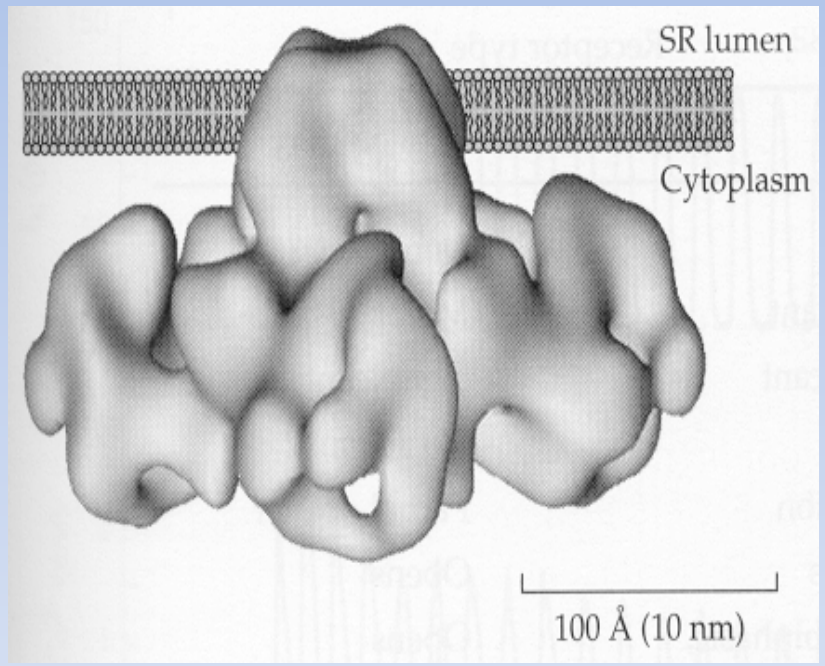
Structure of a voltage-gated K channel obtained by 3-dimensional reconstruction of multiple EM images



EM reconstruction of Ryanodine receptor (SR Ca release channel)



RyR viewed from cytoplasm



RyR viewed from side

Subunits and their assembly

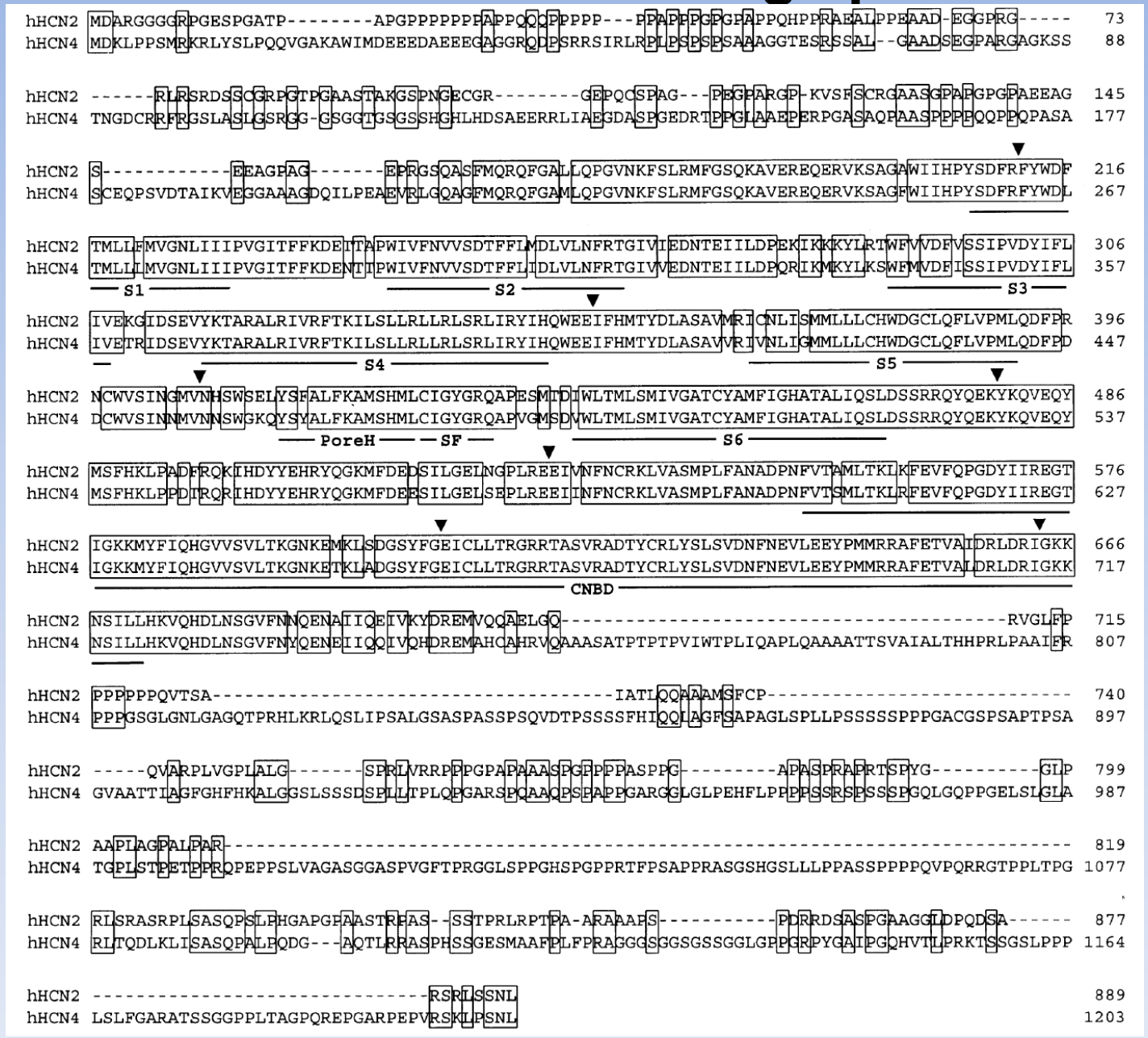
Ion channels subunits

α-subunits form ion conduction pore
Accessory subunits (β, γ, δ are usually smaller in size)

α-subunit size:

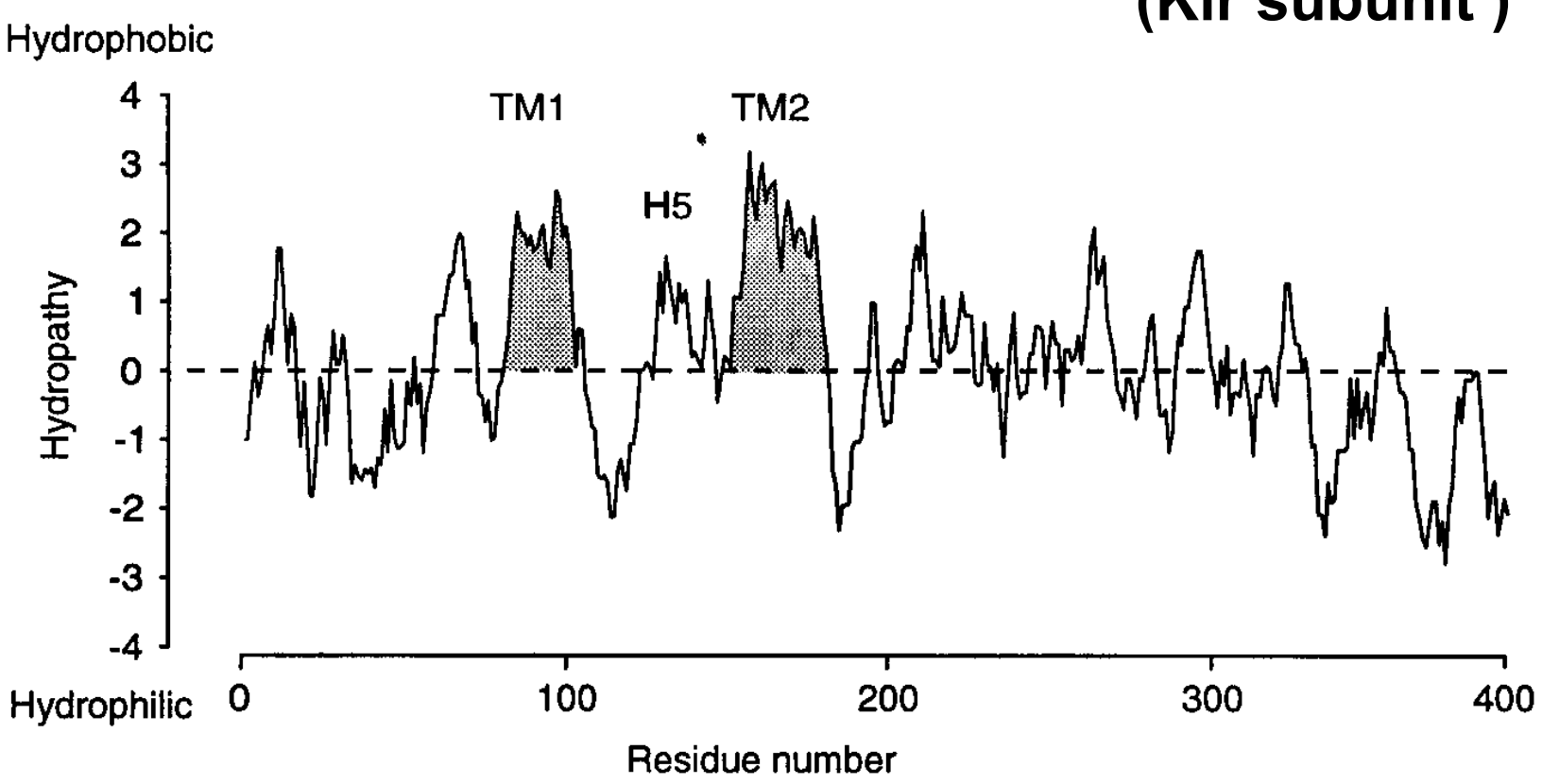
300 amino acids (inward rectifier K channels)
5000 amino acids (Ca release channel of SR)

Channel subunits are large proteins



General structure determined by hydropathy plots

Transmembrane (TM) domains have α–helical structure and are more hydrophobic than intracellular or extracellular domains

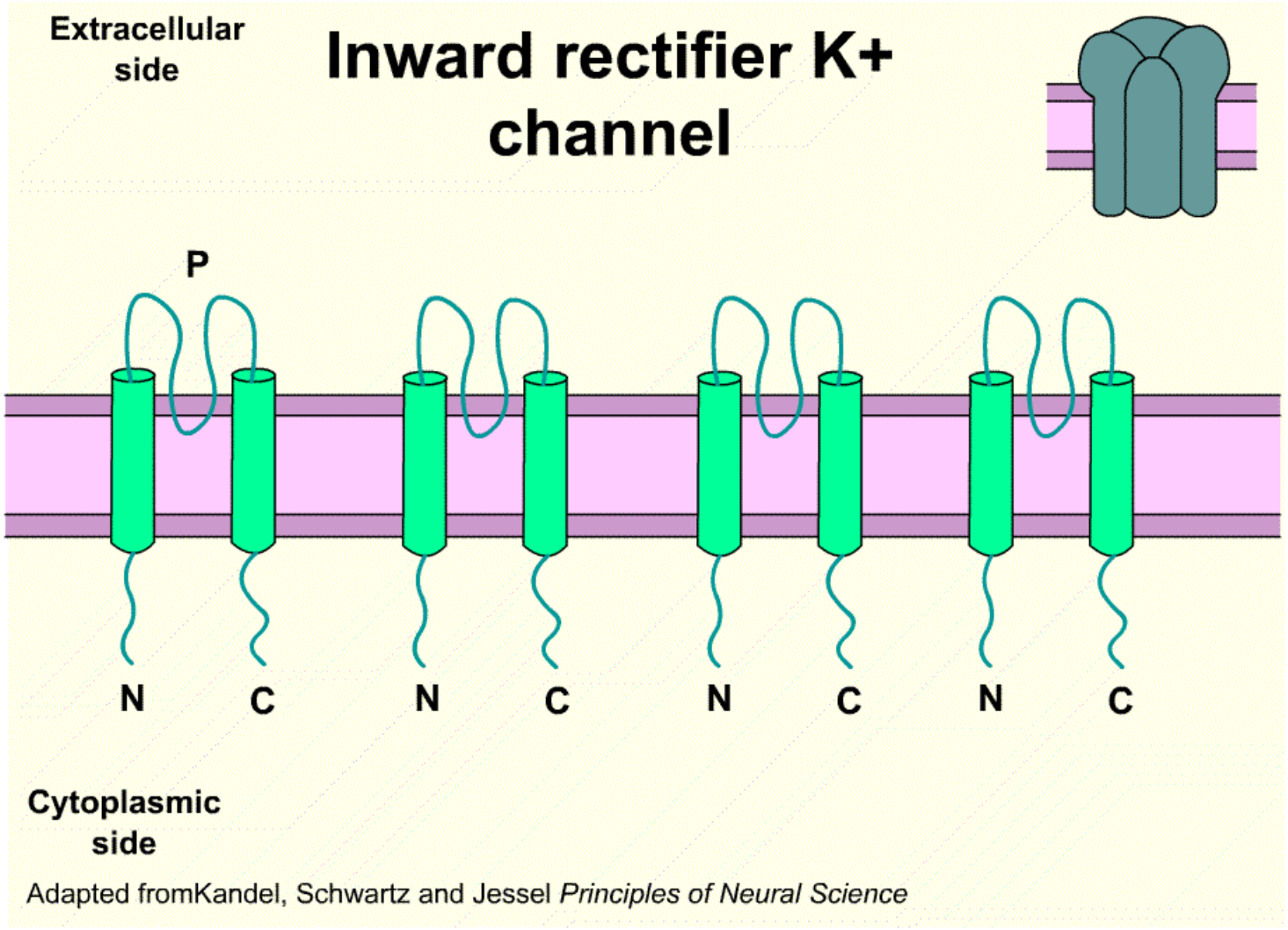


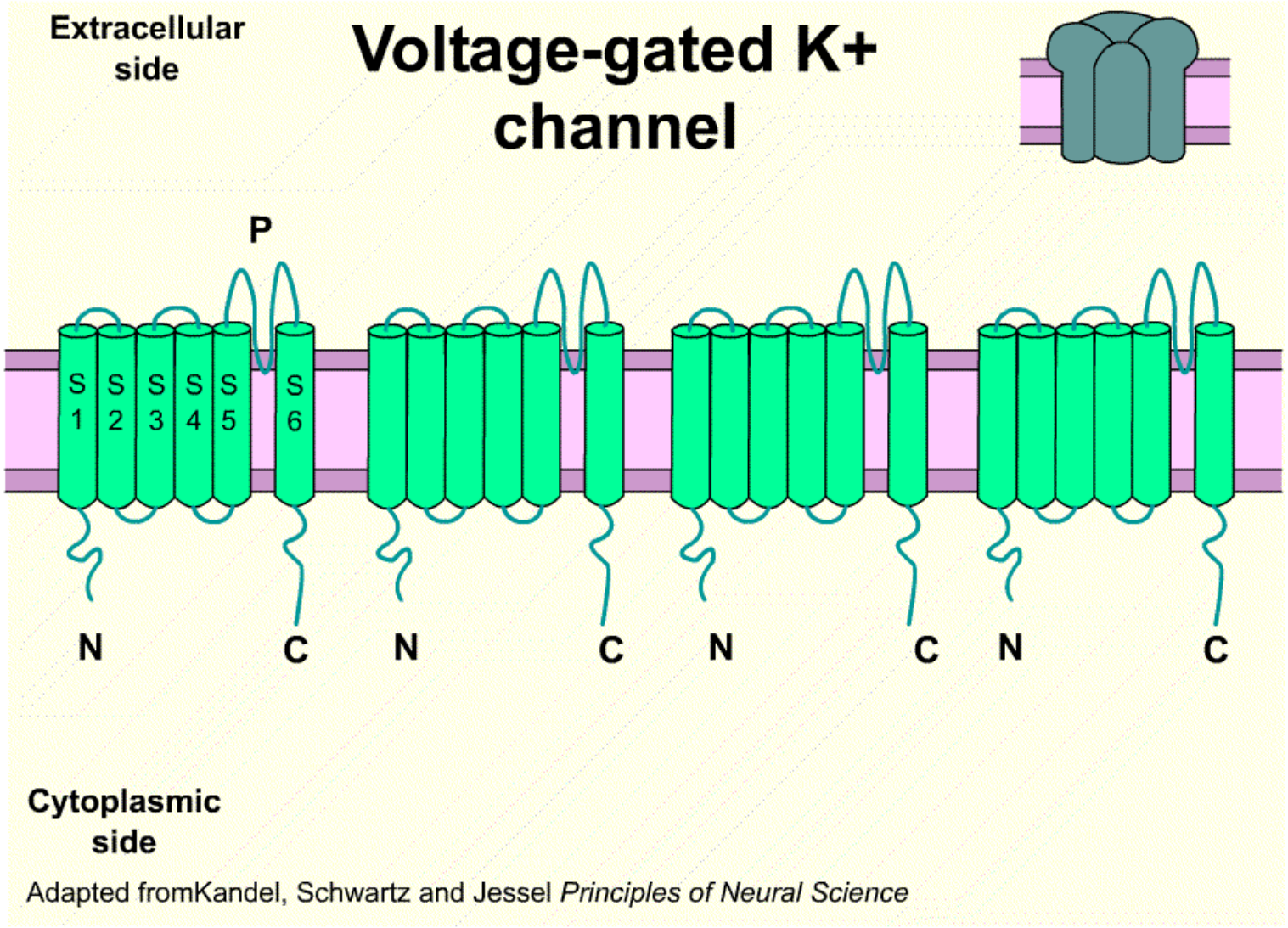
Alpha helix: 3.6 residues/turn; 5.41 Angstroms/turn

Plasma membrane thickness ~ 34 Angstroms

~23 amino acids/transmembrane domain

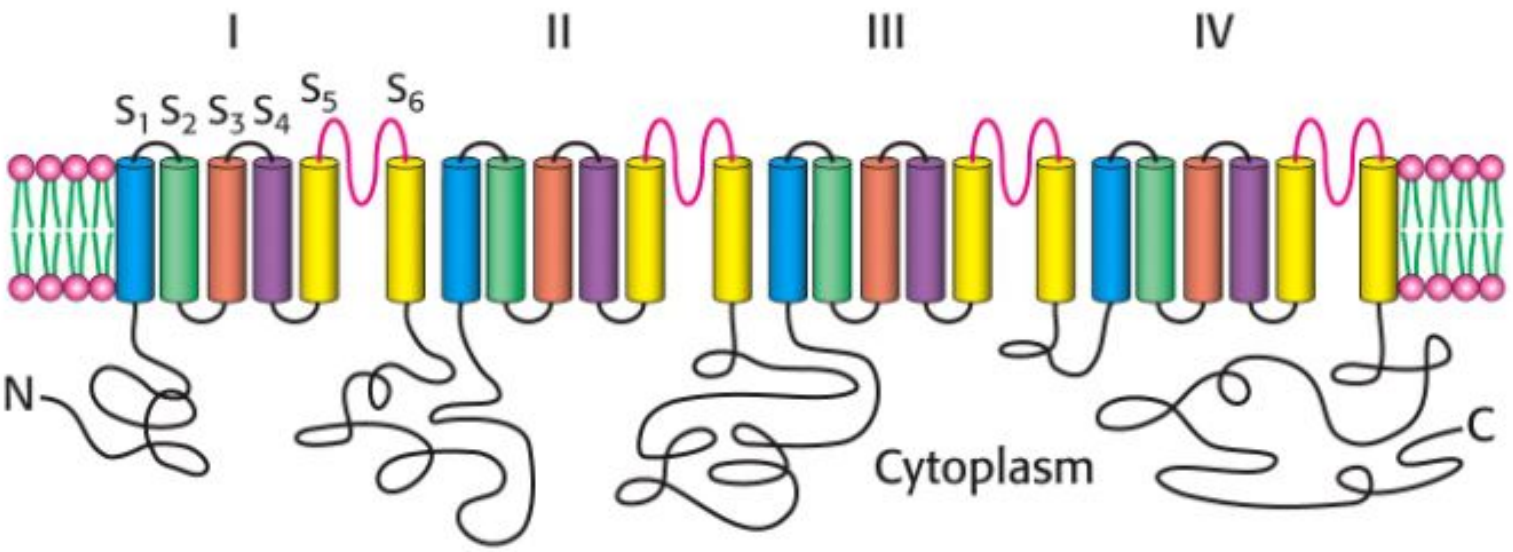
A primitive channel: Kir

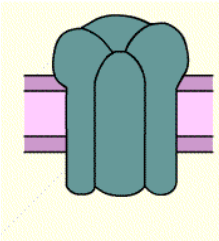




Adapted fromKandel, Schwartz and Jessel *Principles of Neural Science*

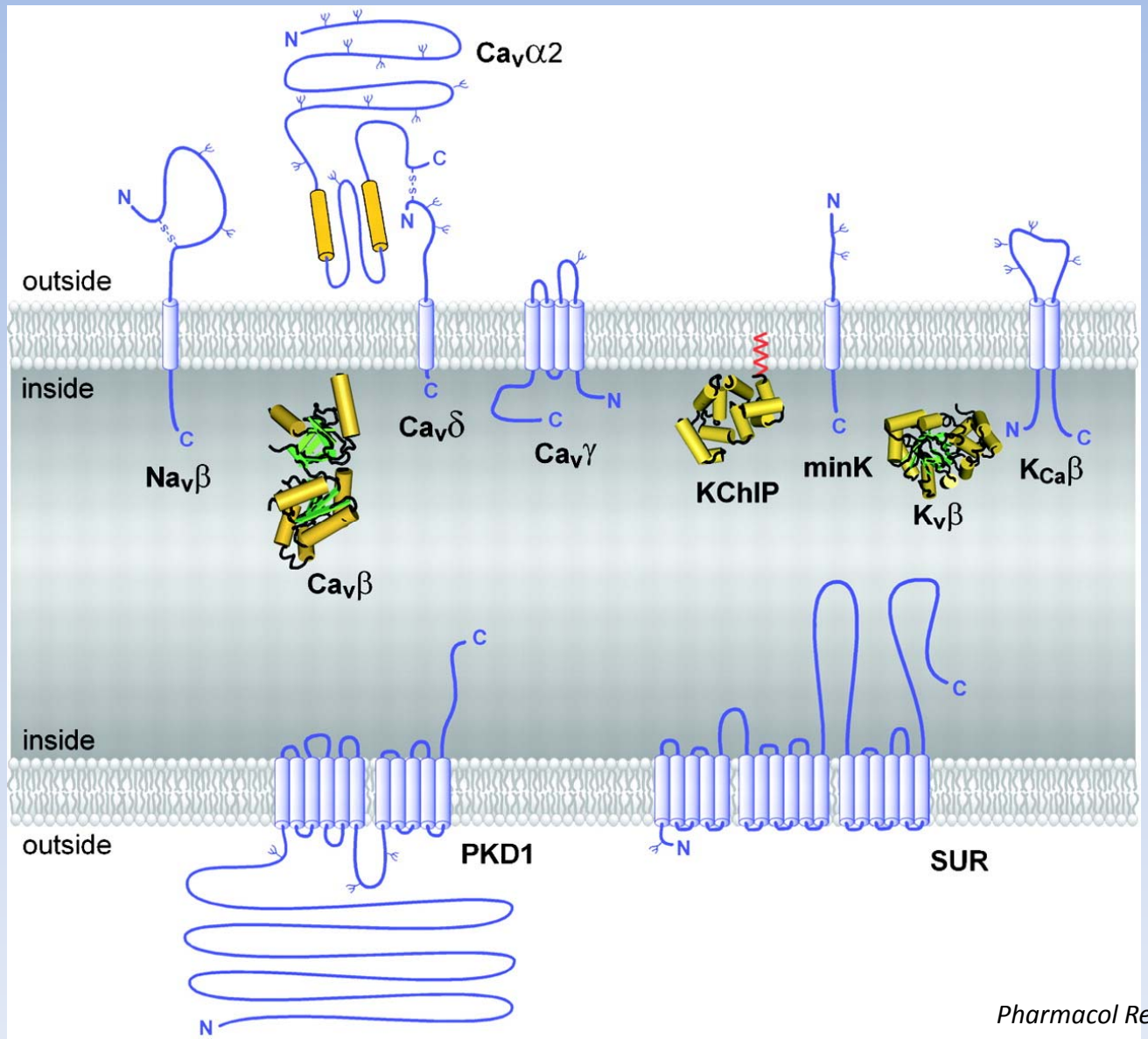
Voltage-gated Na and Ca channel structure



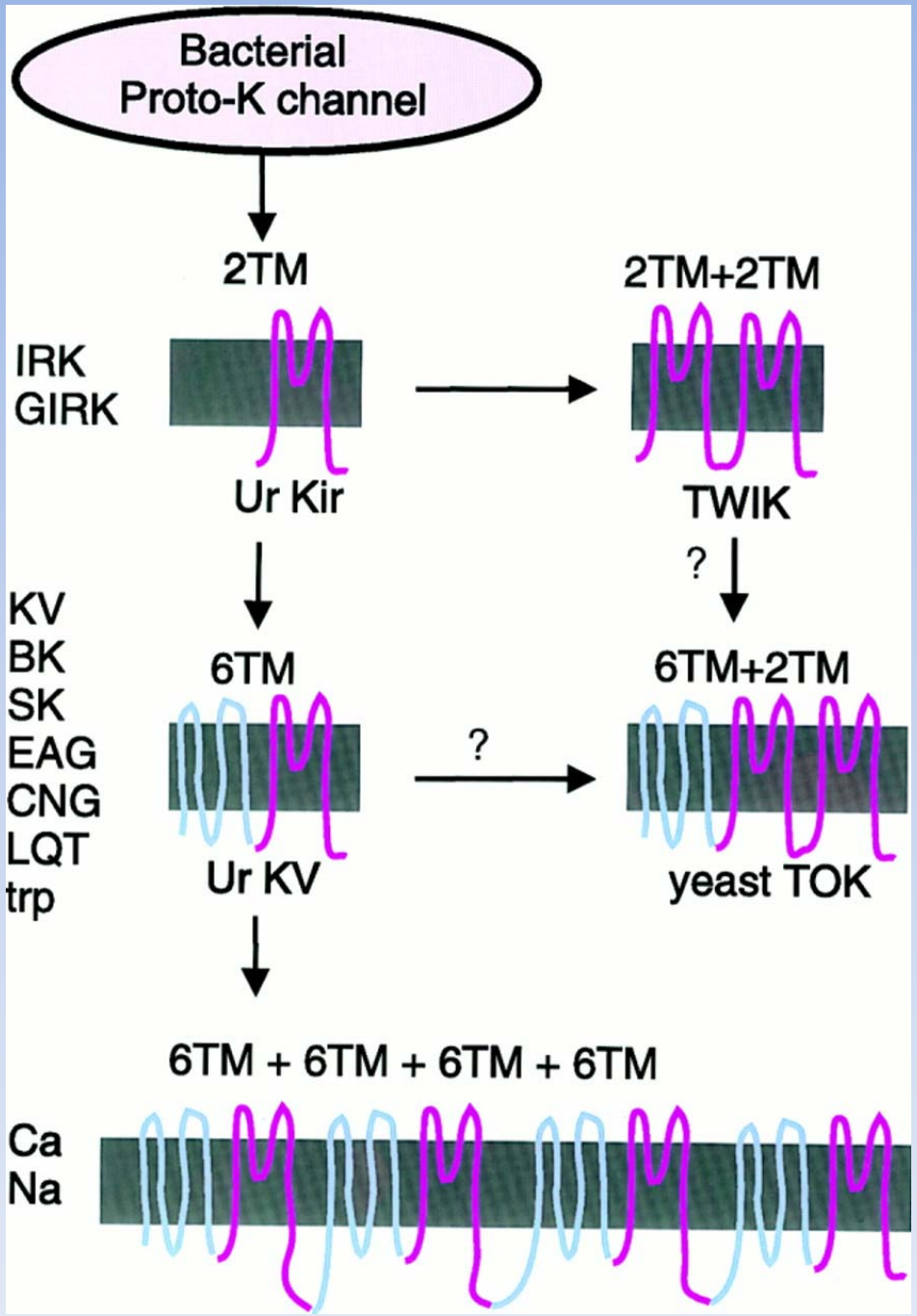


Four motifs (I – IV) in a *single* protein

Auxiliary subunits of the voltage-gated ion channel superfamily

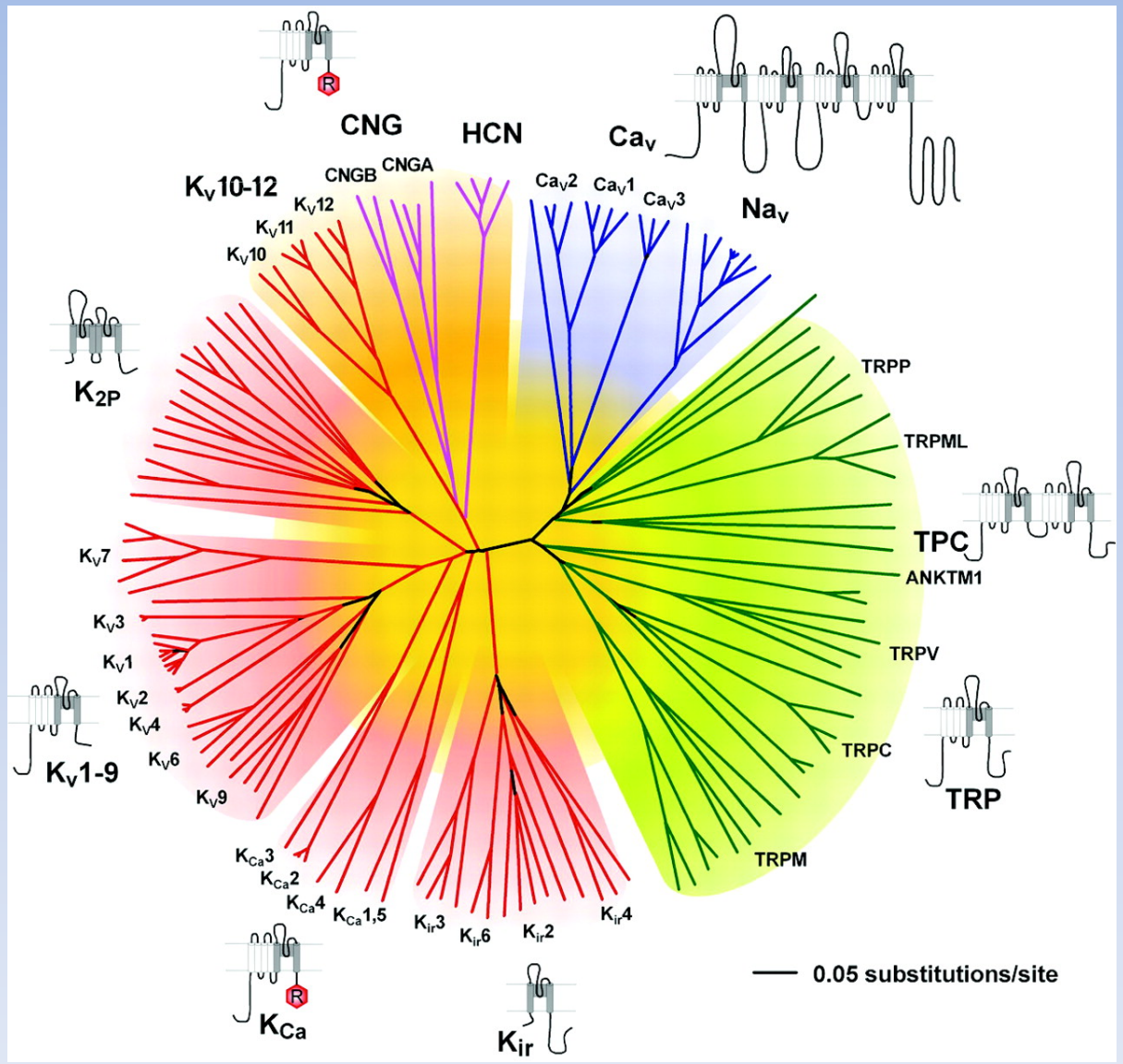


Pharmacol Rev 57:387-395, 2005



Likely Evolution pattern for the Superfamily of Voltage-Gated Channels

Amino acid relationships of the minimal pore regions of the voltage-gated ion channel superfamily (143 types)



