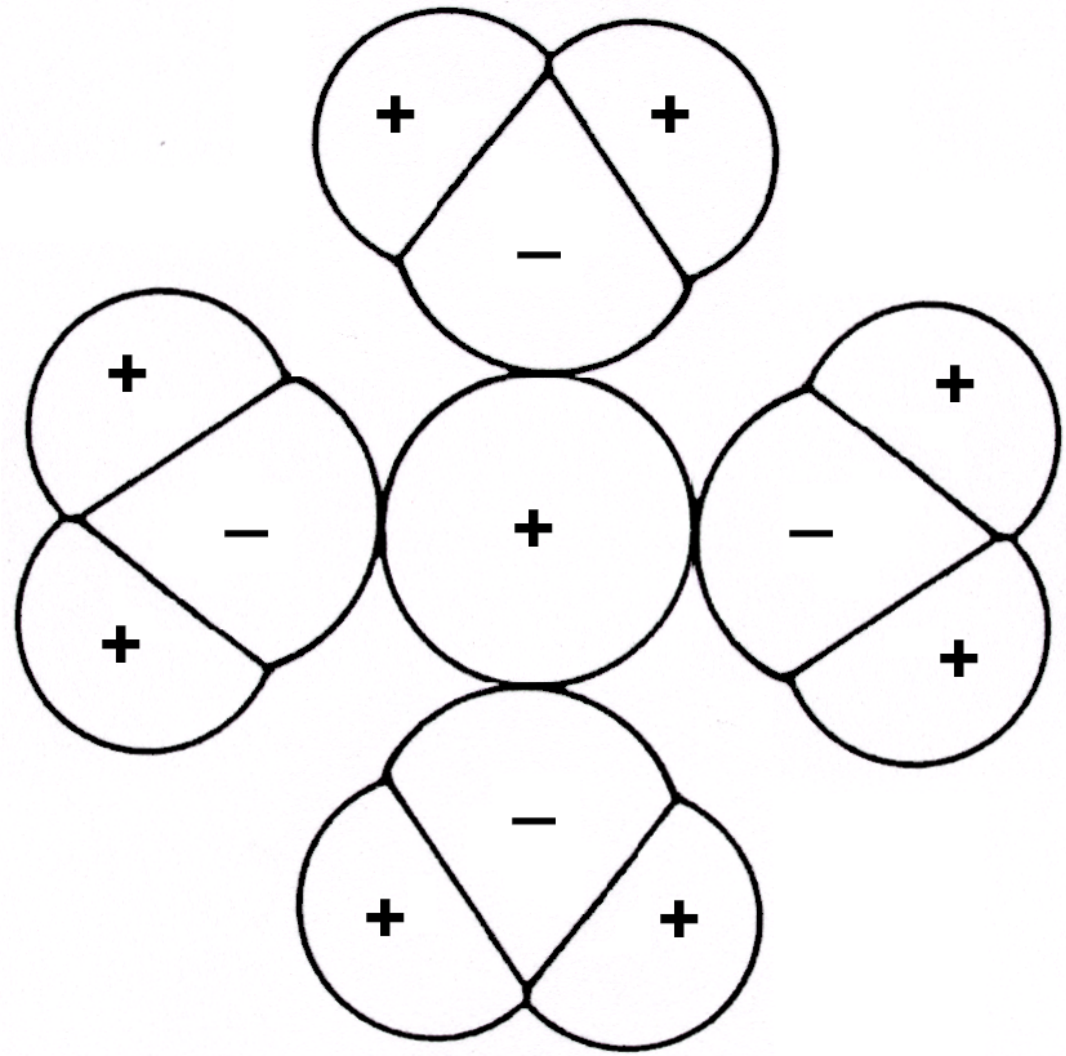
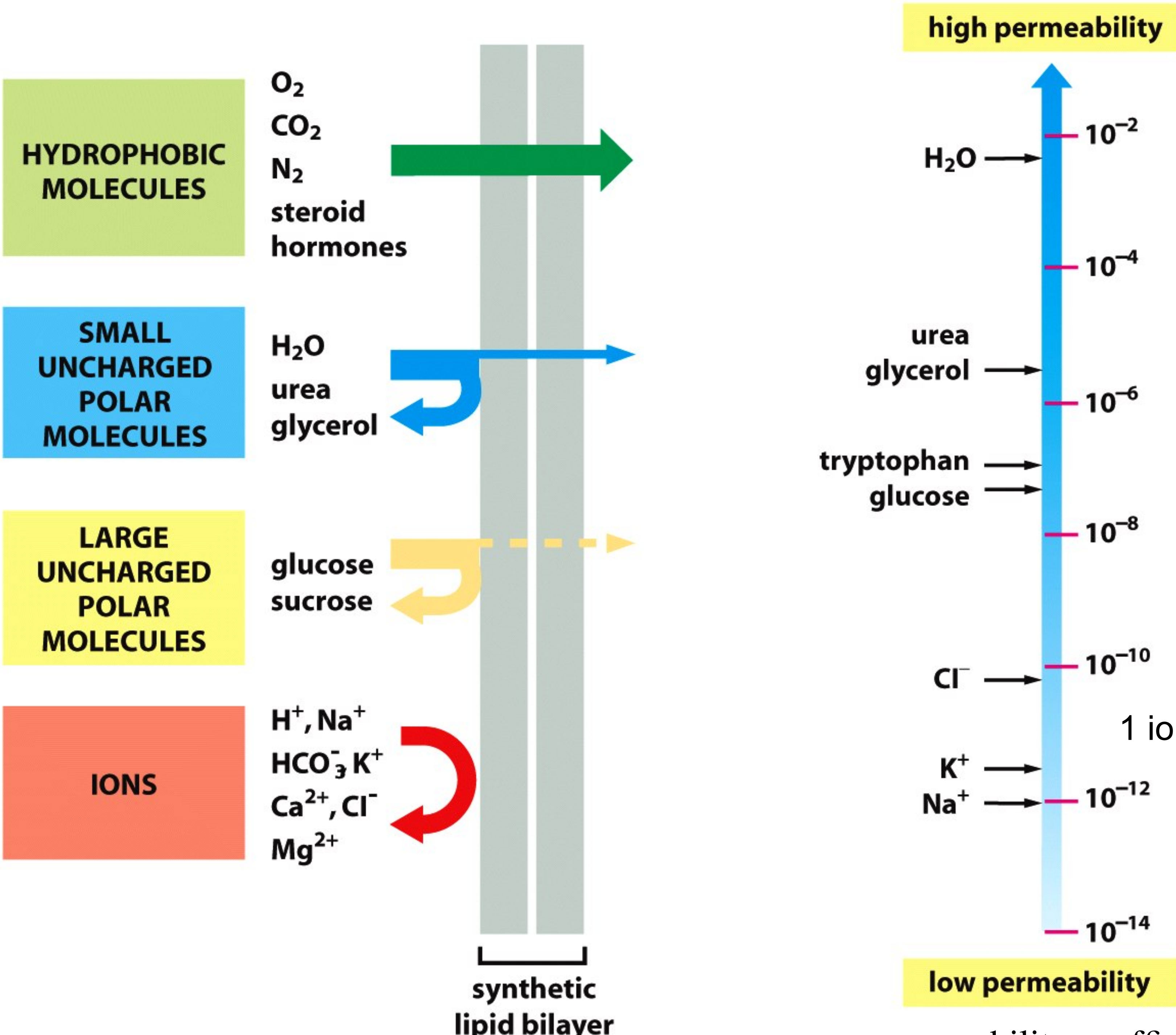
- properties of membranes
- selectivity of $K^+$ channels
- $K^+$ channel gating

# Ions are stable in water






1 ion every ~10 hours

permeability coefficient (cm/sec)

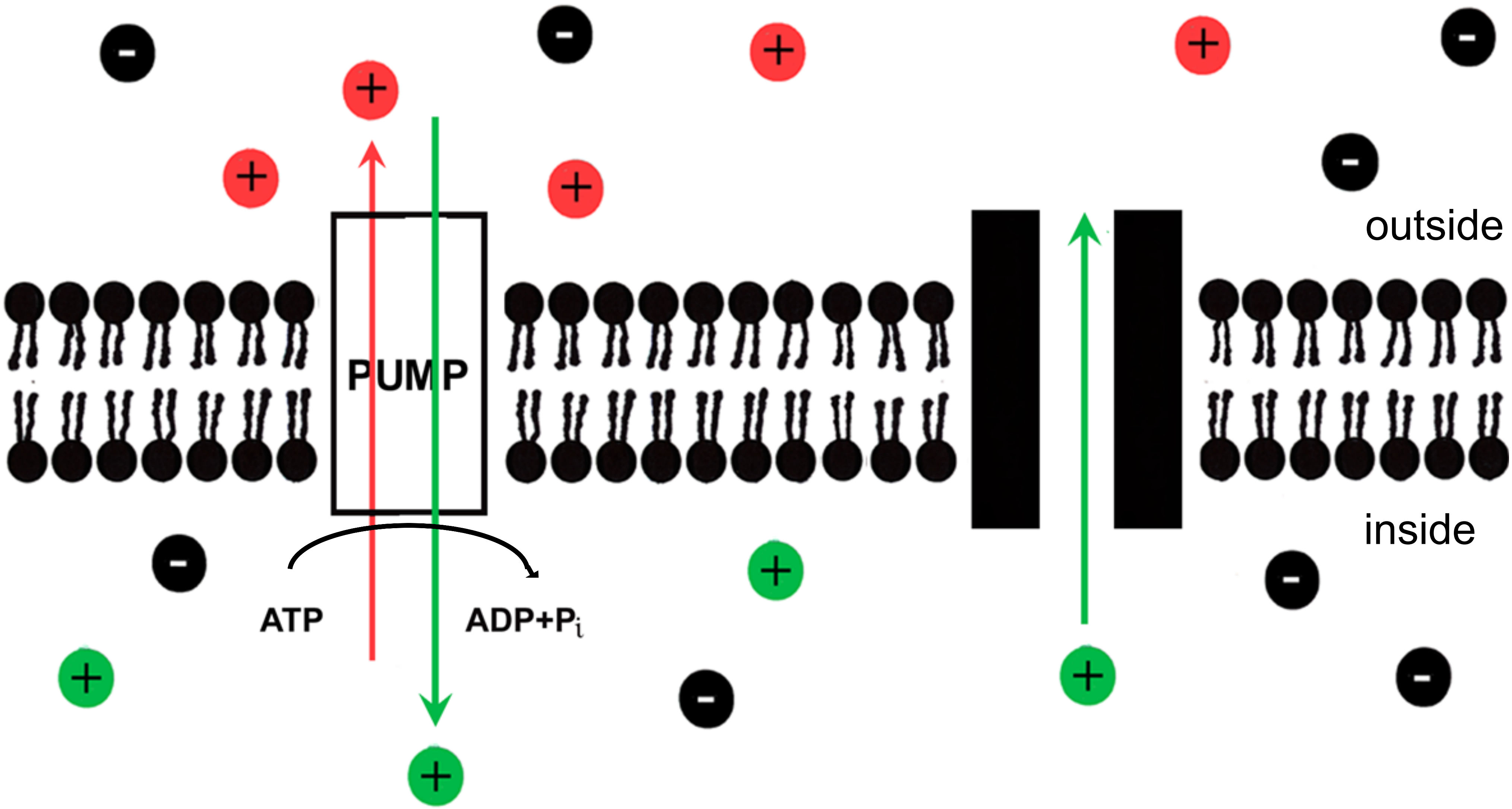
Figure 11-1 *Molecular Biology of the Cell* (© Garland Science 2008)

**Table 11–1 A Comparison of Ion Concentrations Inside and Outside a Typical Mammalian Cell**

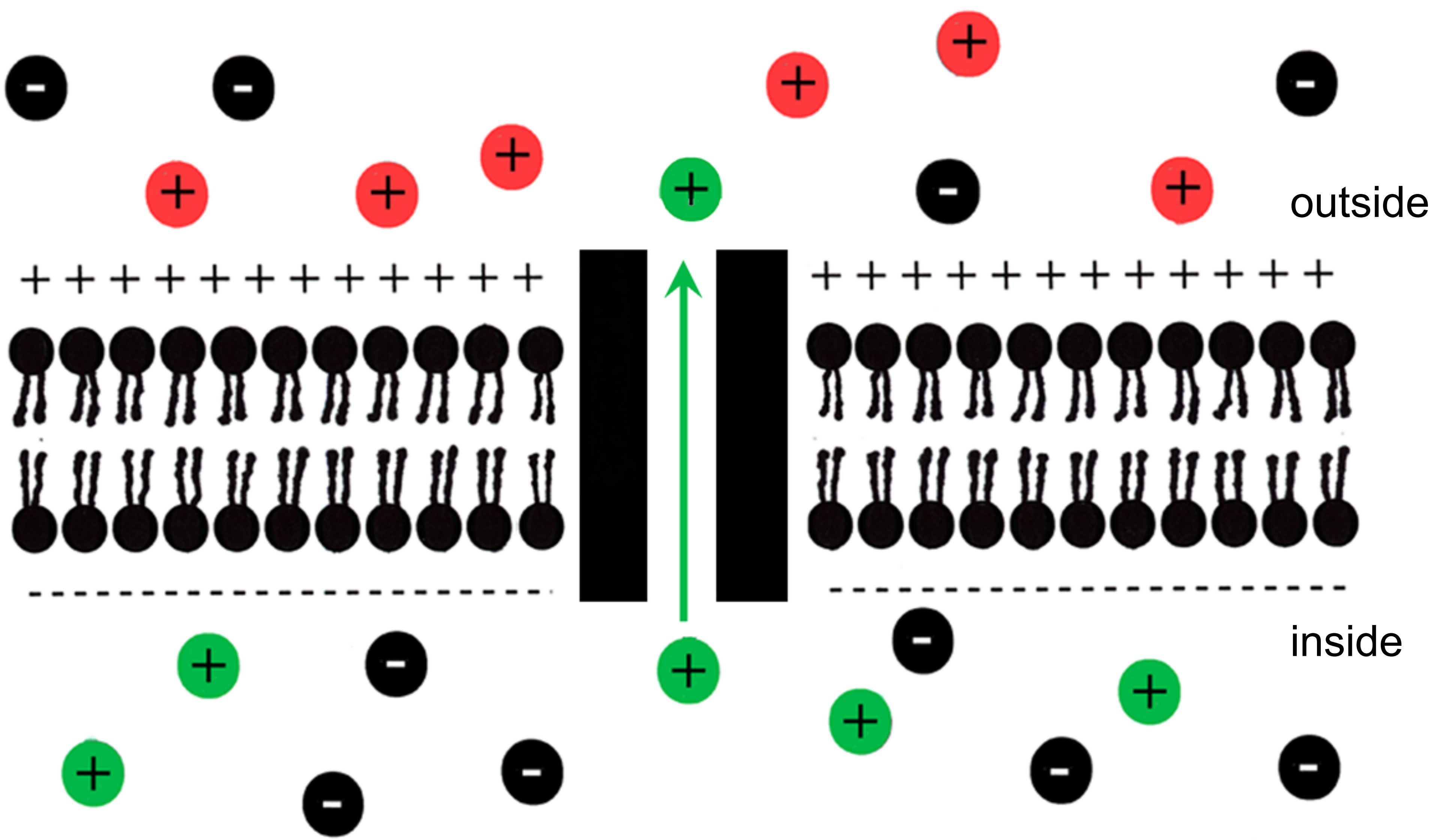
| COMPONENT | INTRACELLULAR CONCENTRATION (mM) | EXTRACELLULAR CONCENTRATION (mM) |
|---|---|---|
| **Cations** | | |
| $Na^+$ | 5–15 | 145 |
| $K^+$ | 140 | 5 |
| $Mg^{2+}$ | 0.5 | 1–2 |
| $Ca^{2+}$ | $10^{-4}$ | 1–2 |
| $H^+$ | $7 \times 10^{-5}$ ($10^{-7.2}$ M or pH 7.2) | $4 \times 10^{-5}$ ($10^{-7.4}$ M or pH 7.4) |
| **Anions*** | | |
| $Cl^-$ | 5–15 | 110 |

*The cell must contain equal quantities of positive and negative charges (that is, it must be electrically neutral). Thus, in addition to $Cl^-$, the cell contains many other anions not listed in this table; in fact, most cell constituents are negatively charged ($HCO_3^-$, $PO_4^{3-}$, proteins, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.). The concentrations of $Ca^{2+}$ and $Mg^{2+}$ given are for the free ions. There is a total of about 20 mM $Mg^{2+}$ and 1–2 mM $Ca^{2+}$ in cells, but both are mostly bound to proteins and other substances and, for $Ca^{2+}$, stored within various organelles.

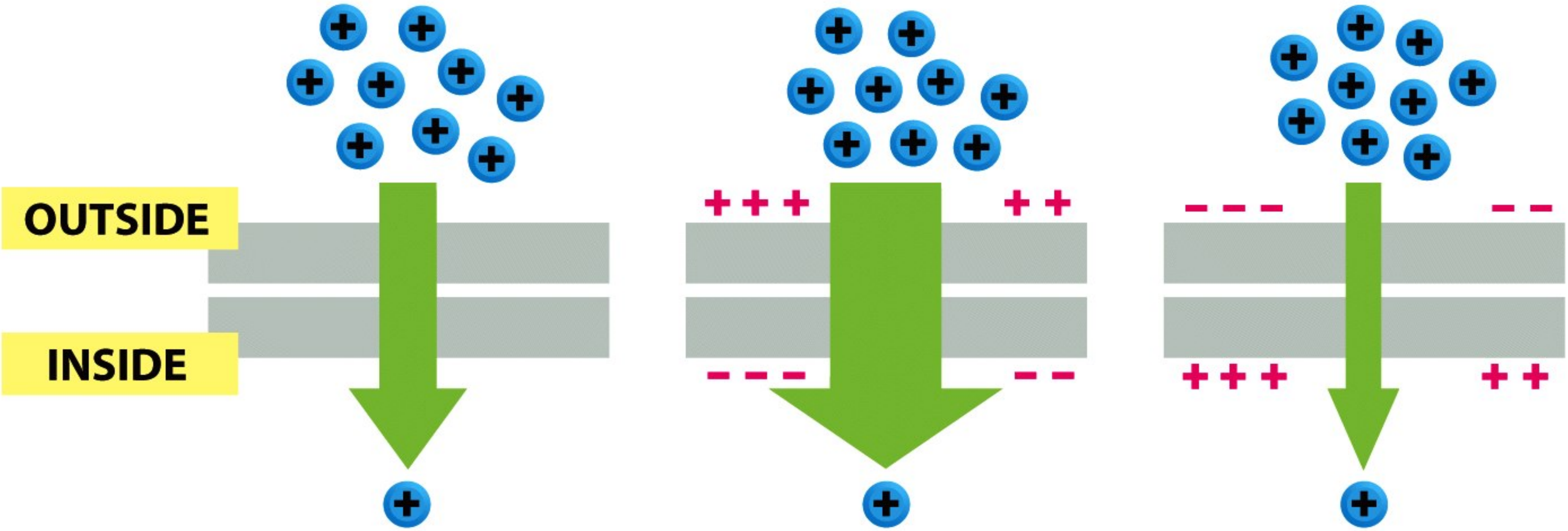
# Pumps build ion gradients, ion channels dissipate gradients



# Ion channels electrically polarize the cell membrane



# Electrochemical gradient

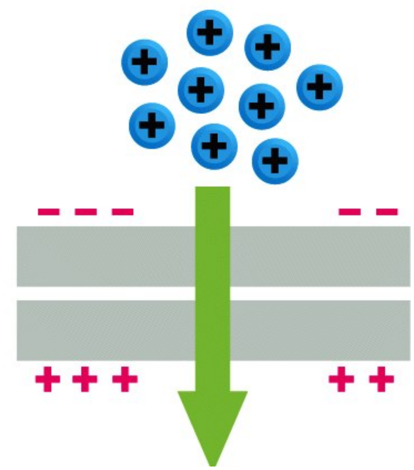



electrochemical gradient with no membrane potential

electrochemical gradient with membrane potential negative inside

electrochemical gradient with membrane potential positive inside

# Equilibrium (Nernst) potential



$$V = \frac{RT}{zF} \ln \frac{C_o}{C_i} = (58\text{mV}) \log \frac{C_o}{C_i}$$

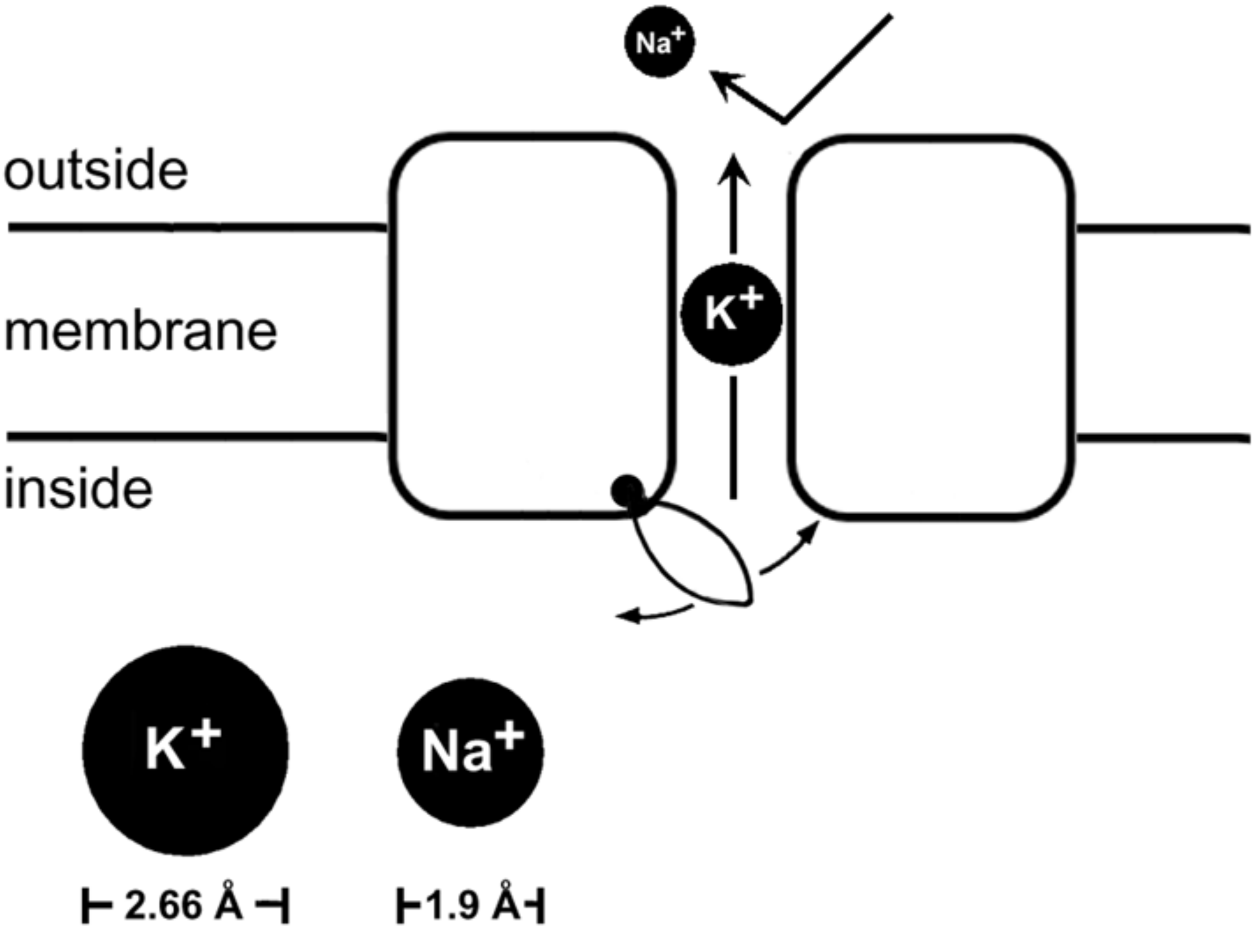
Nernst equation

(at room temp, for a monovalent cation)

- *V* = the equilibrium potential in volts (internal potential minus external potential)
- $C_o$ and $C_i$ = outside and inside concentrations of the ion, respectively
- *R* = the gas constant (2 cal $mol^{-1}$ $K^{-1}$)
- *T* = the absolute temperature (K)
- *F* = Faraday's constant ($2.3 \times 10^4$ cal $V^{-1}$ $mol^{-1}$)
- *z* = the valence (charge) of the ion

Not to be confused with the resting potential of a cell!

# Ion channels exhibit three basic properties

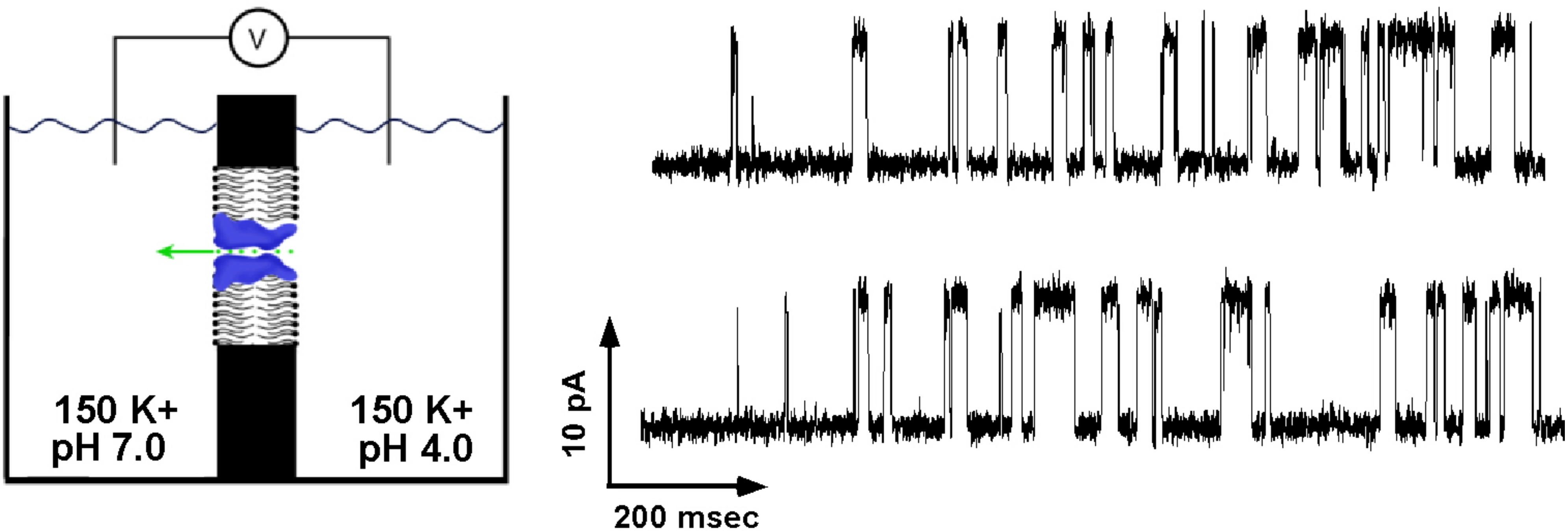



(1) High conduction rates: $K^+$ ions flow across $K^+$ channels at rates of $10^7$ - $10^8$ ions per sec.

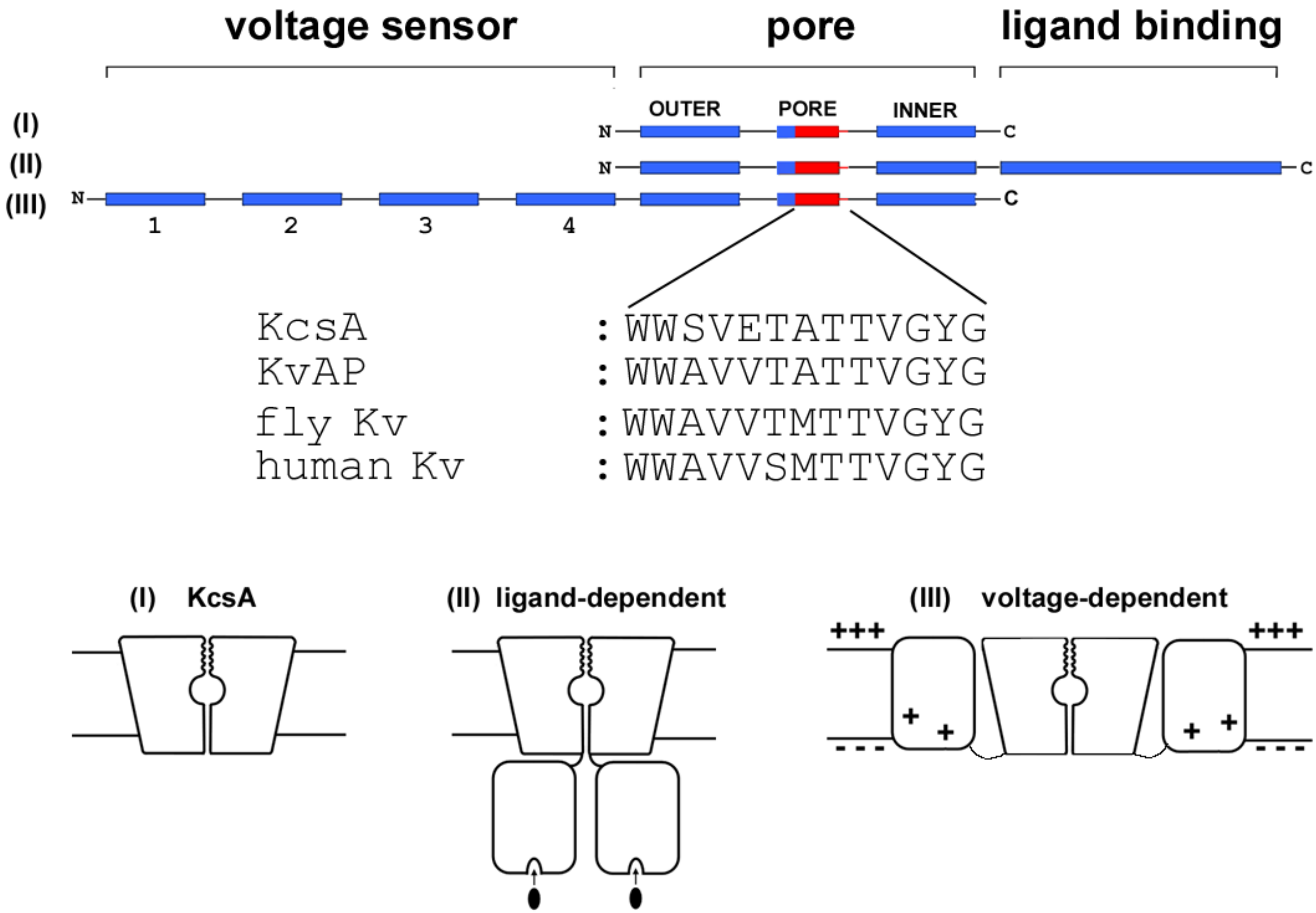
(2) Selectivity: The smaller $Na^+$ ion is essentially excluded.

(3) Gating: conduction is turned on and off by regulated conformational changes that open and close the pore.

