RUTGERS
New Jersey Medical School

What do we (not) know about voltage-gated ion channels?

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Essential functions of membranes.

Transporters
Aquaporins
H^+ channels

Compartmentalization
(separation)

Connexins ?

Function

Of Matter

Across Membrane
(Signal Transduction)

Transport

Of Information
(Communication)

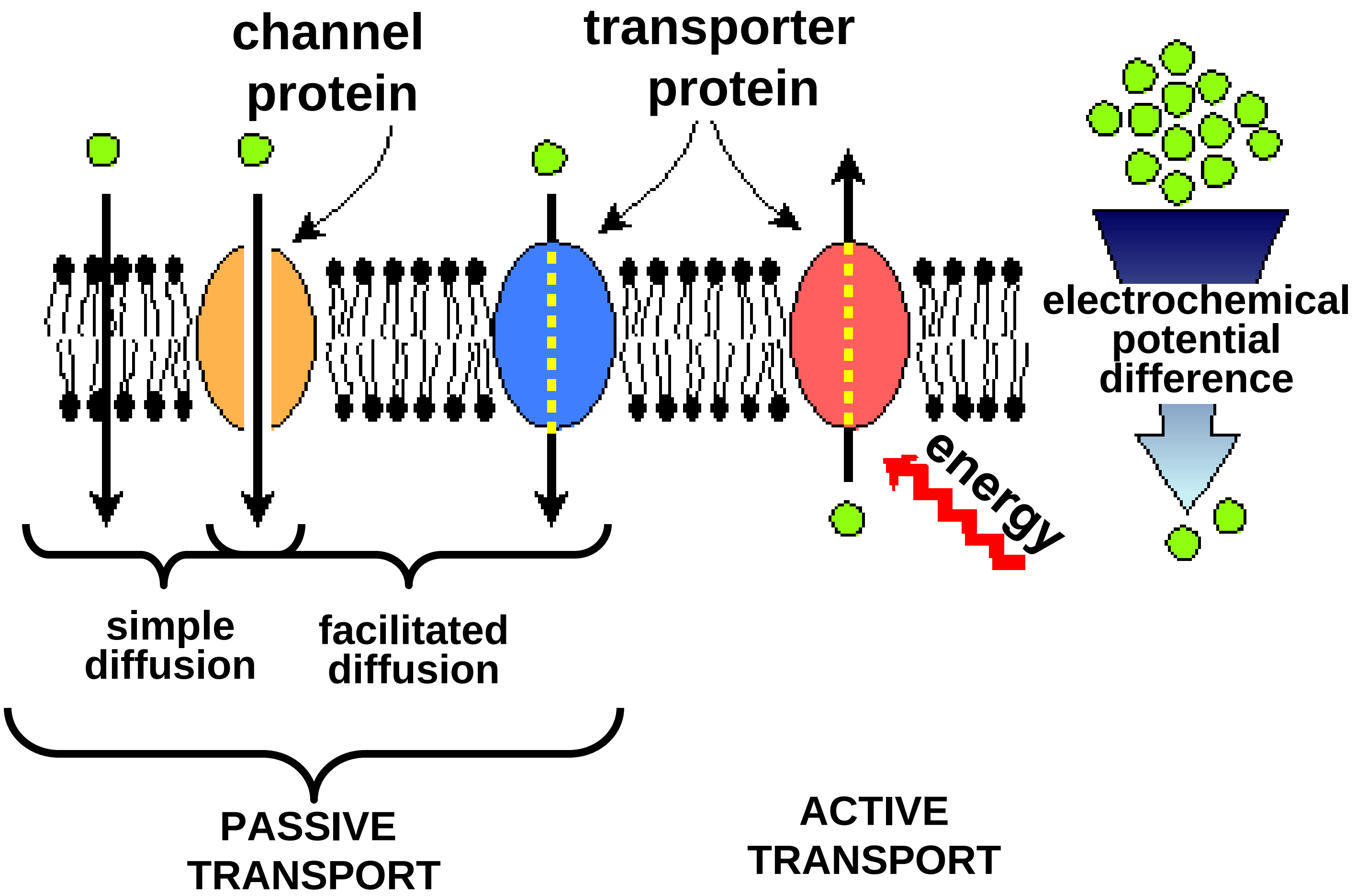
At a distance
(Excitability)

Receptors:
- Ion channels
- GPCRs
- integrins (status of extracellular matrix)
- enzyme-coupled (e.g., guanylyl cyclases, various kinases/phosphotases)

Ionic compositions inside and outside a typical mammalian cell

	Extracellular Conc	Intracellular Conc
Na^+	145 mM	15 mM
K^+	4 mM	140 mM
Cl^-	100 mM	7 mM
Ca^{2+}	2 mM	10^{-8} M
Mg^{2+}	1 mM	1 mM
P_i	2 mM	40 mM
H^+	10^{-7} M	10^{-7} M
Protein	0.2 mM	4 mM

Membrane transport systems

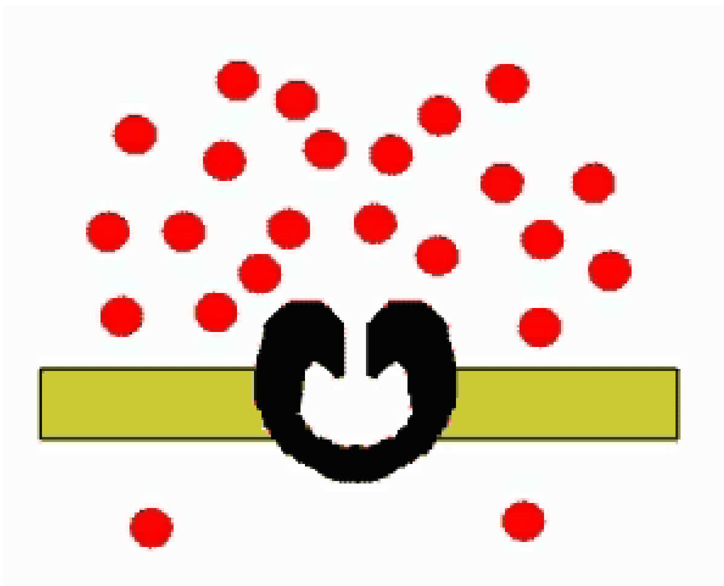


A transporter does “facilitated diffusion”

Concentration gradient:

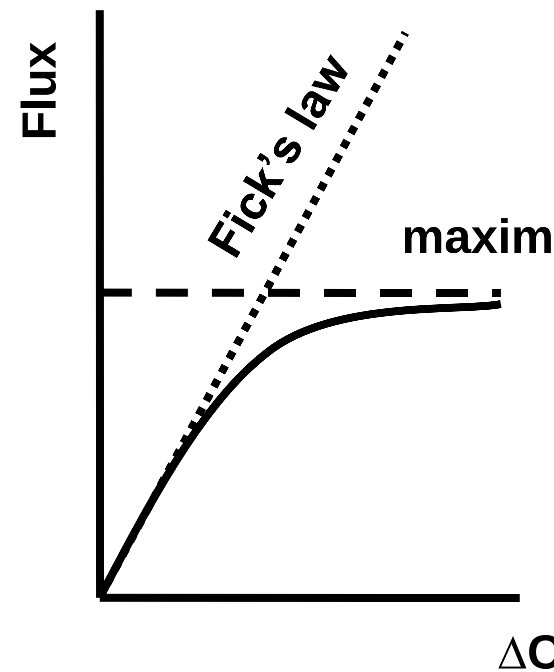
High

Low



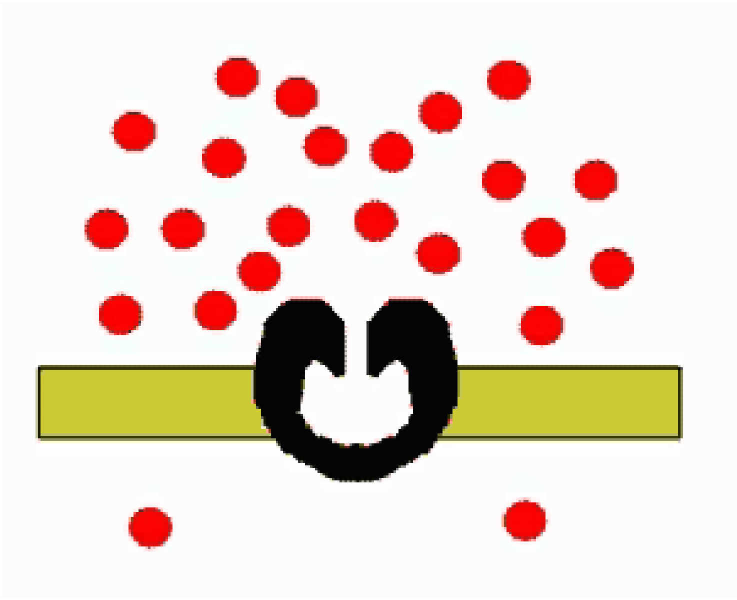
Three major differences between facilitated diffusion and simple diffusion:

- **saturation,**
- **specificity**
- **competition**

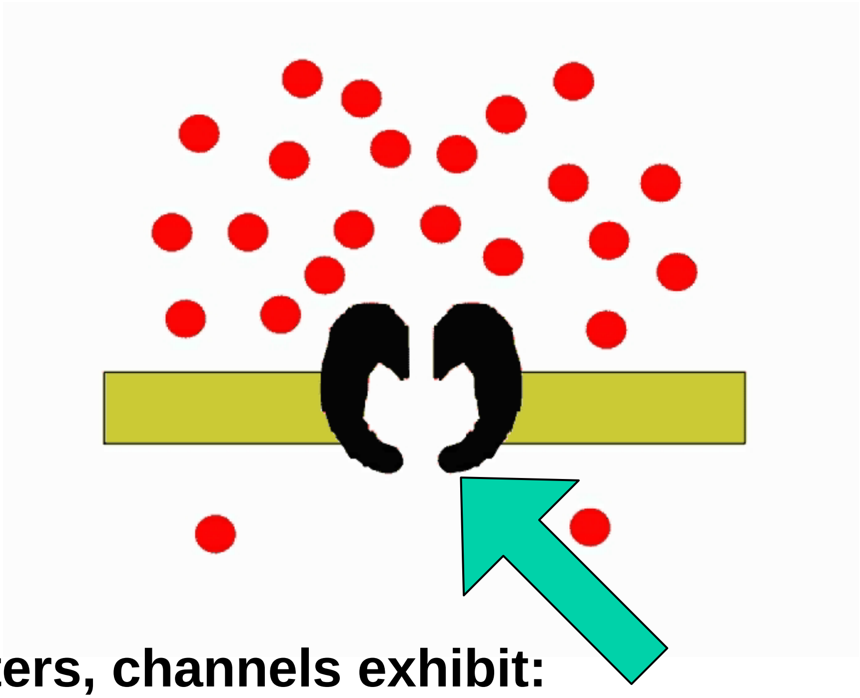


Channels rapidly change permeability of membranes because they pass solutes through water filled pore

Carrier



Channel

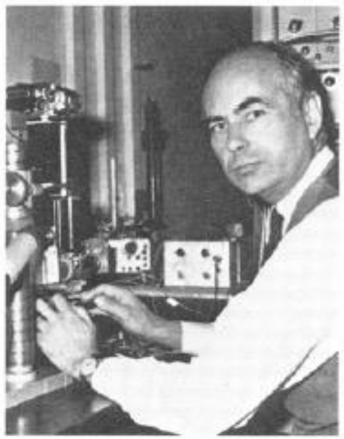


As facilitated diffusion transporters, channels exhibit:

- **saturation**
- **specificity**
- **competition**

Hodgkin and Huxley proposed that ion channels are elementary excitable elements in nerve membranes