Pharmacol Rev 57:387-395, 2005

The “Holy Grail – Part II”

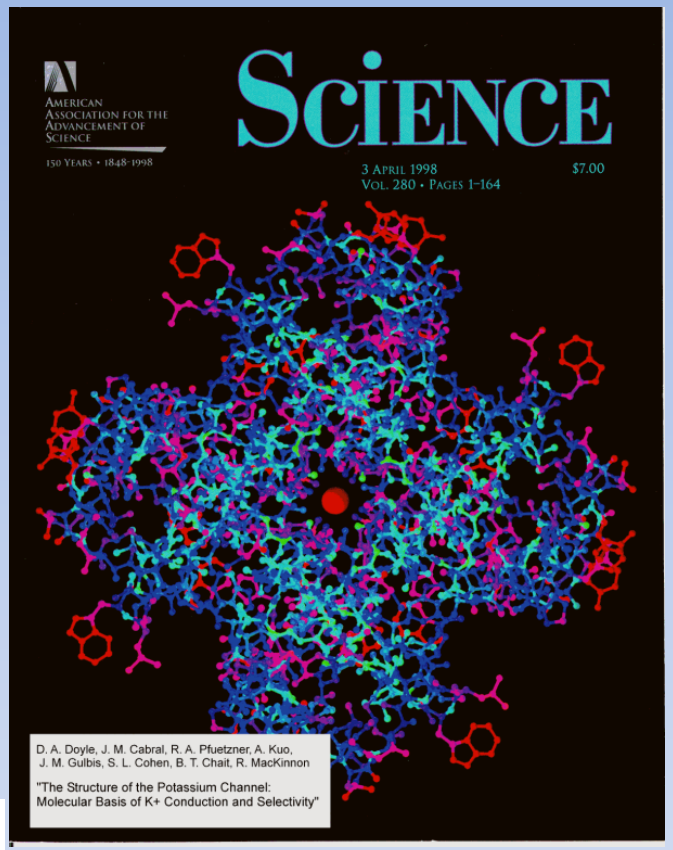
(Clay Armstrong)

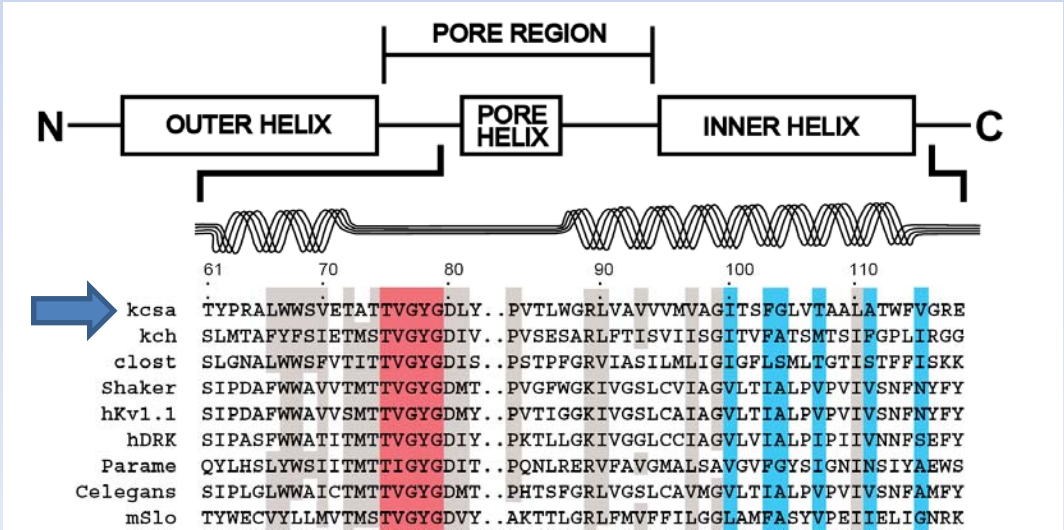
RESEARCH ARTICLES

The Structure of the Potassium Channel: Molecular Basis of K^+ Conduction and Selectivity

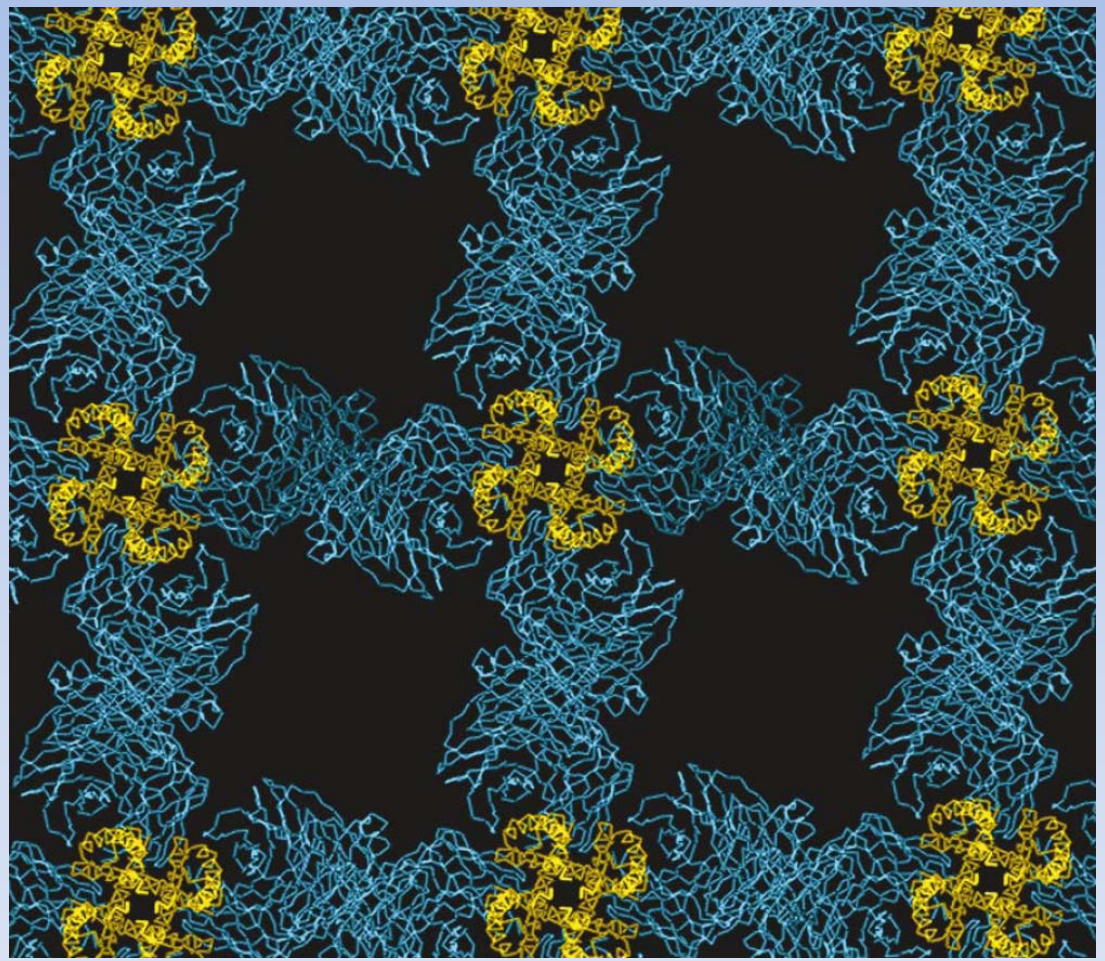
Declan A. Doyle, João Morais Cabral, Richard A. Pfuetzner, Anling Kuo, Jacqueline M. Gulbis, Steven L. Cohen, Brian T. Chait, Roderick MacKinnon*

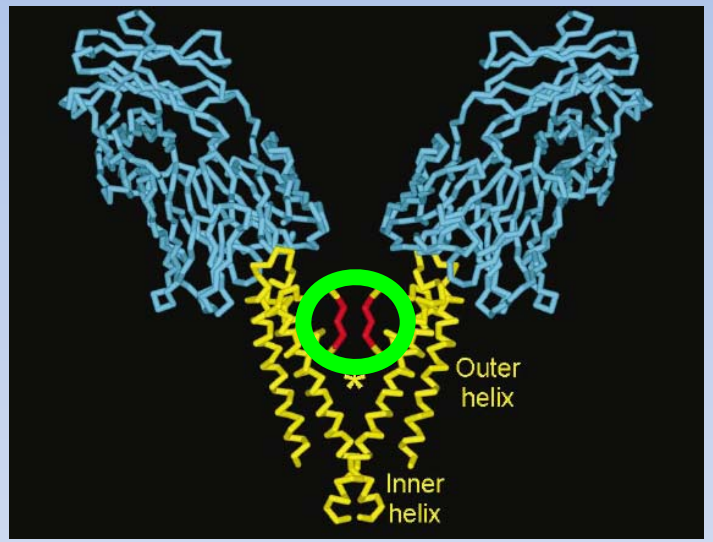
SCIENCE • VOL. 280 • 3 APRIL 1998

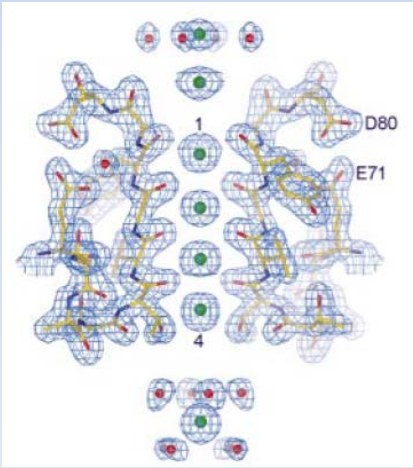




KcsA channel co-crystallized with an antibody Fab fragment to stabilize structure & enhance x-ray resolution







2.0 Angstrom resolution!

Zhou et al (2001) Nature

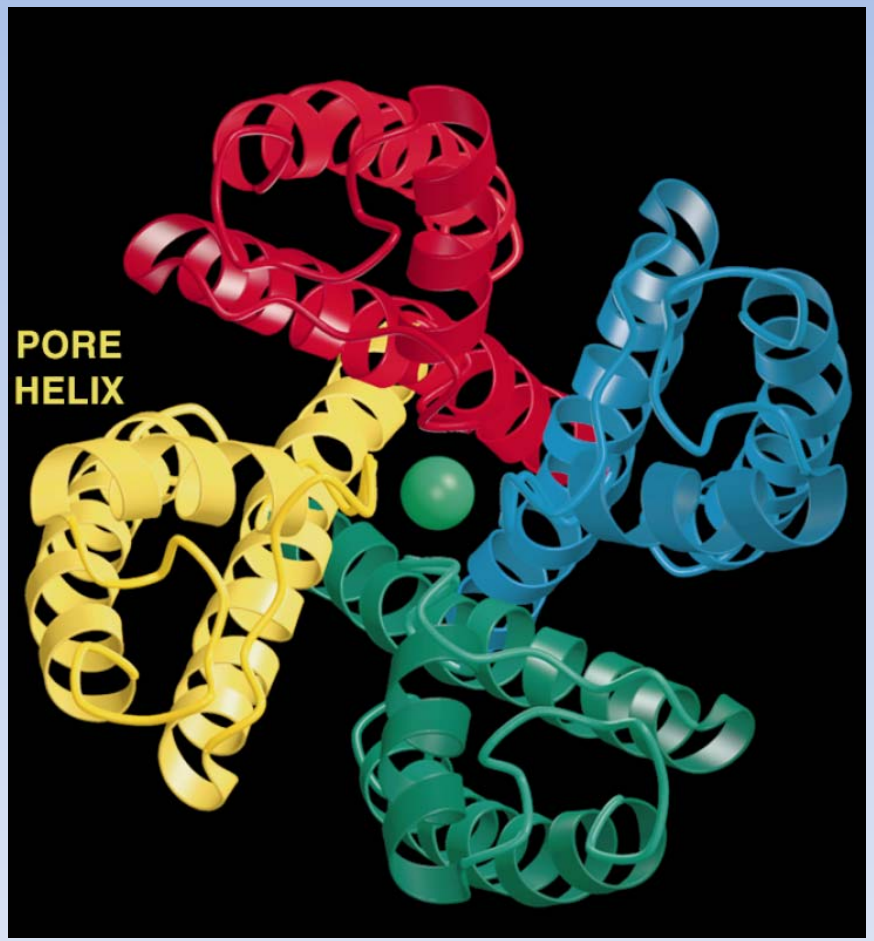
X-ray crystal structures were first obtained from bacterial channels

- KcsA: 2 TM domains/subunit
  - Activated by protons
- MthK: 2 TM domains/subunit
  - Activated by intracellular Ca^{2+}
- KvAP: 6 TM domains/subunit
  - Activated by voltage

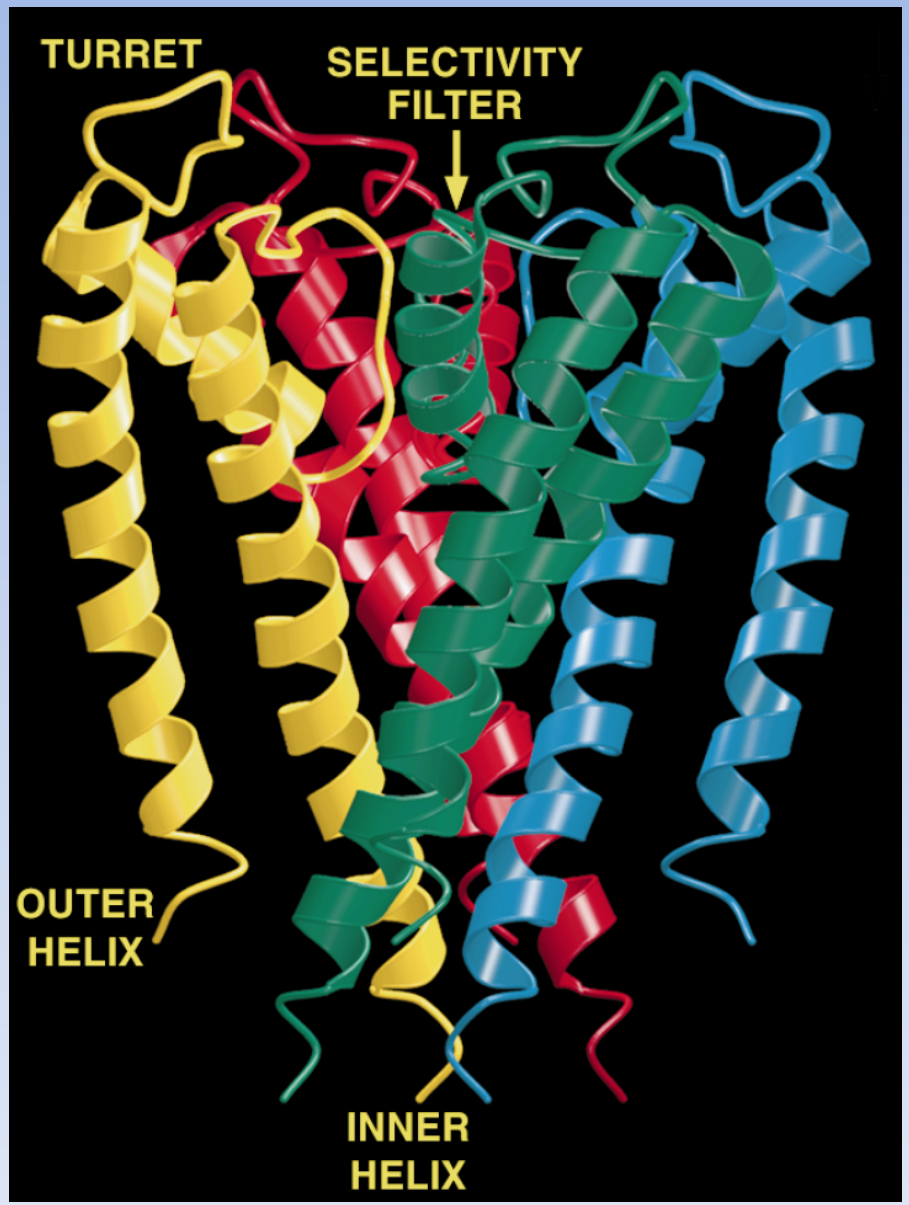
All structures solved in Rod MacKinnon's lab at Rockefeller Univ
(Nobel Prize in Chemistry, 2003)

Doyle et al (1998) Science *280*, 69

KcsA bacterial K channel

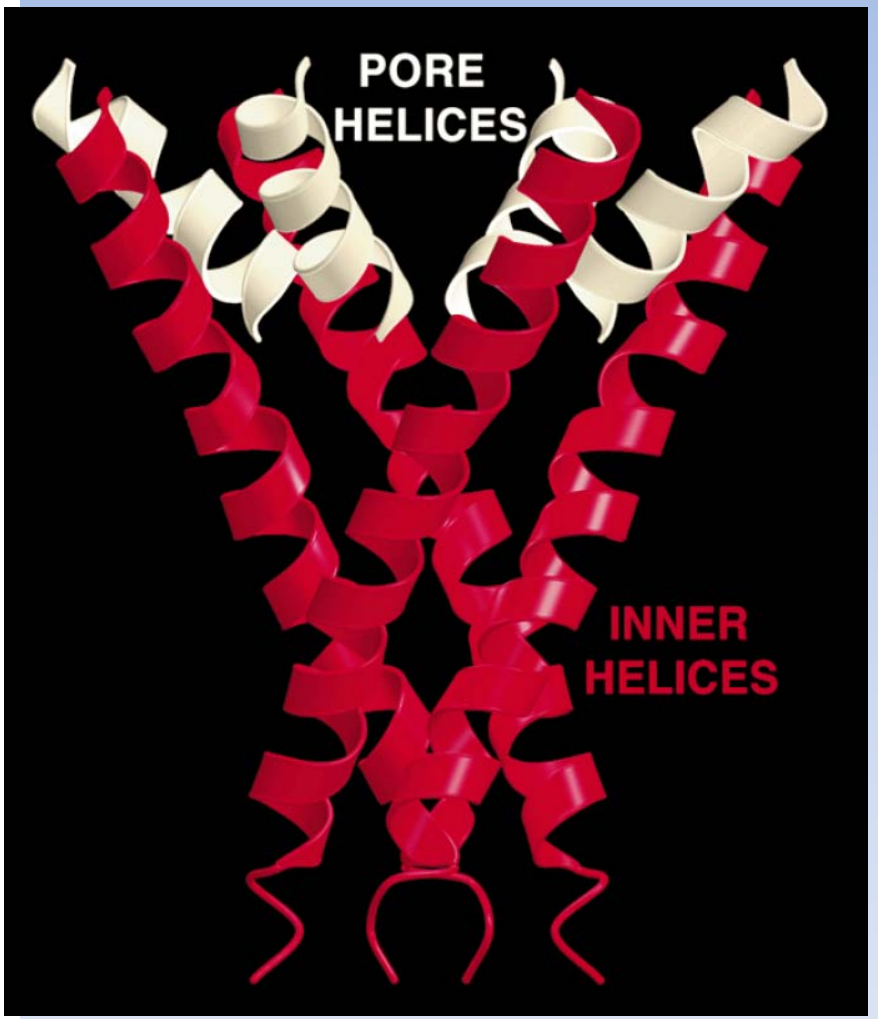


View from extracellular side

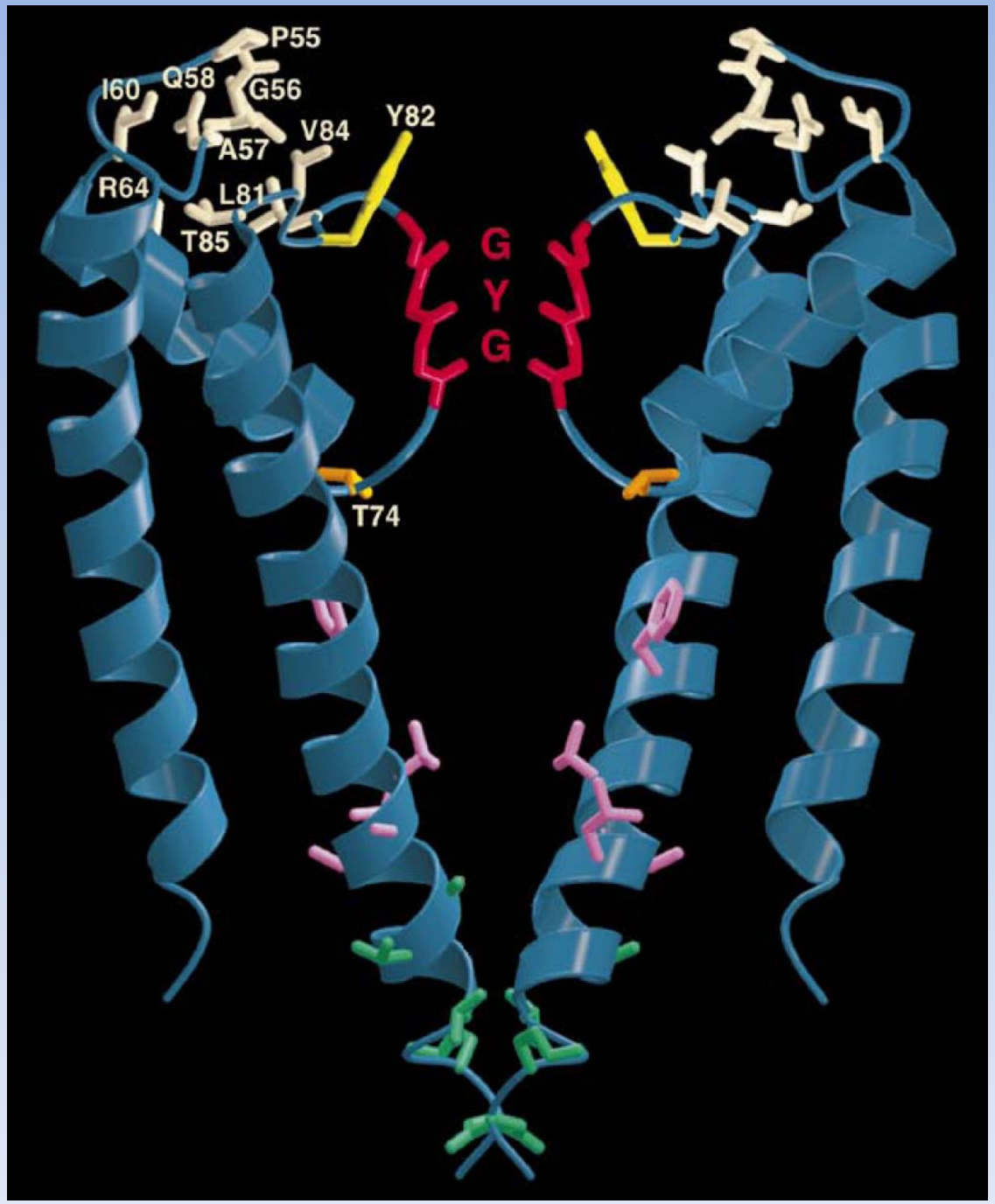


Side view – within membrane

Inner helices form “inverted teepee” structure



Doyle et al (1998) Science *280*, 69



Mutations in *Shaker* that affect function are mapped onto KcsA structure

White: agitoxin2, charybdotoxin binding

Yellow: external TEA binding
Mustard (T74): internal TEA binding

pink: accessible by intracellular ligand only when channel is open

Green: accessible by intracellular ligand when channel is open or closed

GYG – required for K selectivity

Doyle et al (1998) Science *280*, 69

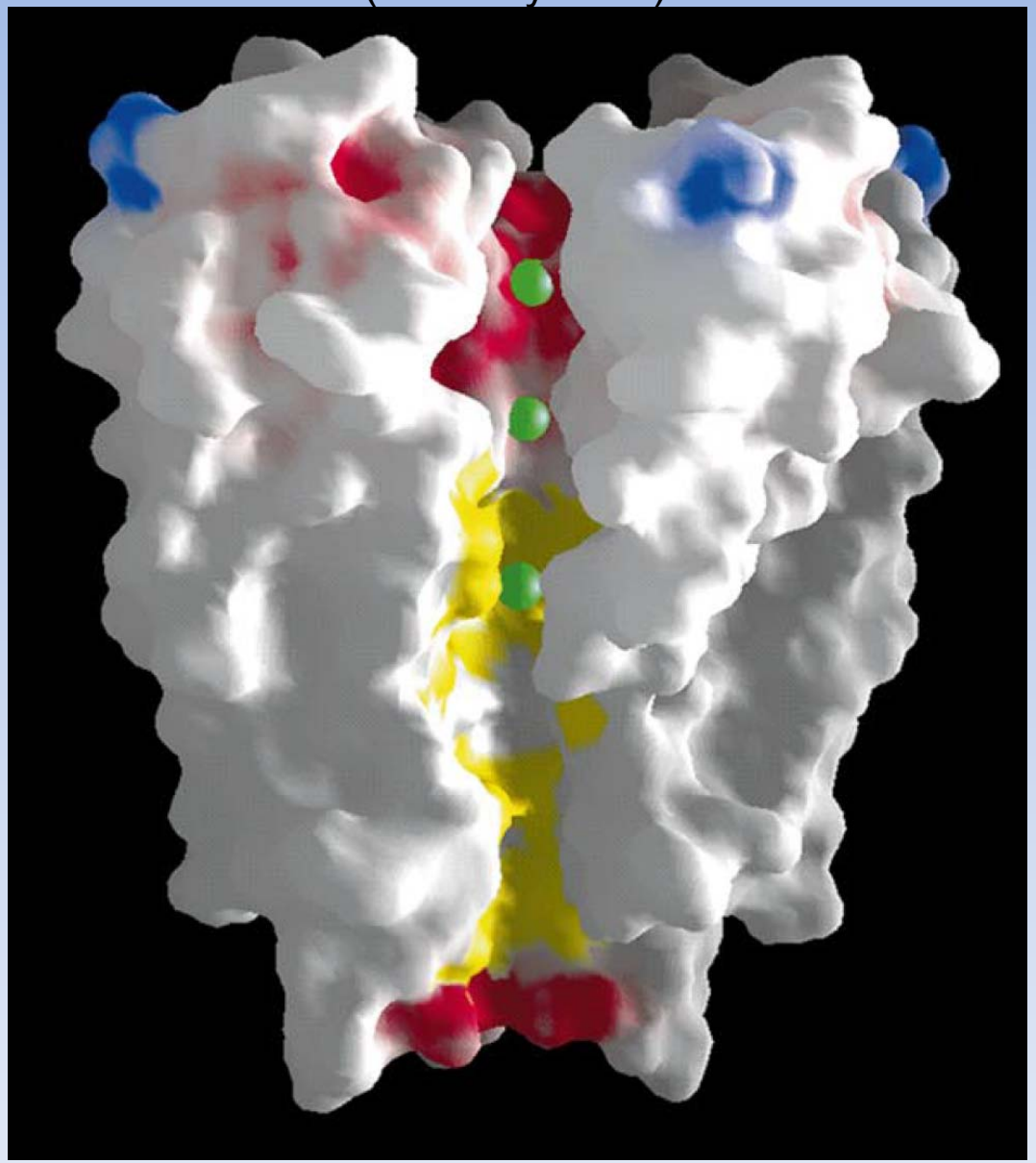
Molecular surface of KcsA and contour of the pore