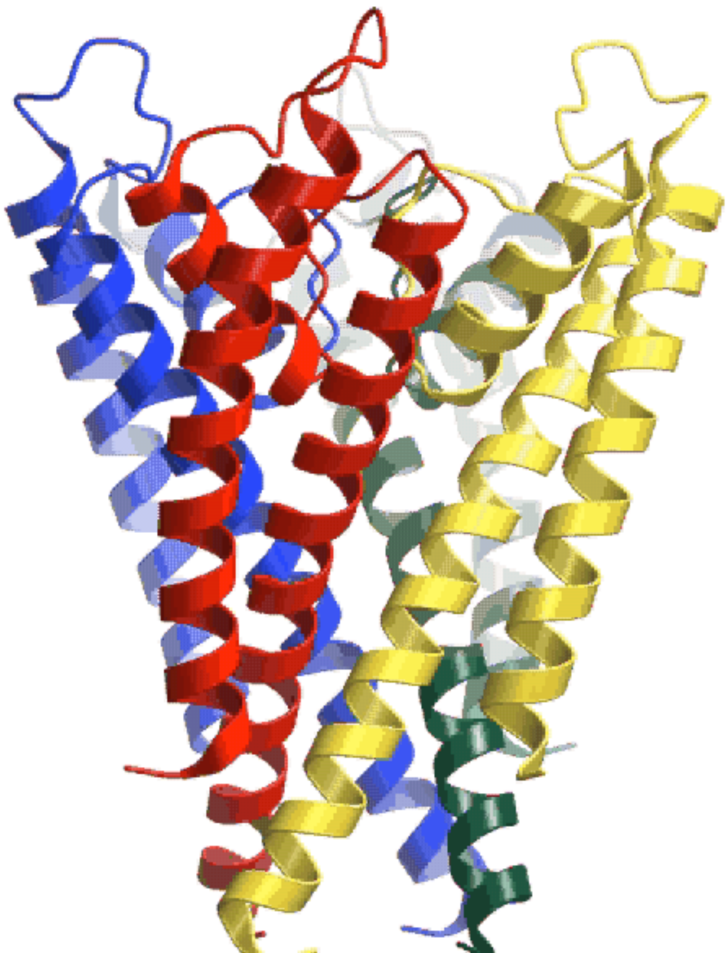
# Conduction through a single $K^+$ channel



# Classification of $K^+$ channels

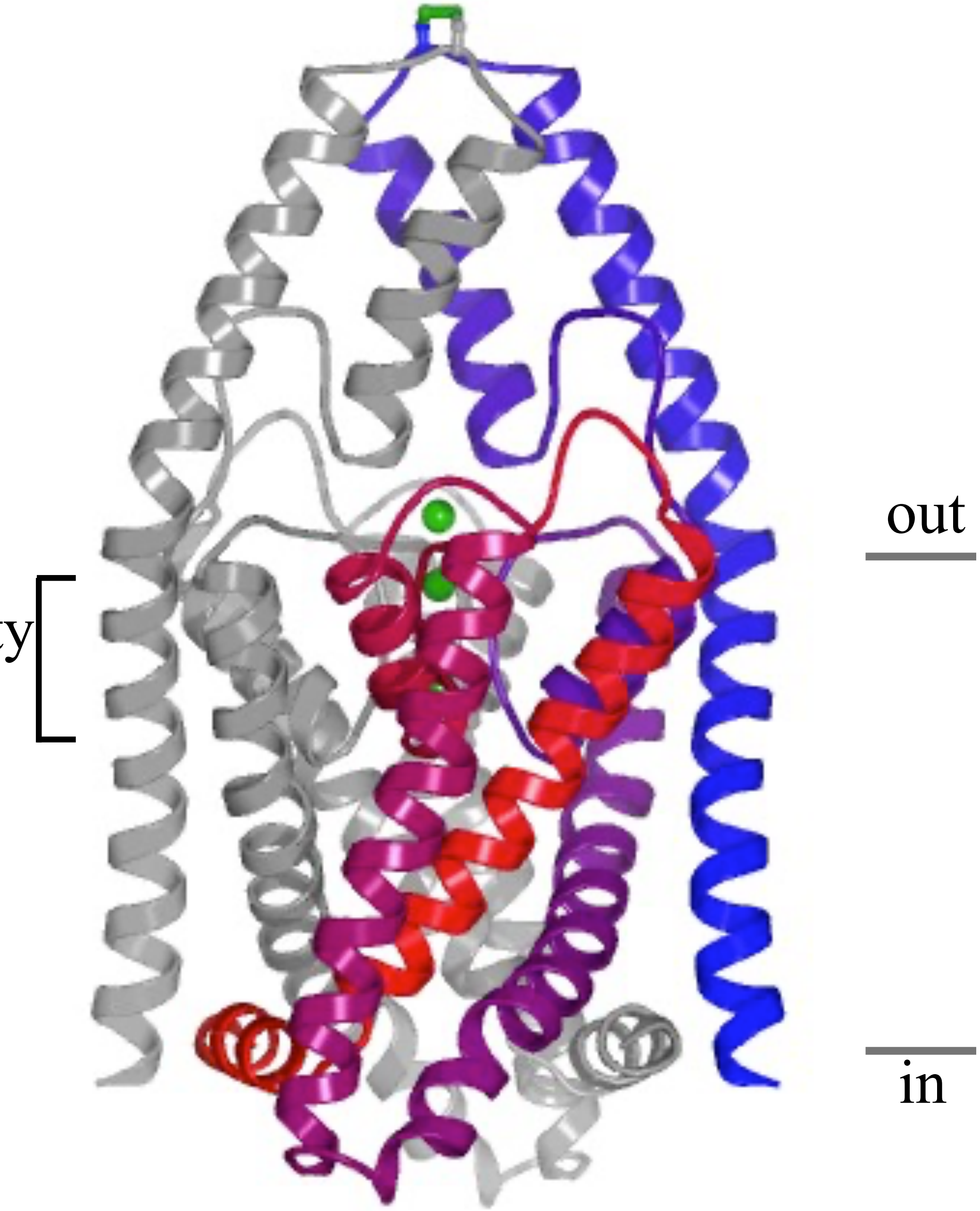


prokaryotic KcsA



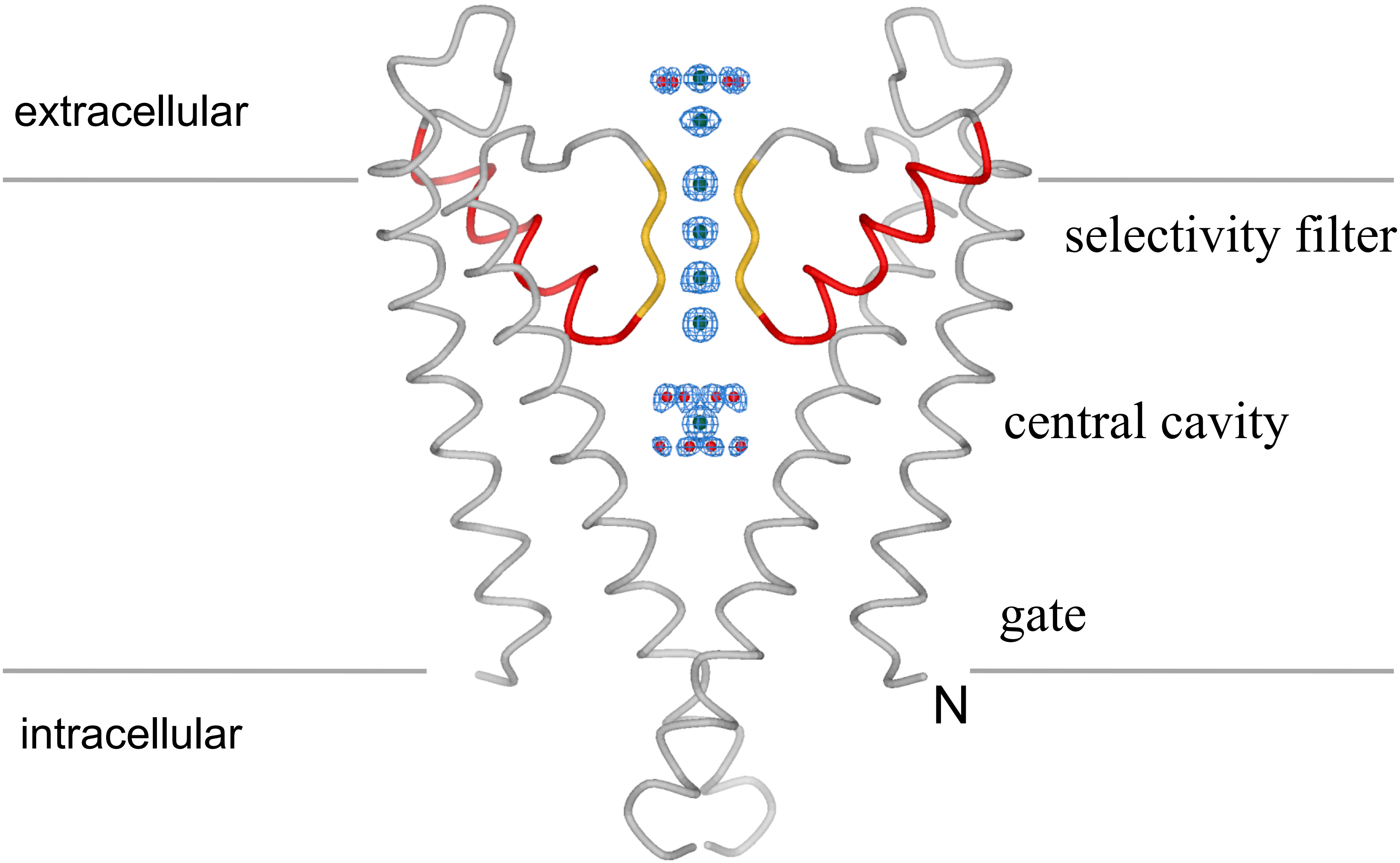
D.A. Doyle et al., *Science* (1998)

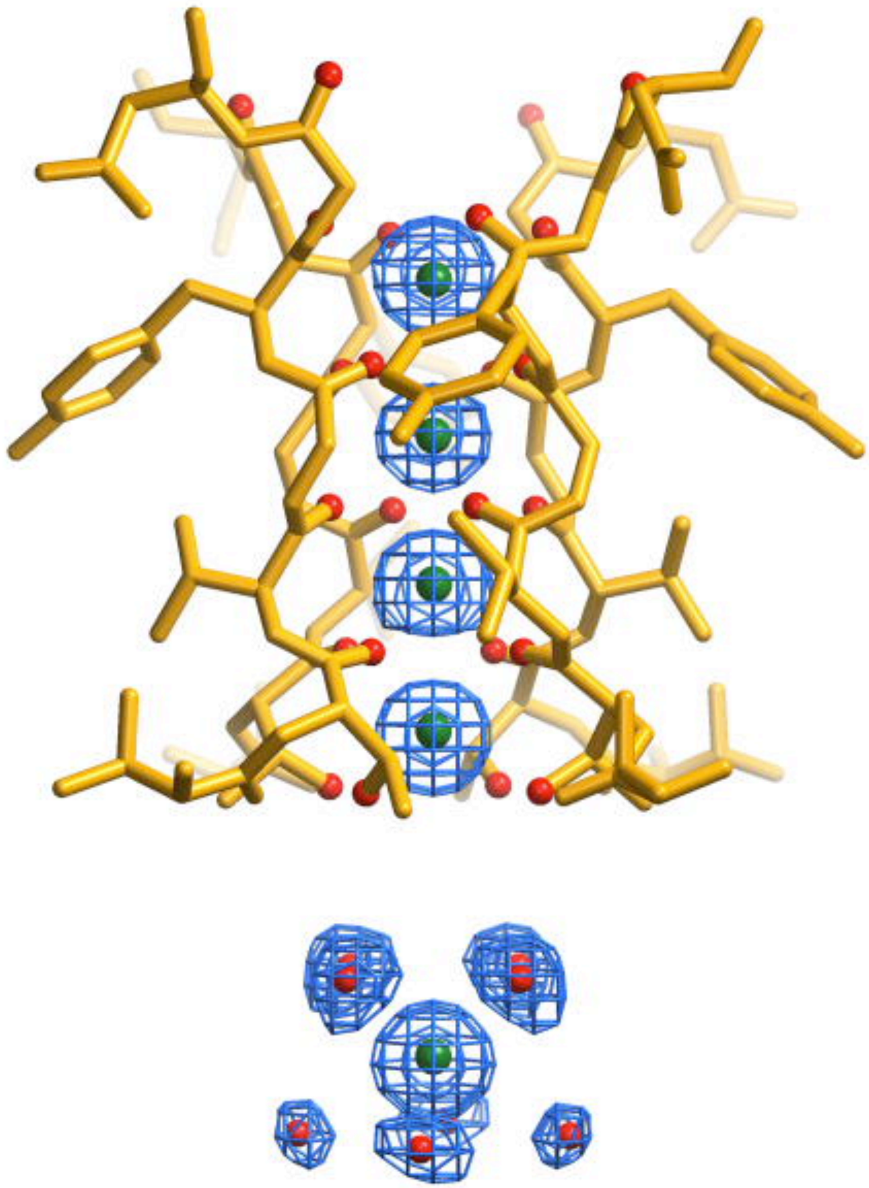
human K2P1




Miller & Long, *Science* (2012)

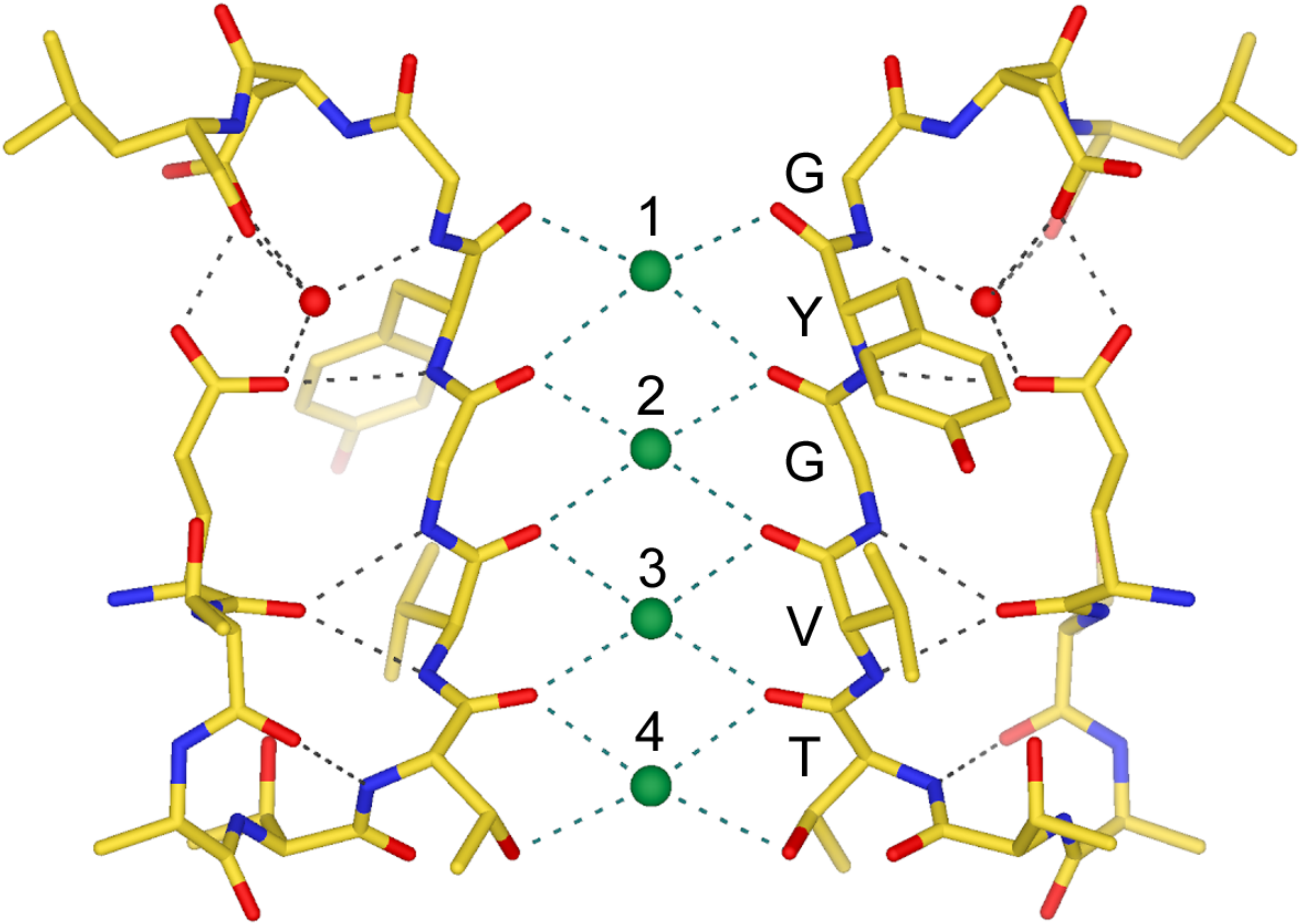
# Two subunits of a $K^+$ channel



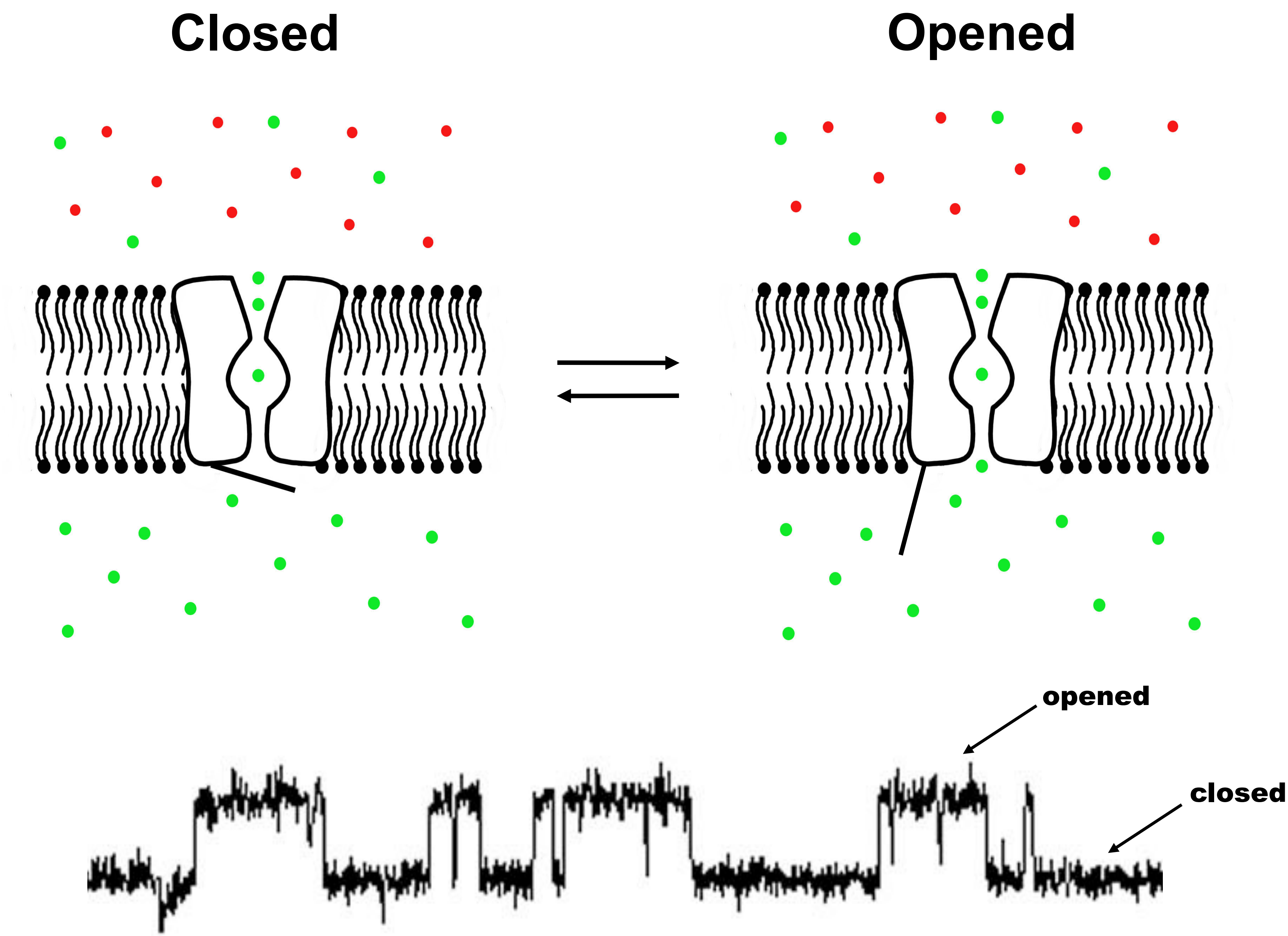


Zhou et al. Nature (2001) vol. 414 (6859) pp. 43-8

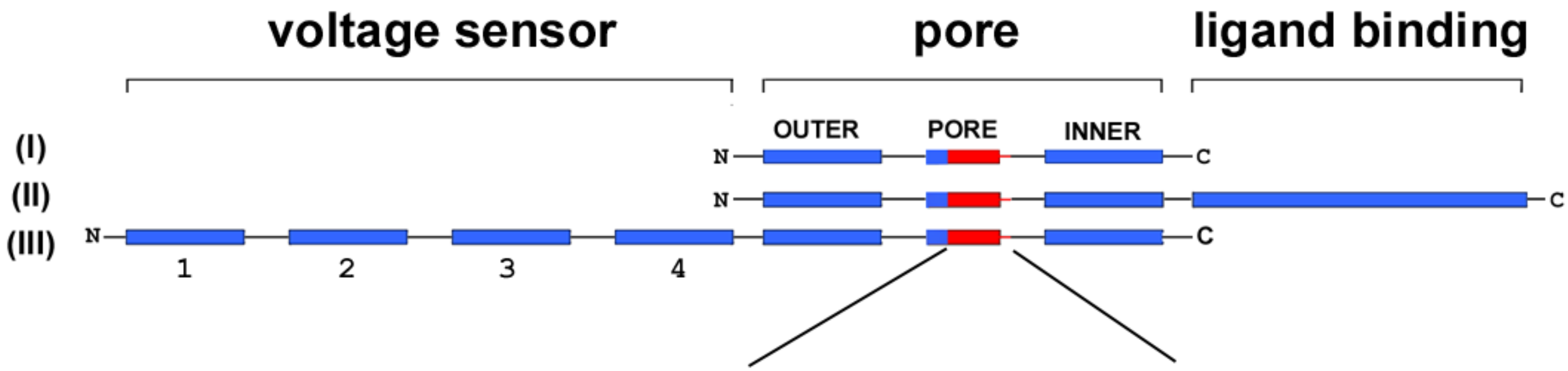
# Carbonyl oxygen atoms coordinate $K^+$ ions



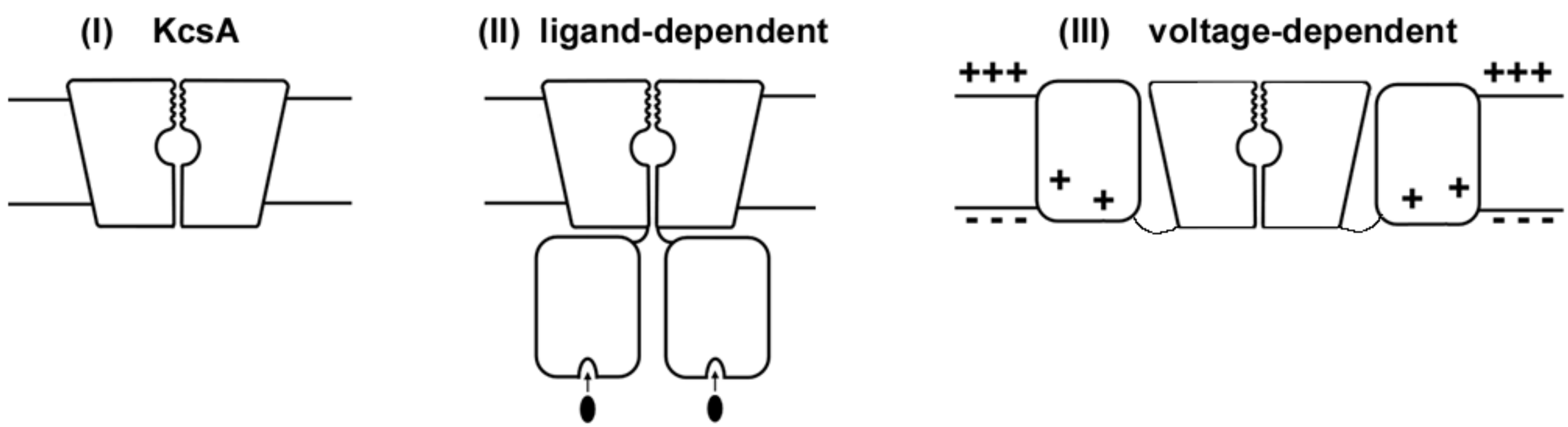
# K+ channel gating



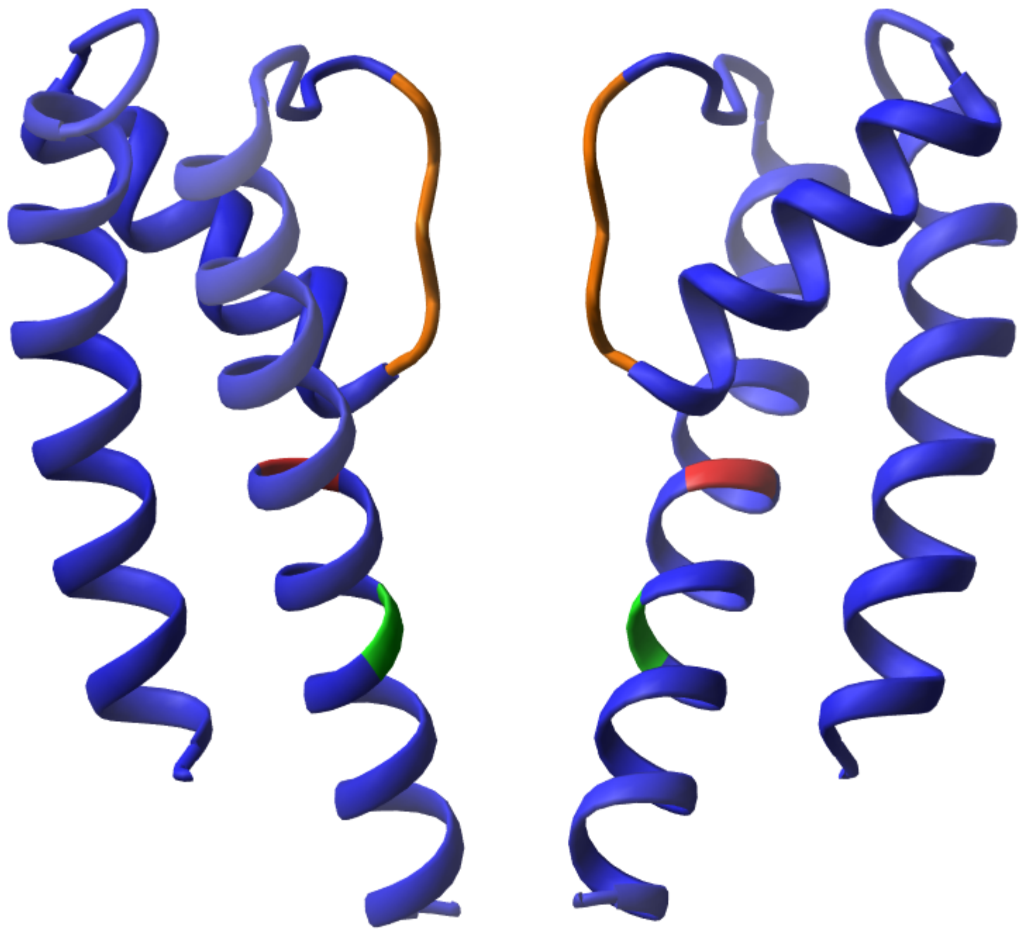
# Classification of $K^+$ channels




| | |
|---|---|
| KcsA | : WWSVETATTVGYG |
| KvAP | : WWAVVTATTVGYG |
| fly Kv | : WWAVVTMTTVGYG |
| human Kv | : WWAVVSMTTVGYG |



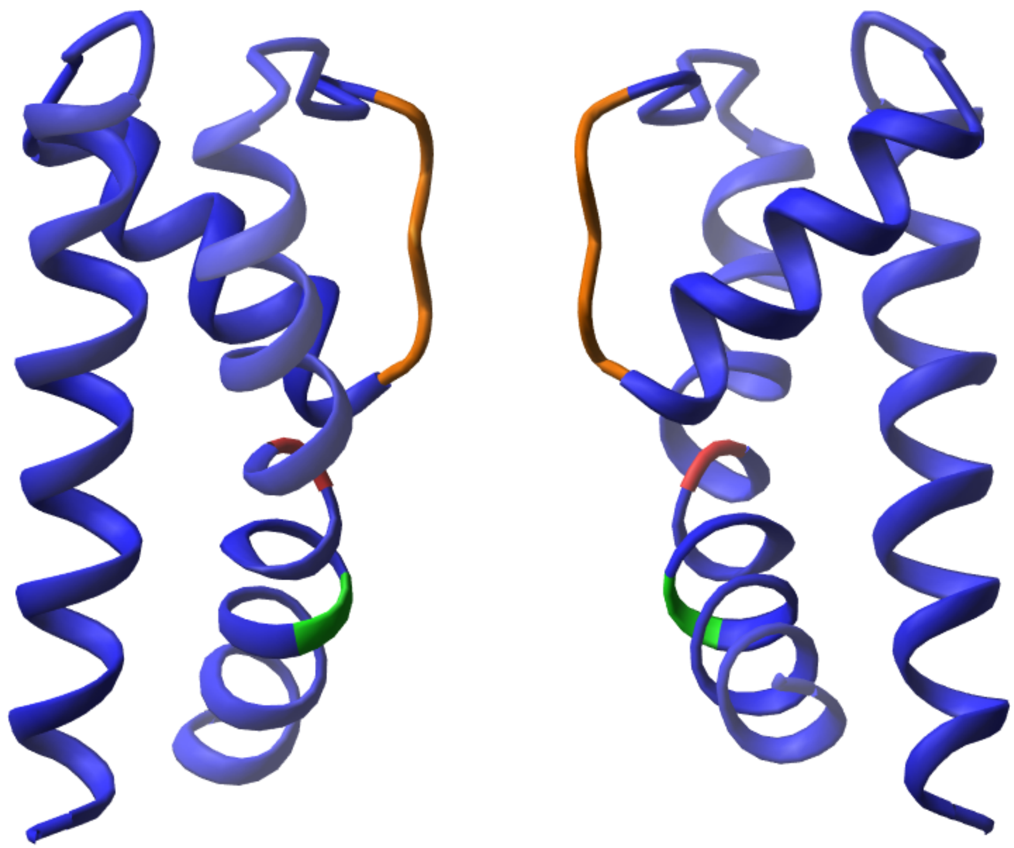
# Mechanics of pore gating



(Closed)

KcsA: Doyle et al. Science (1998) vol. 280 (5360) pp. 69-77

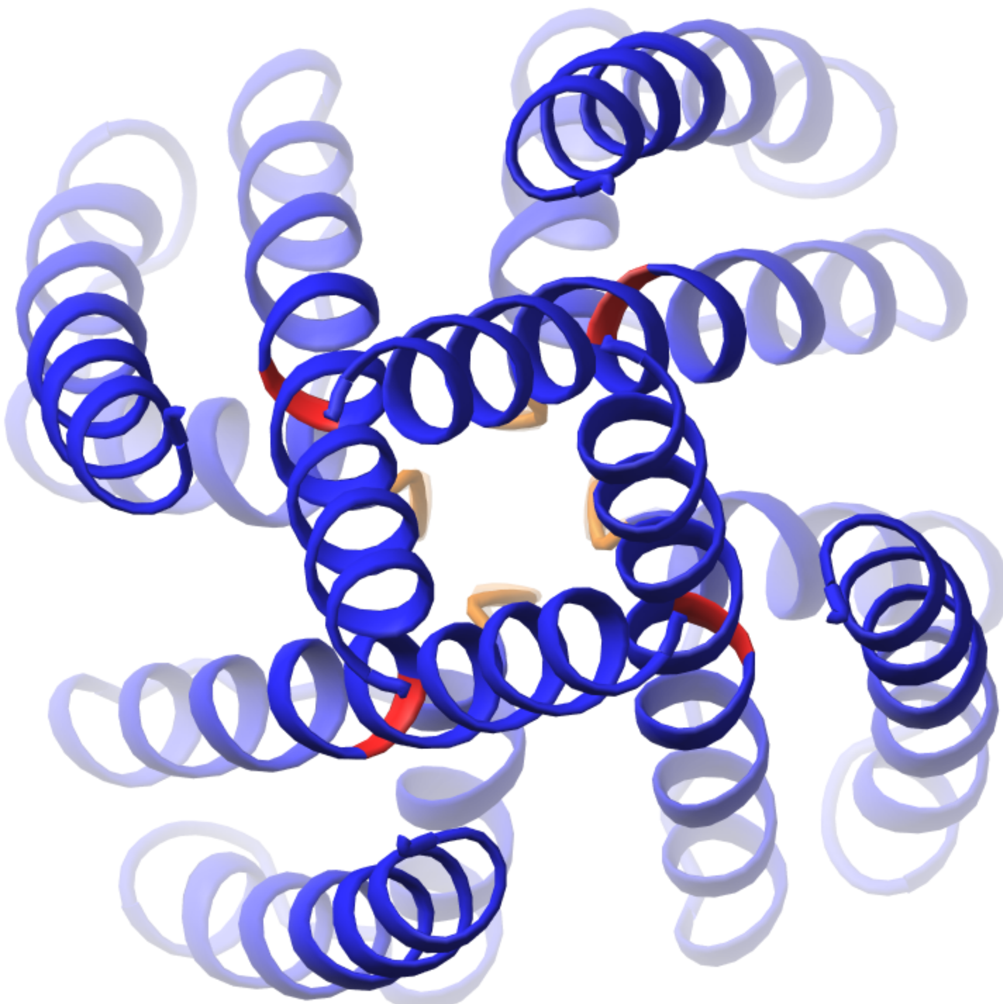
# Mechanics of pore gating



(Opened)