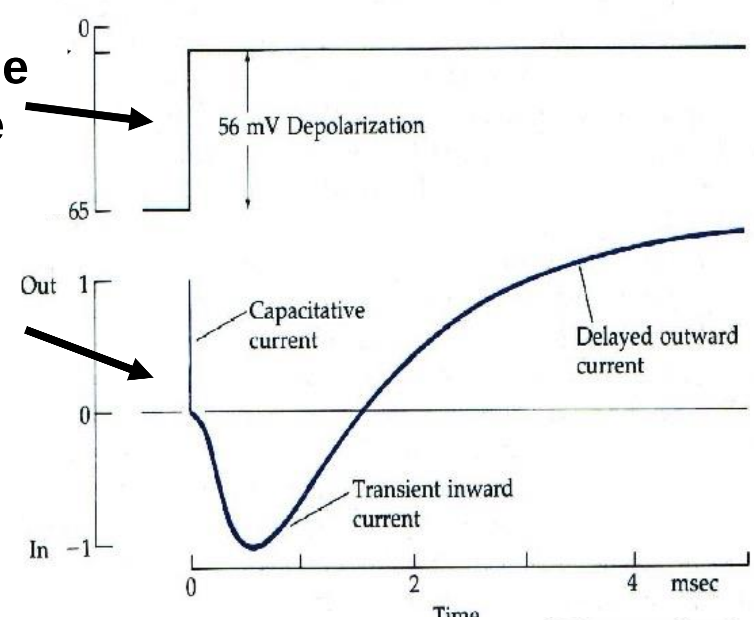
A. L. Hodgkin, 1949



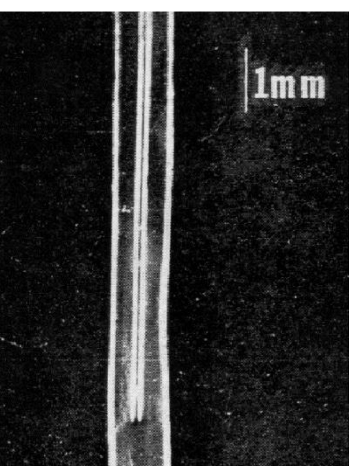
A. F. Huxley, 1974

Voltage pulse

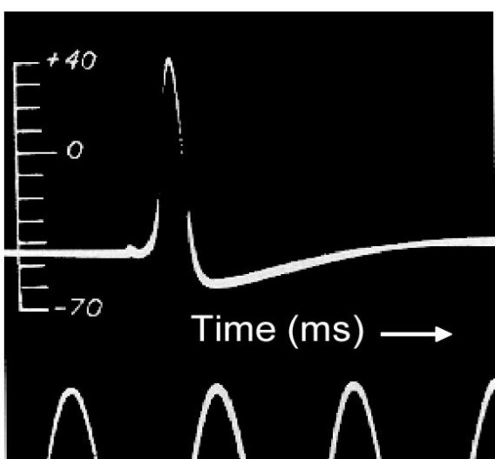
Recorded current



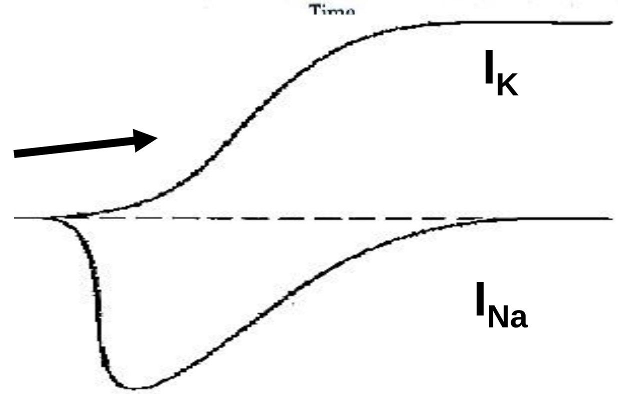
Squid axon with pipette



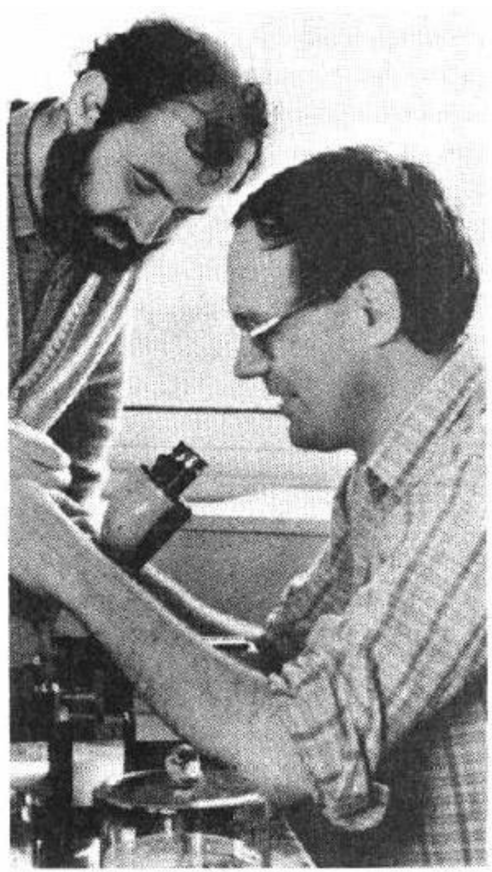
Action potential of squid axon



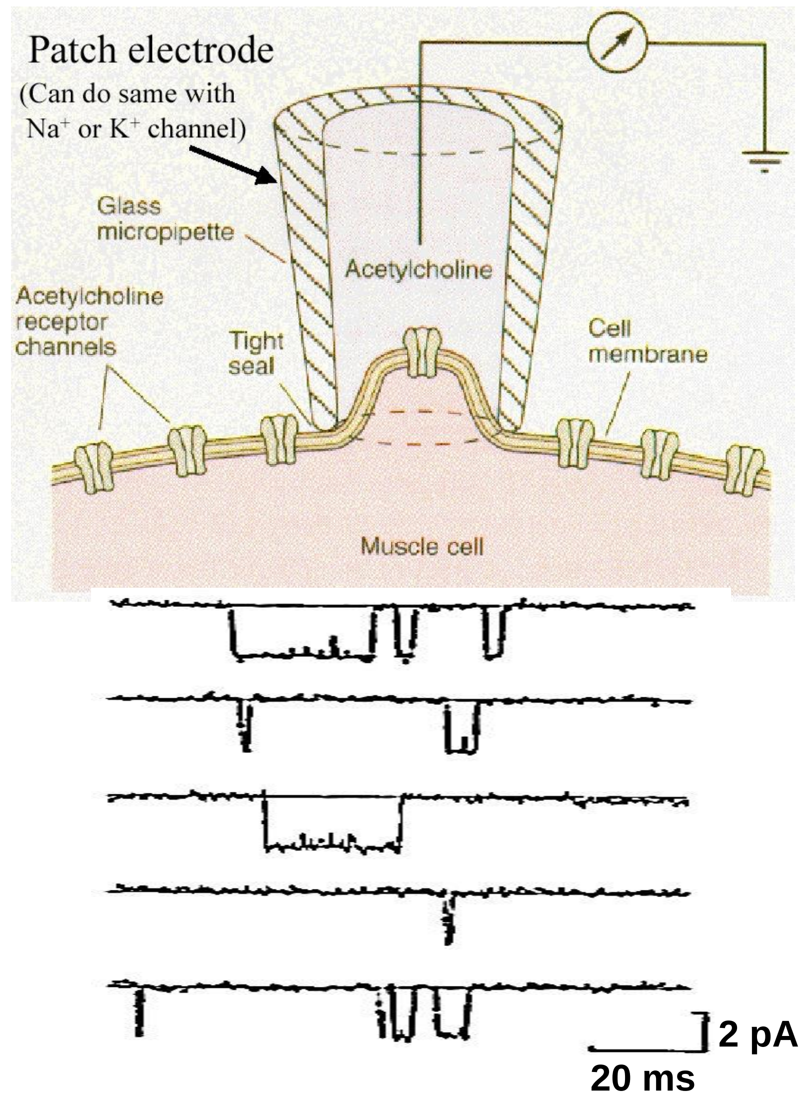
Separation of currents



Neher and Sakmann recorded currents through single channels



Erwin Neher (left) and Bert Sakmann in their laboratory (1985).

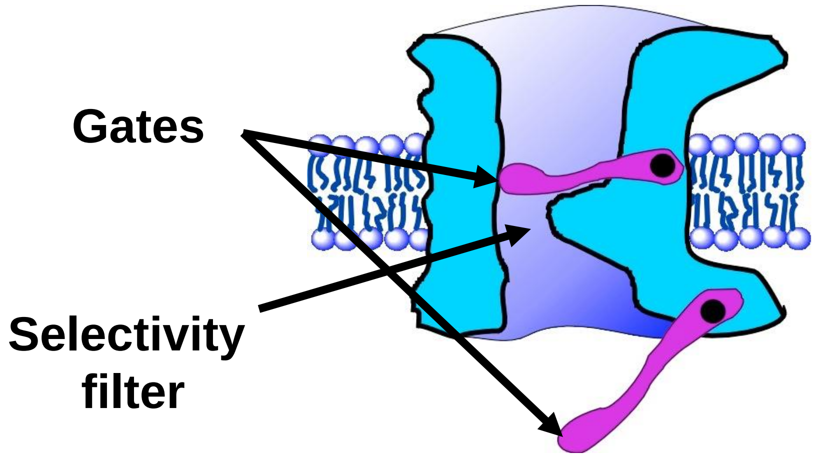


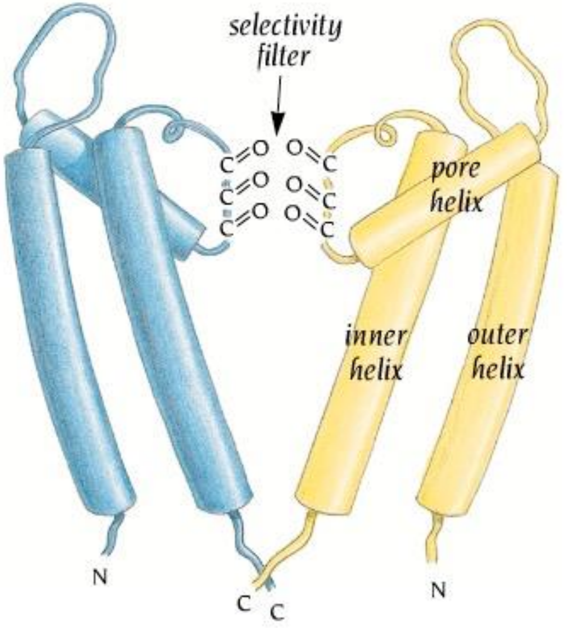
MacKinnon and others began the understanding of channel functions based on their structural principles

Clay Armstrong, 1999

Bertil Hille, 1999

Rod MacKinnon, 1999

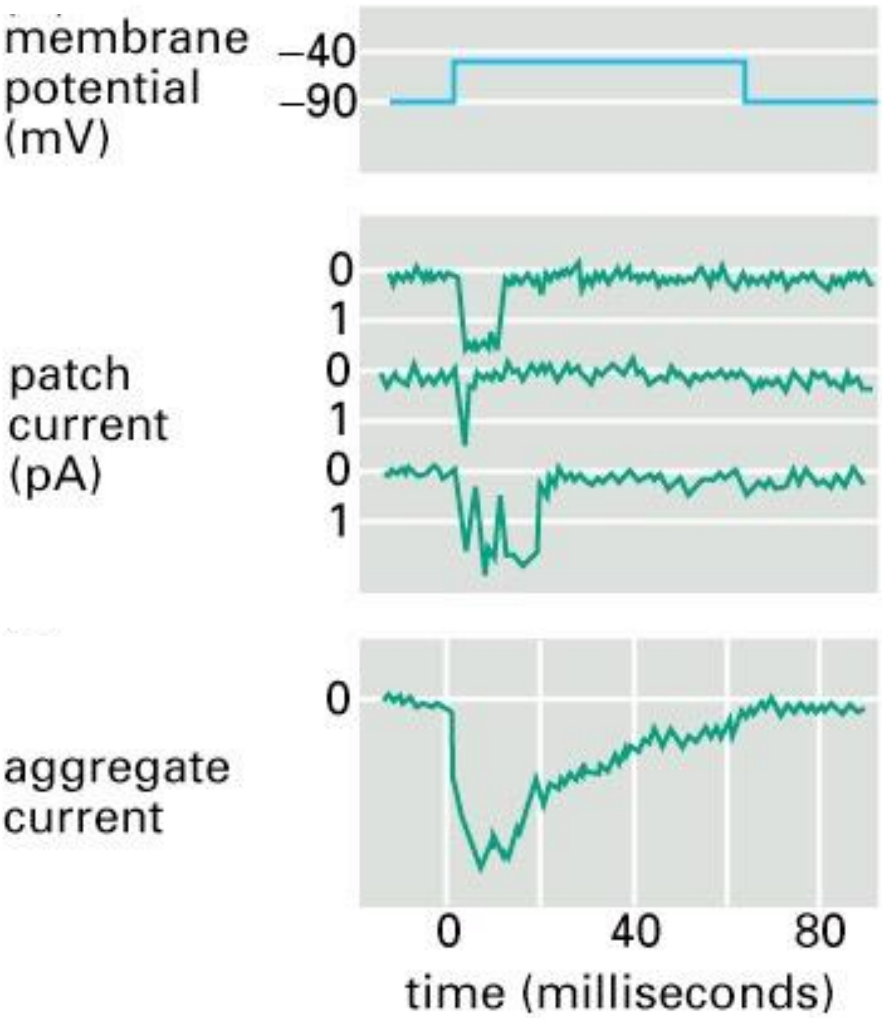




Functional features of channels:

- **Gating mechanism (rapid control of opening)**
- **Conductance/Selectivity (permeability)**
- **Regulation (slow control of opening)**
- **Sensitivity to drugs (pharmacology)**

Total current through one type of channels is the sum of single channel currents



$$\mathbf{I} = \mathbf{N} \times \boldsymbol{i}_{\mathbf{s.ch}}\mathbf{(V, [ion])} \times \mathbf{P_O(V,t)}$$