(cutaway view)

Blue
Basic (+) residues

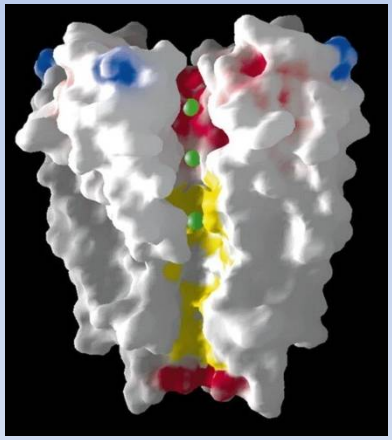
Yellow:
Hydrophobic residues

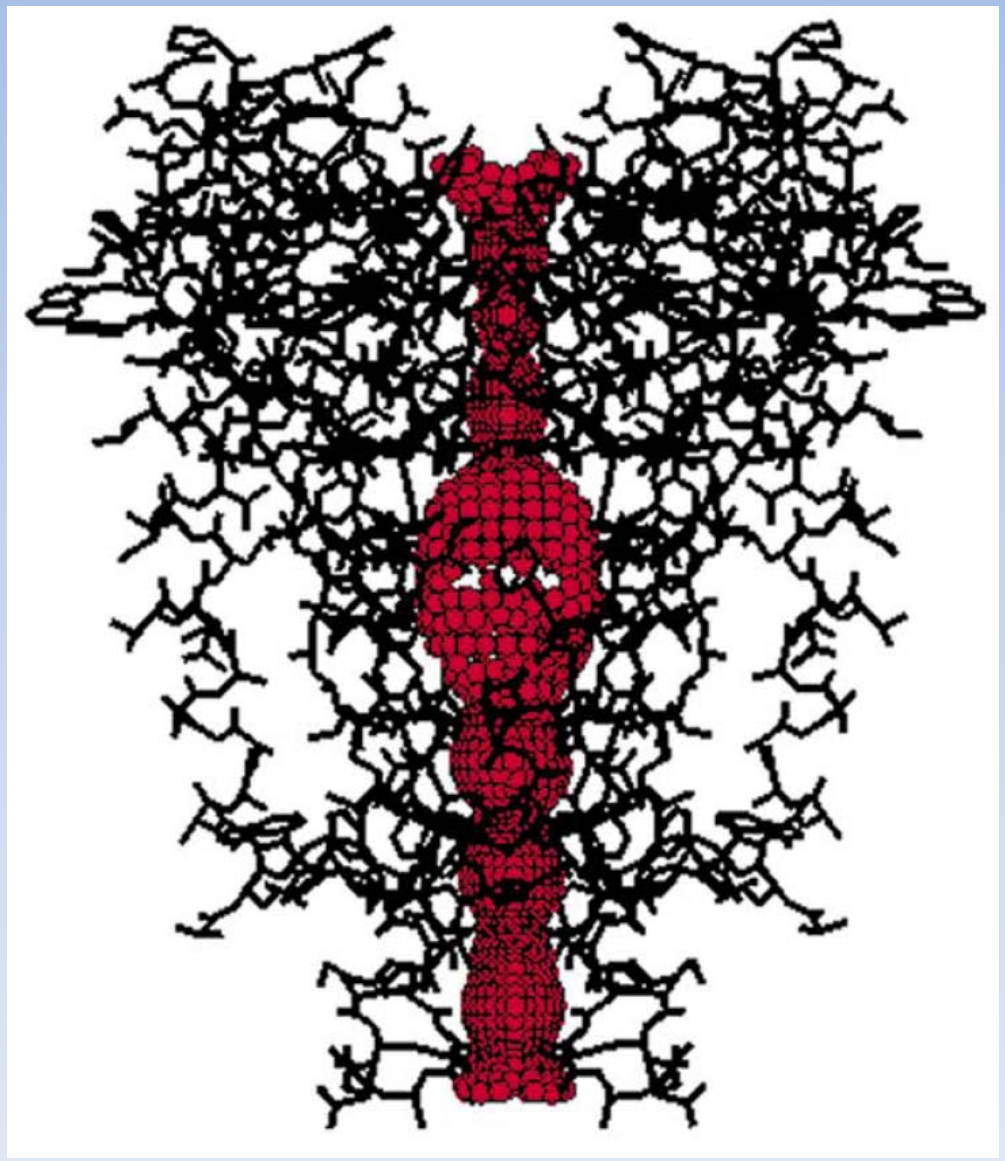
Red:
Acidic (-) residues



K^+

Representation of the inner pore based on nearest van der Waals protein contact





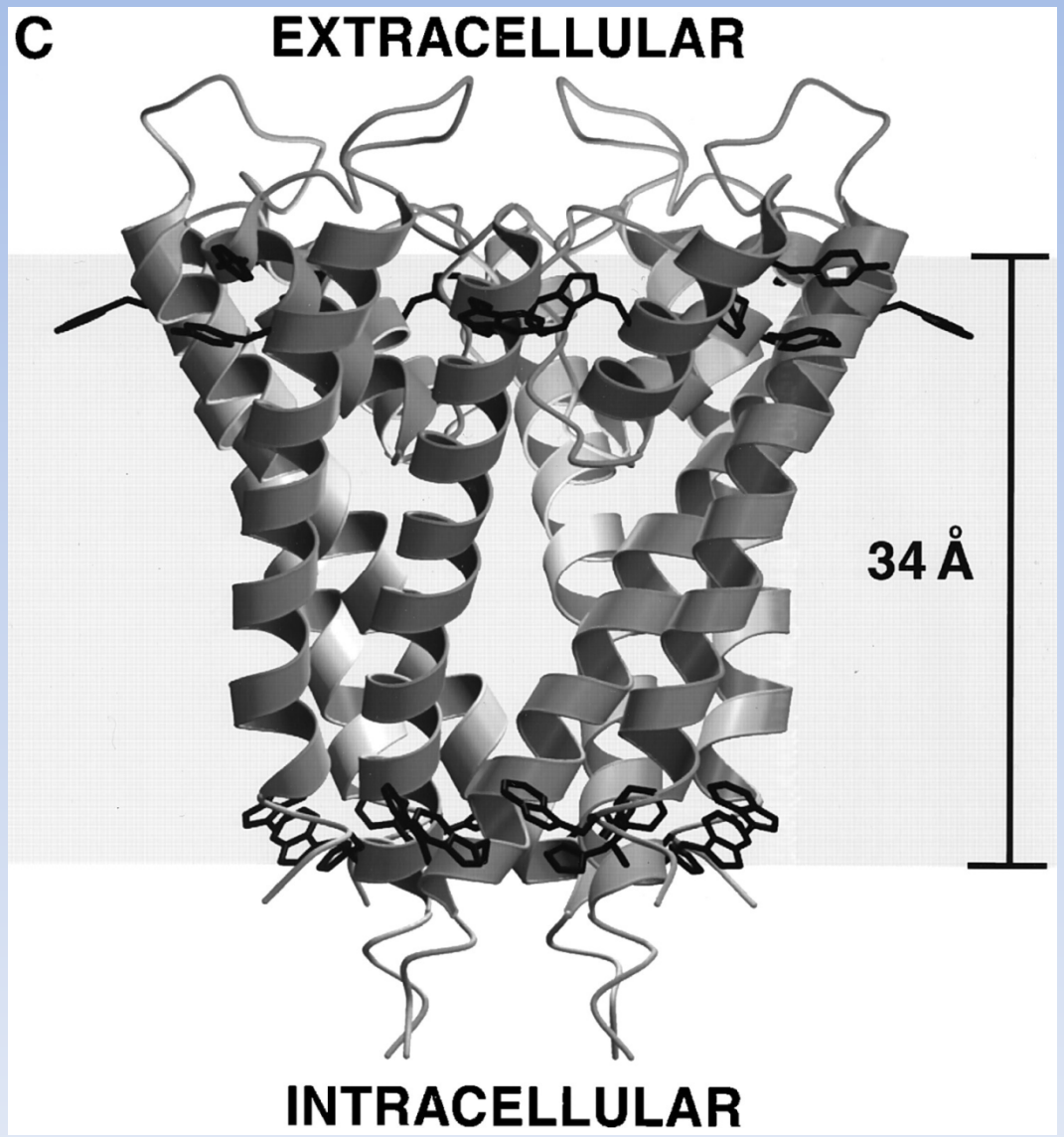
Outer vestibule

Selectivity Filter

Central cavity

Outer vestibule

Ring of aromatic amino acids define the membrane-facing surface

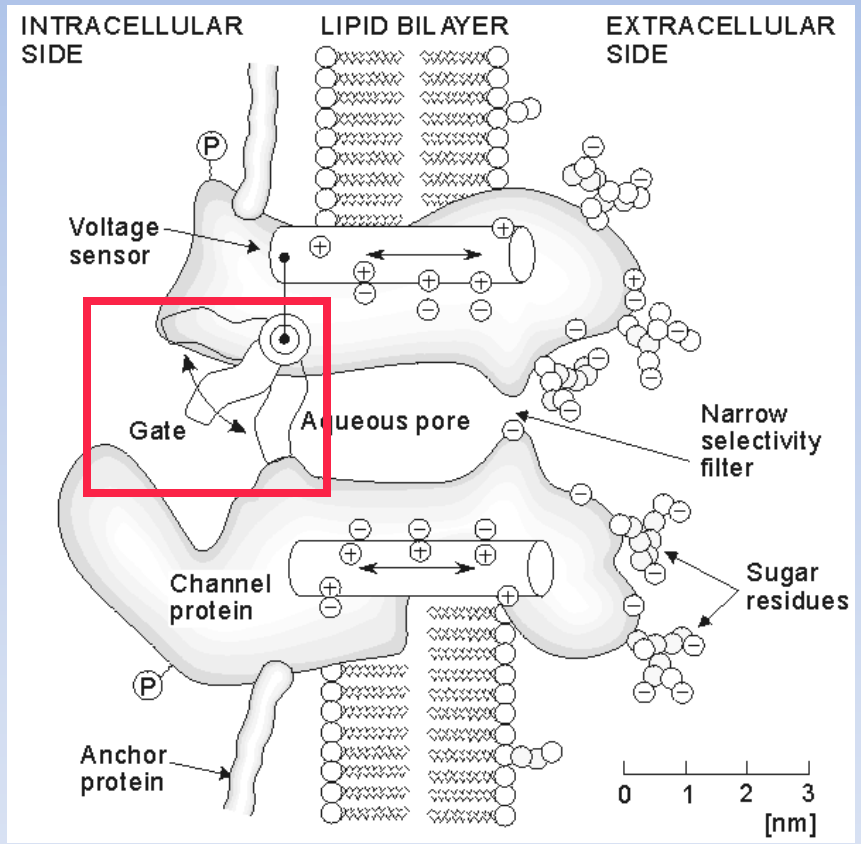


Doyle et al (1998) Science *280*, 69

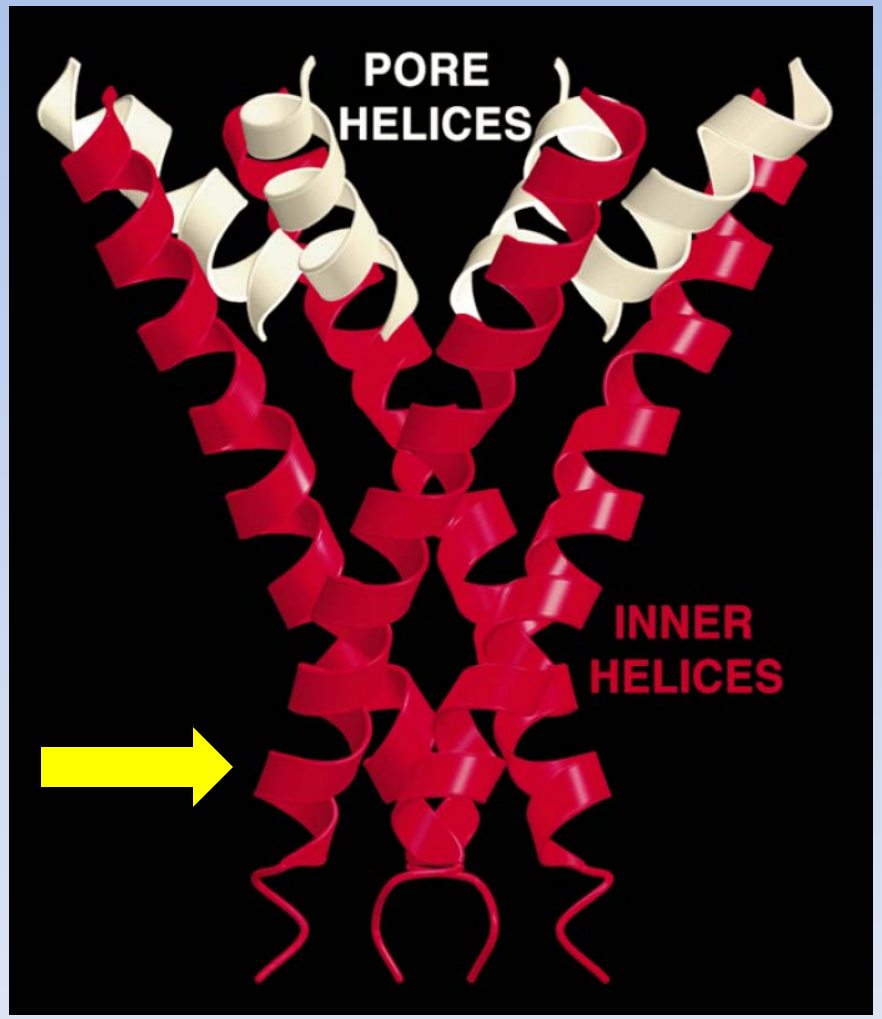
Activation gate

Gates

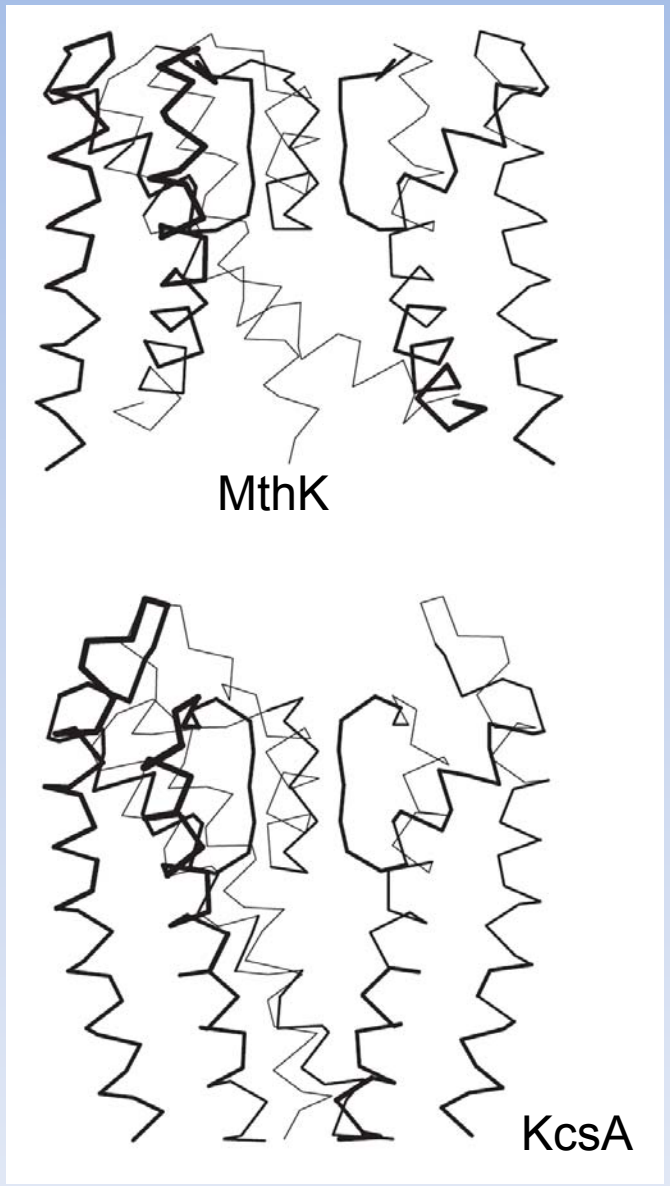
- Activation
- Inactivation



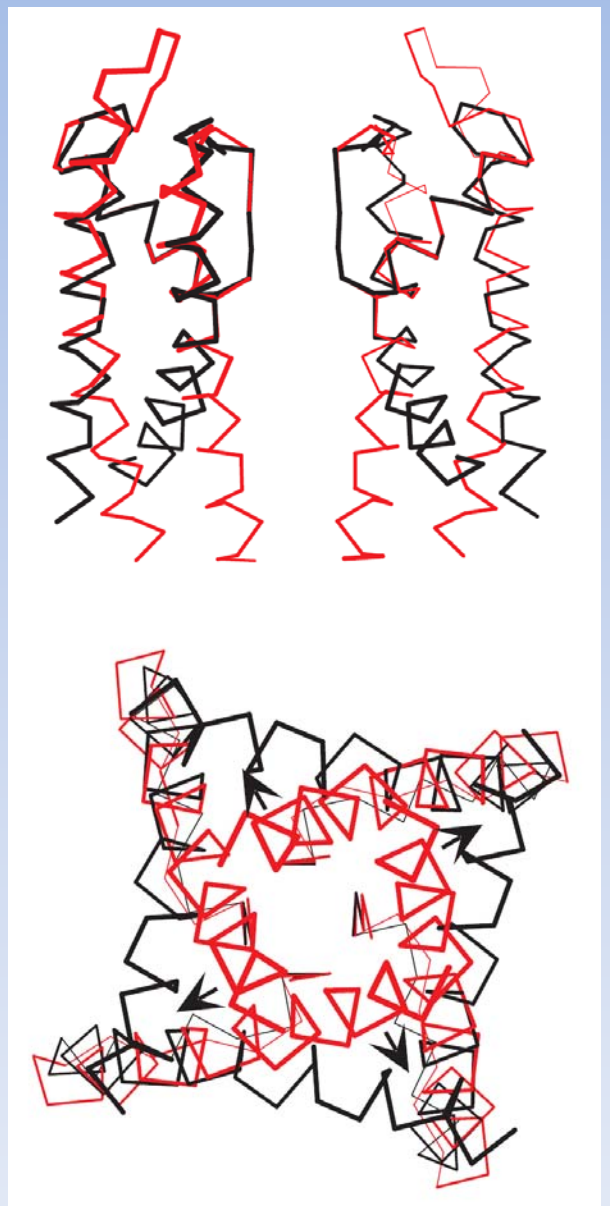
Bundle crossing of inner helices defines the “activation gate”



Open vs closed state of bacterial K channels



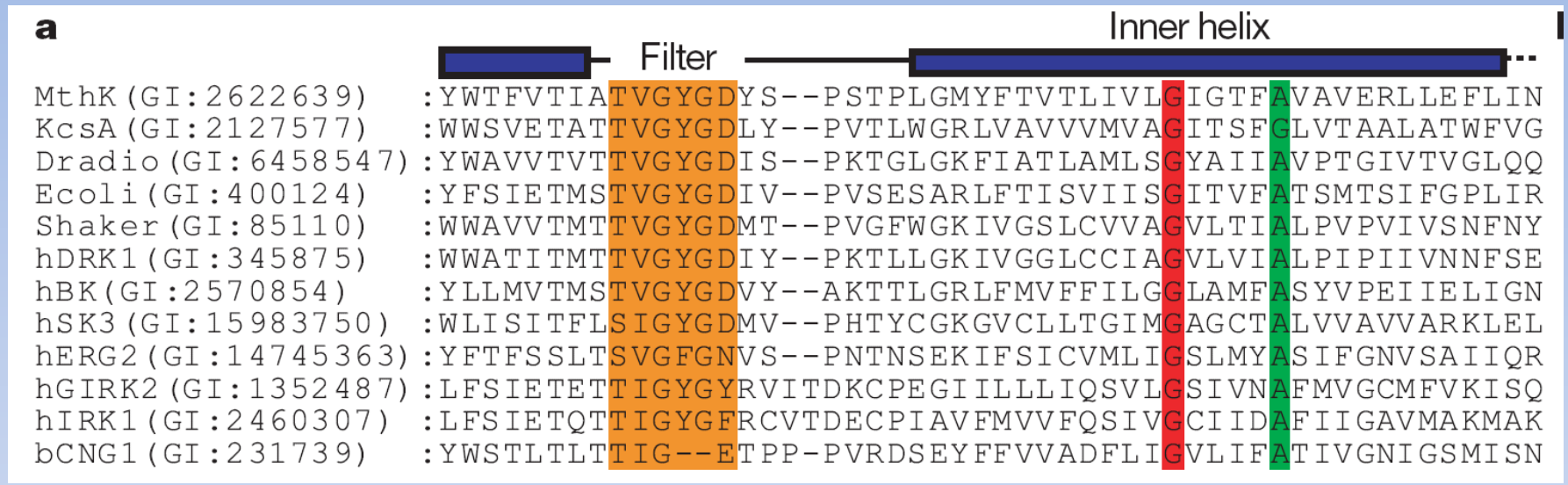
KcsA MthK



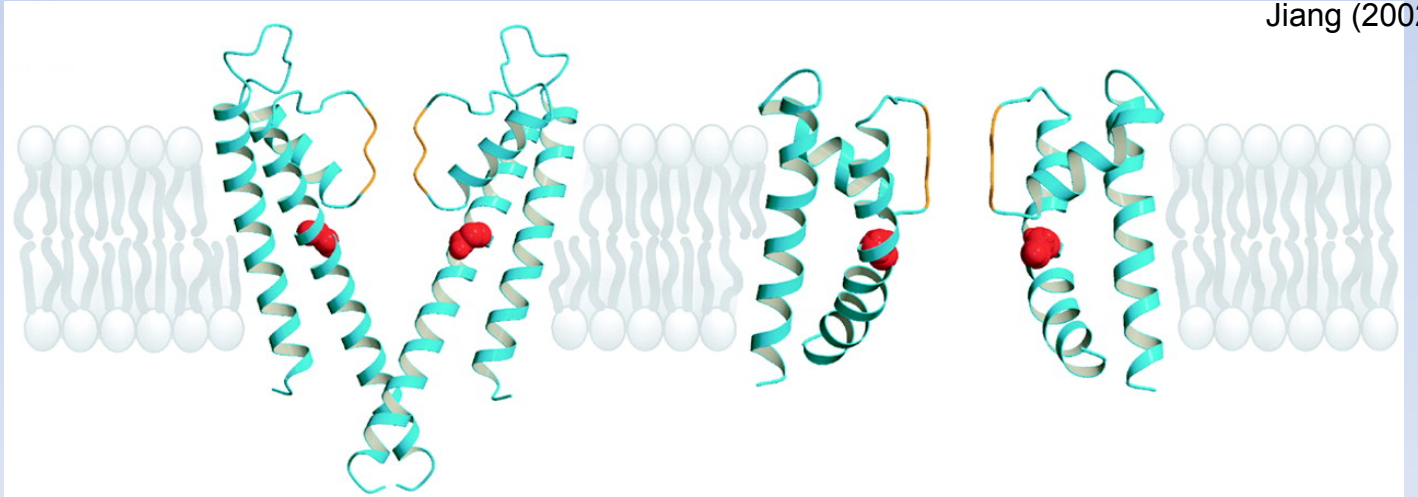
Side view

View from cytoplasm

Channel opening: inner helices bend at “glycine hinge”

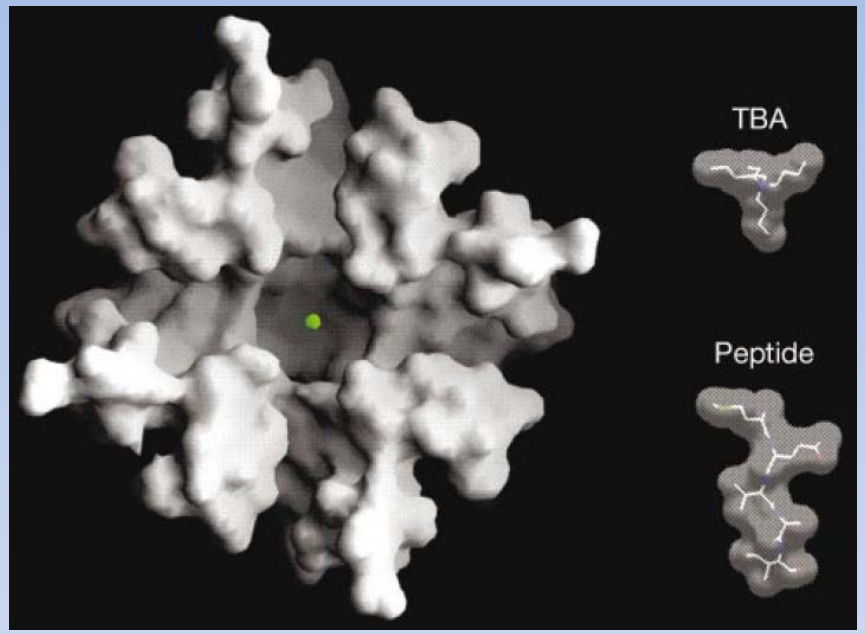


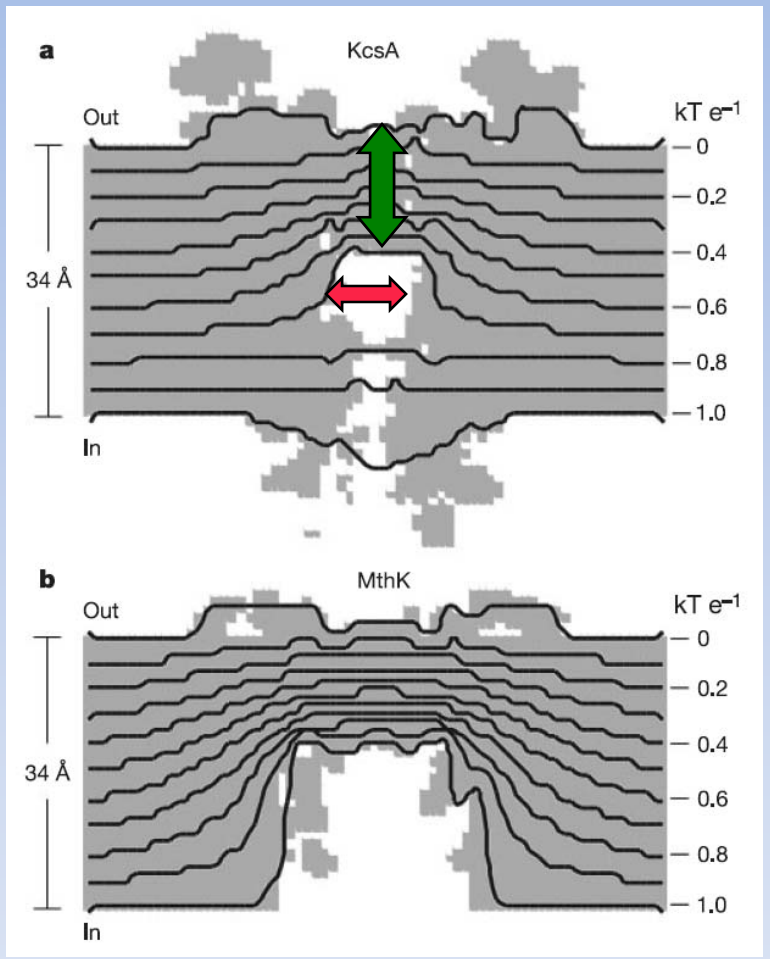
Jiang (2002) Nature **417**: 523



Pharmacol Rev 57: 387-395, 2005

Molecular surface of the MthK pore viewed from the intracellular solution





12 Å

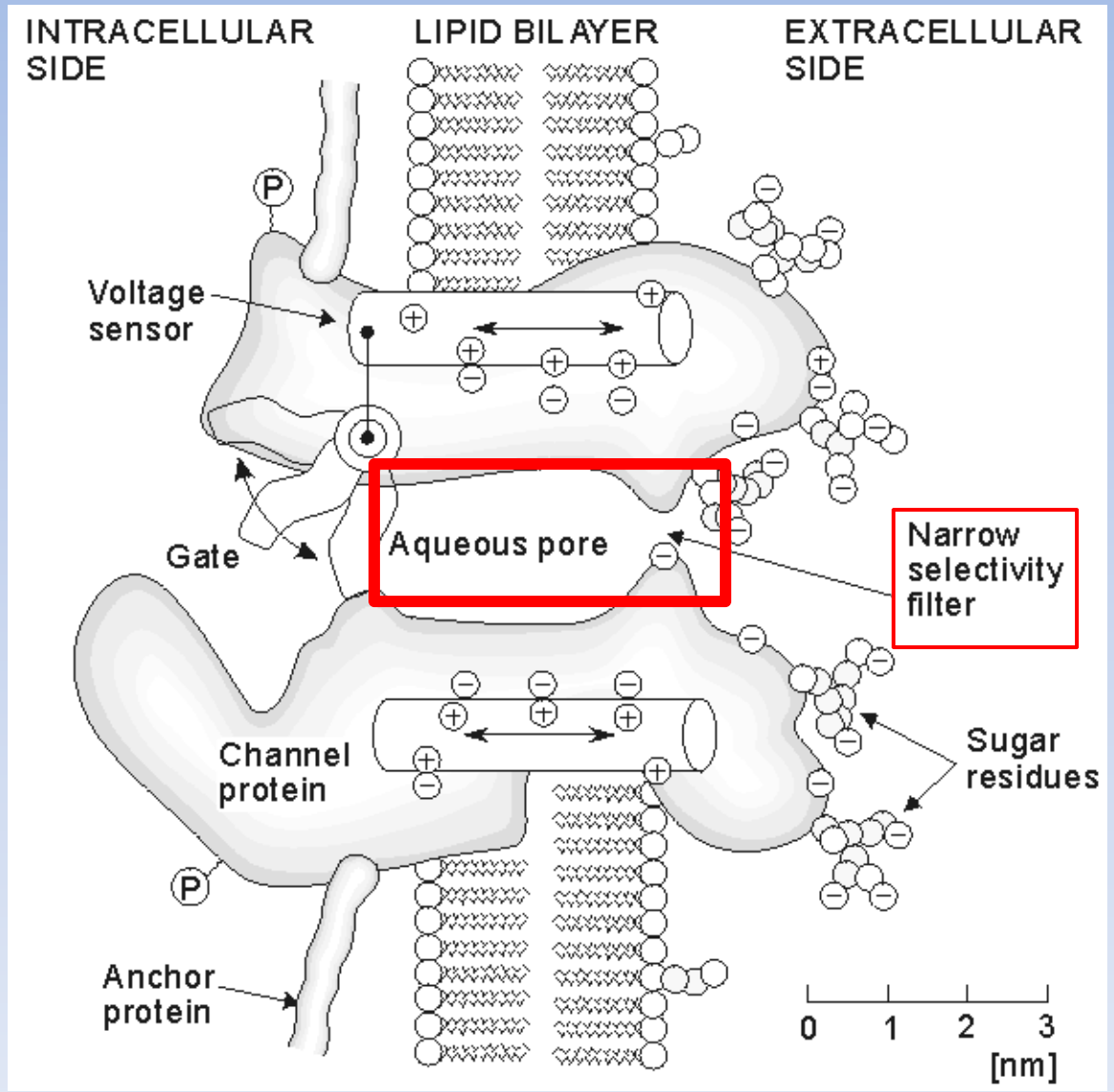
10 Å

The membrane electric potential across the pore changes on opening. Electrostatic contour plots for KcsA (**a**) and MthK (**b**) in a membrane.
grey region: protein or membrane (dielectric constant 2)
white regions: aqueous solution (dielectric constant 80)