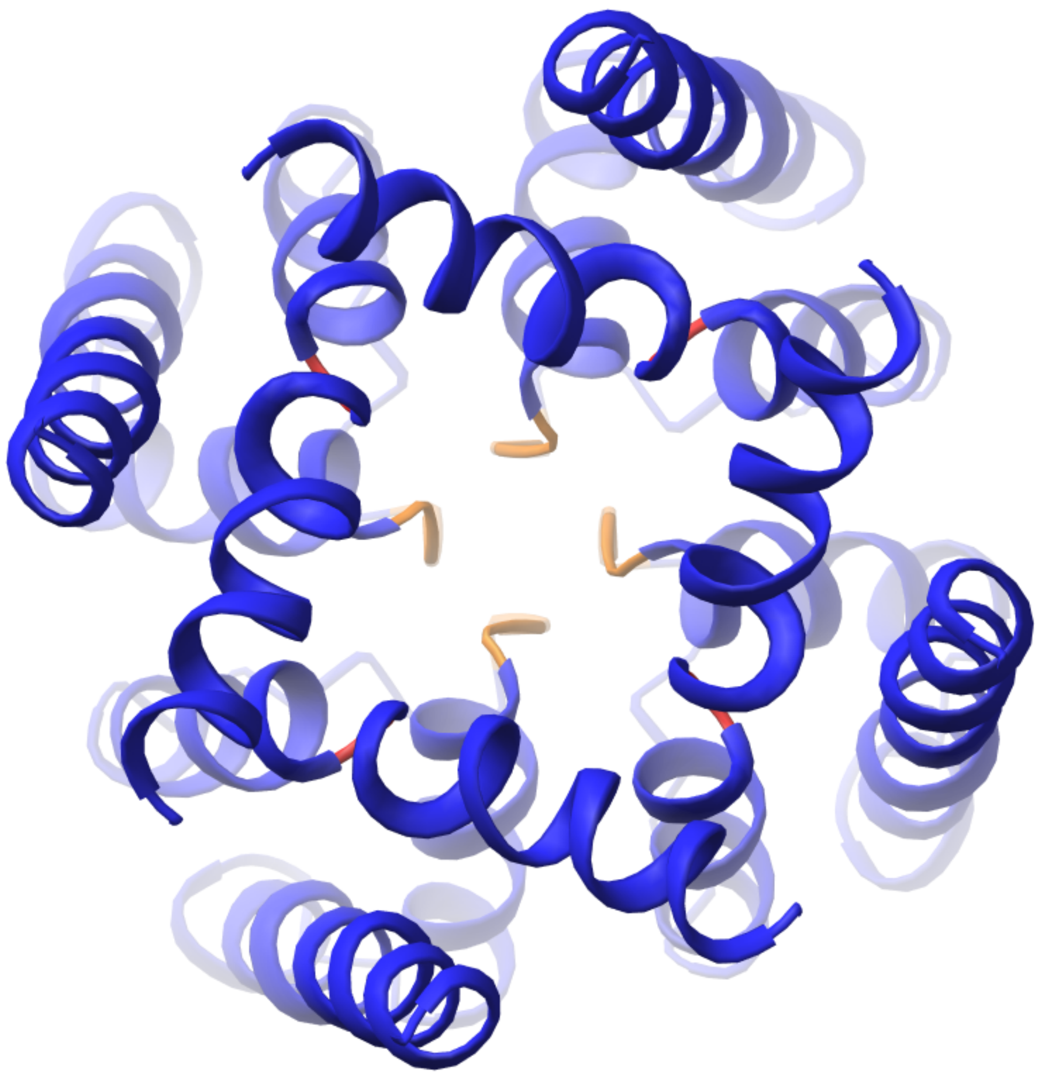
MthK: Jiang et al. Nature (2002) vol. 417 (6888) pp. 523-6

# Mechanics of pore gating



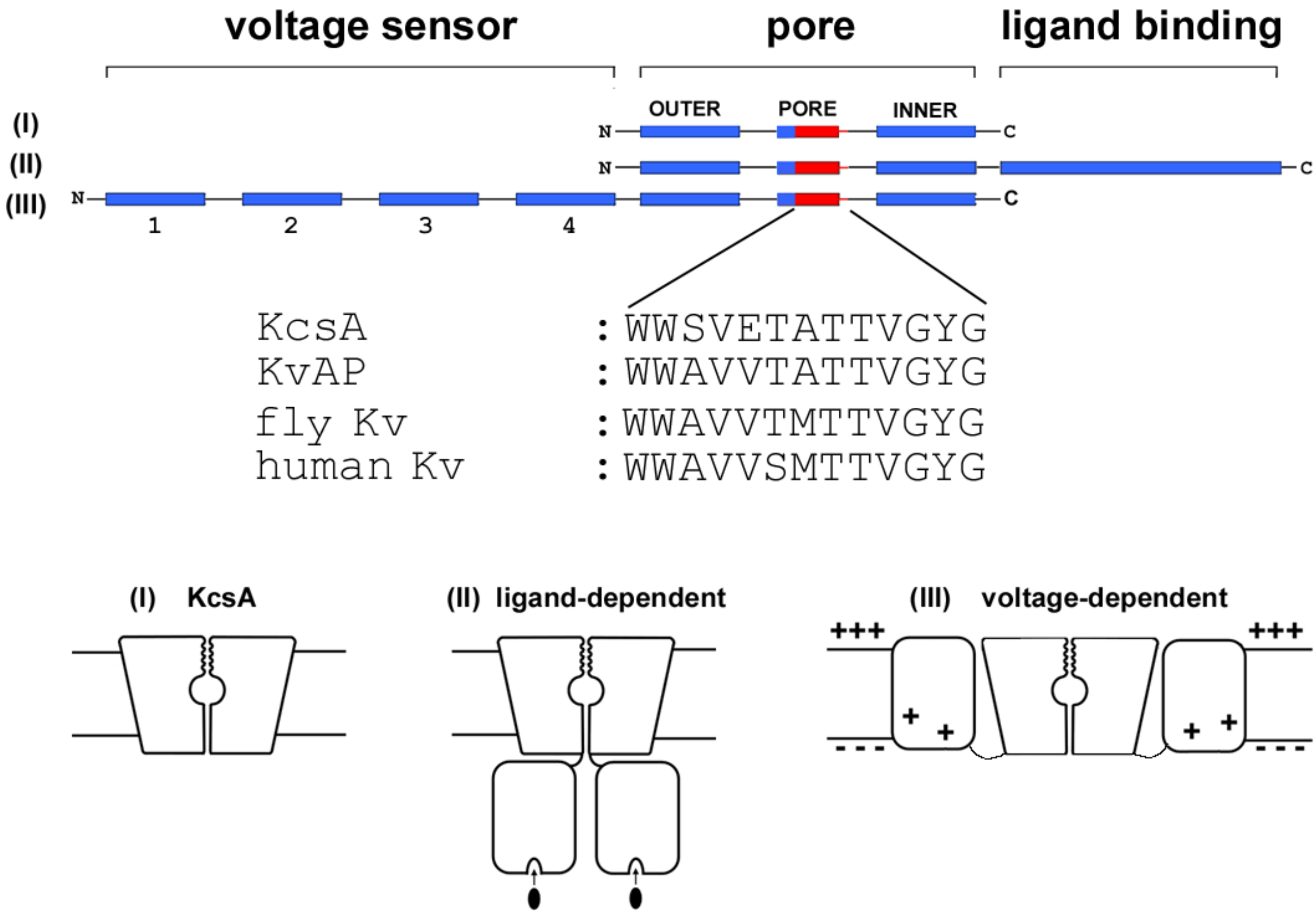
(Closed)

# Mechanics of pore gating



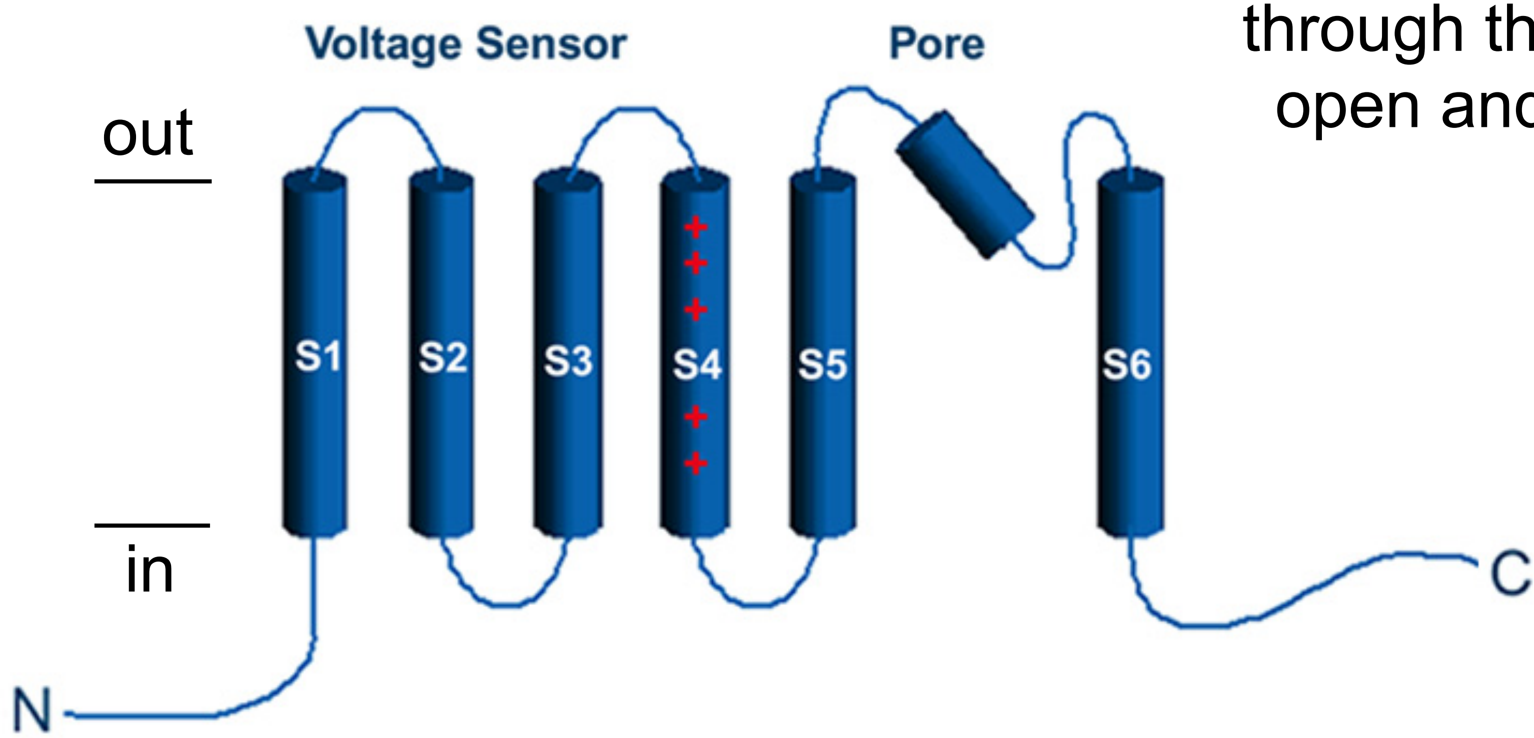
(Opened)

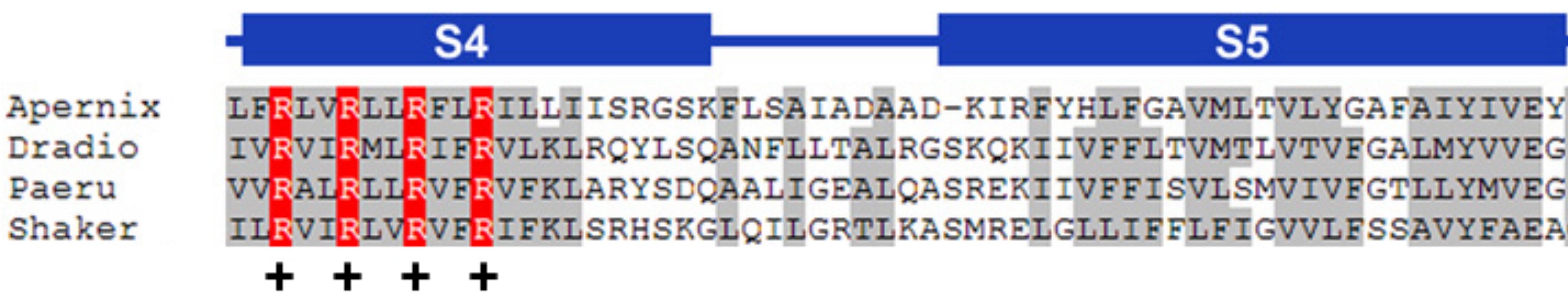
# Classification of K+ channels



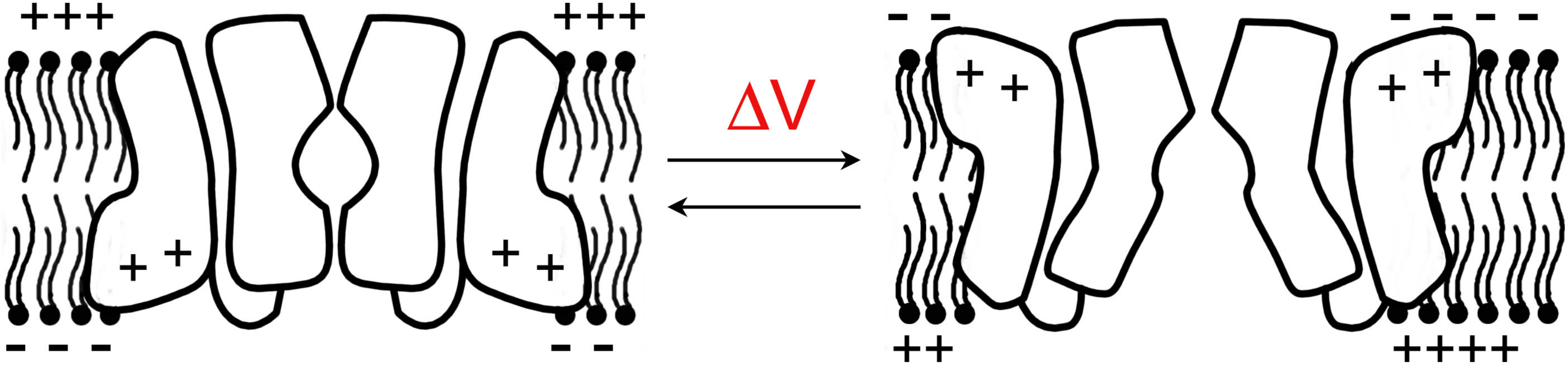
# Voltage-dependent $K^+$ channels

the voltage sensor moves through the electric field to open and close the pore



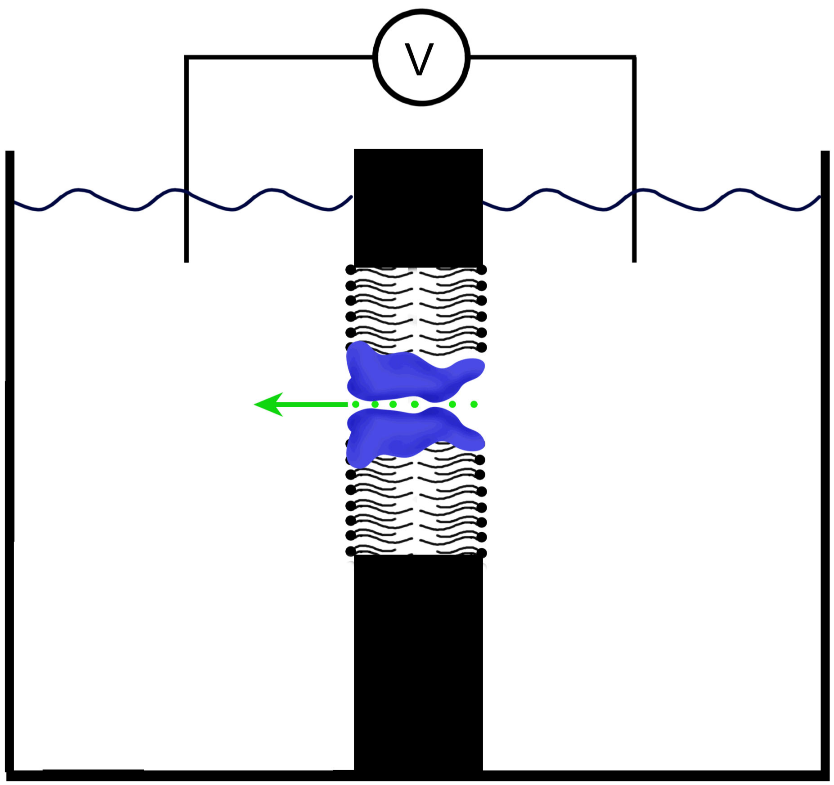
# the voltage sensor moves through the electric field to open and close the pore

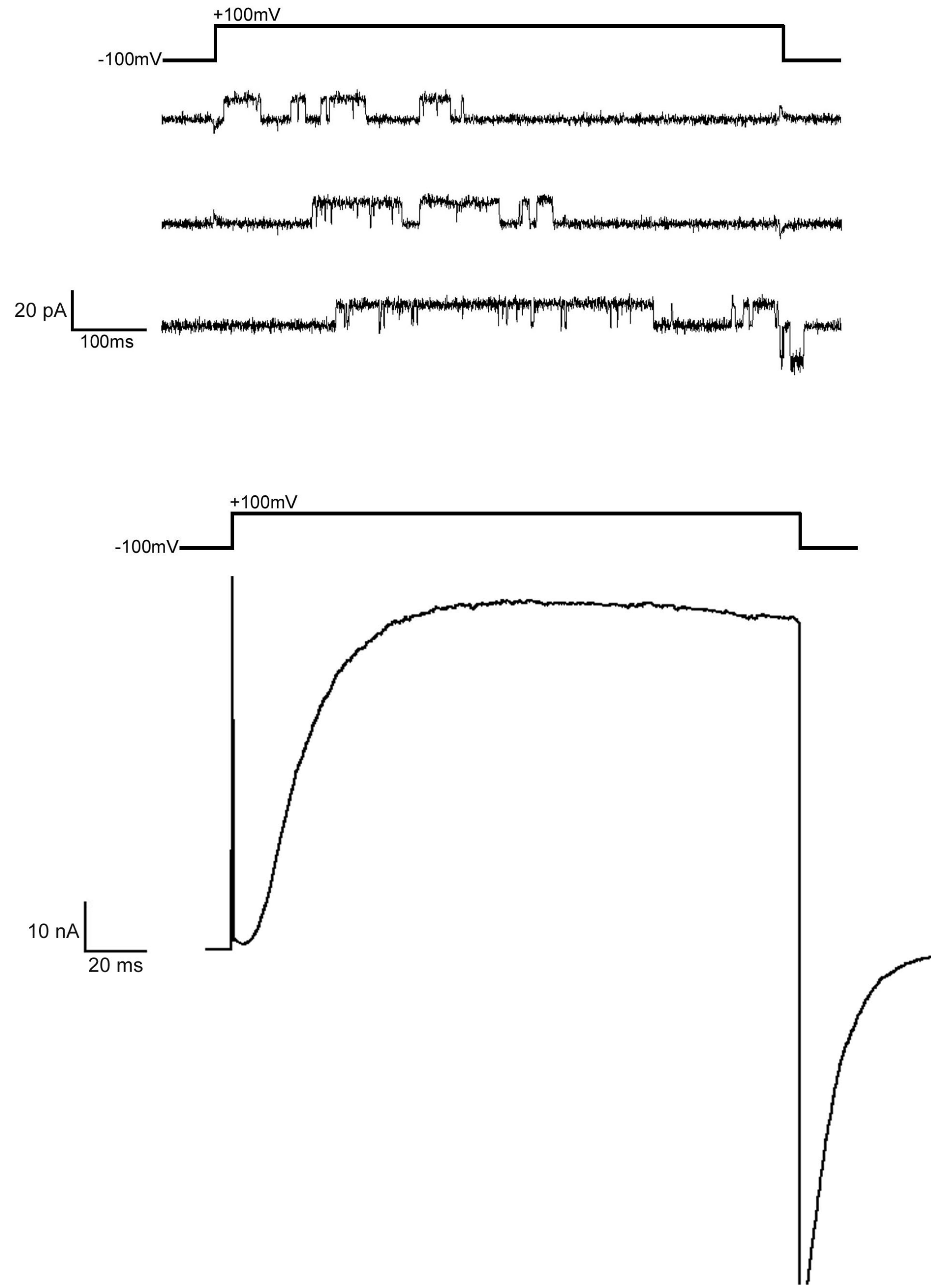



closed
(negative inside)

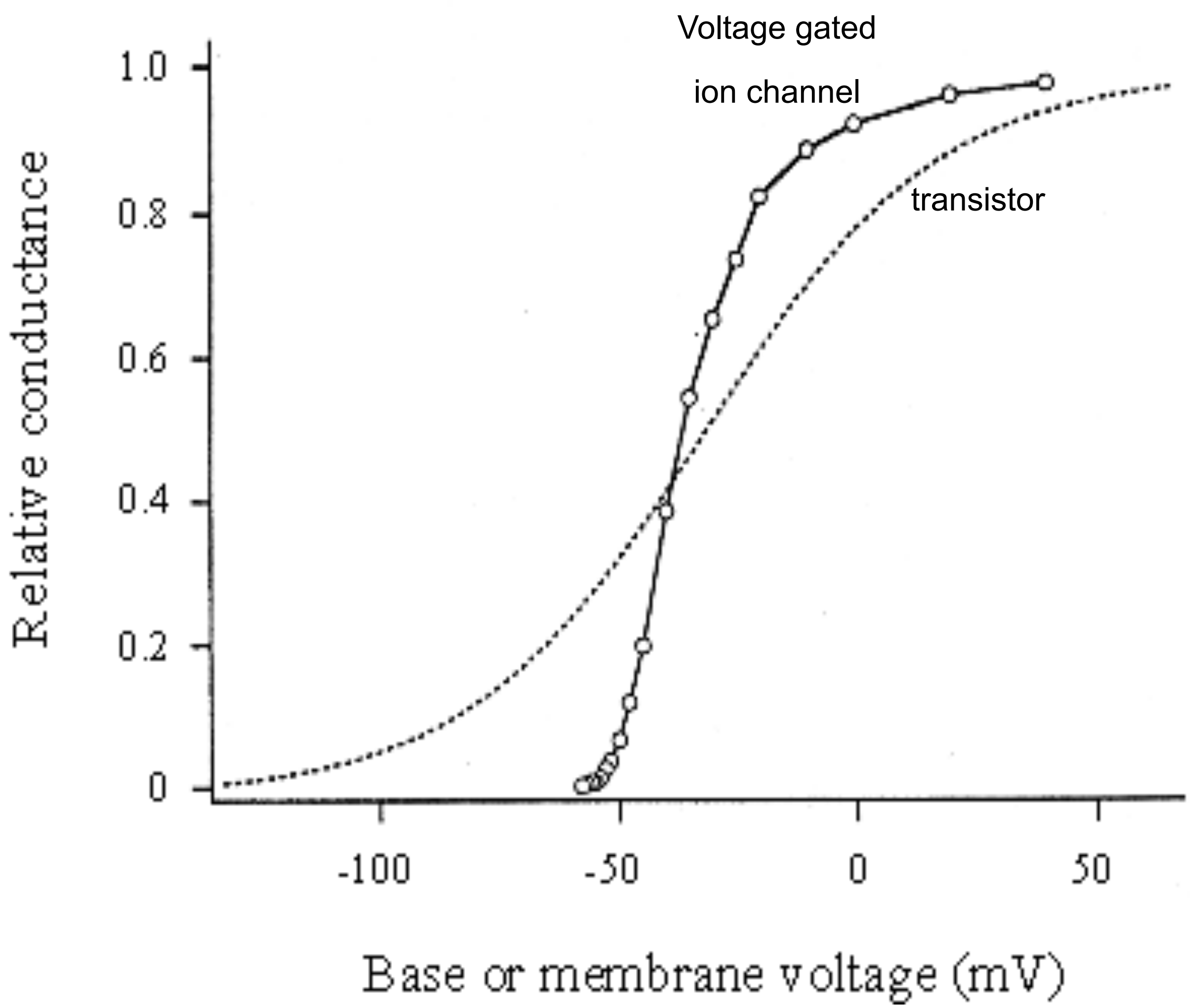
open
(positive inside)

# Voltage-dependent K⁺ channels



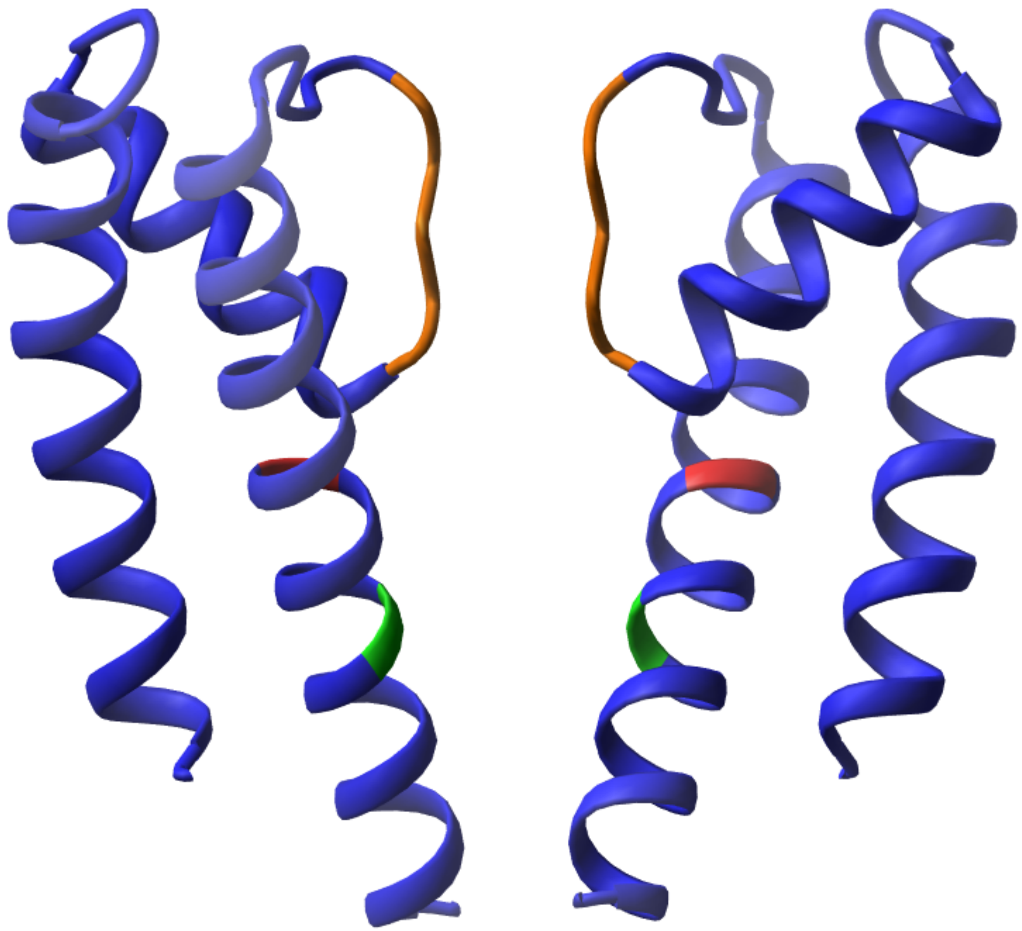



# Comparing a voltage-dependent $K^+$ channel to a bipolar transistor



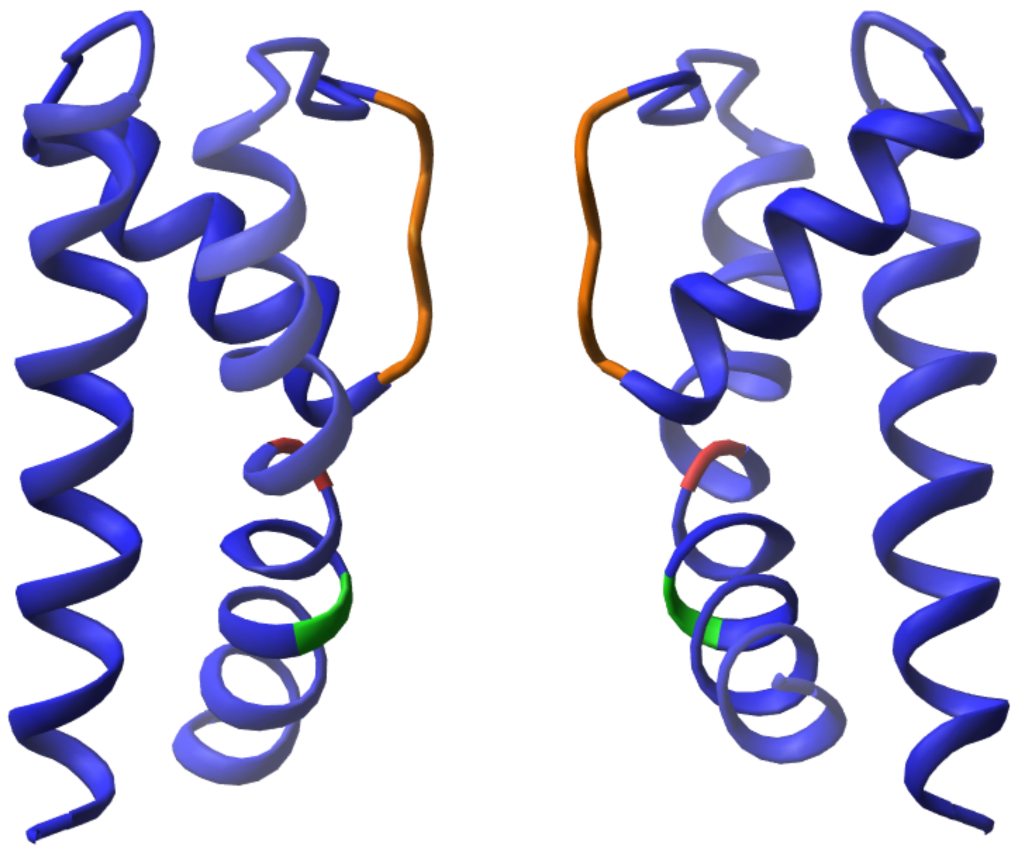

Sigworth, FJ (1994) *Q Rev Biophys* 27:1-40

# Mechanics of pore gating



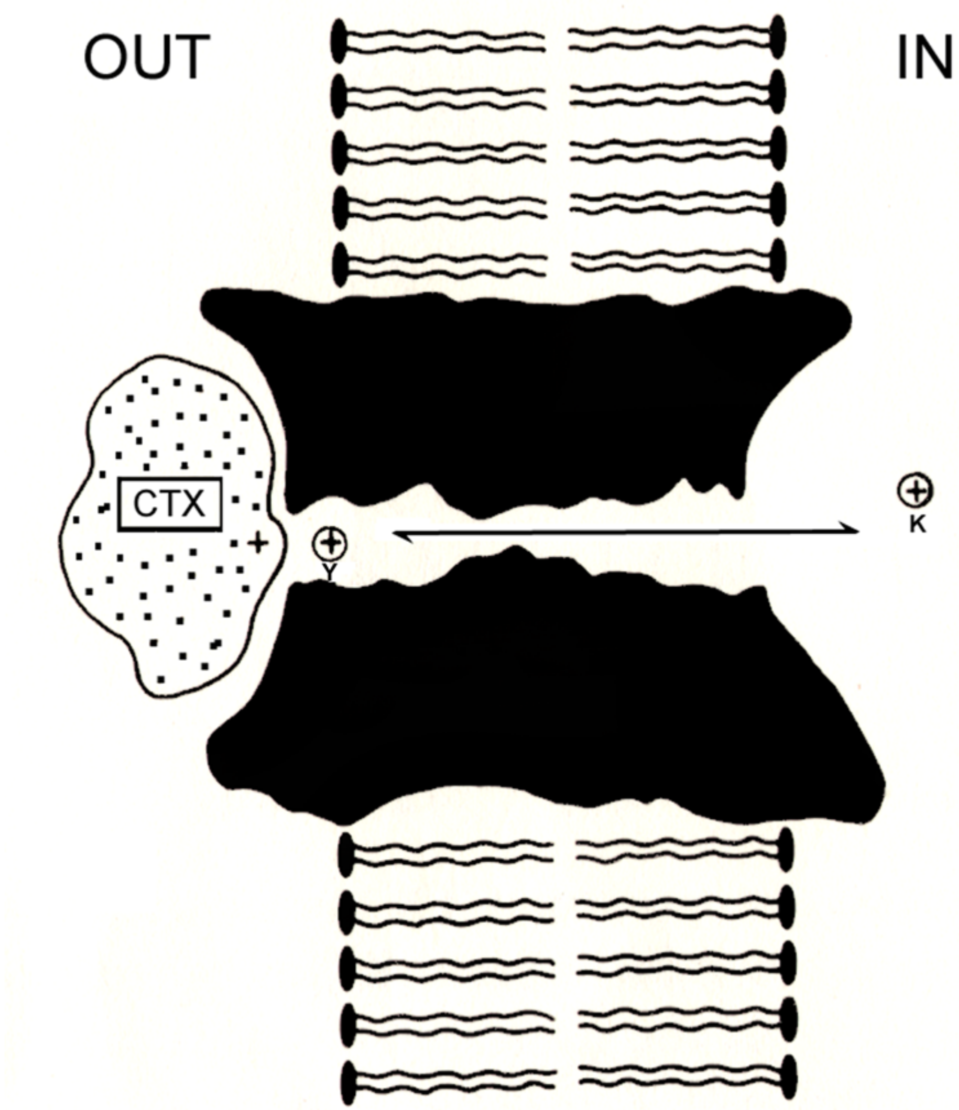
(Closed)