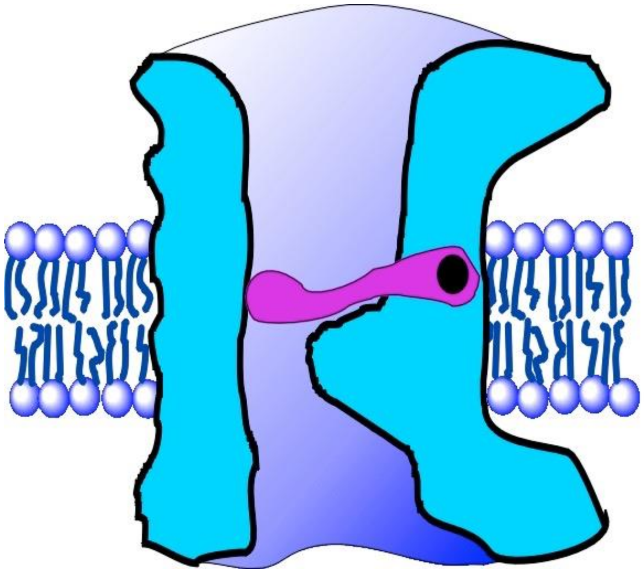
# of functional channels

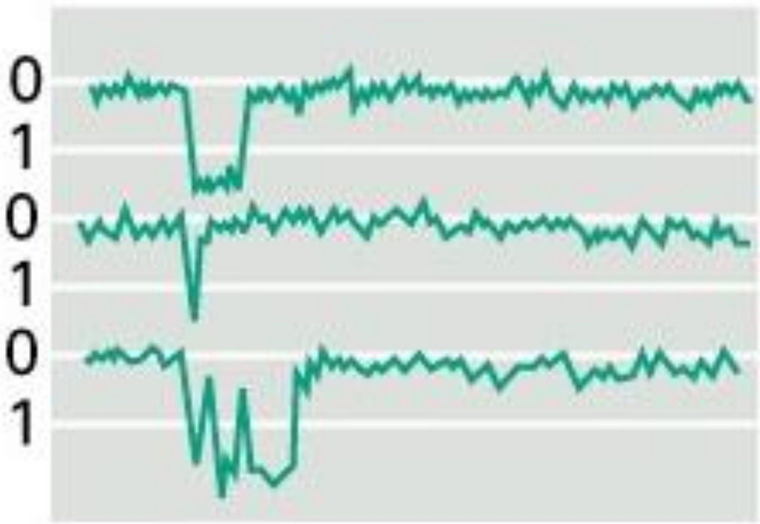
current trough open single channel

open probability

In many cases, single channel conductance and gating are independent

Current through a single channel changes from zero to a certain value



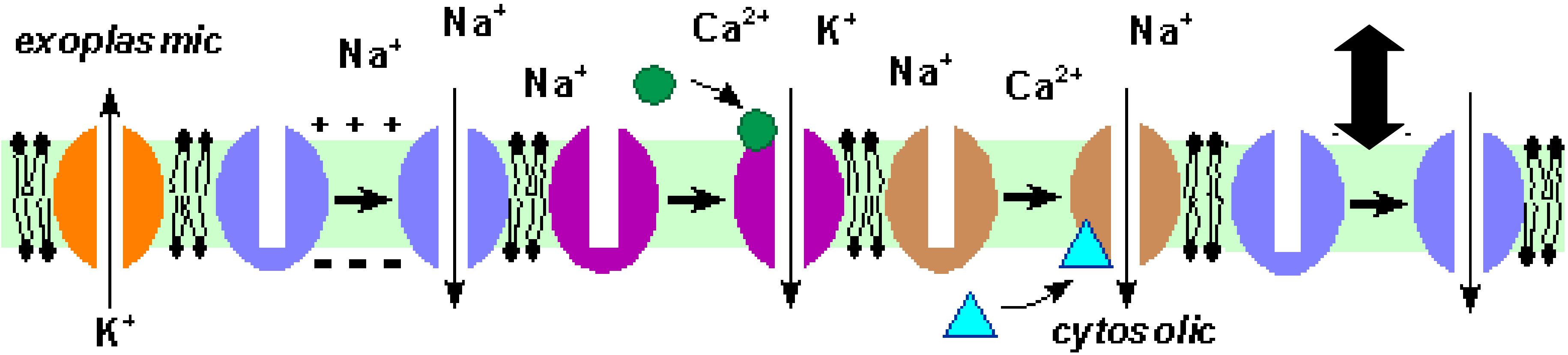


$$I = N \times i_{s.ch}(V, [ion]) \times P_O(V,t)$$

open probability

Gate represents conformational change in one region.
Transition between Closed and Open states is much faster than the dwell time in the states.

Common mechanisms of channel opening (gating):



Resting: always open

Voltage-gated: responds to change in membrane potential

Extracellular ligand-gated: responds to extracellular neuro-transmitter

acetylcholine, glycine, γ-aminobutyrate

Intracellular ligand-gated: responds to intracellular signal trans-duction event

cAMP, cGMP, ATP, Ca^{2+}, Gα , Gβγ, inositol-P_3

Mechano-sensitive: responds to mechanical stress (change in membrane surface tension).

Current through already open channels does not depend depend on time and approximately follows Ohm's Law

$$\boldsymbol{i}_{\text{s.ch}} = \mathbf{g}_{\text{ion}} \times \textbf{\textit{E.P.D.}}$$

single channel conductance

Electrochemical Potential Difference, a.k.a, Driving Force

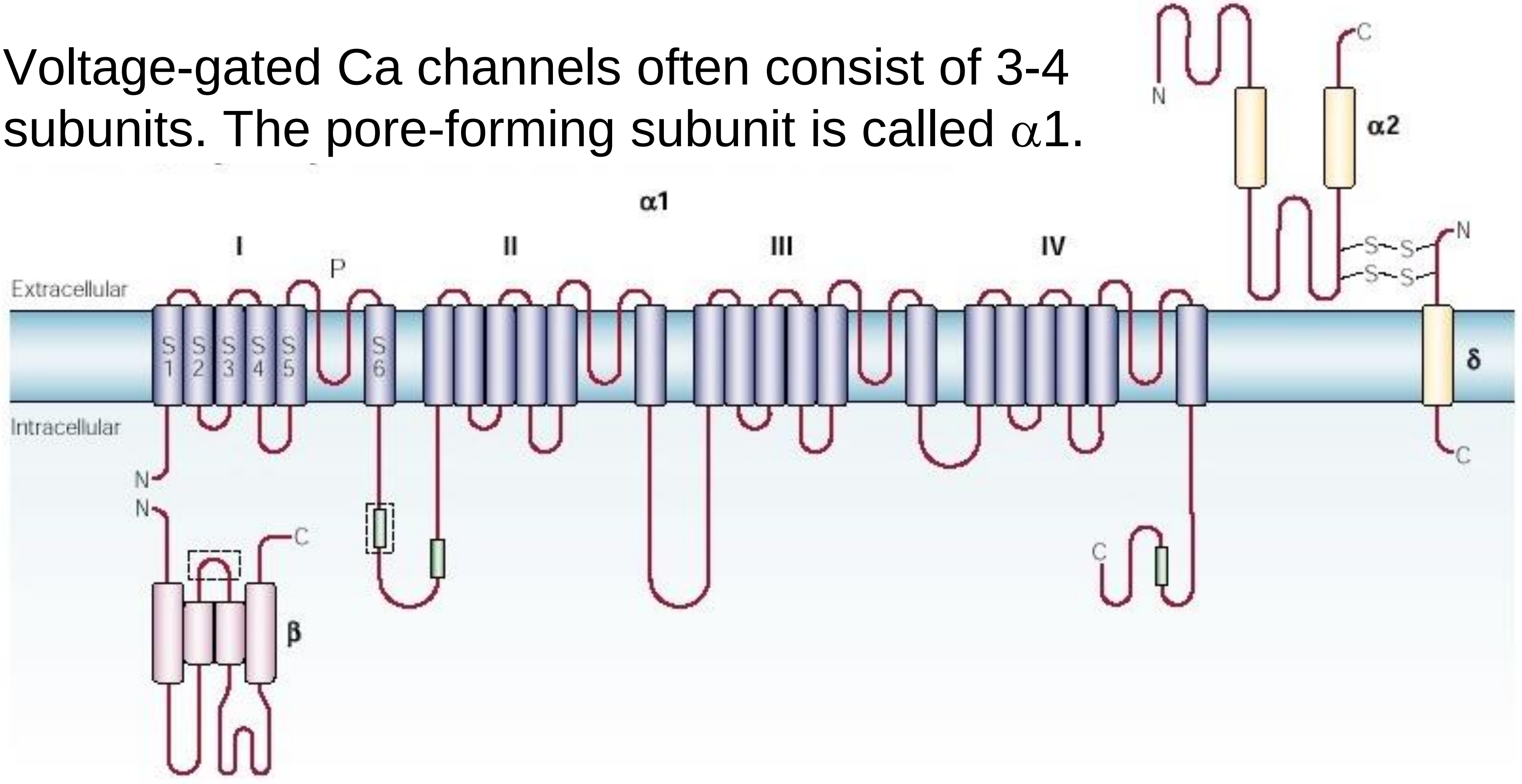
$$\boldsymbol{i}_{\text{s.ch}} = \mathbf{g}_{\text{ion}} \times (\mathbf{V} - \mathbf{V}_{\text{equil}}([\text{ion}]))$$

equilibrium (Nernst) potential

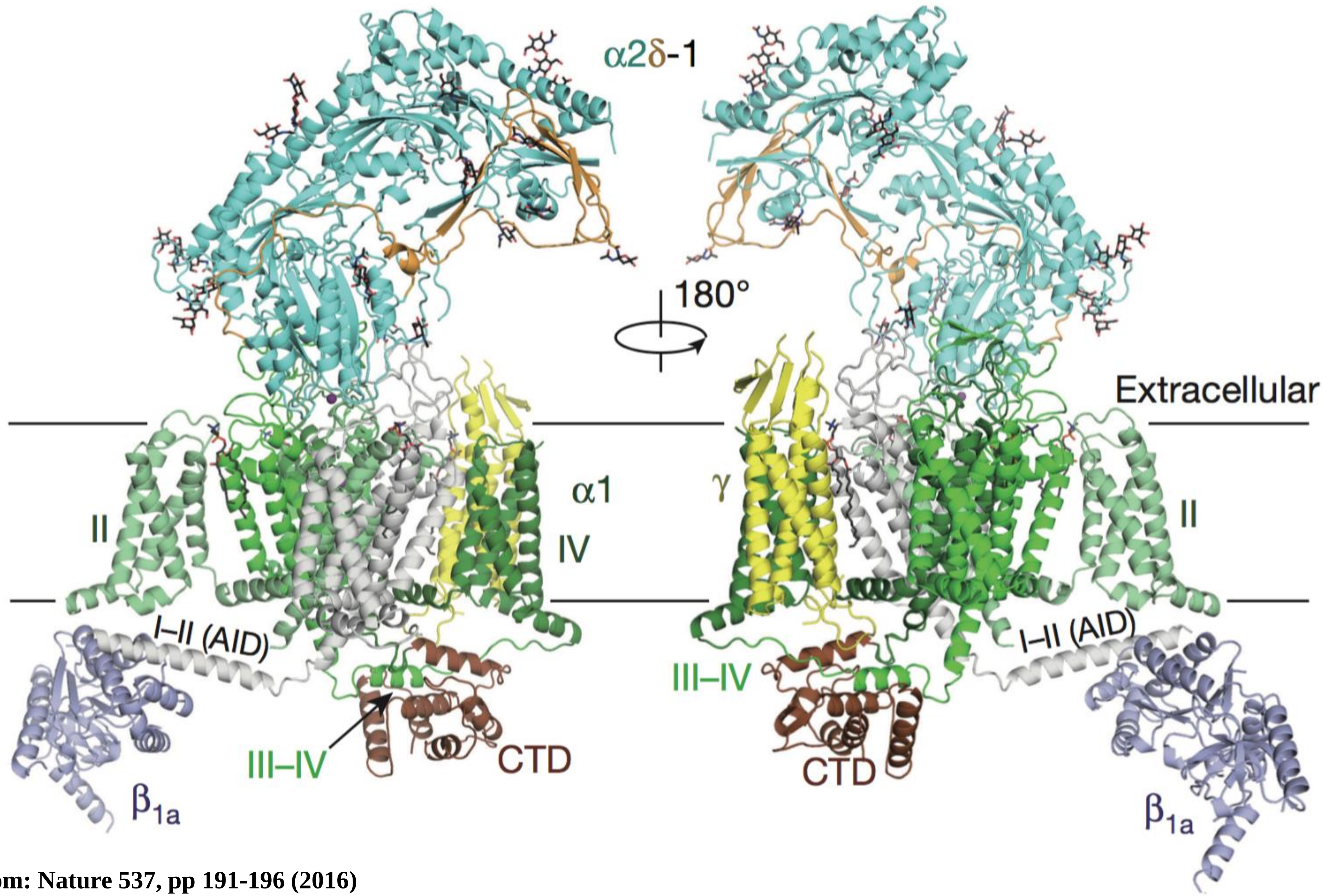
- properties of the selectivity filter (pore) do not depend on the flux
- the net driving force for ions that permeate is a linear function of voltage (empirical law, not valid when $[\text{ion}]_{out}$ is very different from $[\text{ion}]_{in}$, or when the pore of the channel is asymmetrical)

Voltage-gated channels are complex molecules:

Voltage-gated Ca channels often consist of 3-4 subunits. The pore-forming subunit is called α1.