Jiang (2002) Nature **417**: 523

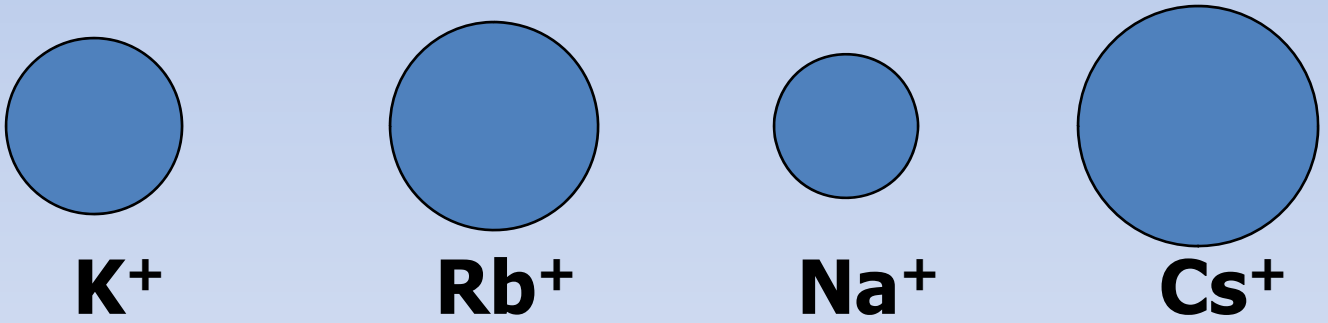
Ion Selectivity

Selectivity filter



Selective ion permeability

Permeability in K channels

	K^+	Rb^+	Na^+	Cs^+
	high	**high**	**low**	**very low**
				
Dehydrated Radius: (Angstroms)	**1.31**	**1.48**	**0.95**	**1.69**

1. How can a channel be selectively permeable to one cation vs another?

Radius of Na^+ is 0.95 Ang, K^+ is 1.31 Ang
-yet K channels selects for K^+ over Na^+ by a factor of 1000-10,000

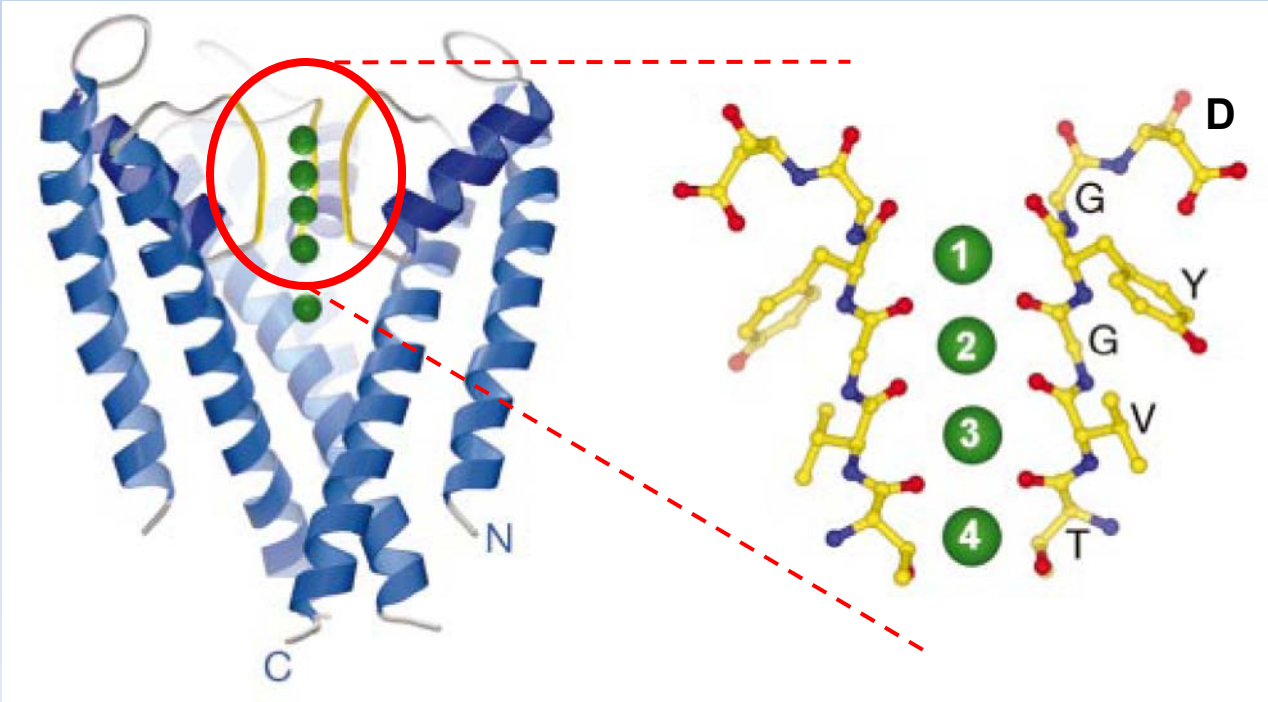
2. How can a channel be highly selective, yet paradoxically have an ion throughput rate near the diffusion limit (100 million ions/sec)?

High selectivity suggests high affinity binding to channel
- this would be expected to slow the ion throughput rate

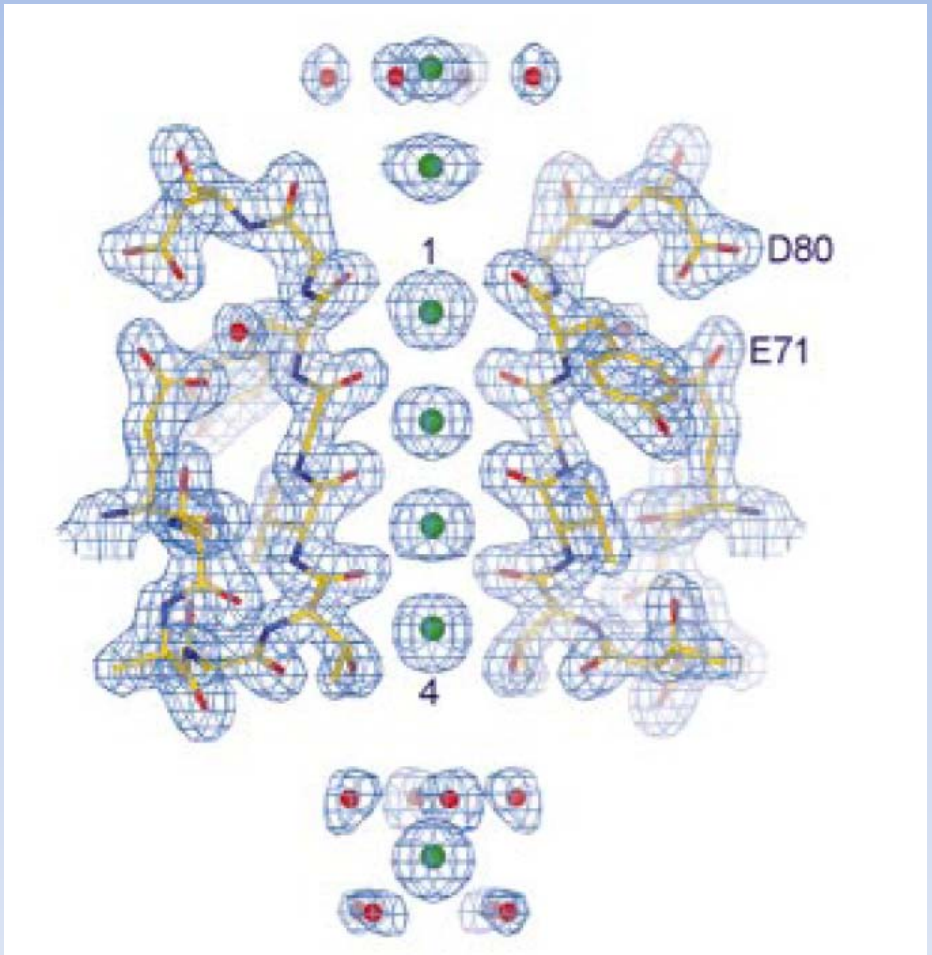
Apparent dilemma solved by x-ray crystallography of a bacterial K-selective channel, KcsA

Selectivity filter of KcsA channel

● K^+

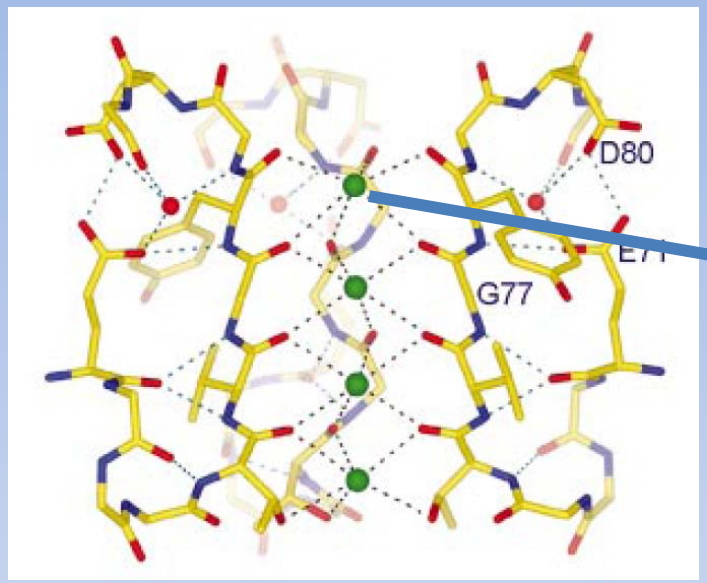


K^+ ions inside the filter are dehydrated

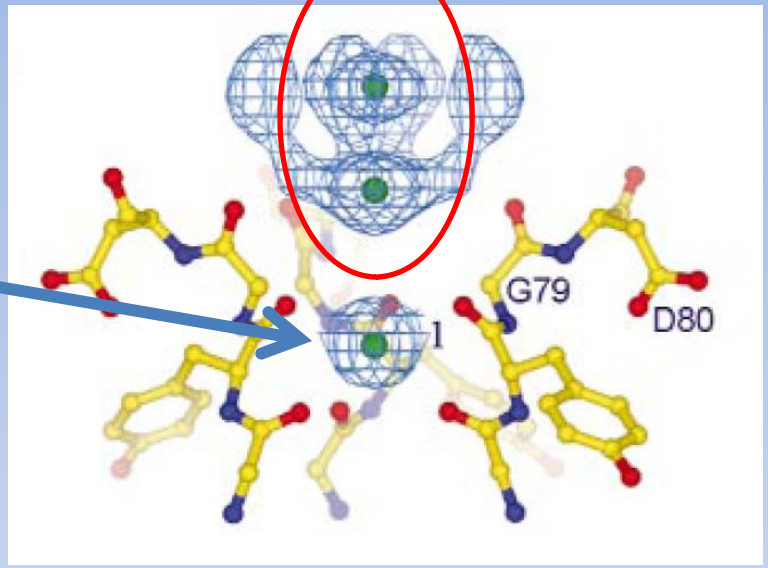


- H_2O
- K^+

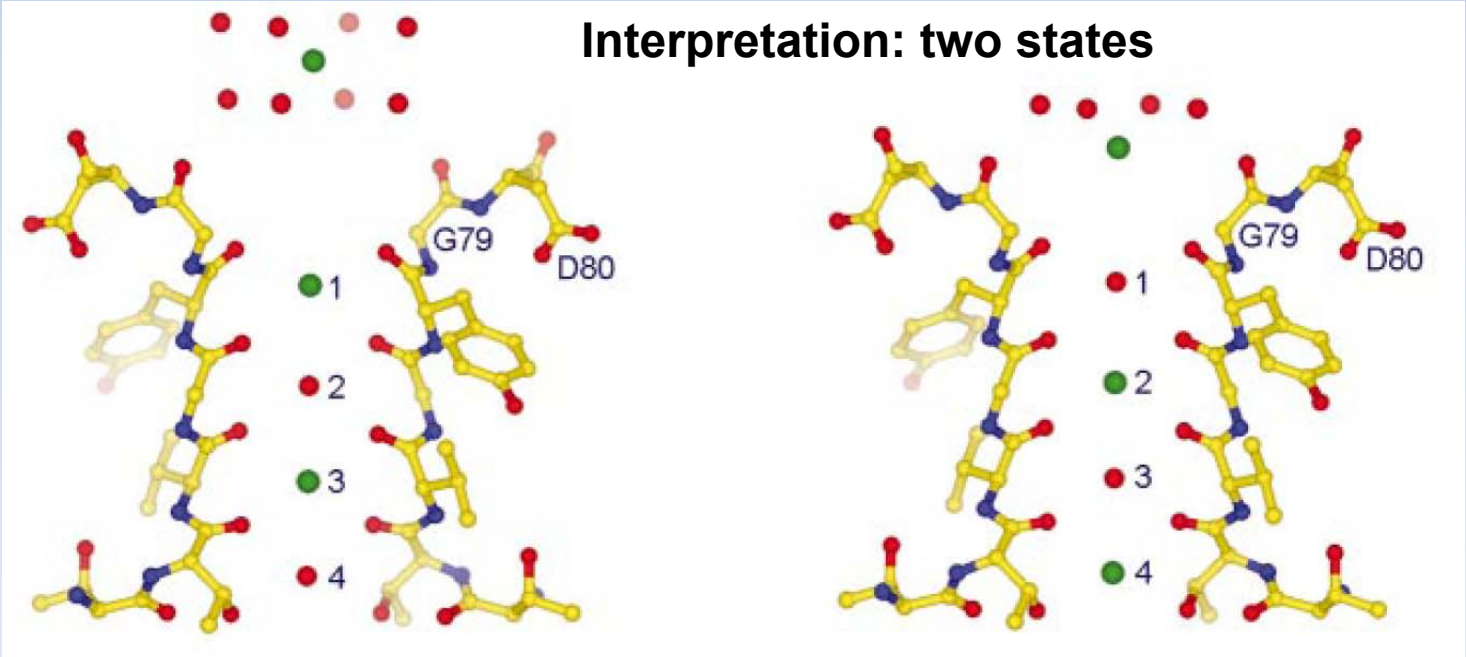
K ion dehydration at the extracellular pore entryway and ion hopping



2 K+ ions



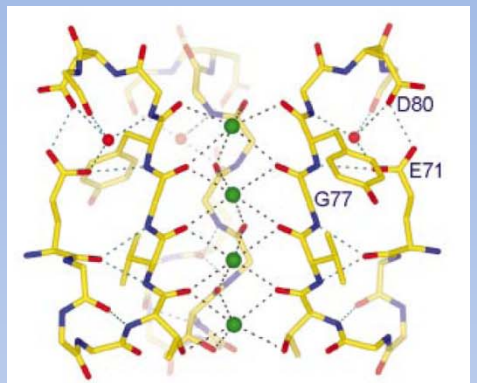
Interpretation: two states



Zhou et al (2001)
Nature, 414:43

Selectivity filter of KcsA channel crystallized in high and low [K^+]

High K structure (conducting)

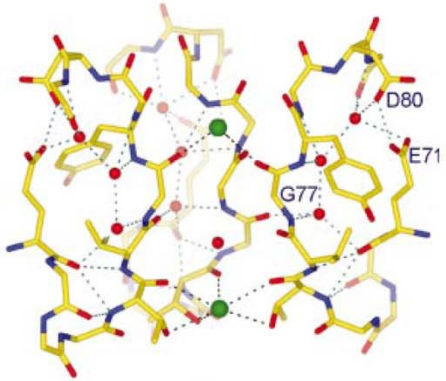


1,3

or

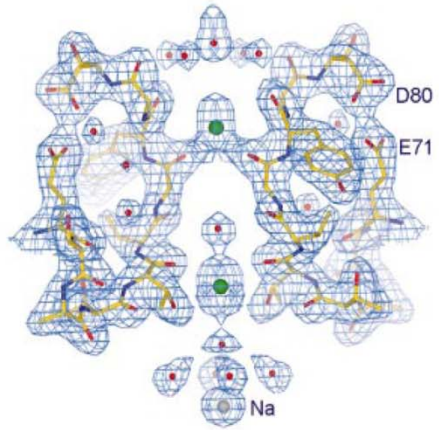
2,4

Low K structure (nonconducting)



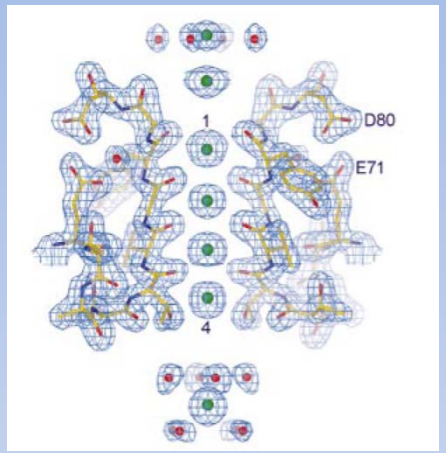
1,4

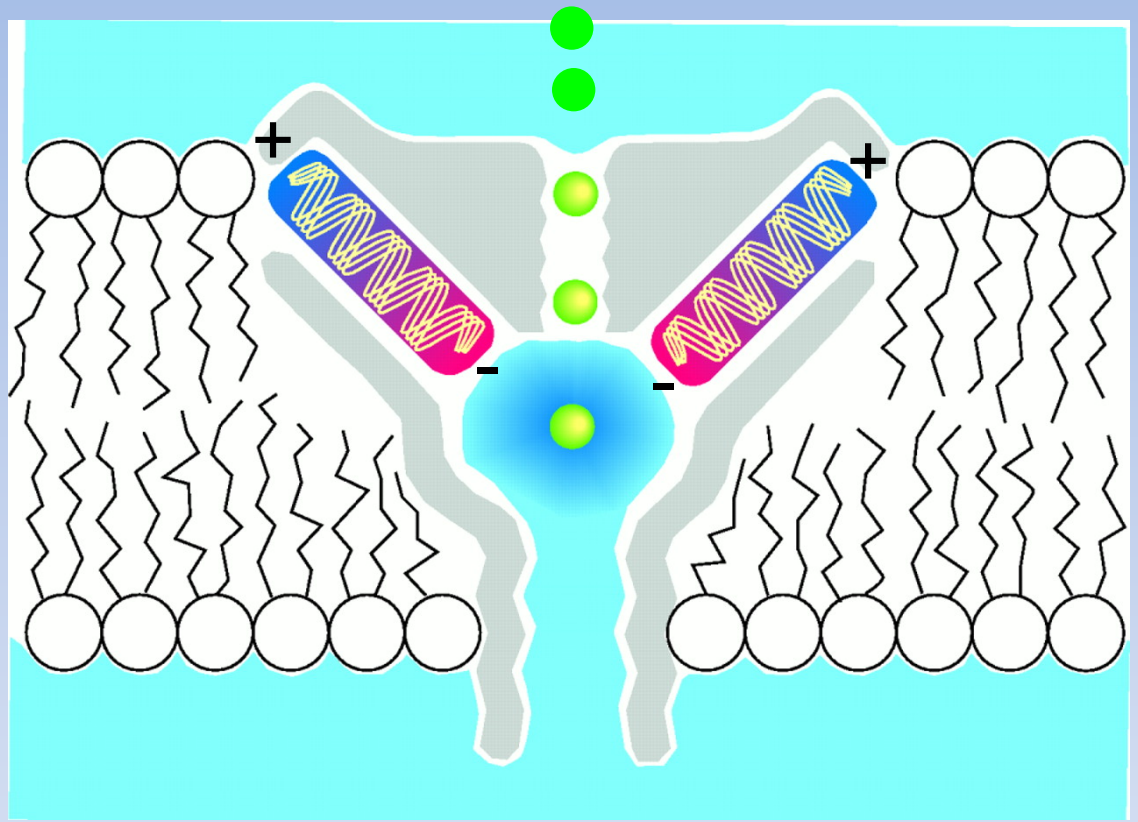
Electron density map in low K



Zhou et al (2001)
Nature 414:43

Two mechanisms by which K^+ channel stabilizes a cation in the middle of the central cavity

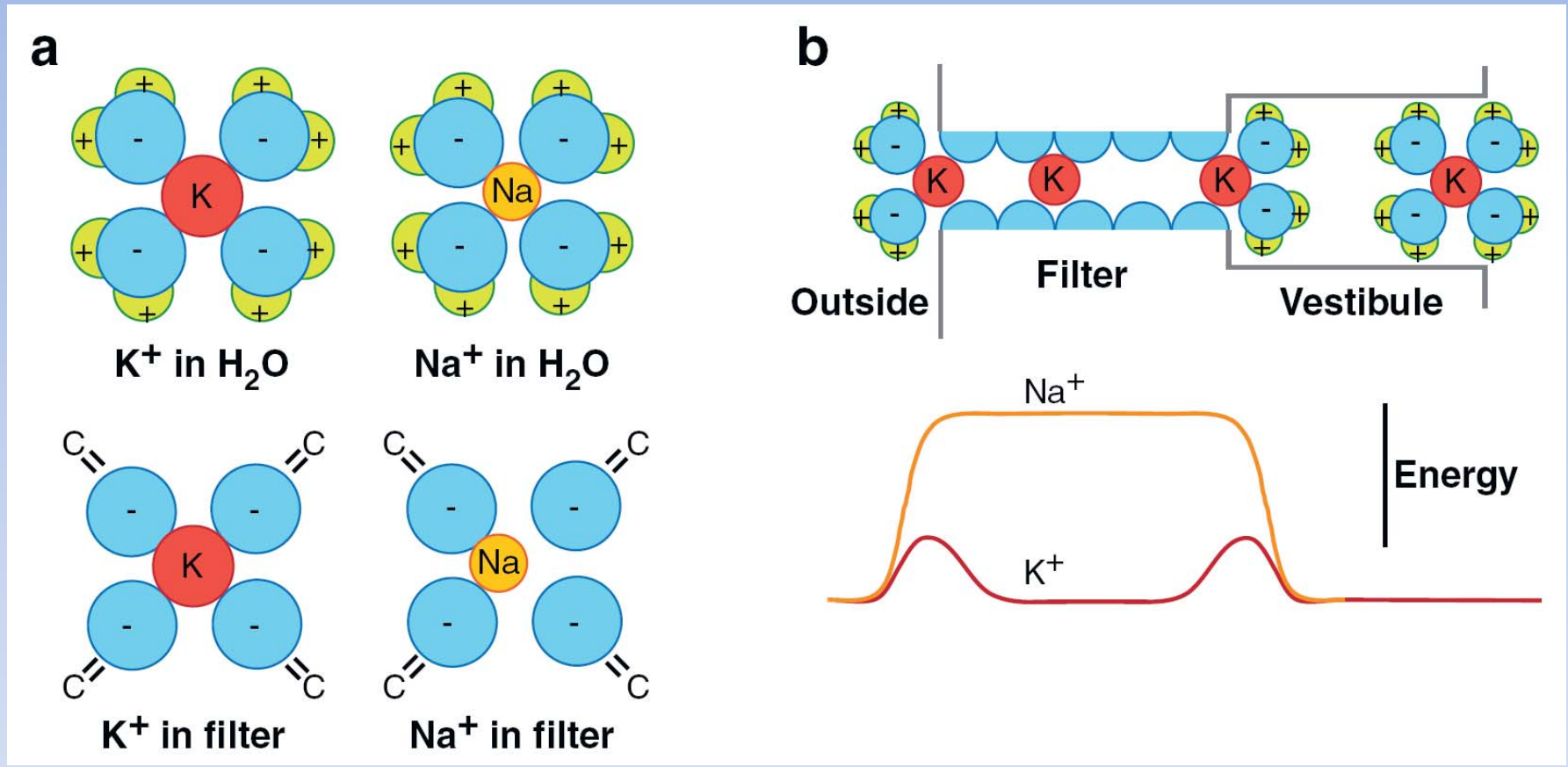




(1) a large aqueous cavity stabilizes a single K^+ in the hydrophobic membrane interior.

(2) oriented pore helices point their partial negative charge (carboxyl end) towards the cavity where a cation is located.

Why is K^+ favored over Na^+?