# Mechanics of pore gating



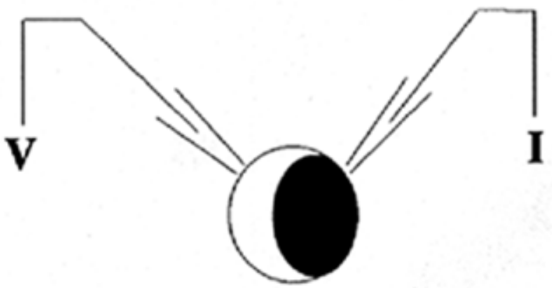
(Opened)

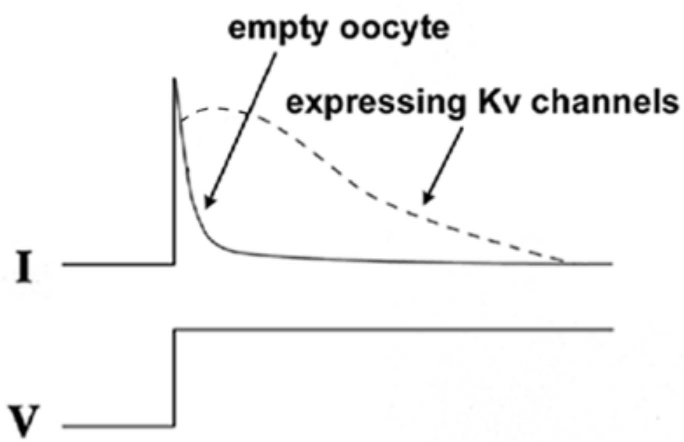
# Scorpion toxins plug K+ channels



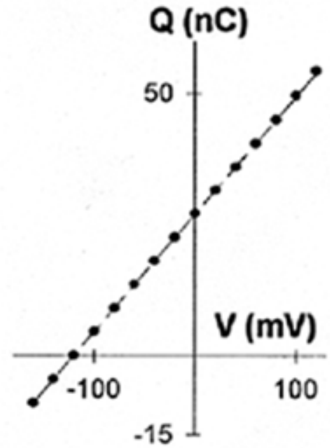
paper

# Gating charges

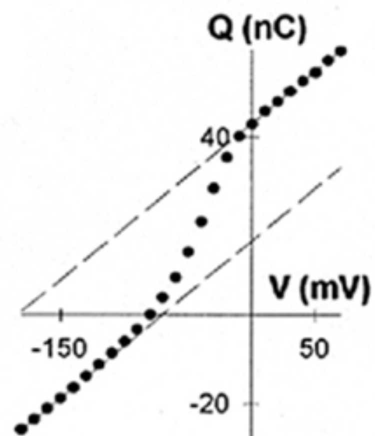


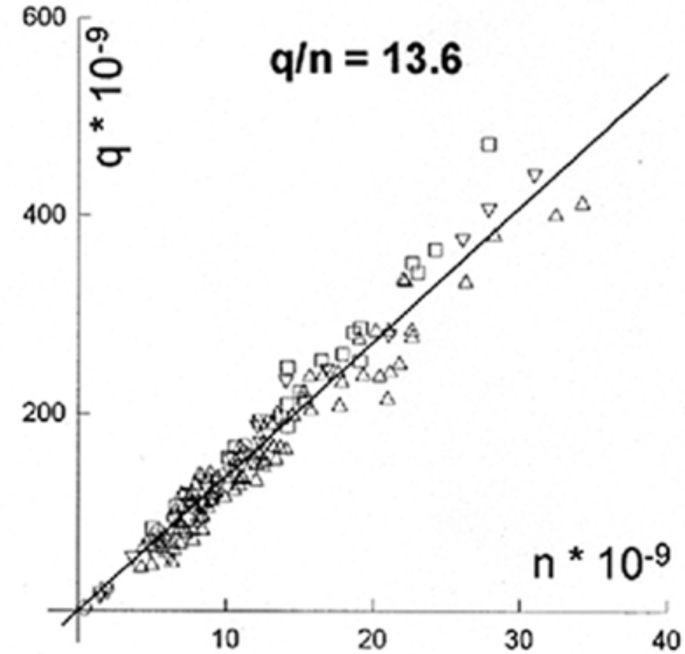




**empty oocyte**




**expressing Kv**

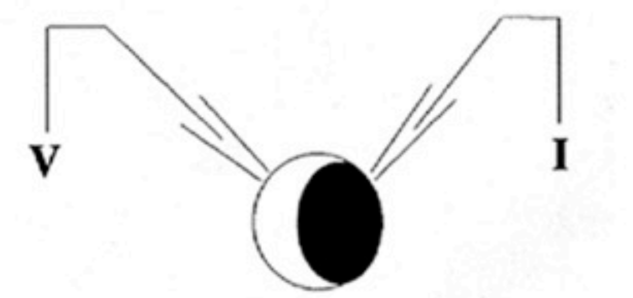


# using oocytes to express channels for electrophysiology

Xenopus oocyte

Linearize plasmid DNA
Transcribe RNA (T7 RNA polymerase)
Inject into oocyte, wait a few days

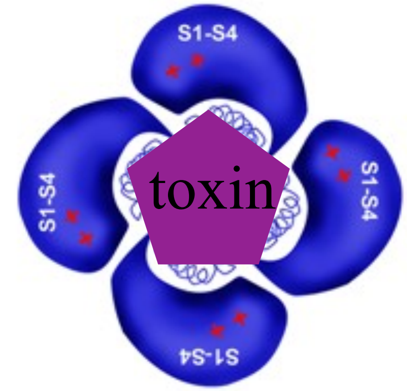


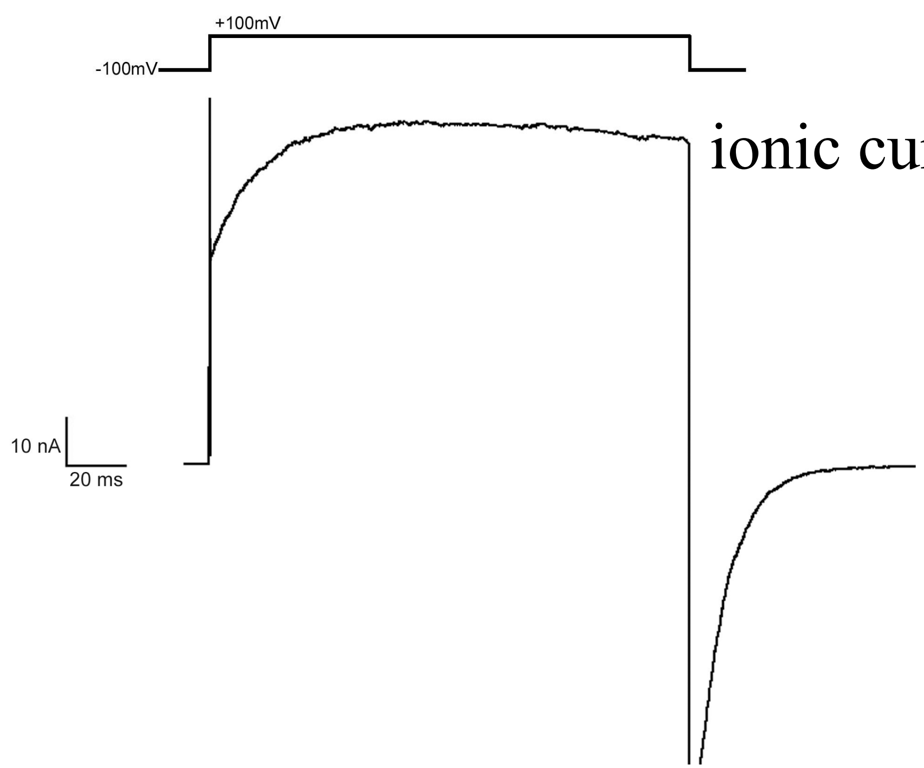

Two-electrode voltage clamp

Measure voltage inside the cell (V).

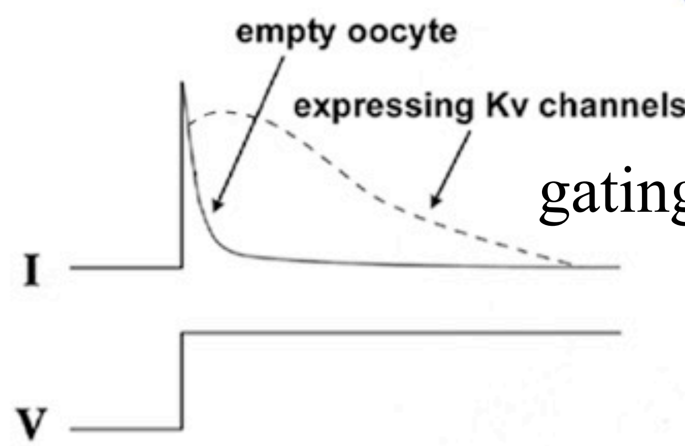
Use an amplifier to inject (or subtract) current (I) into the cell so as to keep the voltage at the voltage setpoint that you desire.

Voltages inside the cell are measured with respect to outside (ground).




ionic current




gating current

# Paper

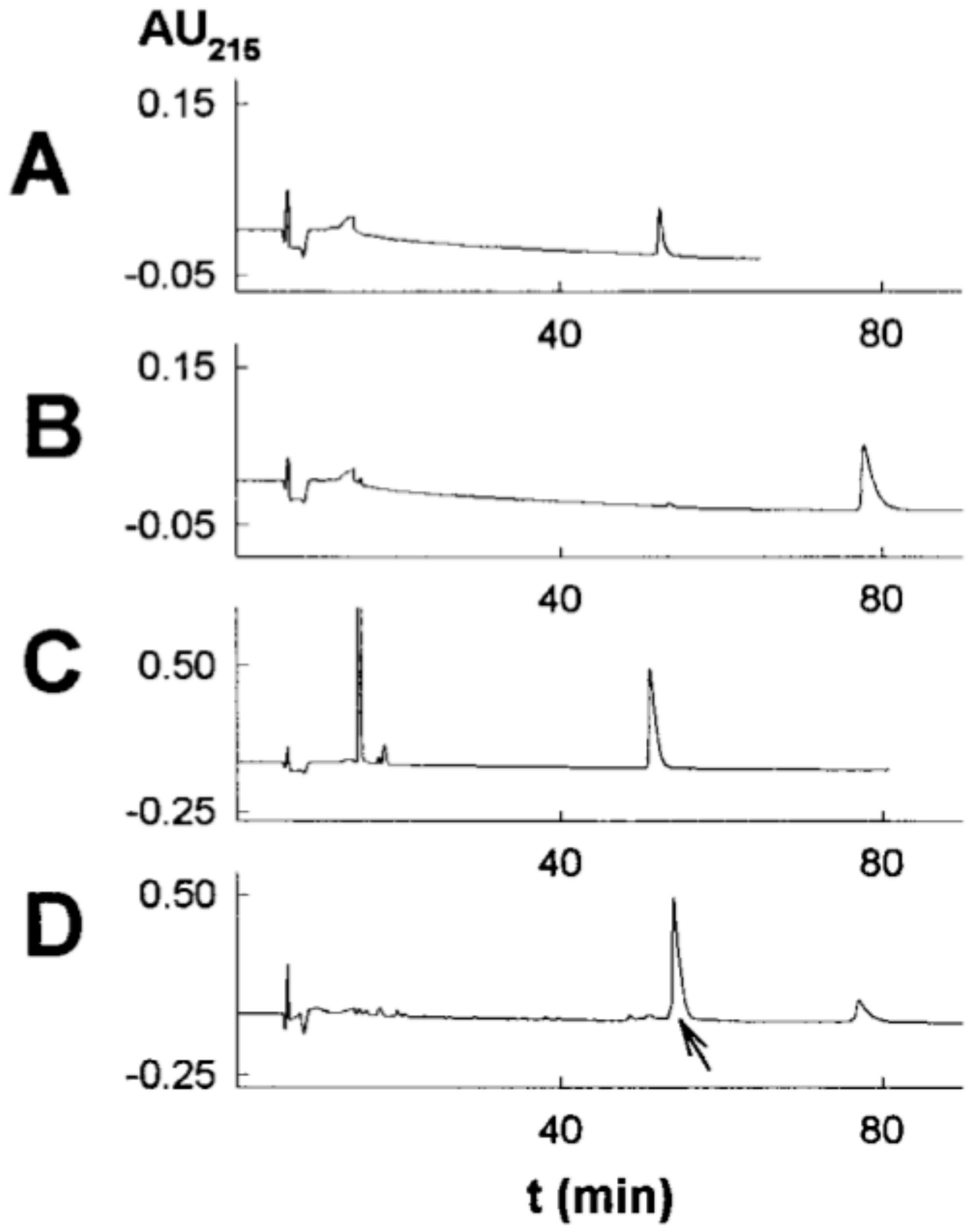
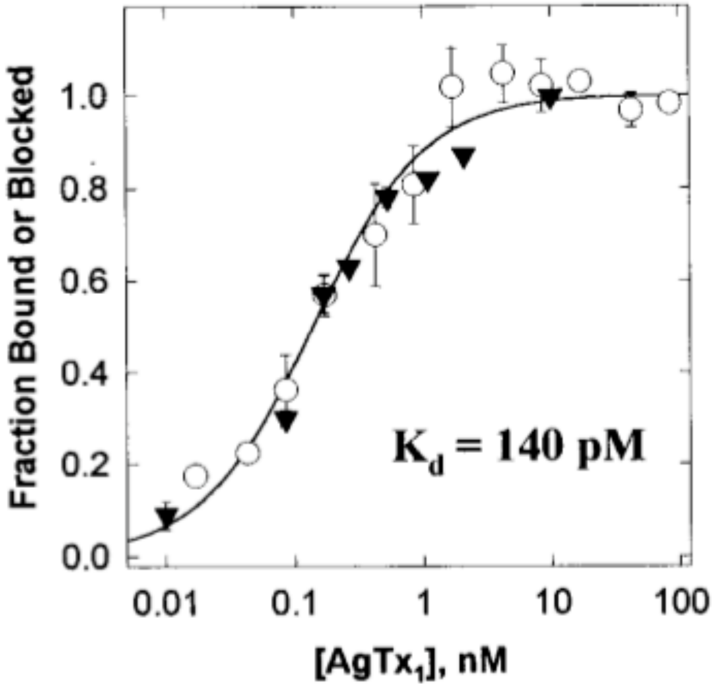


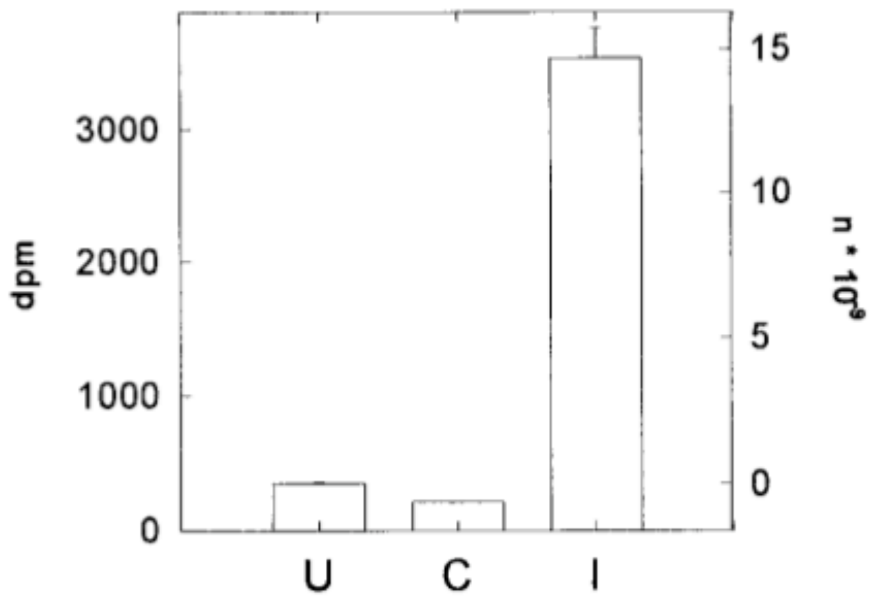

Figure 1. Synthesis and Purification of [$^{3}$H]NEM-Conjugated $AgTX_1$ D20C

A

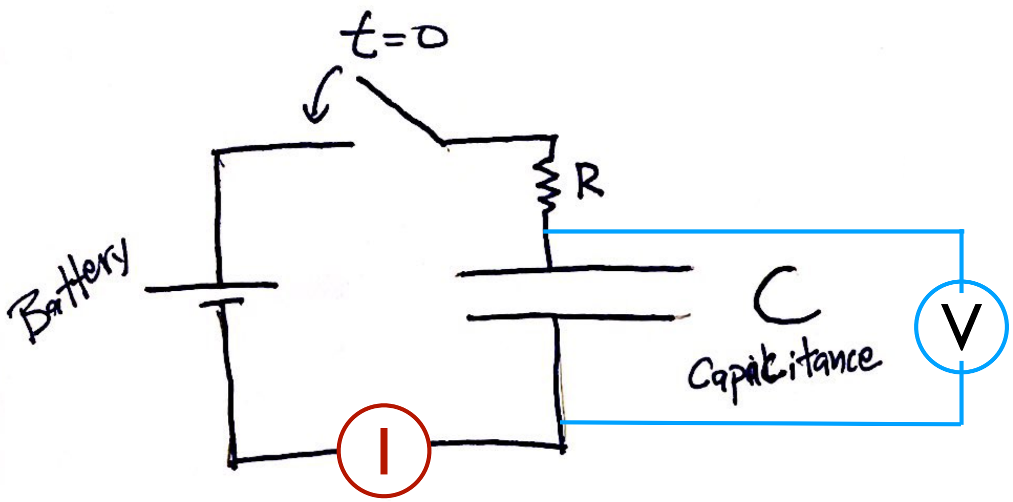


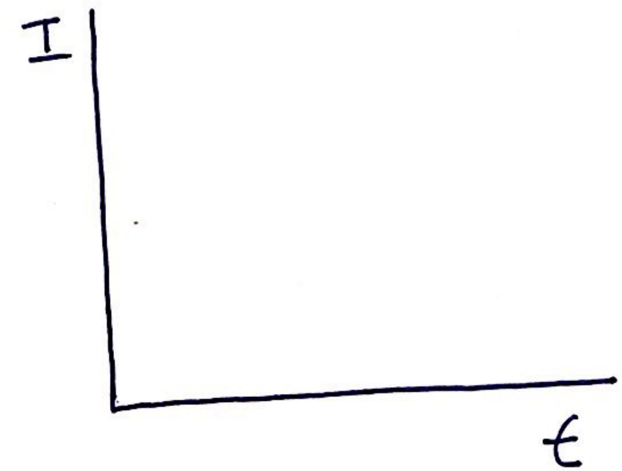
Fraction Bound or Blocked
1.0
0.8
0.6
0.4
0.2
0.0
K_d = 140 pM
0.01
0.1
1
10
100
[AgTx1], nM


B



dpm
3000
2000
1000
0
15
10
5
0
n * 10^9
U
C
I



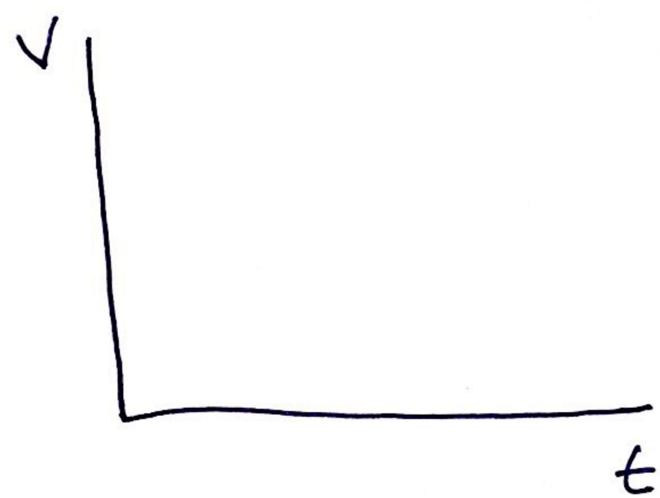



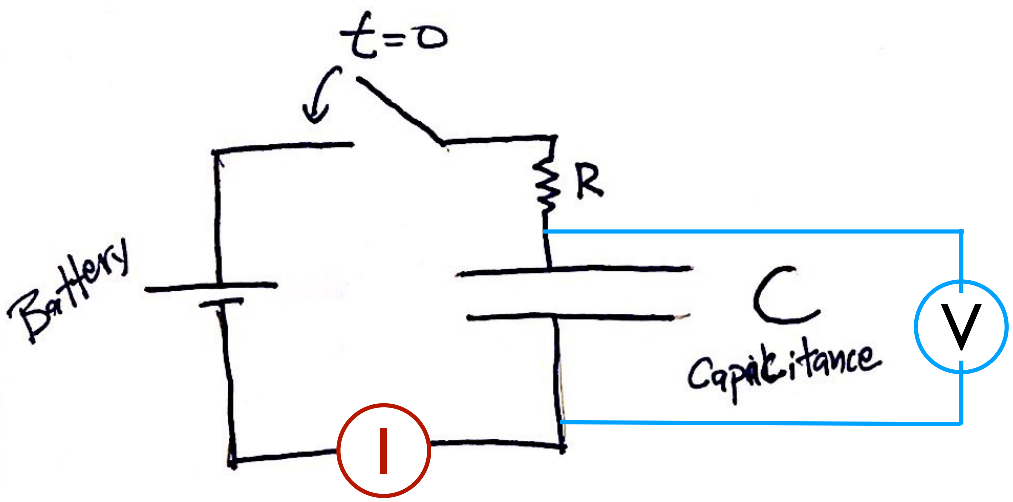

$$Q = CV$$

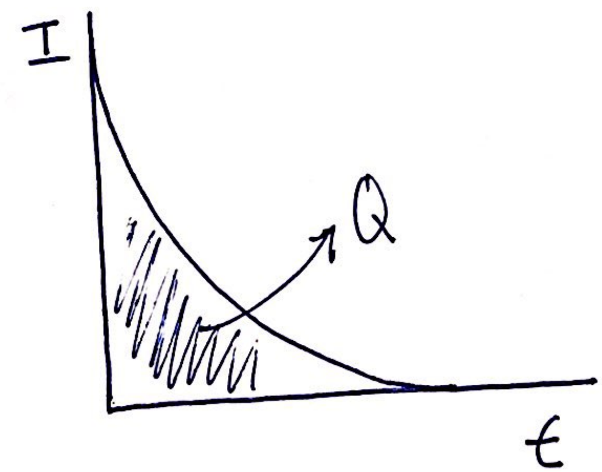
$$V = IR$$

$$I = \frac{dQ}{dt}$$

$$\hookrightarrow \quad Q = \int I(t)\,dt$$






$$Q = CV$$

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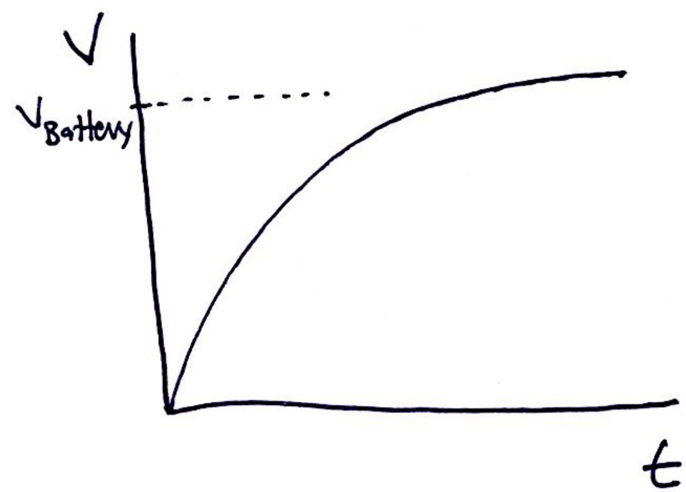
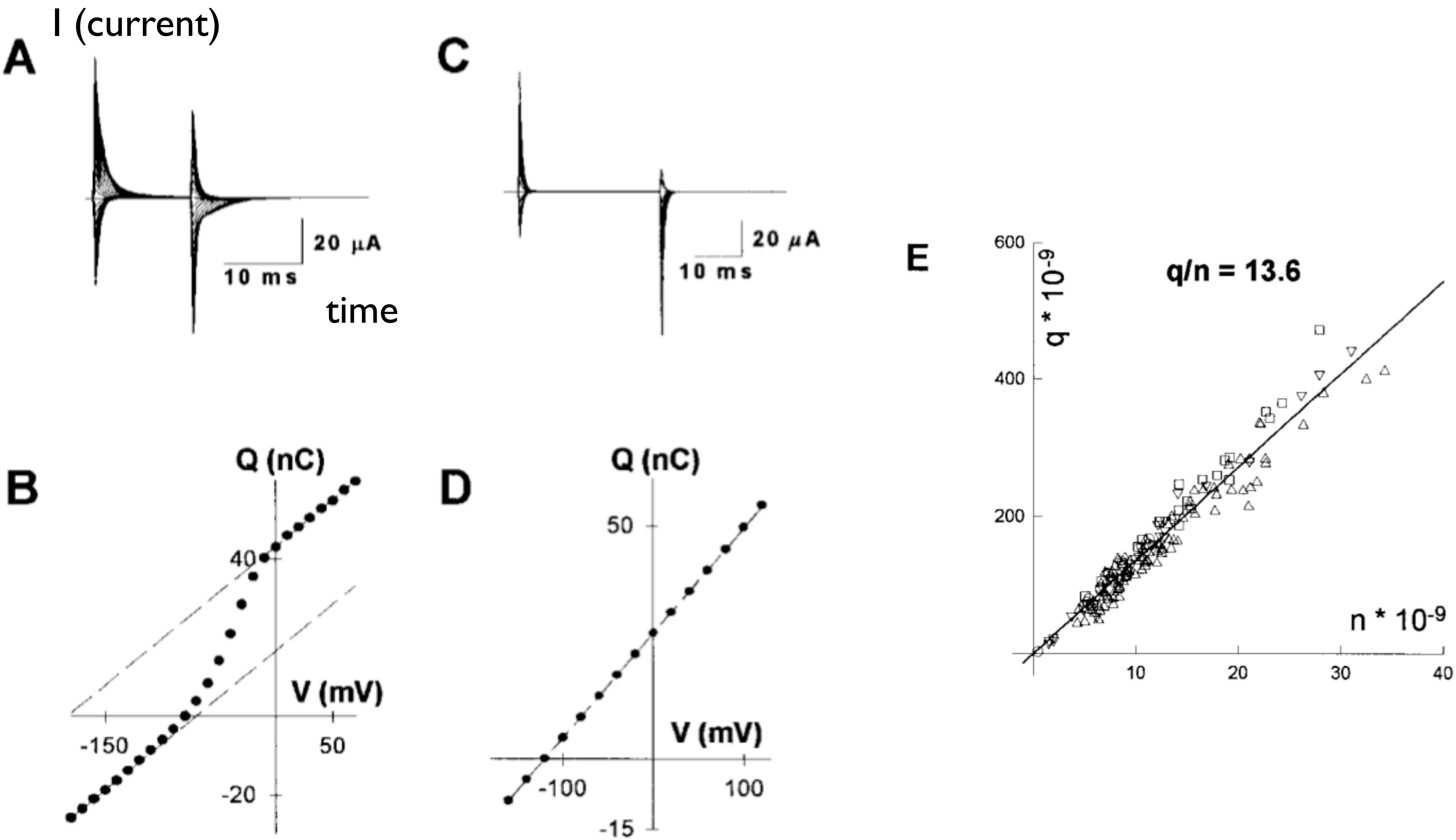
$$I = \frac{dQ}{dt}$$

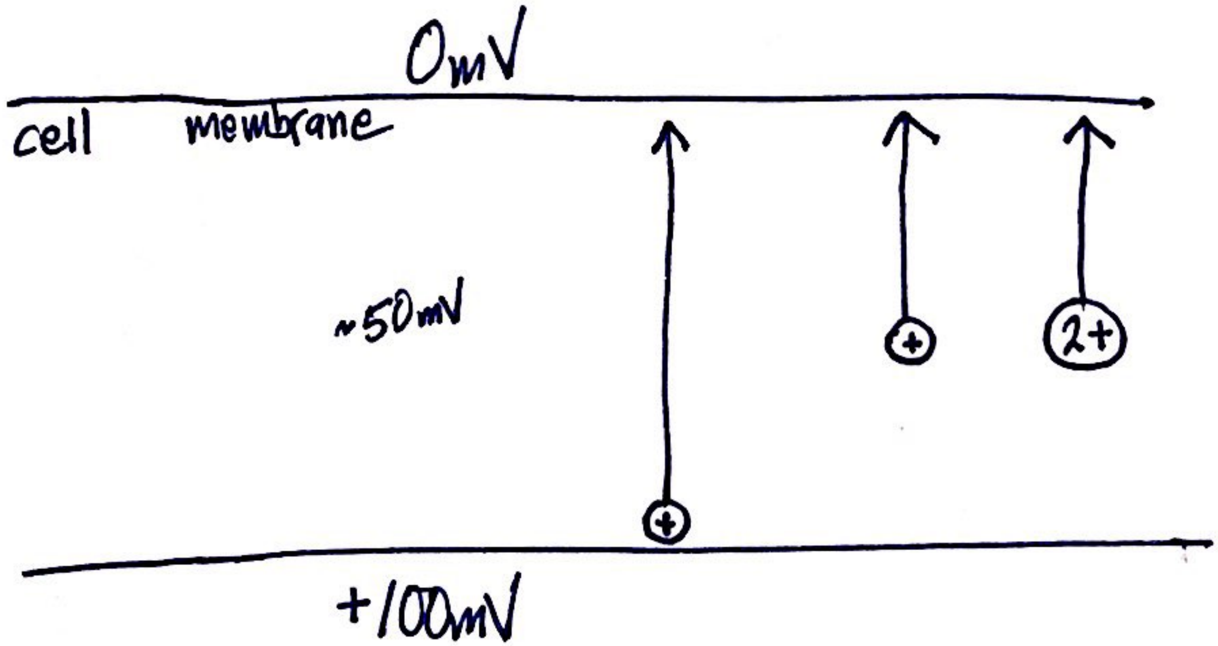
$$\hookrightarrow \quad Q = \int I(t)\,dt$$






$$Q = \int I(t)\,dt$$

$$1\ C = 6.24 \cdot 10^{18}\ e$$


0mV
cell membrane
~50mV
+
2+
+
+100mV

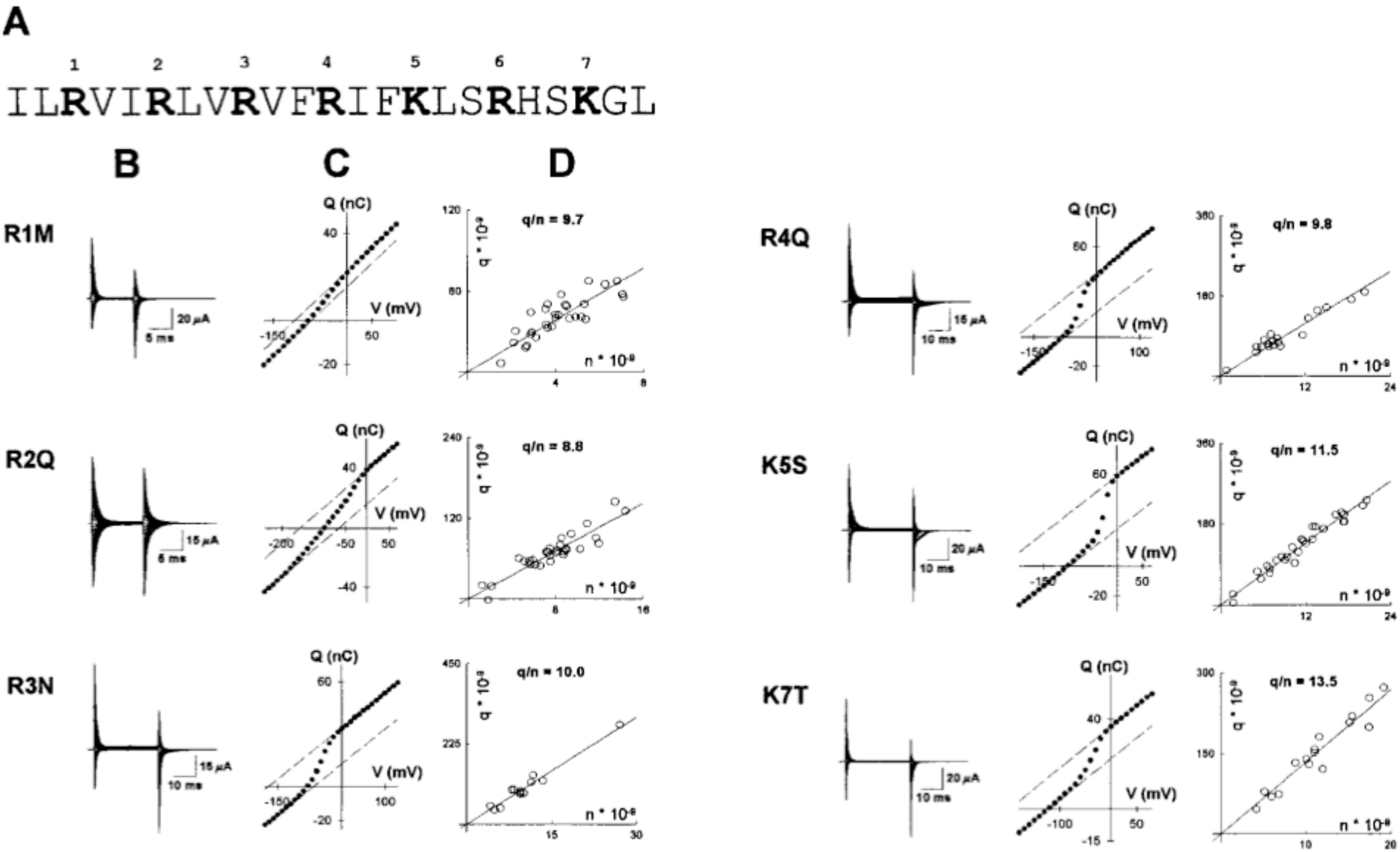



Figure 4. Determination of Gating Charge for Charge-Neutralizing Mutations in the S4 Segment

