

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: 1.5×10^{-17} A

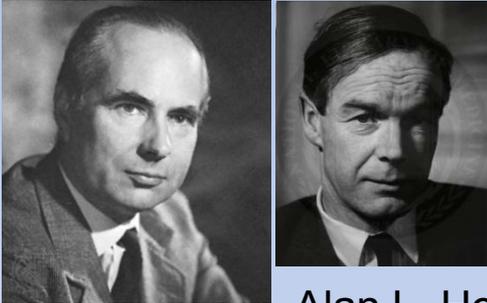
frame of reference:

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

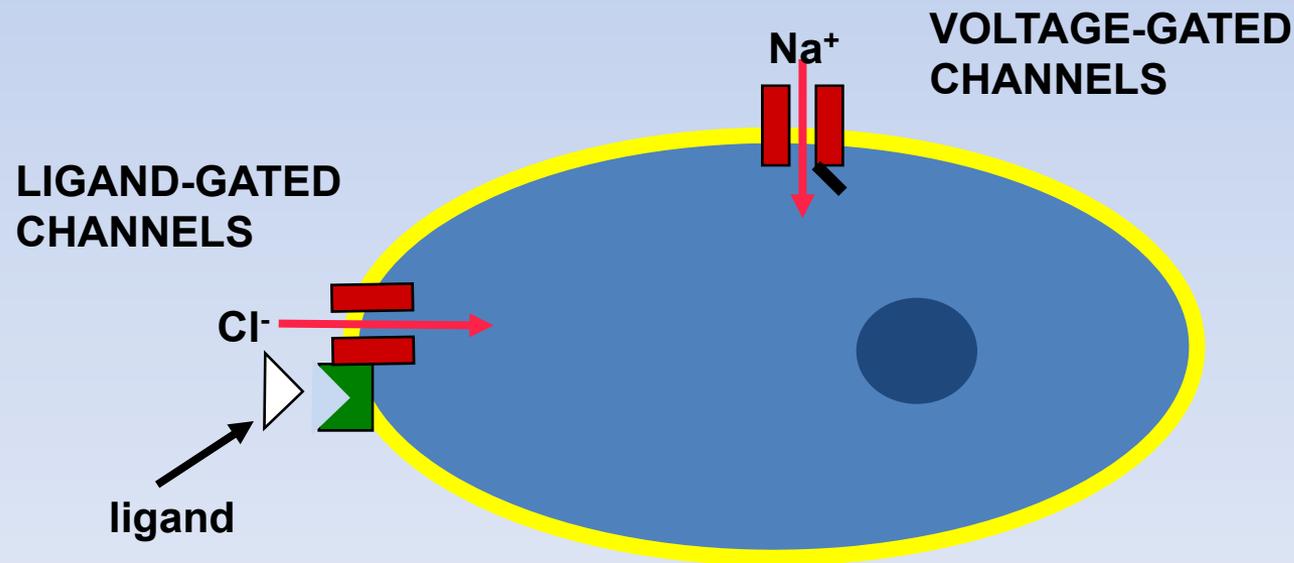
1) **Voltage-gated:** based on ion selectivity
(*K, Na, Ca, Cl channels*)

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*

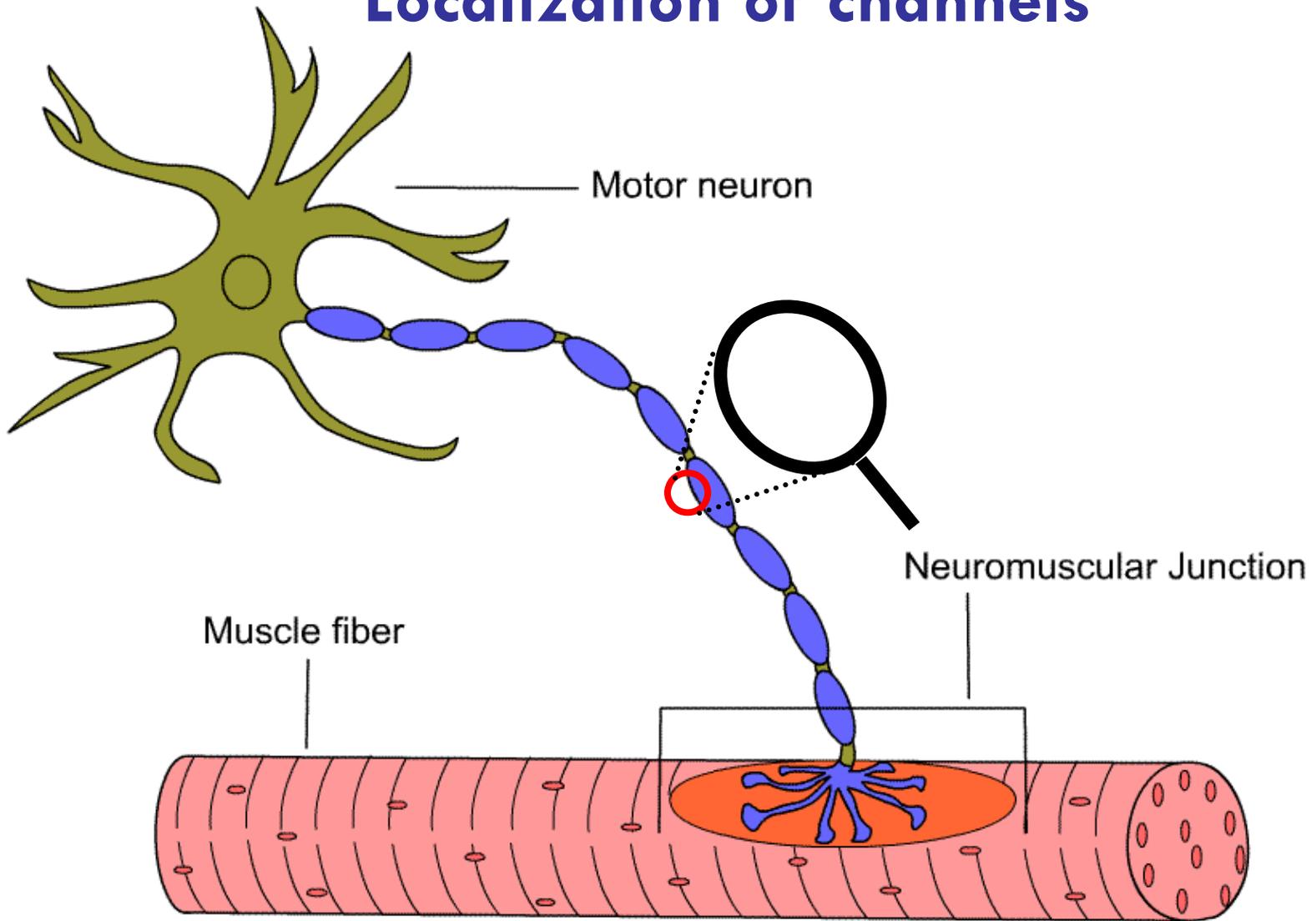
Oscillators: *pacemaker channels of the heart and central neurons*

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

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

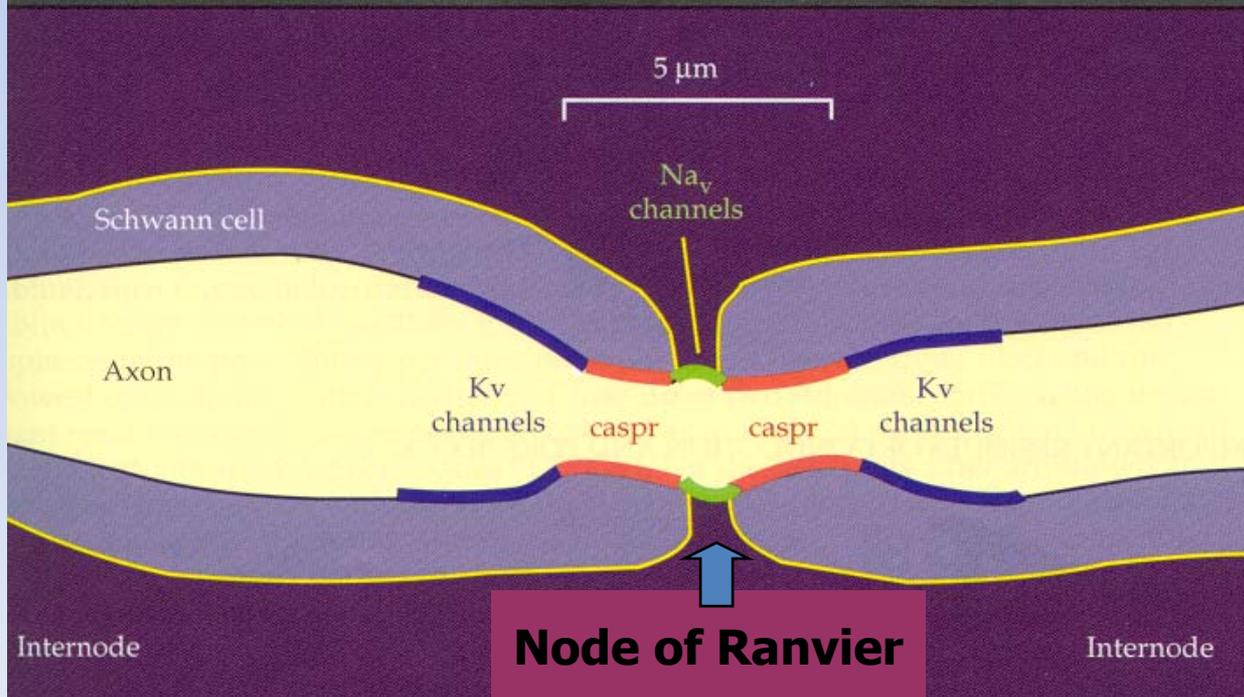
**Ion channels can be
highly localized**

Localization of channels



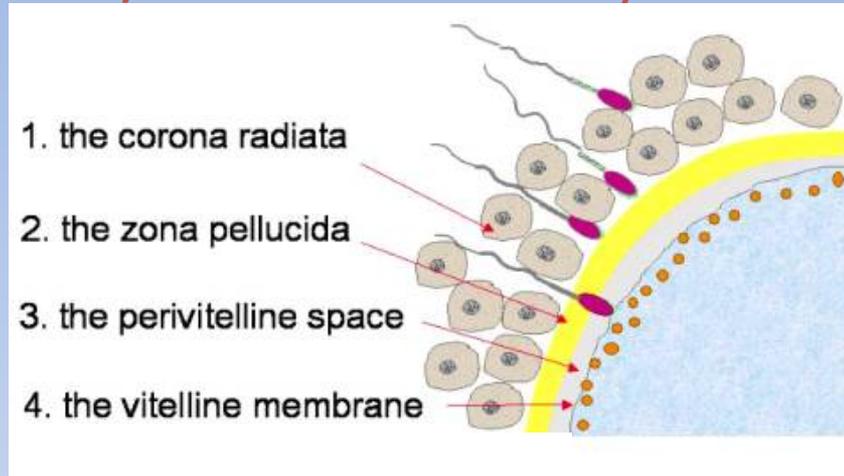
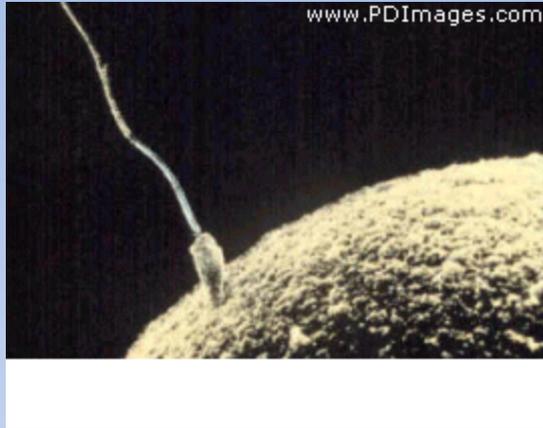
Adapted from: Kandel, Schwartz, and Jessell. *Principles of Neural Science*

Site-specific membrane targeting of ion channels

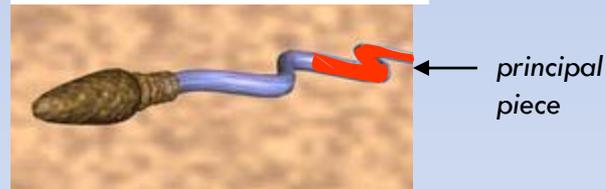


CatSper Ca channels

(6TM domains/subunit like Kv channels)



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

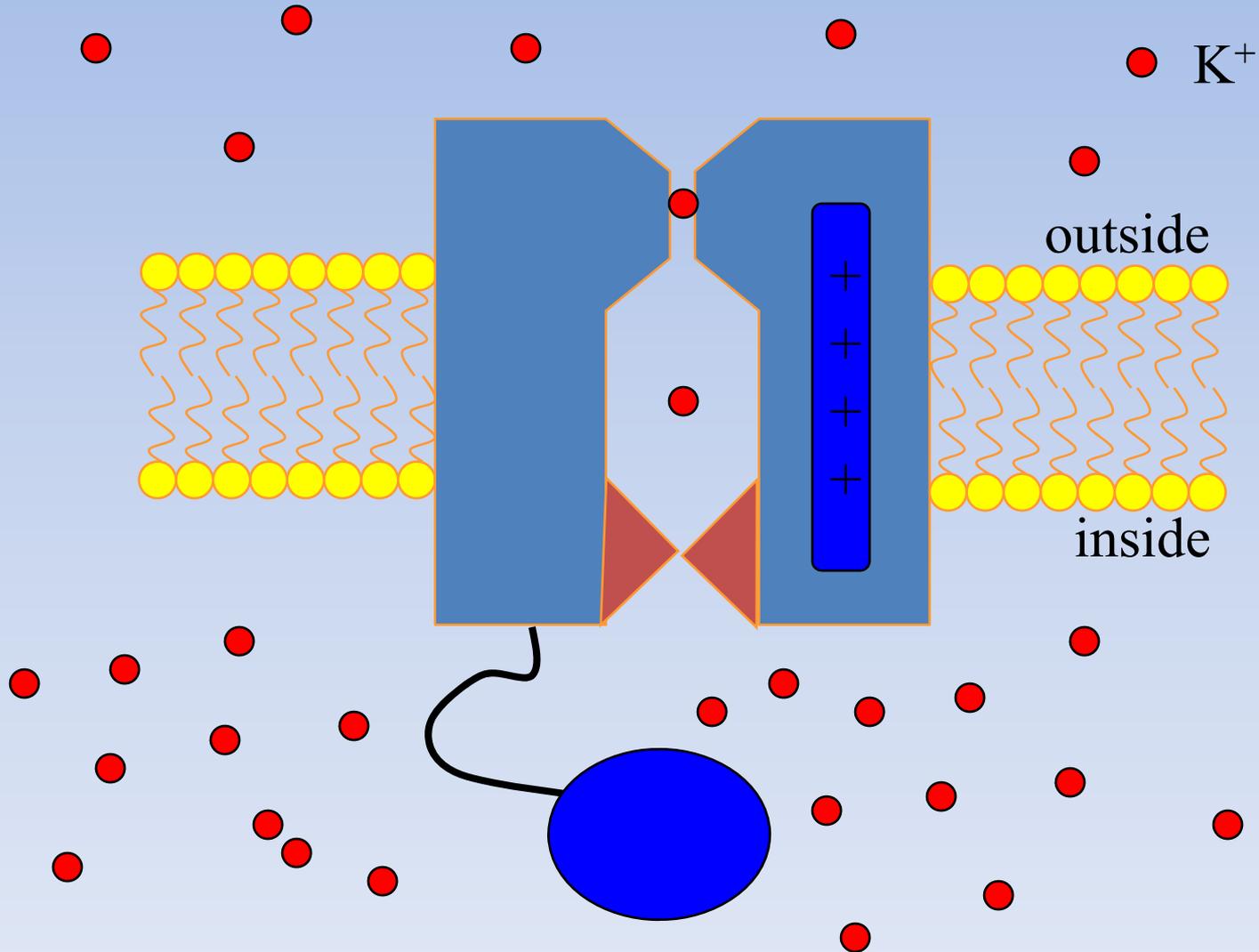


Sperm lacking CatSper are poorly motile (**no hyperactivity** during capacitation phase) and are unable penetrate zona pellucida and fertilize egg.

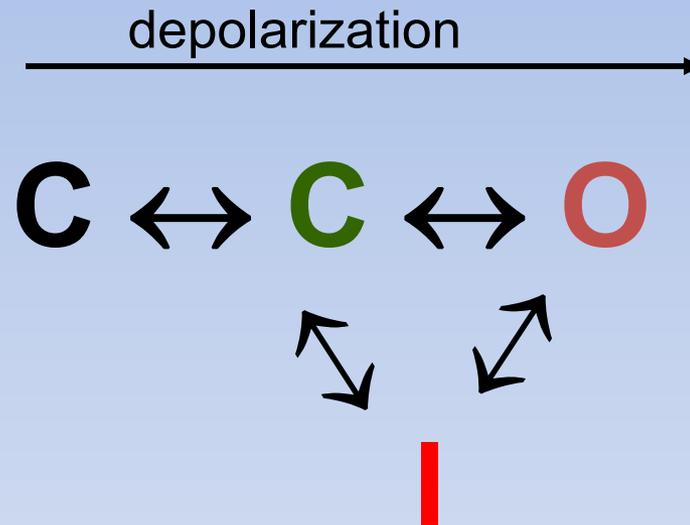
Genetic knock out of CatSper makes male mice infertile
- target for new contraceptive drugs?

Channel Gating: closed-open-inactivated

CLOSED



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



TERMINOLOGY:

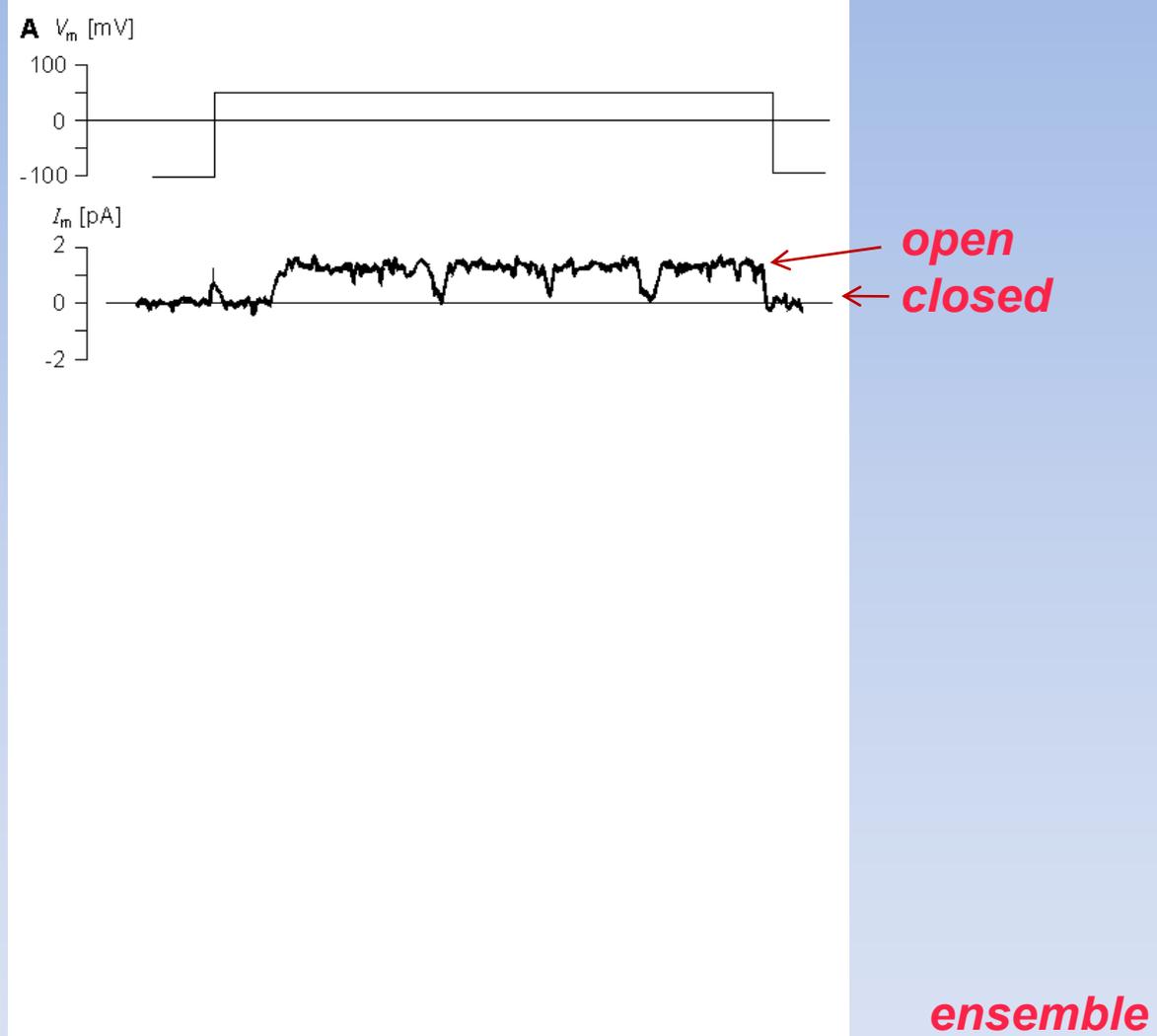
Activation: **C** → **O**

Deactivation: **O** → **C**

Inactivation: **C** → **I**; **O** → **I**

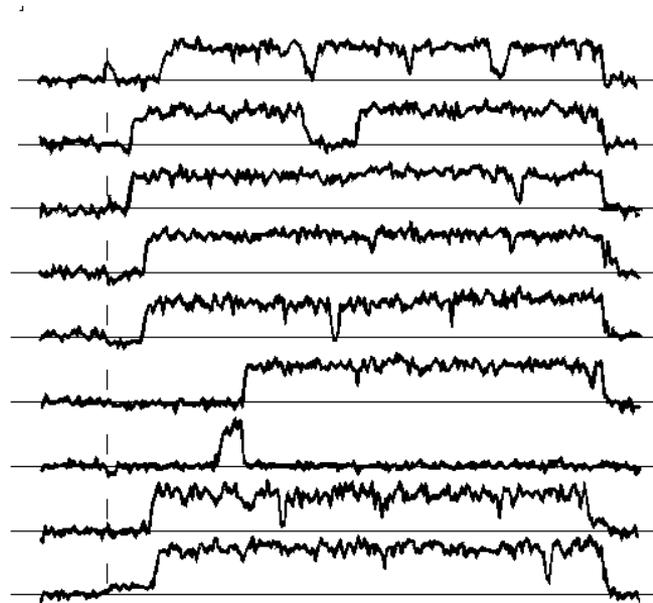
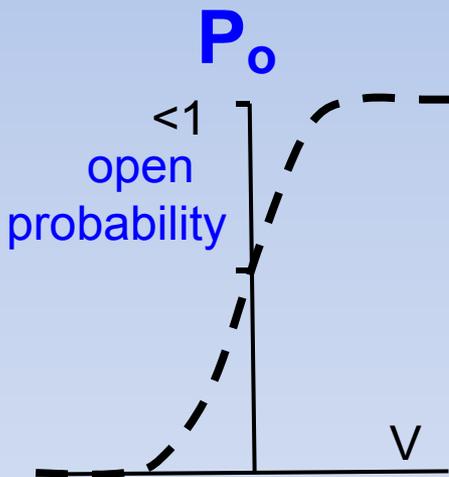
Recovery from inactivation: **I** → **C**; **I** → **O**