Armstrong (2007) *Ann Rev Physiol* 69:1-18

The energy for K^+ in water and the selectivity filter is similar (~79 kcal/M)

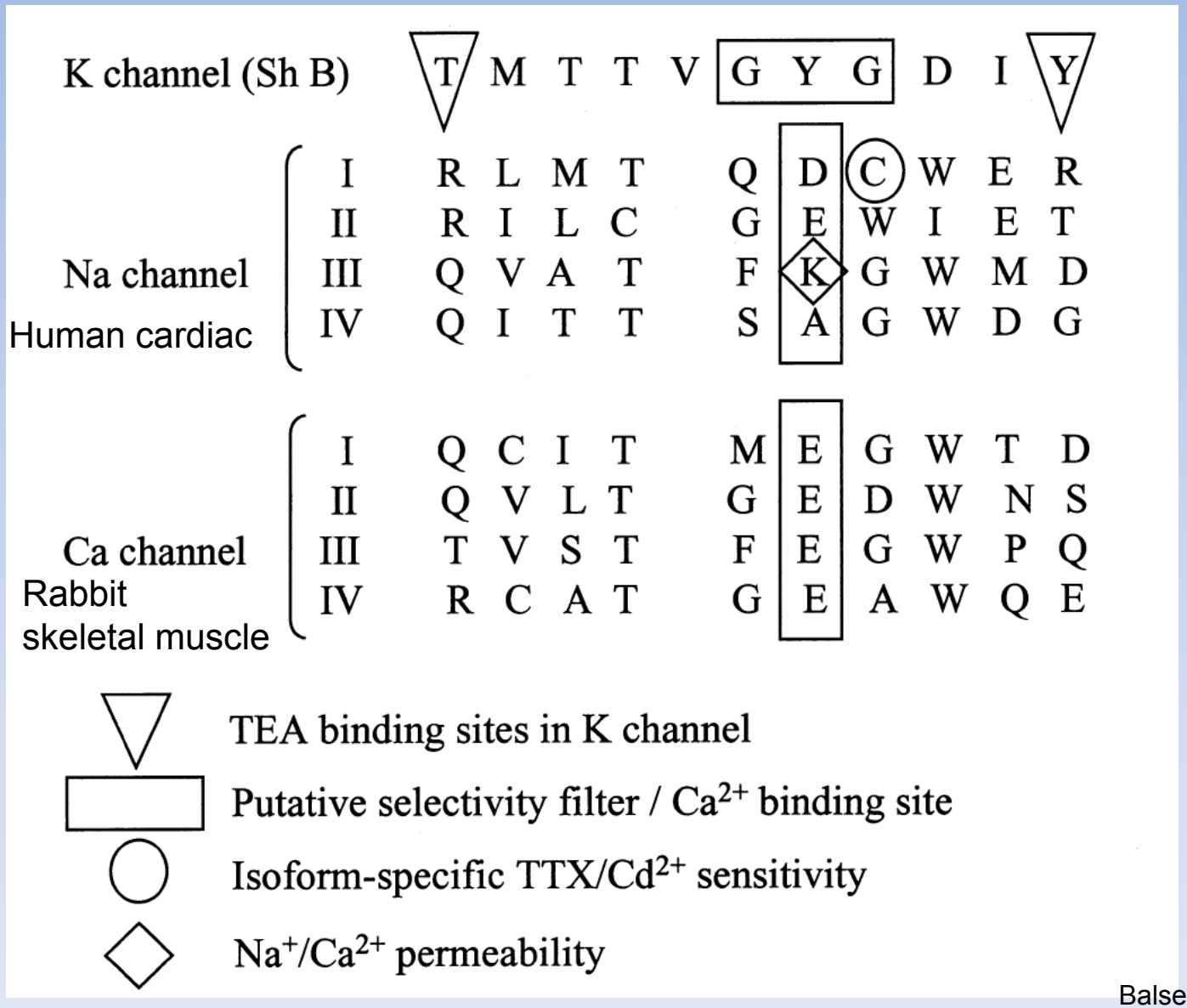
Coordination of Na^+ in selectivity filter is energetically unfavorable (lower binding affinity than K^+)

Summary: High selectivity and high permeation rate

10^8 ions/sec – selectivity filter must allow K ion to dehydrate, enter and cross filter within ~10 ns

- High selectivity:
  - Multiple ion occupancy: optimized geometry of K binding sites (1,3/2,4) in the narrow selectivity filter (customized oxygen cages)
- High permeation: electrostatic repulsion between adjacent K ions (4 M equivalent local concentration)
- A central cavity that is lined by hydrophobic residues
  - with plenty of water and central K+ stabilized by pore helix dipoles

Ion selectivity in Na and Ca channels

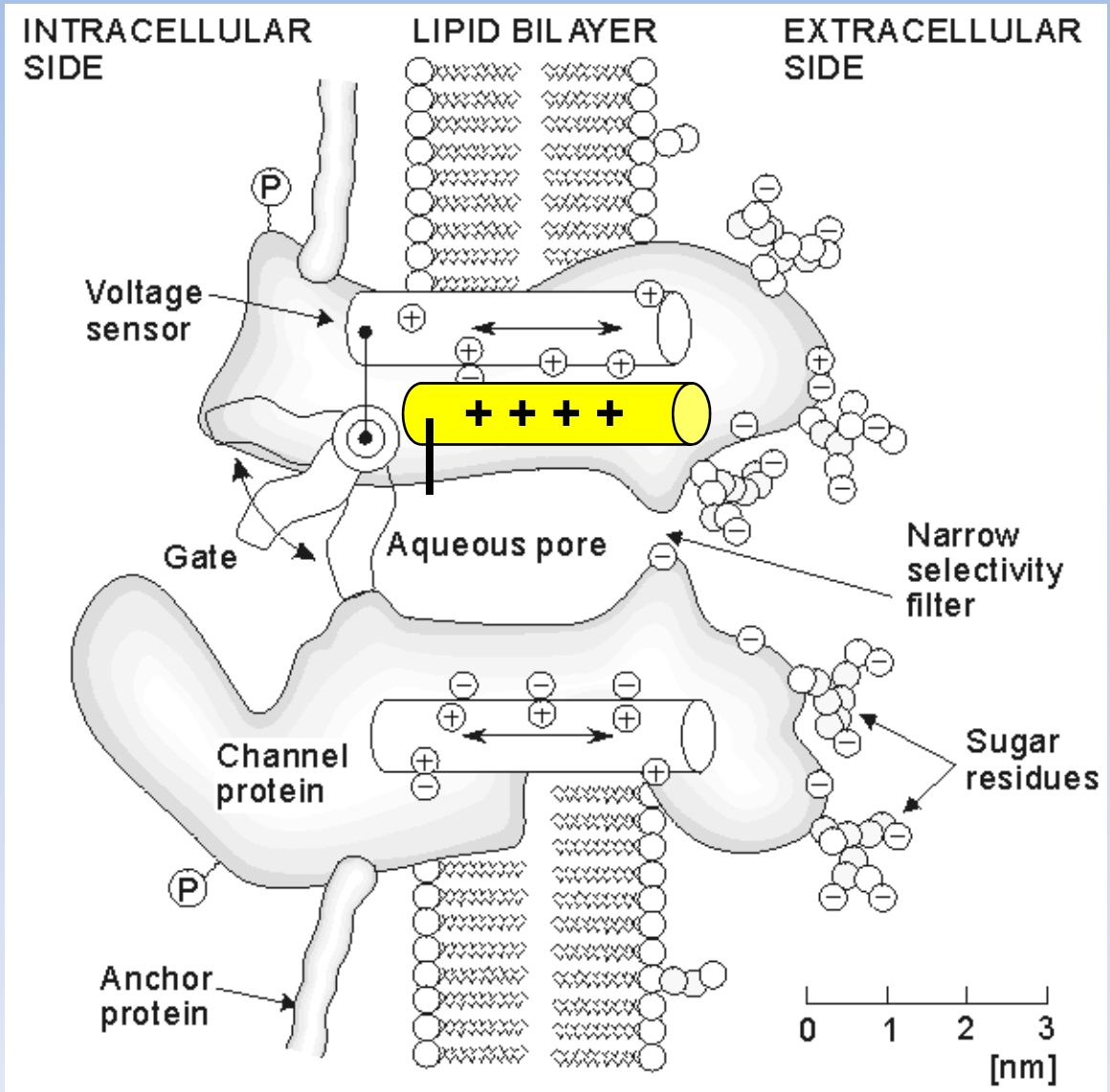


Balser (1999)
Cardiovasc Res 42:327

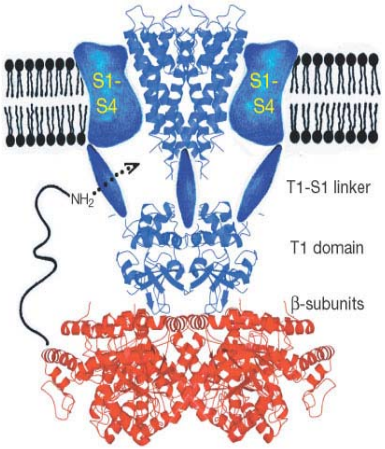
Voltage sensing

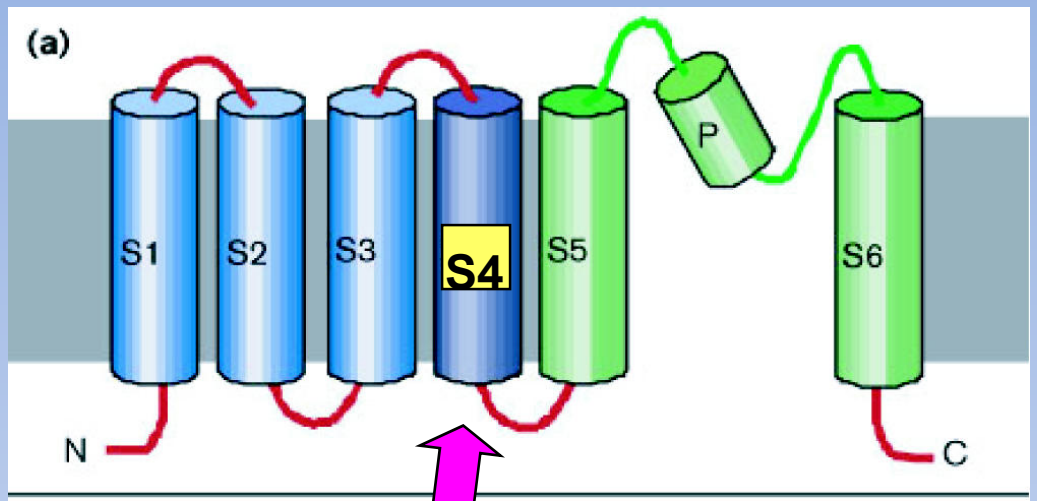
VSD: the voltage sensor domain

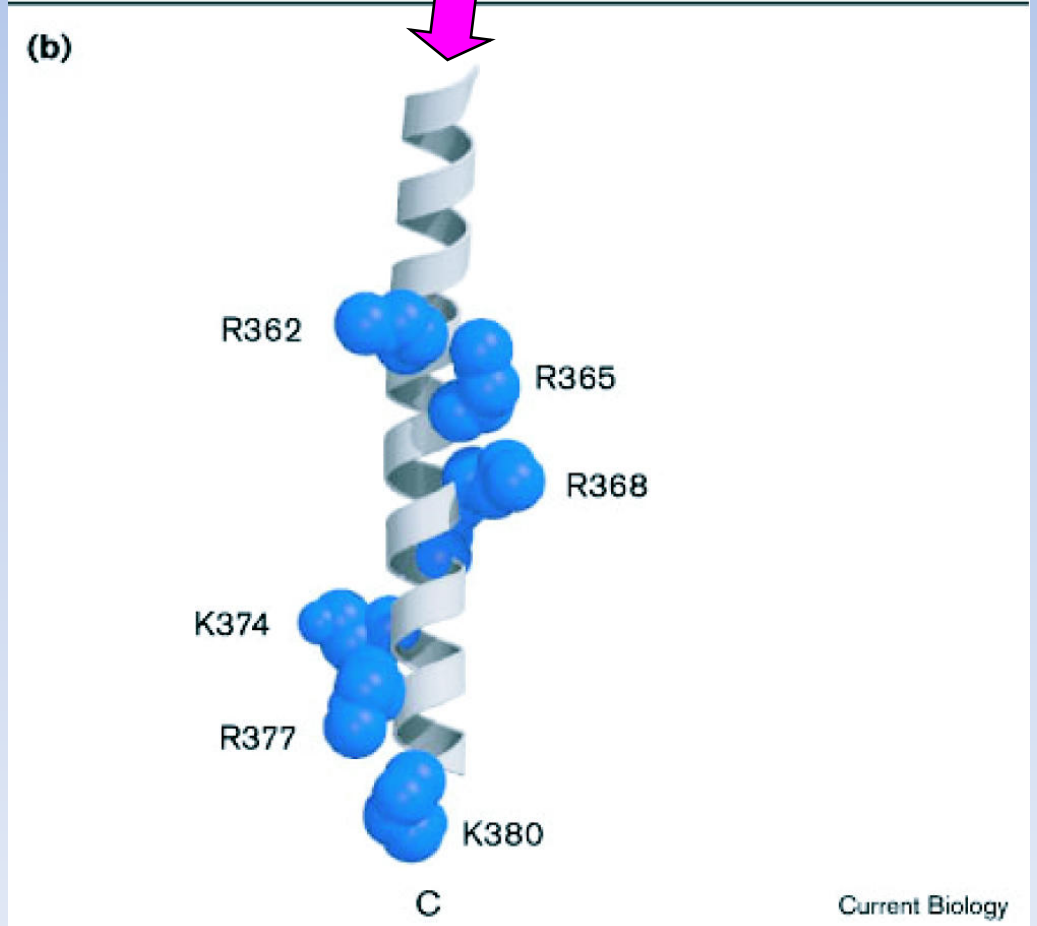
Voltage sensor



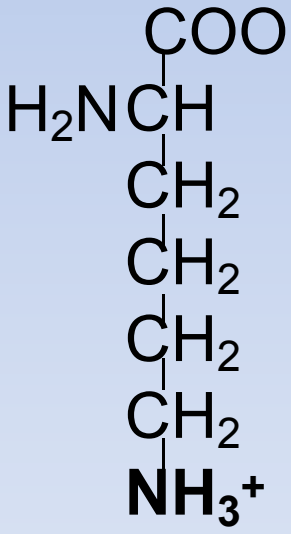
Voltage-gated ion channel = pore domain + VSD



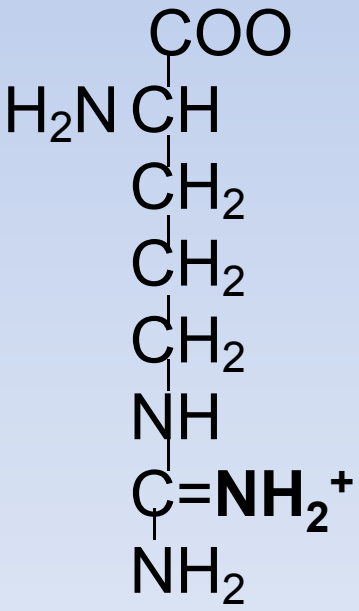




S4 domain is the primary voltage sensor

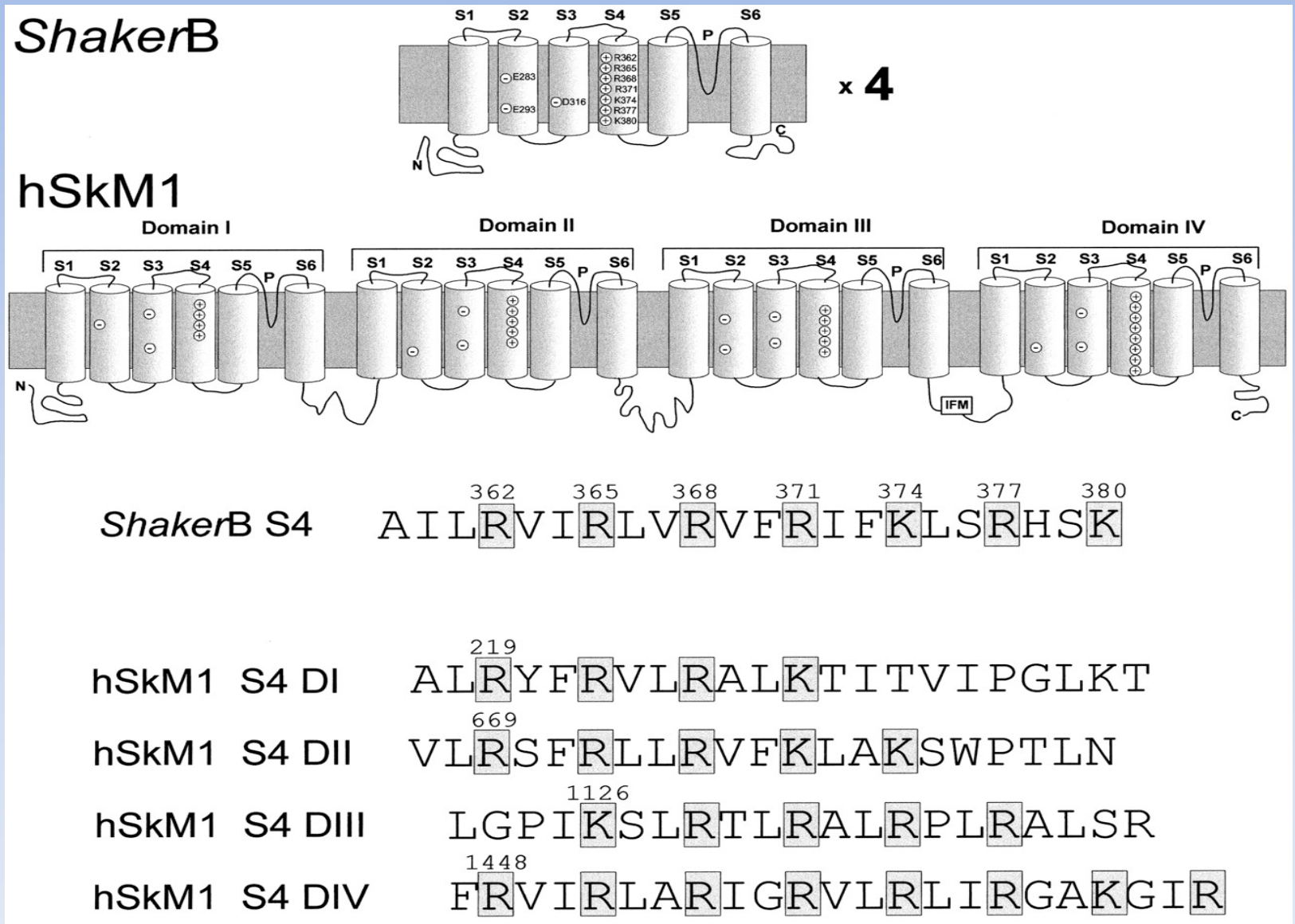


Lysine (K)

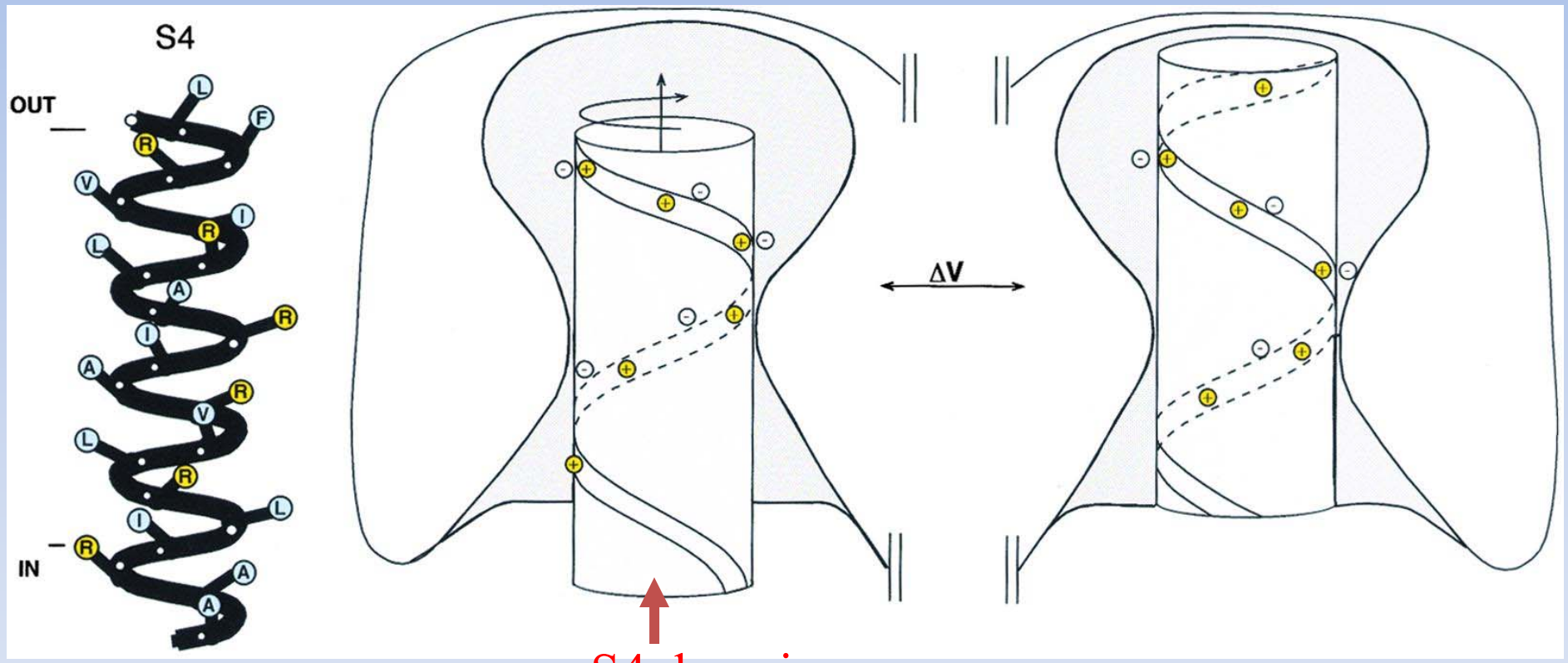


Arginine (R)

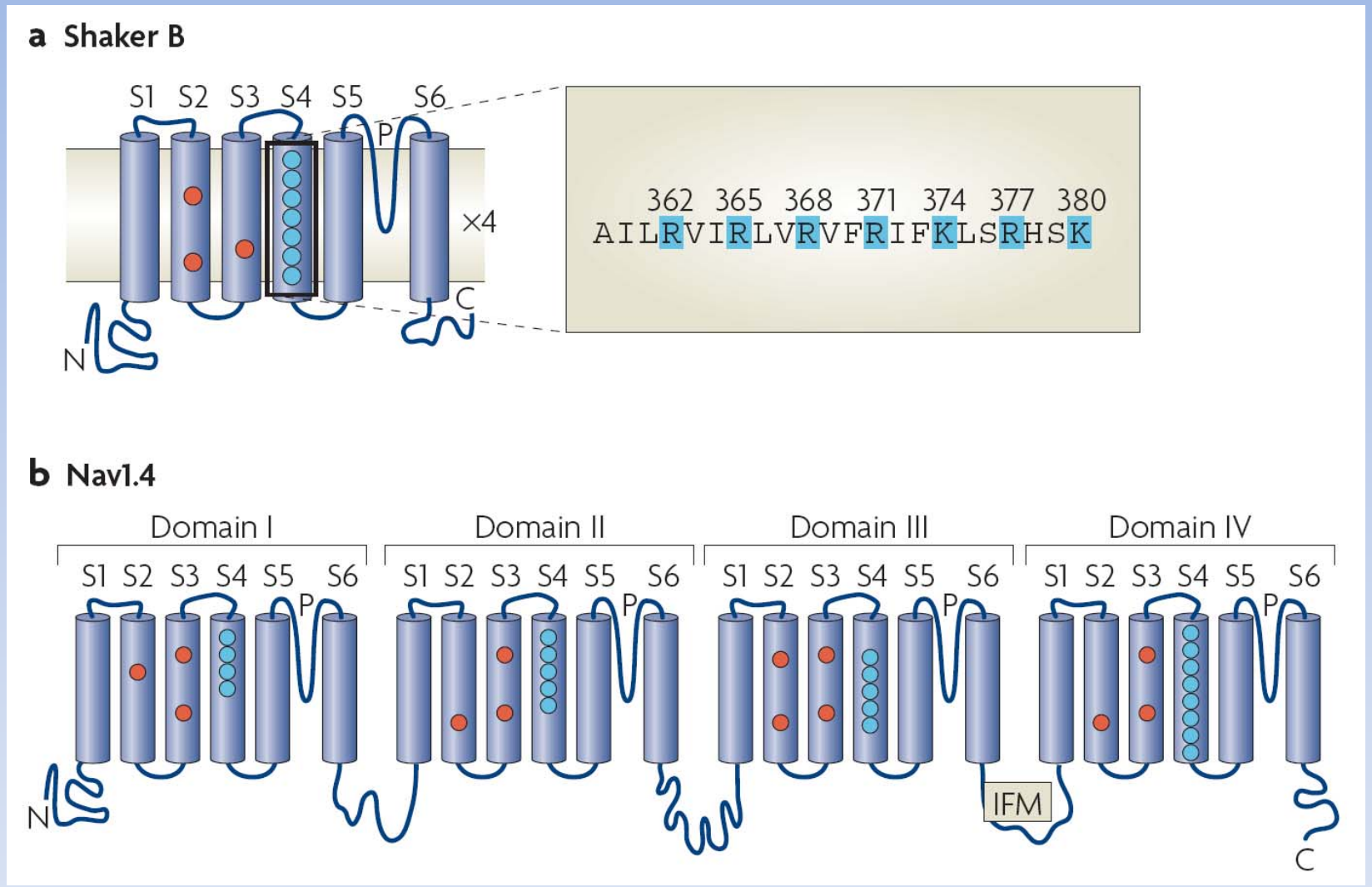
S4 domains from different channels are similar but not identical



Helical screw motion model of voltage sensor



VSD = S2/S3/S4

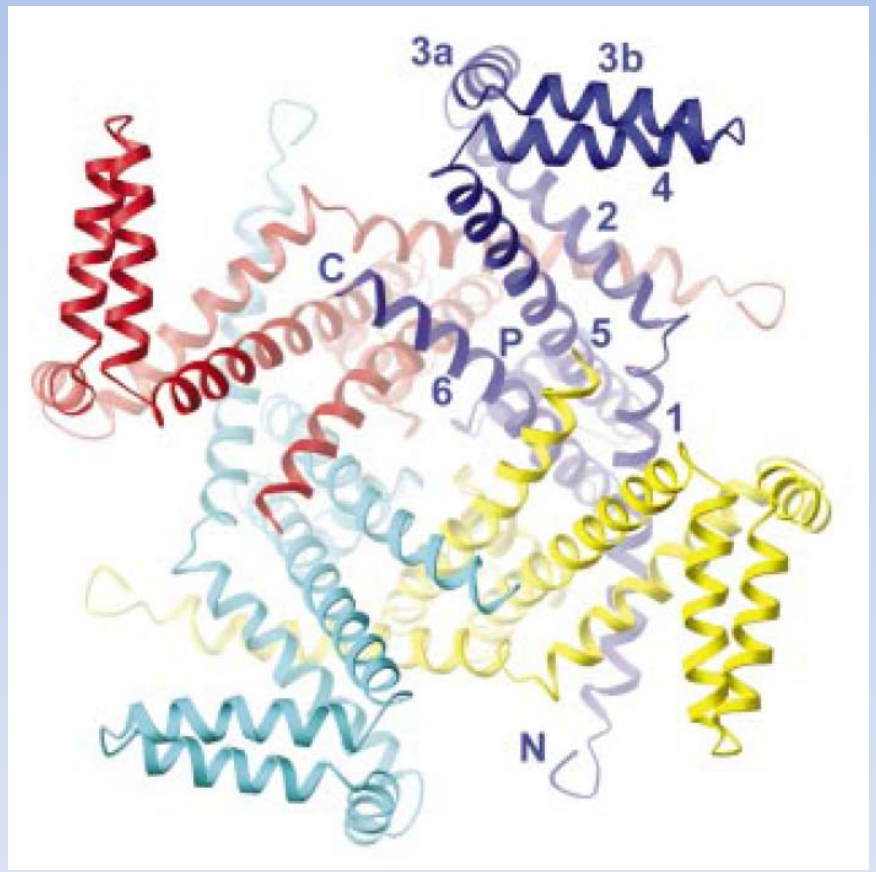


- Salt bridge forms between acidic residues in S2/S3 (red spheres) and basic residues of S4 (blue spheres)
- Consistent with helical screw motion

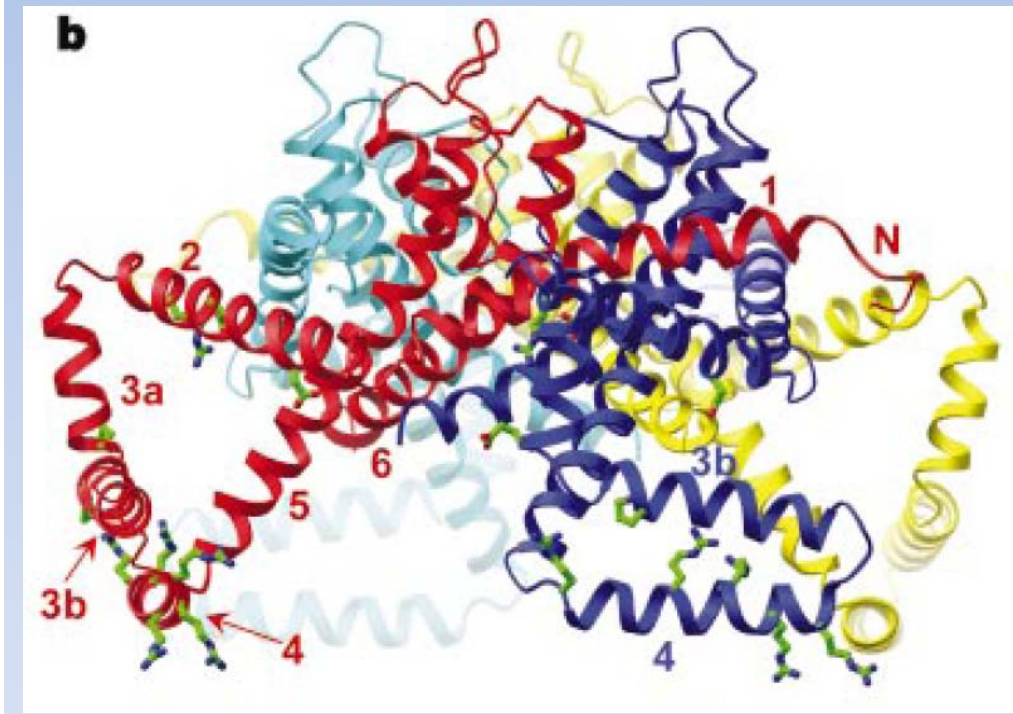
Bezanilla (2008)
Nature Reviews Mol Cell Biol 9:323