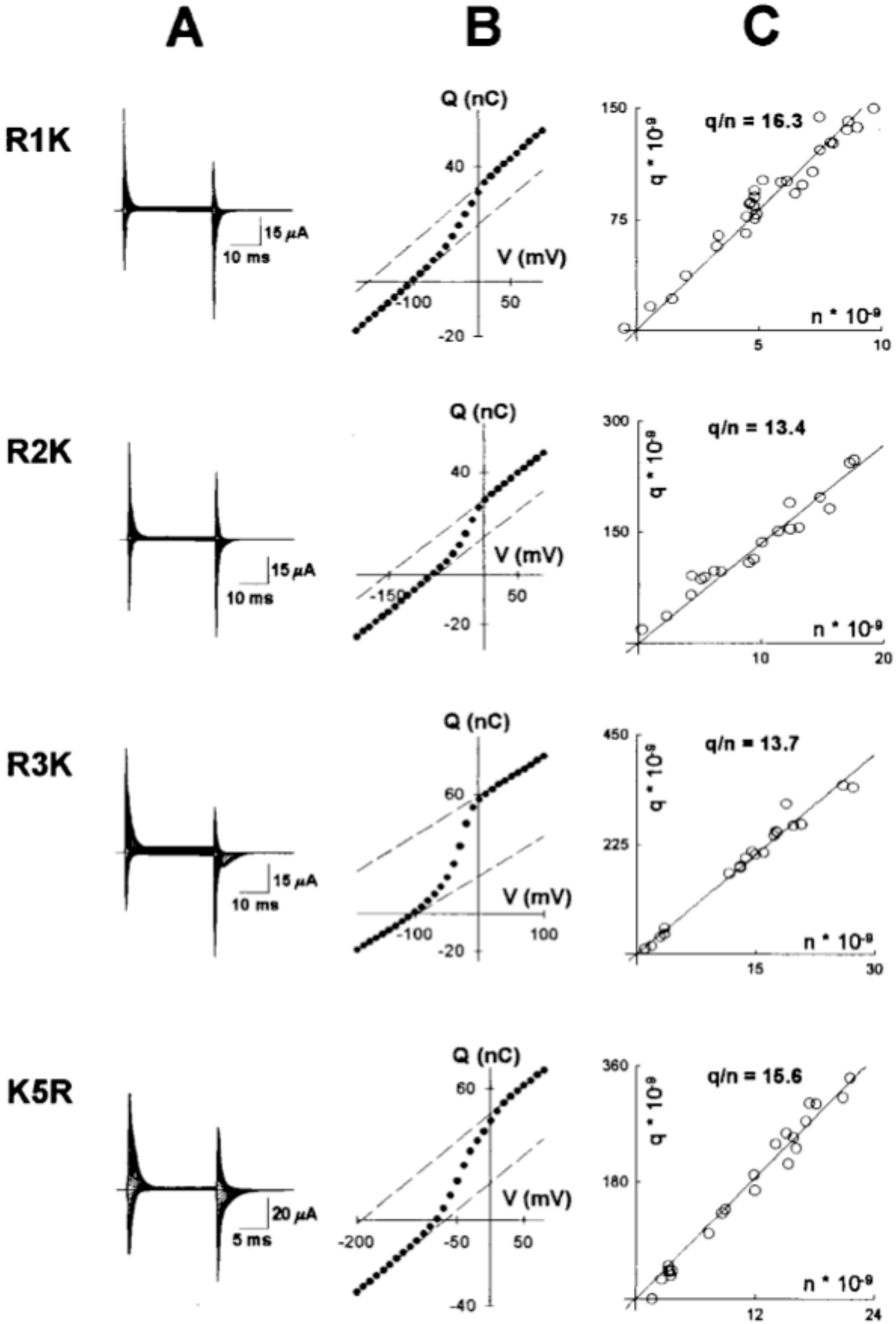



**Figure 5. Determination of Gating Charge for Charge-Conserving Mutations in the S4 Segment**

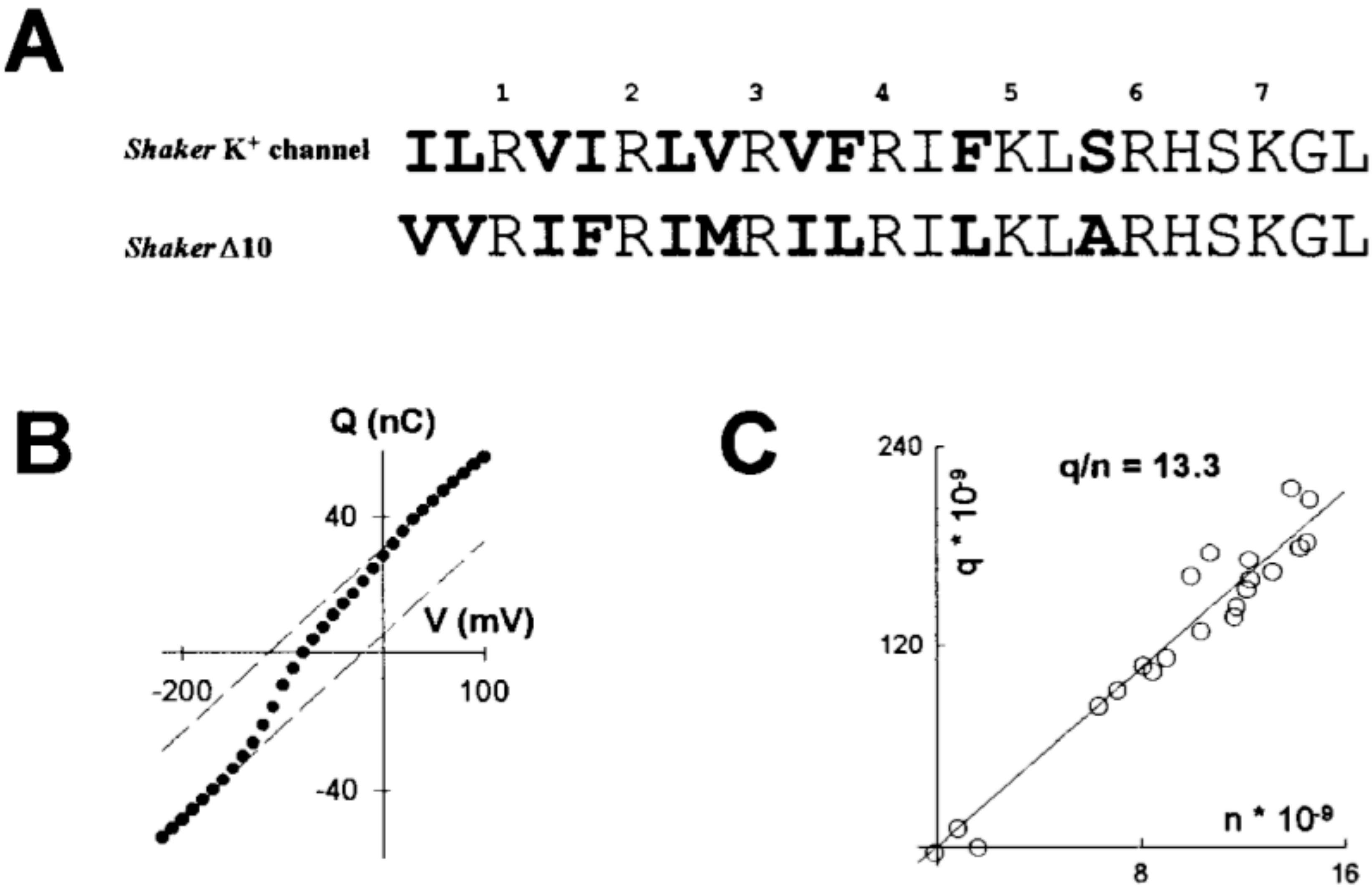



Figure 6. Contribution of Nonbasic Residues in the S4 Segment to Gating Charge

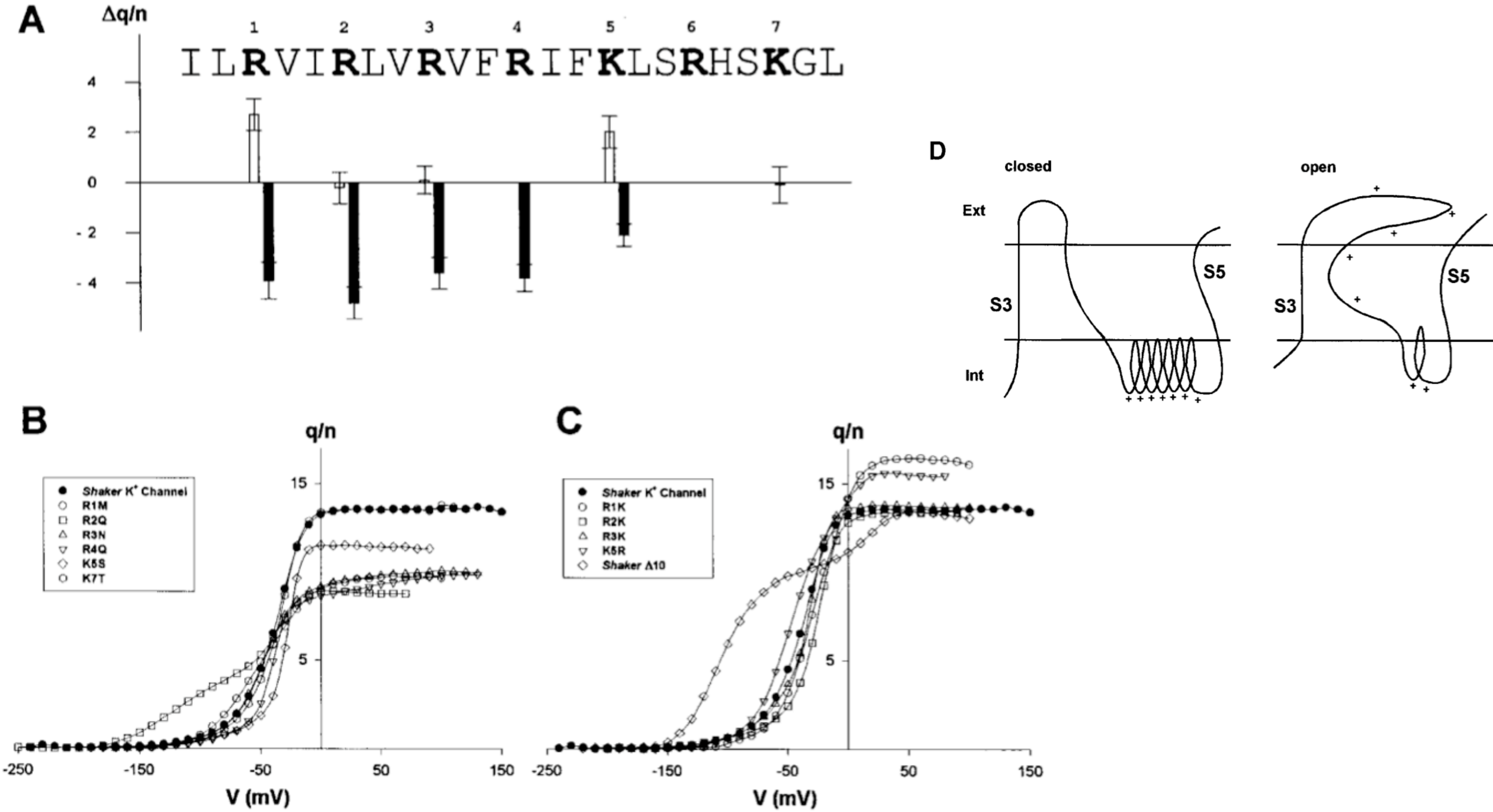
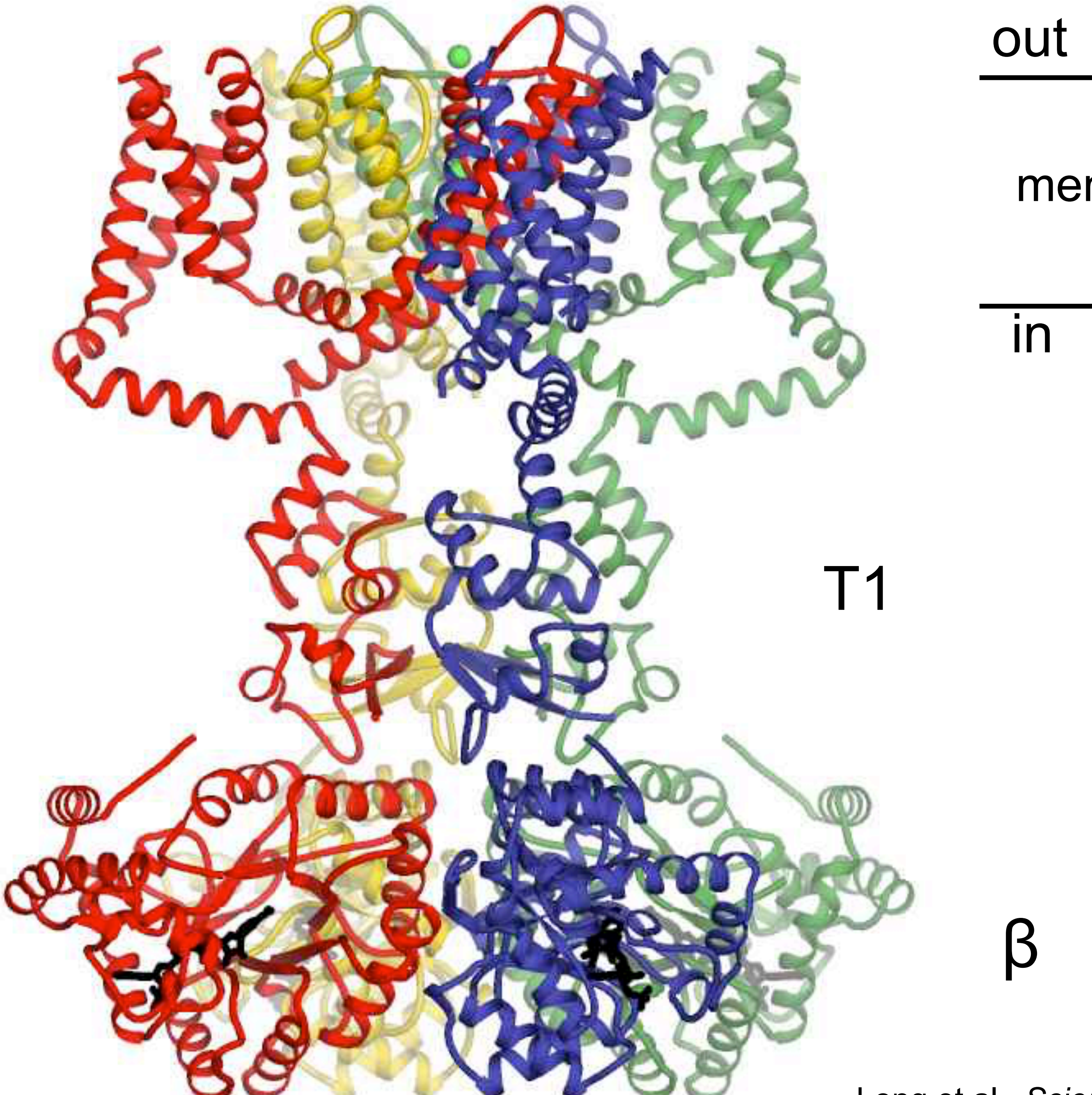
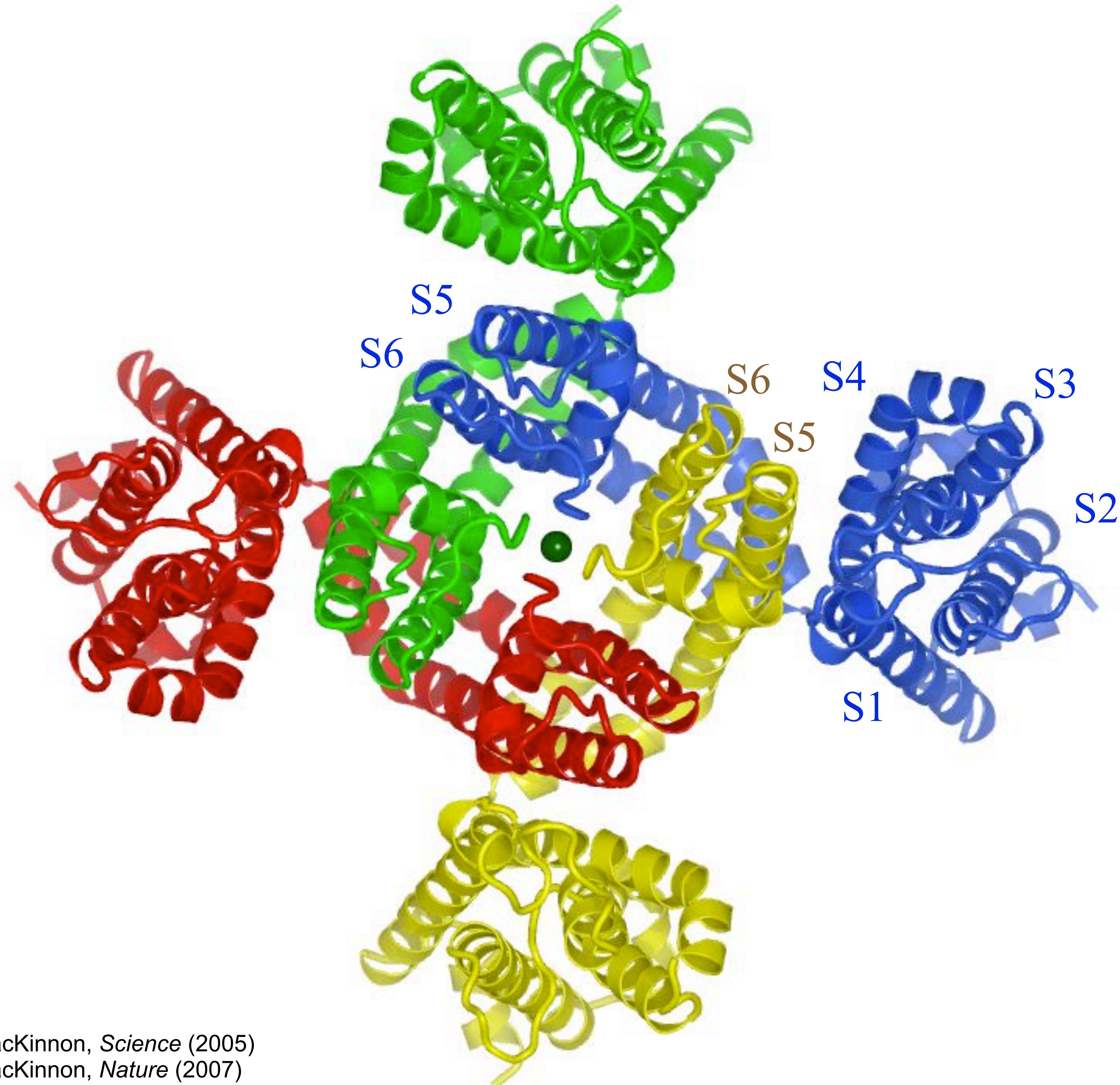



Figure 7. Summary

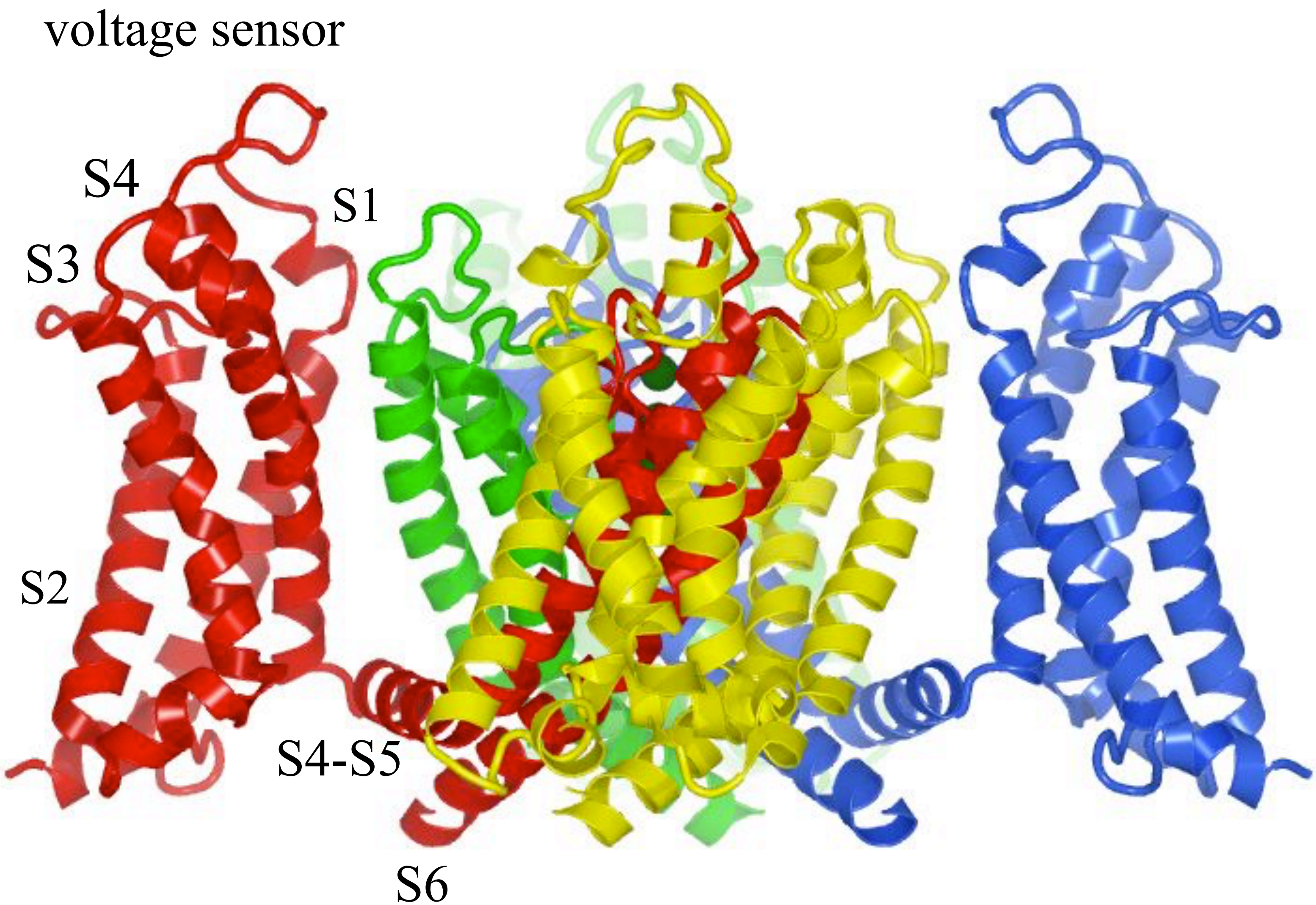
# Kv1.2 - $\beta_2$ crystal structure at 2.9Å




Long et al., *Science* (2005)

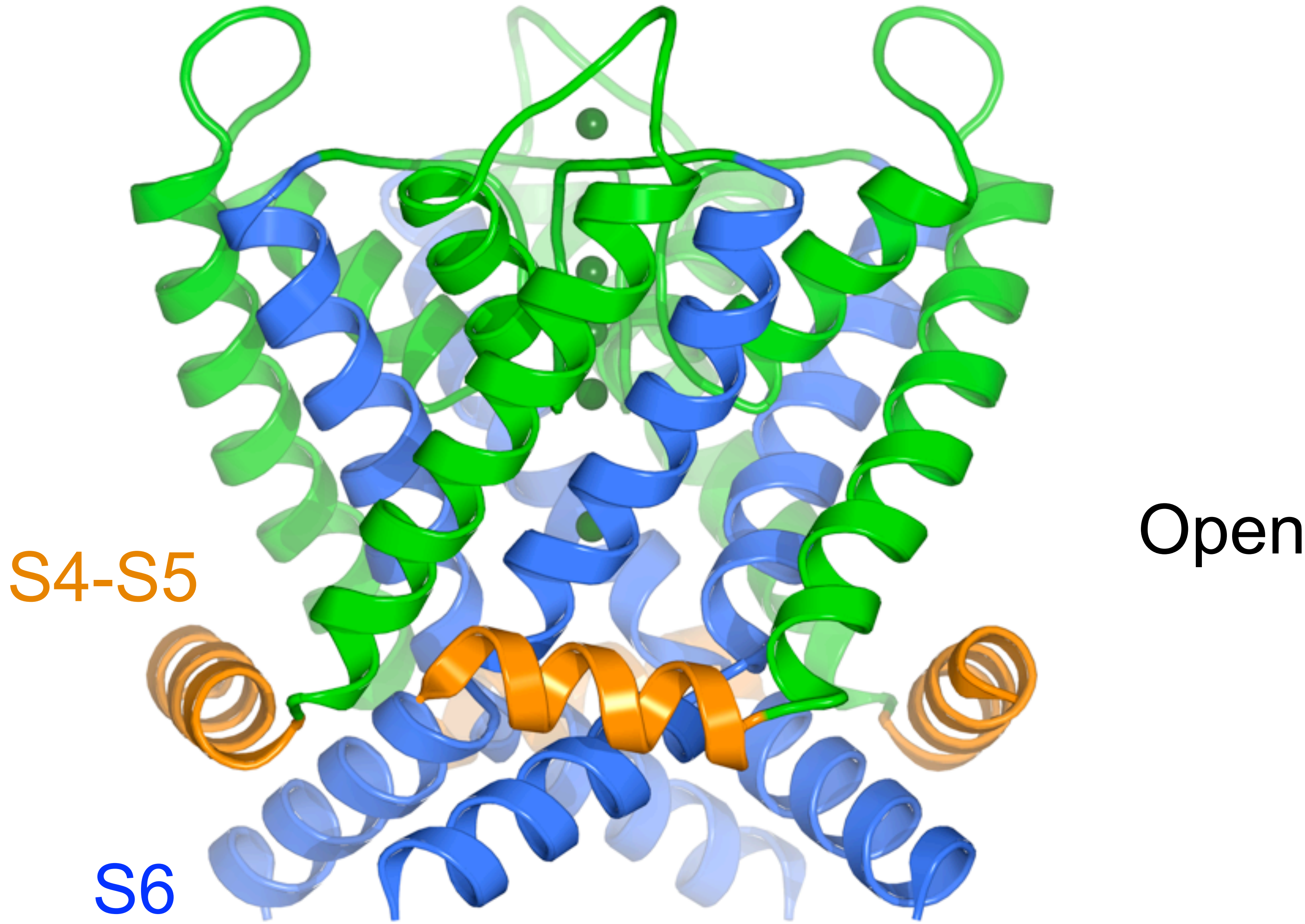



Long et al. & MacKinnon, *Science* (2005)
Long et al. & MacKinnon, *Nature* (2007)