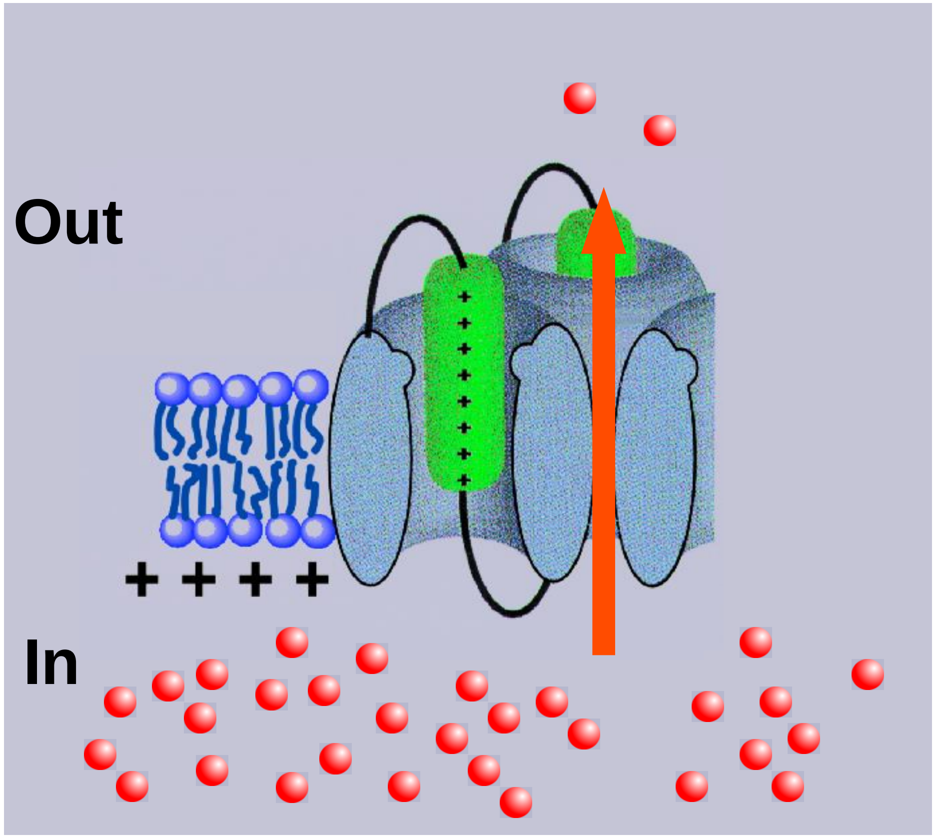


A 3.6Å structure of CaV1.1 channel :



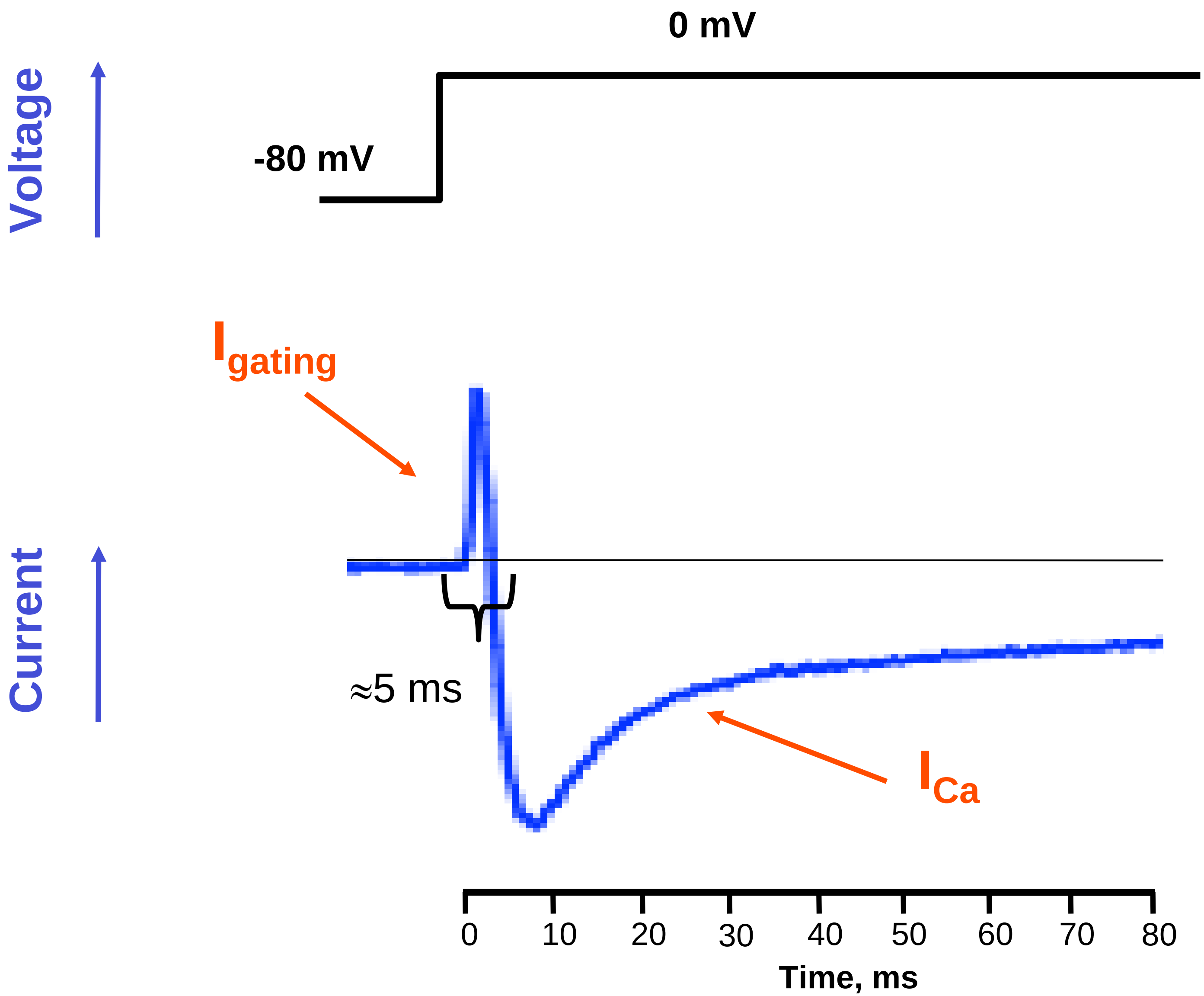
From: Nature 537, pp 191-196 (2016)

Voltage-dependent channels have voltage sensor

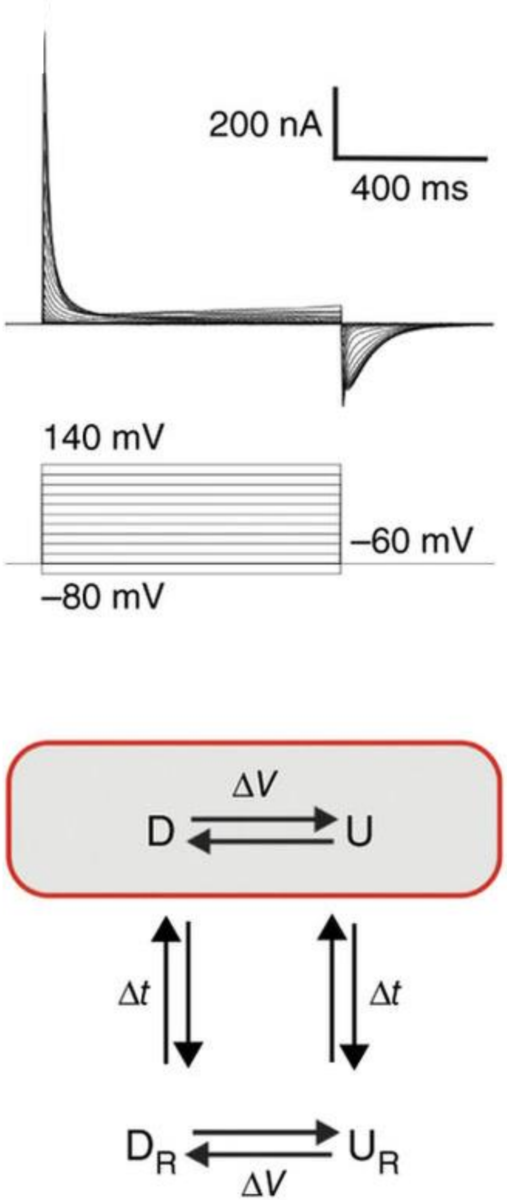


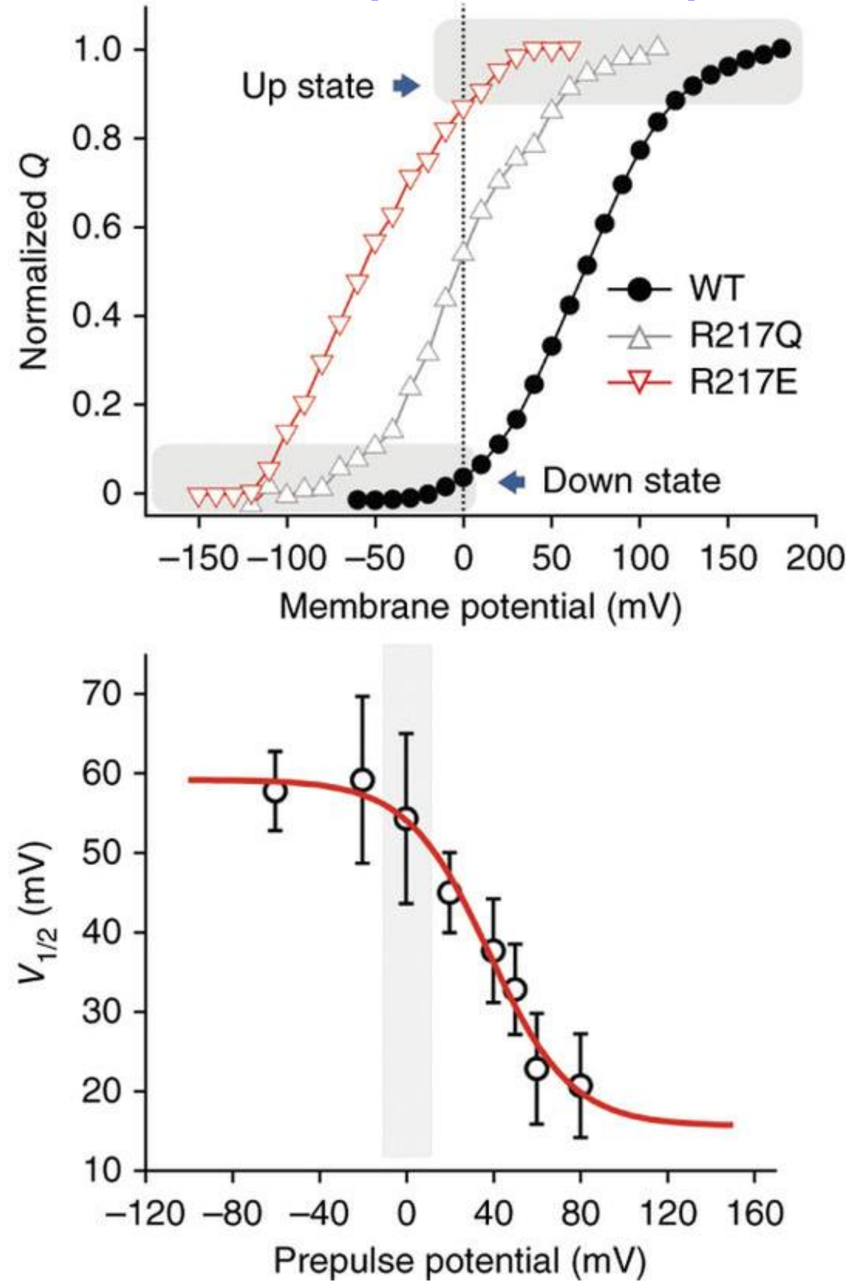
Movement of charged moieties of the channel causes the ion-selective pore to open during depolarization. Rapid movement of relatively small quantity of ions dramatically affects transmembrane voltage.

Gating current in voltage gated Ca^{2+} channels



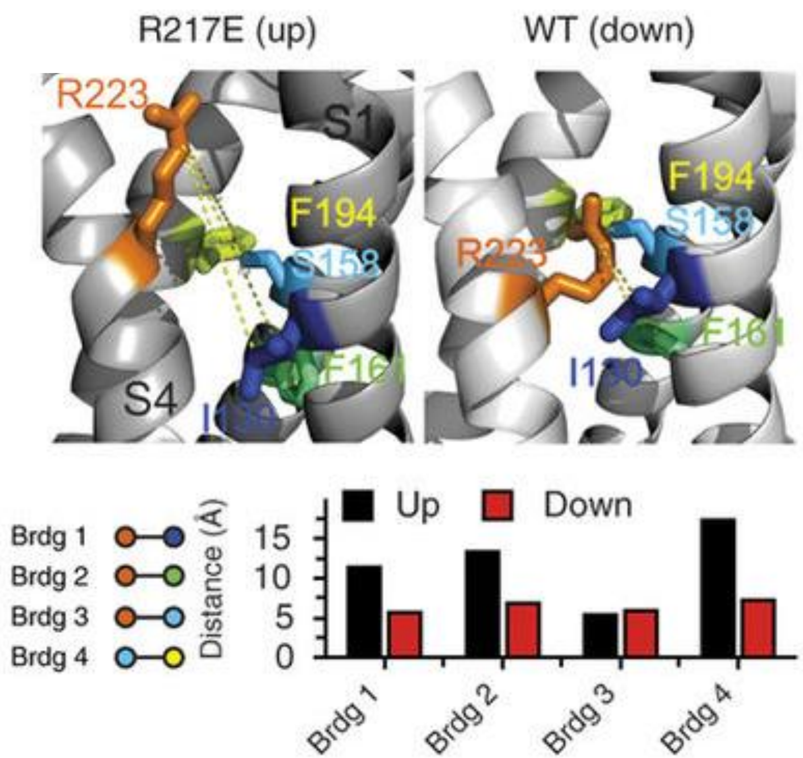
Gating charge movement has complex V-dependence