A different view of VSD: based on structure of KvAP, a voltage-gated K^+ channel

(from thermophilic archaebacteria, *Aeropyrum pernix*)



Top view



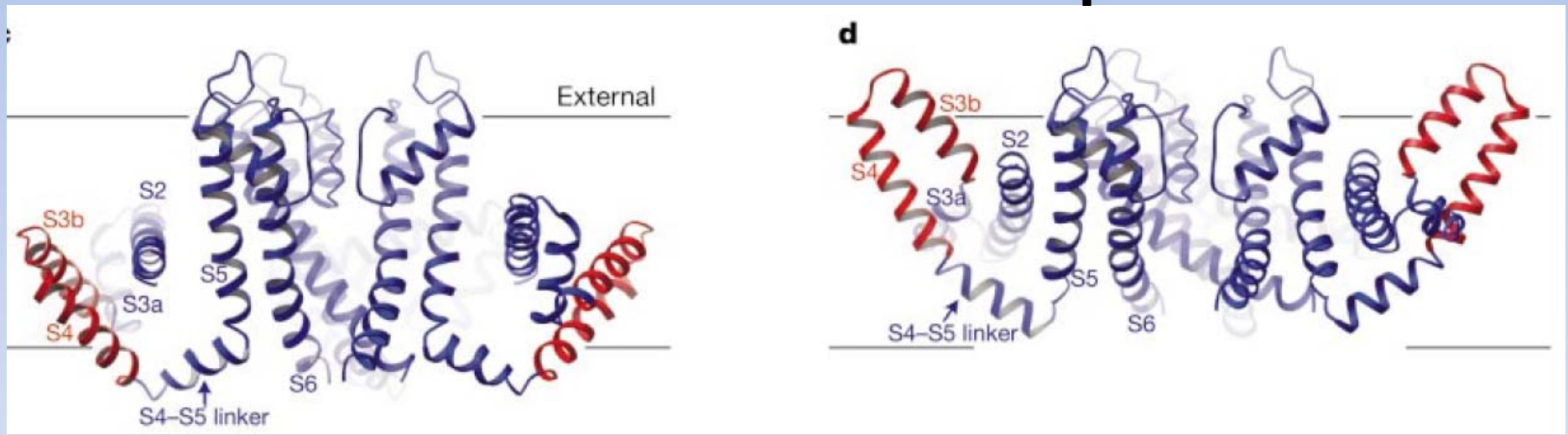
side view

Jiang et al (2003) Nature

KvAP channel

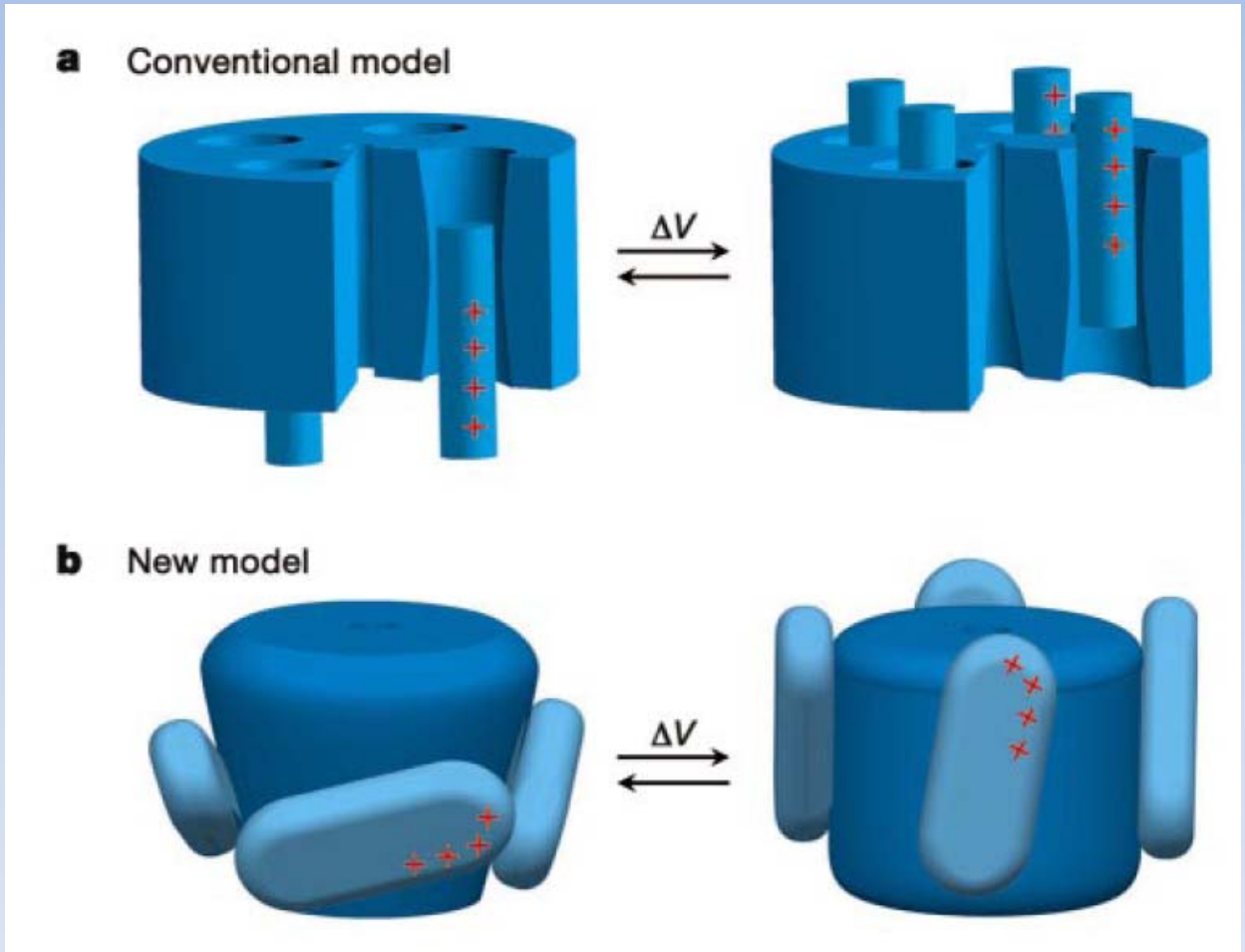
"Paddle" model of voltage sensor movement

Closed **Open**



Jiang et al (2003)
Nature. 423:42

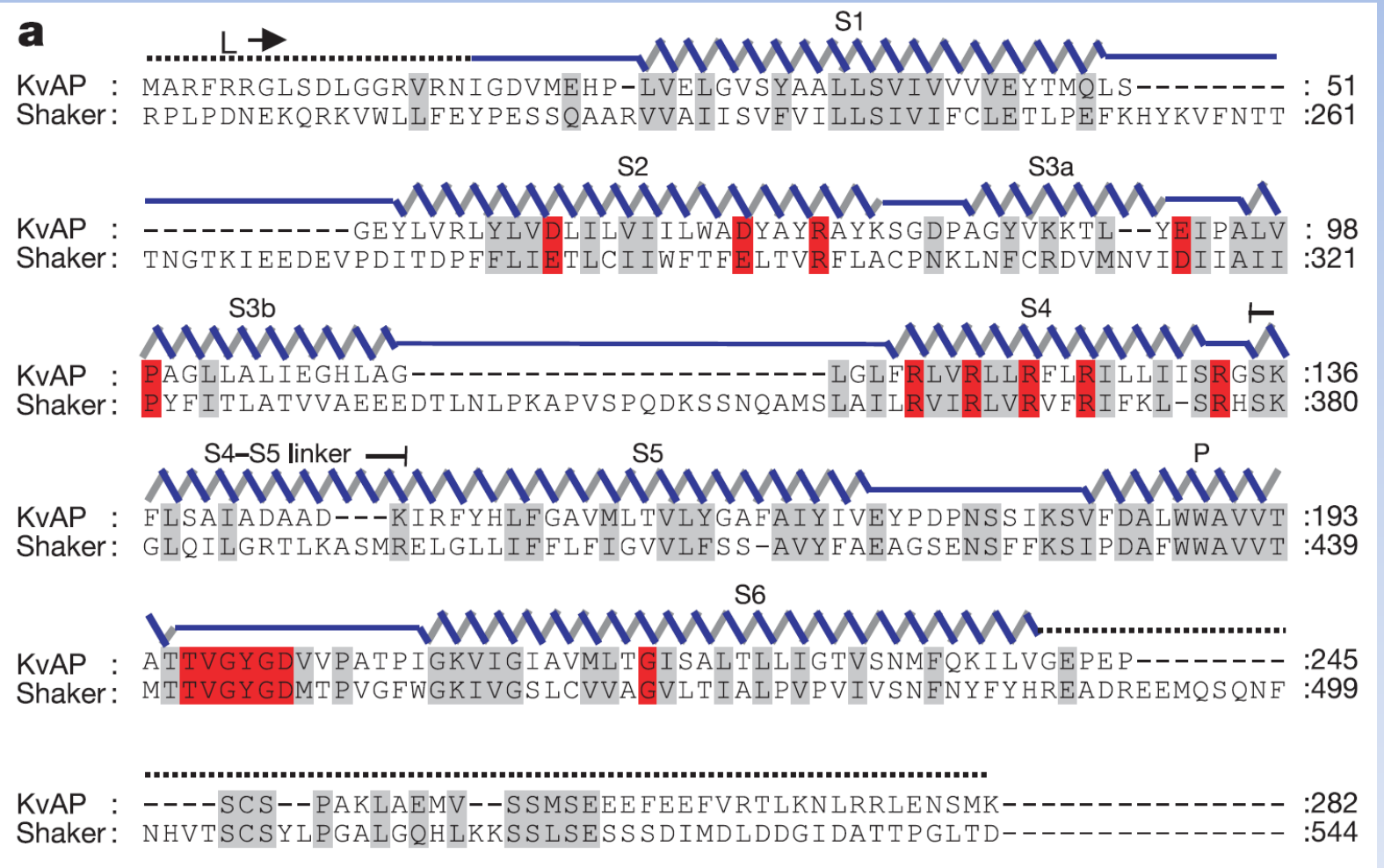
controversy



Shaker

KvAP

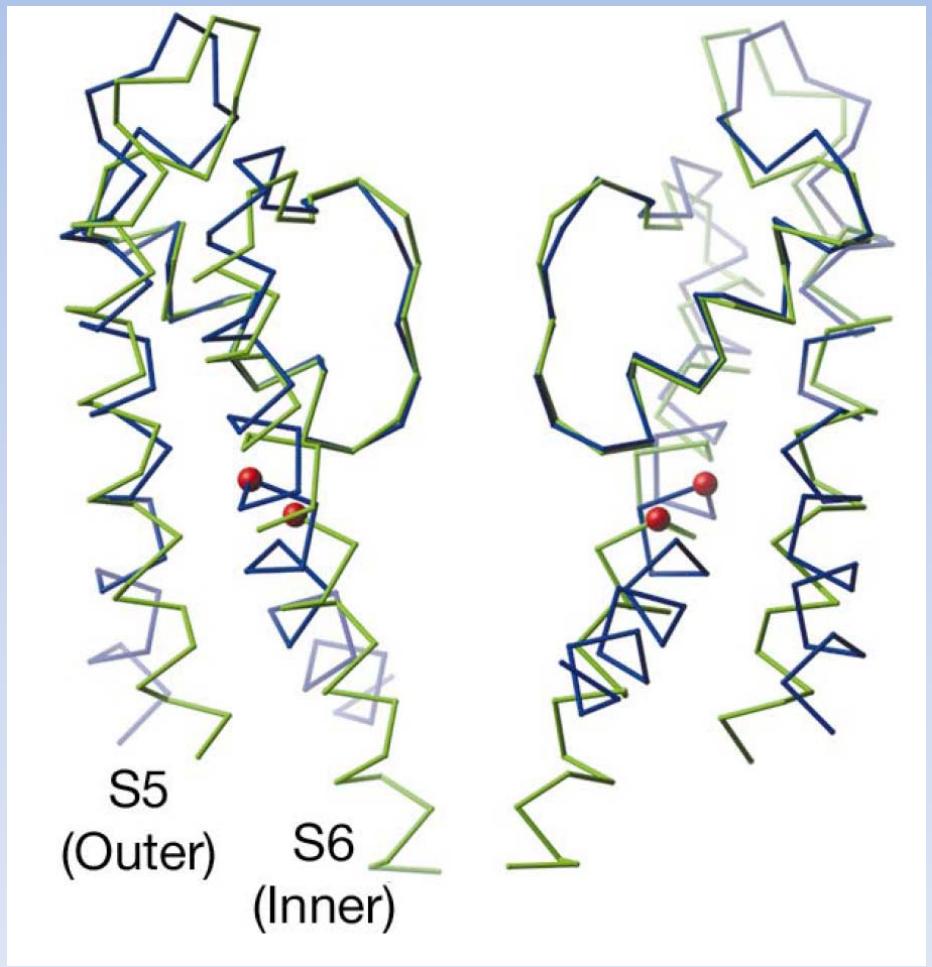
KvAP sequence is similar to Shaker

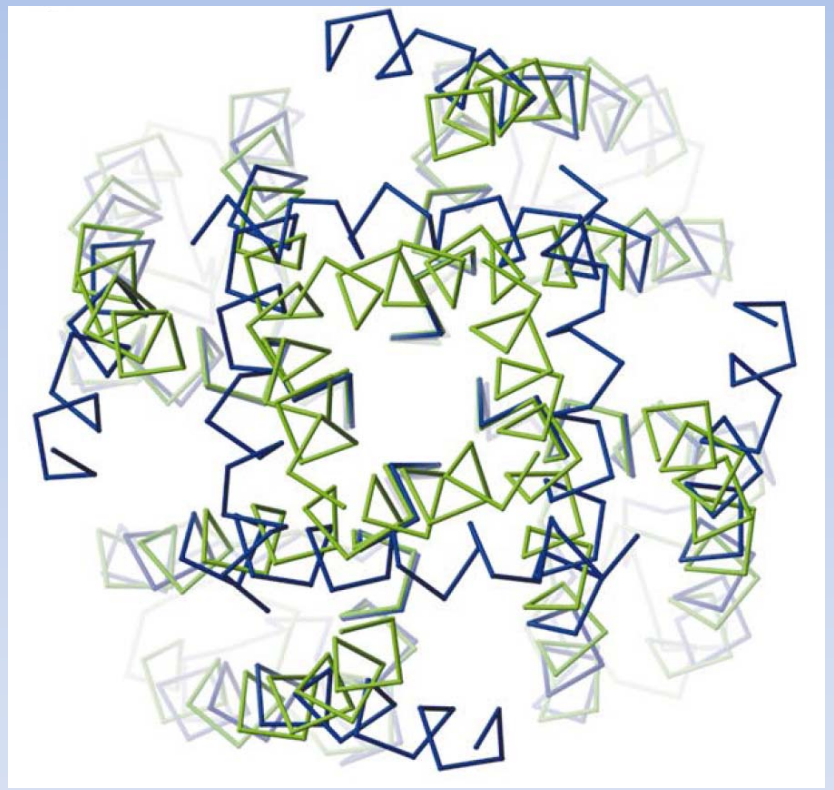


Jiang et al (2003) Nature

Comparison of KvAP and KcsA pore domain

KcsA: green KvAP: blue

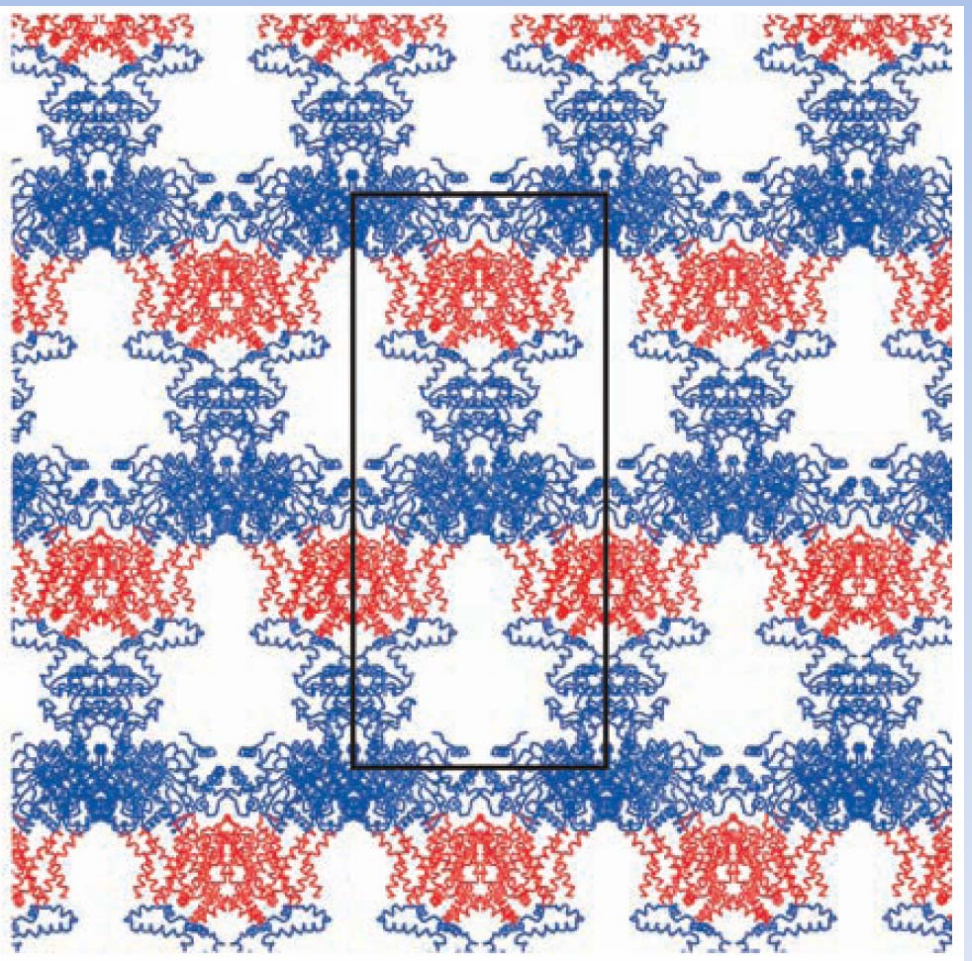


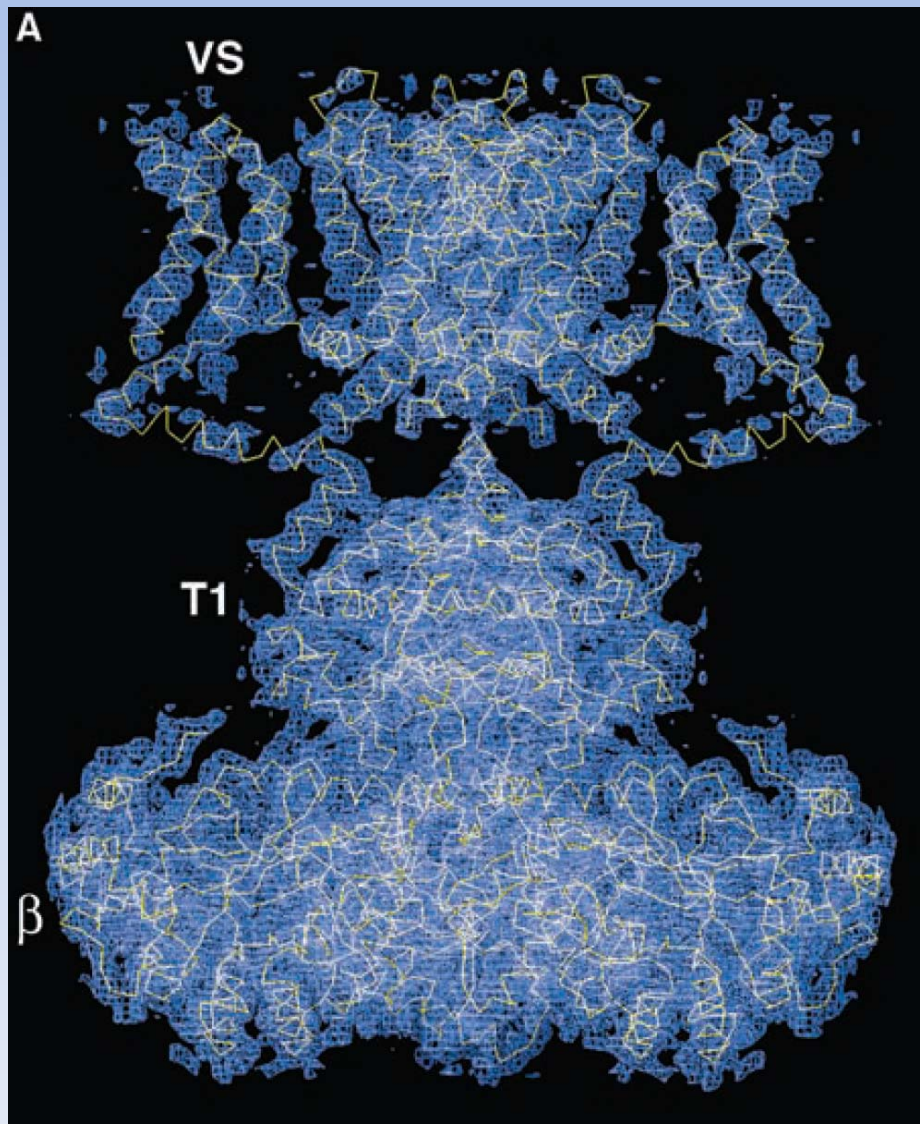


Glycine hinge

Jiang et al (2003) Nature

Electron density and crystal lattice of the Kv1.2–ß2 subunit complex, a mammalian K channel

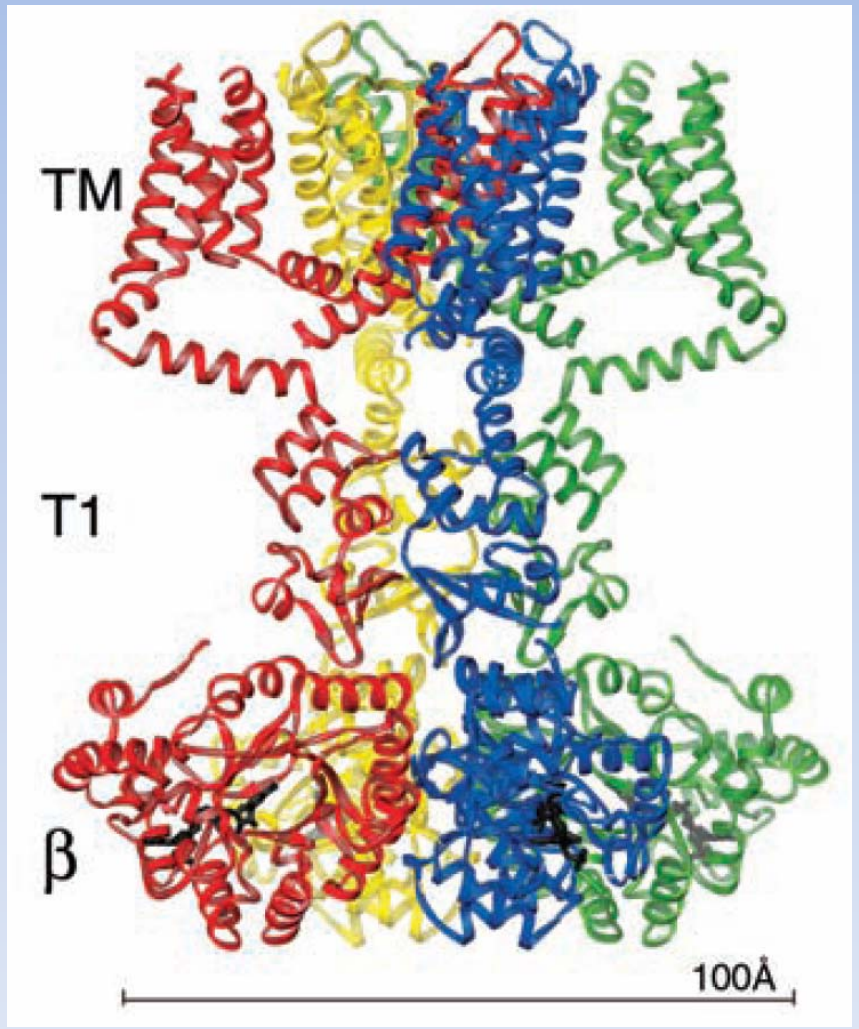


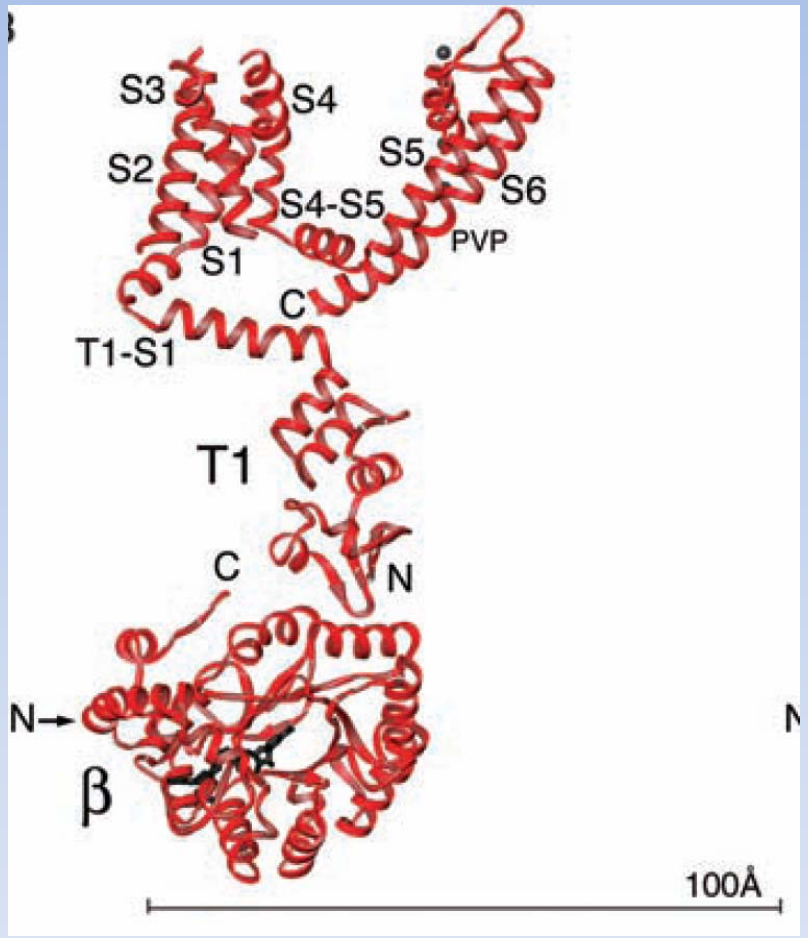


Long et al (2005) Science

The Kv1.2–ß$_2$ subunit channel complex

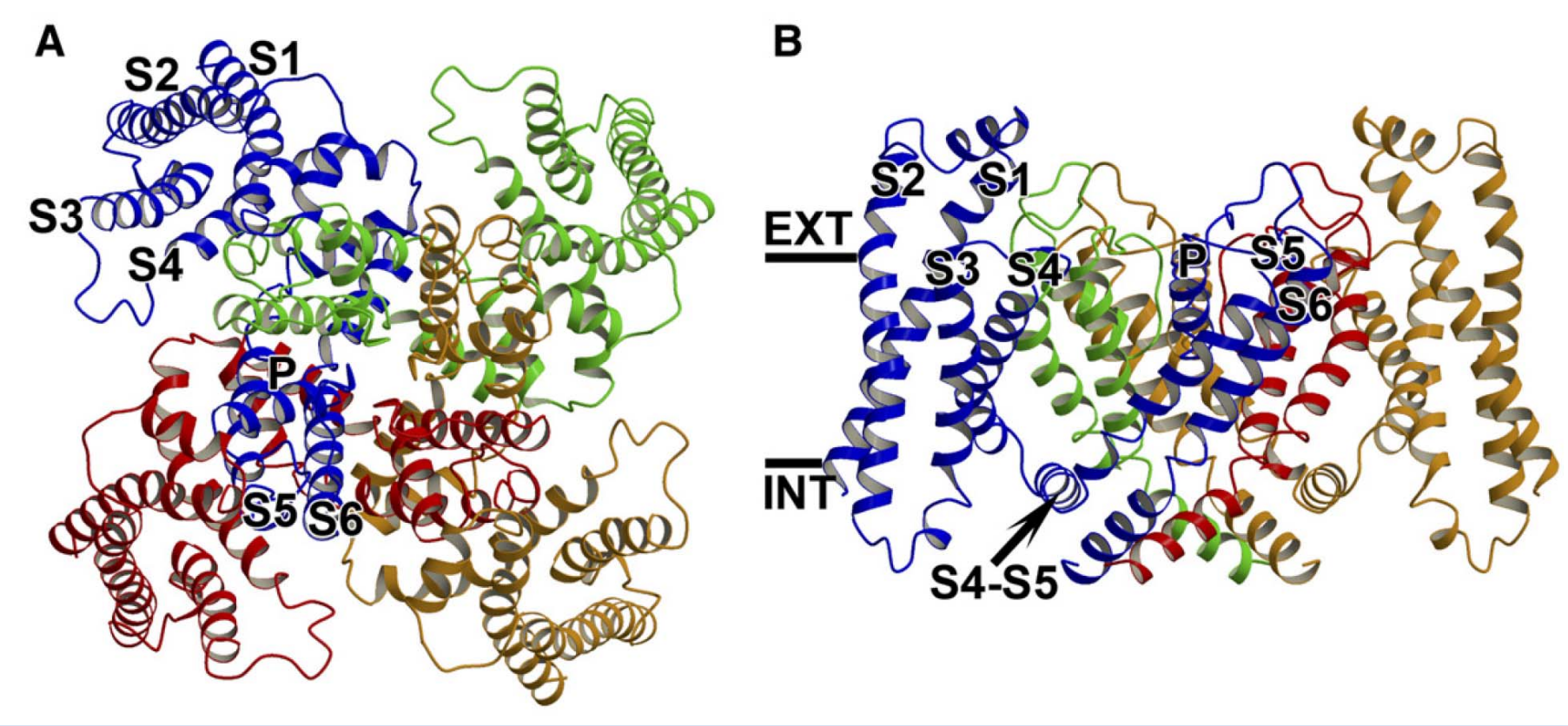
(back to the traditional VSD model)