Single channel currents sum to generate whole cell currents



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

N = total # of channels in cell



i single channel current amplitude

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

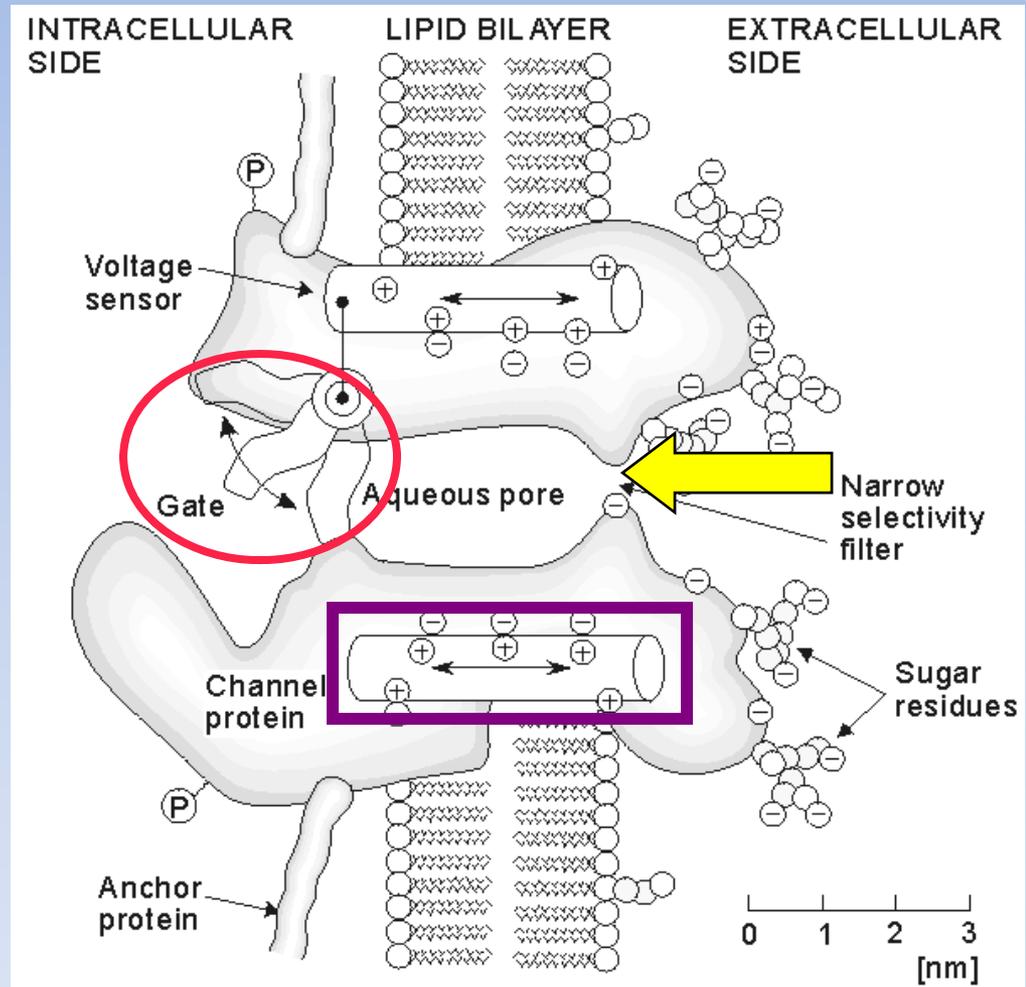
Channel structure

Transmembrane,
extra- & intra-cellular
domains

Gates

Pore and
selectivity filter

Voltage sensor



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

```

GAT CTG AAG TTC CAA GTG CGA GTG GCT TTC BCT TTC CGT ATT CGC GTC CAT
Asp Leu Lys Phe Gln Val Arg Val Ala Phe Ala Phe Arg Ile Arg Val His

15      30      45
TTC CGT TTC GGT TTC GTT GGA AAG CTA GAG CEC TGC TCC CAT CEC CAC AGT TTC
Phe Arg Phe Gly Phe Val Gly Lys Leu Glu Arg Cys Cys His Arg His Ser Phe

60      75      90
TTC GAT CCG AAC CCG ATT TGG GAA ACA GCC GCC AAG ATG ACC ATG TGG CAG AGT
Phe Asp Arg Asn Arg Ile Trp Glu Thr Ala Ala Lys Met Thr Met Trp Gln Ser

120     135     150
GTC GGC ACG AGC GCA TGG CTC CCA TGG ATG AAG CTG TGC GCA TCG TCC ACA ACG
Gly Gly Arg Ser Ala Trp Leu Pro Trp Met Lys Leu Met Ala Ser Ser Thr Arg

165     180     195     210
AGC GCG CCA CAC GGA GAA CGT TCA GAG TCA GTC CCG TTC CAA CBA CCG CAA CCT
Ser Ala Pro His Gly Glu Arg Ser Glu Ser Val Arg Phe Gln Arg Ala Gln Pro

225     240     255     270
GAA CCA GTC TTT GCC CAA ATT GAG CAG TCA ABA CBA ABA ACG GCG GCG TGG TCA
Glu Pro Val Phe Ala Gln Ile Glu Gln Ser Arg Arg Arg Arg Gly Gly Trp Ser

285     300     315
TGG CTT TGG TGC GGA CCG CAA CAC TTT GAA CCC ATT CCT CAC GAT GAT GAT TCT
Trp Leu Trp Cys Gly Pro Gln His Phe Glu Pro Ile Pro His Asp Asp Ser

330     345     360     375
GCG AAA AGA GTC GTT ATA AAT ATA AAT GTA ACG GGA TTA ACG TTT GAG ACA CAA
Ala Lys Arg Val Val Ile Asn Ile Asn Val Ser Gly Leu Arg Phe Glu Thr Gln

390     405     420
CTA CGT ACG TTA AAT CAA TTC CCG GAC ACG CTG CTT GGG GAT CCA GCT CCG ABA
Leu Arg Thr Leu Asn Gln Phe Pro Asp Thr Leu Leu Gly Asp Pro Ala Arg Arg

435     450     465     480
TTA CCG TAC TTT GAC CCG CTT AGA AAT GAA TAT TTT TTT GAC CBT ABT CBA CCG
Leu Arg Tyr Phe Asp Pro Leu Arg Asn Glu Tyr Phe Phe Asp Arg Ser Arg Pro

495     510     525     540
AGC TTC GAT GCG ATT TTA TAC TAT TAT CAG ABT GGT GCG CCA CTA CCG ABA CCG
Ser Phe Asp Ala Ile Leu Tyr Tyr Gln Ser Gly Gly Arg Leu Arg Arg Pro

555     570     585     600
GTC AAT GTC CCT TTA GAC GTA TTT ABT BAA BAA ATA AAA TTT TAT BAA TTA BBT
Val Asn Val Pro Leu Asp Val Phe Ser Glu Ile Lys Phe Tyr Glu Leu Gly

615     630     645
GAT CAA GCA ATT AAT AAA TTC AGA GAG GAT BAA BGC TTT ATT AAA BGG BAA BAA
Asp Gln Ala Ile Asn Lys Phe Arg Glu Asp Glu Gly Phe Ile Lys Glu Glu Glu

660     675     690
AGA CCA TTA CCG GAT AAT GAG AAA CAG ABA AAA GTC TGG CTG TCC TTC GAG TAT
Arg Pro Leu Pro Asp Asn Glu Lys Gln Arg Lys Val Trp Leu Ser Phe Glu Tyr

705     720     735     750