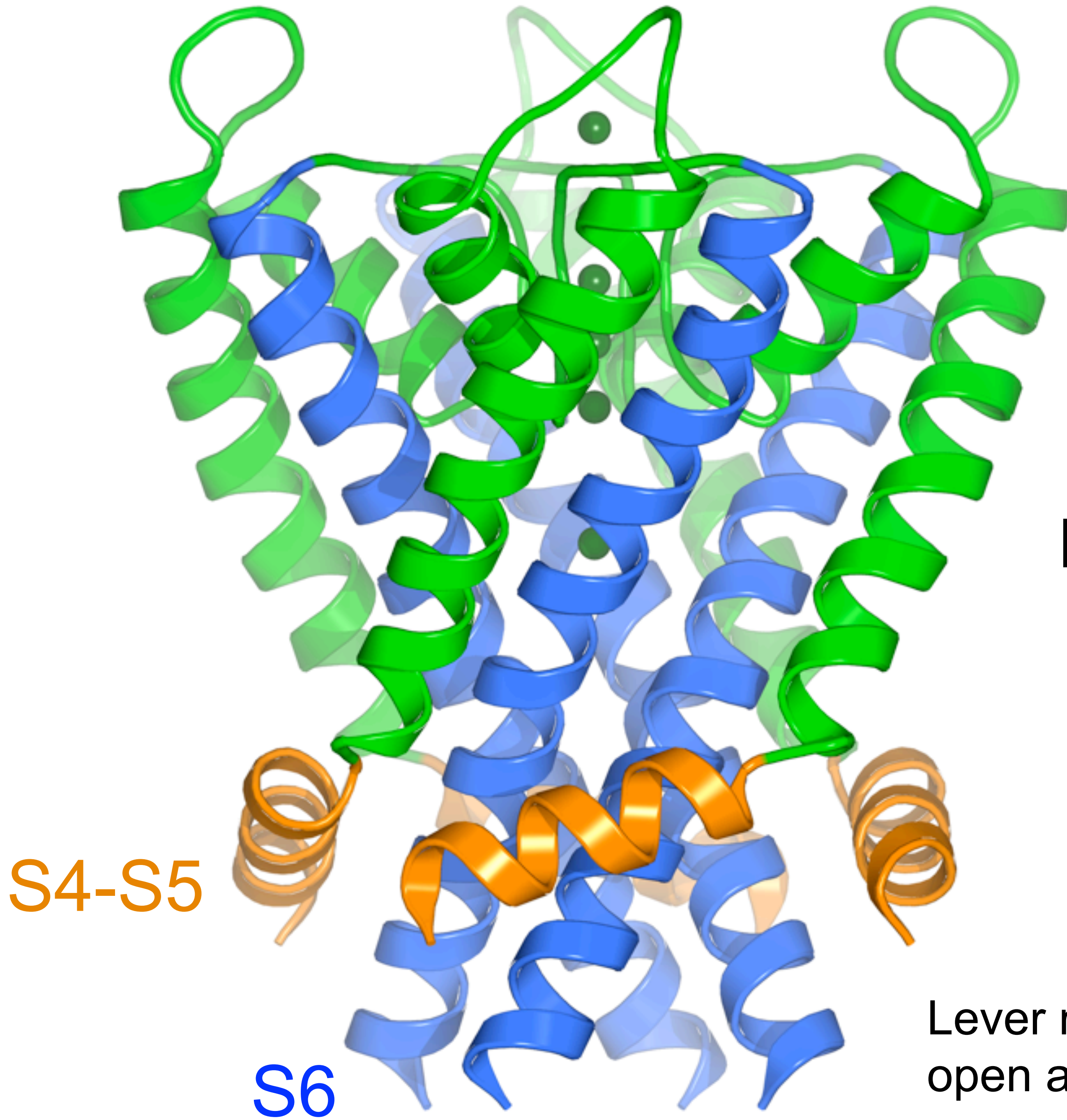



The S4-S5 helix couples the voltage sensor to the pore

# role of the S4-S5 linkers

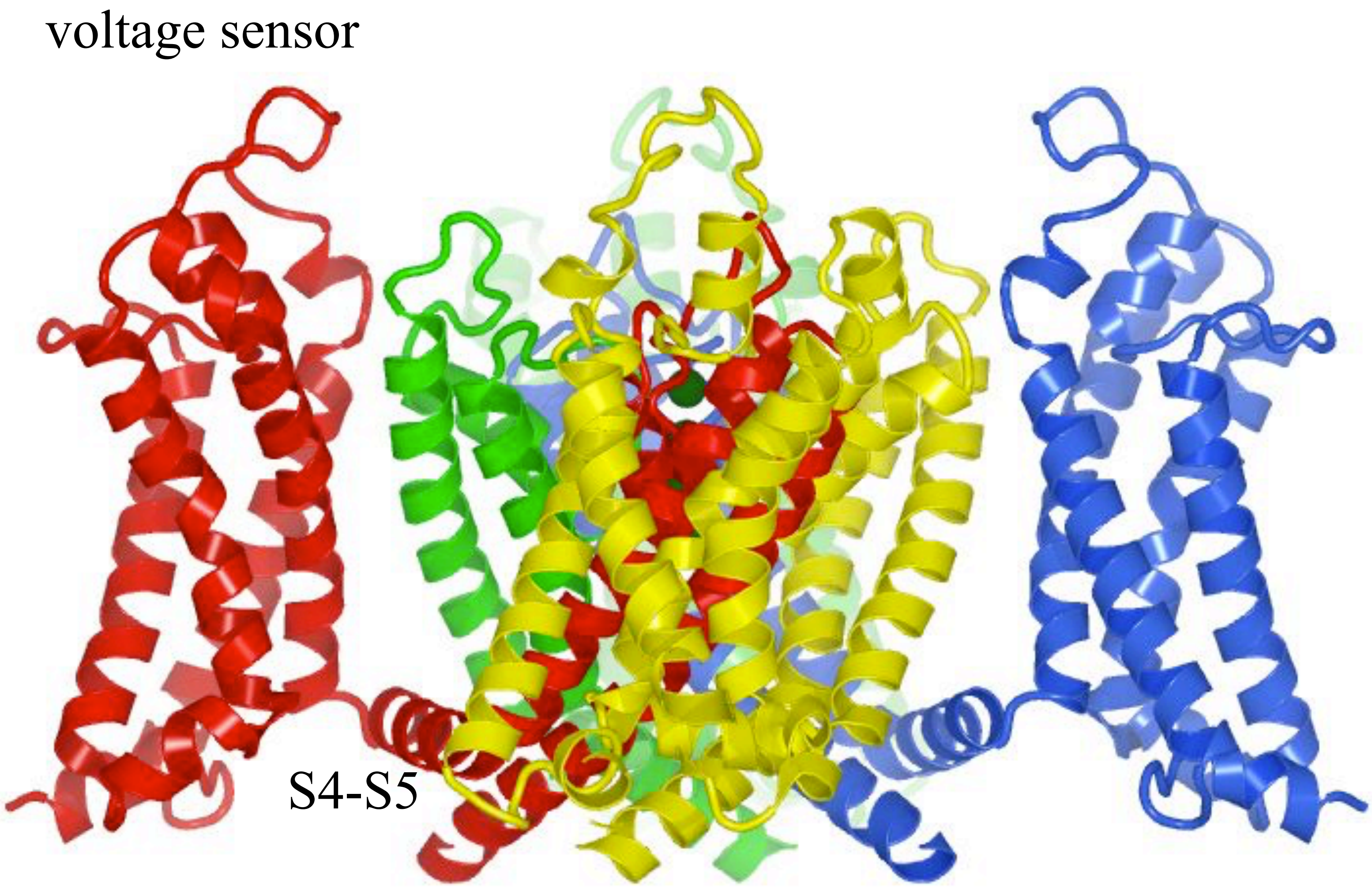


# role of the S4-S5 linkers




Hypothetical closed

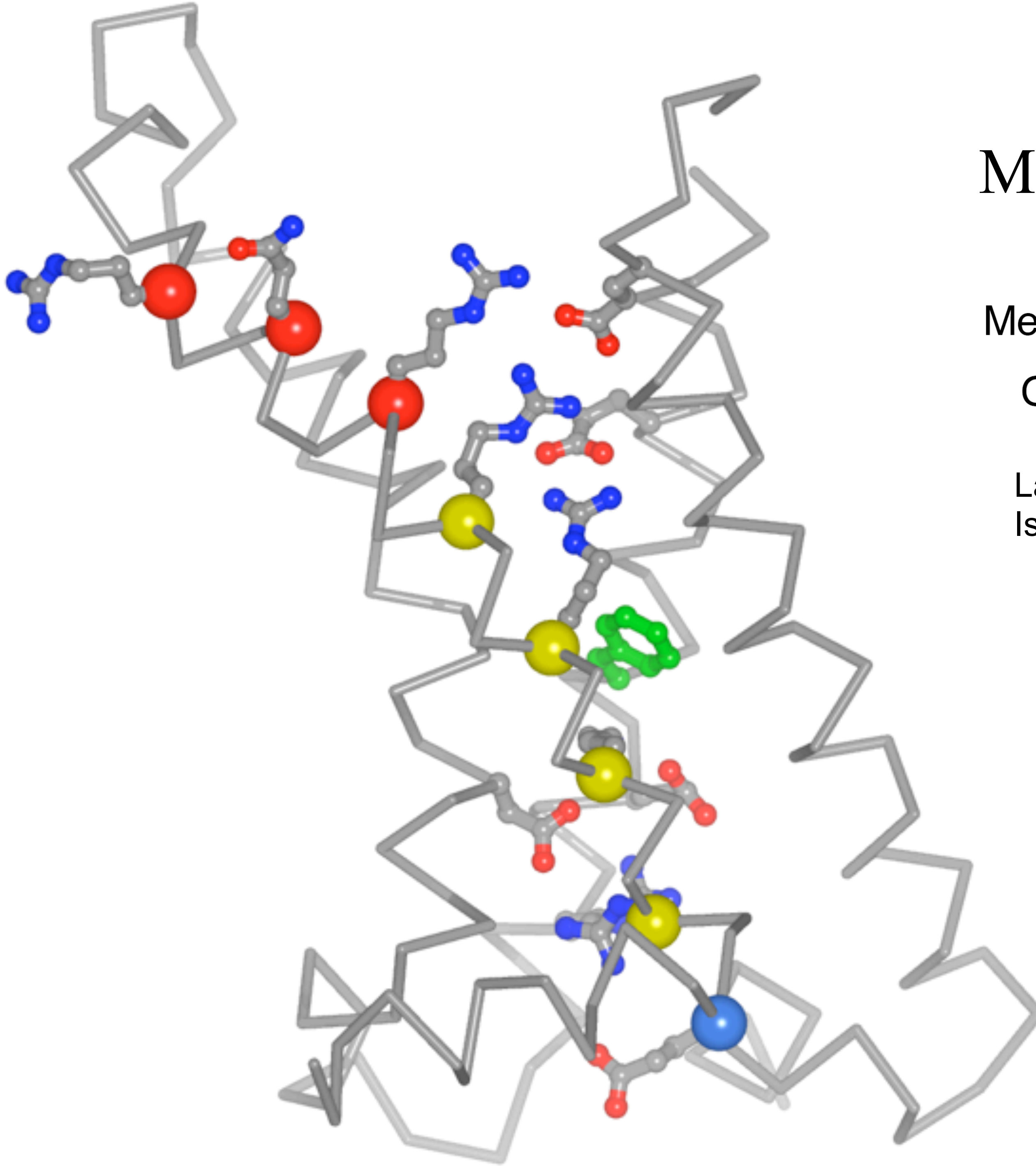
Lever mechanism to open and close the pore



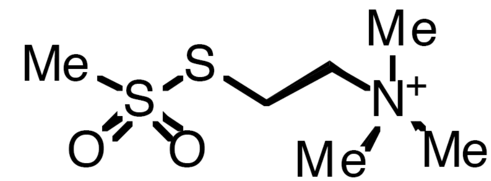

Linkage between S4 and S4-S5

# Open state MTS reactivity

extracellular

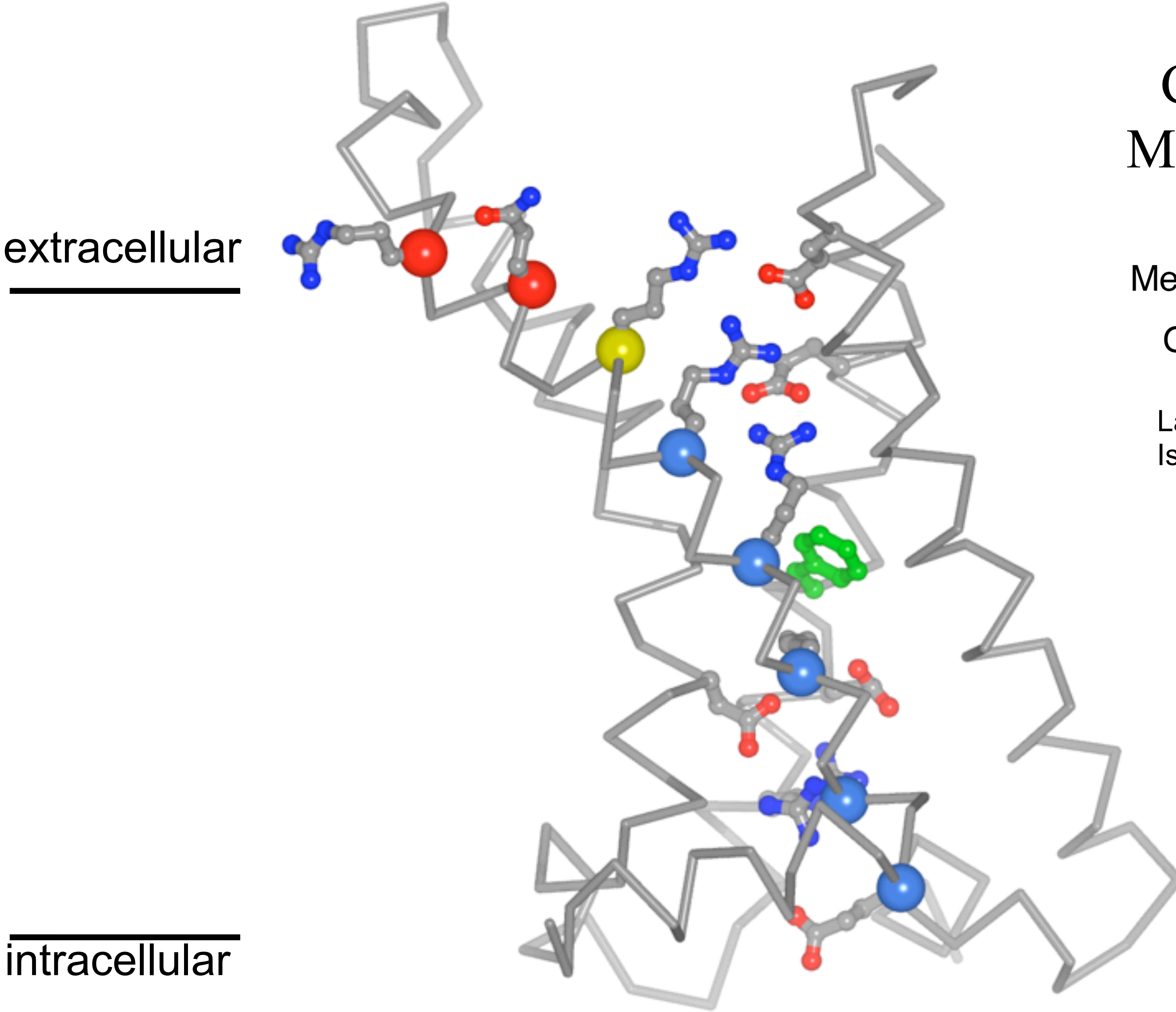


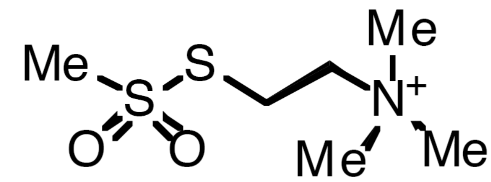
intracellular



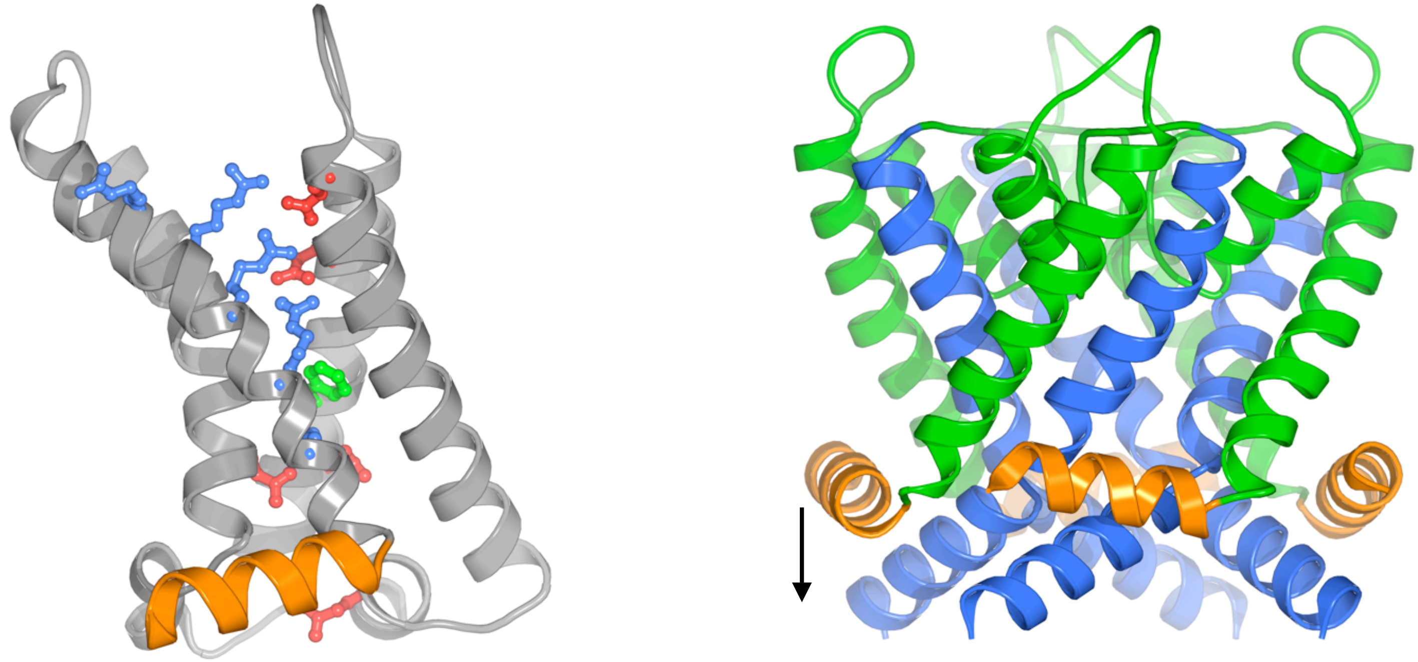

Larsson et al. &
Isacoff, *Neuron* (1996)

# Closed state MTS reactivity

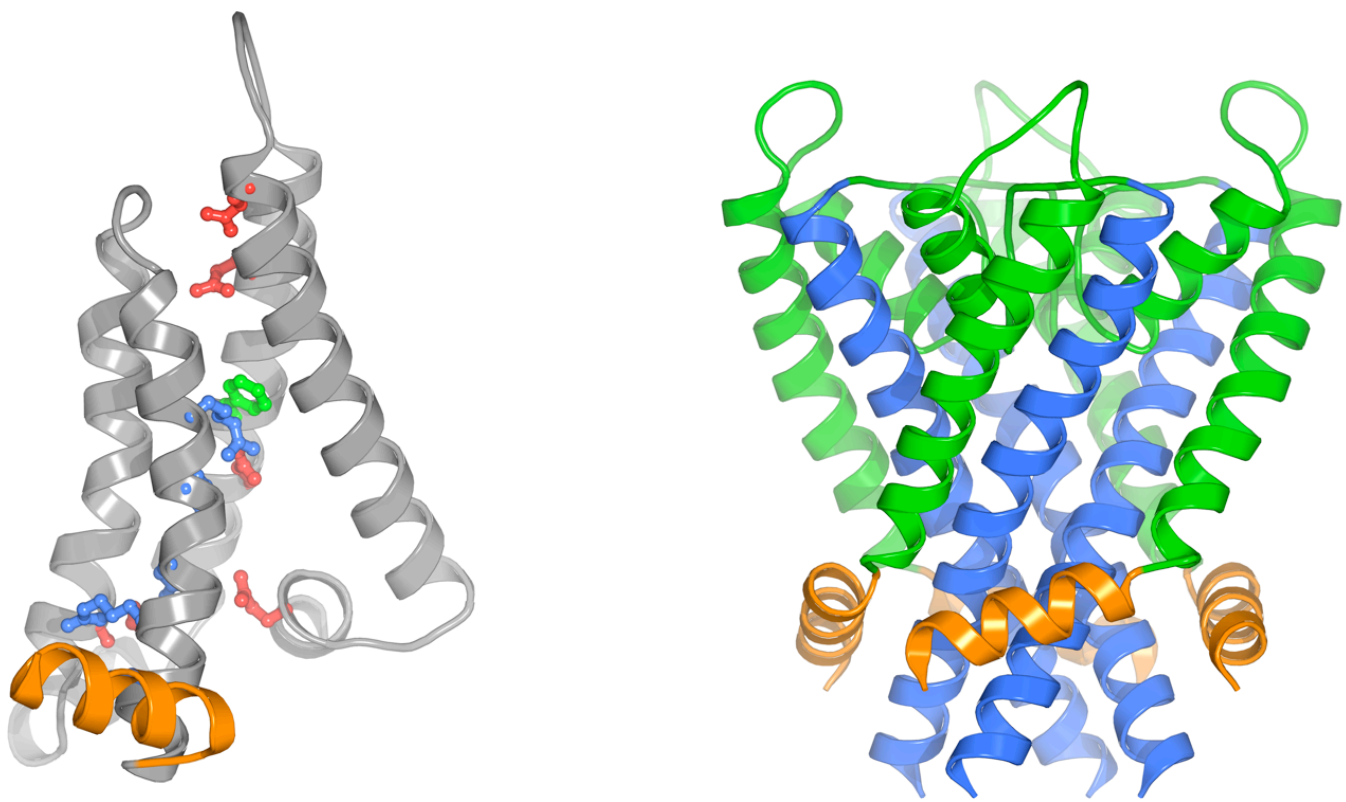



Larsson et al. & Isacoff, *Neuron* (1996) open



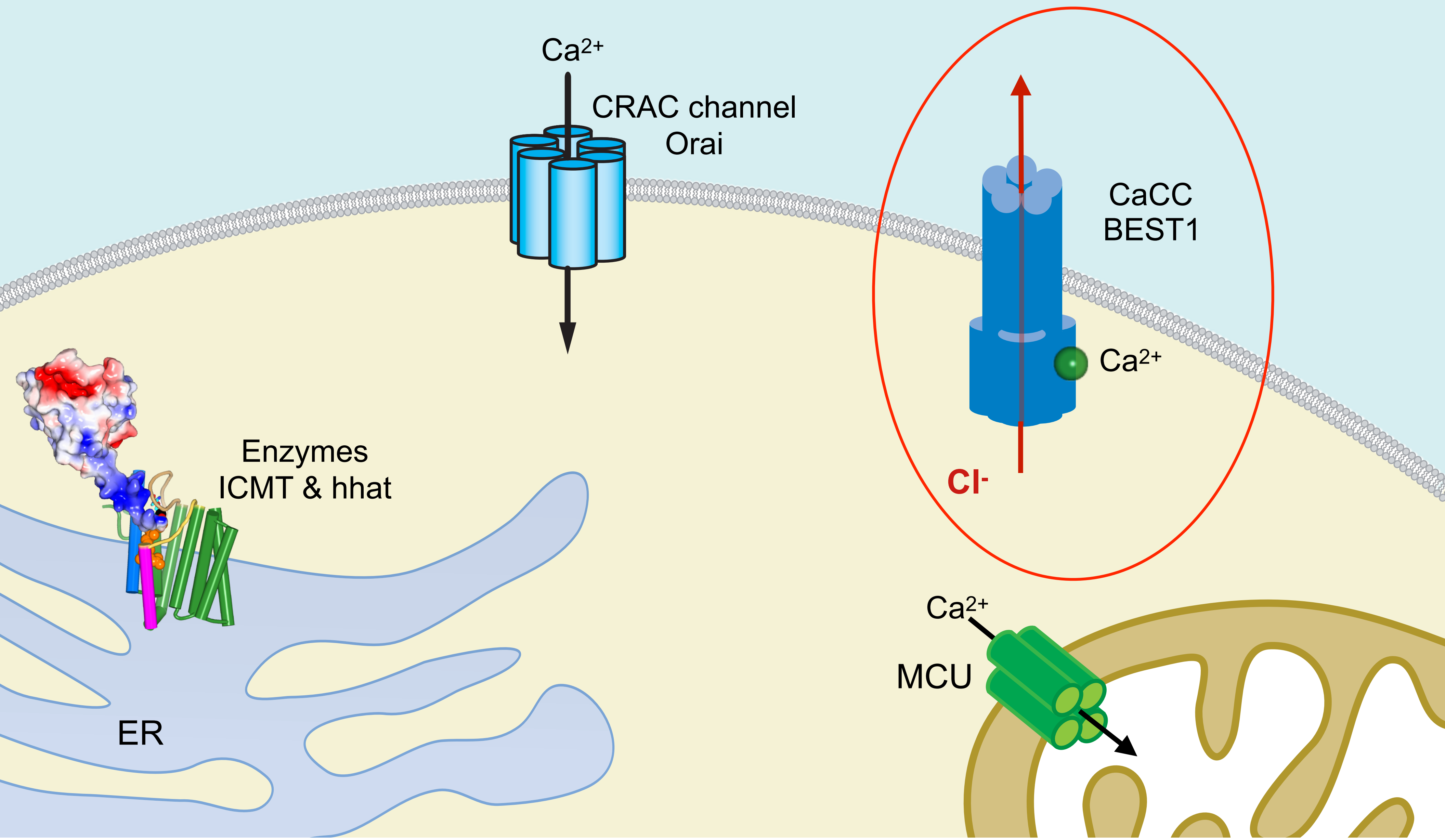
hypothetical
closed



# Long Lab

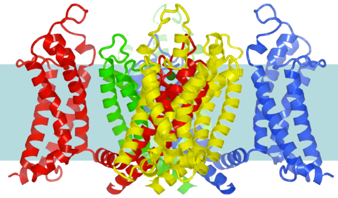
## Membrane enzymes

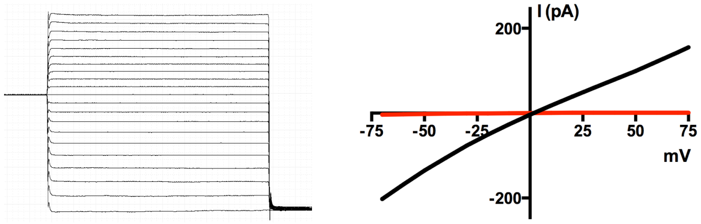
## Ion channels in $Ca^{2+}$ signaling



# Stephen Long - ion channels in calcium signaling and membrane enzymes

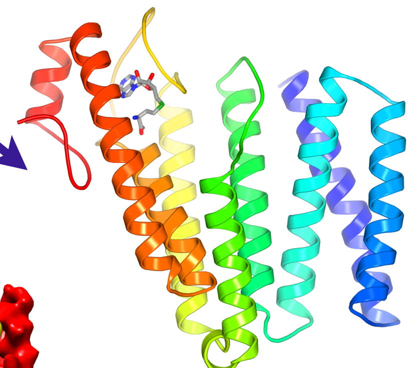
## structural biology, electrophysiology, enzymology, drug discovery



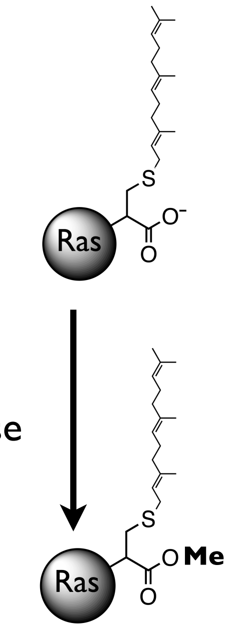



Vaisey, Miller & Long, *PNAS* (2016)
Vaisey, & Long, *JGP* (2018)

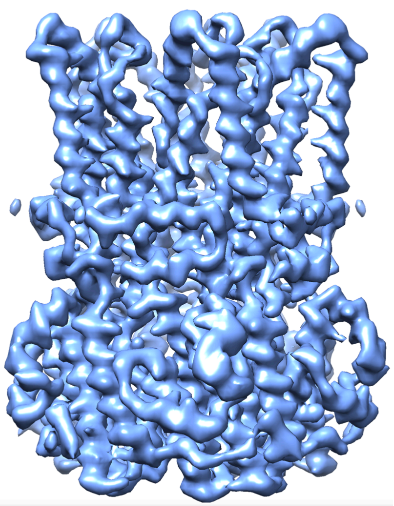
## membrane enzymes



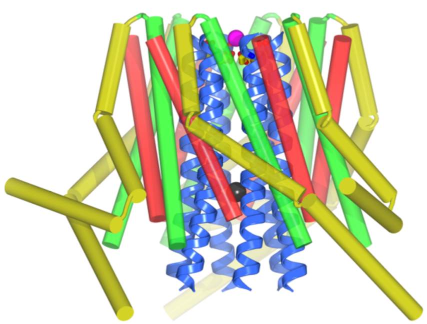
ICMT
methyltransferase
Diver et al.,
*Nature* (2018)



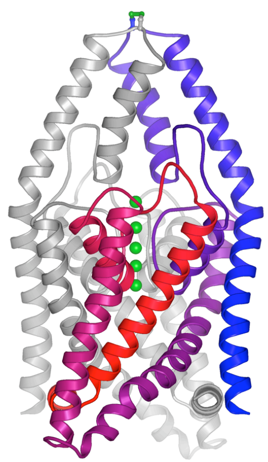

## ion channels



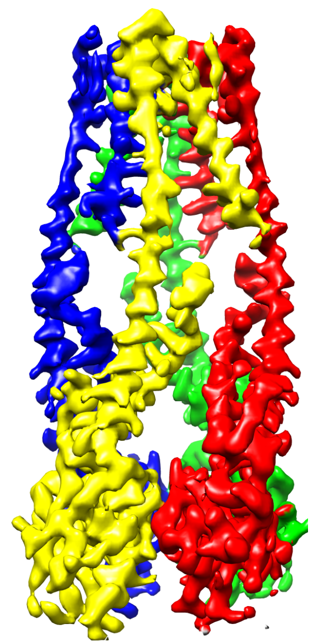
Calcium-Activated
Chloride Channel
Dickson et al., *Nature* (2014)
MIller*, Vaisey*, & Long, *eLife* (2019)



Calcium Channel Orai
Hou et al., *Science* (2012)



Potassium Channel K2P1
Miller & Long, *Science* (2012)

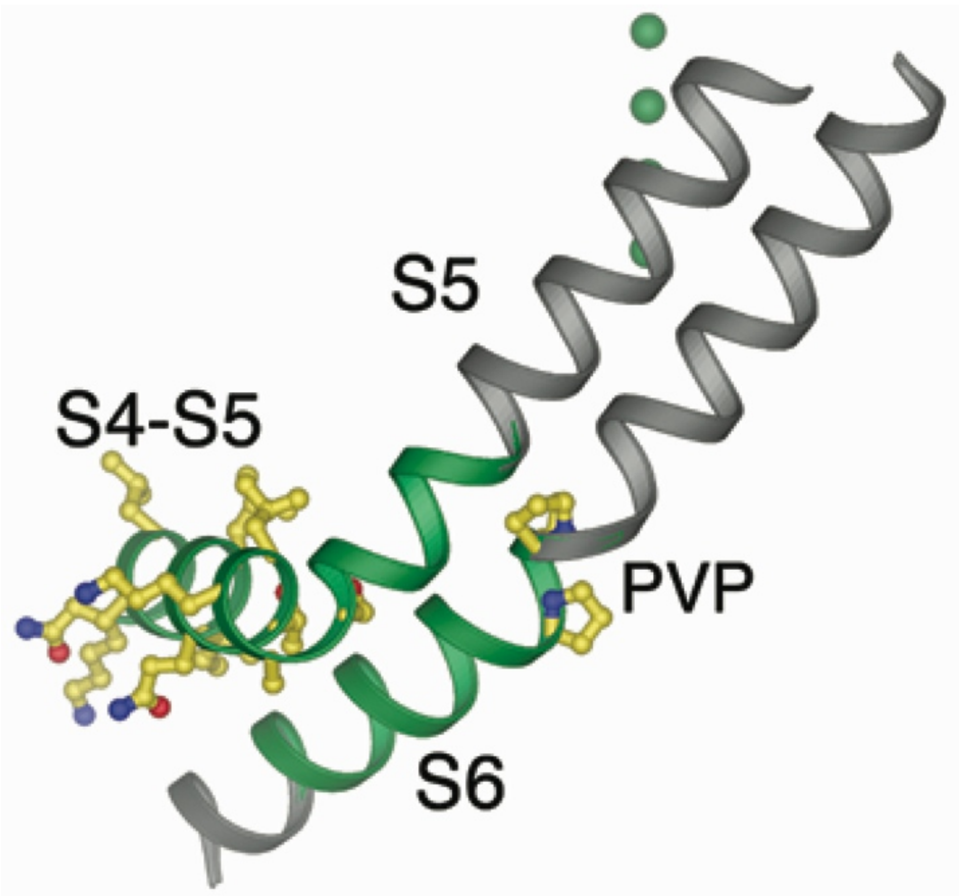


MCU Calcium channel
Baradaran, Wang et al. & Long
*Nature* (2018)

Research opportunities
discovery
molecular mechanism
physiology
anticancer drug development

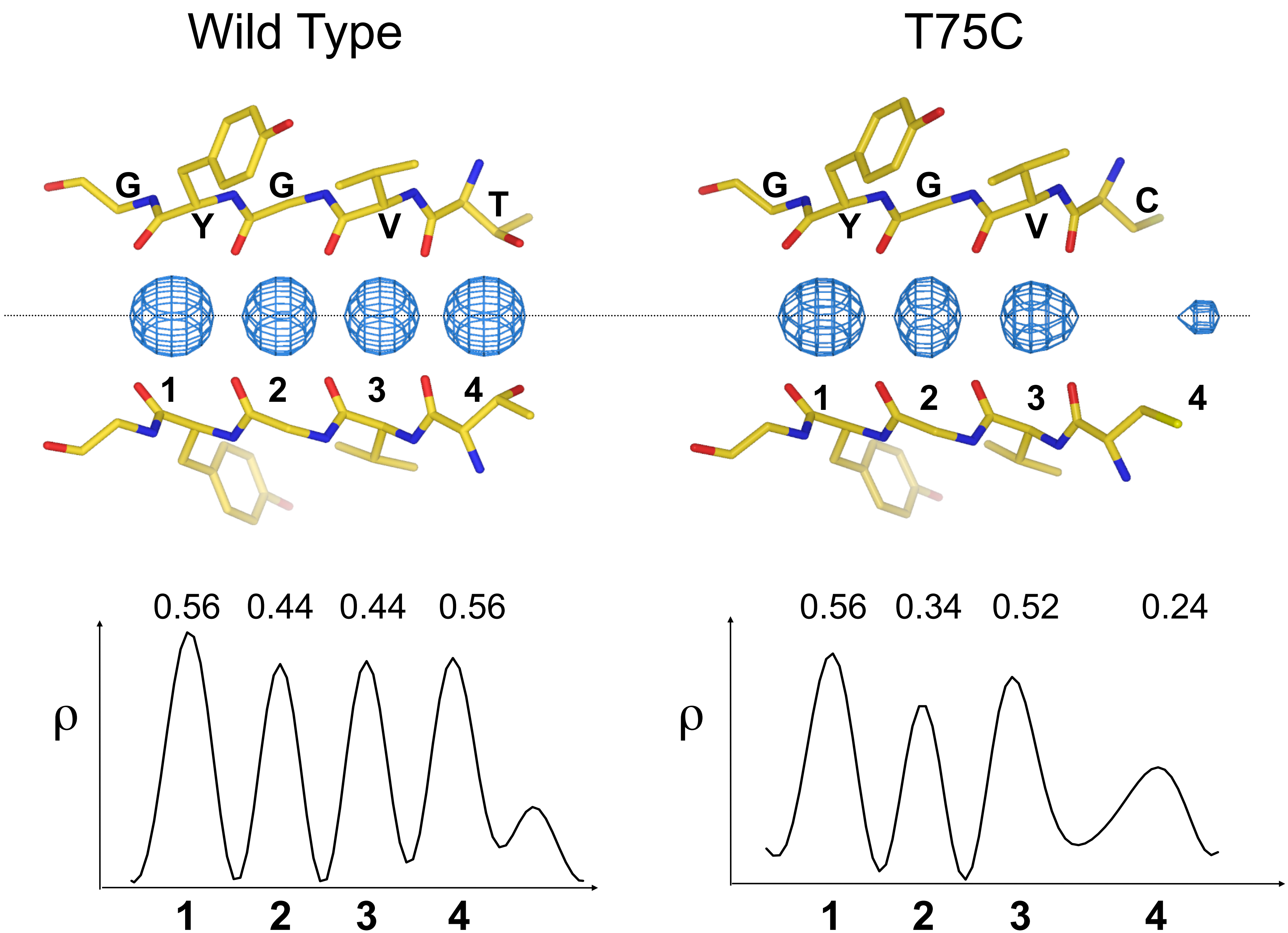
Red lettering denotes work by graduate students