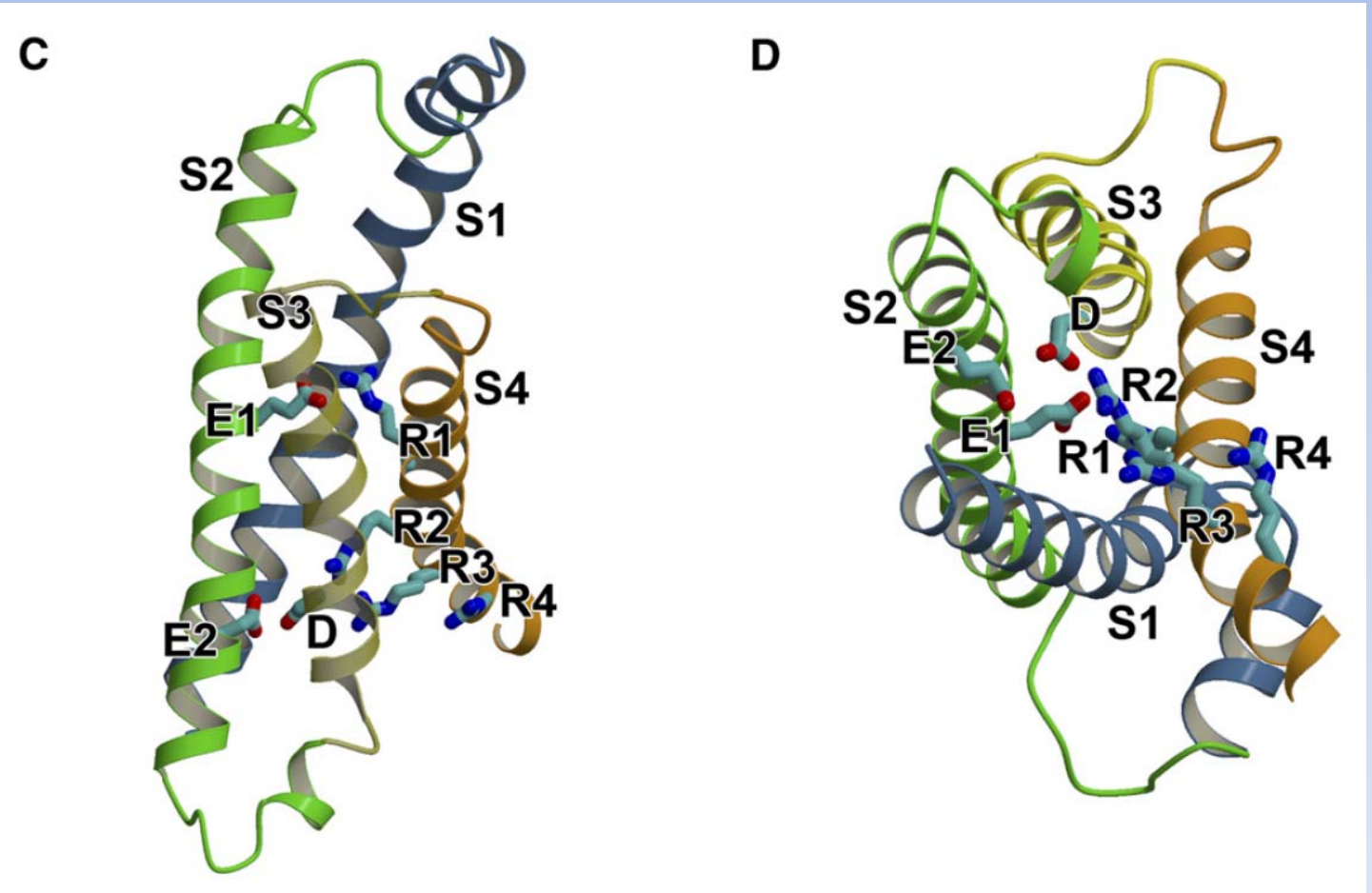
Long et al (2005) Science

Model of Kv1.2
in the closed state



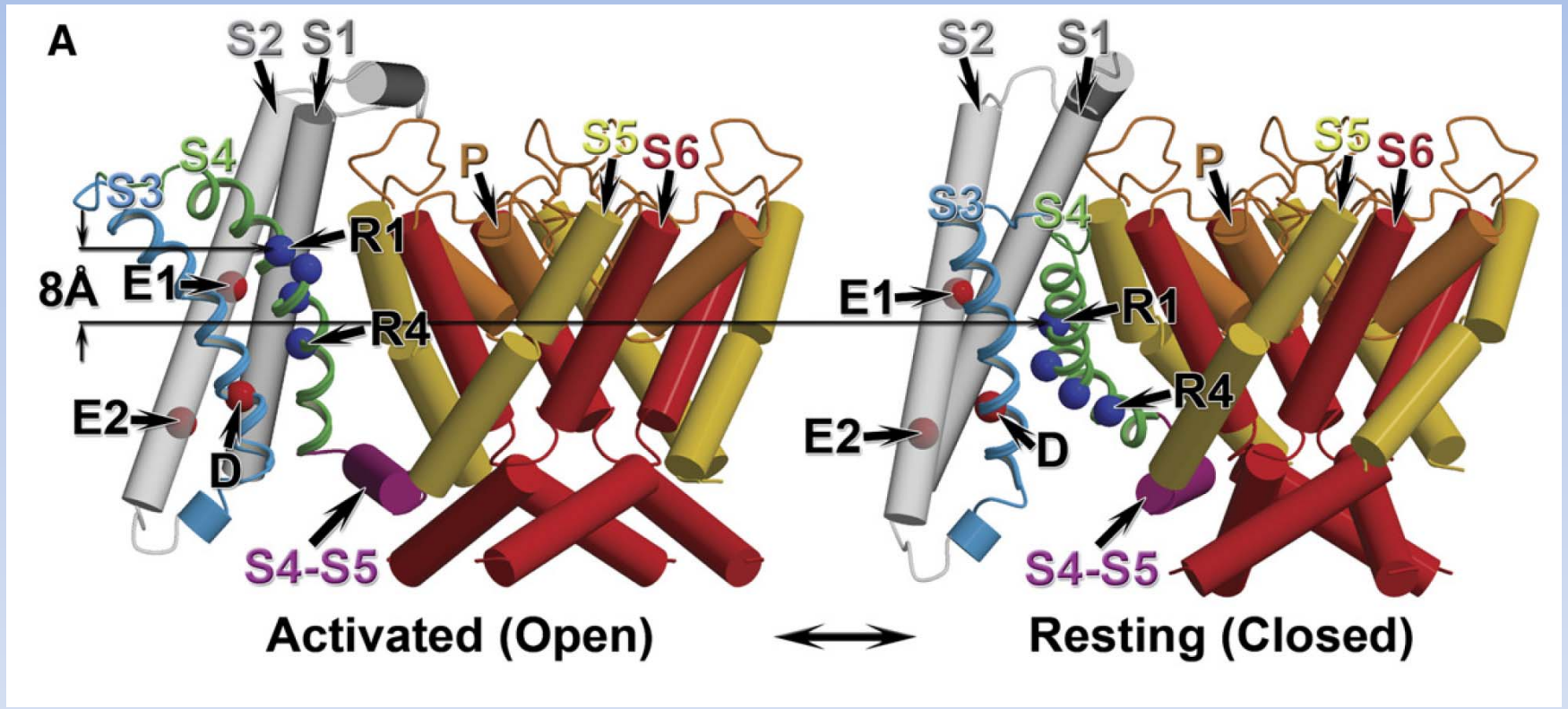
Pathak et al (2007)
Neuron 56:124

Model of Kv1.2 VSD in the closed state: salt bridges form between basic residues in S4 and acidic residues in S2/S3



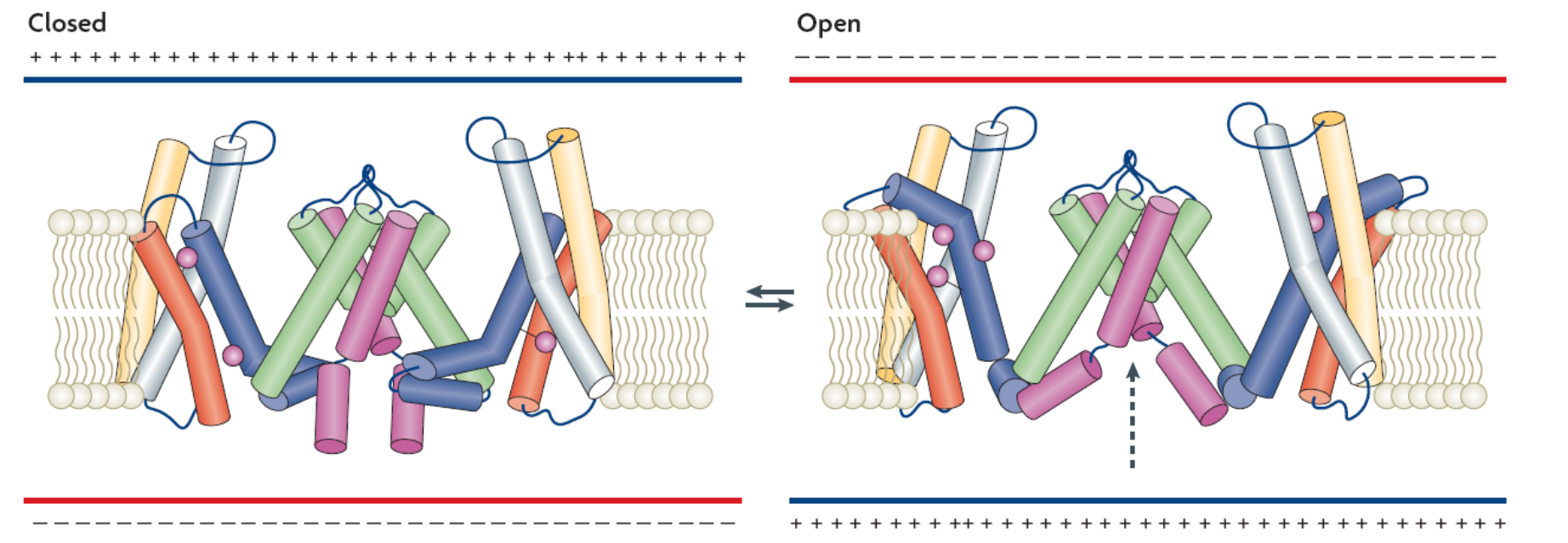
Side view

view from intracellular side



Pathak et al (2007)
Neuron 56:124

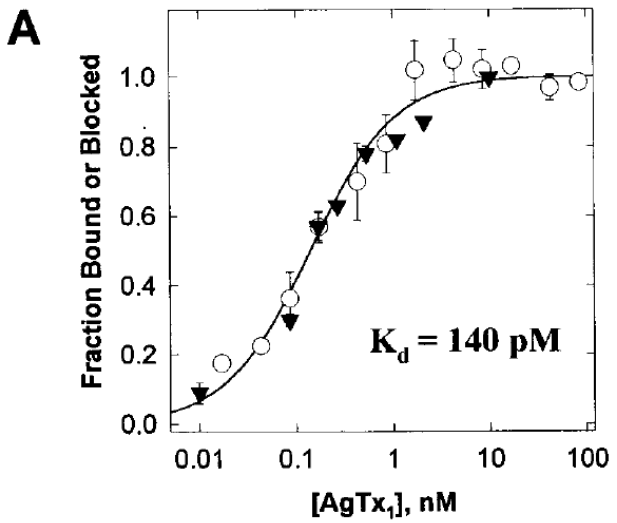
Model of S4 movements in a Kv channel

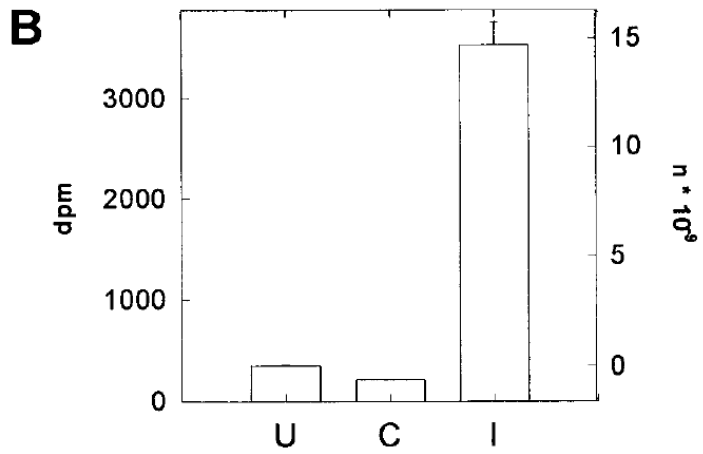


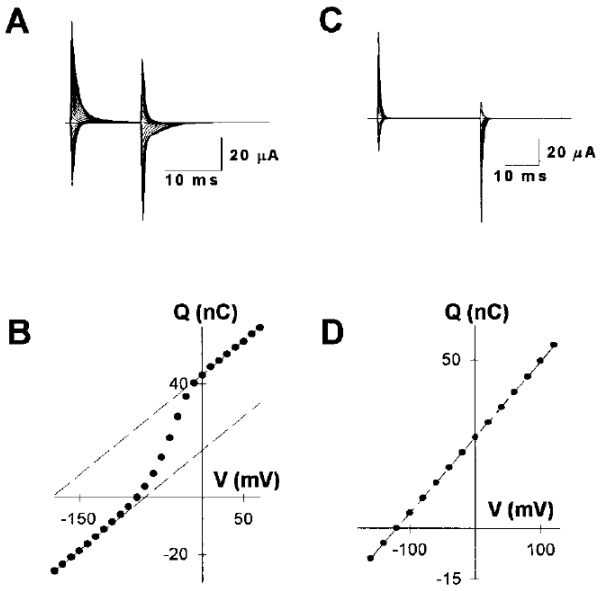
(only two subunits shown)

Bezanilla (2008)
Nature Reviews Mol Cell Biol 9:323

Contribution of the S4 domain to gating charge in *Shaker* K channels







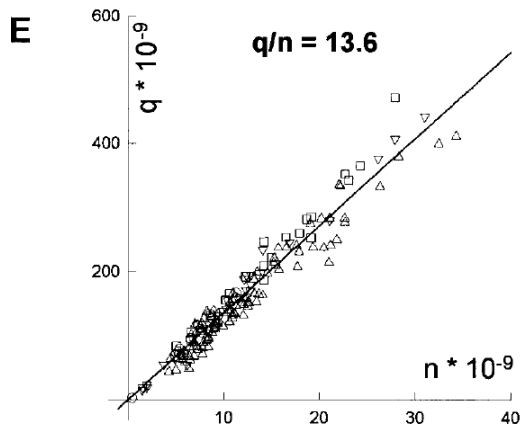


Figure 3. Determination of the Gating Charge of the *Shaker* K^+ Channel

Aggarwal and MacKinnon (1996) Neuron **16**, 1169

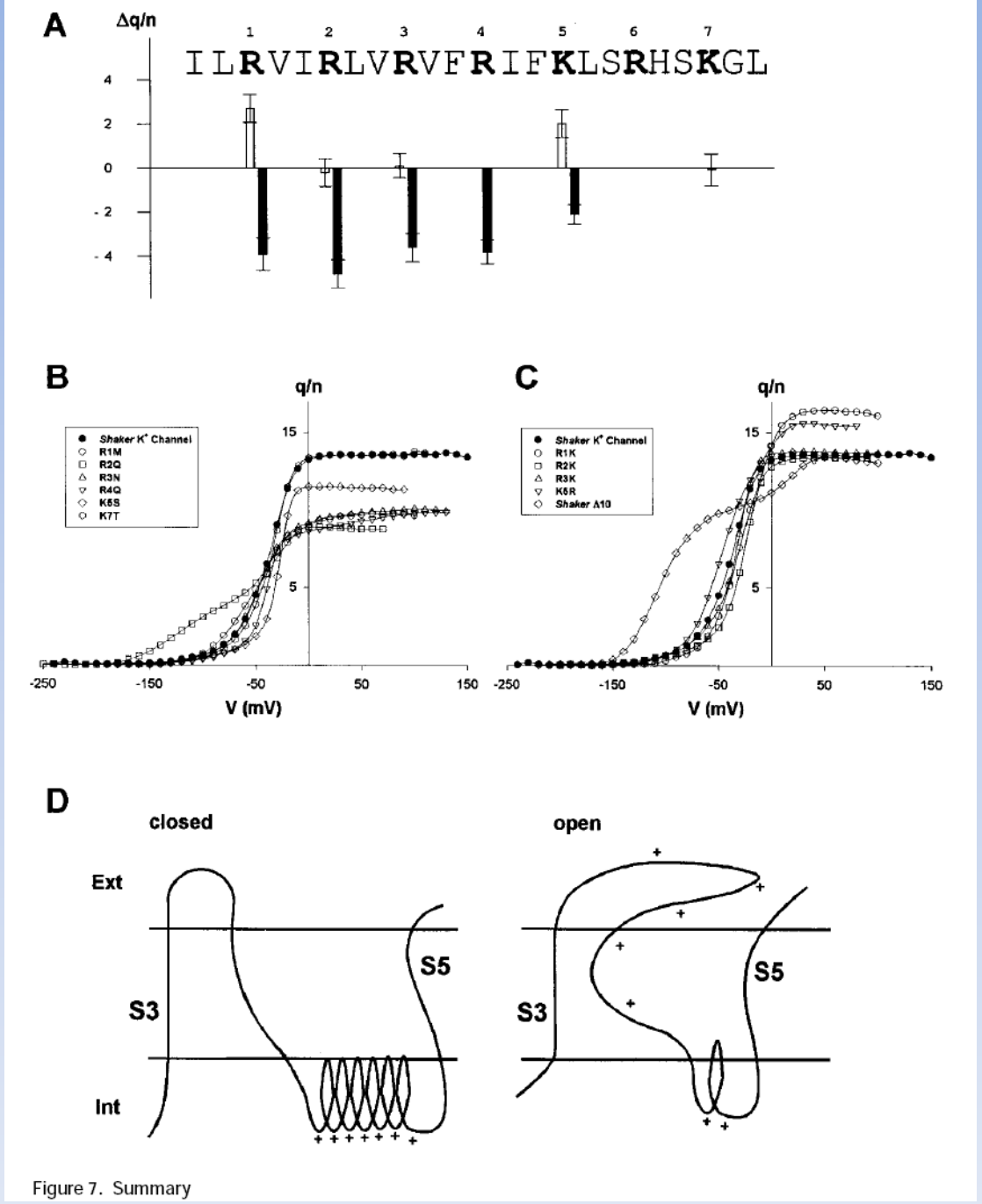


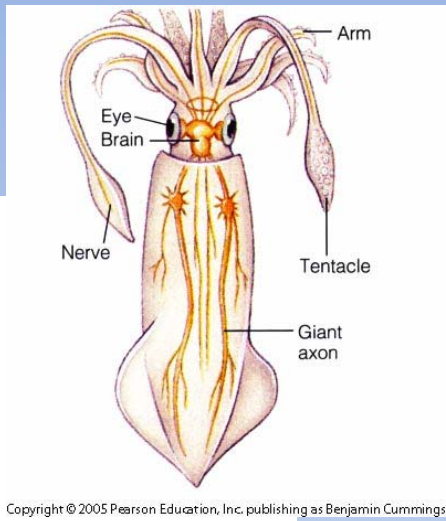
Figure 7. Summary

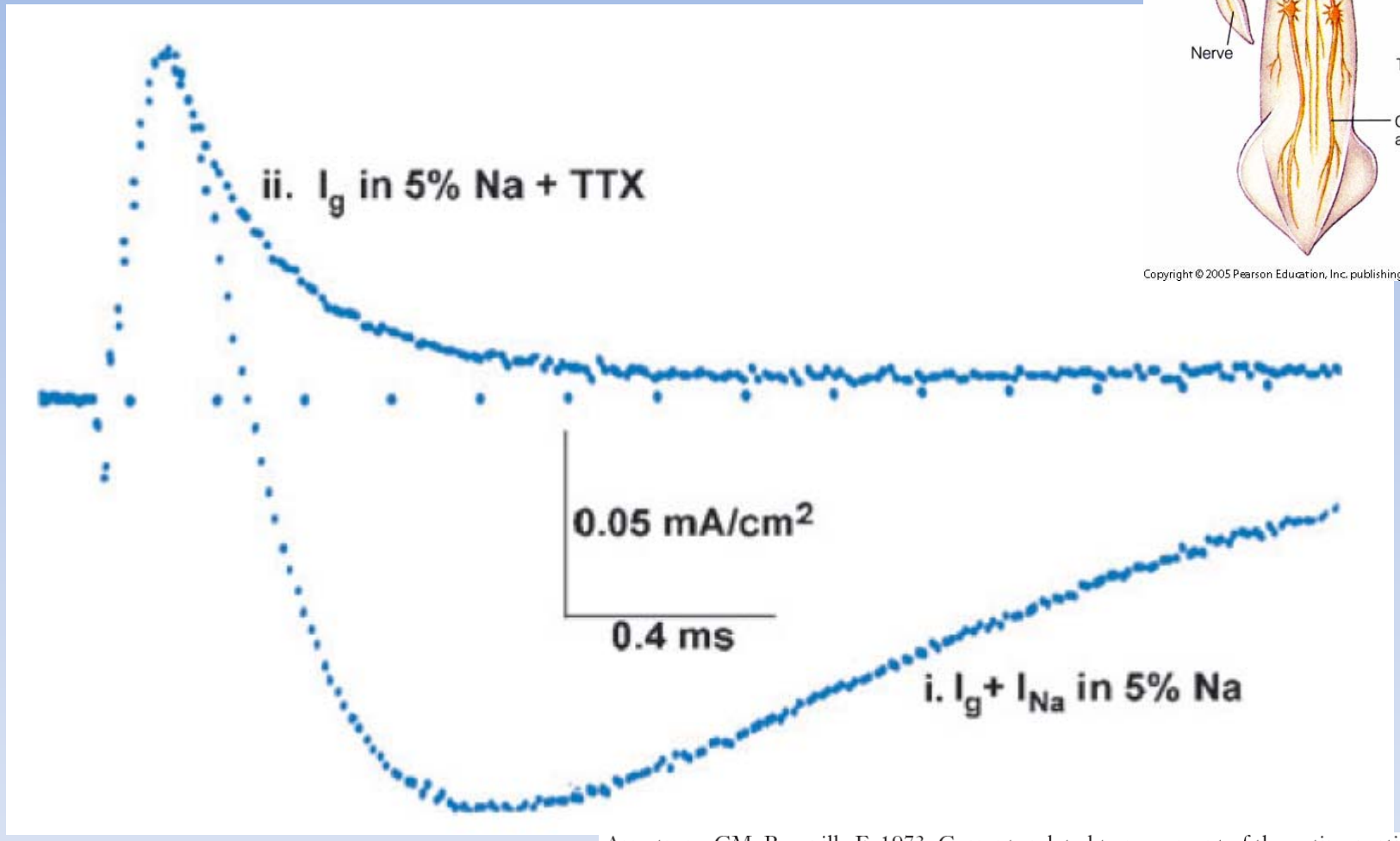
Aggarwal and MacKinnon (1996) Neuron **16**, 1169

Voltage-activated ion channels respond to changes in membrane voltage by coupling the movement of charges to channel opening. A K^+ channel-specific radioligand was designed and used to determine the origin of these gating charges in the *Shaker* K^+ channel. Opening of a *Shaker* K^+ channel is associated with a displacement of 13.6 electron charge units. Gating charge contributions were determined for six of the seven positive charges in the S4 segment, an unusual amino acid sequence in voltage-activated cation channels consisting of repeating basic residues at every third position. Charge-neutralizing mutations of the first four positive charges led to large decreases ($\sim$4 electron charge units each) in the gating charge; however, the gating charge of *Shaker* Δ10, a *Shaker* K^+ channel with 10 altered nonbasic residues in its S4 segment, was found to be identical to the wild-type channel. These findings show that movement of the NH_2-terminal half but not the CO_2H-terminal end of the S4 segment underlies gating charge, and that this portion of the S4 segment appears to move across the entire transmembrane voltage difference in association with channel activation.