CCA GAA AGT TCG CAA GCC GCC AGA GTT GTA GCC ATA ATT ABT GTA TTT GTT ATA
Pro Glu Ser Ser Gln Ala Ala Arg Val Val Ala Ile Ile Ser Val Phe Val Ile

765     780     795
TTG CTA TCA ATT GTT ATA TTT TGT CTA GAA ACA TTA CCC GAA TTT AAG CAT TAC
Leu Leu Ser Ile Val Ile Phe Cys Leu Glu Thr Leu Pro Glu Phe Lys His Tyr

810     825     840     855
AAG GTG CGT ACG AAT CAA GCG AAA ECT CAG GAC CTC CAA GGG ATA CAA ATC CAT
Lys Val Arg Thr Asn Gln Ala Lys Pro Gln Asp Leu Gln Gly Ile Gln Ile His

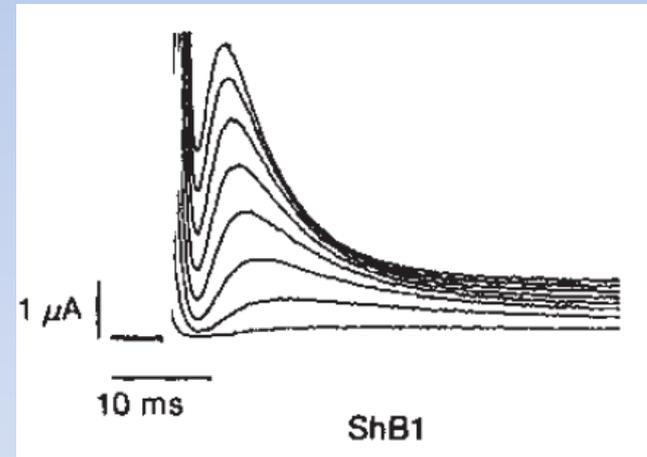
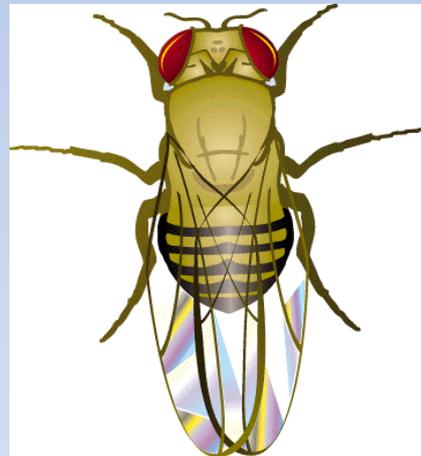
870     885     900     915
ATT TTC CTT TCC TTT TCT TTT TCC TGT GTT TCT GTG TGG CAT ACT TTC ACG TGT
Ile Phe Leu Ser Phe Ser Phe Ser Cys Val Ser Val Trp His Thr Phe Arg Cys

930     945     960
TCA ATA CAA CAA CAA ATG GCA CAA AAA TCC CCG AAG CCG GAG TGG CCT GAC ATC
Ser Ile Gln Gln Gln Met Ala Gln Lys Ser Arg Lys Pro Glu Trp Pro Asp Ile

975     990     1005     1020
CAG ATC CTT TCC TTC CTT ATA GAA ACG ITA TGT ATT ATT TGG TTT CAT TTG AAC
Gln Ile Leu Ser Phe Leu Ile Glu Thr Leu Cys Ile Ile Trp Phe His Leu Asn
    
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and Mark A. Tanouye
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Pasadena, California 91125

Cell (1987) 50:405



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

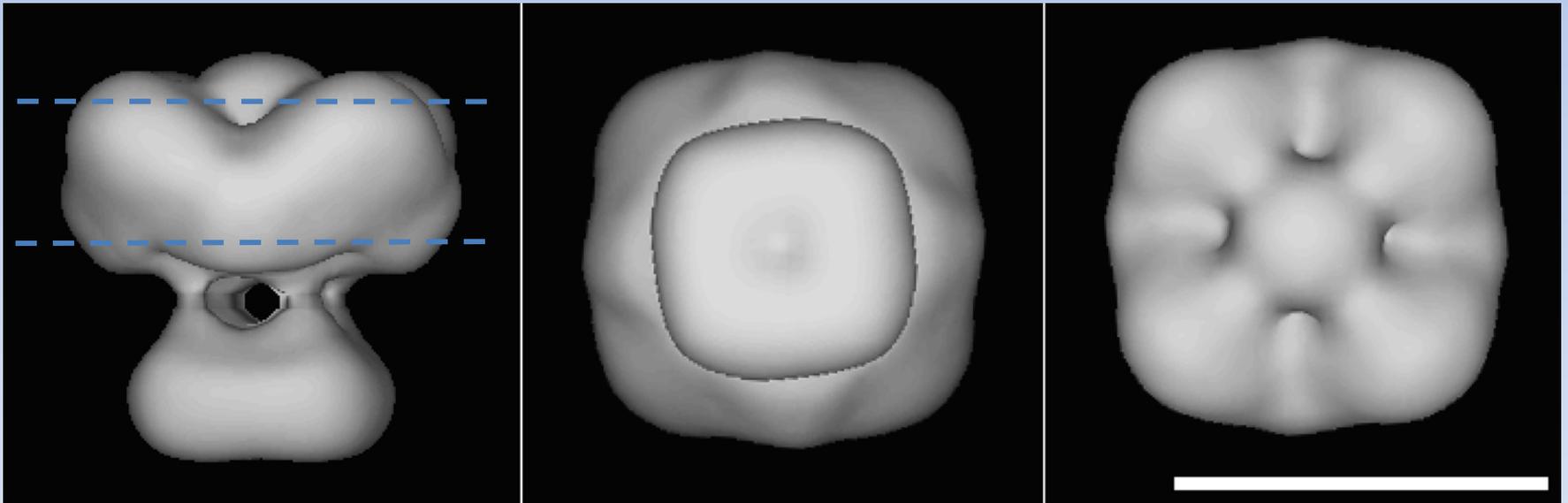
Channel Structure

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

Side view

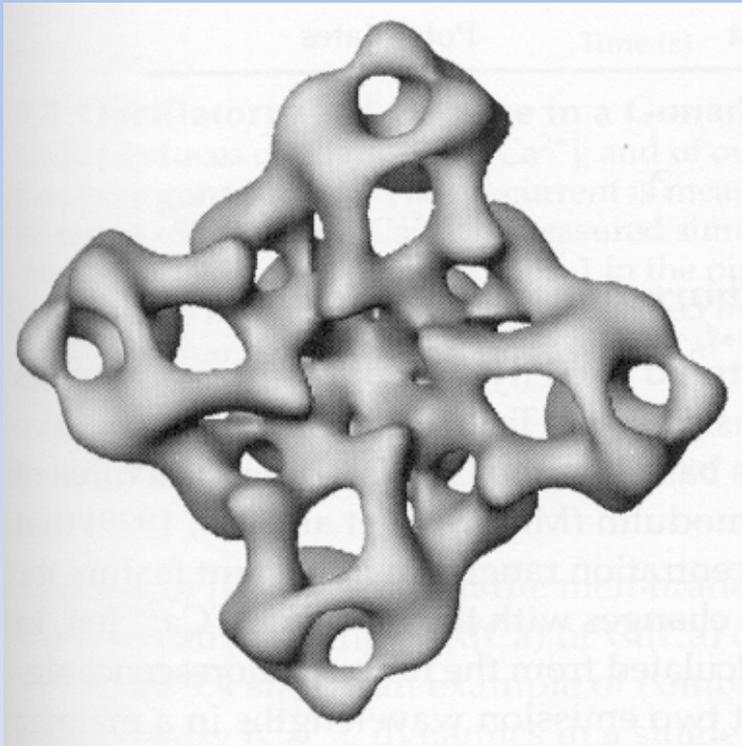
cytoplasmic face

extracellular face

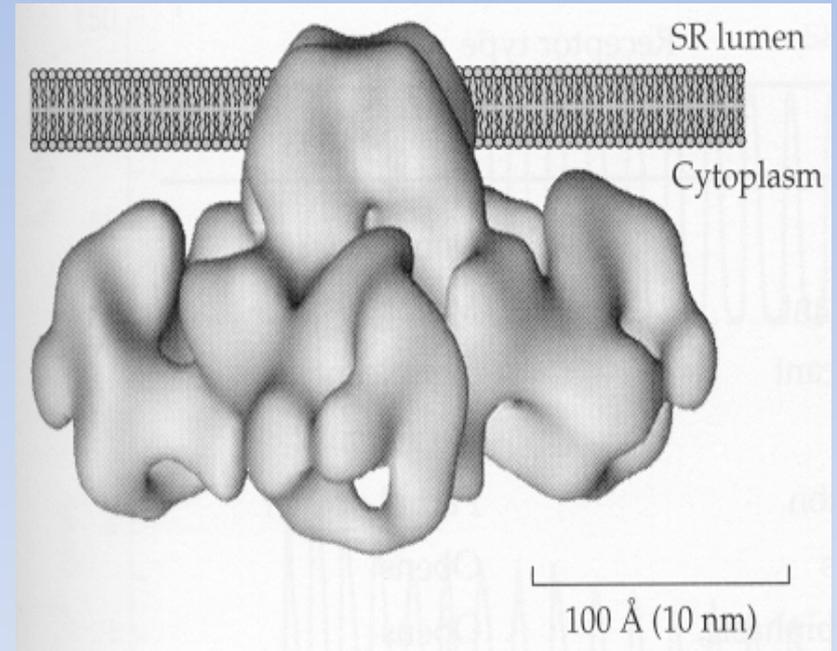


100 Angstroms

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)

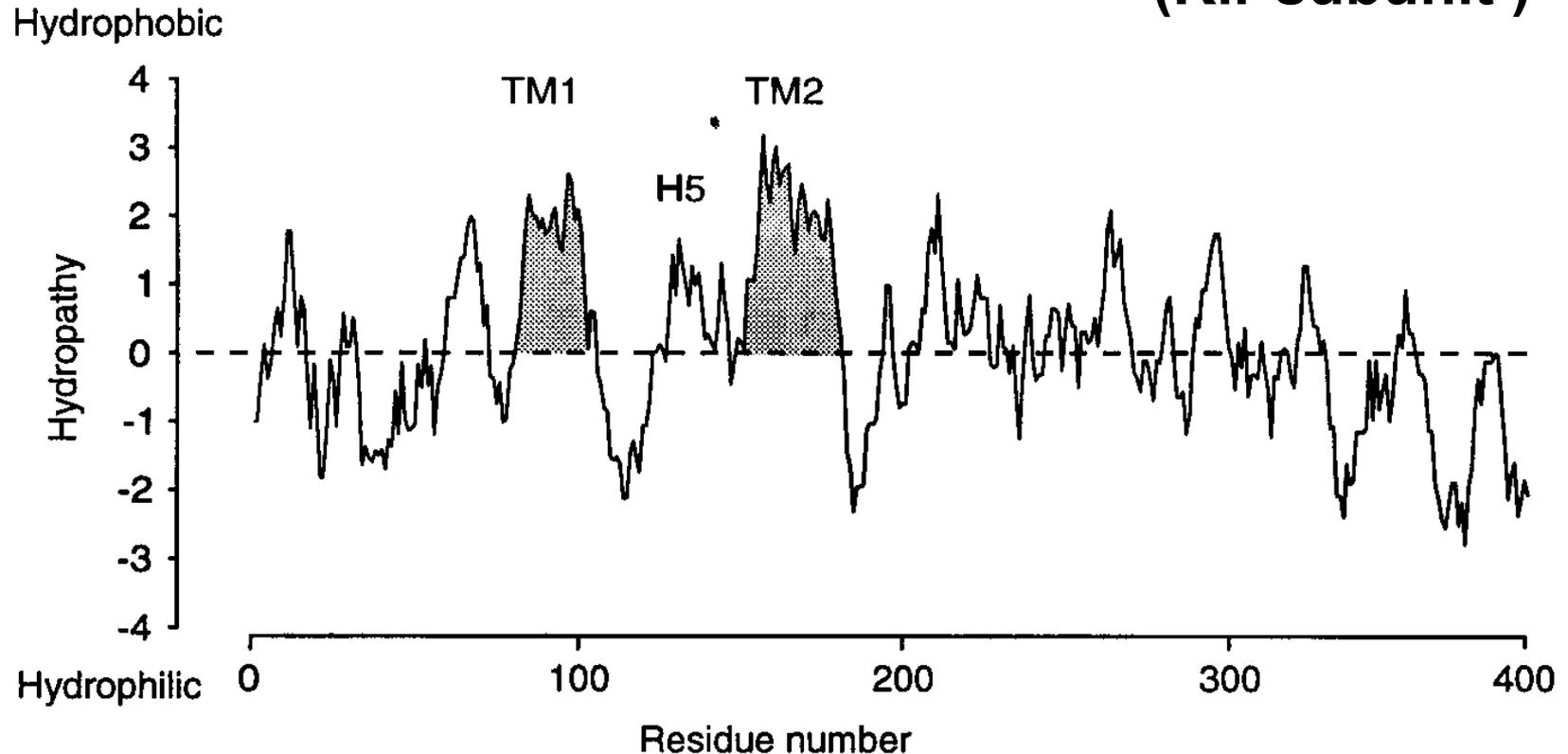
Channel subunits are large proteins

hHCN2	MDARGGGGRPGESPGATP-----APGPPPPPPAPPQQPPPPP--PPAPPPGGPGEAPPQHPRAEALPPEAAD-EGGPRG-----	73
hHCN4	MDKLPSPMRKRLYSLSLPOQVGAKAWIMDEEEDAEEEGAGGRQPPSRRSIRLRPLPSPPSAAGGTESSSAL--GAADSEGPARGAGKSS	88
hHCN2	-----RLRSRDSSCGRPGTPGAASTAKGSPNGECGR-----GEPQCSFAG--PEGFARGP-KVSFSCRGAASGFAPGPGFAEEAG	145
hHCN4	TNGDCRRFRGSLASLGSRRGG- GSGGTGSGSSHGHLHDSABERRLIAEGDASPGEEDRTPPGLAAPERPGASAQFAASPPPEQQPPQPASA	177
hHCN2	S-----EAGFAG-----EPRGSQASFMQRQFGALLQPGVNKFSLRMFGSQKAVEREQERVKSAGAWIIHPYSDFRFYWDF	216
hHCN4	SCEQPSVDTAIKVEGGAAAGDQILPEAEVRLGQAGFMQRQFGAMLQPGVNKFSLRMFGSQKAVEREQERVKSAGFWIIHPYSDFRFYWDL	267
hHCN2	TMLLFMVGNLIIIPVGITFFKDEITAPWIVFNVVSDTFFLIDLVLNFRFTGIVIEDNTEIILDPEKIKKKYLRTWFWVDFVSSIPVDYIFL	306
hHCN4	TMLLFMVGNLIIIPVGITFFKDEITAPWIVFNVVSDTFFLIDLVLNFRFTGIVIEDNTEIILDPEKIKKKYLRTWFWVDFVSSIPVDYIFL	357
	----- S1 ----- S2 ----- S3 -----	
hHCN2	IVEKGIIDSEVYKTARALRIVRFTKILSLRLLRLLSRLIRYIHQWEEIFHMTYDLASAVMRIENLISMMLLLCHWDGCLQFLVPMLQDFPR	396
hHCN4	IVETRIIDSEVYKTARALRIVRFTKILSLRLLRLLSRLIRYIHQWEEIFHMTYDLASAVMRIENLISMMLLLCHWDGCLQFLVPMLQDFPD	447
	----- S4 ----- S5 -----	
hHCN2	NCWVSINGMVNHWSSELYSFALFKAMSHMLCIGYGRQAPESMTDILWLTMSLMIVGATCYAMFIGHATALIQSLDSSRRQYQEKYKQVEQY	486