# S4-S5 linker interacts with S6



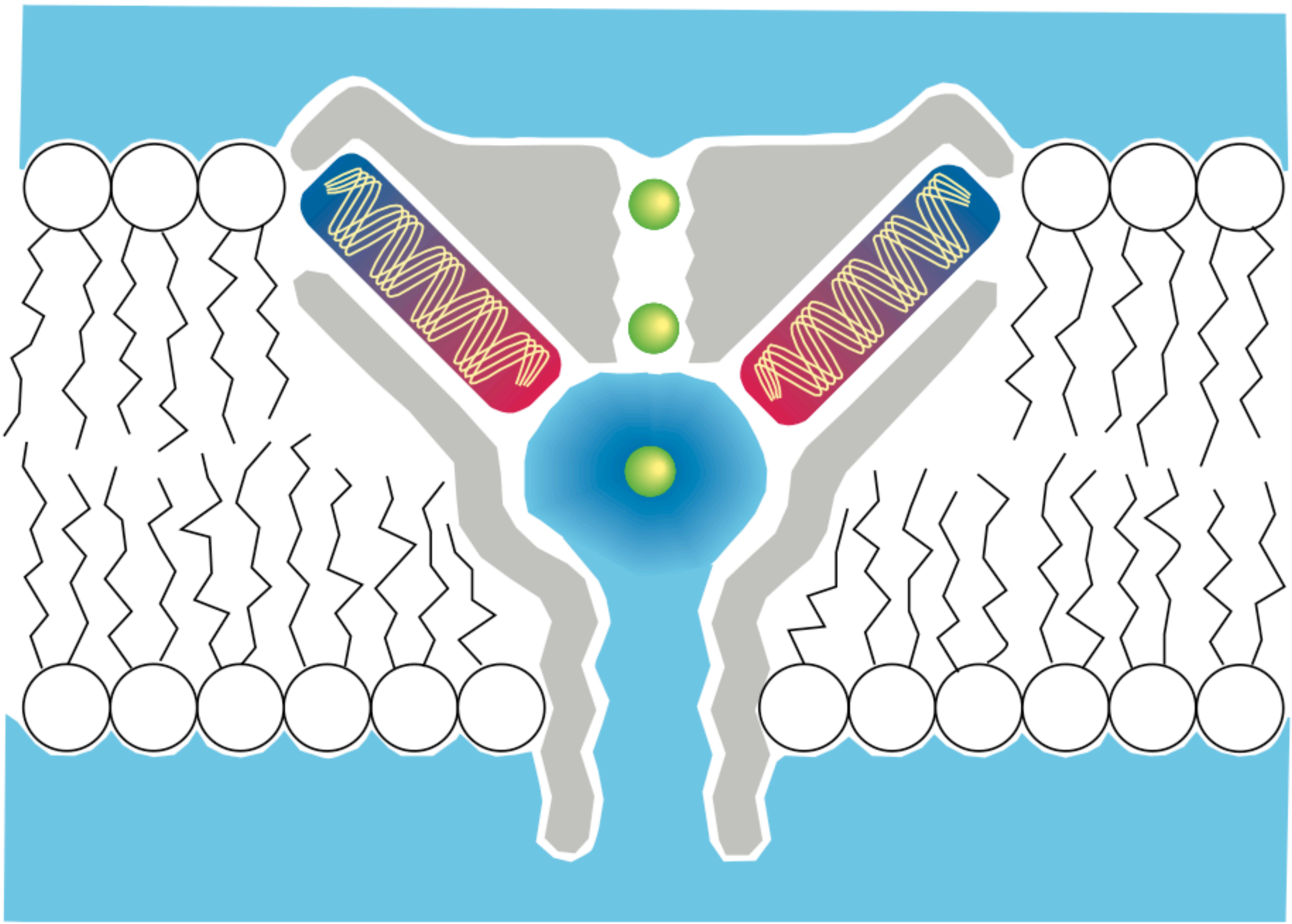

Zhe Lu et al., *Nature* (2001)
Zhe Lu et al., *J. Gen. Physiol.* (2002)

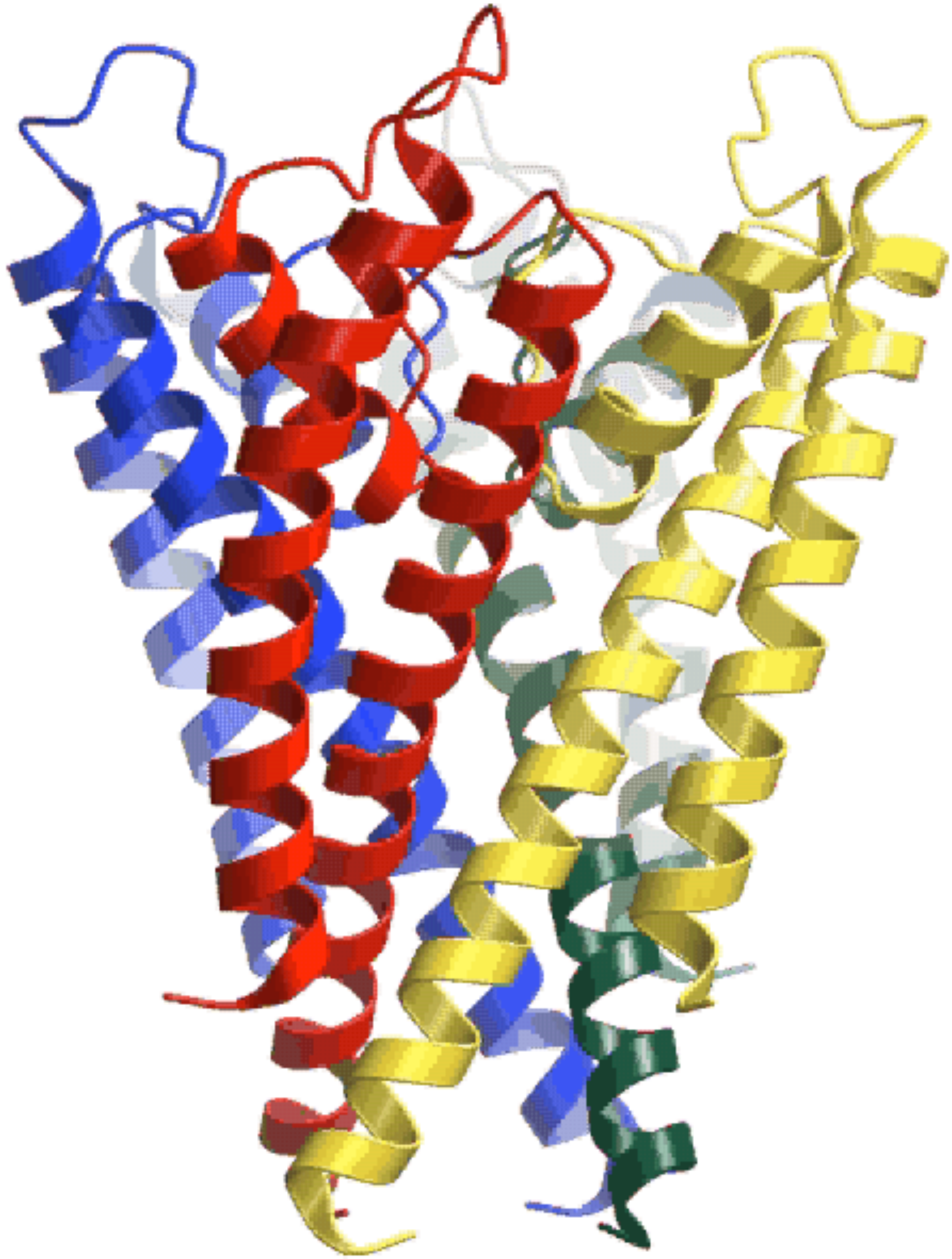
# Mutation of position 4 alters occupancy at 2 and 4




Zhou and MacKinnon. Journal of Molecular Biology (2003) vol. 333 (5) pp. 965-75 & (2004) vol. 338 (4) pp. 839-46

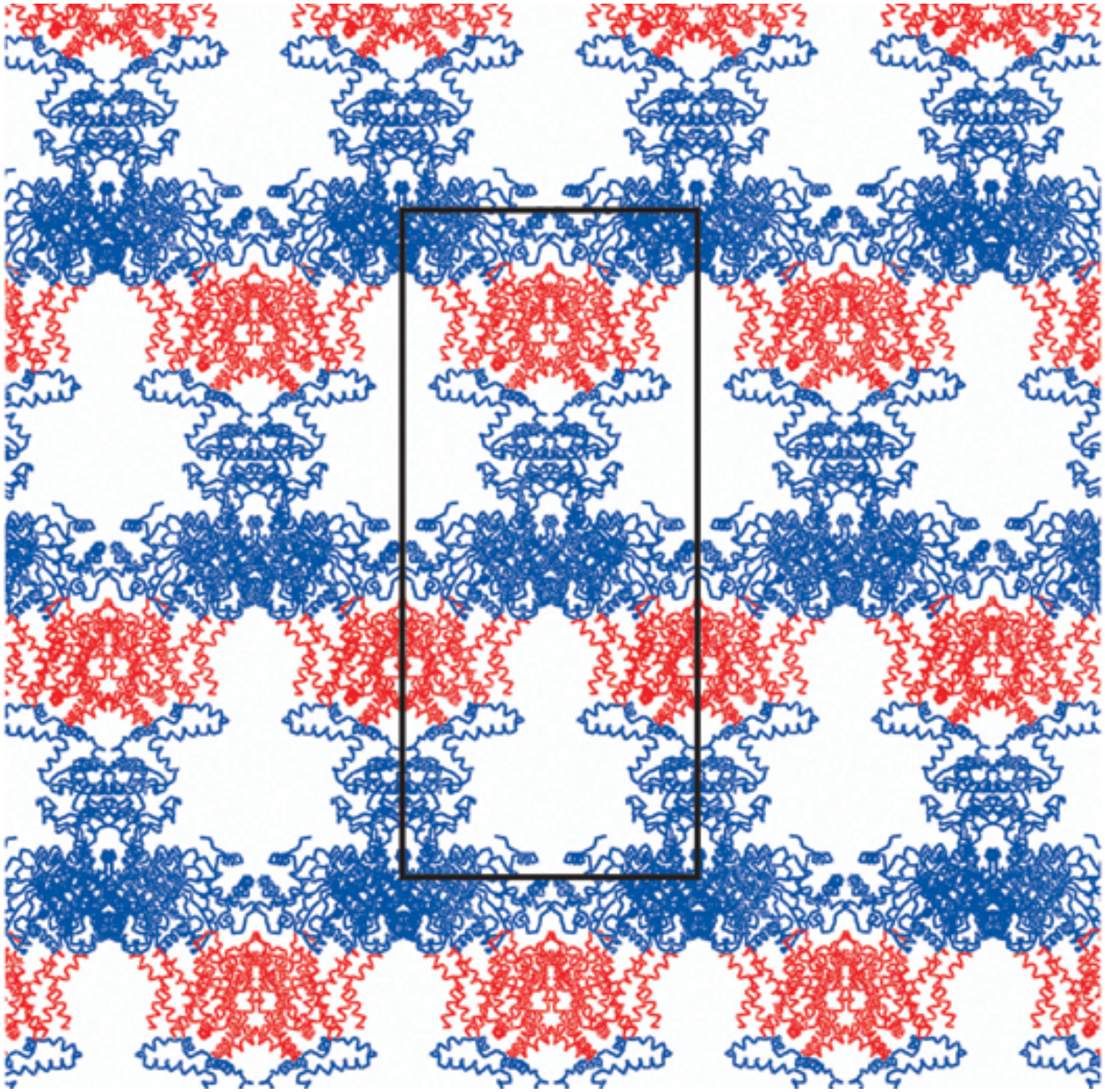
# Helix dipole may help stabilize $K^+$ at center of membrane





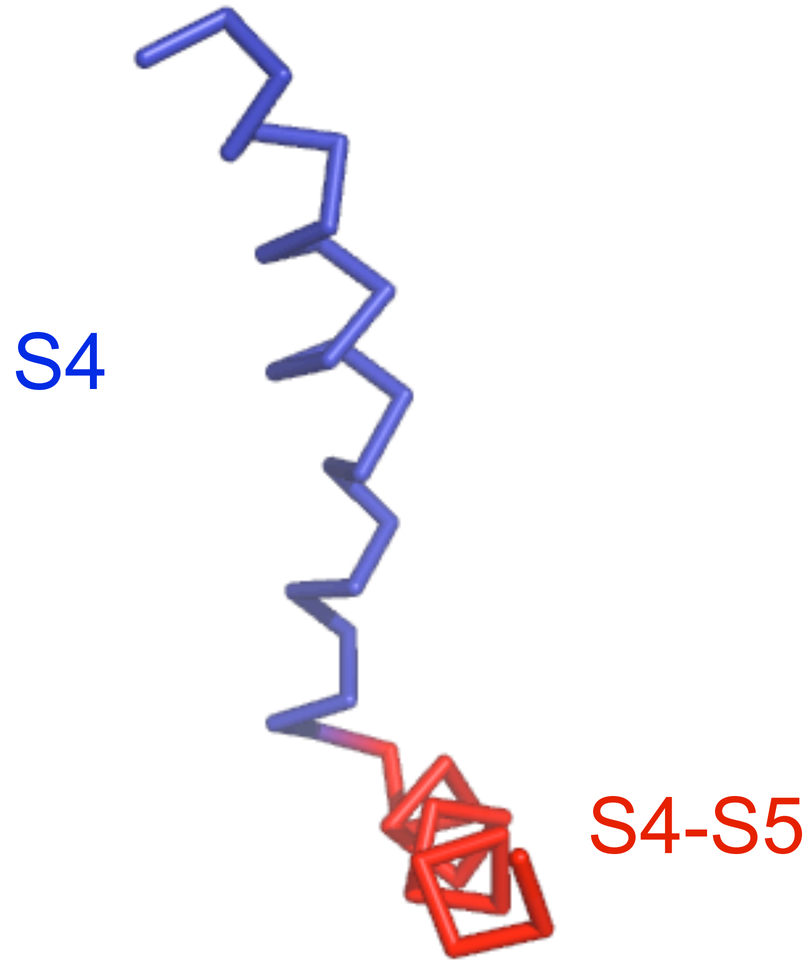
Doyle et al. Science (1998) vol. 280 (5360) pp. 69-77