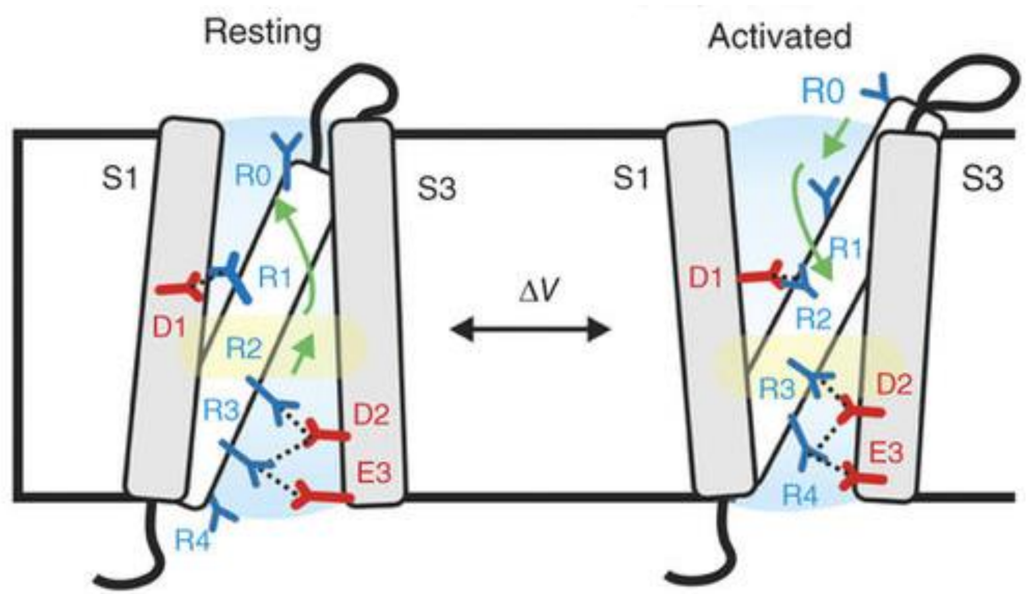




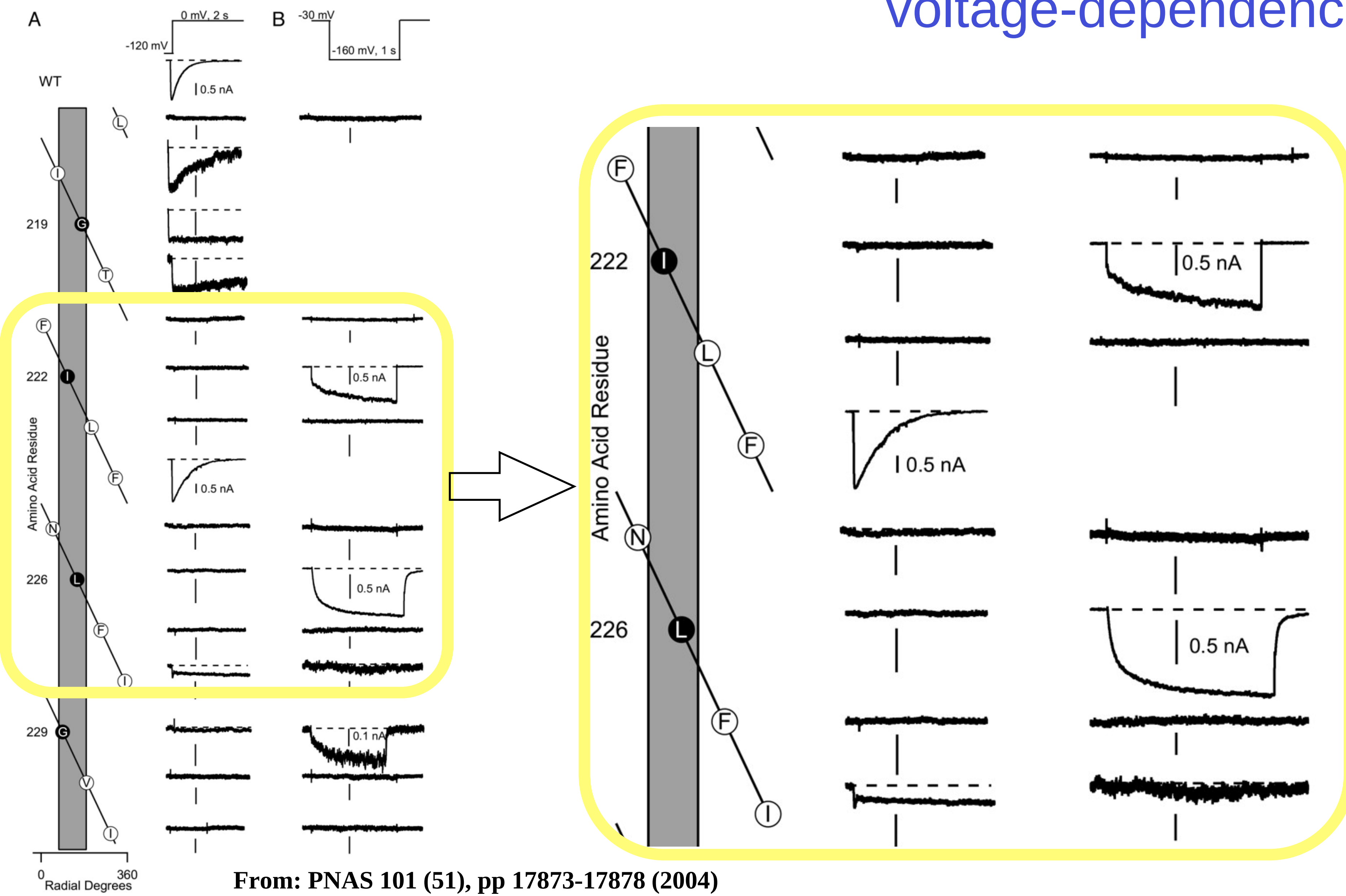
From: Nat. Struct. Mol. Biol. 21, pp 244–252 (2014)

Voltage sensor displacement and corresponding charge



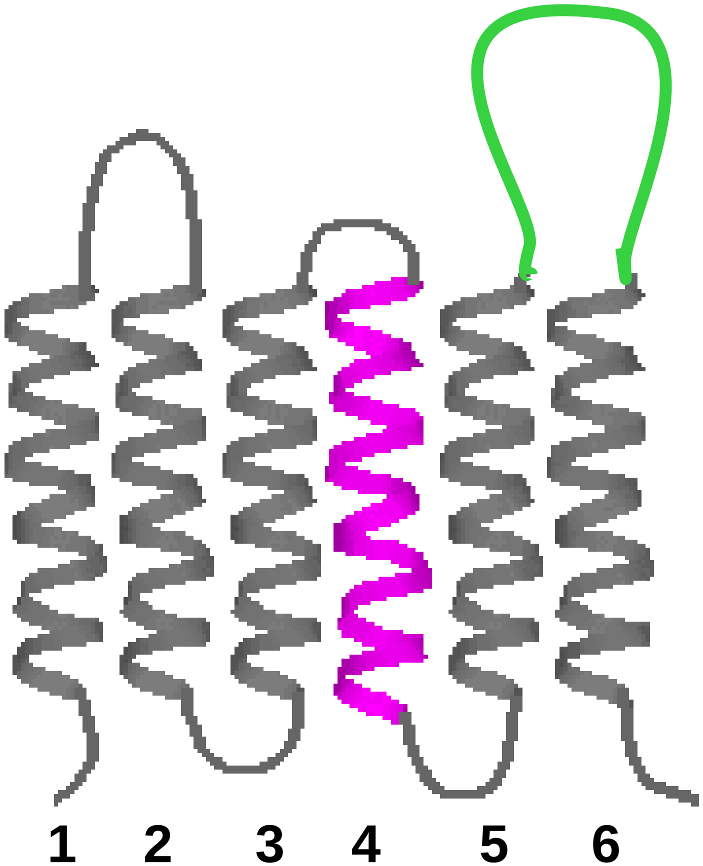


Mutations at the gate can reverse polarity of voltage-dependence



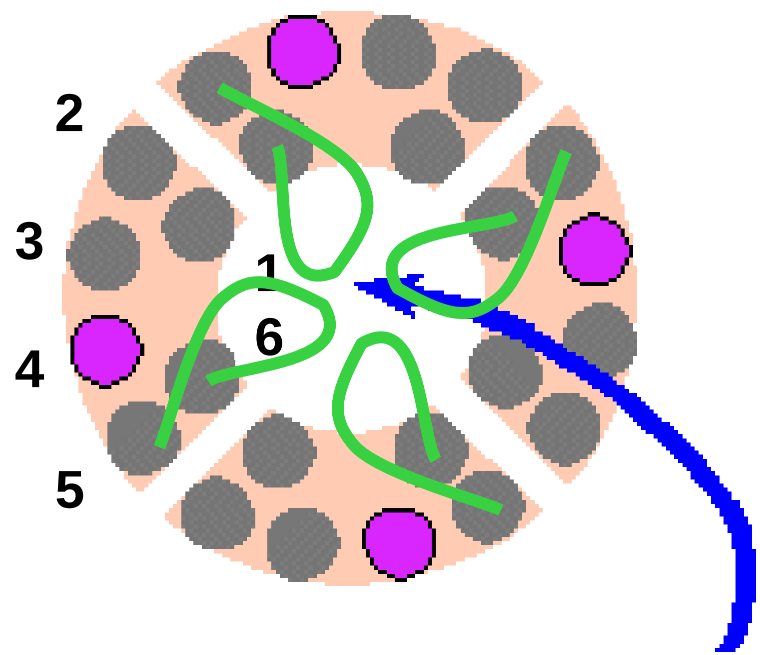
From: PNAS 101 (51), pp 17873-17878 (2004)

Common structural features of “classic” voltage-gated ion channels

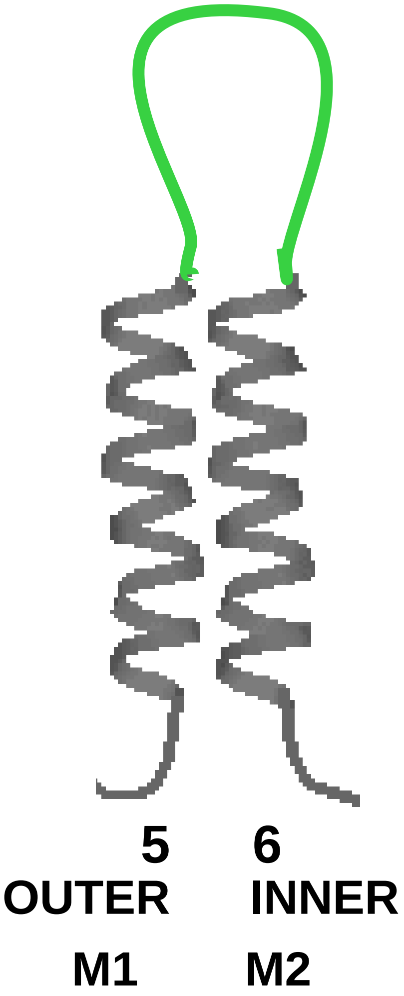


6 membrane spanning α-helixes
about 600 amino acids

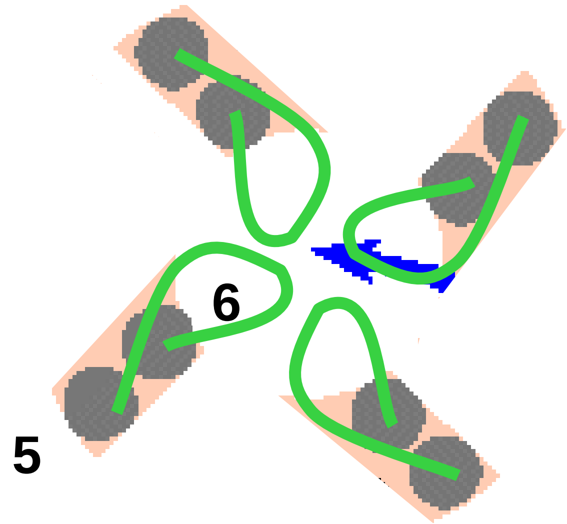
Voltage-gated K channel is a tetramer:



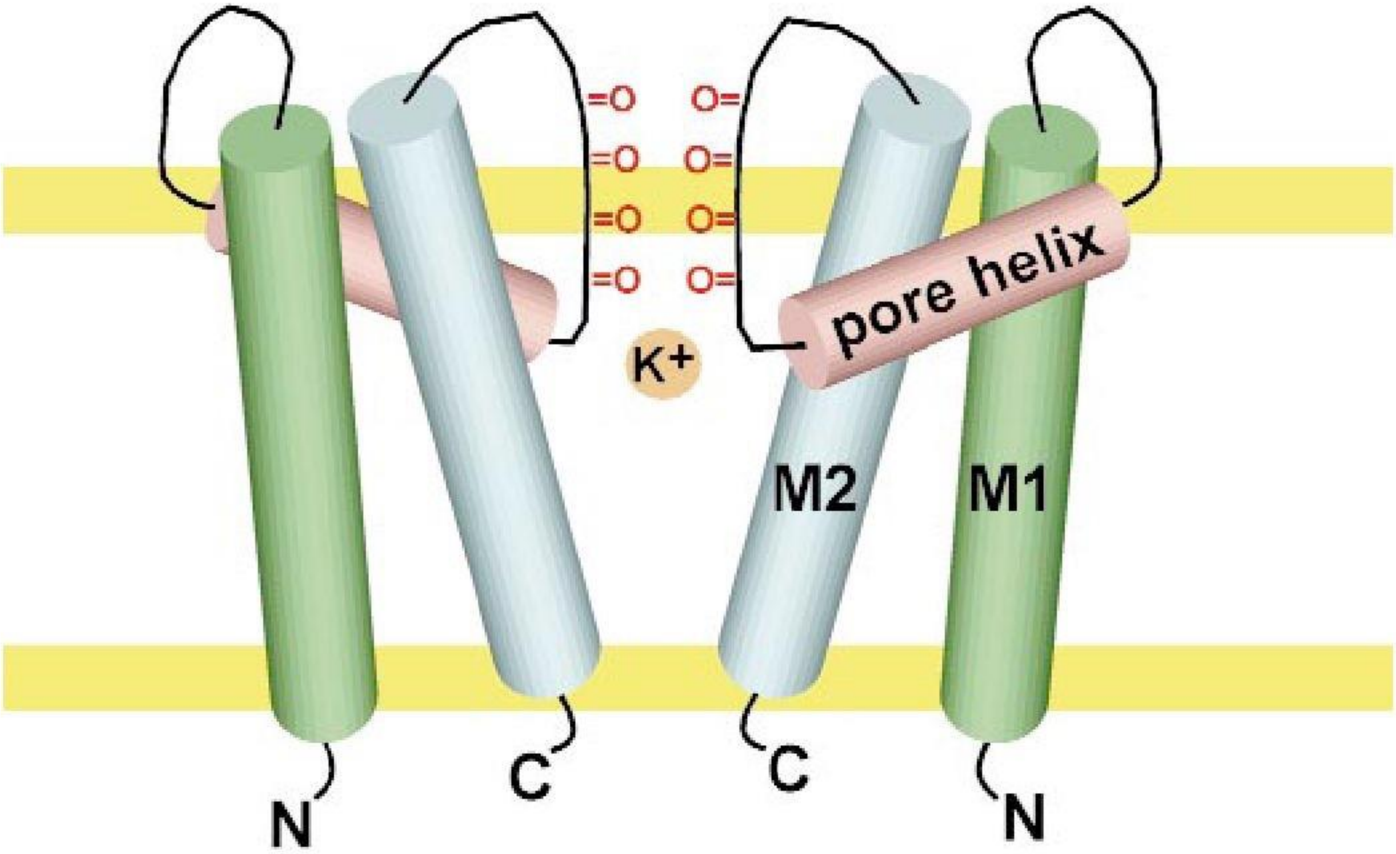
All K+ channels have similar selectivity filter