hHCN4	DCWVSINMNVNNSWGKQYSYALFKAMSHMLCIGYGRQAPVMSDILWLTMSLMIVGATCYAMFIGHATALIQSLDSSRRQYQEKYKQVEQY	537
	----- PoreH ----- SF ----- S6 -----	
hHCN2	MSFHKLPADEFQKIDHDYIEHRYQGKMFDESSILGELINGPLREEIIVNFNCRKLVASMPLFANADPNFVTAAMLTKLRFEVFQPGDYIIREGT	576
hHCN4	MSFHKLPPDITQRIDHDYIEHRYQGKMFDESSILGELISEPLREEIIVNFNCRKLVASMPLFANADPNFVTSMLTKLRFEVFQPGDYIIREGT	627
hHCN2	IGKKMYFIQHGVS SVLTGKNKEMKLS DGSYFGEICLLTRGRRTASVRADTYCRLYSLSDVNFNEVLEEYPMRRRAFETVALDRLDRIGKK	666
hHCN4	IGKKMYFIQHGVS SVLTGKNKEMKLS DGSYFGEICLLTRGRRTASVRADTYCRLYSLSDVNFNEVLEEYPMRRRAFETVALDRLDRIGKK	717
	----- CNBD -----	
hHCN2	NSILLHKVQHDLNSGVFNQENATIQEIVKYDREMVOQAEALGQ-----RVGLFPP	715
hHCN4	NSILLHKVQHDLNSGVFNQENETIQEIVKYDREMAHCAHRVQAAASATPTPTPVIWTPLIQAPLQAAAATTSVAIALTHHPRLPAAIFR	807
hHCN2	PPPPPPQV TSA-----IATLQQAAAMSFPC-----	740
hHCN4	PPPGSGLGNL GAGQTPRHLKRLQSLIPSA LGSASPASSPSQVDT P SSSSFH IQQLAGFSAPAGLSPLL P SSSSSPPPGACGSPSAPT PSA	897
hHCN2	-----QVARPLVGPLALG-----SPRLVRRPPPGPAPAAASPGPPPPASPPG-----APASPRAPRTSRYG-----GLP	799
hHCN4	GVAATTIAGFGHFHKLALGGSLSSSDSPILTFLOPGARSPQAQPSHAPPFGARGGLGLPEHFLPPPSSRSPSSSFGQLGQPPELSI GLA	987
hHCN2	AAPLAGPALPAR-----	819
hHCN4	TGPLSTPETEPRQPEPPSLVAGASGGASPVGFTPRGGLSPPGHSPGPPRTFPSPAPPRASGSHGSLLLPPASSPPPPQVPQRRTGTPPLTPG	1077
hHCN2	RLSRASRPLSASQPSLPHGAPGFAASTREAS--SSTPRLRPTFA-ARAAAFS-----PDRRDSASPGAAGGLDPQDSA-----	877
hHCN4	RLTQDLKLSASQPALFHODG---AOTLRASPHSSGESMAAFELFPRAGGSGSGSSGGLGPPGRPYGATPGHVTLPKRTSSGSLPPP	1164
hHCN2	-----RSRLSSNL	889
hHCN4	LSLFGARATSSGGPPLTAGPQREPGARPEPVRSKLPSNL	1203

General structure determined by hydropathy plots

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

(Kir subunit)

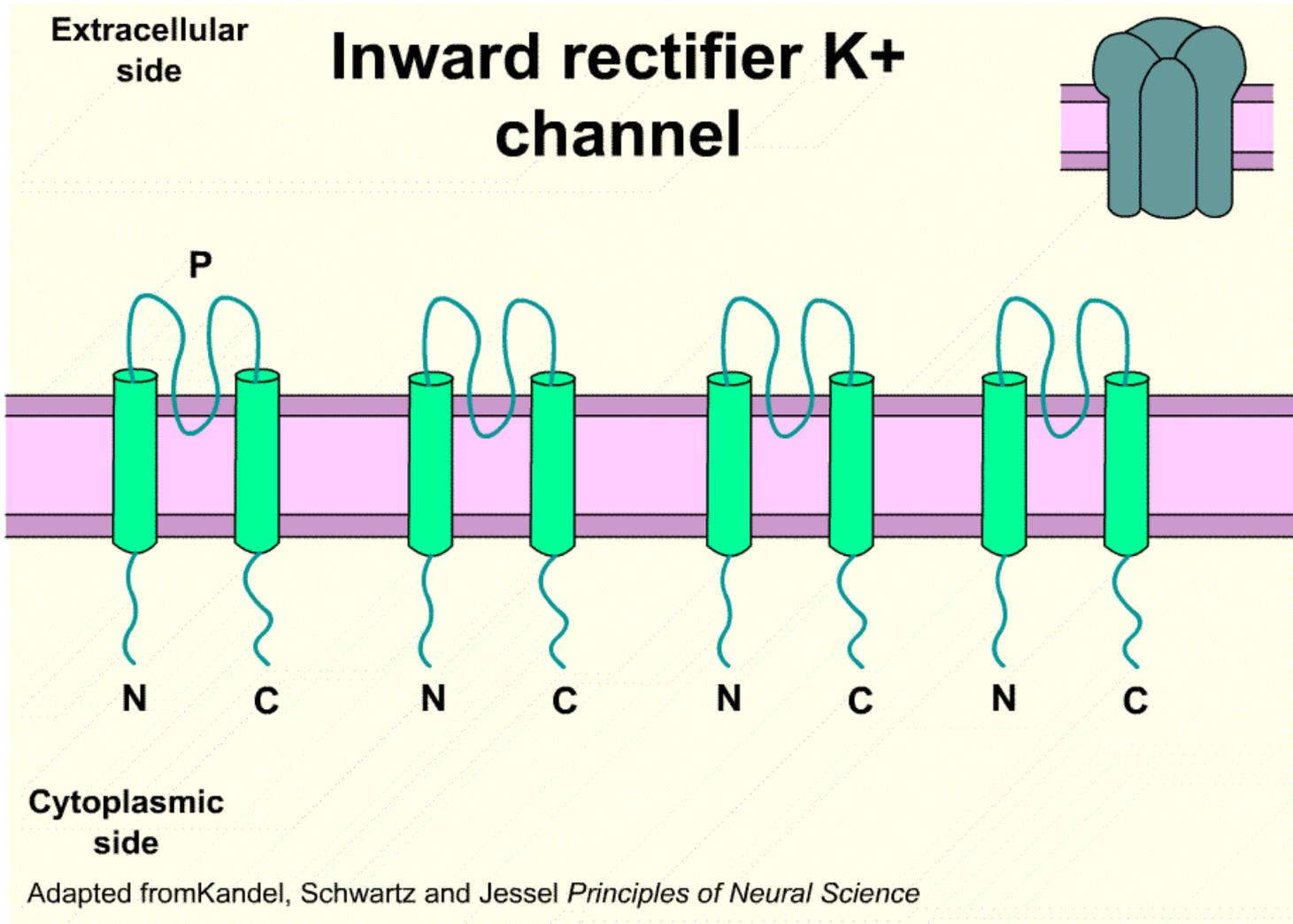


Alpha helix: 3.6 residues/turn; 5.41 Angstroms/turn

Plasma membrane thickness ~ 34 Angstroms

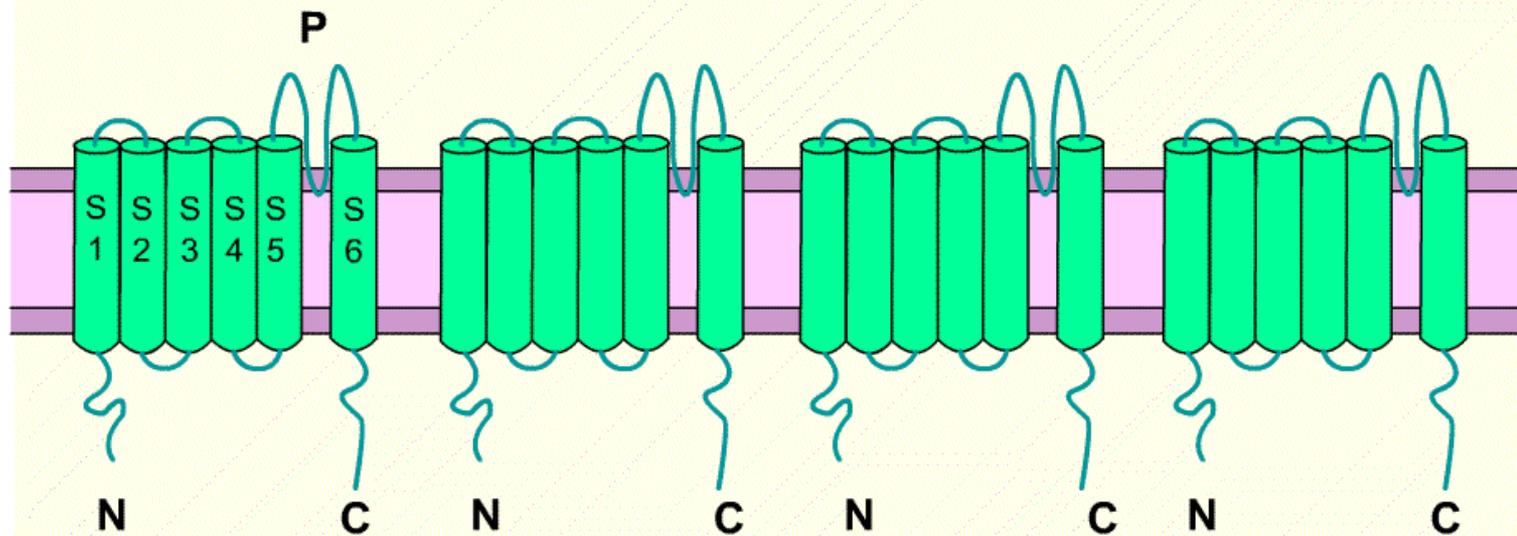
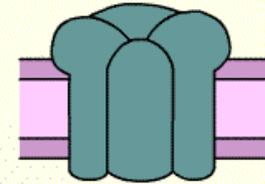
~23 amino acids/transmembrane domain

A primitive channel: Kir



Extracellular
side

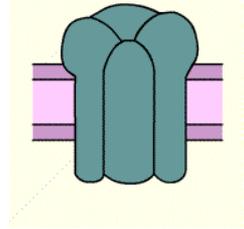
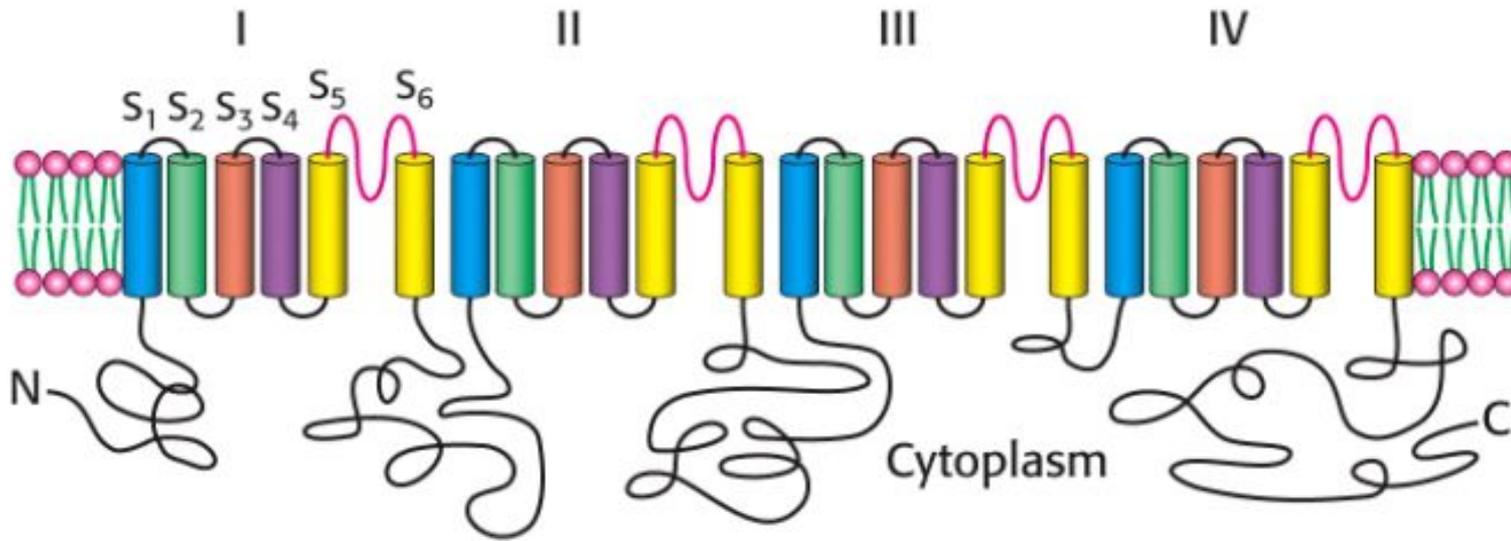
Voltage-gated K⁺ channel



Cytoplasmic
side

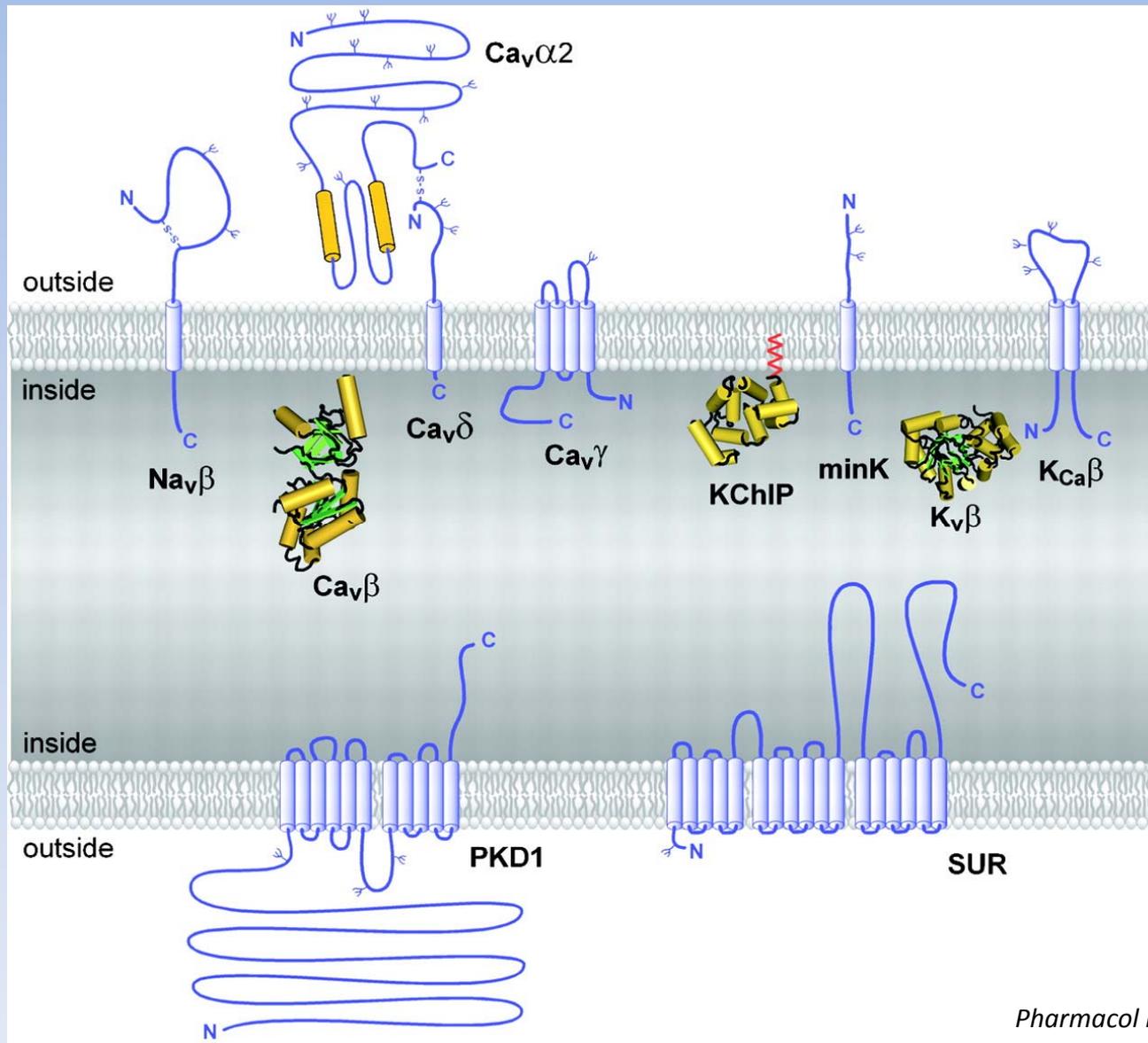
Adapted from Kandel, Schwartz and Jessel *Principles of Neural Science*

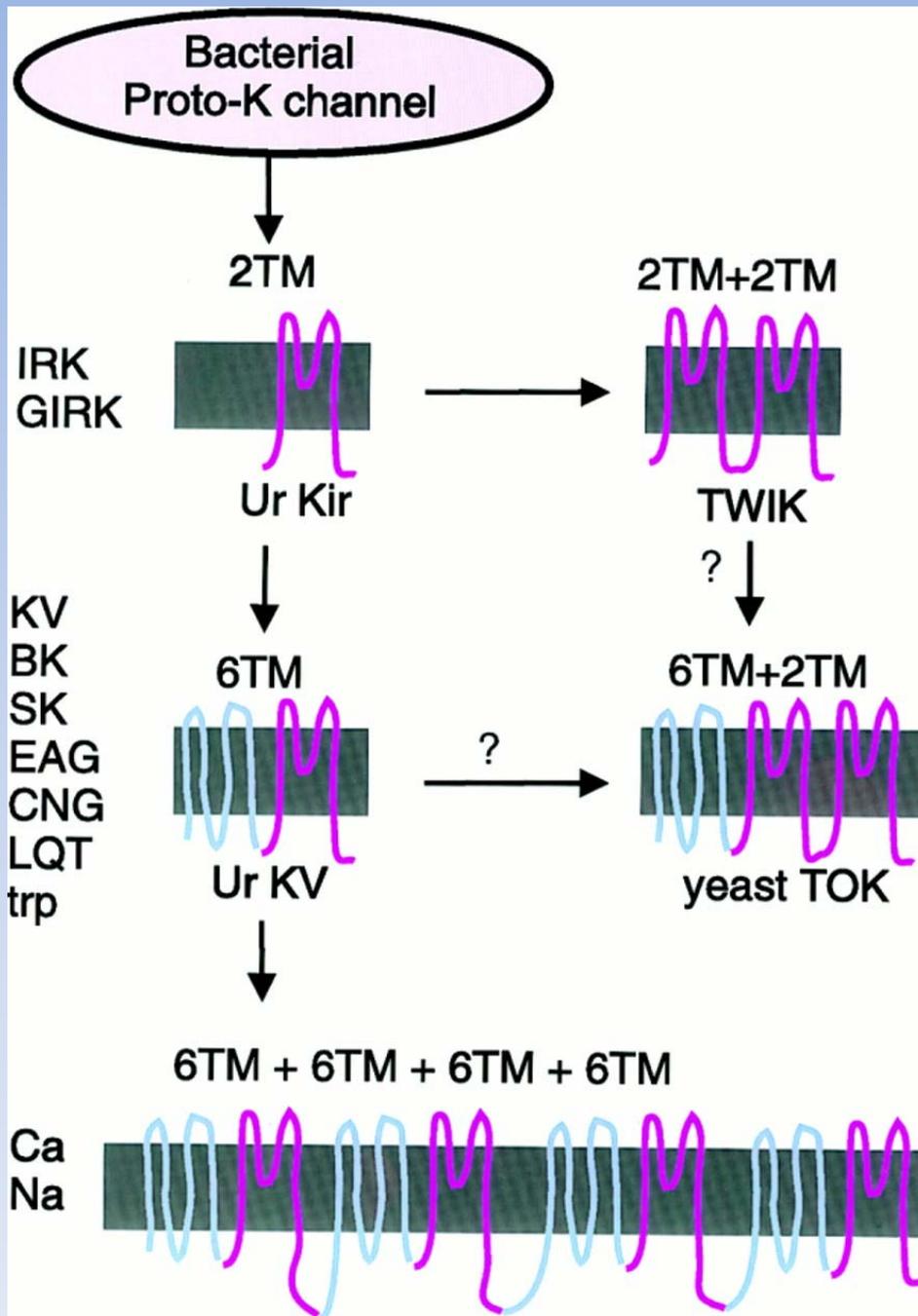
Voltage-gated Na and Ca channel structure



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

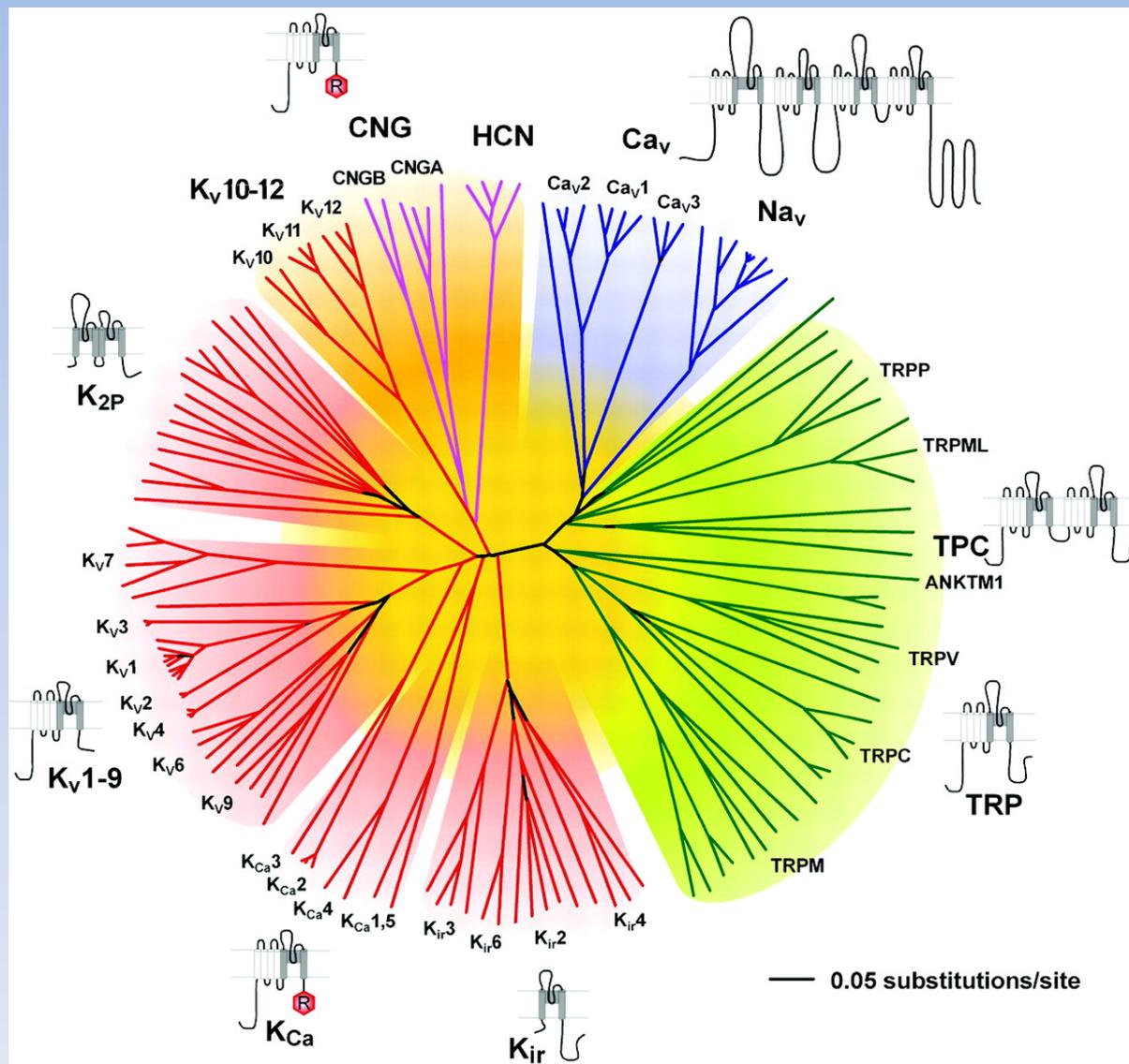
Auxiliary subunits of the voltage-gated ion channel superfamily





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)



The “Holy Grail – Part II”

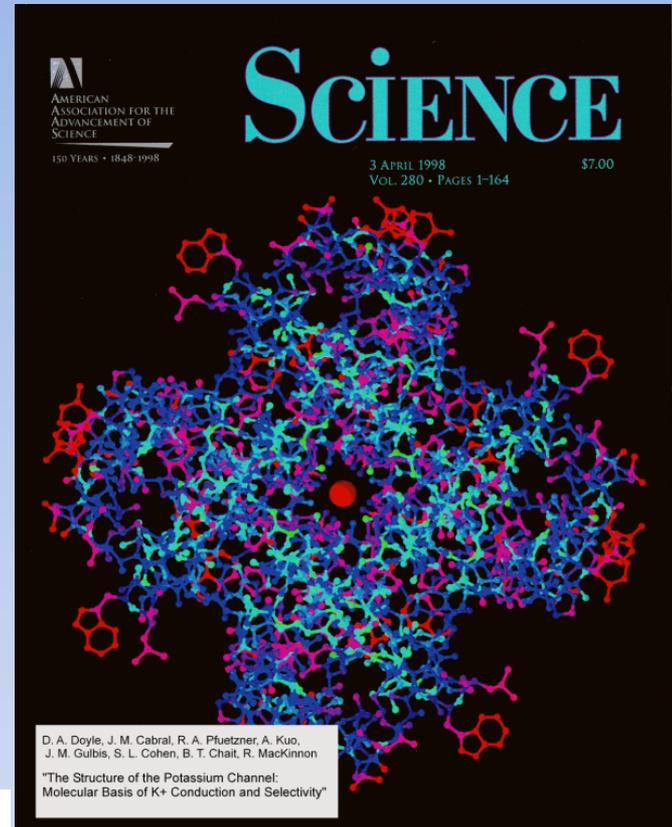
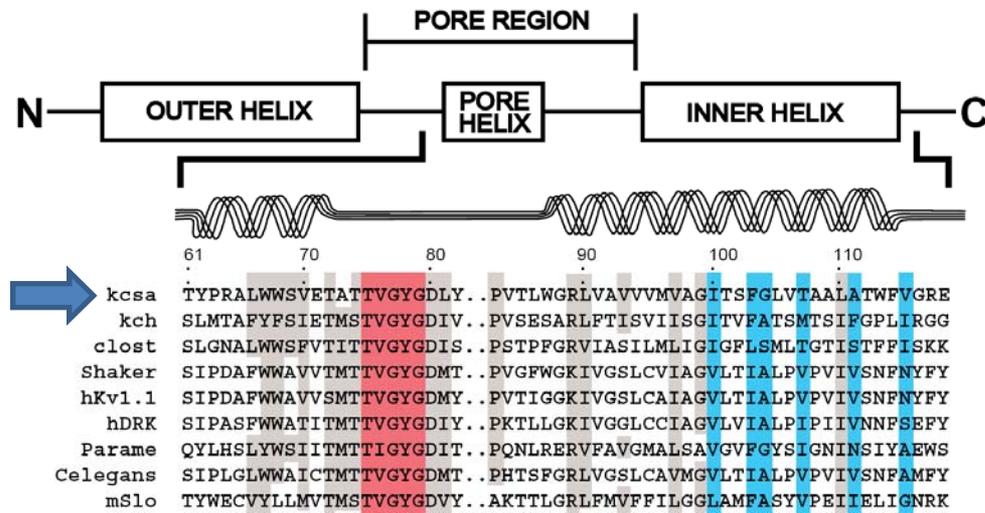
(Clay Armstrong)

RESEARCH ARTICLES

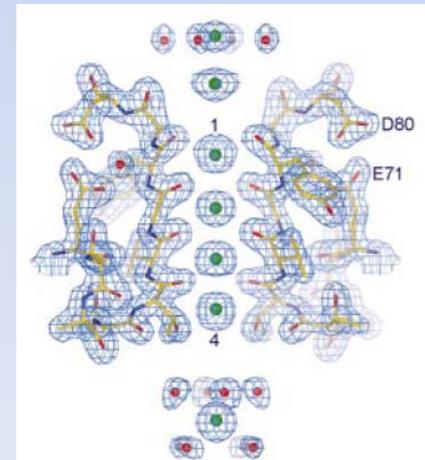
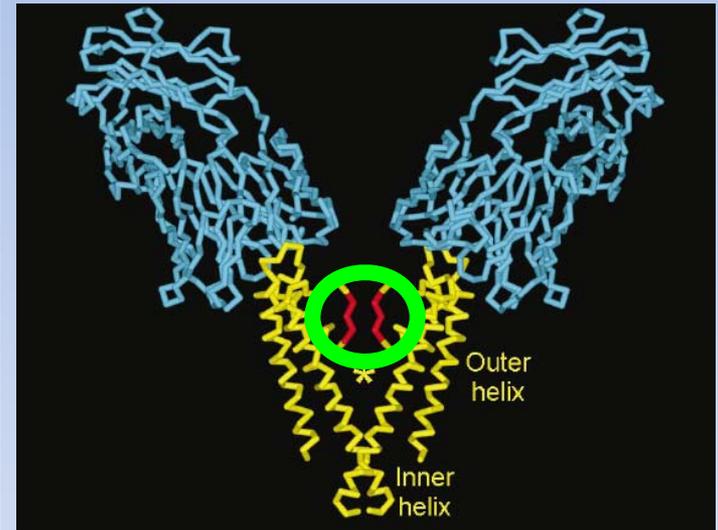
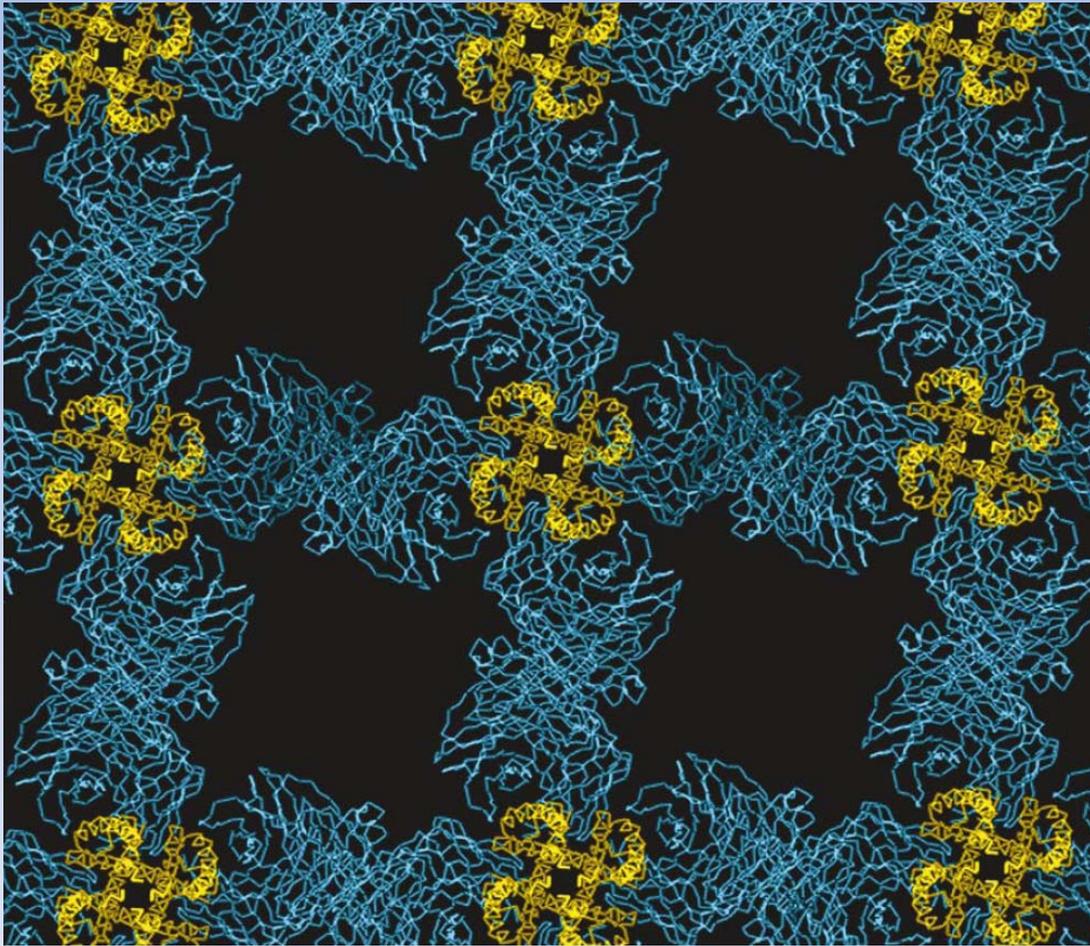
The Structure of the Potassium Channel: Molecular Basis of K⁺ Conduction and Selectivity

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!