Aggarwal and MacKinnon (1996) Neuron **16**, 1169

First recording of gating current (I_g) for Na channels in a squid giant axon

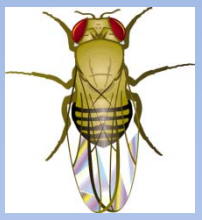




Armstrong CM, Bezanilla F. 1973. Currents related to movement of the gating particles of the sodium channels. *Nature* 242:459–61

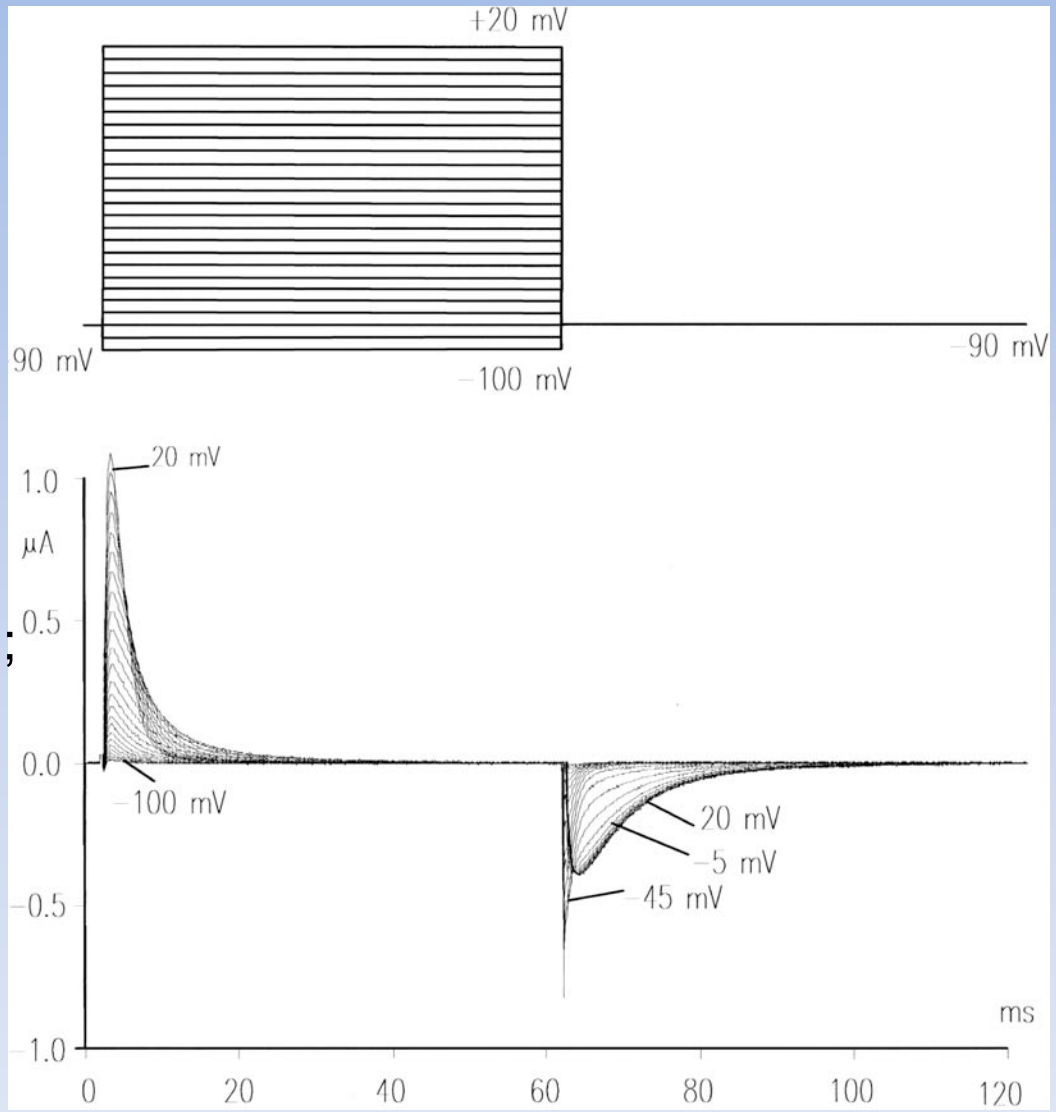
I_K was eliminated by removing all K^+, I_{Na} was reduced by lowering [Na^+].
I_{cap} was removed by subtraction, then eliminated with tetrodotoxin (TTX)

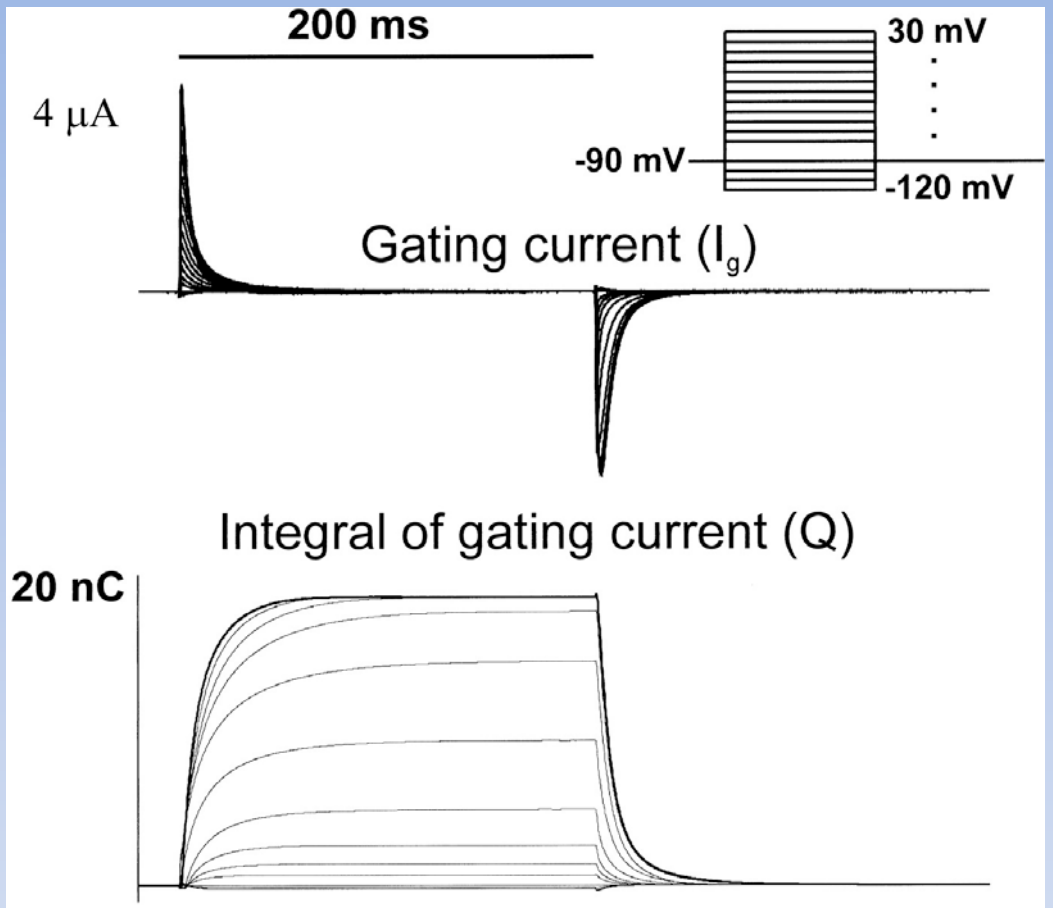
Gating currents of cloned *Shaker* K channel



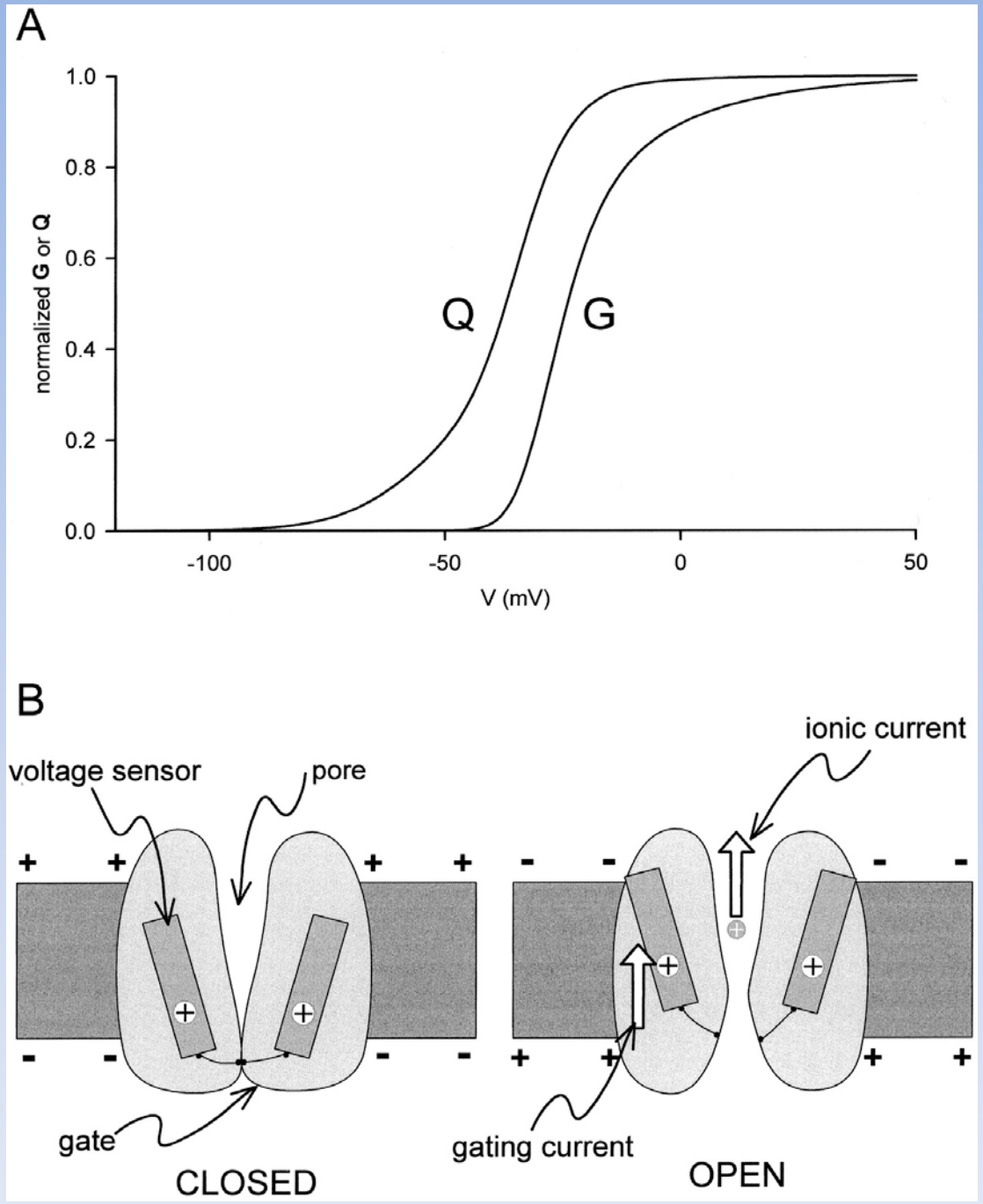
Voltage pulse protocol

Gating currents (Ionic currents blocked; or use nonconducting channels)



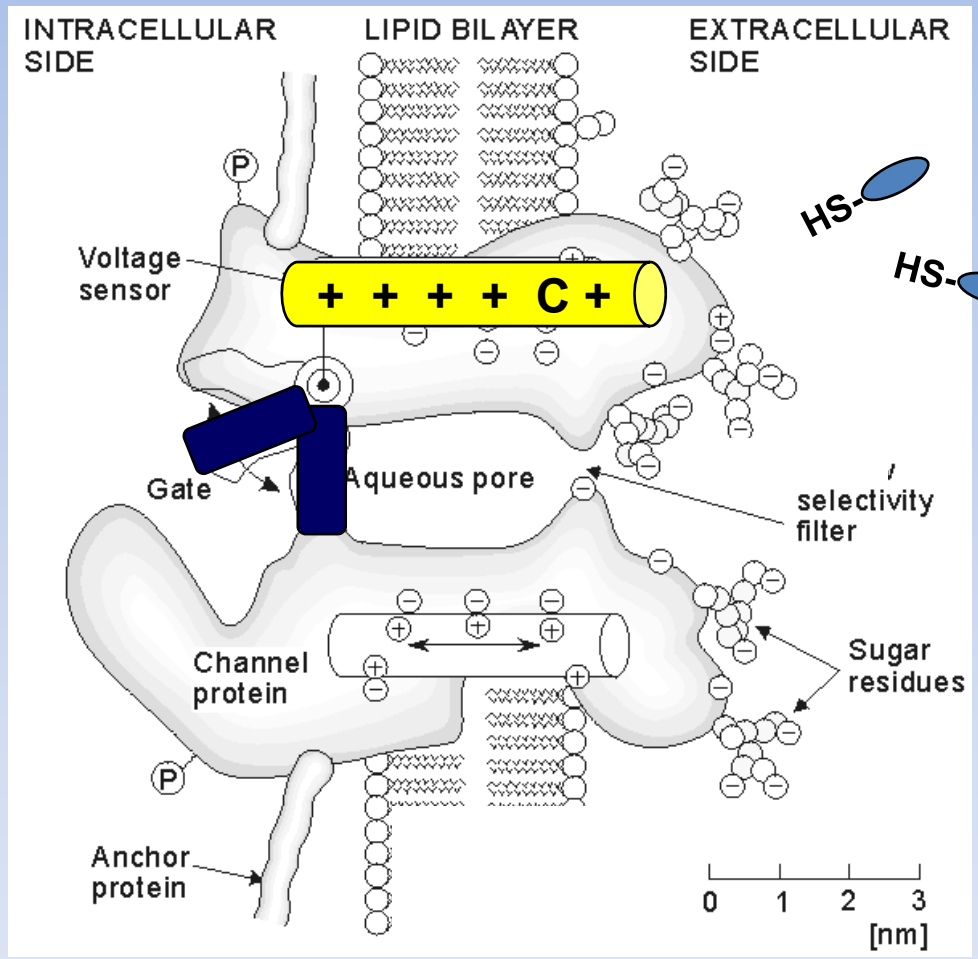


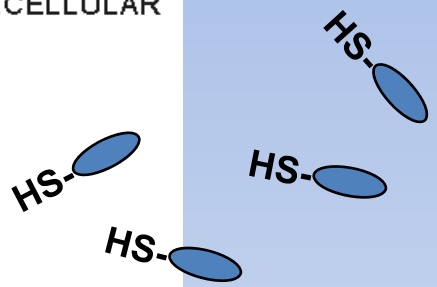
Integrate gating current to obtain charge (Q)



Q = charge
(gating current)
G = conductance
(ionic current)

“Accessibility” of residues mutated to Cys used to determine extent of S4 movement during channel activation

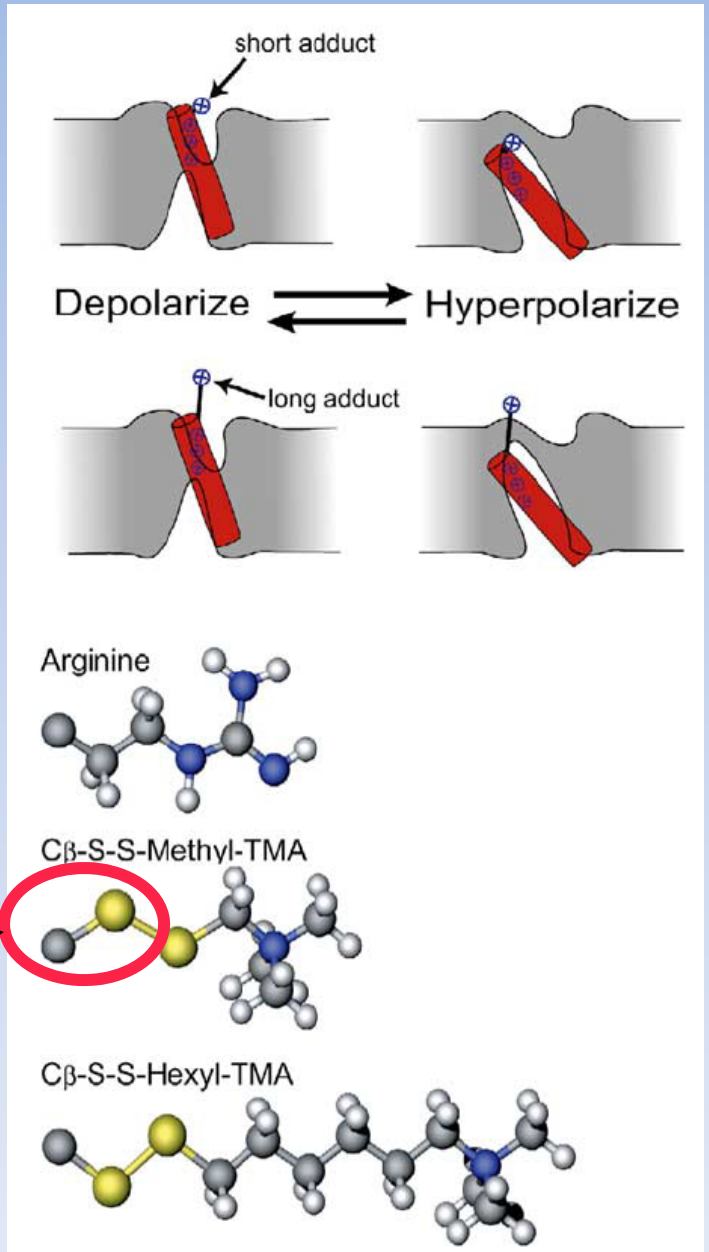




In closed state,the Cys residues can not be modified by Cys reactive agent (MTSET, HS-)

In open state, Cys residue *can* be modified

Experiment to probe for extent of S4 domain movement during gating of Shaker K channel



-SH of Cys residue

Ahern and Horn (2005)
Neuron 48:25-29

Intramembrane charge displacement

- Measurement of total charge

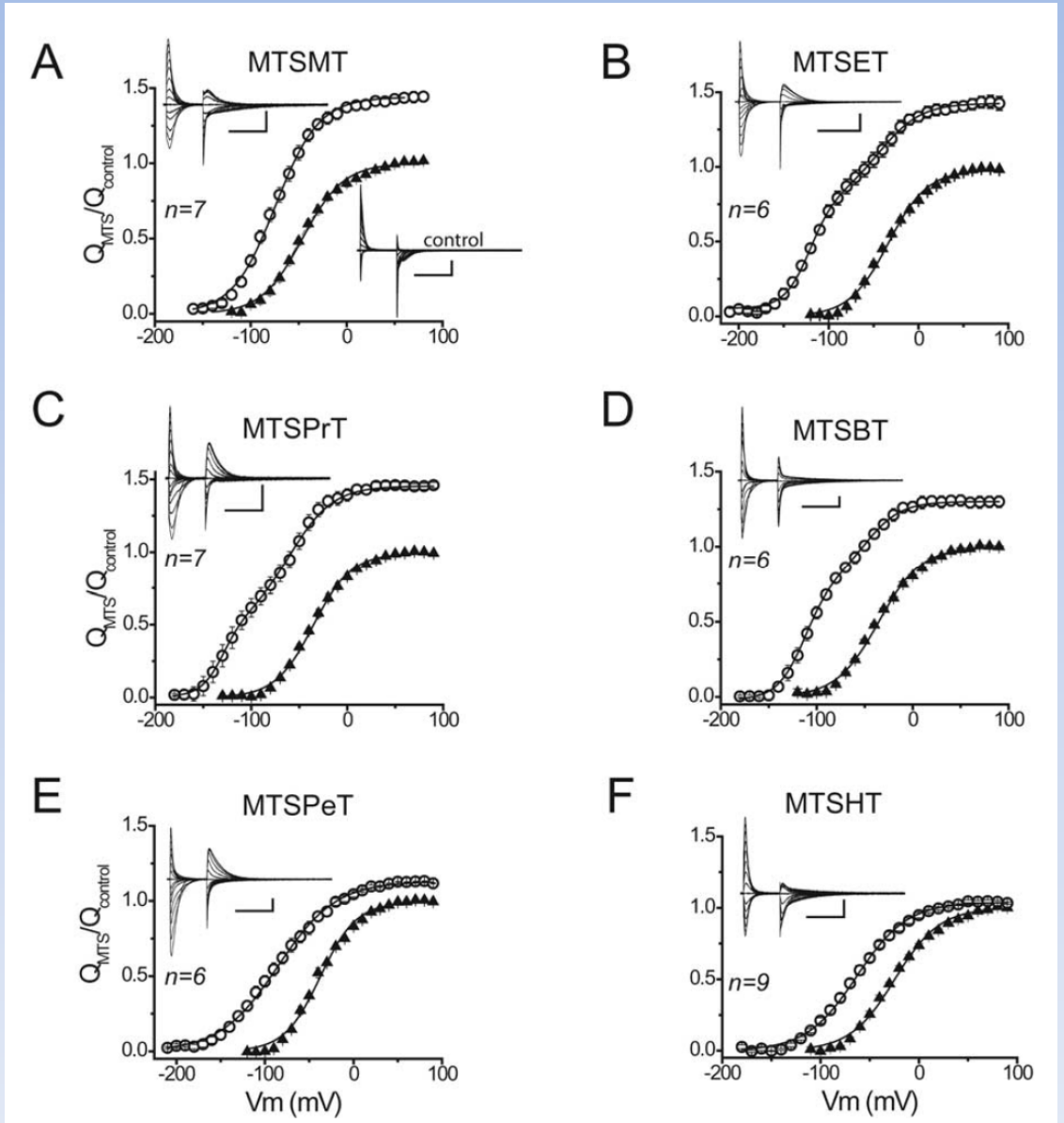
  moved (Q_{tot})/# of channels = 13 e^o/channel

Mutate a single Arg group (R362) – outermost charged a.a. in S4 domain of *Shaker* K channel

……reduces Q/channel to 9 e^o/channel

(1 e^o/subunit of homotetramer)

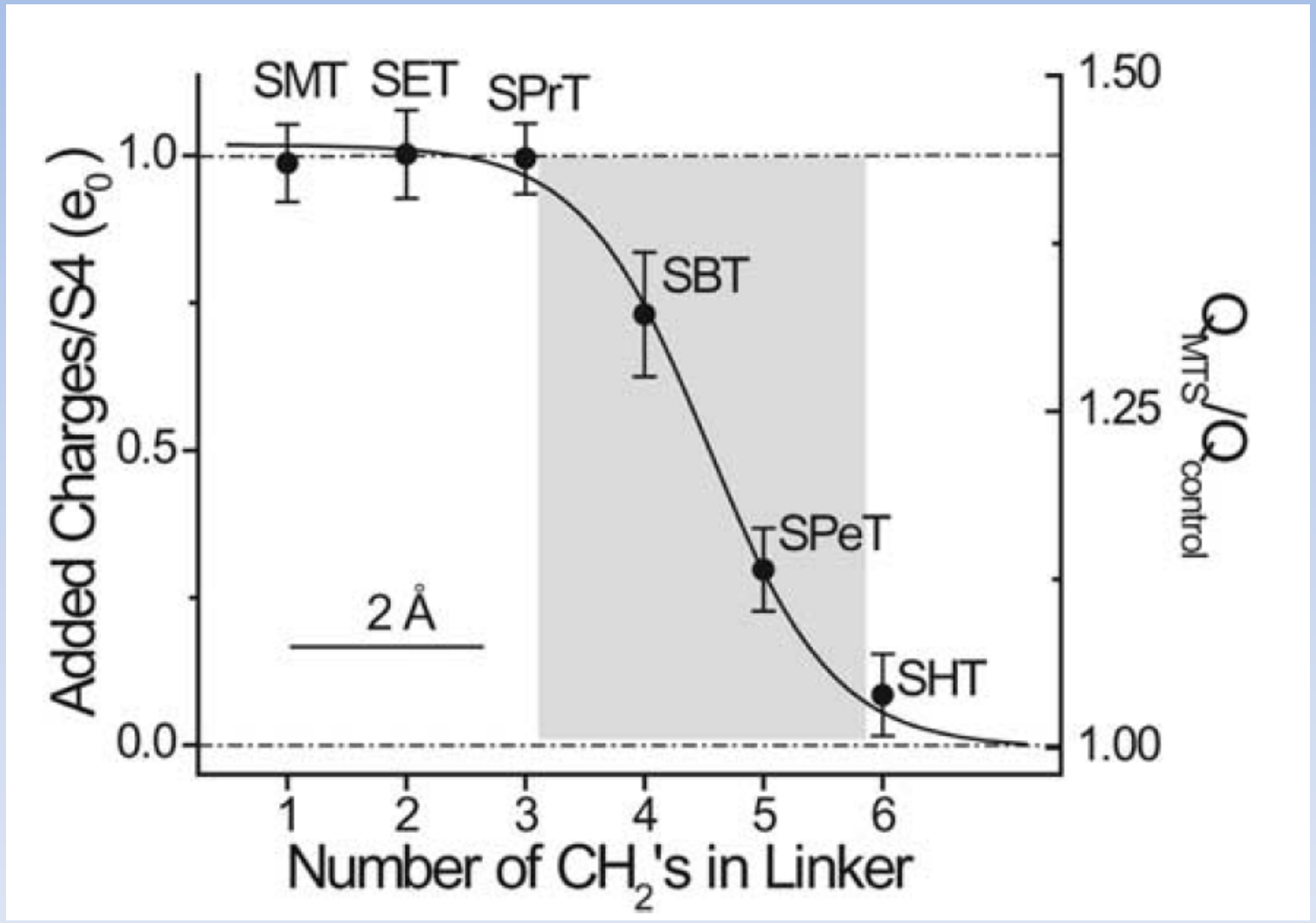
MTS modification of R362C affects gating currents and Q_{tot} of *Shaker* K channels



44% increase in Qtot

Ahern and Horn (2005)
Neuron 48:25-29

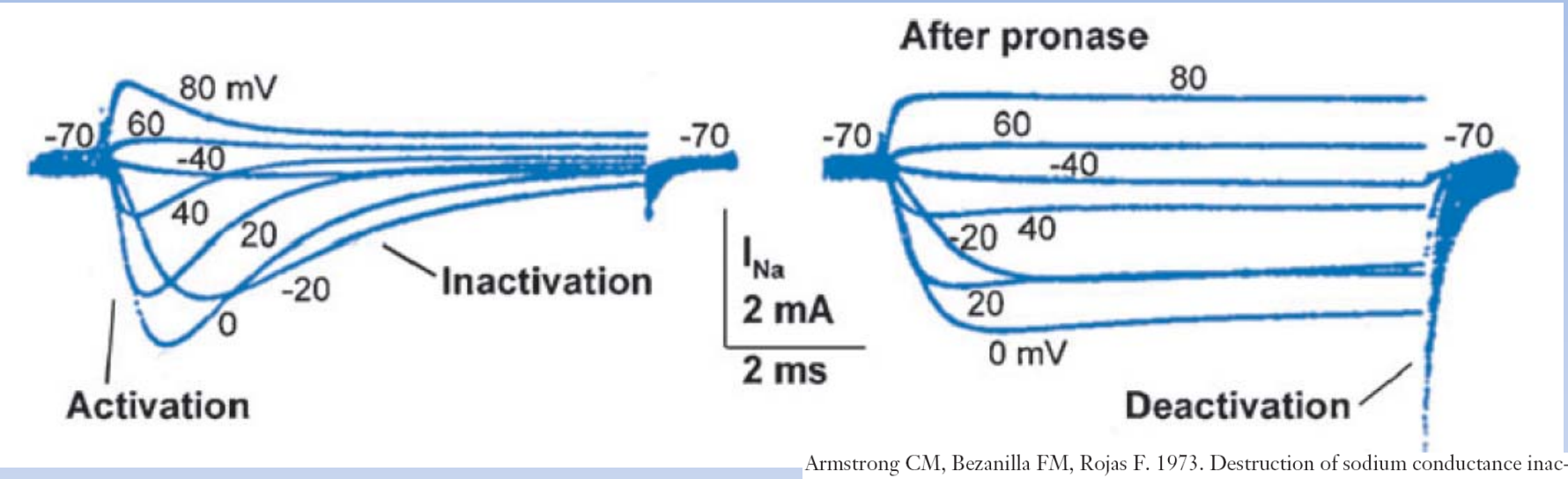
Increasing tether length monotonically decreases charge movement



Ahern and Horn (2005)
Neuron 48:25-29

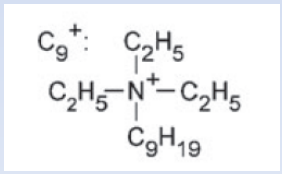
Inactivation gates

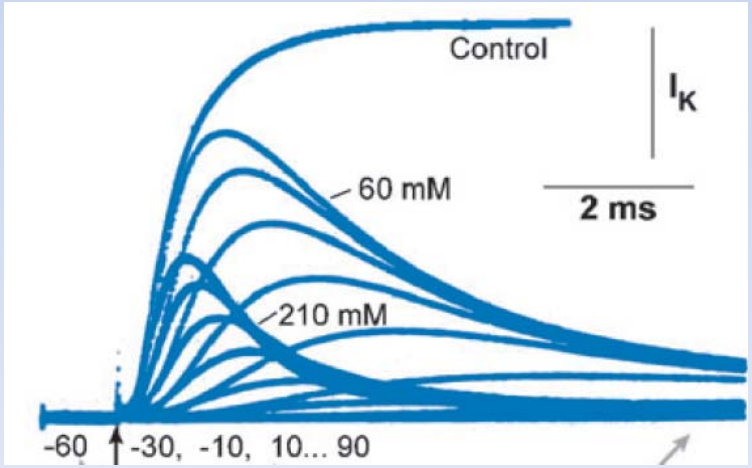
Pronase, a proteolytic enzyme applied internally to squid giant axons eliminates inactivation of Na channels

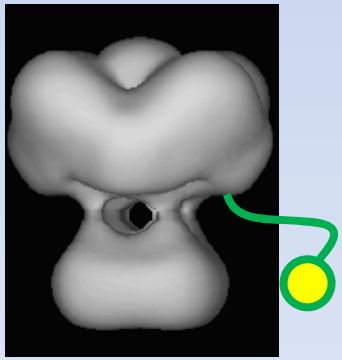


Armstrong CM, Bezanilla FM, Rojas F. 1973. Destruction of sodium conductance inactivation in squid axons perfused with pronase. *J. Gen. Physiol.* 62:375–91

Looks similar to block of K channels by internal C9:



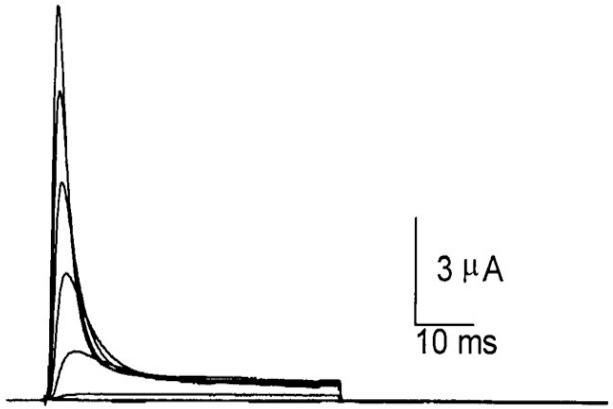




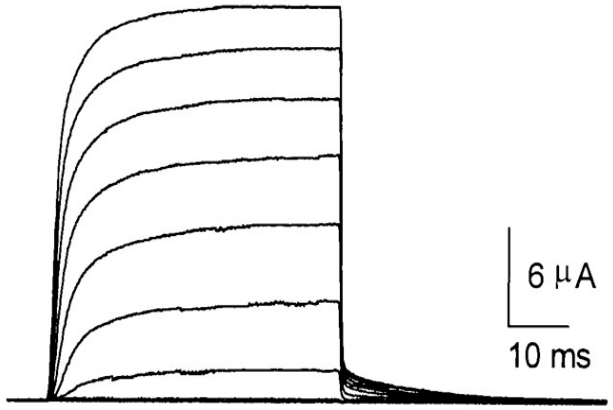
"ball and chain"

Armstrong (2007) *Ann Rev Physiol* 69:1-18

Removal of “ball and chain” from subunit eliminates inactivation in cloned Shaker K channels



Currents from normal channel



Currents from channel with amino terminus removed