Bacterial *KcsA* channel

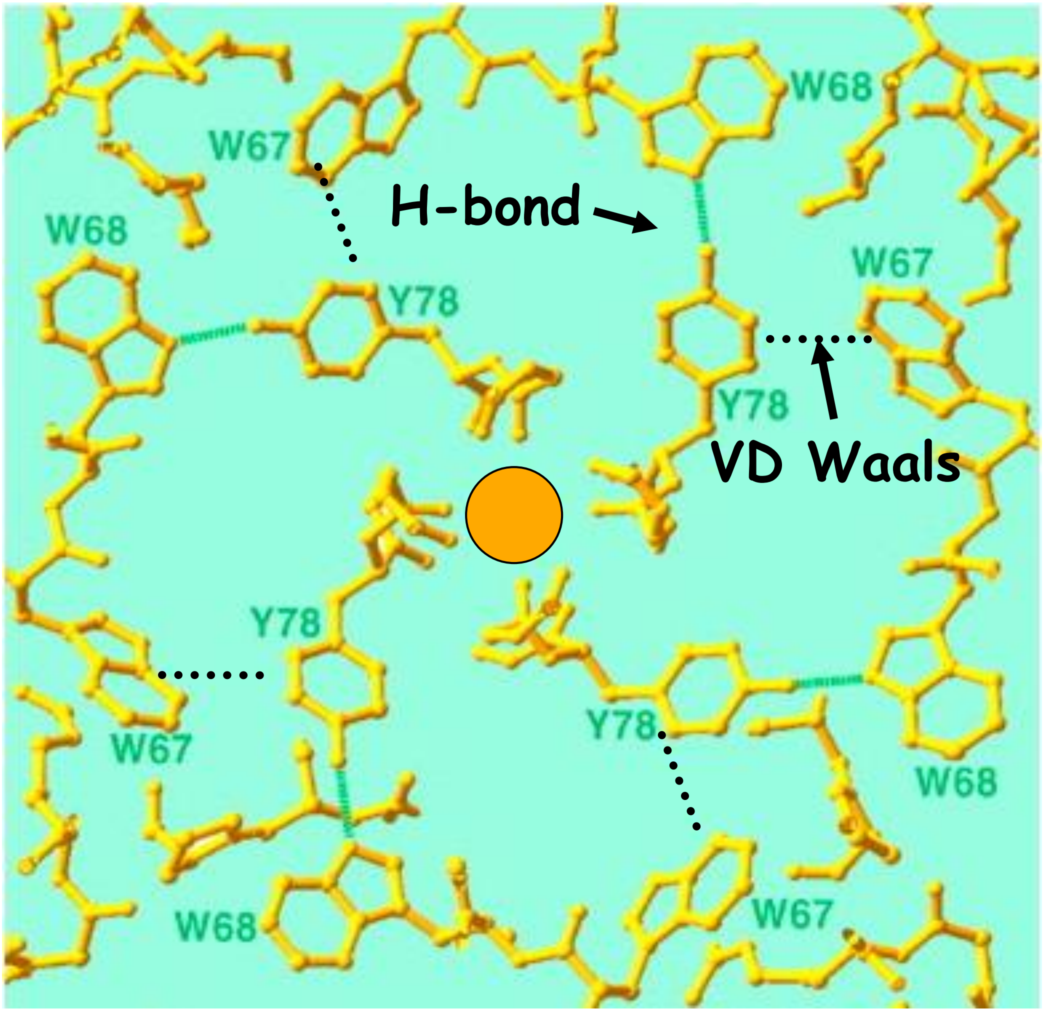


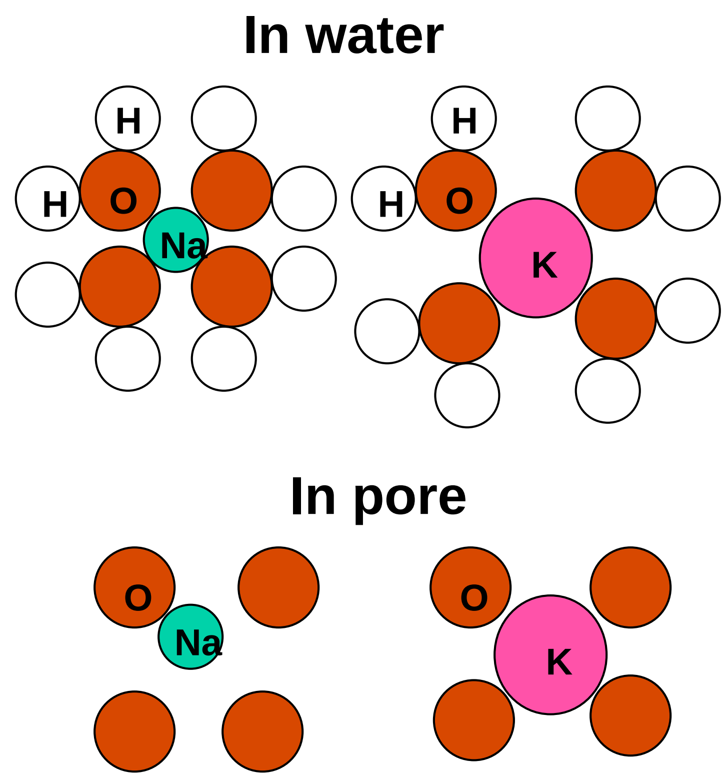
Topology of KcsA K^+ channel:



How do K channels select for K^+ ions?

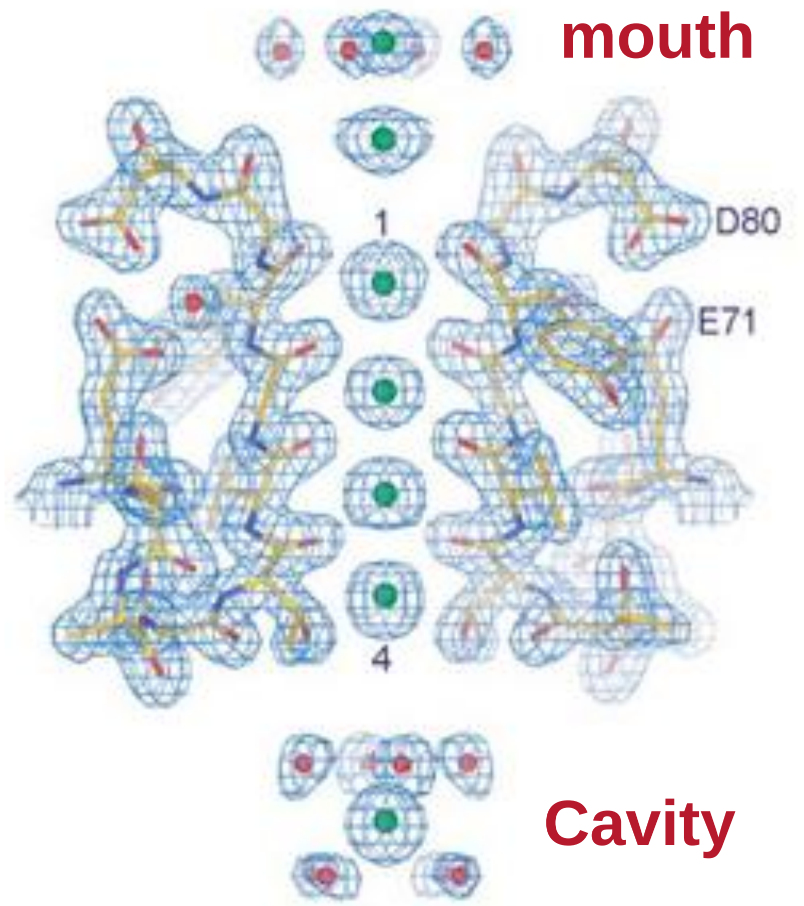
H-bonds of Van der Waals interactions of Tyr residues of GYG sequence with Trp residues of pore helices maintain filter sequence geometry for precise K^+ fit





How do ions manage to go rapidly through channels?

- Conductance ~10^8 ions/sec; each ion must cross selectivity filter within 10 ns
- mapping of pore region shows multiple K^+ positions at external mouth, pore and cavity
- K^+ ions in filter too close (3.2 Å) to be 4 individual K^+ ions - due to charge repulsion
- K^+ distribution represents alternate K^+ positions of equal probability

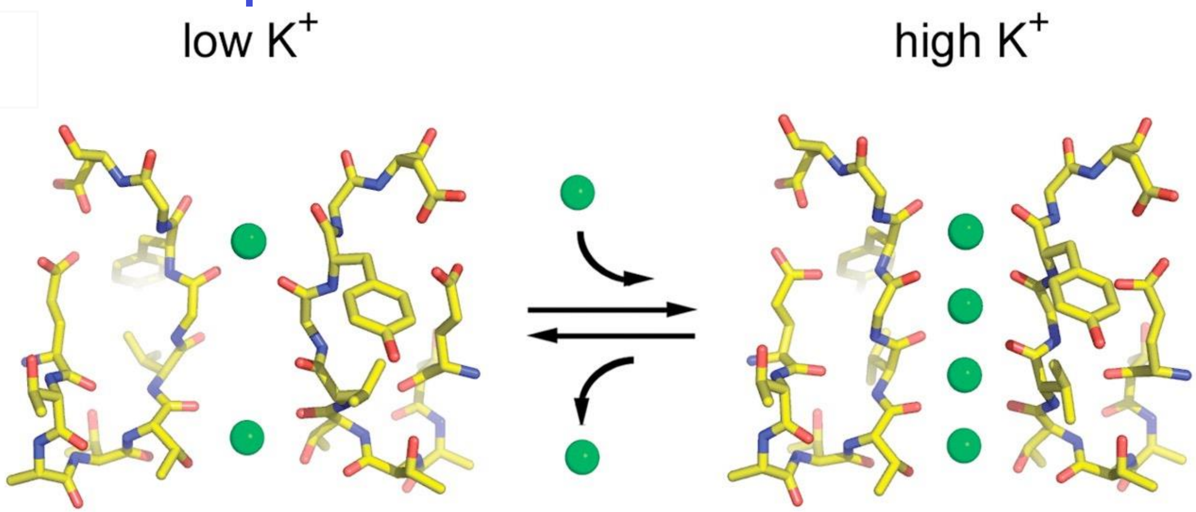


Ion binding and changes of selectivity regions in different transporters

K^+ channel:

Small, hence,

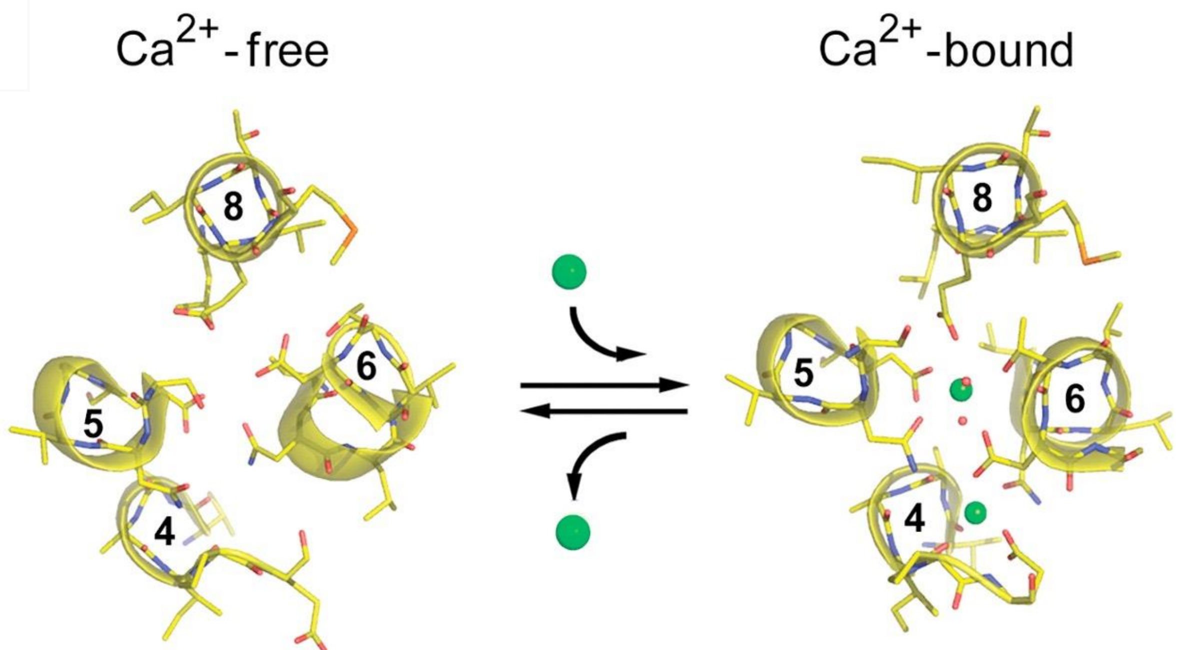
fast movements



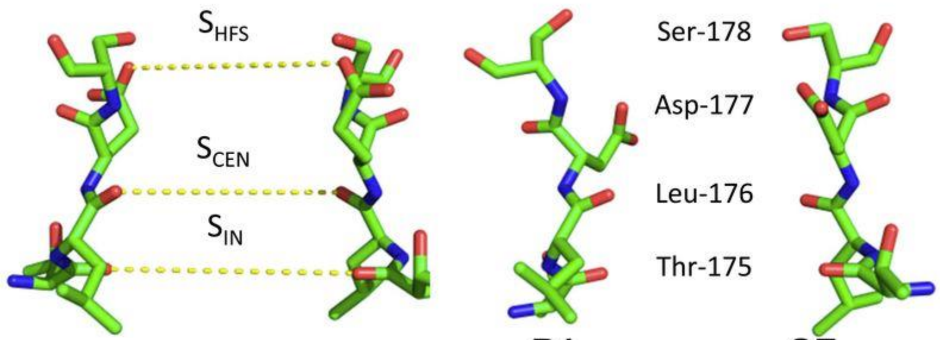
Ca^{2+} ATPase:

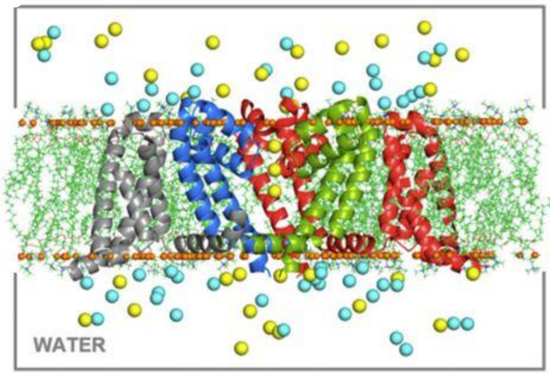
Large, hence,

slow movements



Ca^{2+}, bacterial Na^{+}, and eukaryotic Na^{+}channels select by different mechanisms

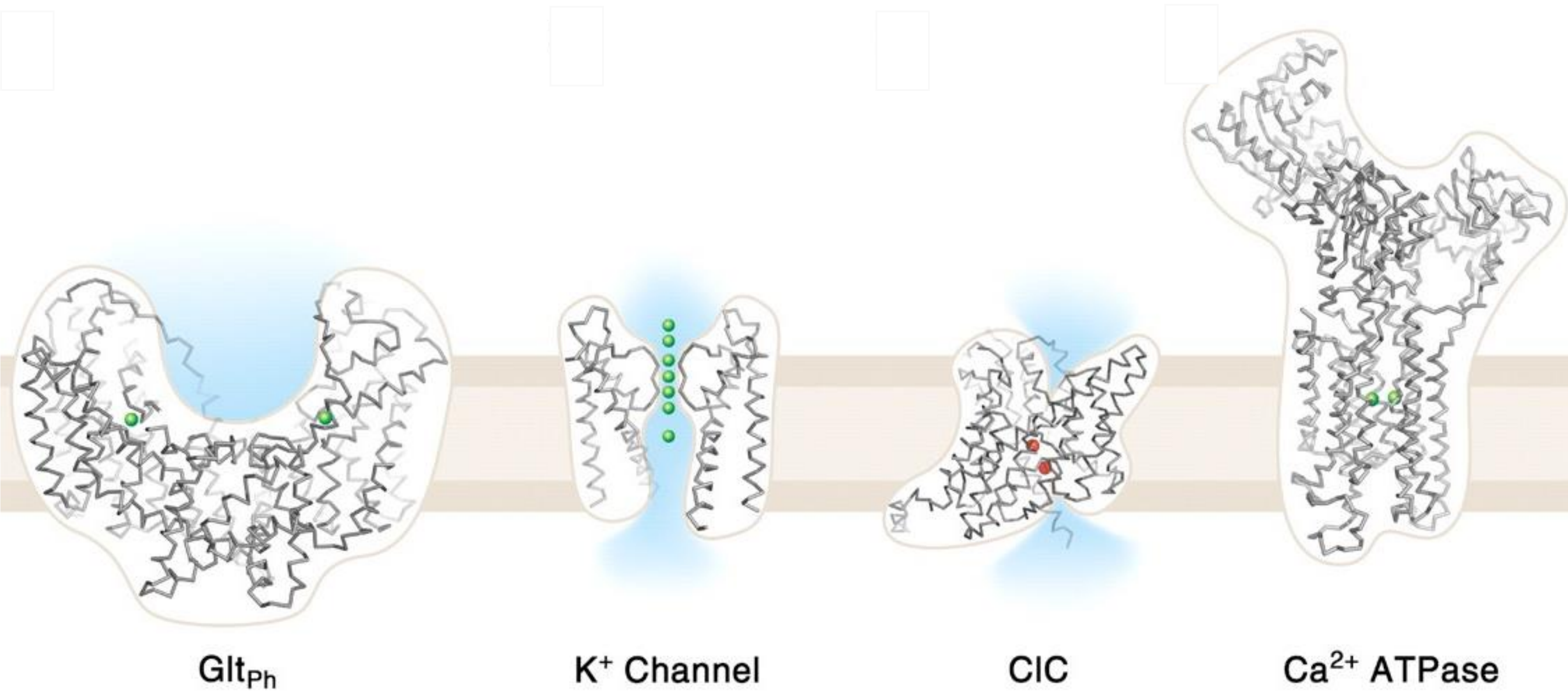




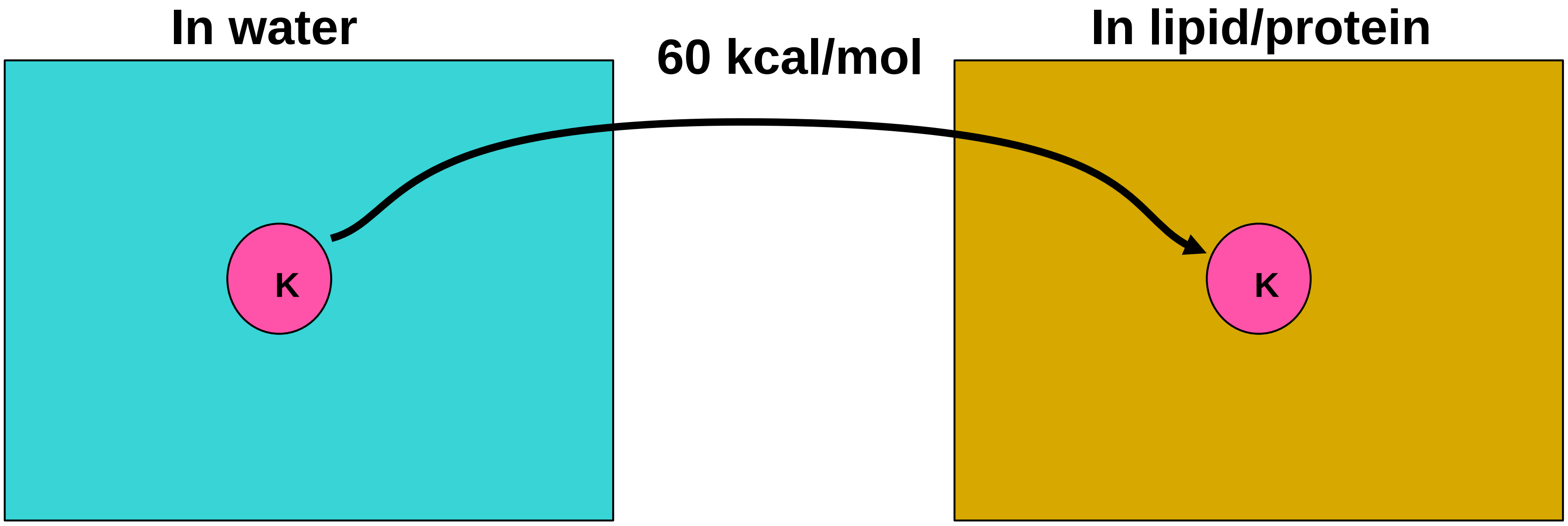
		P1 / **SF** / P2		Ident	Simil	Q_{SF}
NaChBac	173	YFGNLQLSLLTLFQVV**TLESWAS**GVMRPIFAEVWWS	208	100	100	−4
NavAb	159	WFGTLGESFYTLFQVM**TLESWSM**GIVRPLMEVYPYA	194	61	79	−4
NavRh	161	SRWDLGISLITLFQVL**TLSSWET**VMLPMQEIYWWSW	196	55	73	−4
rSkM1-DI	382	SYDTFSWAFLALFRLM**TQDYWEN**LFQLTLRAAGKTY	417	31	69	−5
rSkM1-DII	737	HMNDFFHSFLIVFRIL**TGEWIET**MWDCMEVAGQAMC	772	0	0	
rSkM1-DIII	1119	NYDNVGLGYLSLLQVA**TFKGWMD**IMYAAVDSREKEQ	1154	0	0	
rSkM1-DIV	1510	FNFETFGNSICLFEIT**TSAGWDG**LLNPILNSGPPDC	1545	0	0	
hCav1.2-DI	345	NFDNFAFAMLTVFQCI**TMEGWTD**VLYWMQDAMGYEL	380	41	64	−8
hCav1.2-DII	688	TFDNFPQSLLTVFQIL**TGEDWNS**VMYDGIMAYGGPS	723	47	60	
hCav1.2-DIII	1117	DFDNVLAAMMALFTVS**TFEGWPE**LLYRSIDSHTEDK	1152	36	54	
hCav1.2-DIV	1446	NFQTFPQAVLLPLRCA**TGEAWQD**IMLACMPGKKCAP	1478	0	0	
Shaker	423	NSFFKSIPDAFWWAVVTMT**TV*GYG*D**MTPVGVWGKIV	458	40	60	0
KvAP	177	NSSIKSVFDALWWAVVTAT**TV*GYG*D**VVPATPIGKVI	212	0	0	0

From: JGP 143, pp 157-171 (2014)

Water access to different transporters

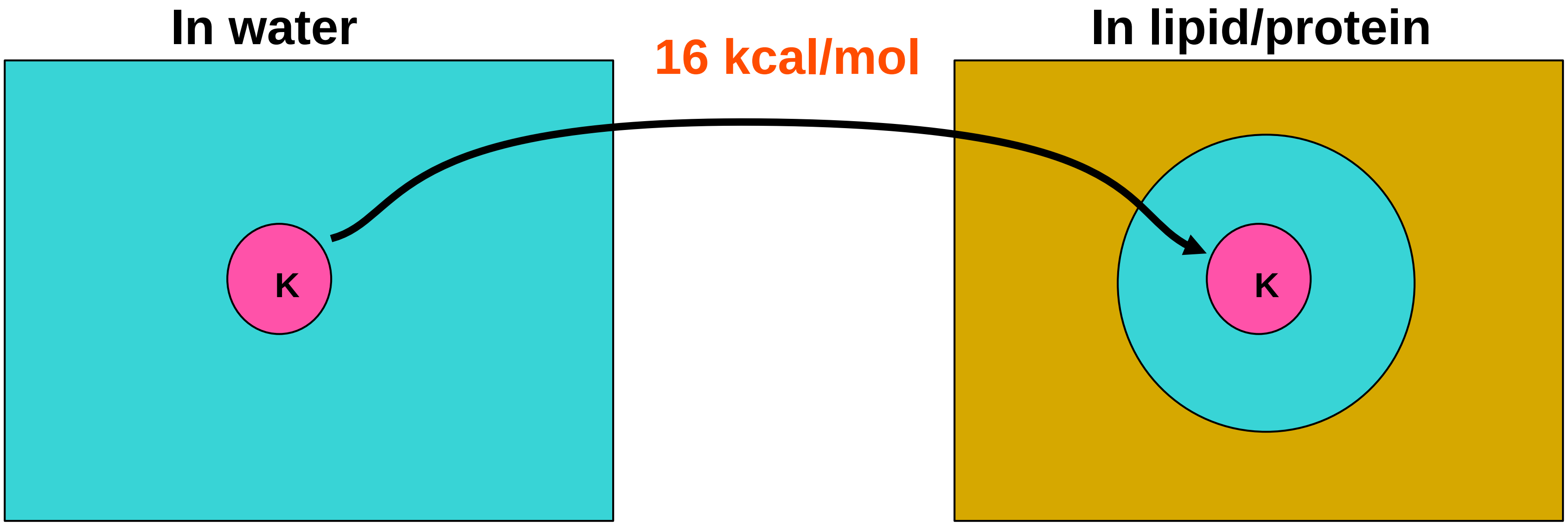


How do channels overcome the destabilizing influence of the low dielectric membrane environment?