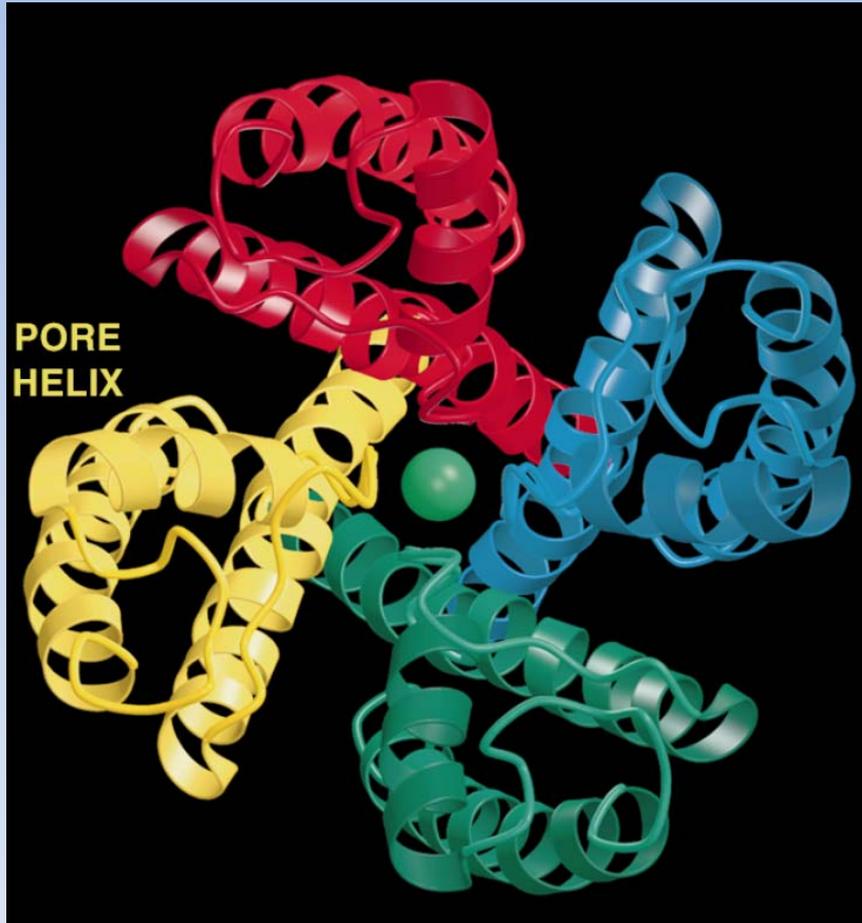
X-ray crystal structures were first obtained from bacterial channels

- 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

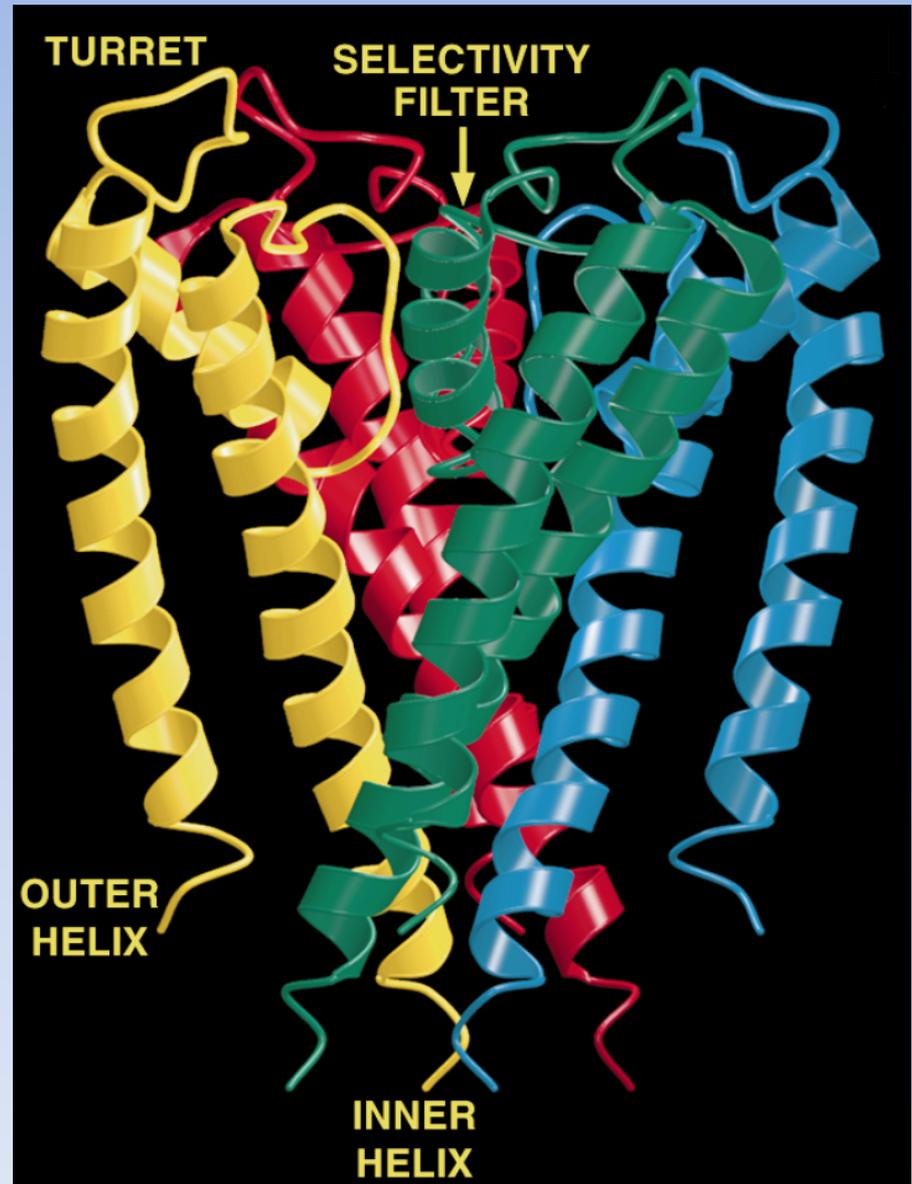
All structures solved in Rod MacKinnon's lab at Rockefeller Univ
(Nobel Prize in Chemistry, 2003)



KcsA bacterial K channel

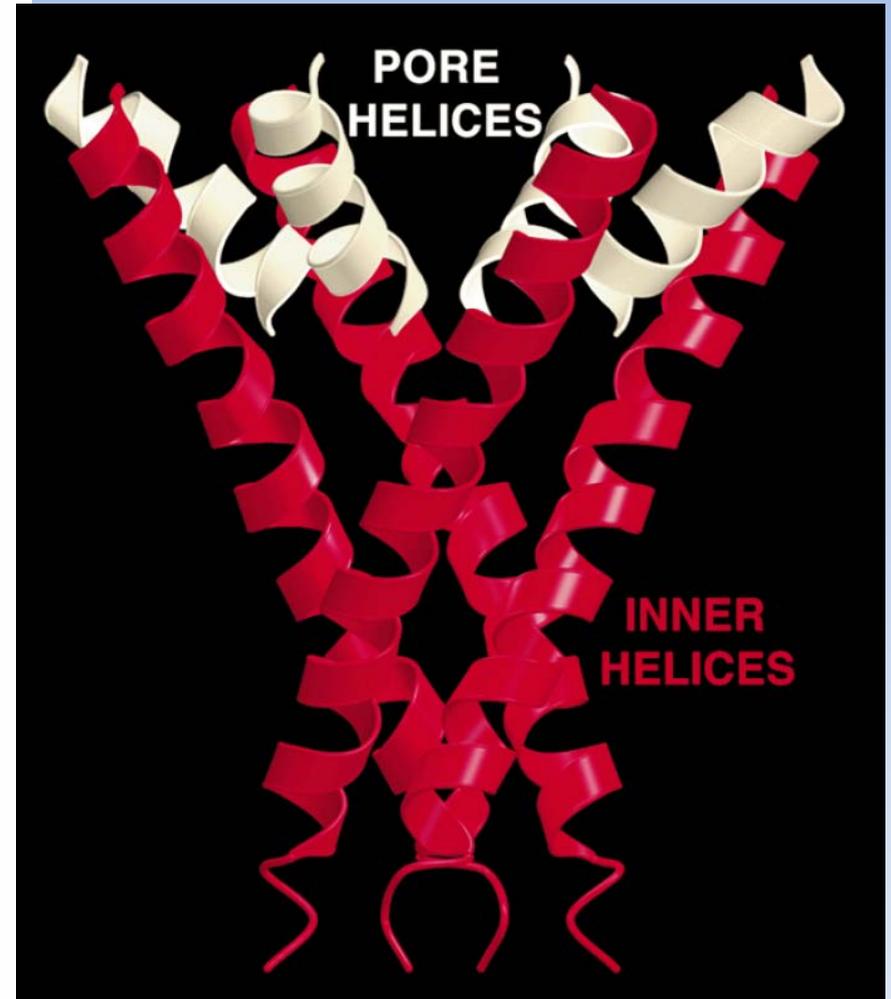


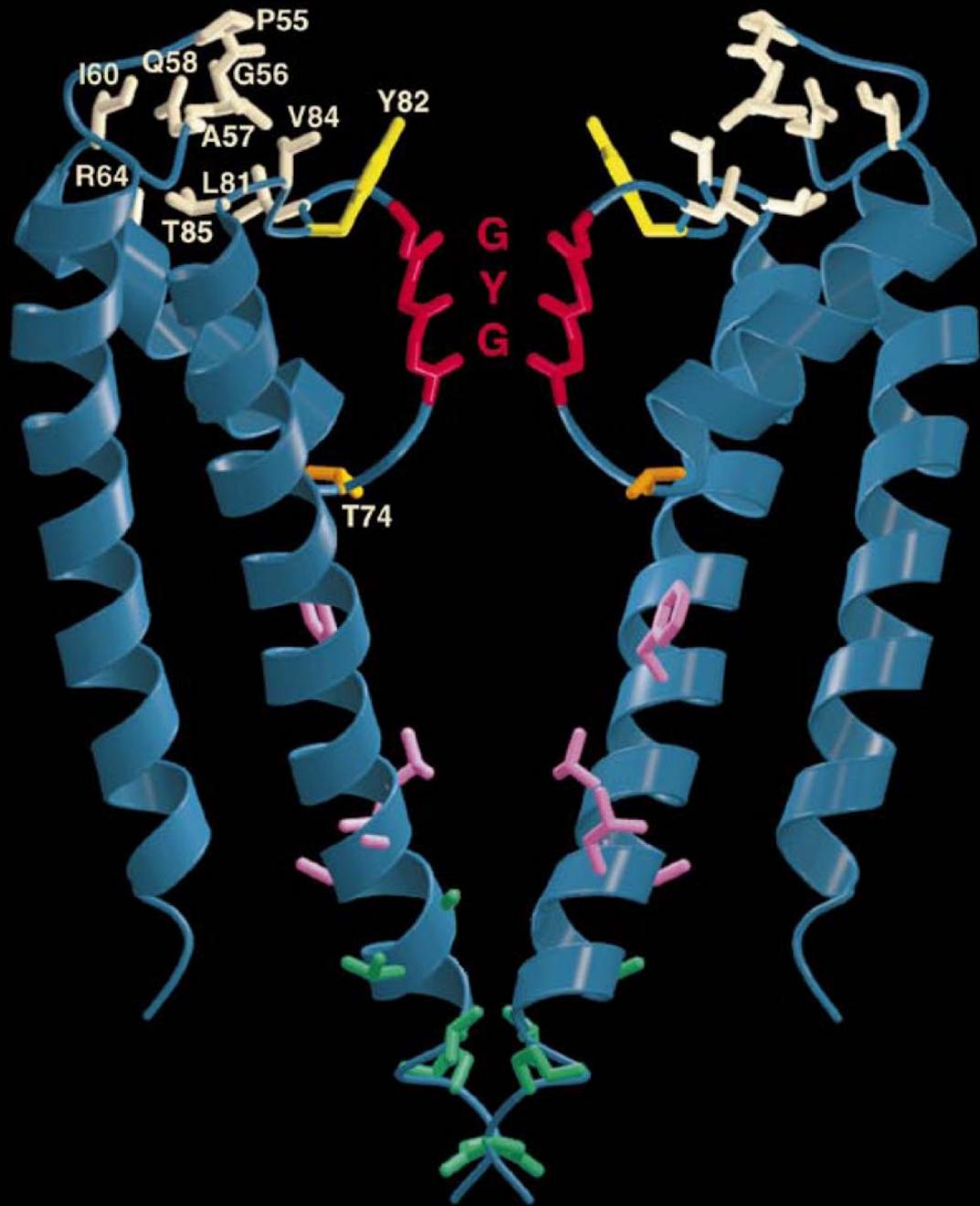
View from extracellular side



Side view – within membrane

Inner helices form “inverted teepee” structure





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

Molecular surface of KcsA and contour of the pore (cutaway view)

Blue

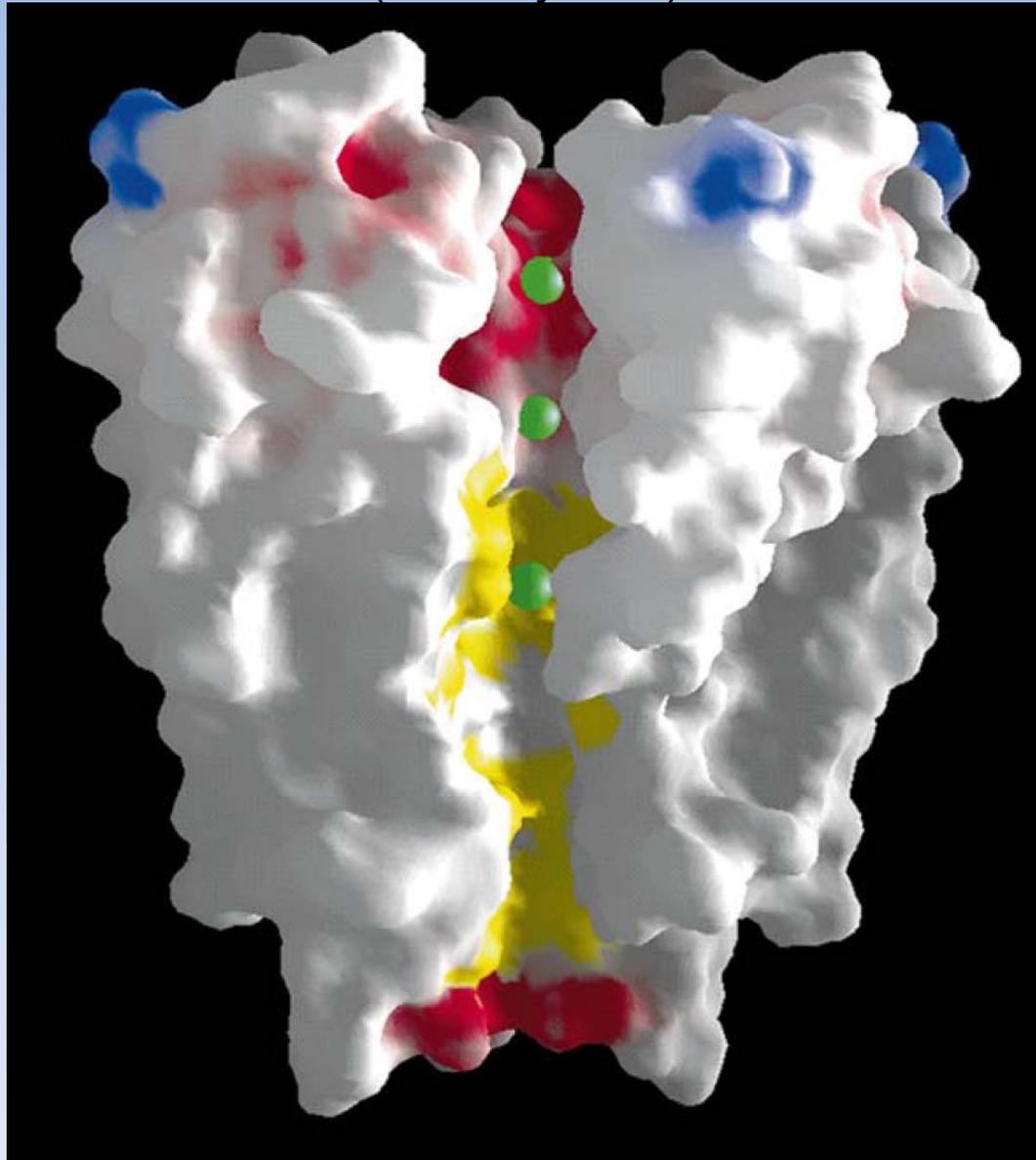
Basic (+)
residues

Yellow:
Hydrophobic
residues

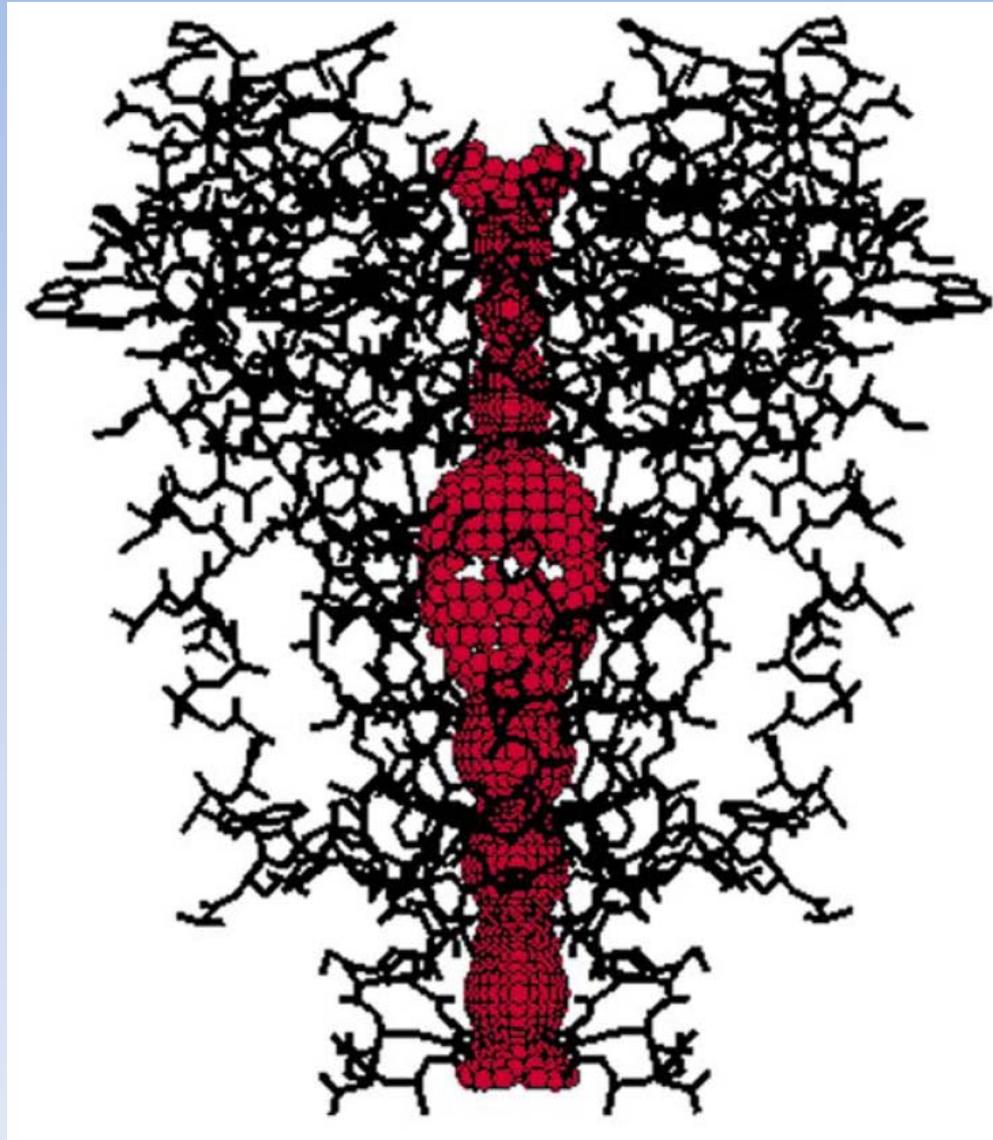
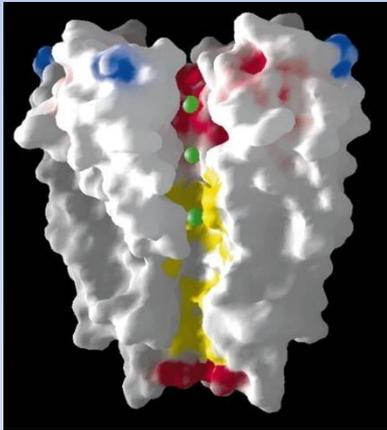
Red:

Acidic (-)
residues

● K⁺



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



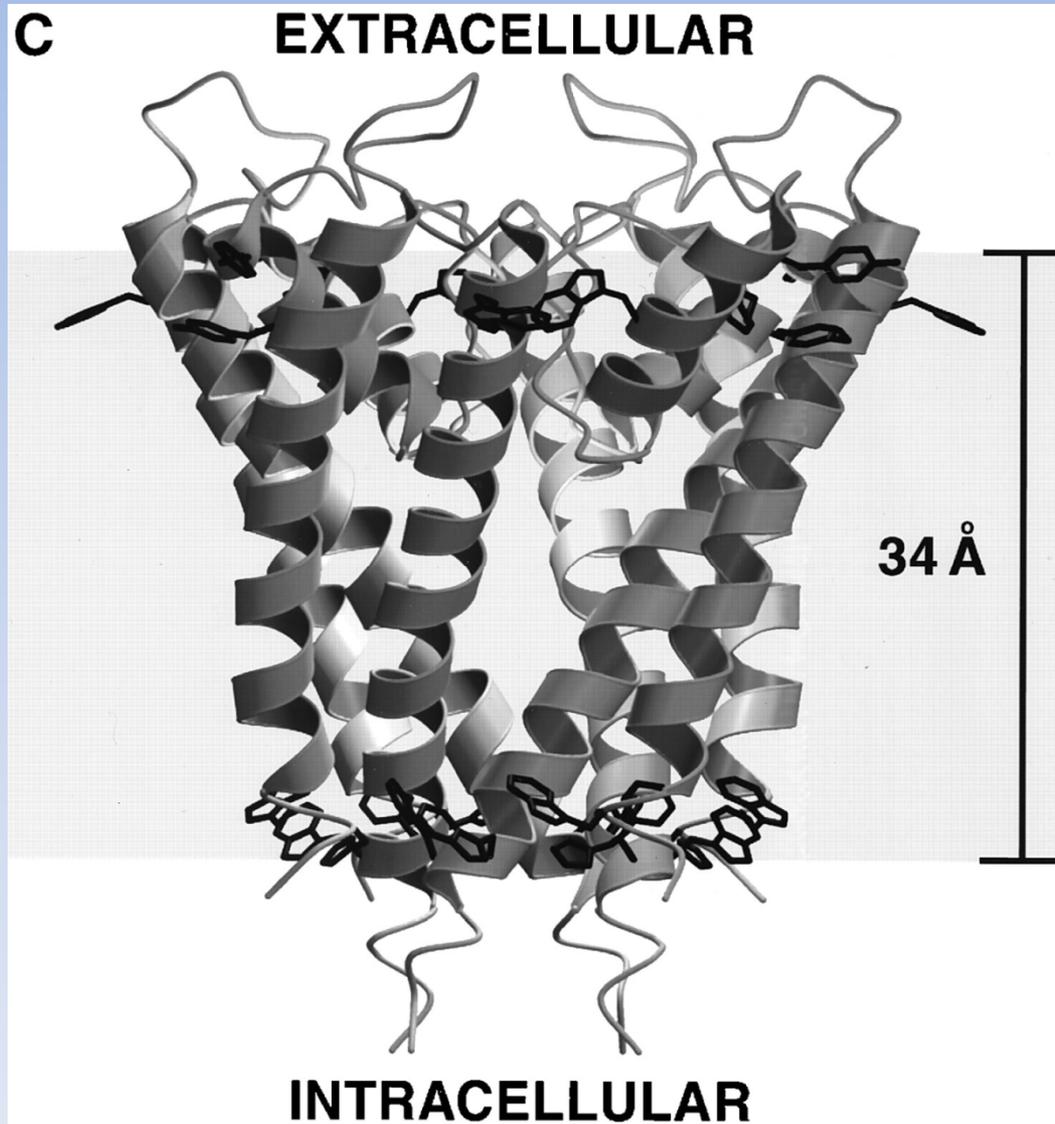
Outer vestibule

Selectivity
Filter

Central cavity

Outer vestibule

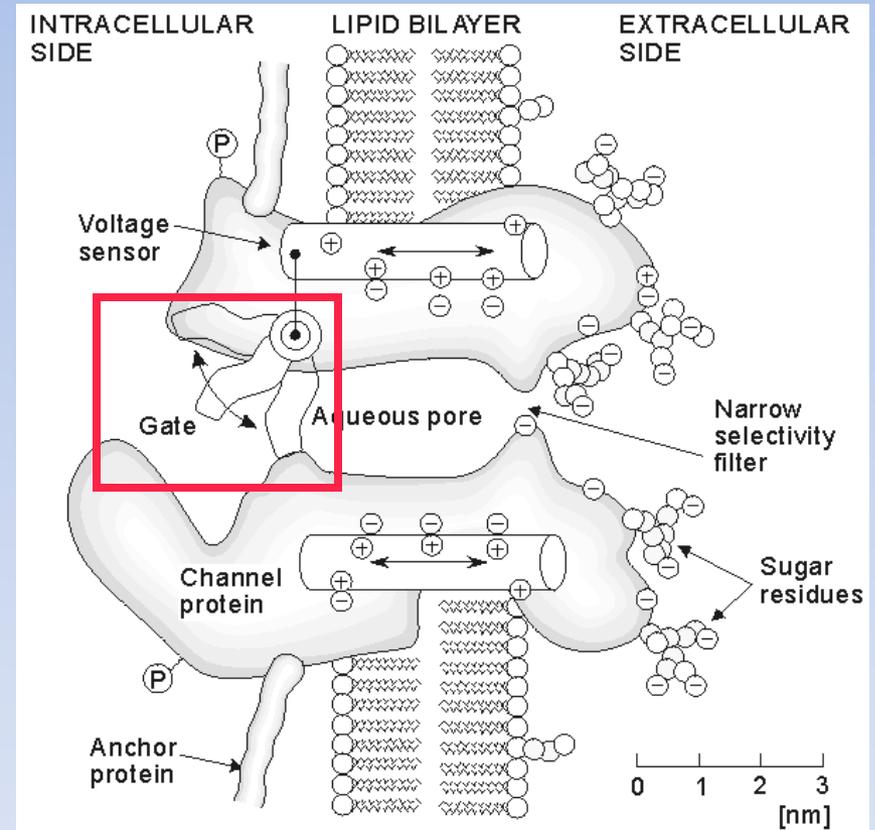
Ring of aromatic amino acids define
the membrane-facing surface



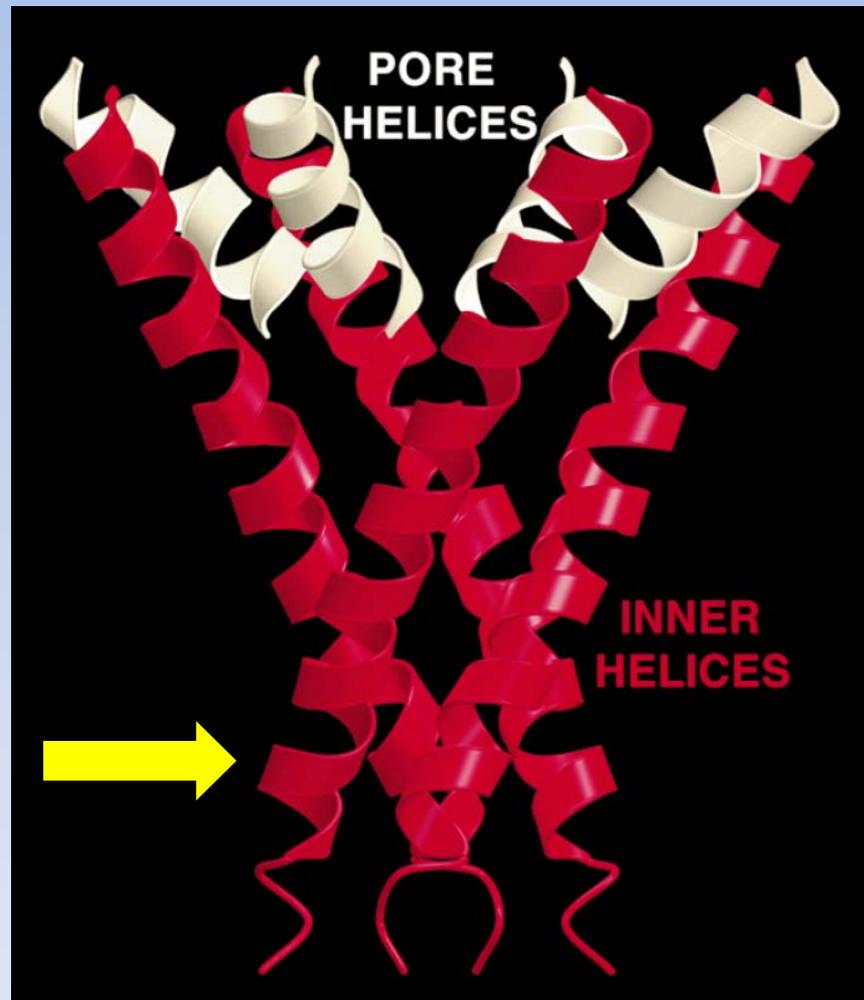
Activation gate

Gates

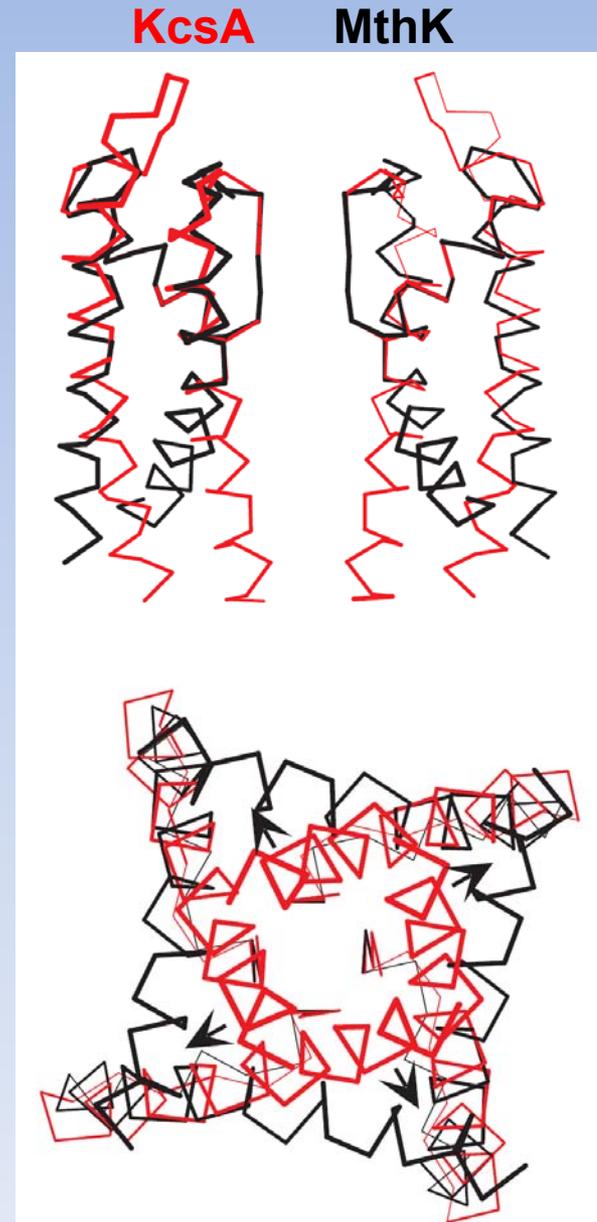
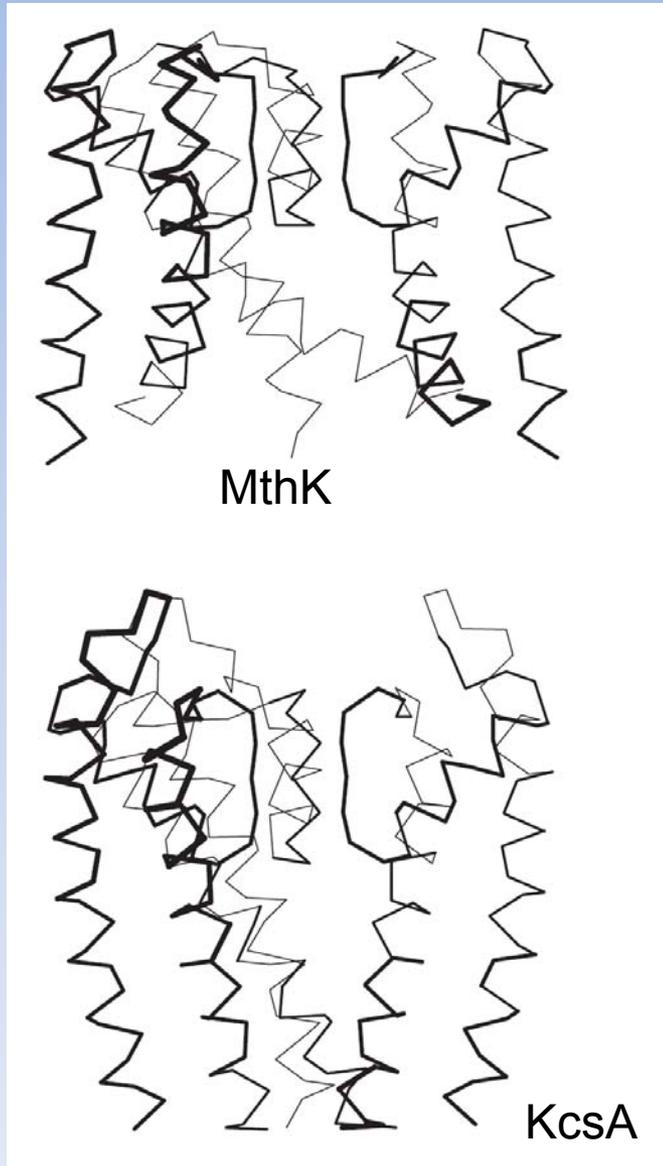
- Activation
- Inactivation



Bundle crossing of inner helices defines the “activation gate”



Open vs closed state of bacterial K channels

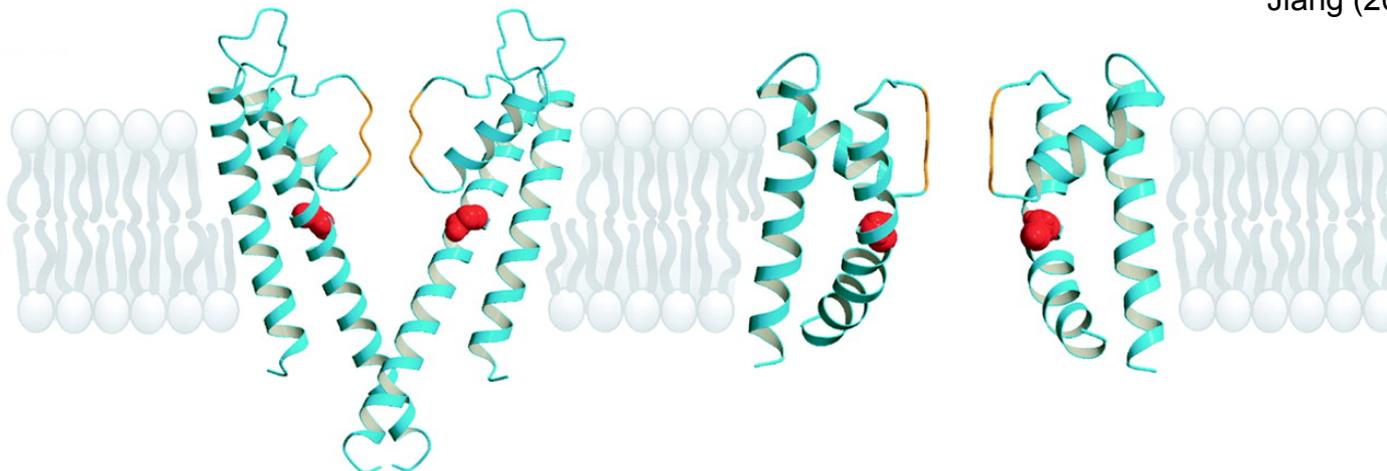


Channel opening: inner helices bend at “glycine hinge”

a

		Filter		Inner helix	
MthK (GI:2622639)	: YWTFVVTIA	TVGYGD	YS--PSTPLGMYFTVTLIVL	GIGTF	AVAVERLLEFLIN
KcsA (GI:2127577)	: WWSVETAT	TVGYGD	LY--PVTWGRLVAVVVMVAG	GITSF	GLVTAALATWFGV
Dradio (GI:6458547)	: YWAVVTVT	TVGYGD	IS--PKTGLGKFIATLAML	GYAII	AVPTGIVTVGLQQ
Ecoli (GI:400124)	: YFSIETMS	TVGYGD	IV--PVSEARLFTISVVIS	GITVF	ATSMTSIFGPLIR
Shaker (GI:85110)	: WWAVVTMT	TVGYGD	MT--PVGFWGKIVGSLCVVA	GVLTI	ALPVPVIVSNFNY
hDRK1 (GI:345875)	: WWATITMT	TVGYGD	IY--PKTLLGKIVGGLCCIA	GVLVI	ALPIPIIVNNFSE
hBK (GI:2570854)	: YLLMVTMS	TVGYGD	VY--AKTTLGRLFMVFFILG	GLAMF	ASYVPEIIEELIGN
hSK3 (GI:15983750)	: WLISITFL	SIGYGD	MV--PHTYCGKGVCLLTGIM	GAGCT	ALVVAVVARKLEL
hERG2 (GI:14745363)	: YFTFSSLT	SVGFGN	VS--PNTNSEKIFSICVMLI	GSLMY	ASIFGNVSAIIQR
hGIRK2 (GI:1352487)	: LFSIETET	TIGYGY	RVITDKCPEGIILLLIQSVL	GSIVN	AFMVGCMFVKISQ
hIRK1 (GI:2460307)	: LFSIETQT	TIGYGF	RCVTDECPIAVFMVVFQSI	VGCID	AFIIGAVMAKMAK
bcNG1 (GI:231739)	: YWSTLTLT	TIG--E	TPP-PVRDSEYFFVVADFLI	GVLIF	ATIVGNI GSMISN

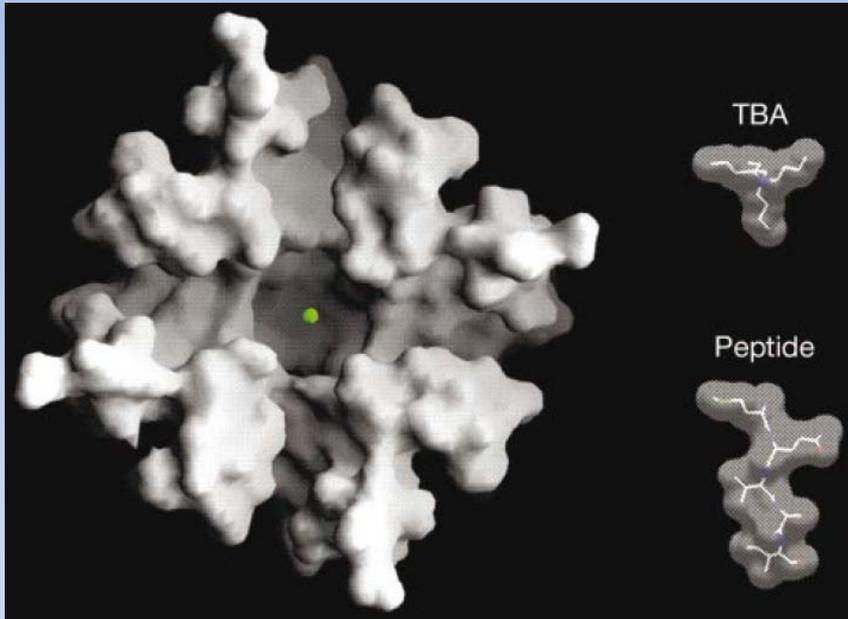
Jiang (2002) Nature **417**: 523



KcsA K⁺ channel
"closed"

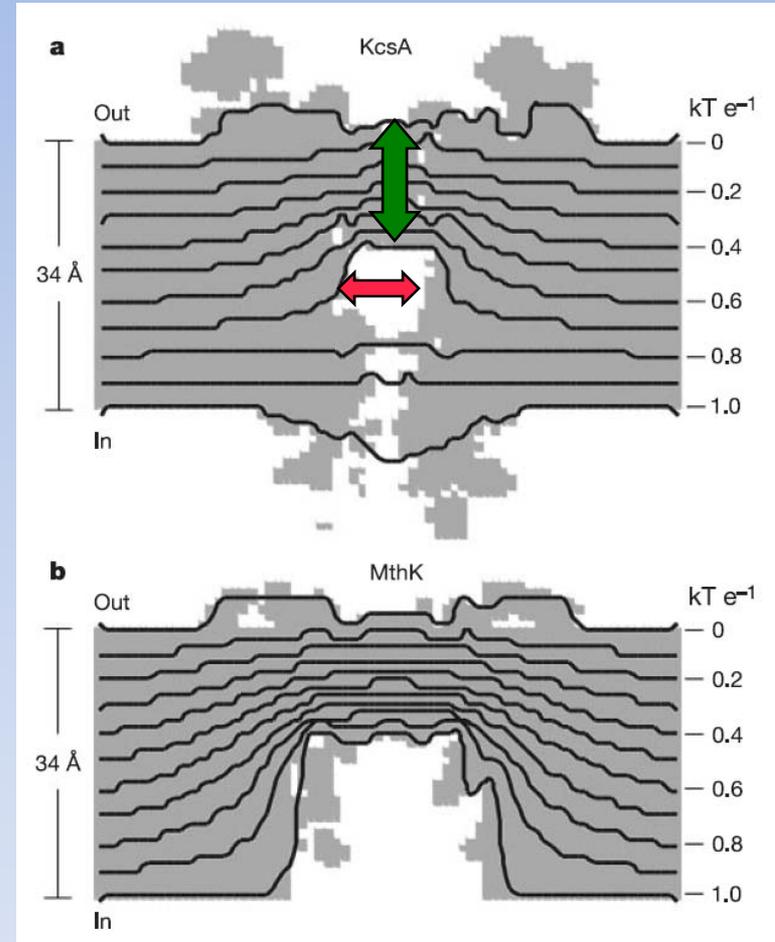
MthK K⁺ channel
"open"

Molecular surface of the MthK pore viewed from the intracellular solution



12 Å

10 Å

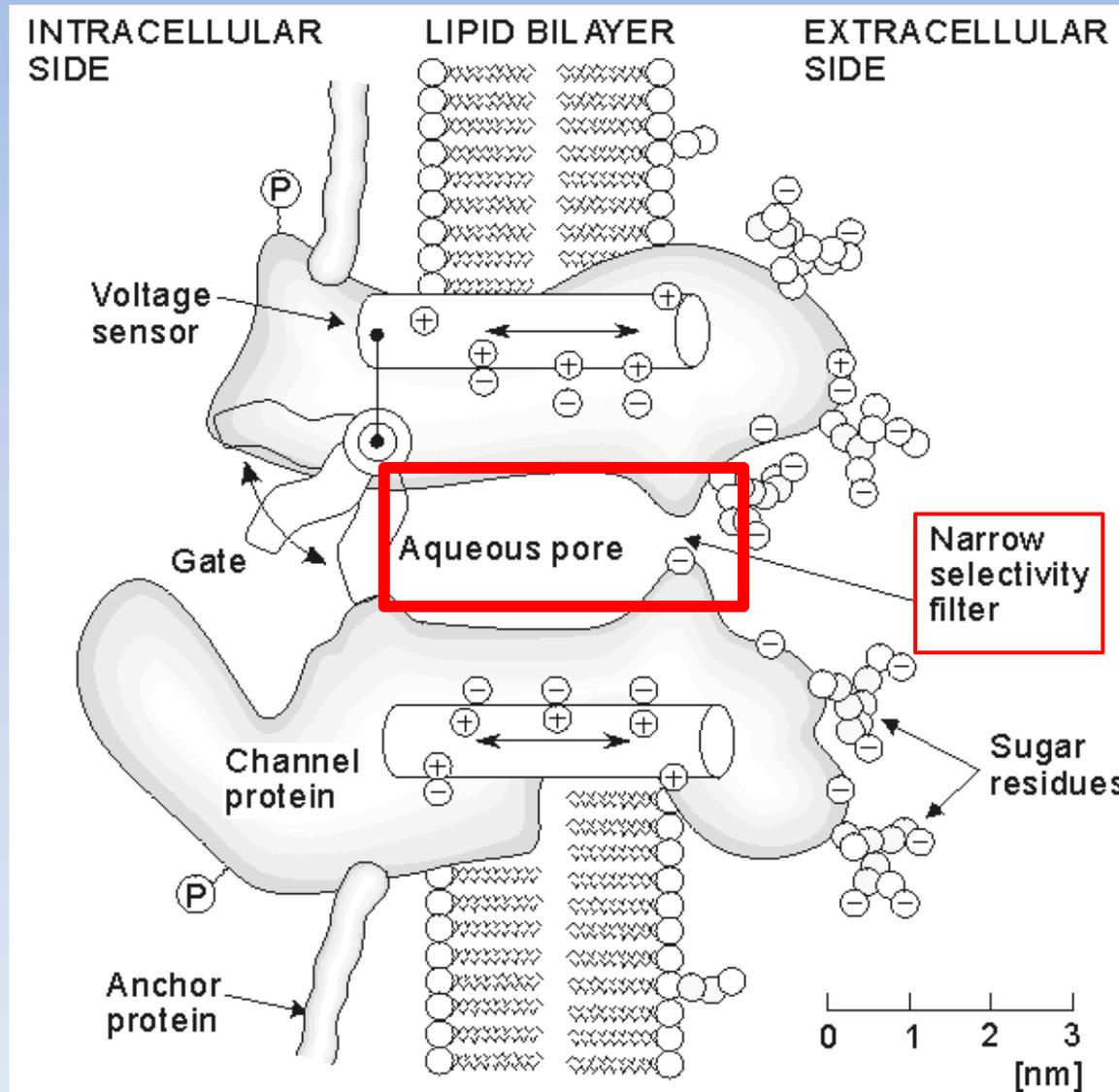


The membrane electric potential across the pore changes on opening. Electrostatic contour plots for KcsA (a) and MthK (b) in a membrane.

grey region: protein or membrane (dielectric constant 2)
white regions: aqueous solution (dielectric constant 80)

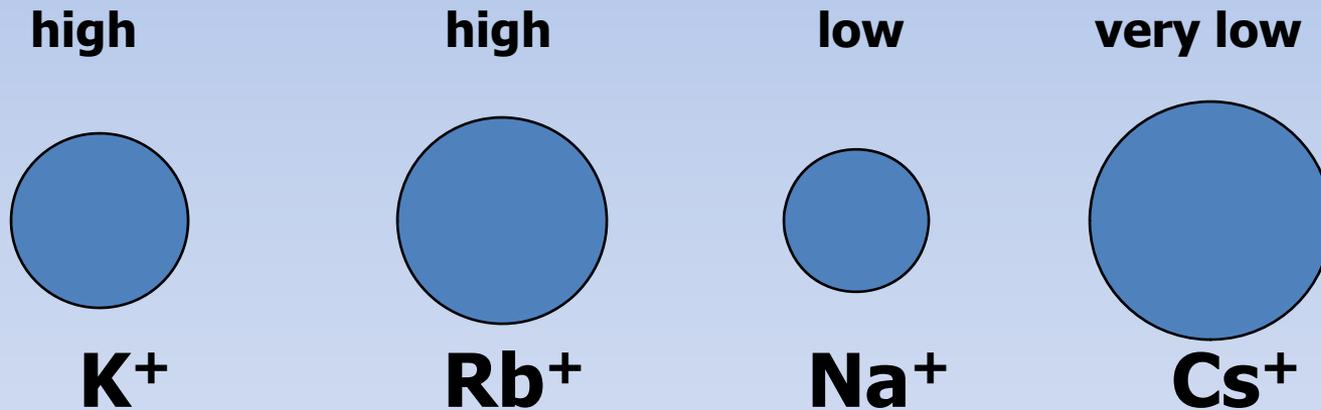
Ion Selectivity

Selectivity filter



Selective ion permeability

Permeability in K channels



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

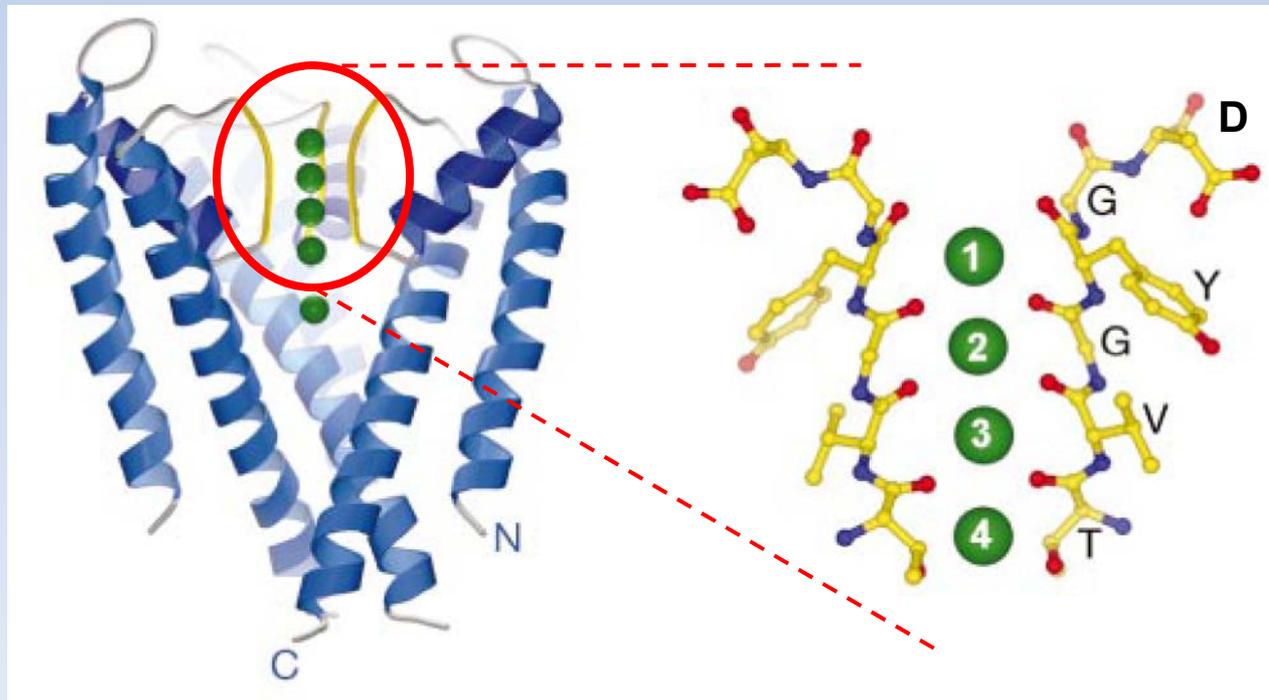
Apparent dilemma solved by x-ray crystallography of a bacterial K-selective channel, KcsA

Selectivity filter of KcsA channel

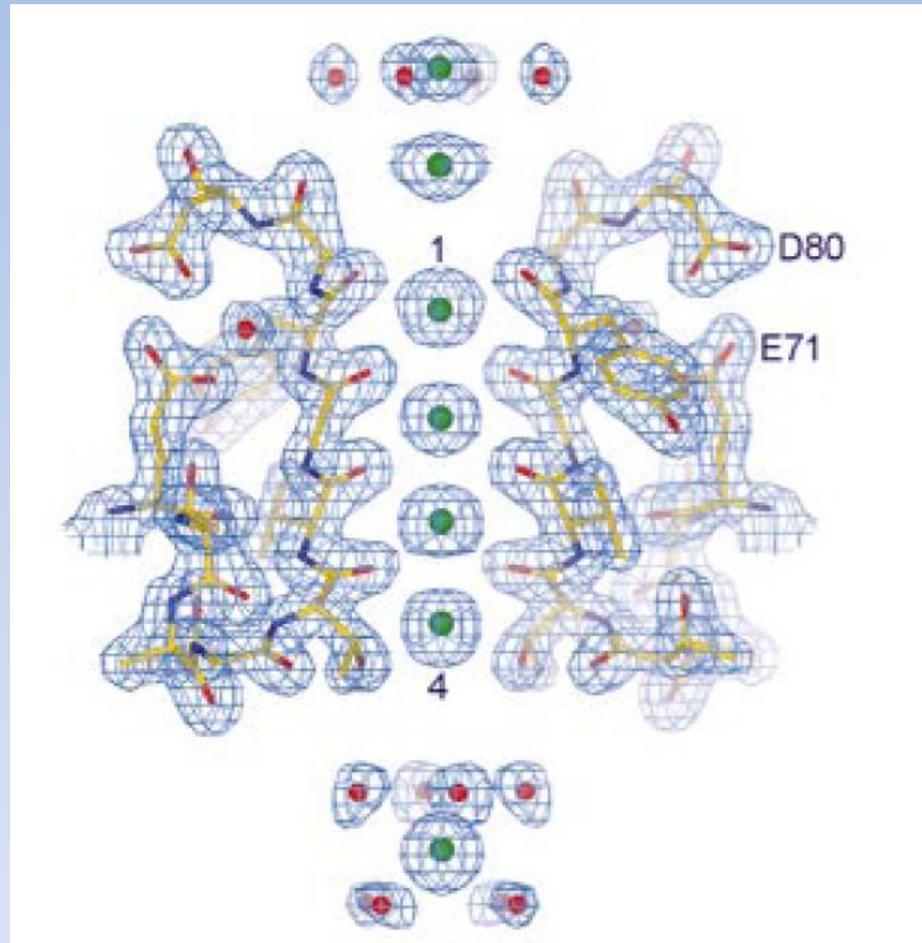
a

	Filter	Inner helix
MthK (GI:2622639)	: YWTFVVTIA TVGYGDYS--PSTPLGMYFTVTLIVL	GIGTF
KcsA (GI:2127577)	: WWSVETAT TVGYGDLY--PVTLLWGRLVAVVVMVA	GITSF
Dradio (GI:6458547)	: YWAVVTVT TVGYGDIS--PKTGLGKFIATLAML	GYAII
Ecoli (GI:400124)	: YFSIETMS TVGYGDIV--PVSESARLFTISVVIIS	GITVF
Shaker (GI:85110)	: WWAVVTMT TVGYGDMT--PVGFWGKIVGSLCVVA	GVLTI
hDRK1 (GI:345875)	: WWATITMT TVGYGDIY--PKTLLGKIVGGLCCIA	GVLVI
hBK (GI:2570854)	: YLLMVTMS TVGYGDVY--AKTTLGRLFMVFFIL	GLAMF

● K⁺



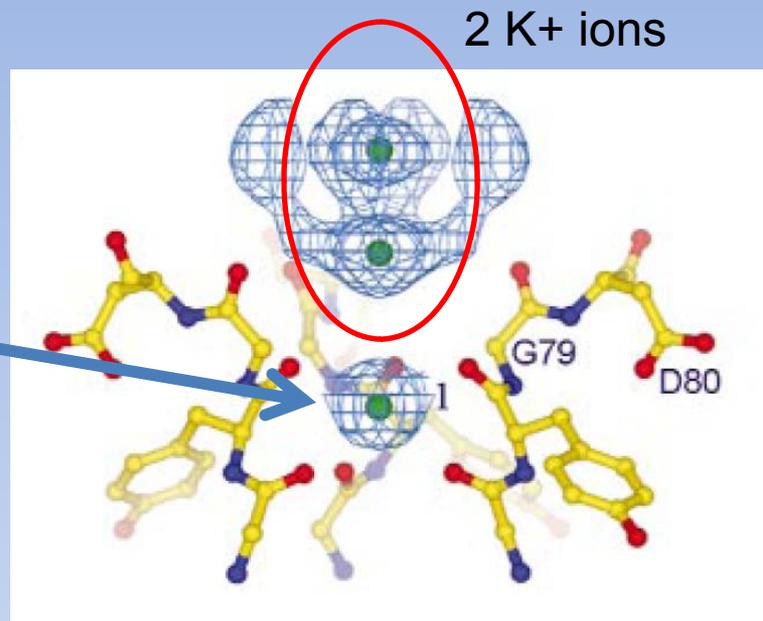
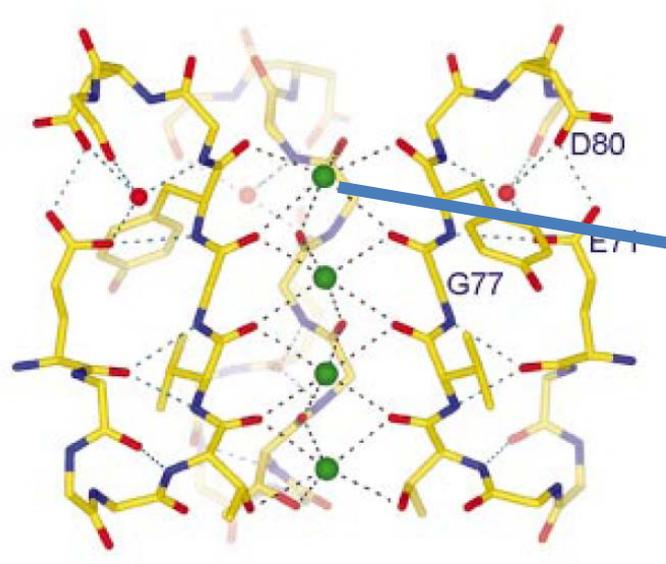
K⁺ ions inside the filter are dehydrated



● H₂O

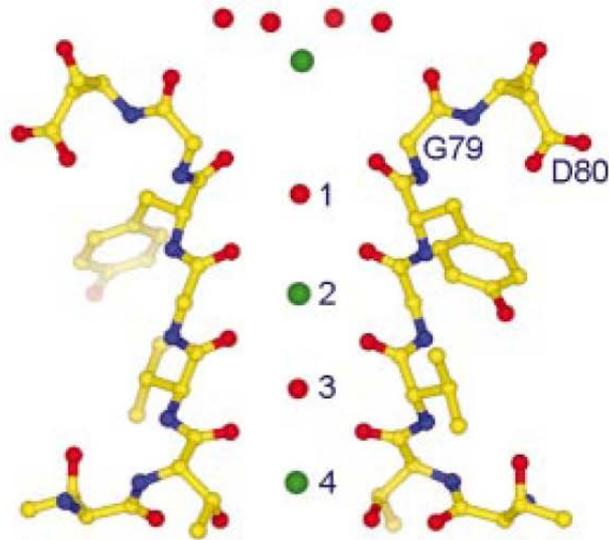
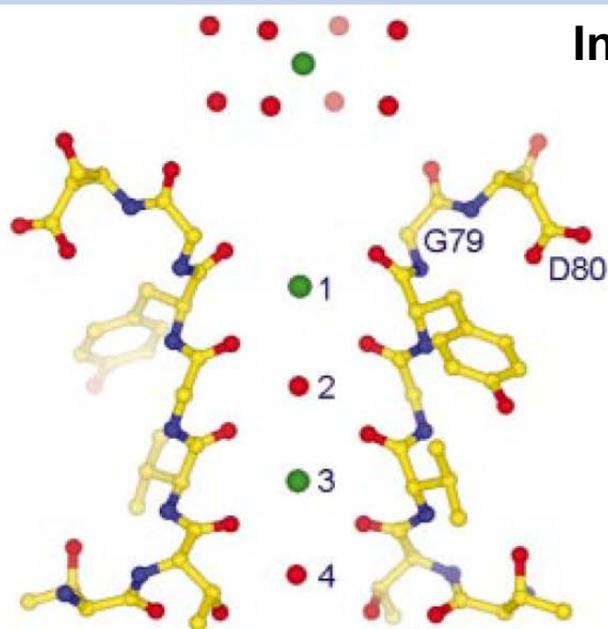
● K⁺

K ion dehydration at the extracellular pore entryway and ion hopping



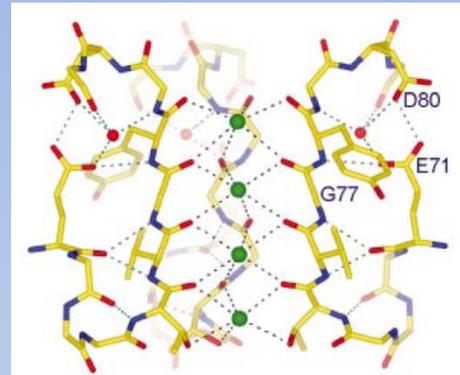
2 K⁺ ions

Interpretation: two states



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

High K structure
(conducting)

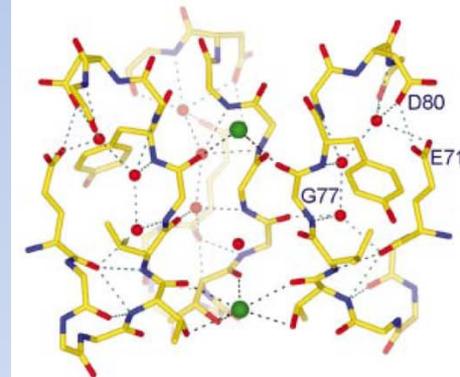


1,3

or

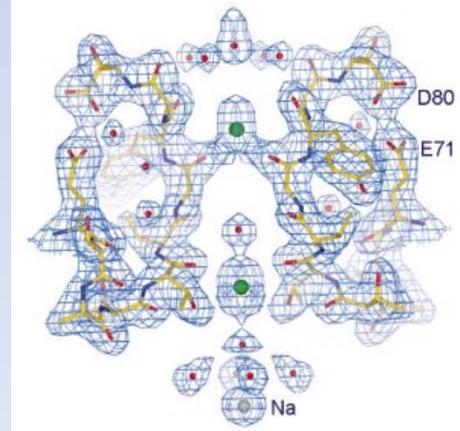
2,4

Low K structure
(nonconducting)

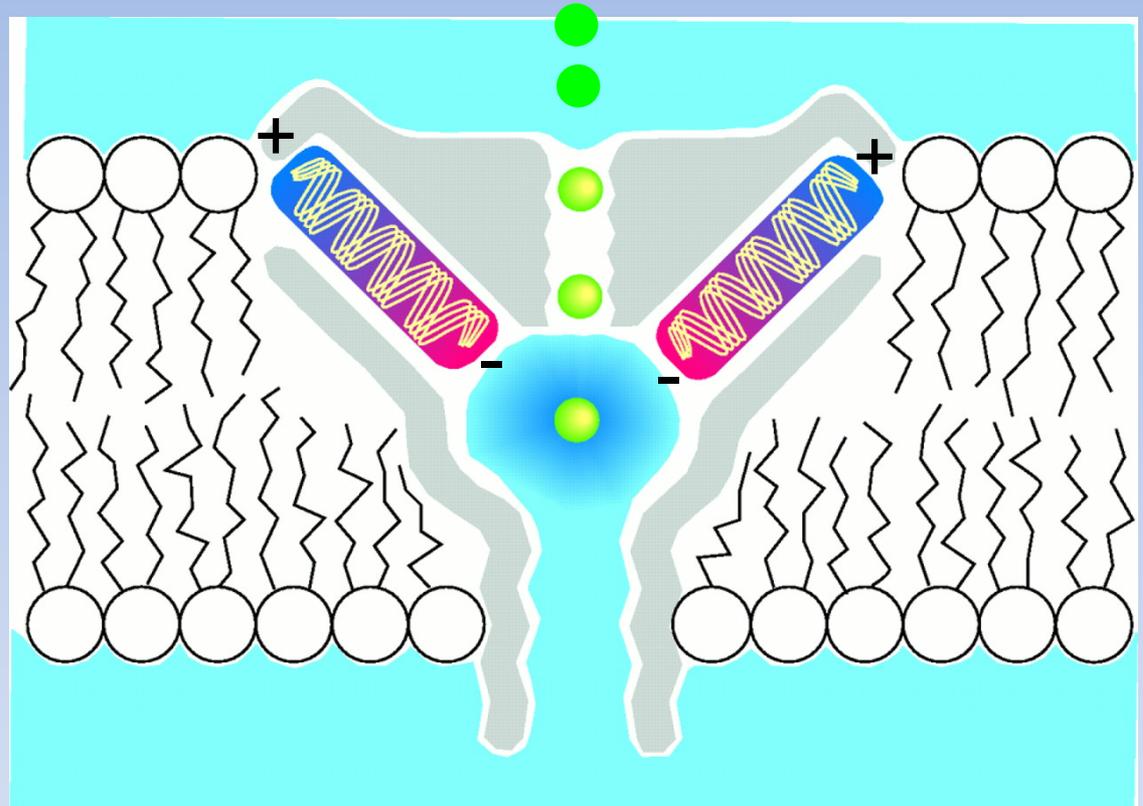
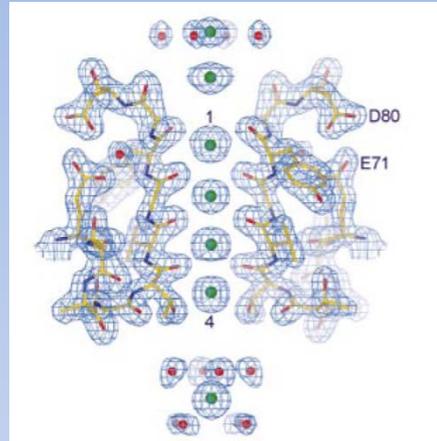


1,4

Electron density
map in low K

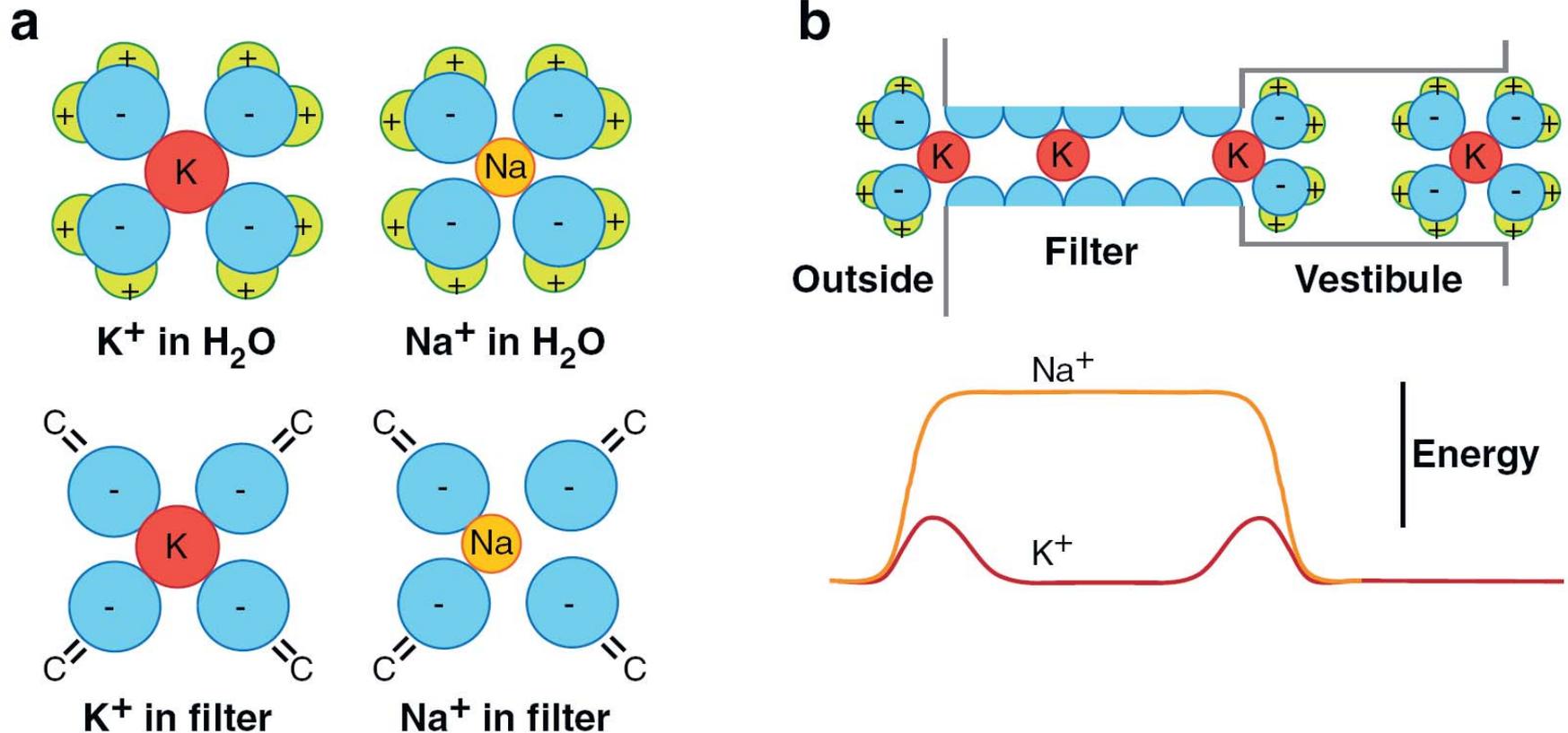


Two mechanisms by which K^+ channel stabilizes a cation in the middle of the central cavity



- (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

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

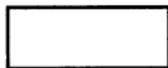
- High selectivity:
 - Multiple ion occupancy: optimized geometry of K binding sites (1,3/2,4) in the narrow selectivity filter (customized oxygen cages)
- High permeation: electrostatic repulsion between adjacent K ions (4 M equivalent local concentration)
- A central cavity that is lined by hydrophobic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

Ion selectivity in Na and Ca channels

K channel (Sh B)		▽	T	M	T	T	V	□	G	Y	G	D	I	▽	Y	
Human cardiac Na channel	I II III IV	[I	R	L	M	T	Q	D	⊙	W	E	R			
			II	R	I	L	C	G	E	W	I	E	T			
			III	Q	V	A	T	F	⬠	K	G	W	M	D		
			IV	Q	I	T	T	S	A	G	W	D	G			
Rabbit skeletal muscle Ca channel	I II III IV	[I	Q	C	I	T	M	E	G	W	T	D			
			II	Q	V	L	T	G	E	D	W	N	S			
			III	T	V	S	T	F	E	G	W	P	Q			
			IV	R	C	A	T	G	E	A	W	Q	E			