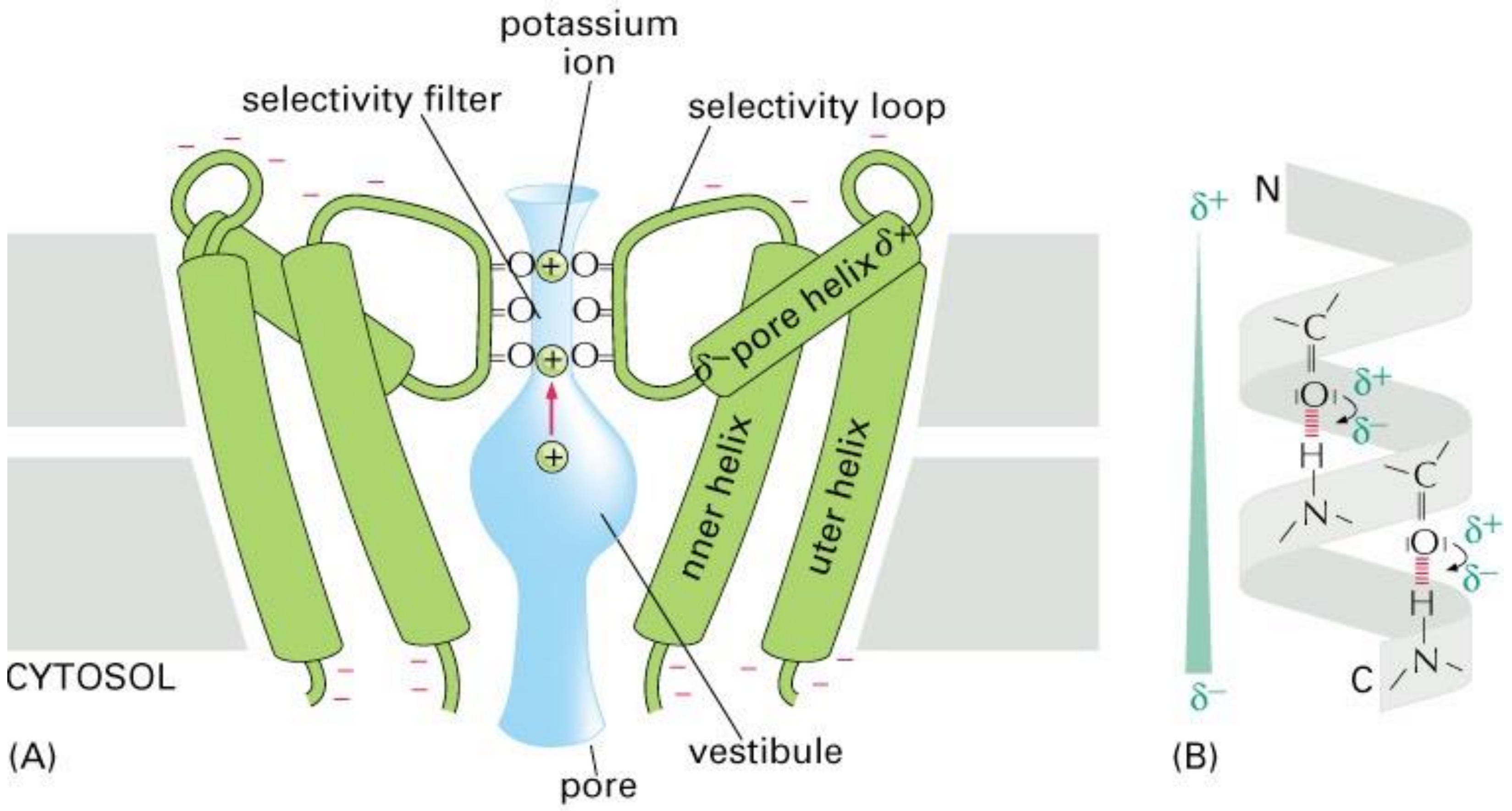
Transfer of an ion from water to environment with low electrical polarizability is energetically prohibited

The water filled cavity significantly lowers energy of the dielectric barrier



Transfer of an ion to water filled cavity (about 20 water molecules) surrounded by medium with low dielectric polarizability is less costly.

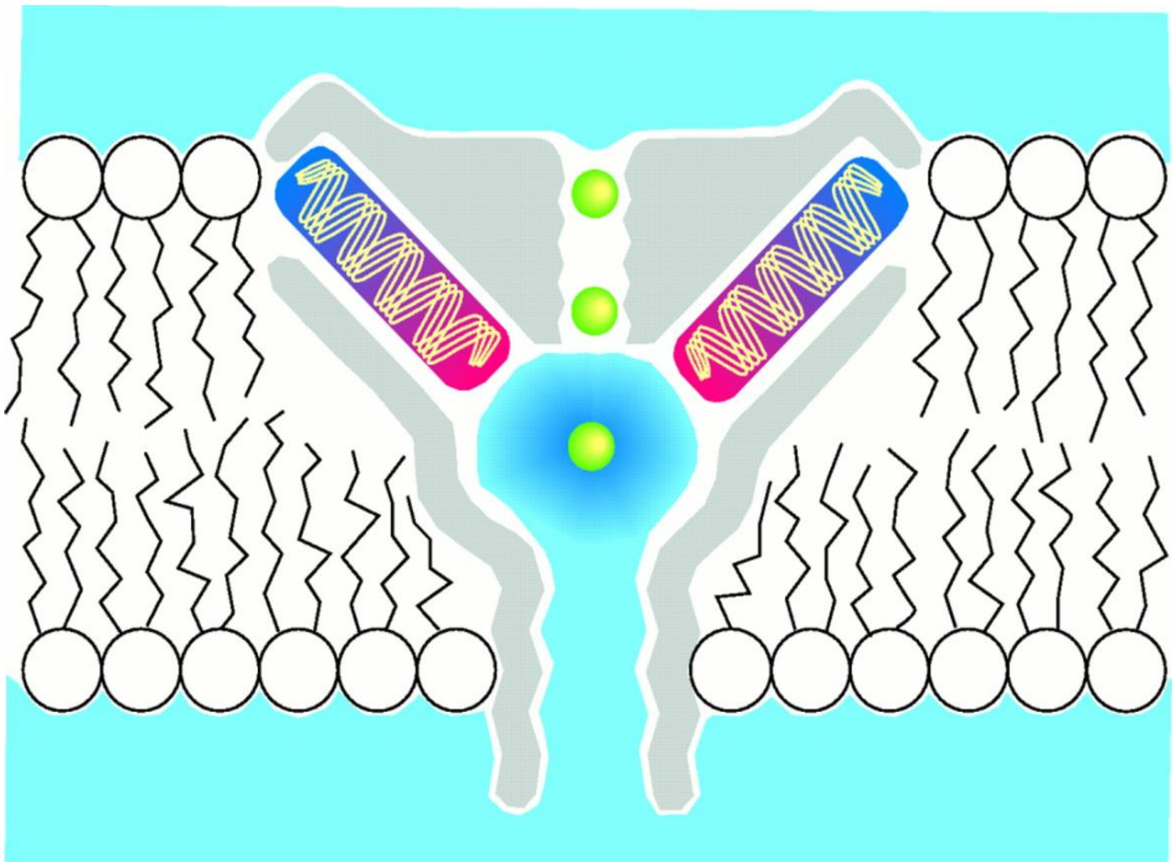
Pore helices may have large electrostatic effect



Polarization of α-helices is shielded in water soluble proteins.

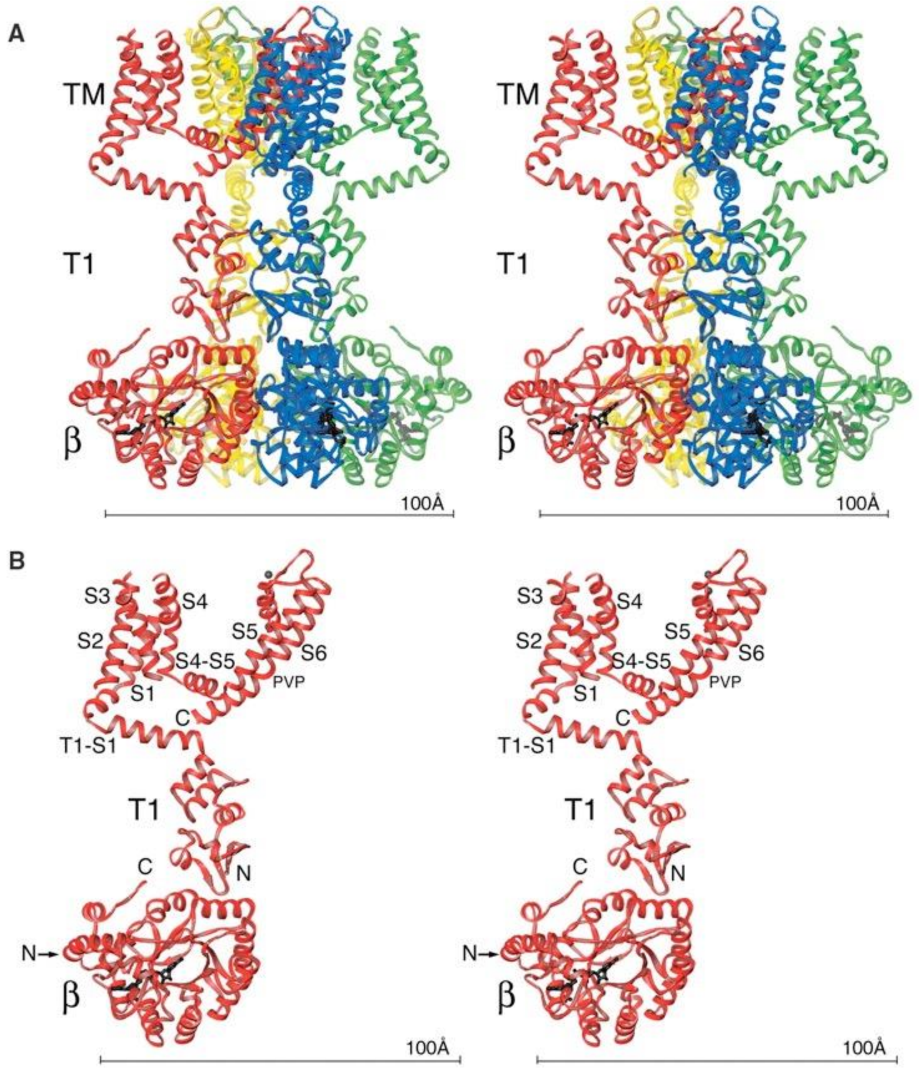
But it is significant in proteins surrounded by lipids.

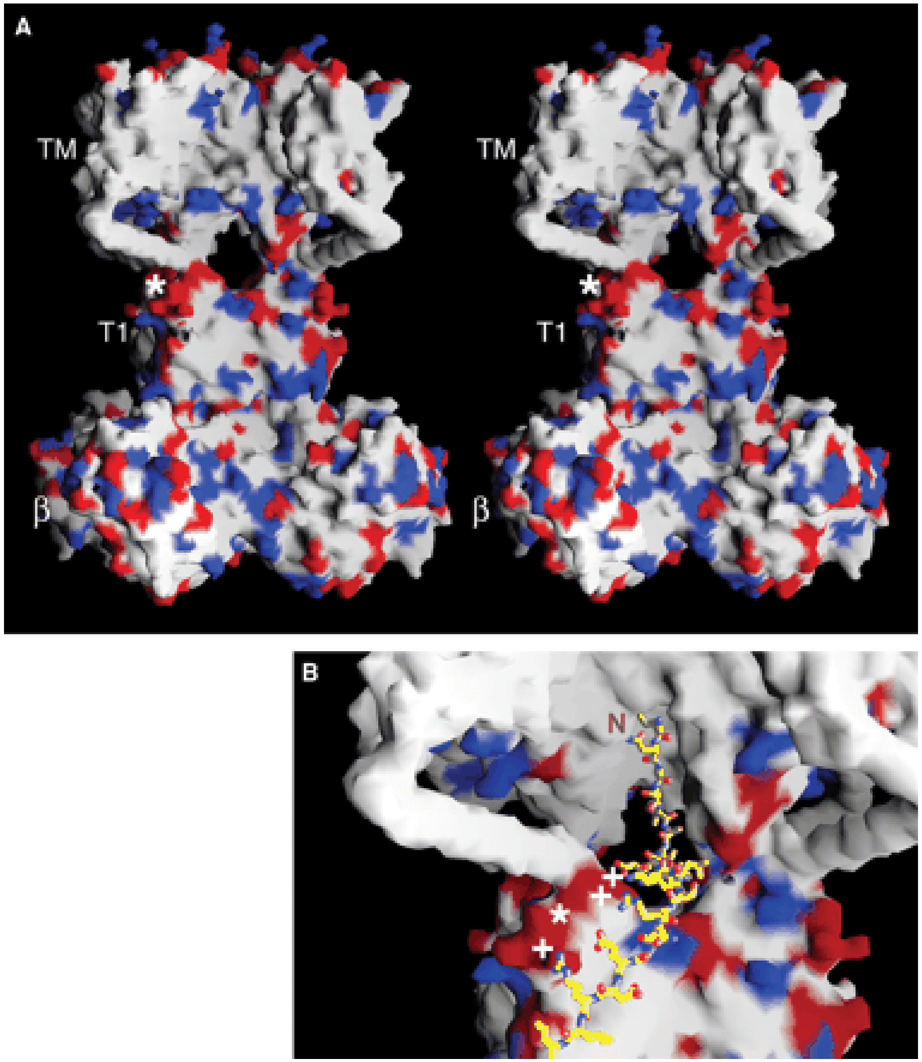
Pore helices further stabilize ions in the membrane



Free energy for transfer of a single K^+ ion from bulk water to the cavity of a channel with two more K^+ in the selectivity filter is –8.5 kcal/mol!

Where is the inactivation gate?





Role of selectivity structure in inactivation gating.