TEA binding sites in K channel



Putative selectivity filter / Ca²⁺ binding site



Isoform-specific TTX/Cd²⁺ sensitivity

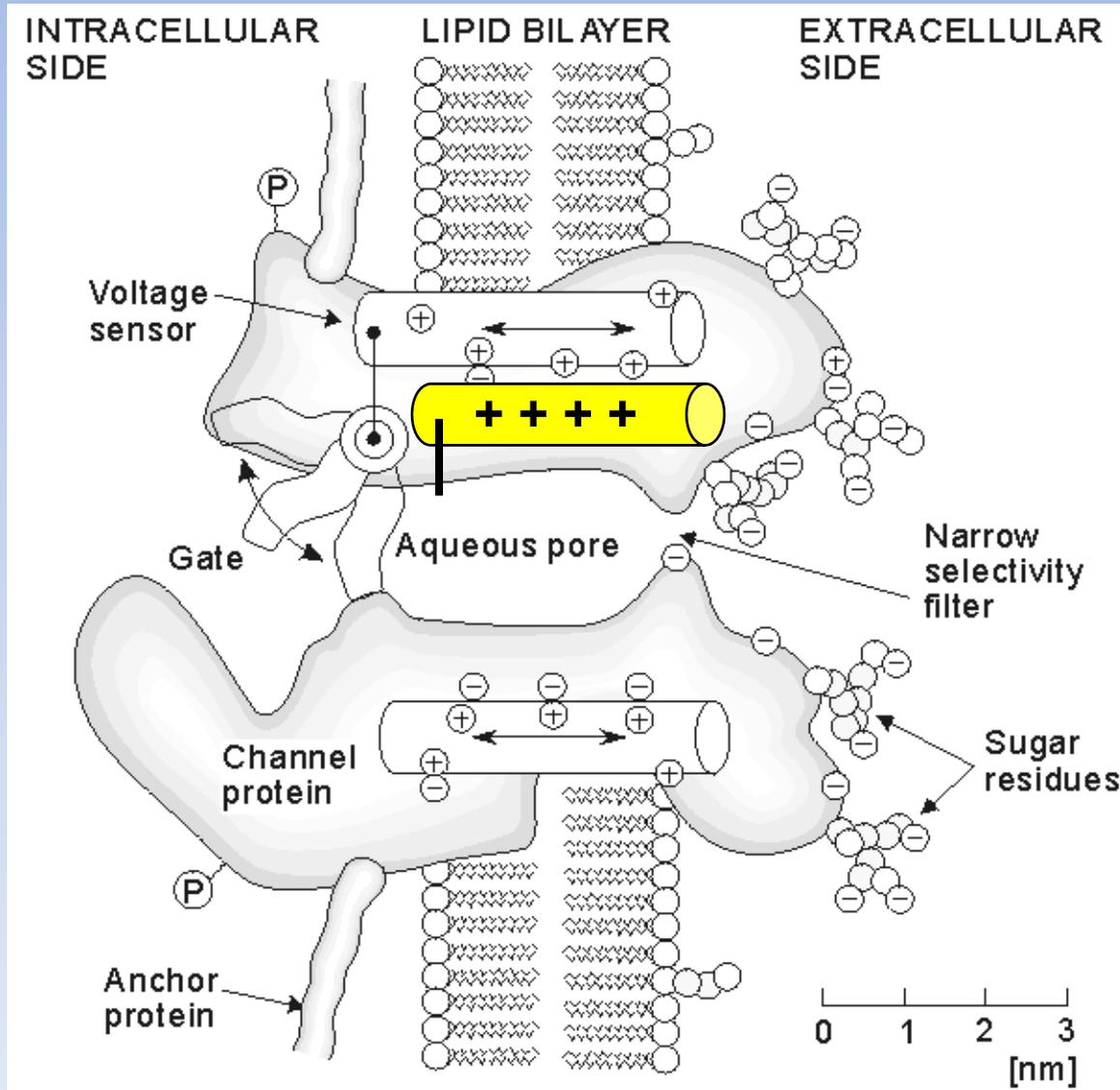


Na⁺/Ca²⁺ permeability

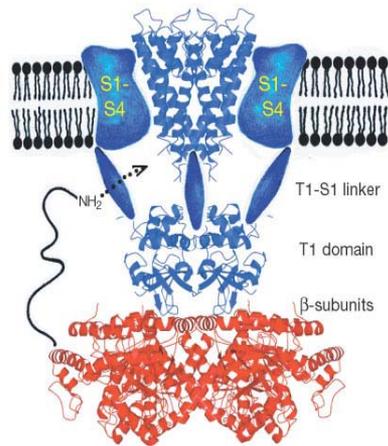
Voltage sensing

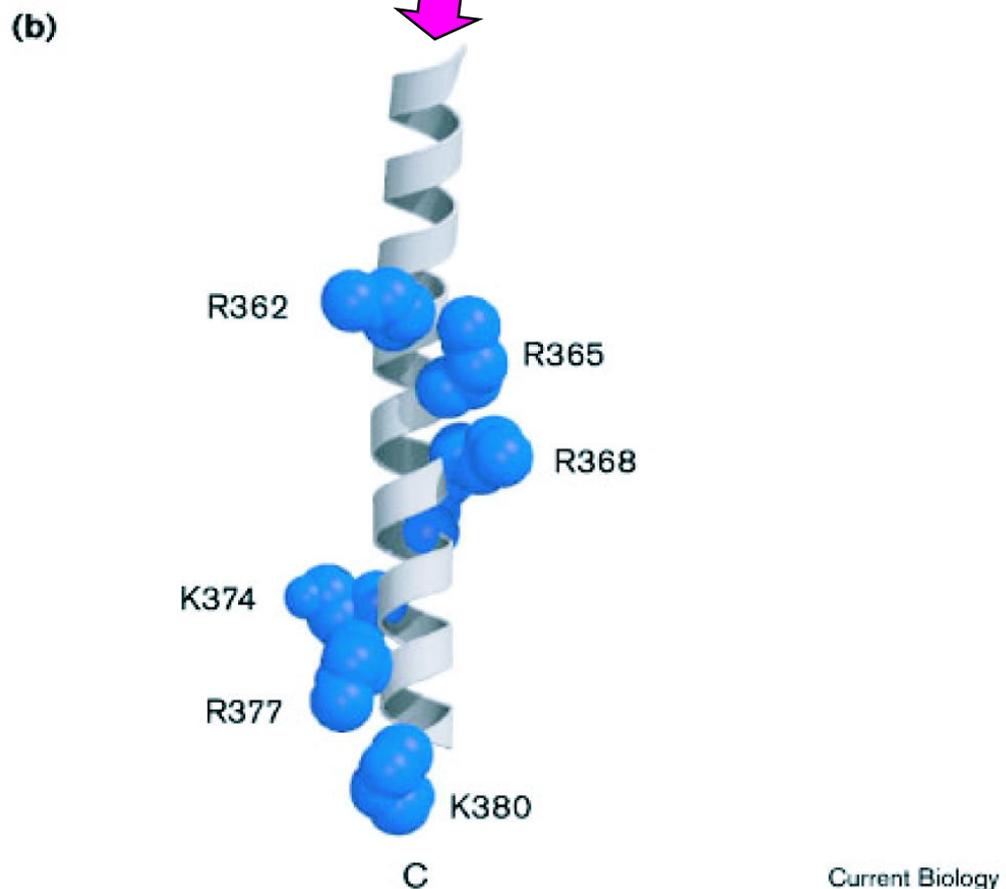
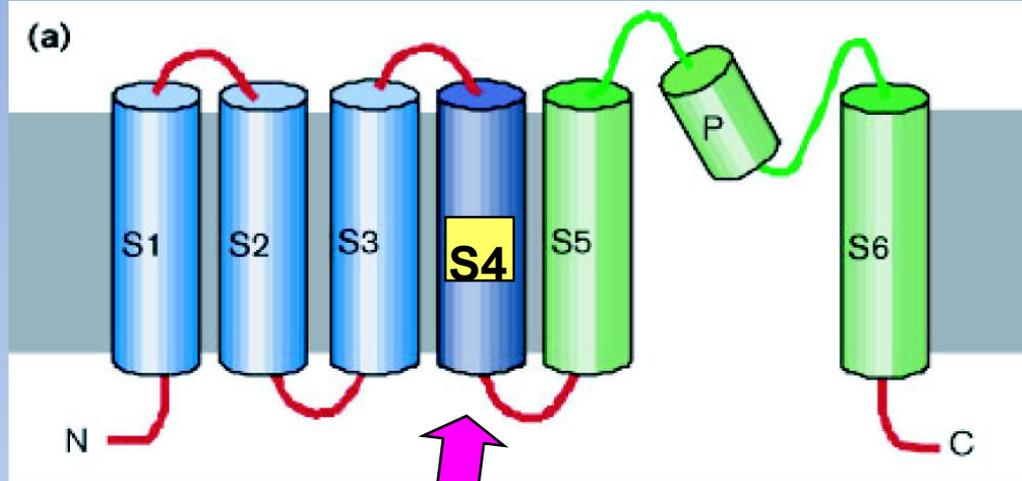
VSD: the voltage sensor domain

Voltage sensor

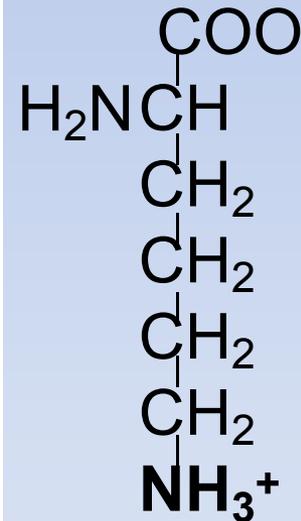


Voltage-gated ion channel = pore domain + VSD

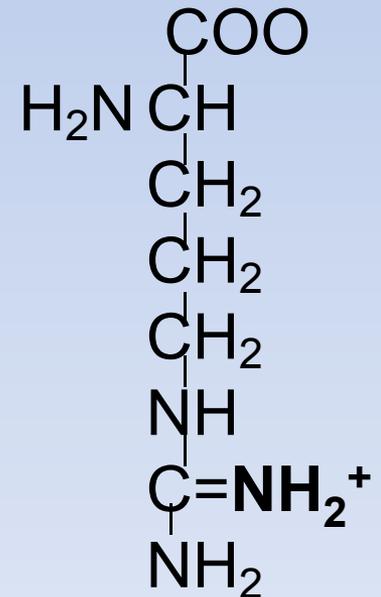




S4 domain is the primary voltage sensor



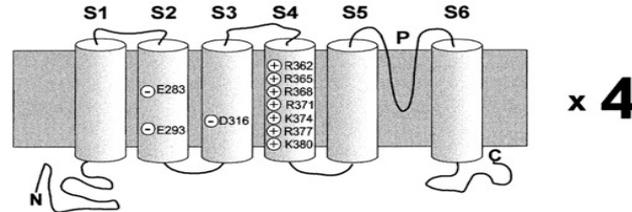
Lysine (K)



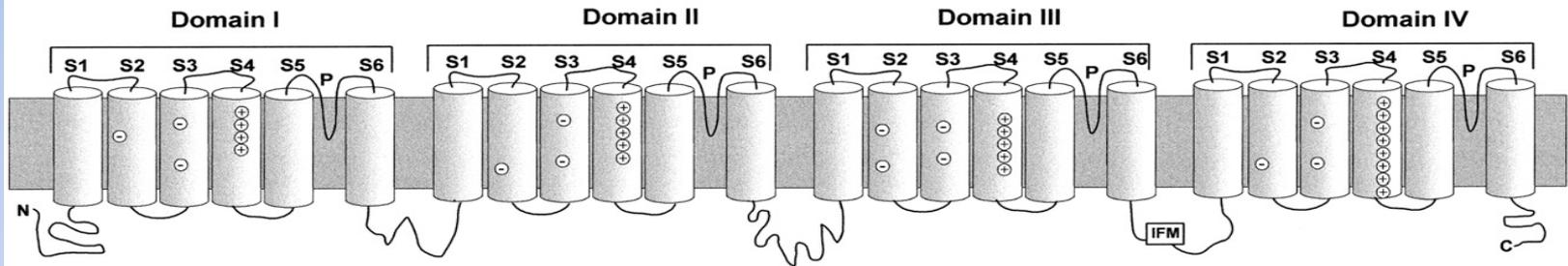
Arginine (R)

S4 domains from different channels are similar but not identical

ShakerB



hSkM1



ShakerB S4 362 365 368 371 374 377 380
 A I L R V I R L V R V F R I F K L S R H S K

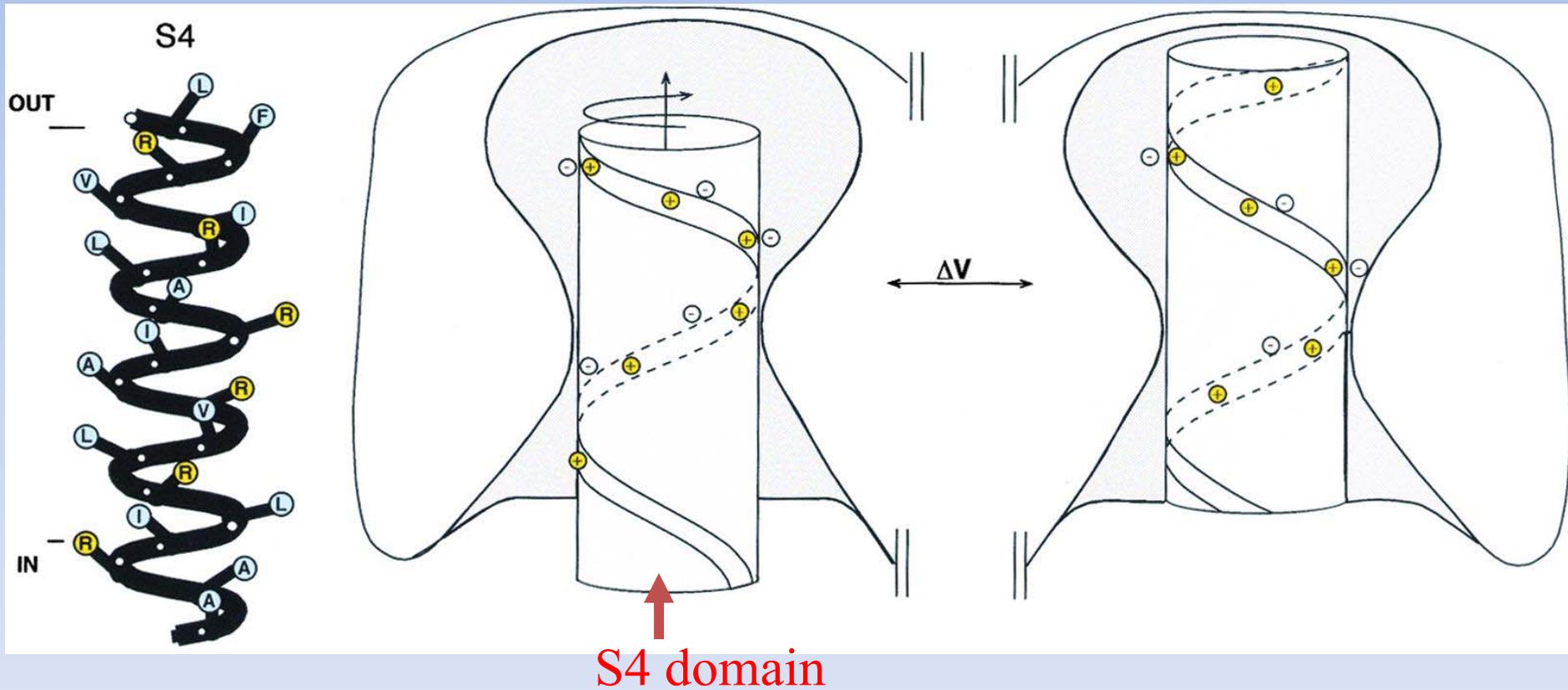
hSkM1 S4 DI 219
 A L R Y F R V L R A L K T I T V I P G L K T

hSkM1 S4 DII 669
 V L R S F R L L R V F K L A K S W P T L N

hSkM1 S4 DIII 1126
 L G P I K S L R T L R A L R P L R A L S R

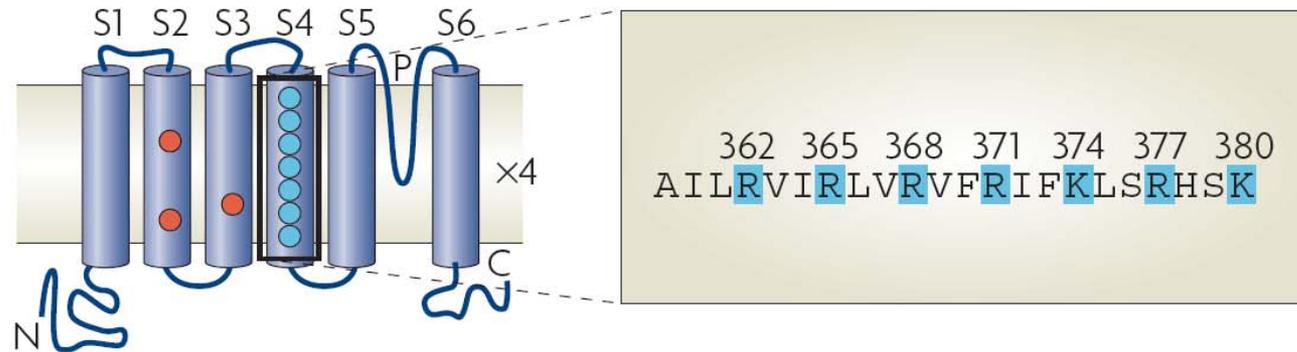
hSkM1 S4 DIV 1448
 F R V I R L A R I G R V L R L I R G A K G I R

Helical screw motion model of voltage sensor

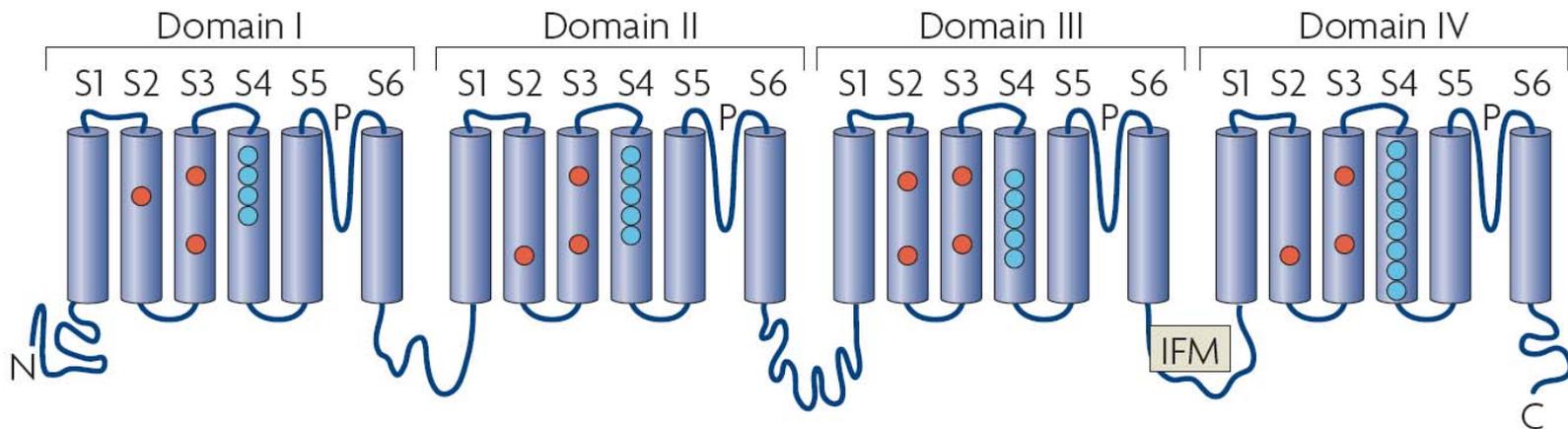


VSD = S2/S3/S4

a Shaker B



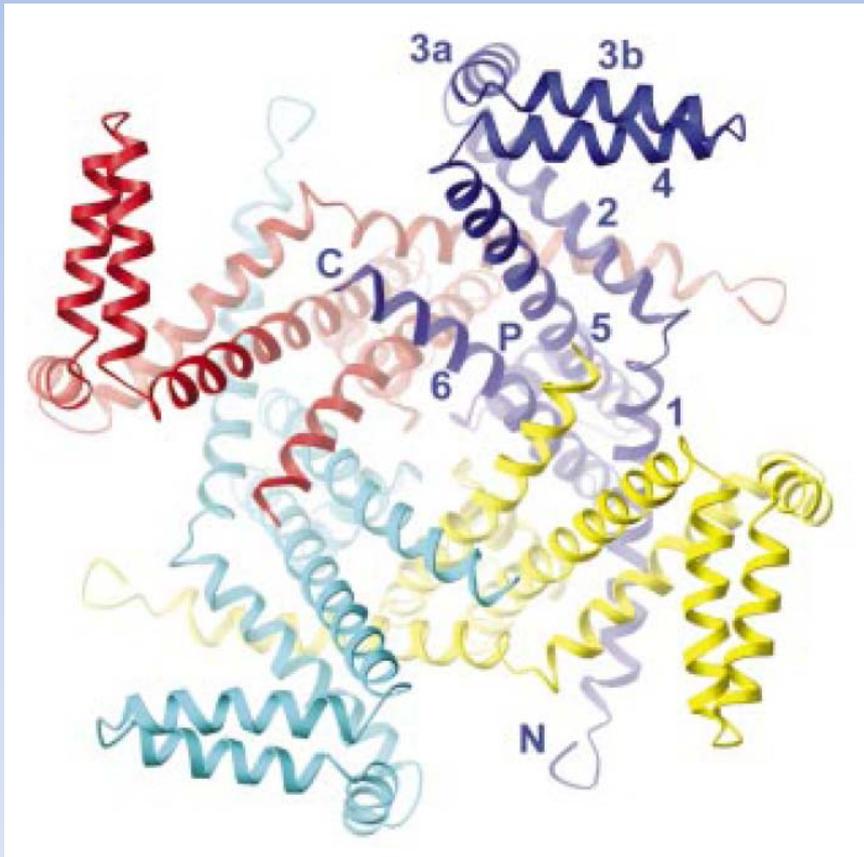
b Nav1.4



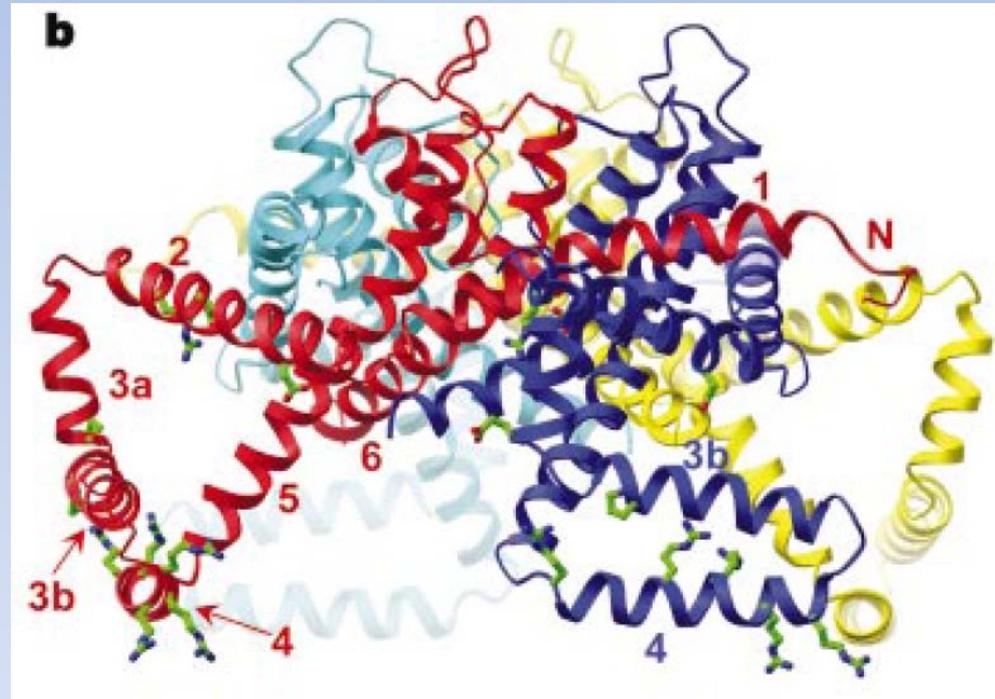
- Salt bridge forms between acidic residues in S2/S3 (red spheres) and basic residues of S4 (blue spheres)
- Consistent with helical screw motion

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

(from thermophilic archaebacteria, *Aeropyrum pernix*)



Top view

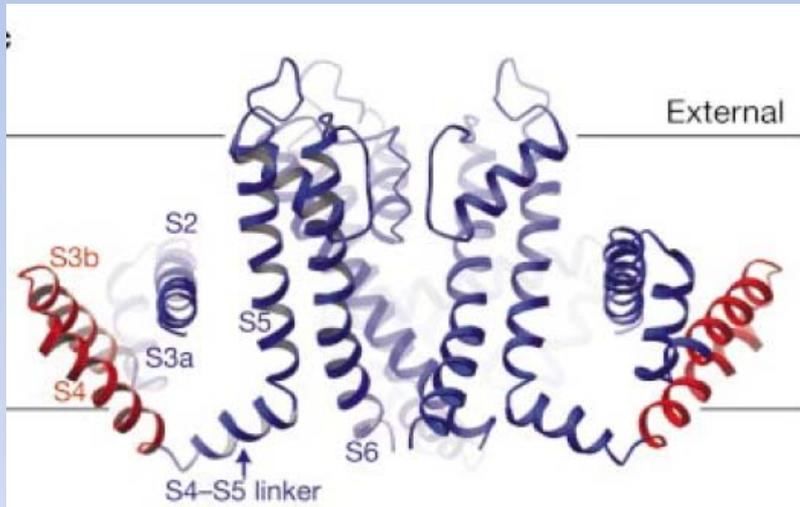


side view

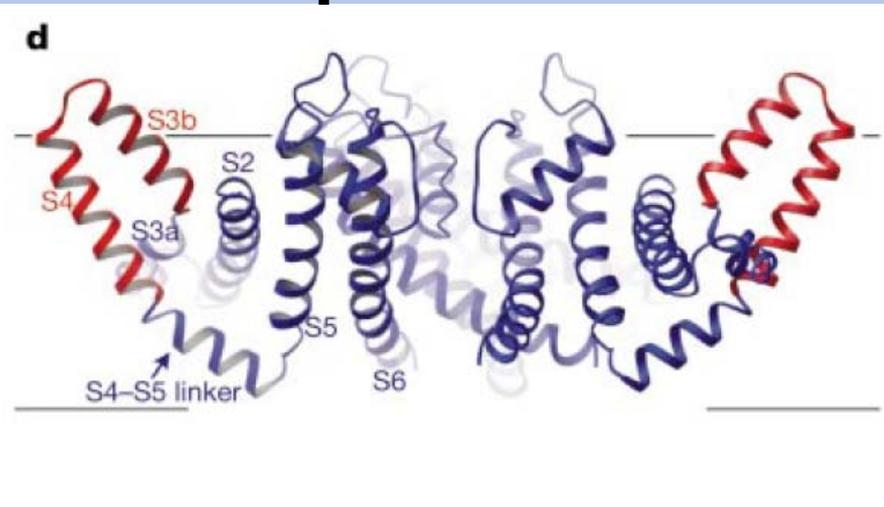
KvAP channel

“Paddle” model of voltage sensor movement

Closed

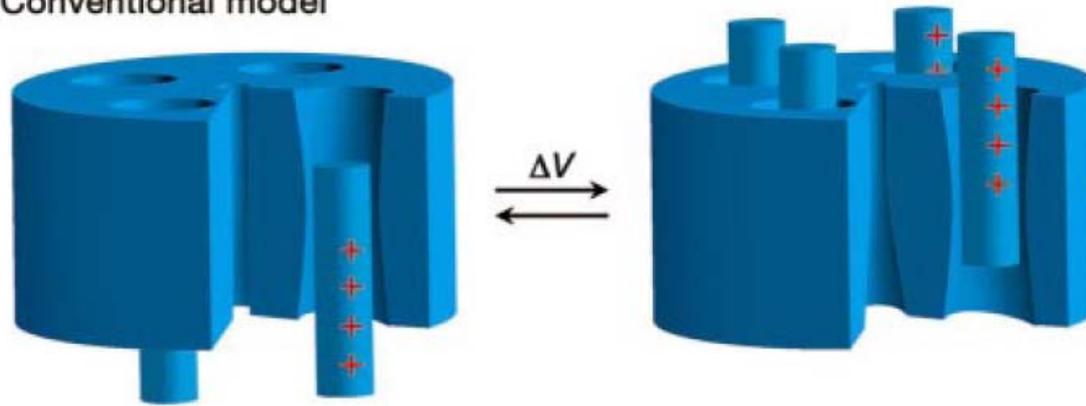


Open



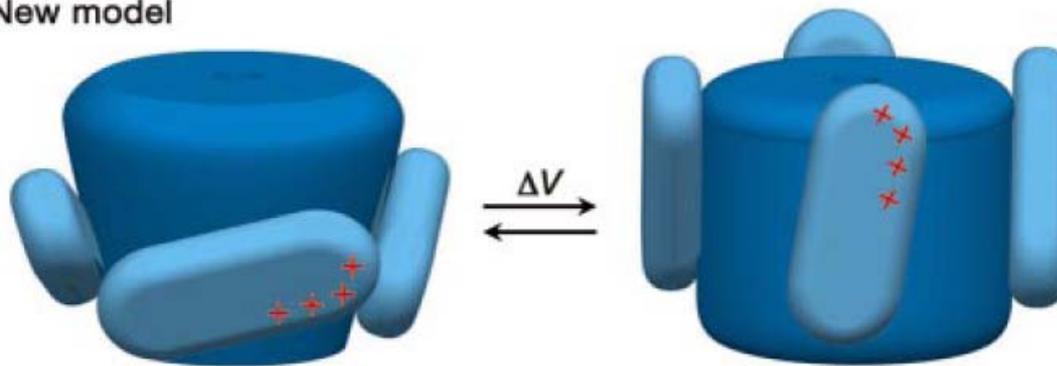
controversy

a Conventional model



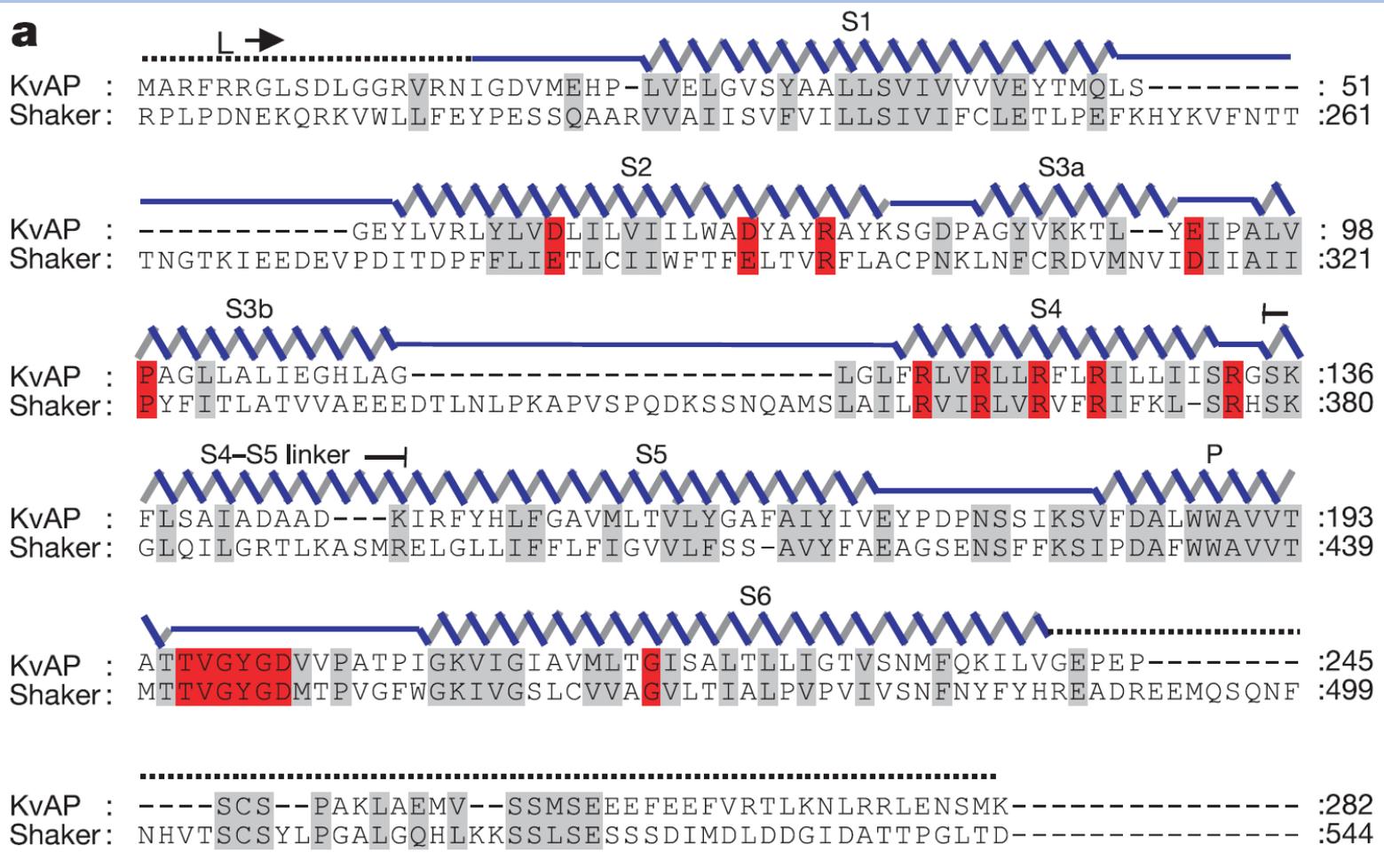
Shaker

b New model



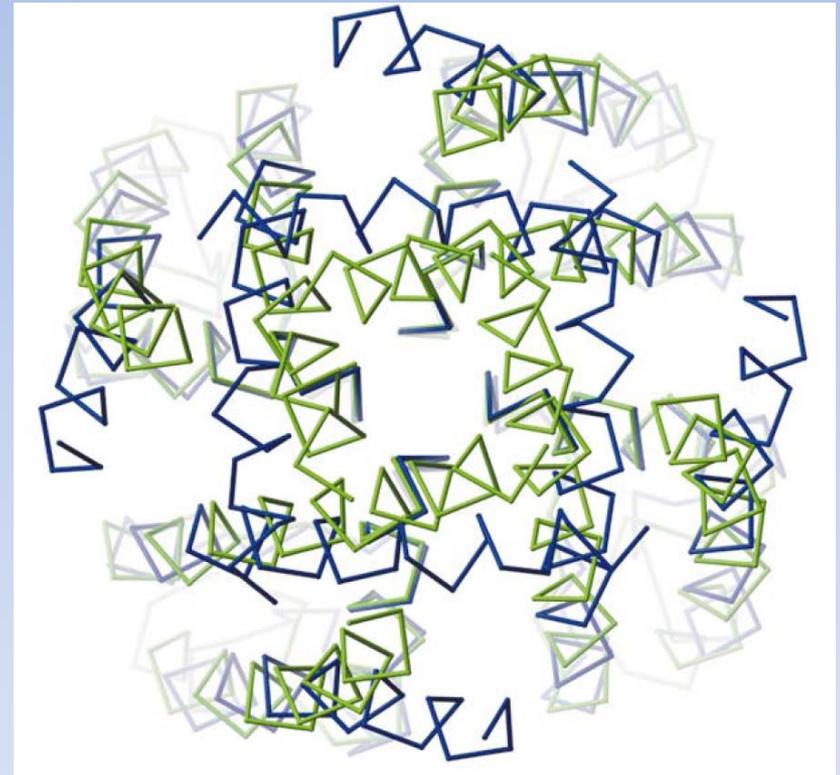
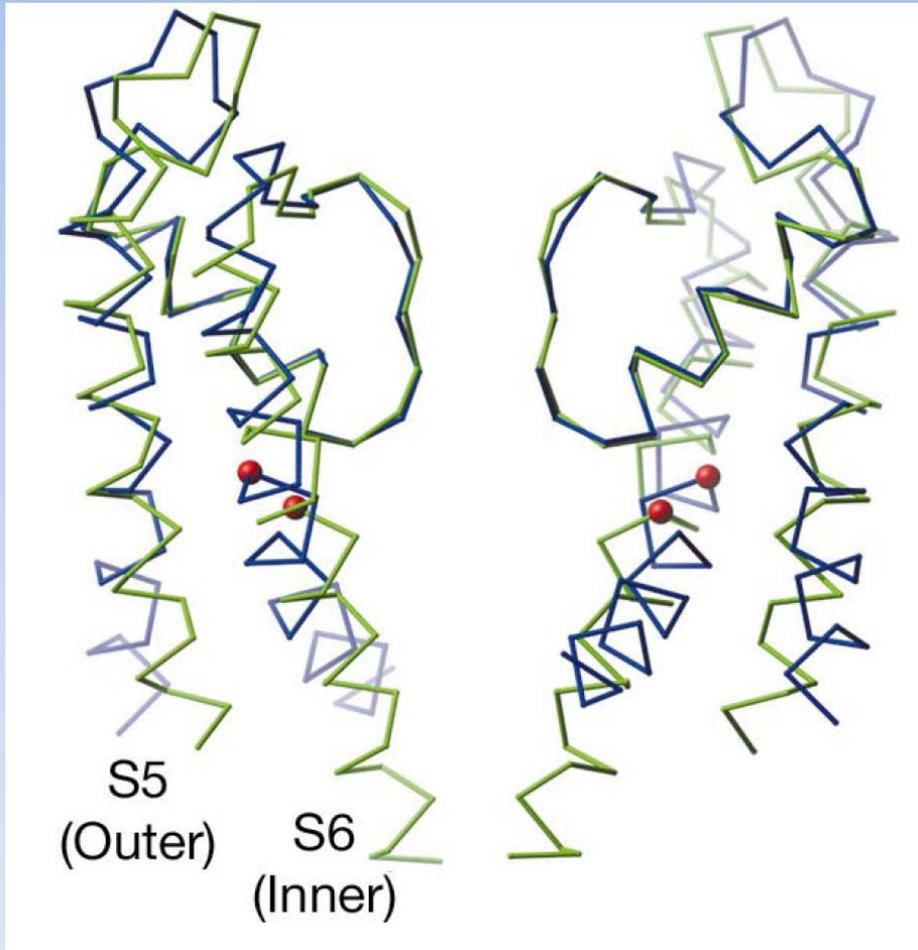
KvAP

KvAP sequence is similar to Shaker



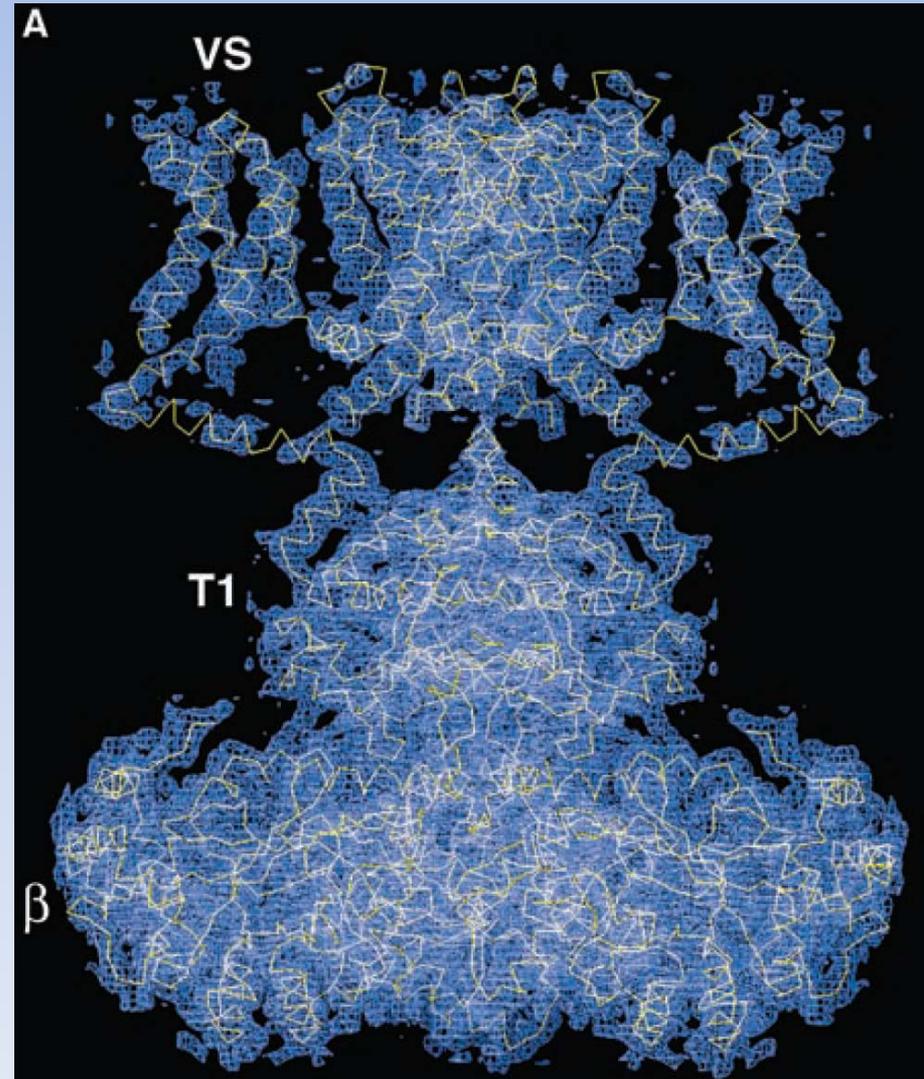
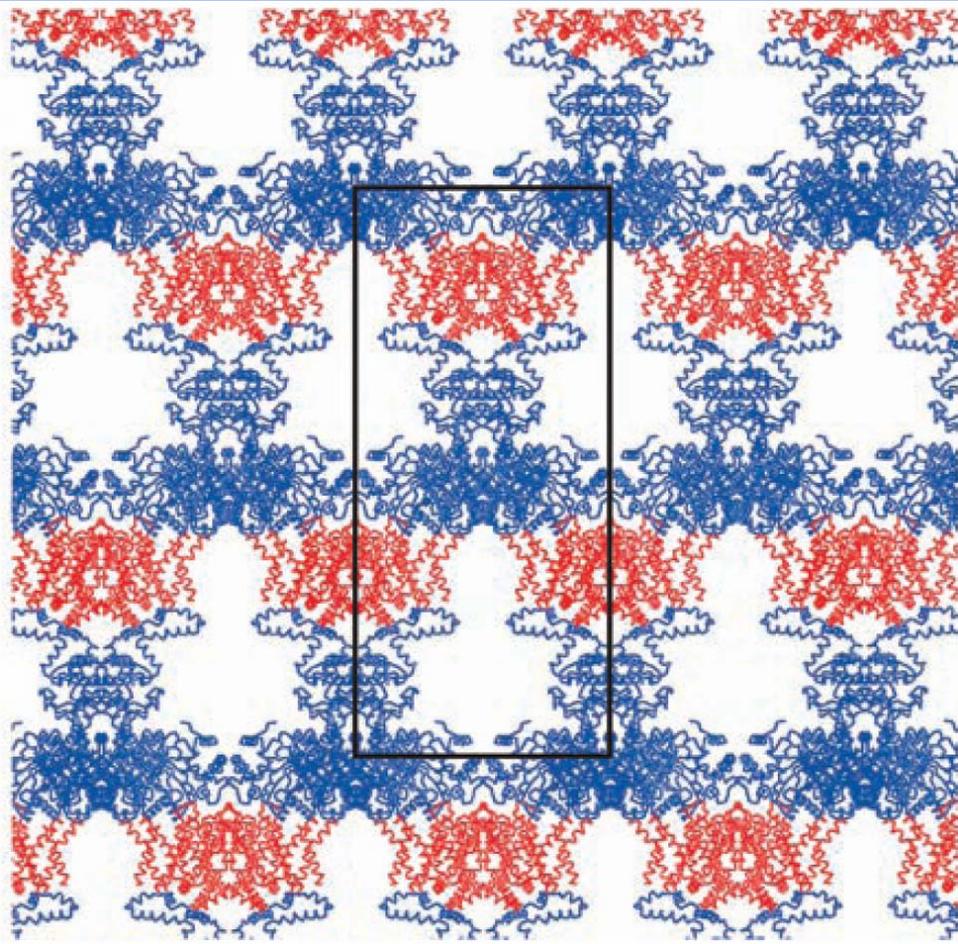
Comparison of KvAP and KcsA pore domain

KcsA: green KvAP: blue



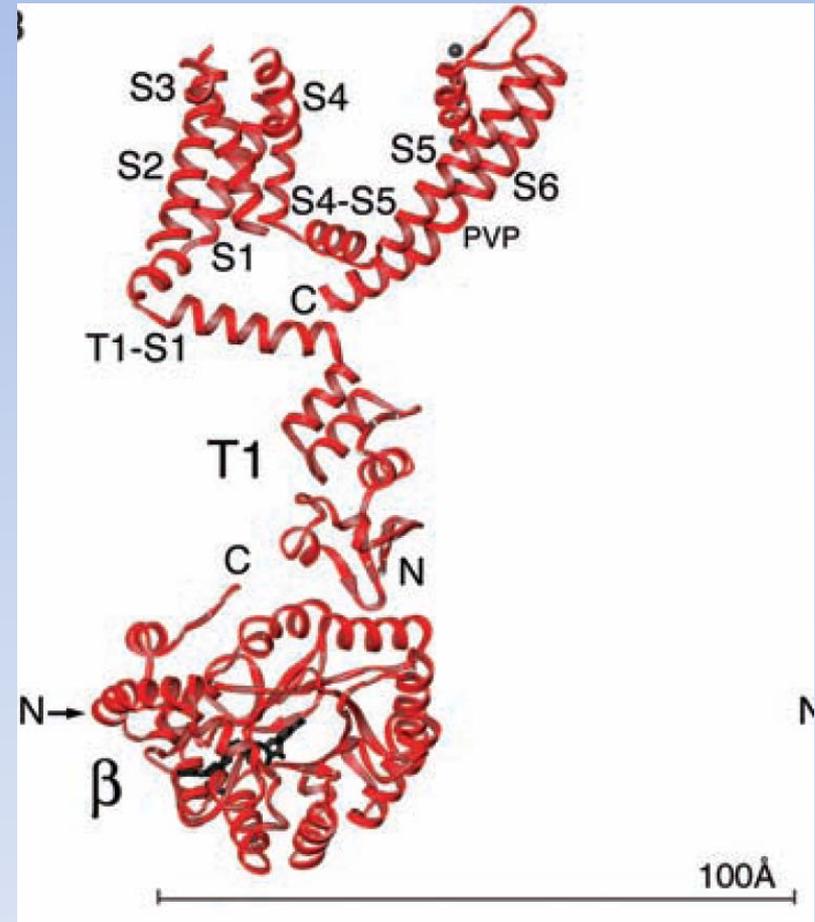
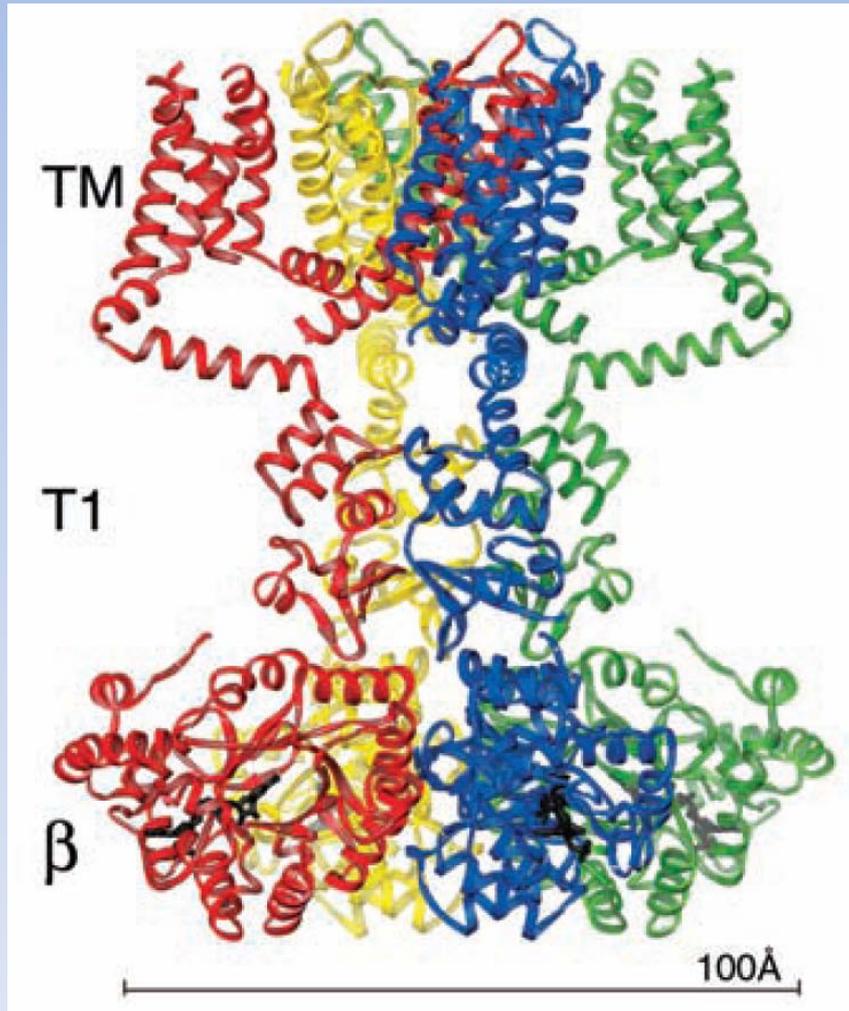
● Glycine hinge

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

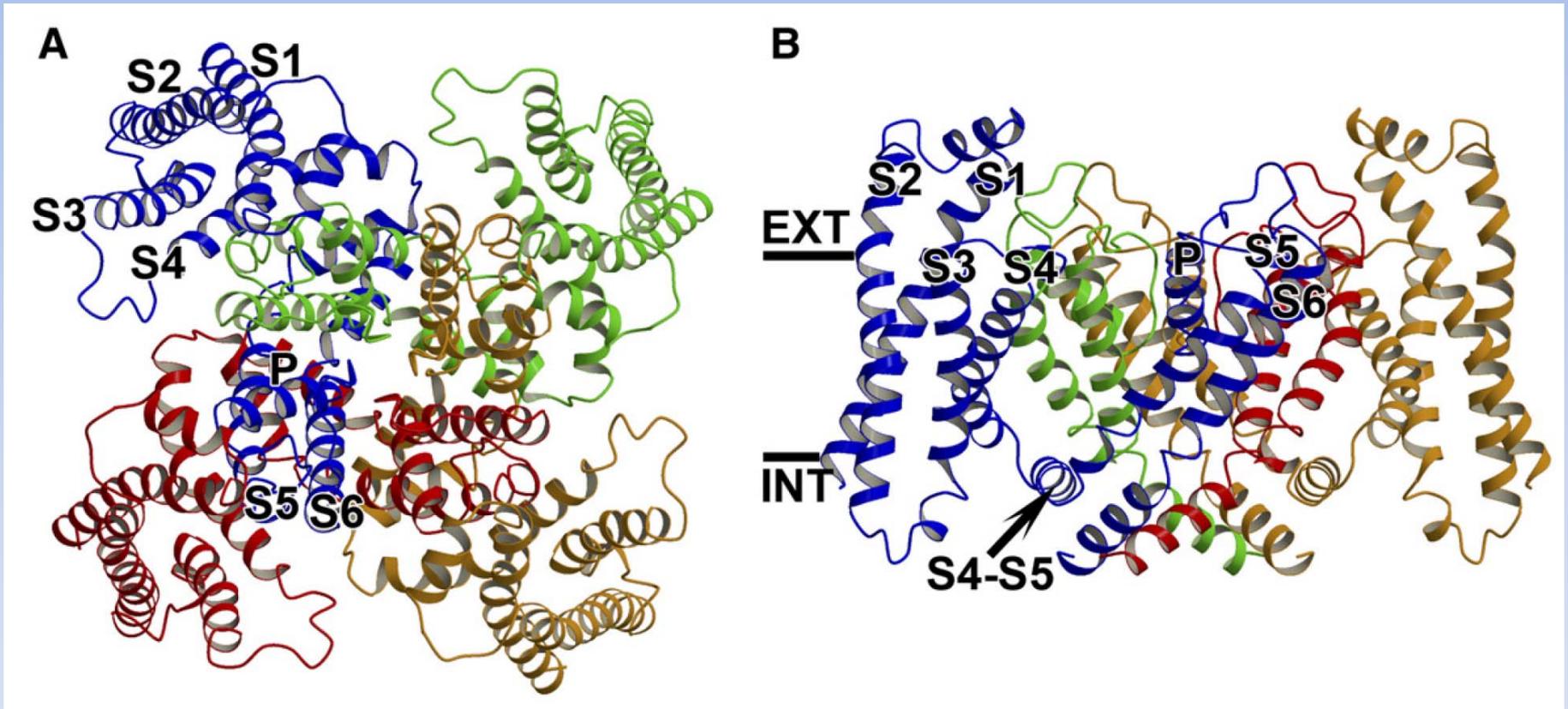


The Kv1.2- β_2 subunit channel complex

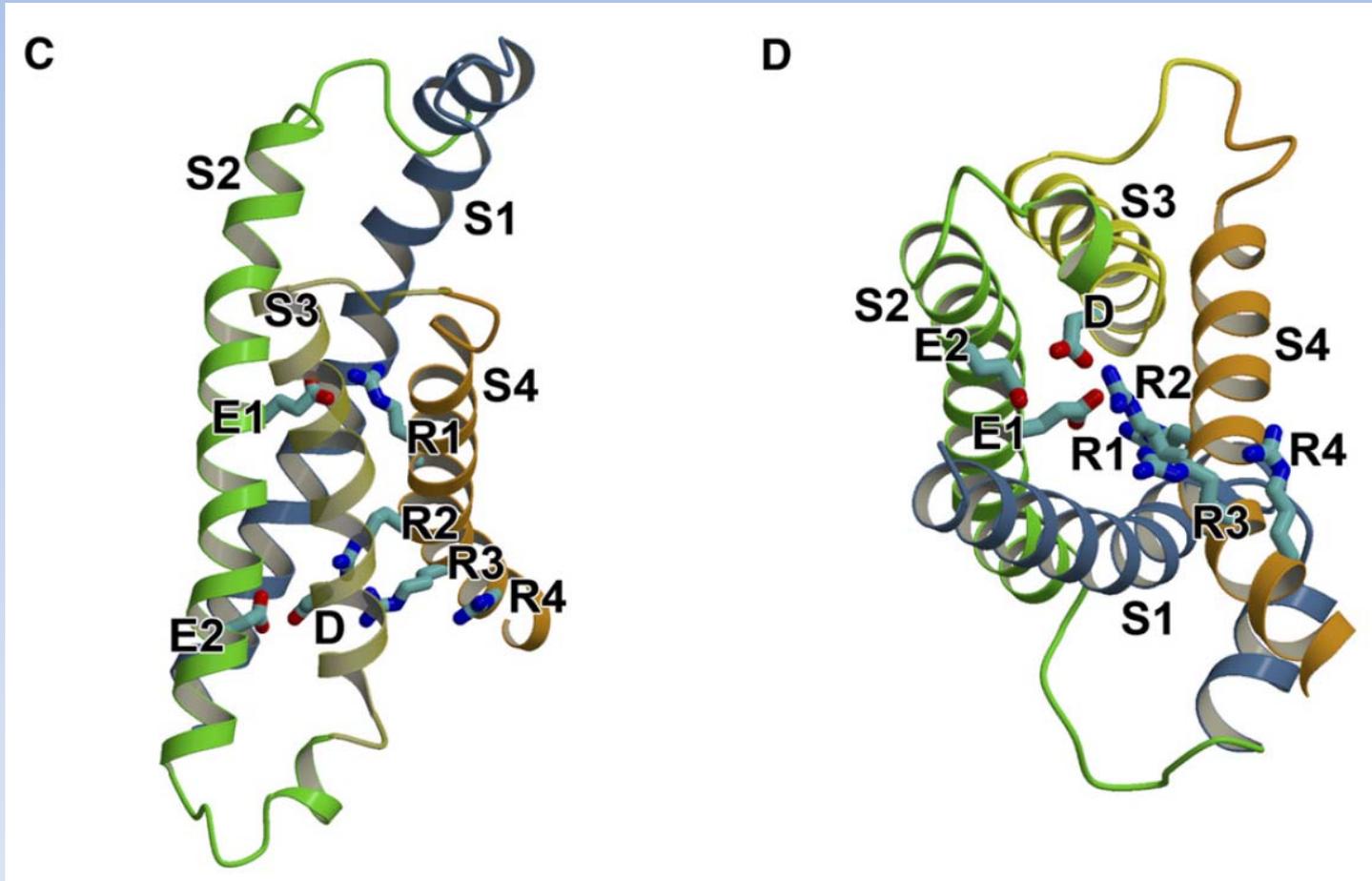
(back to the traditional VSD model)



Model of Kv1.2 in the closed state

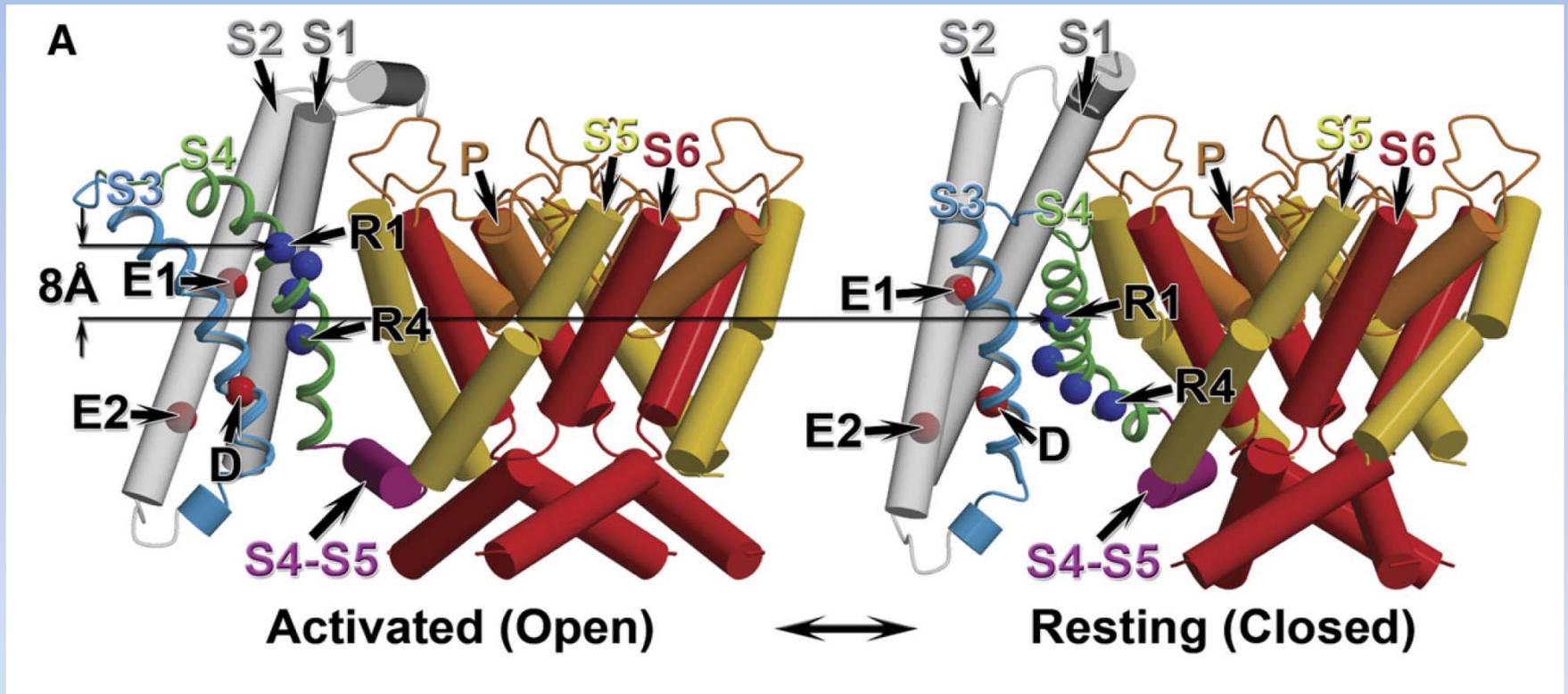


Model of Kv1.2 VSD in the closed state: salt bridges form between basic residues in S4 and acidic residues in S2/S3

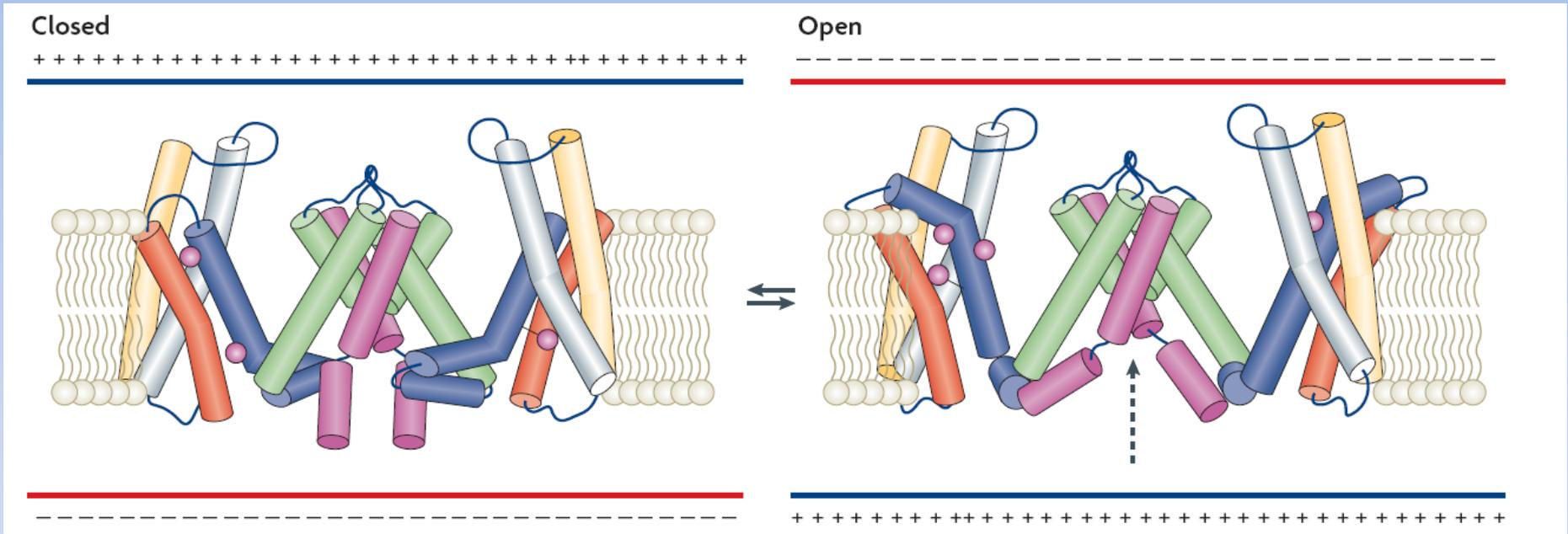


Side view

view from intracellular side



Model of S4 movements in a Kv channel



(only two subunits shown)

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

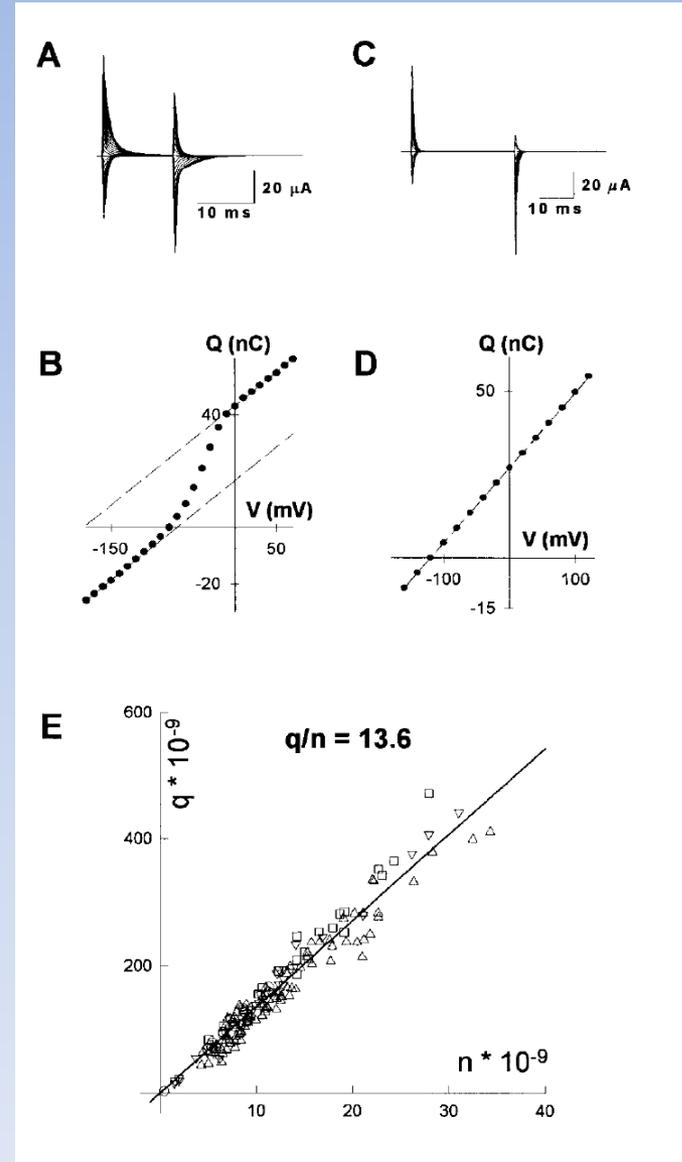
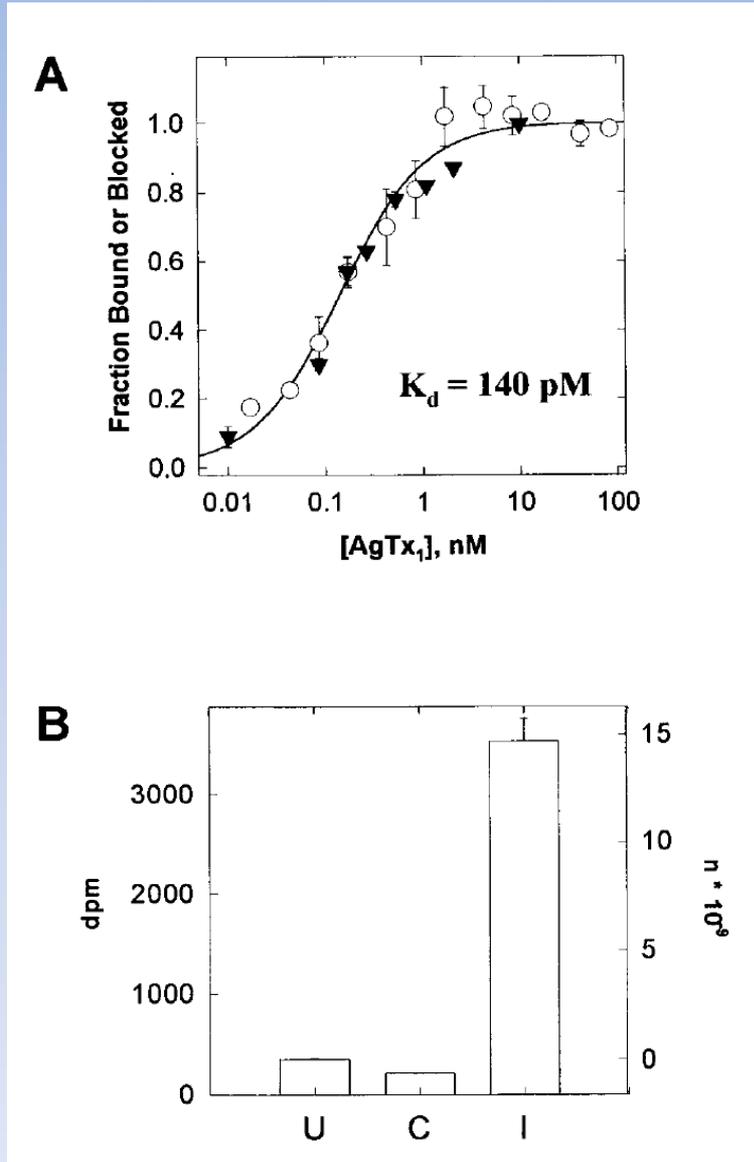


Figure 3. Determination of the Gating Charge of the *Shaker* K⁺ Channel

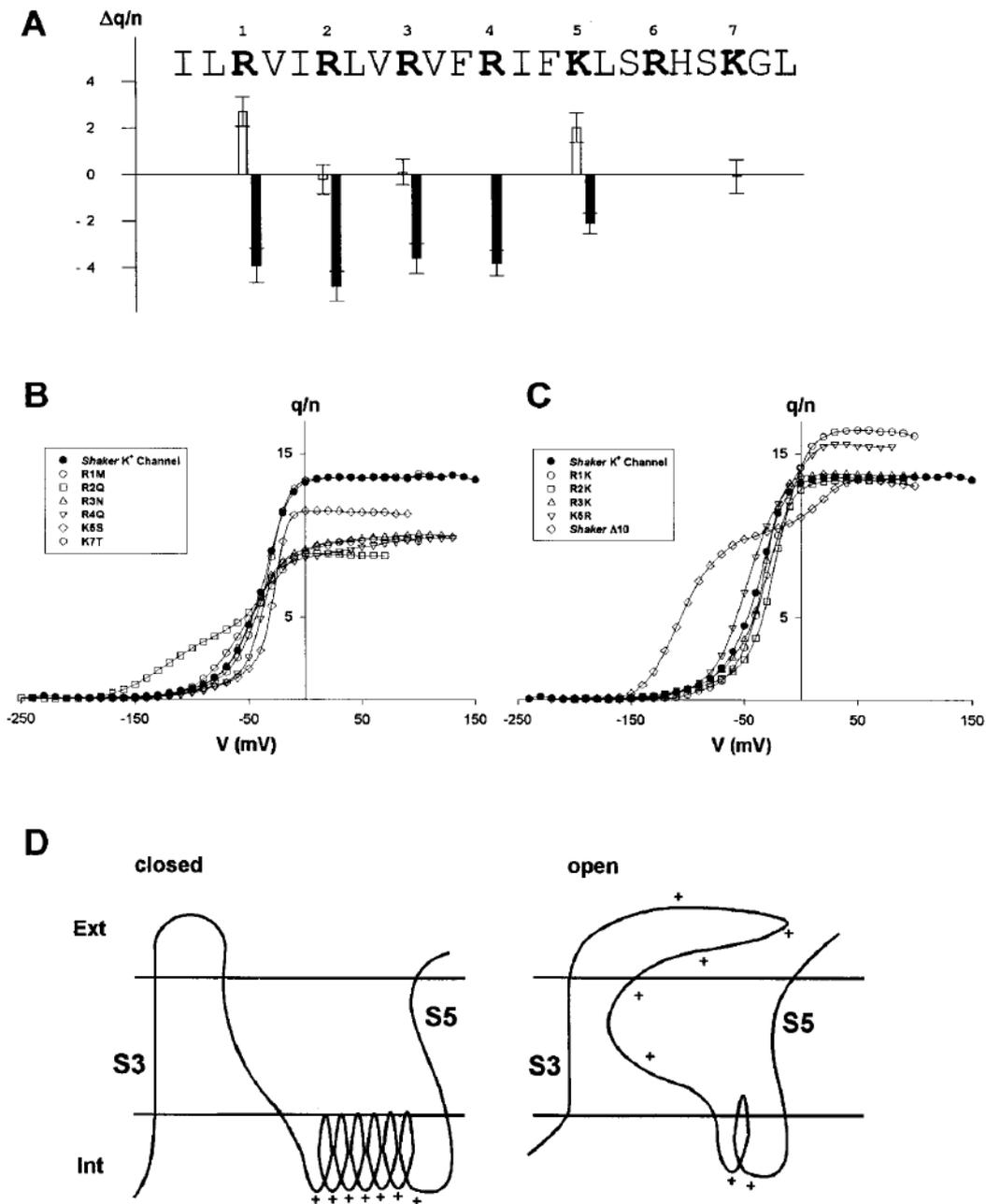
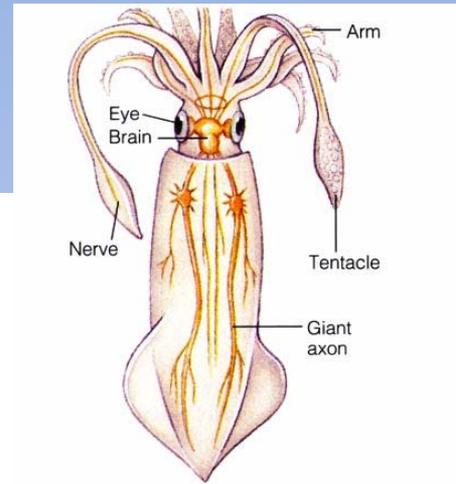


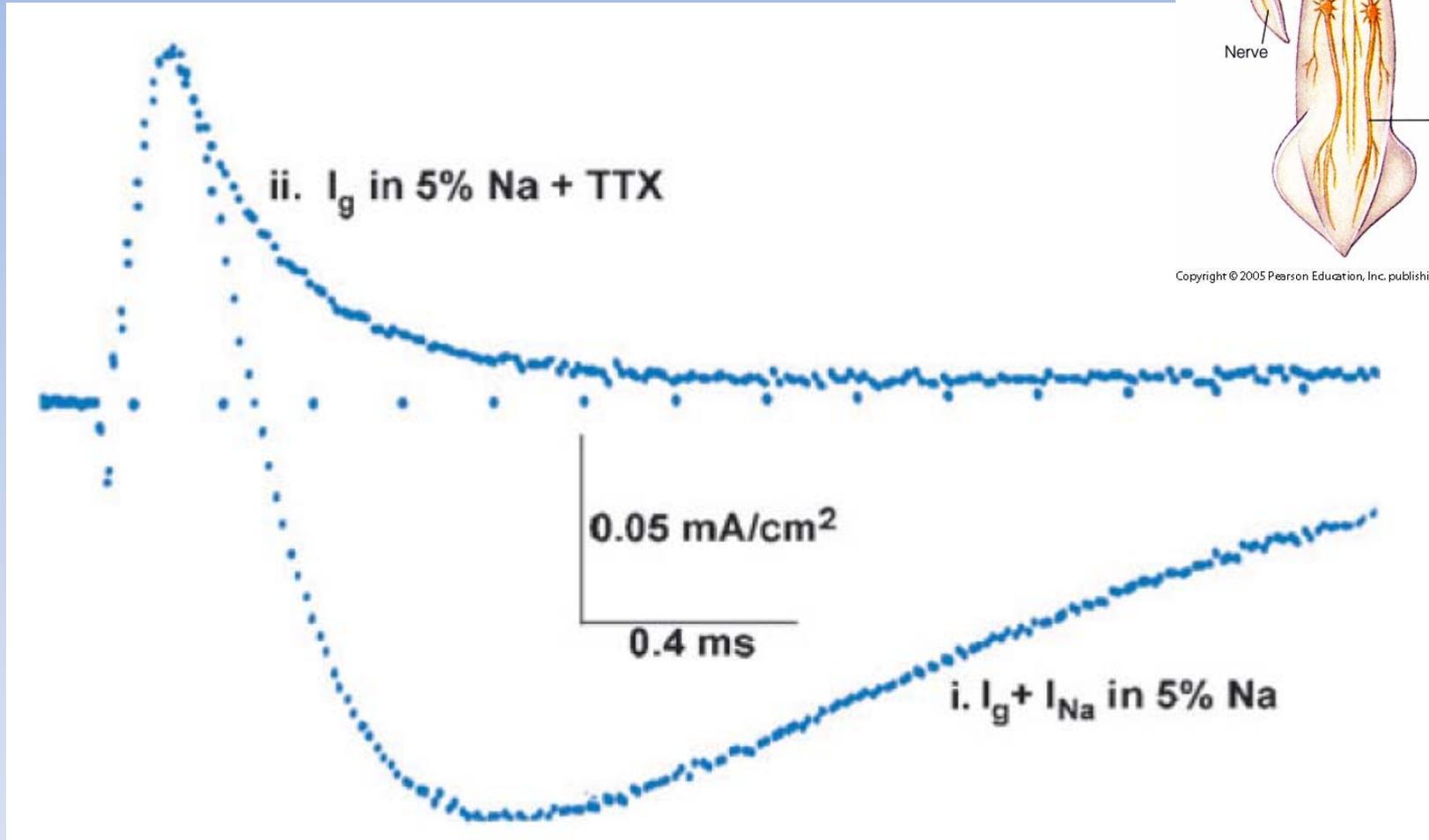
Figure 7. Summary

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 (~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 NH₂-terminal half but not the CO₂H-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.

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



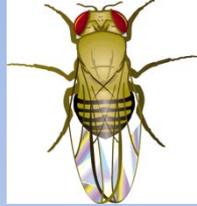
Copyright © 2005 Pearson Education, Inc. publishing as Benjamin Cummings



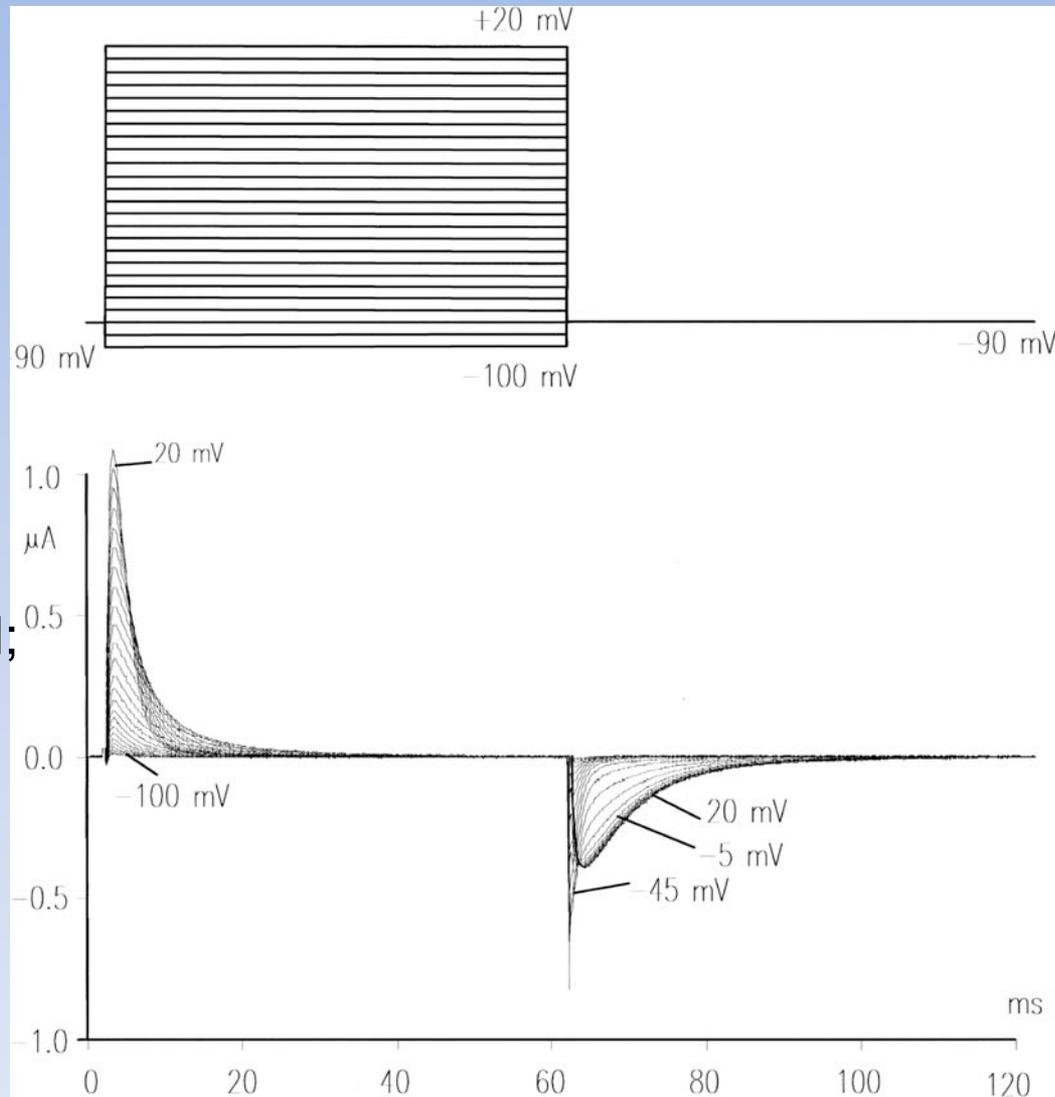
Armstrong CM, Bezanilla F. 1973. Currents related to movement of the gating particles of the sodium channels. *Nature* 242:459–61

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)

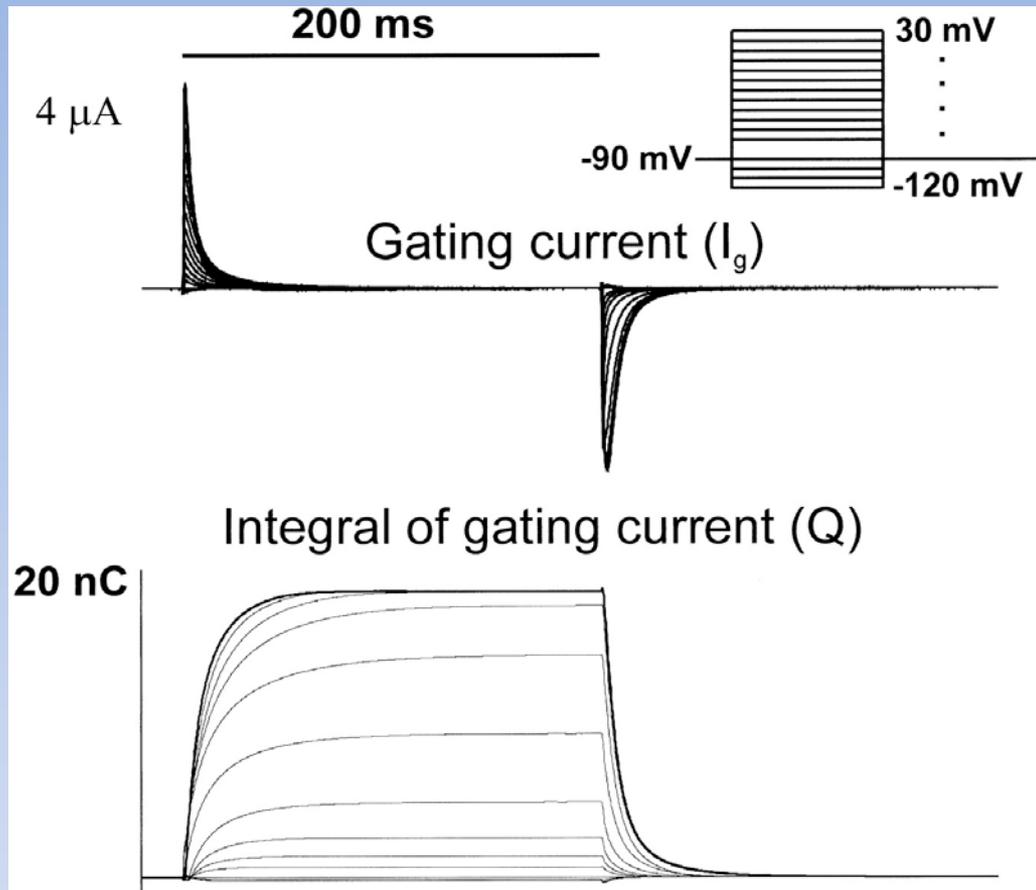
Gating currents of cloned *Shaker* K channel



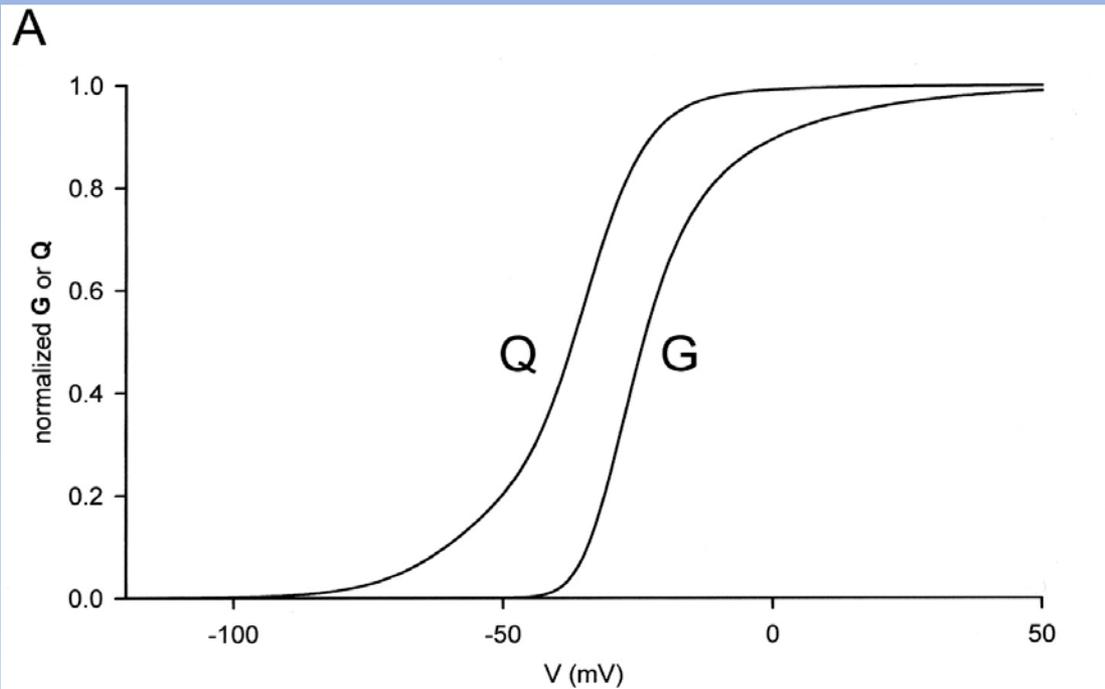
Voltage pulse protocol



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

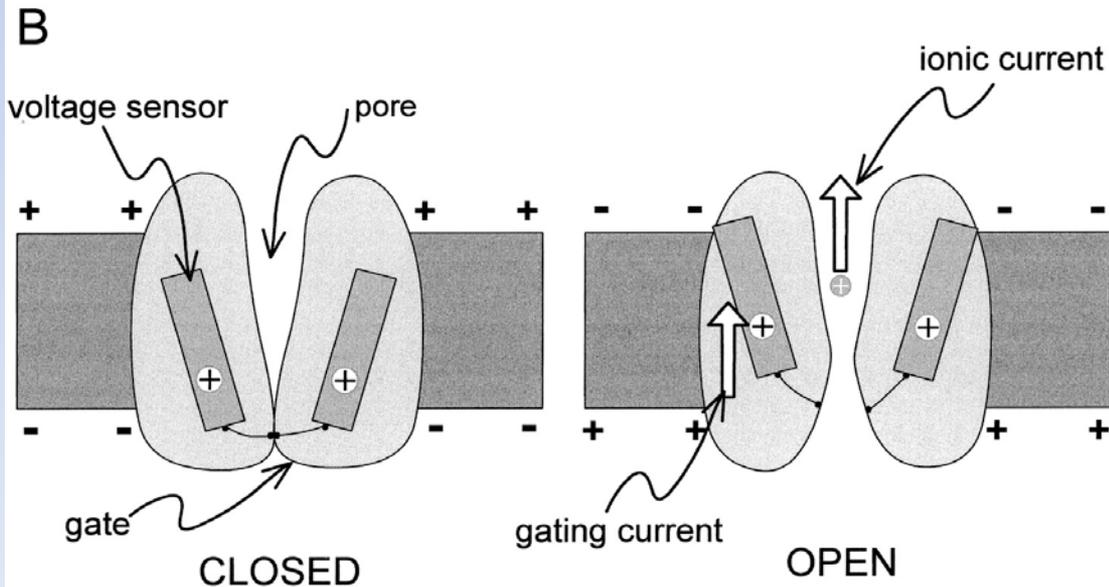


**Integrate gating current
to obtain charge (Q)**

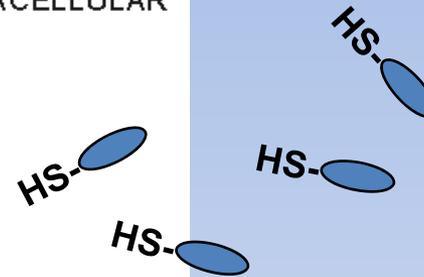
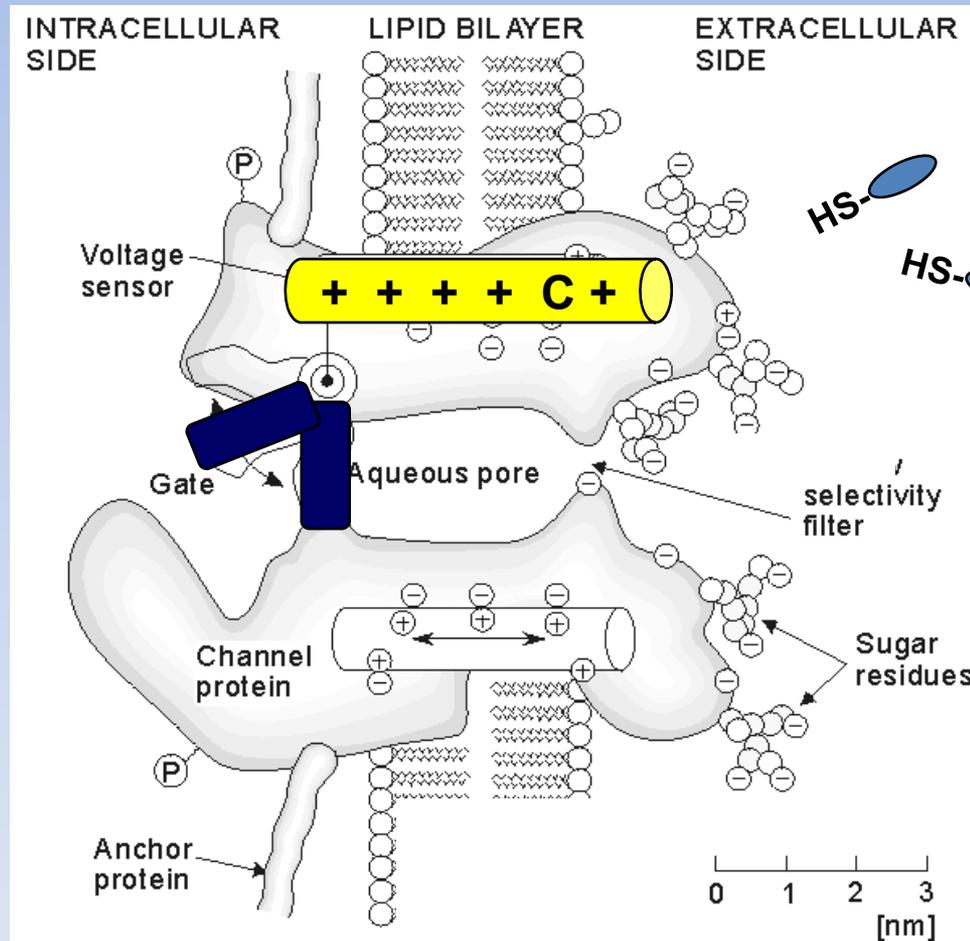


Q = charge
(gating current)

G = conductance
(ionic current)



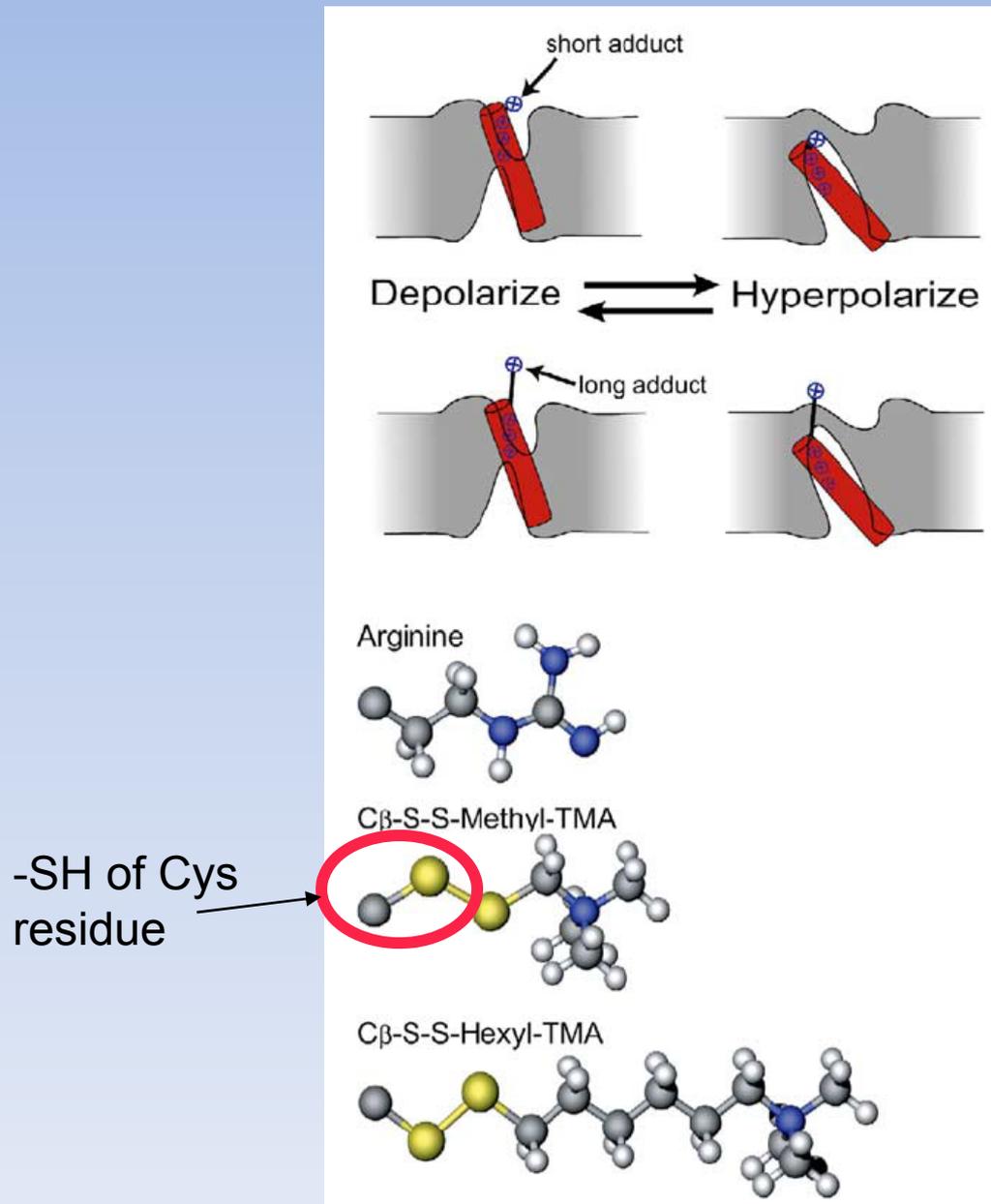
“Accessibility” of residues mutated to Cys used to determine extent of S4 movement during channel activation



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



Intramembrane charge displacement

- Measurement of total charge

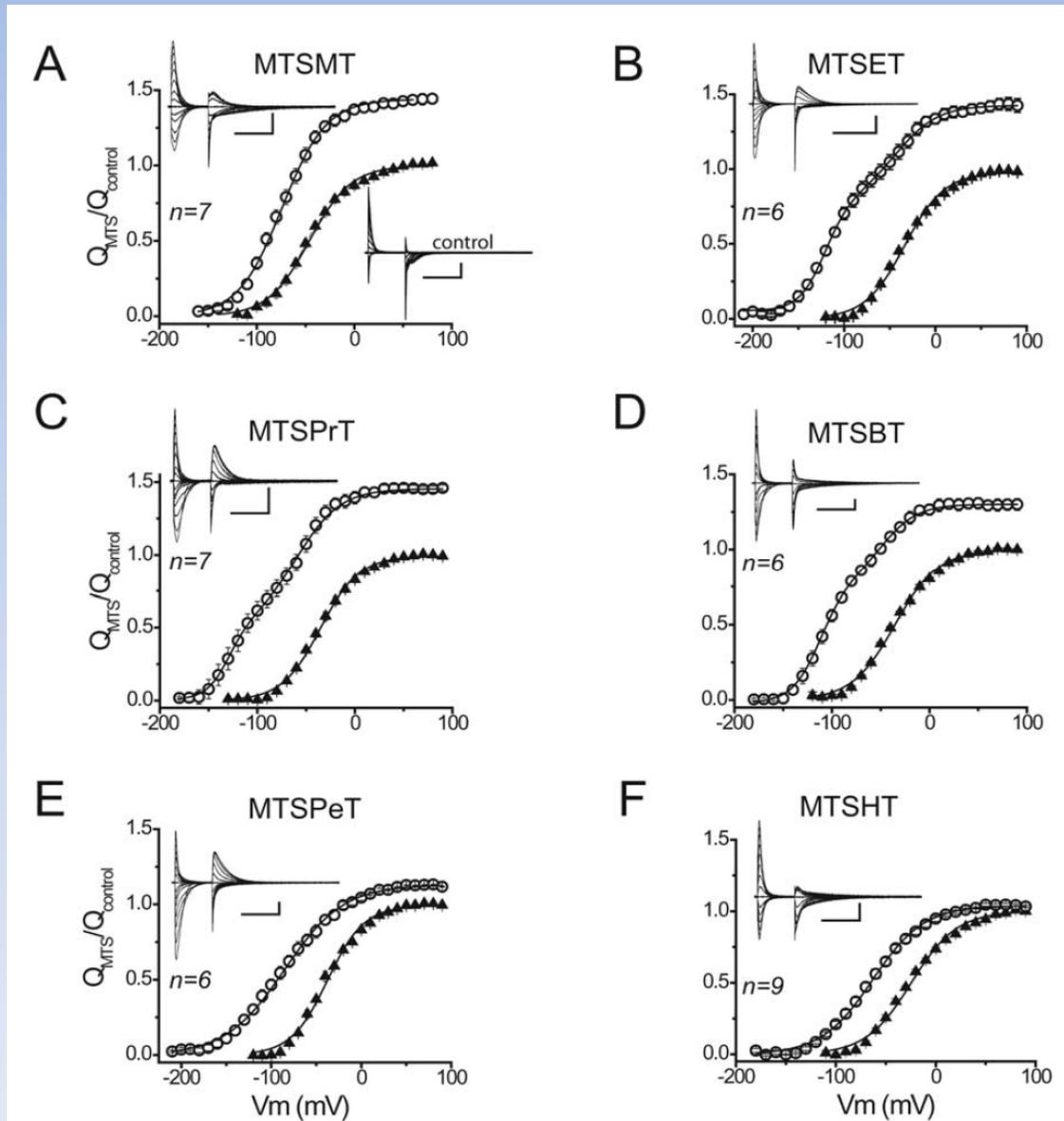
moved $(Q_{\text{tot}})/\# \text{ of channels} = 13 e^0/\text{channel}$

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

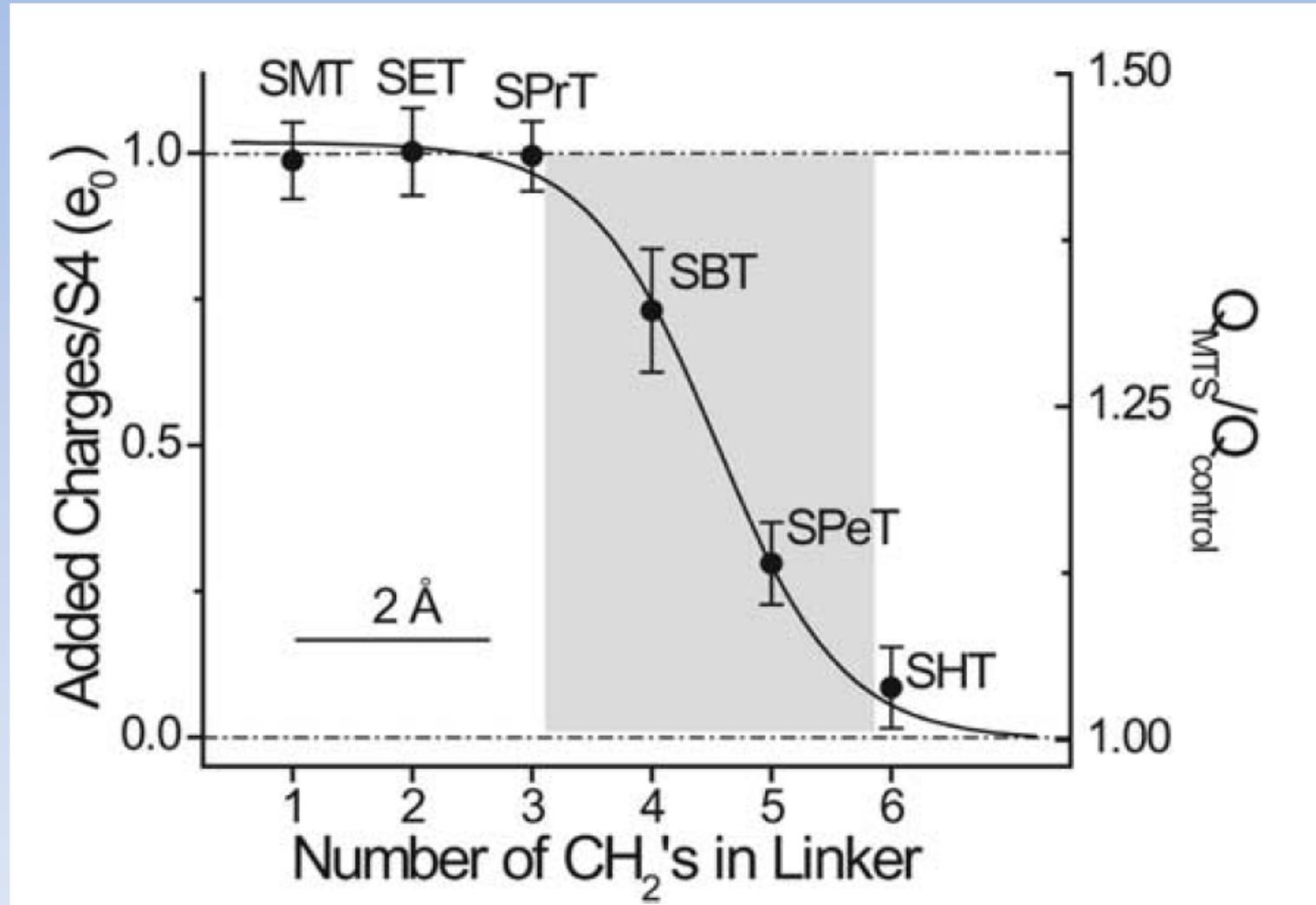
.....reduces Q/channel to $9 e^0/\text{channel}$

($1 e^0/\text{subunit of homotetramer}$)

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

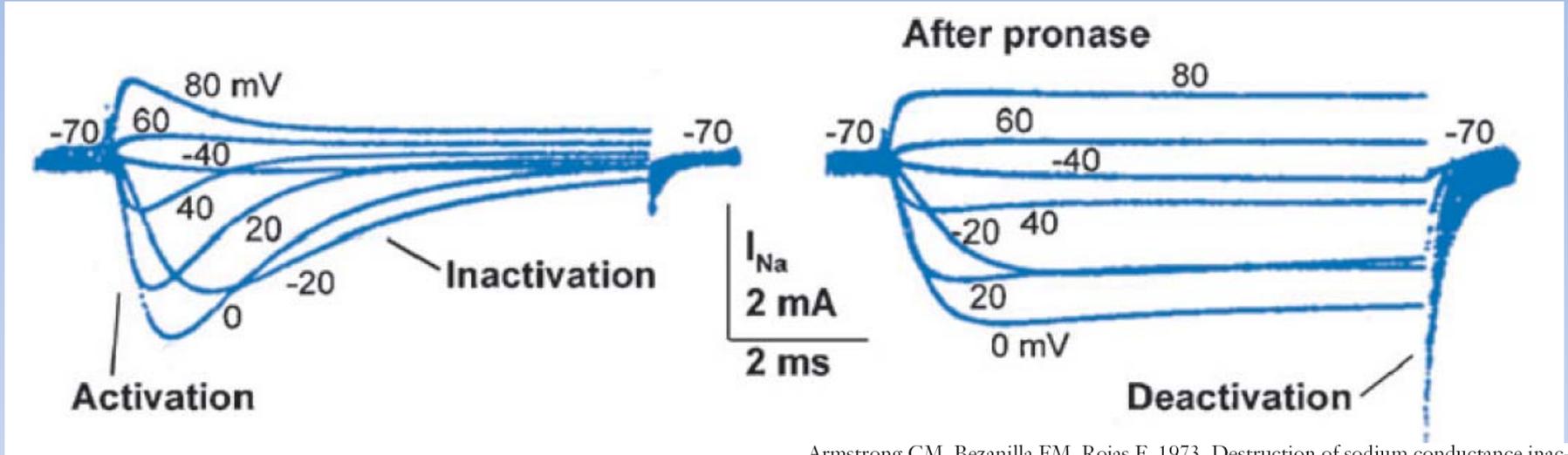


Increasing tether length monotonically decreases charge movement



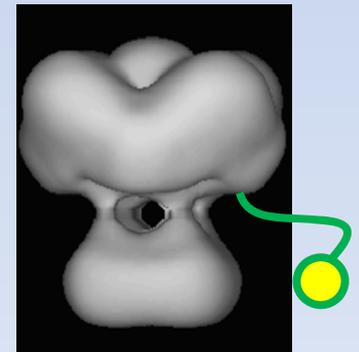
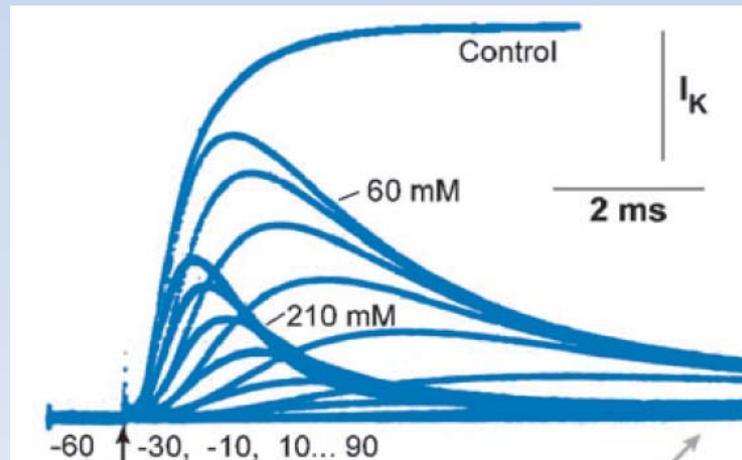
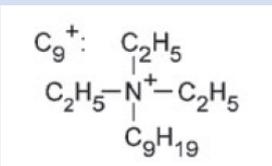
Inactivation gates

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



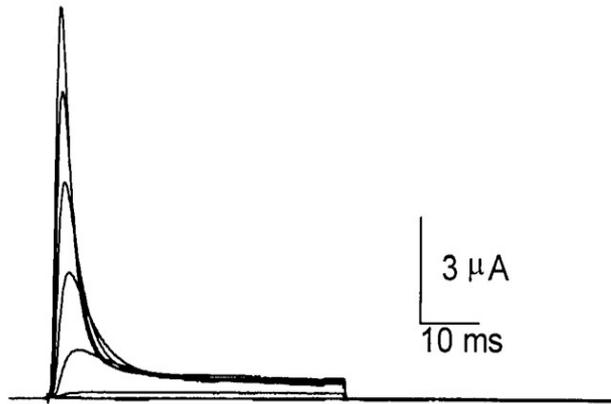
Armstrong CM, Bezanilla FM, Rojas F. 1973. Destruction of sodium conductance inactivation in squid axons perfused with pronase. *J. Gen. Physiol.* 62:375-91

Looks similar to block of K channels by internal C9:

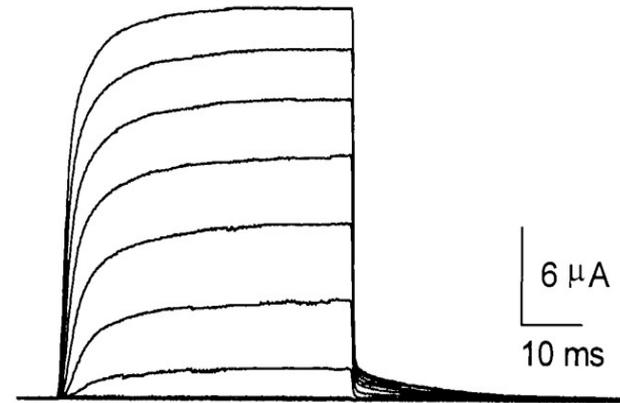


“ball and chain”

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