# Crystal packing of Kv1.2 + β resembles a lipid membrane

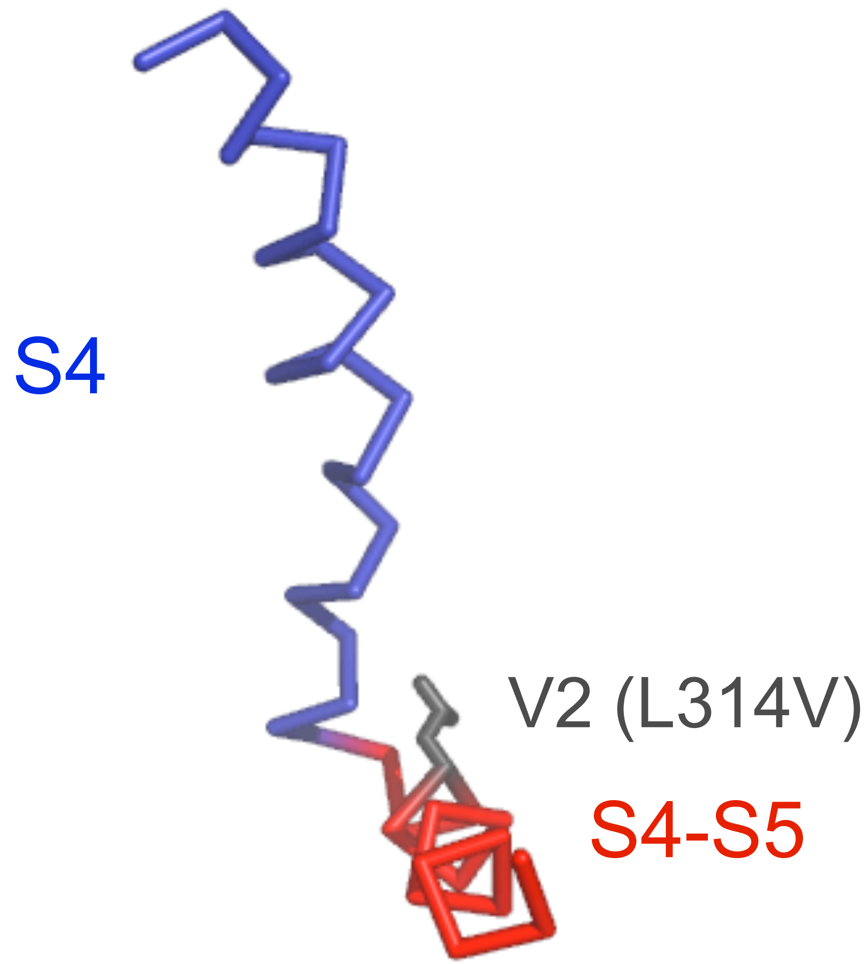


red: membrane-spanning region
blue: intracellular region

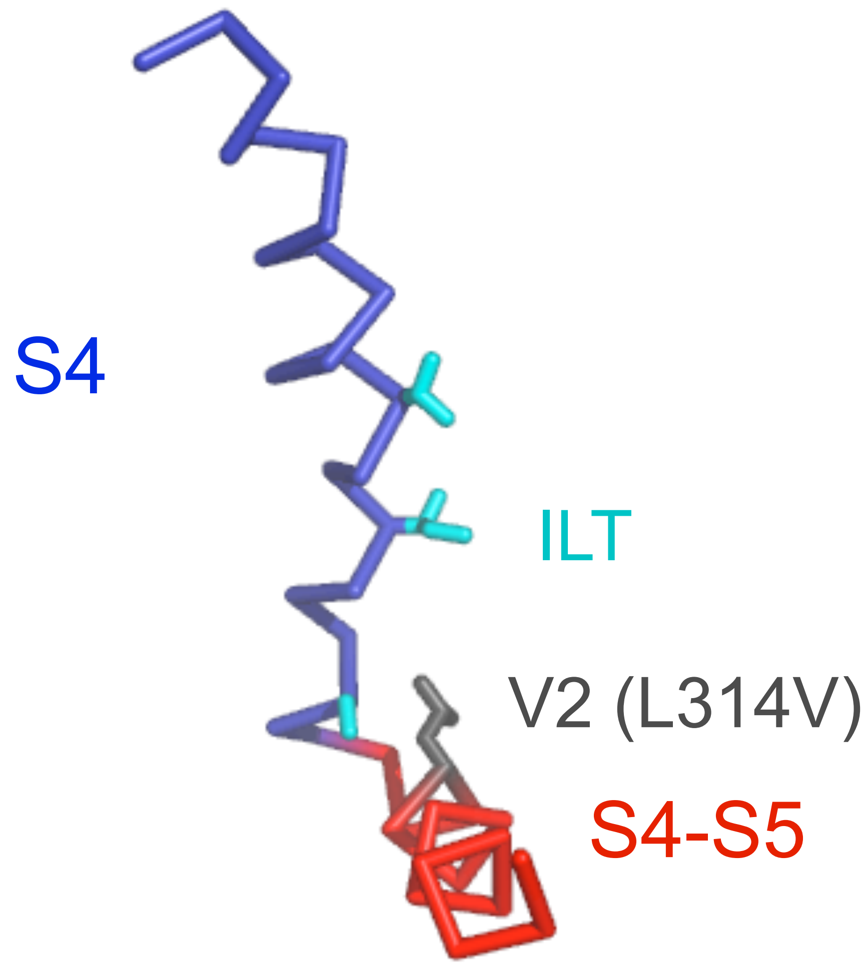
# linkage between S4 and S4-S5



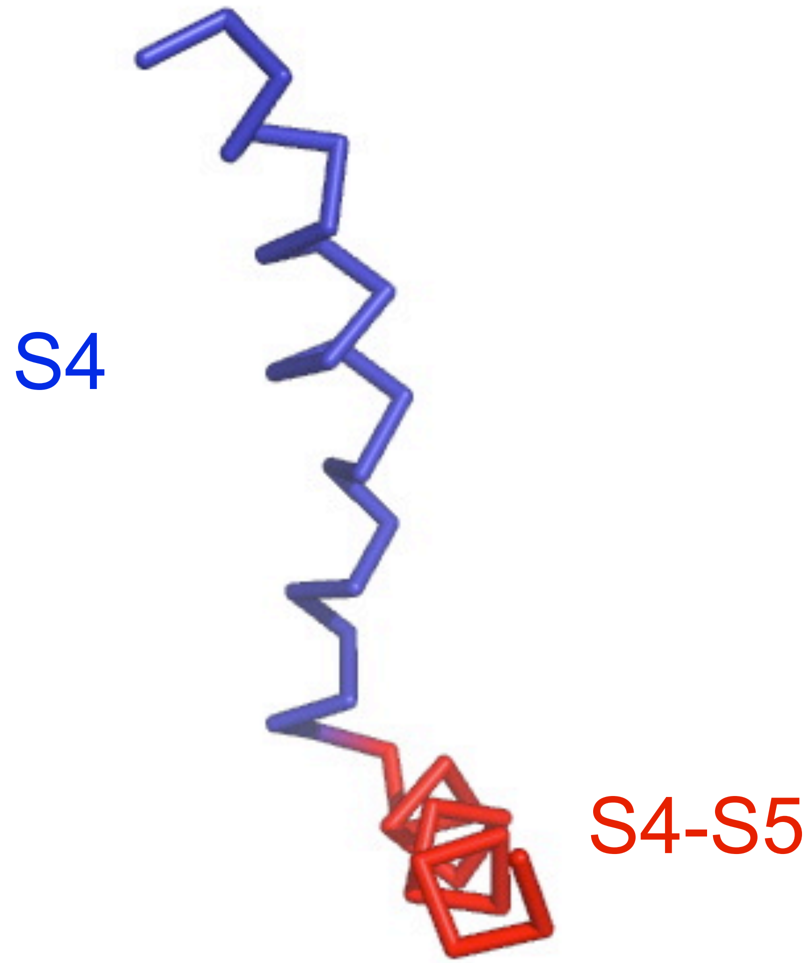
# linkage between S4 and S4-S5

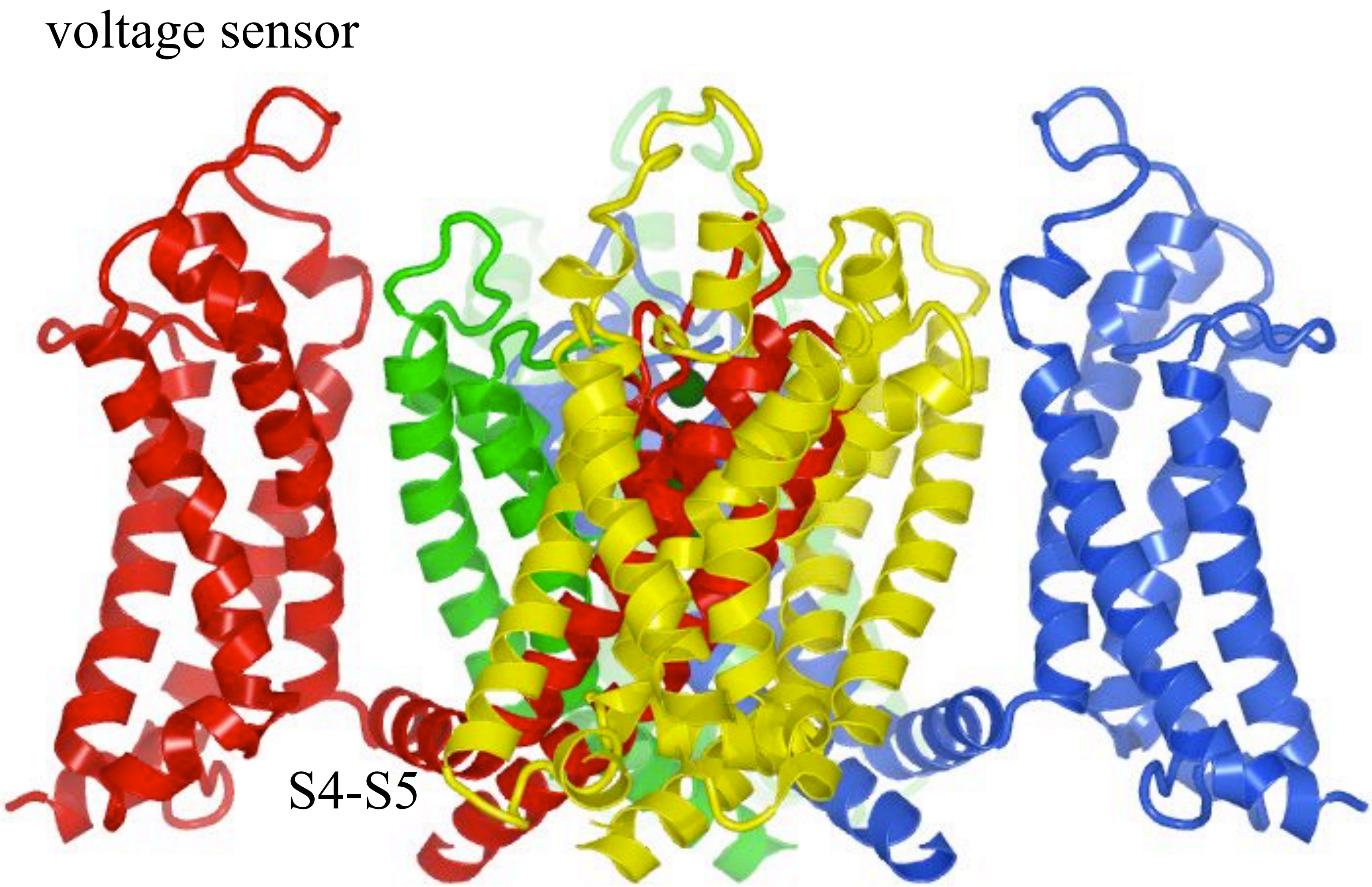


# linkage between S4 and S4-S5

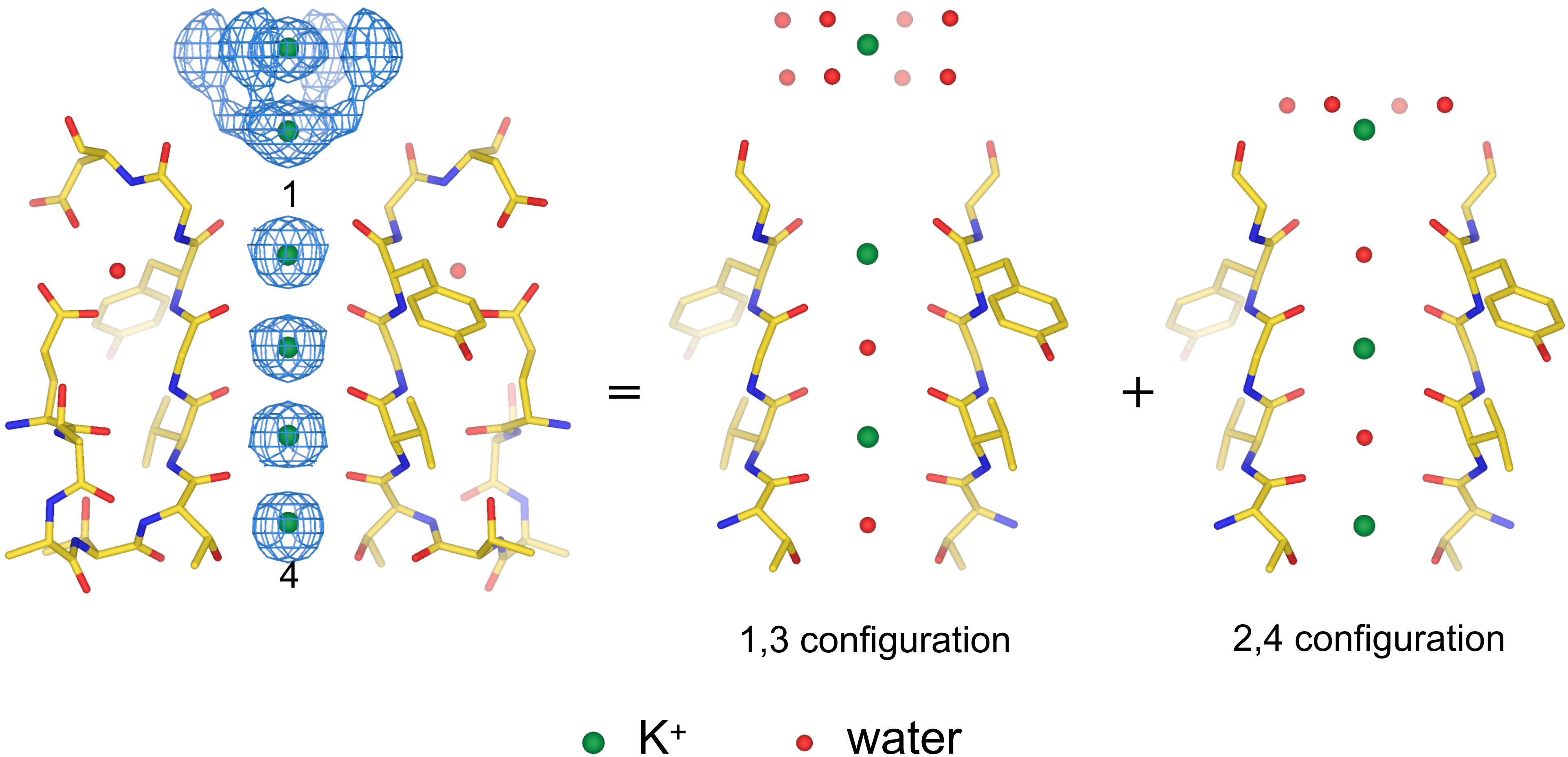


# linkage between S4 and S4-S5

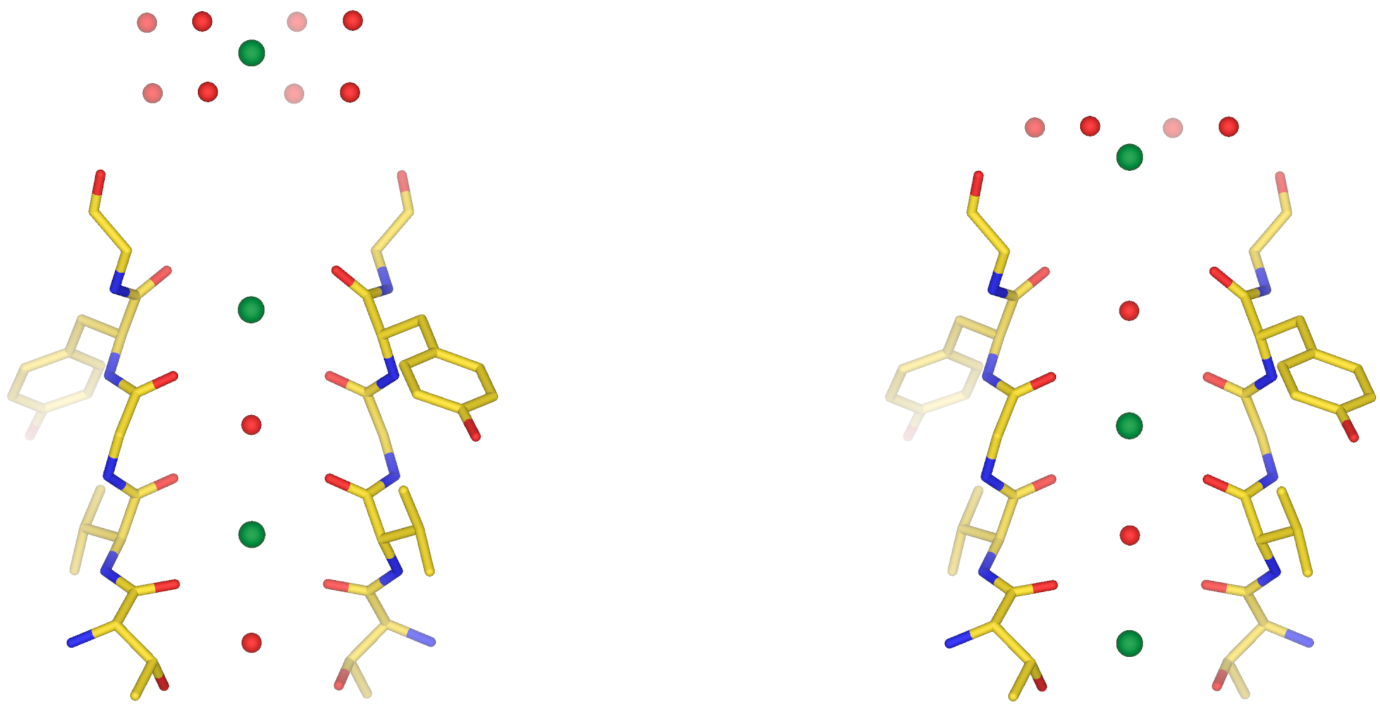



voltage sensor
S4-S5

# 2 $K^+$ ions bind in specific configurations inside the filter



# 2 $K^+$ ions bind in specific configurations inside the filter

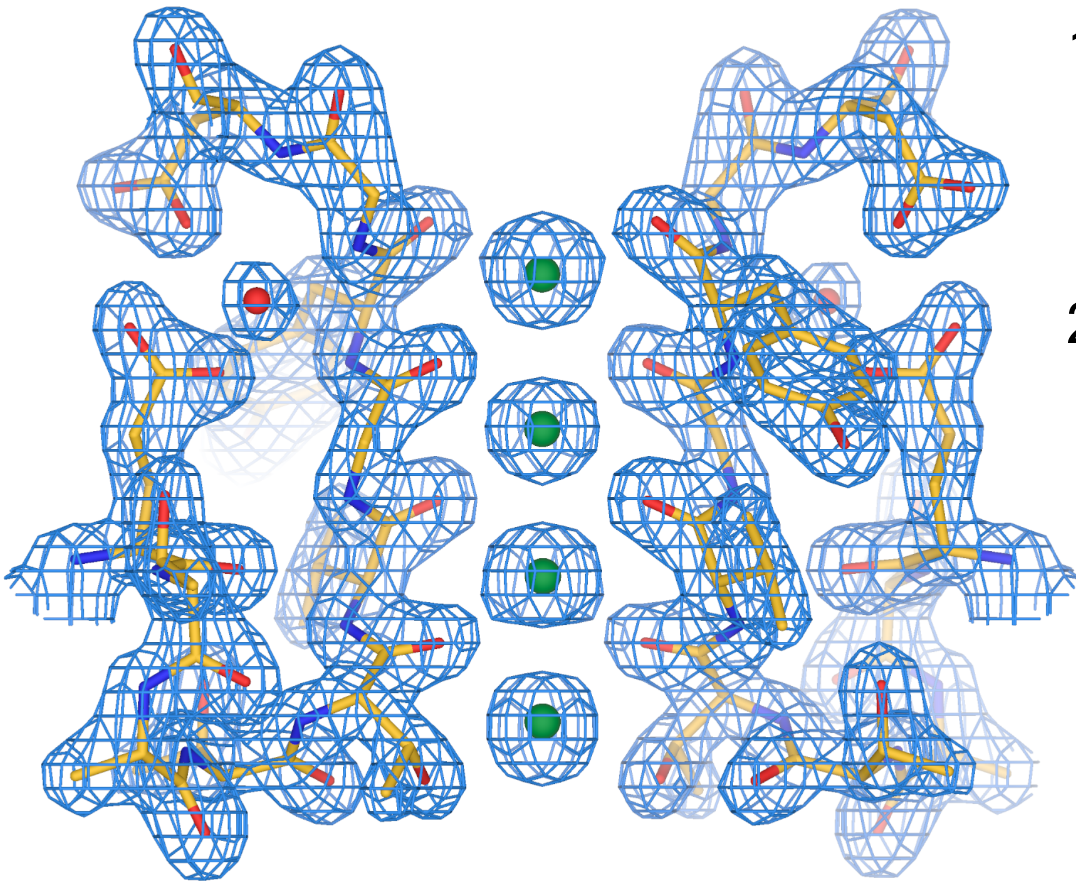


1,3 configuration

2,4 configuration

● $K^+$ ● water

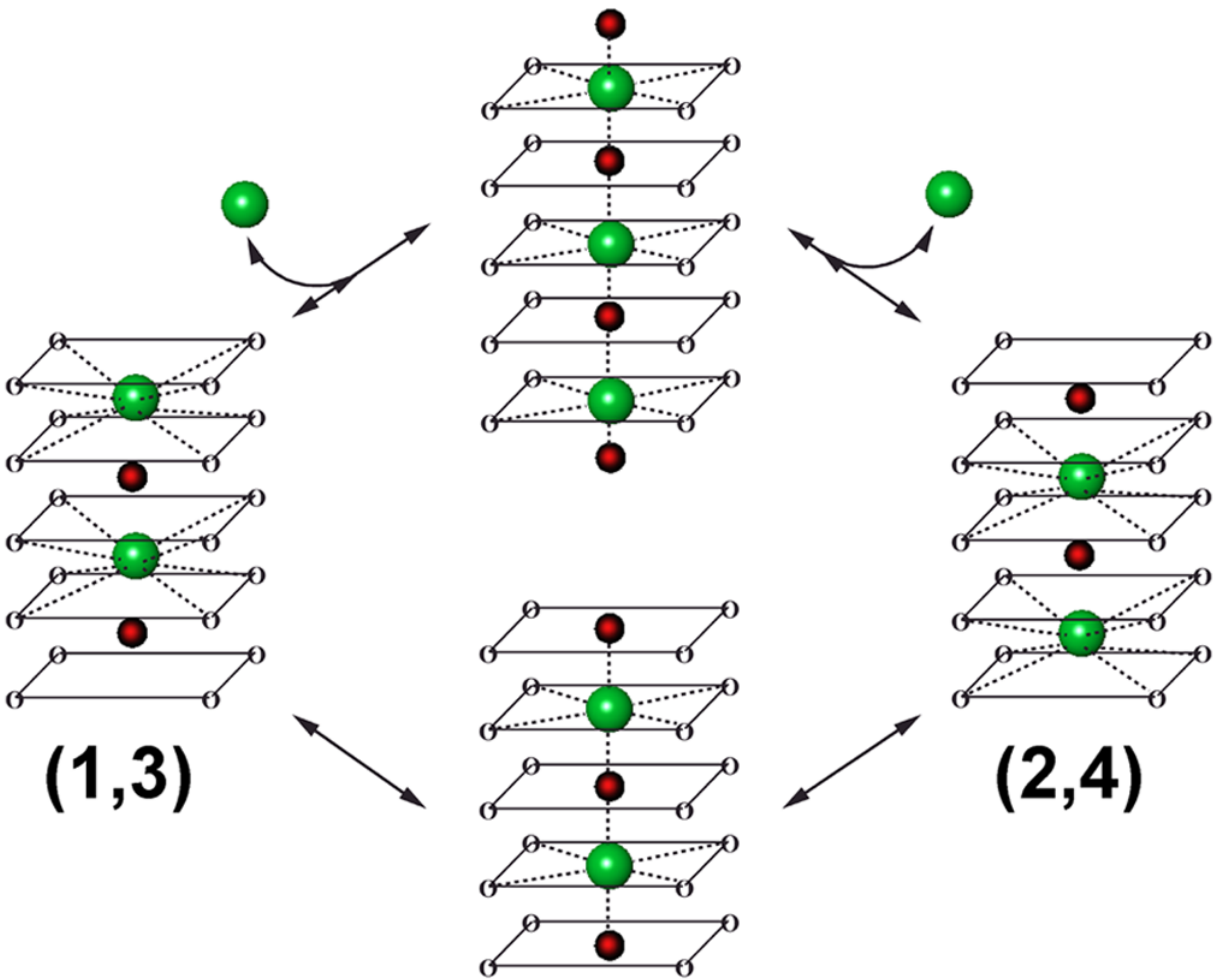
# Principles of high selectivity and high conduction rates



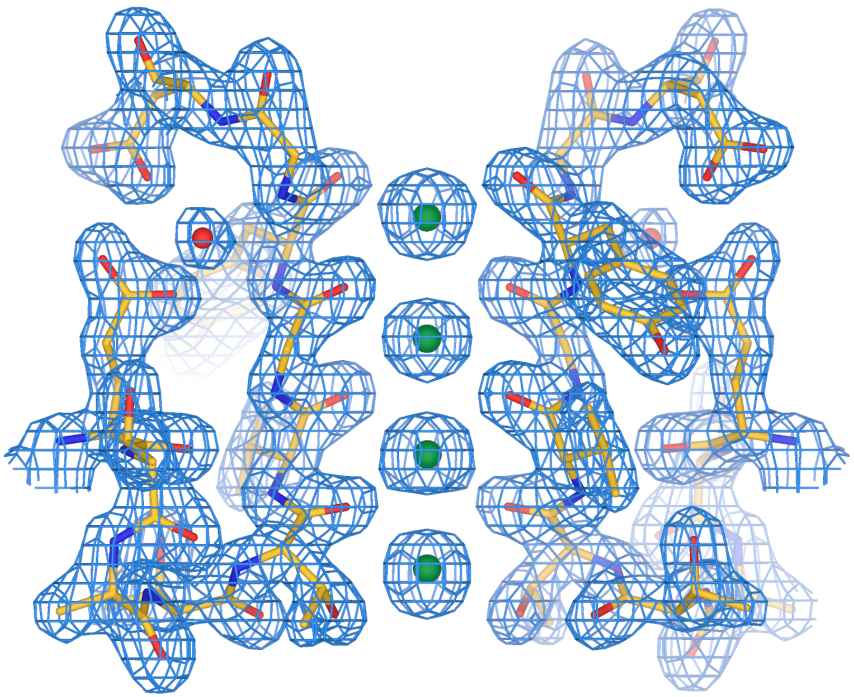
1. 8 oxygen atoms surround $K^+$ ions, forming a selective coordination shell at each site.

2. Sequential sites bind 2 $K^+$ ions in 1,3 or 2,4 configurations. Two ions in close proximity will tend to repel each other, favoring rapid conduction.

# K+ conduction cycle



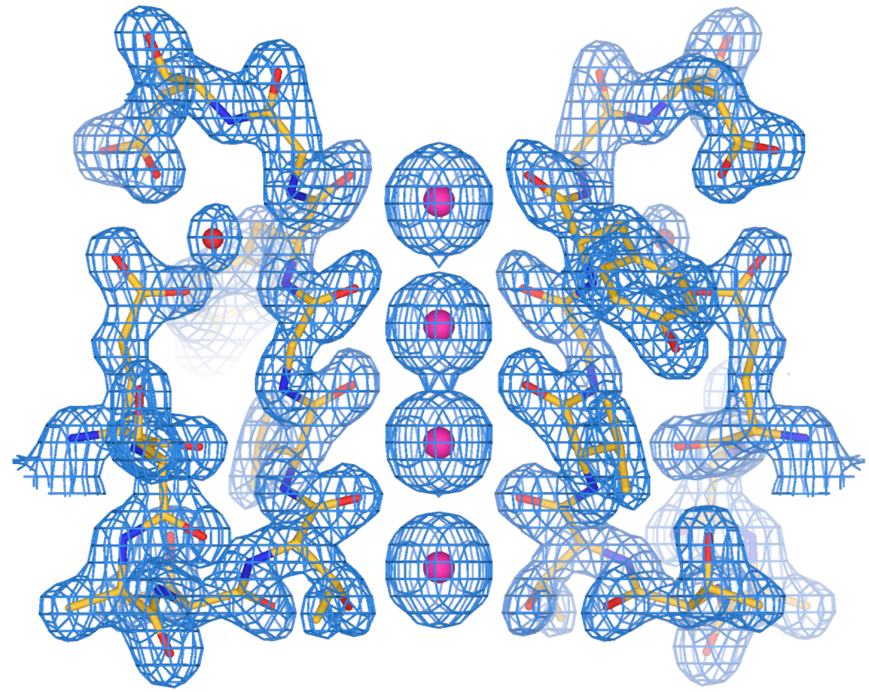
# $Tl^+$ reveals occupancy of individual sites ~ 0.5



resolution 2.0 Å

$K^+$ radius = 1.33 Å

18 $e^-$



resolution 1.9 Å

$Tl^+$ radius = 1.40 Å

80 $e^-$ + anomalous

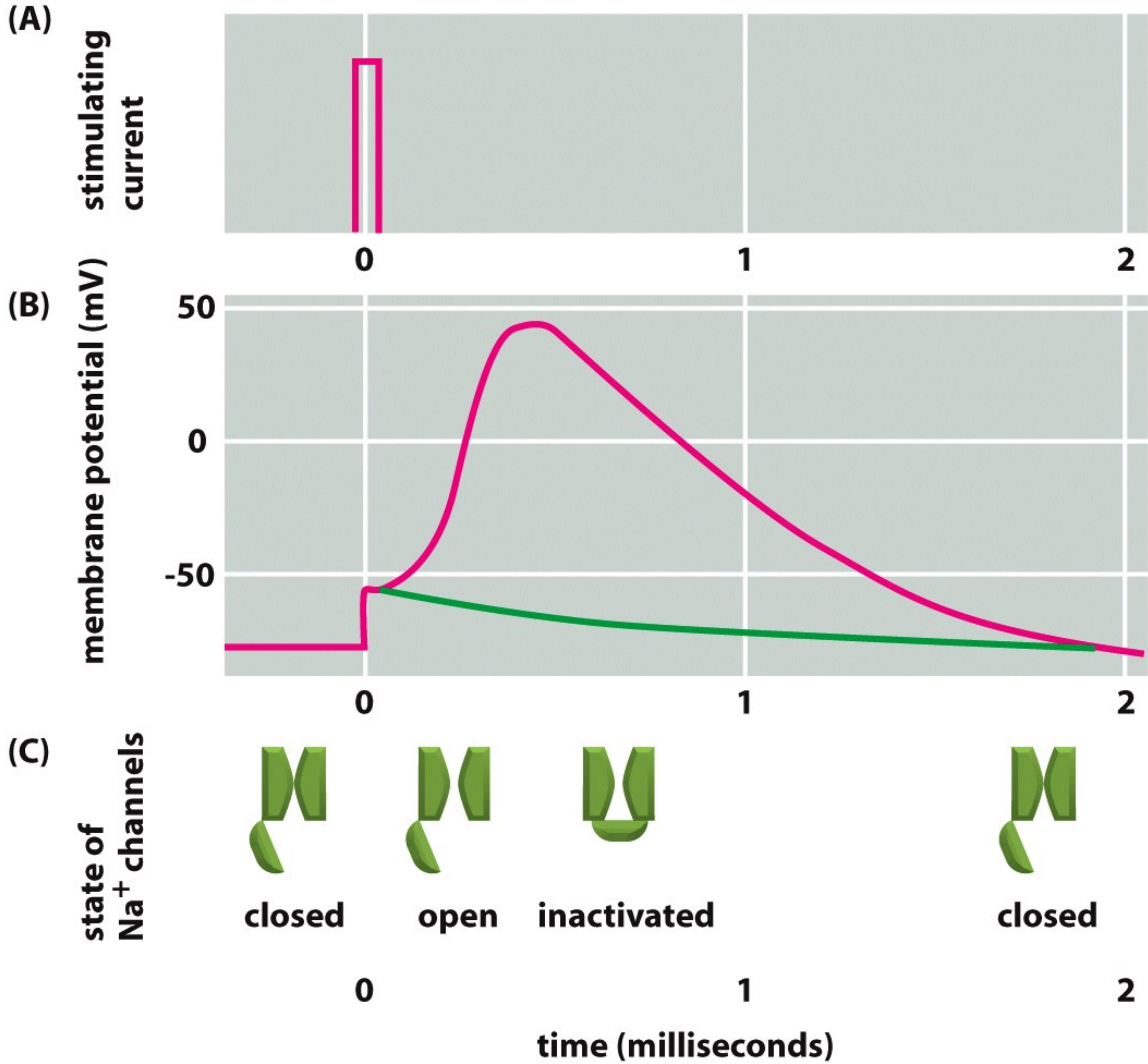



Figure 11-29 *Molecular Biology of the Cell* (© Garland Science 2008)

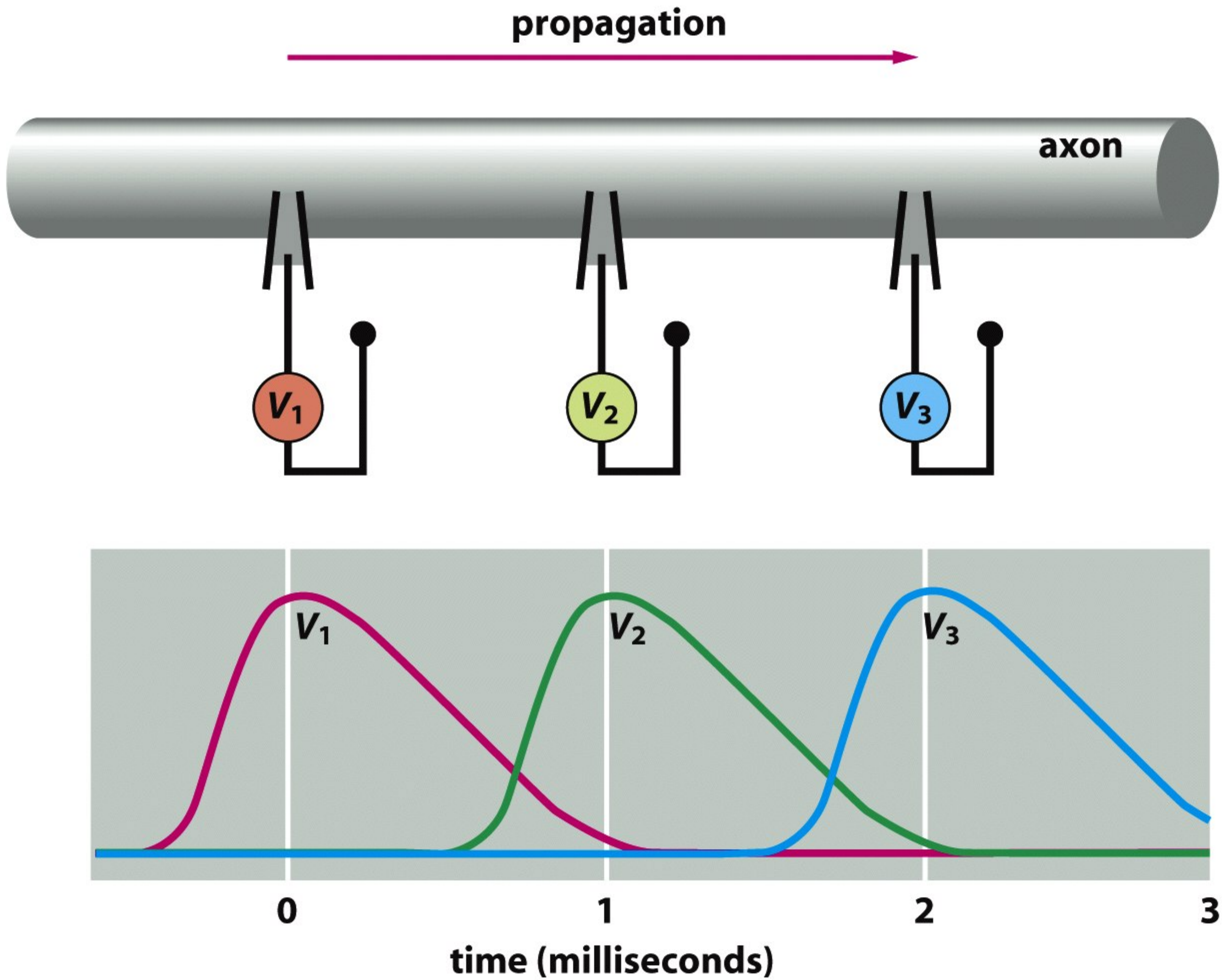



Figure 11-30a *Molecular Biology of the Cell* (© Garland Science 2008)

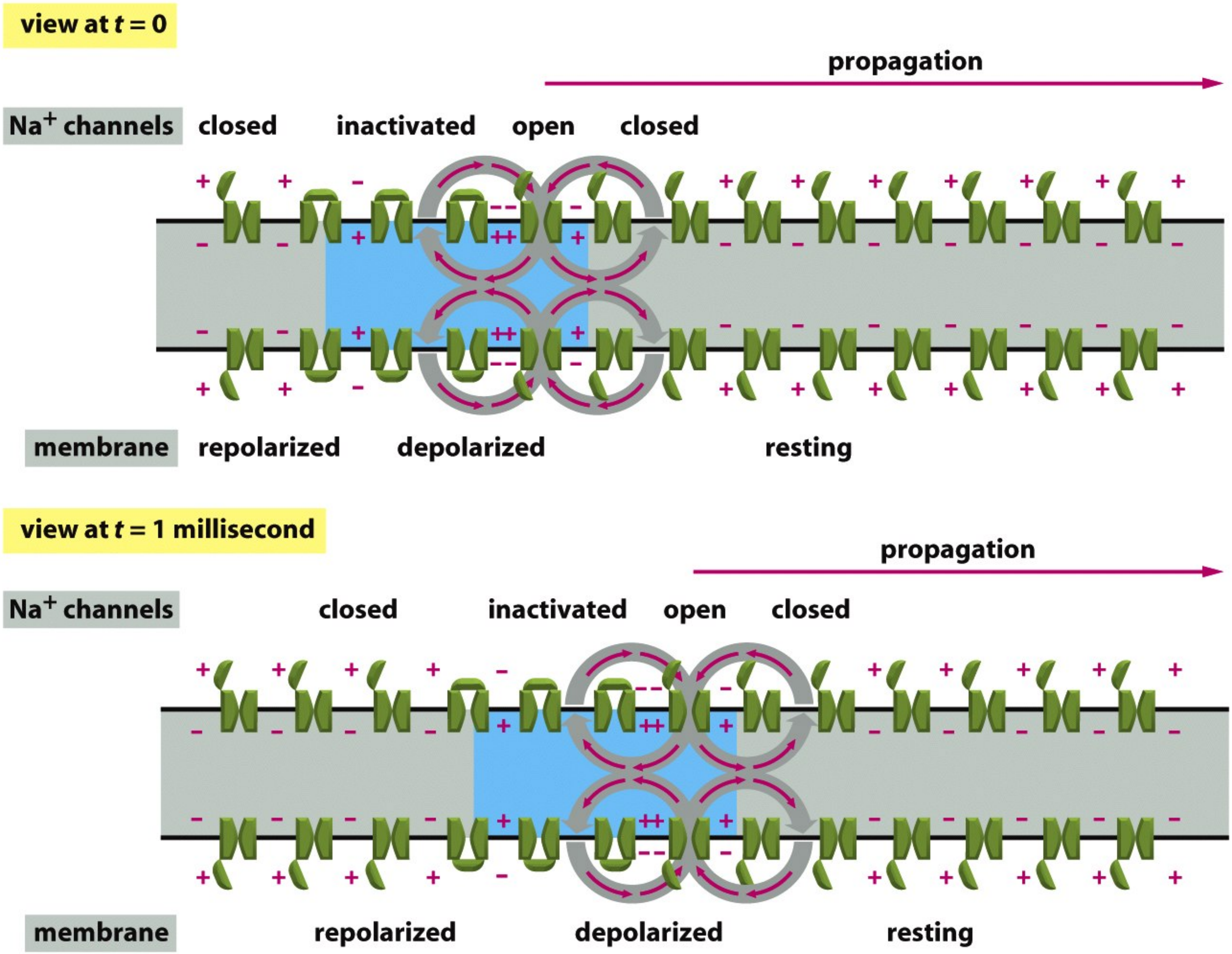



Figure 11-30b *Molecular Biology of the Cell* (© Garland Science 2008)

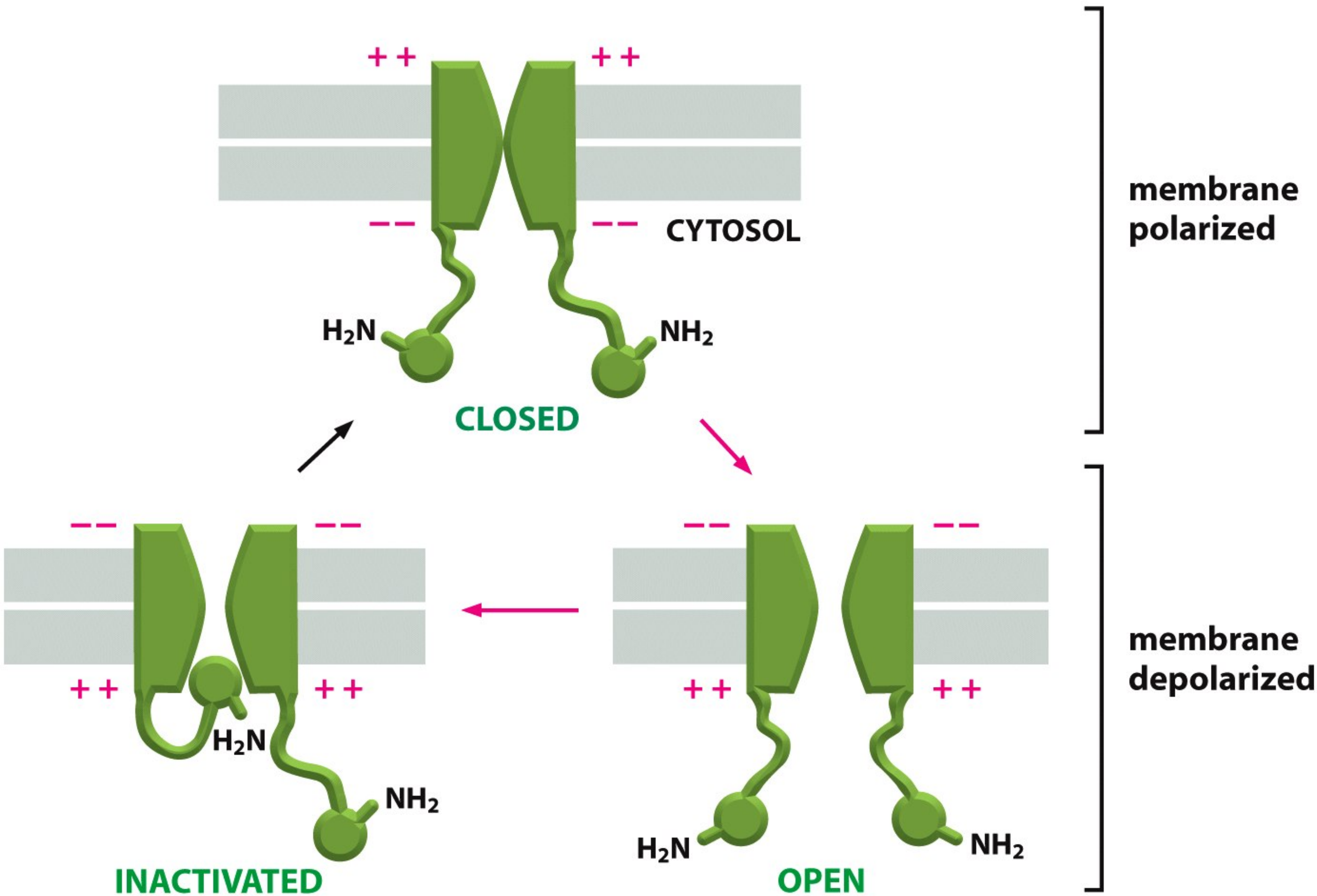
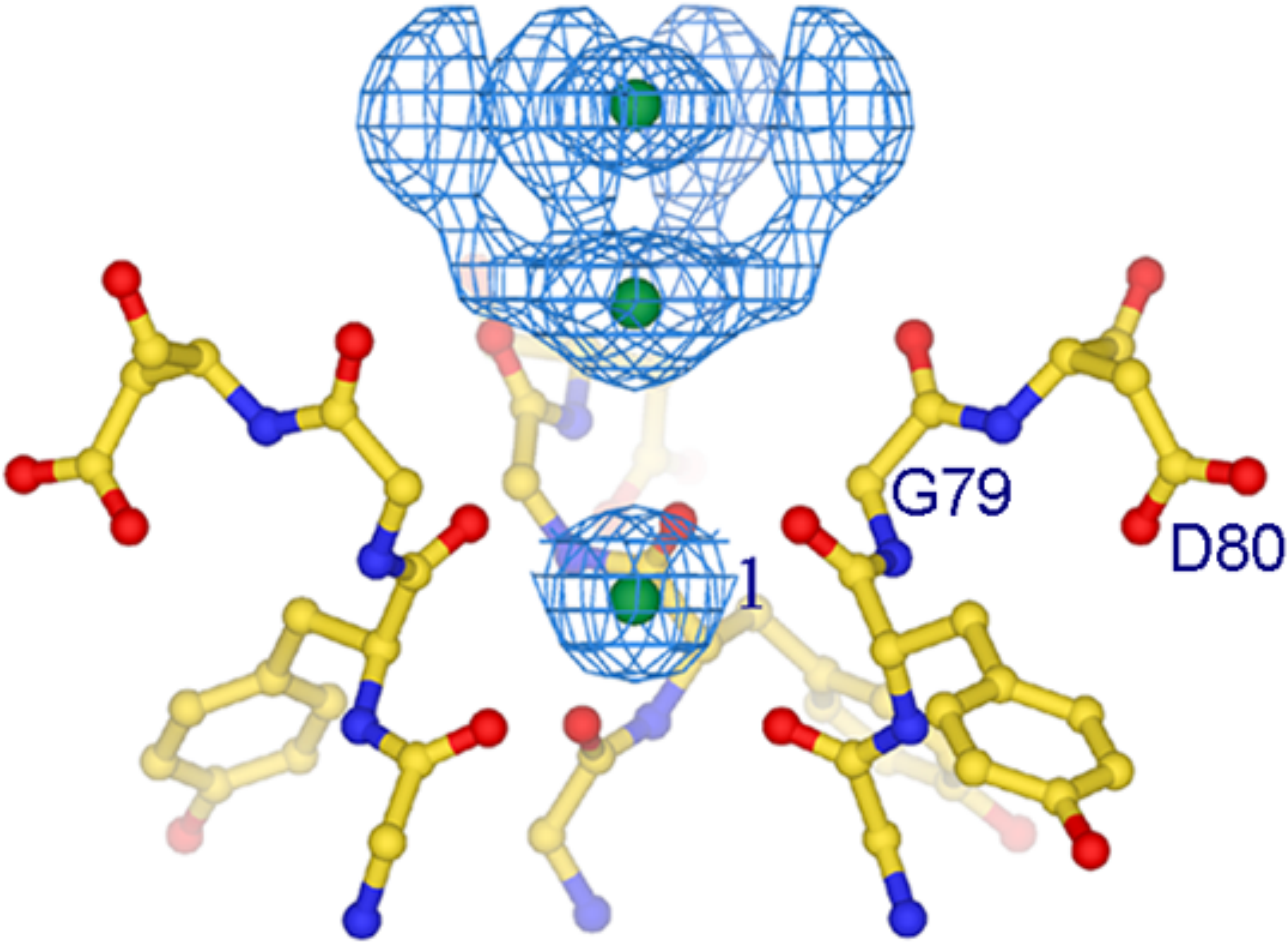
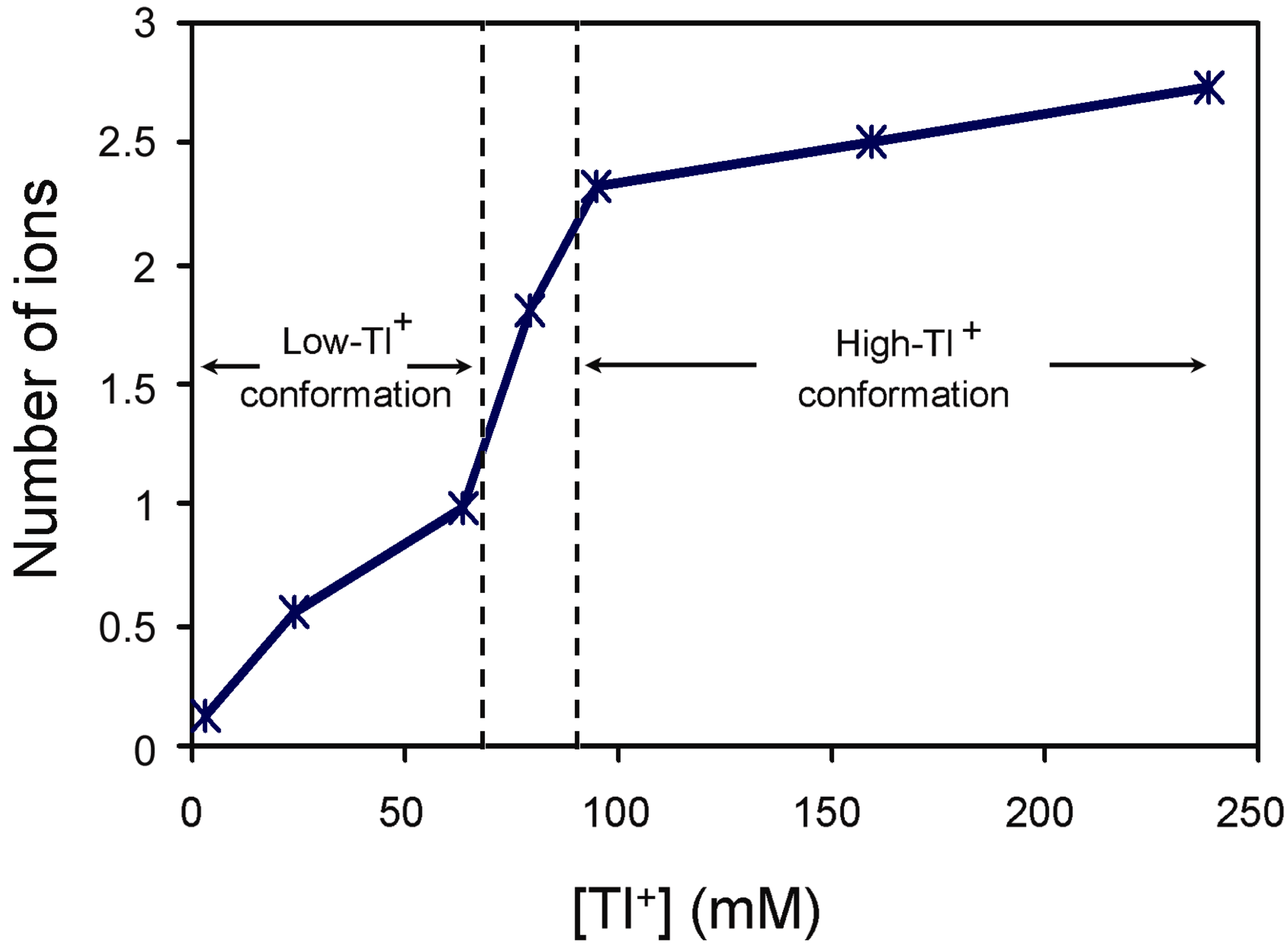


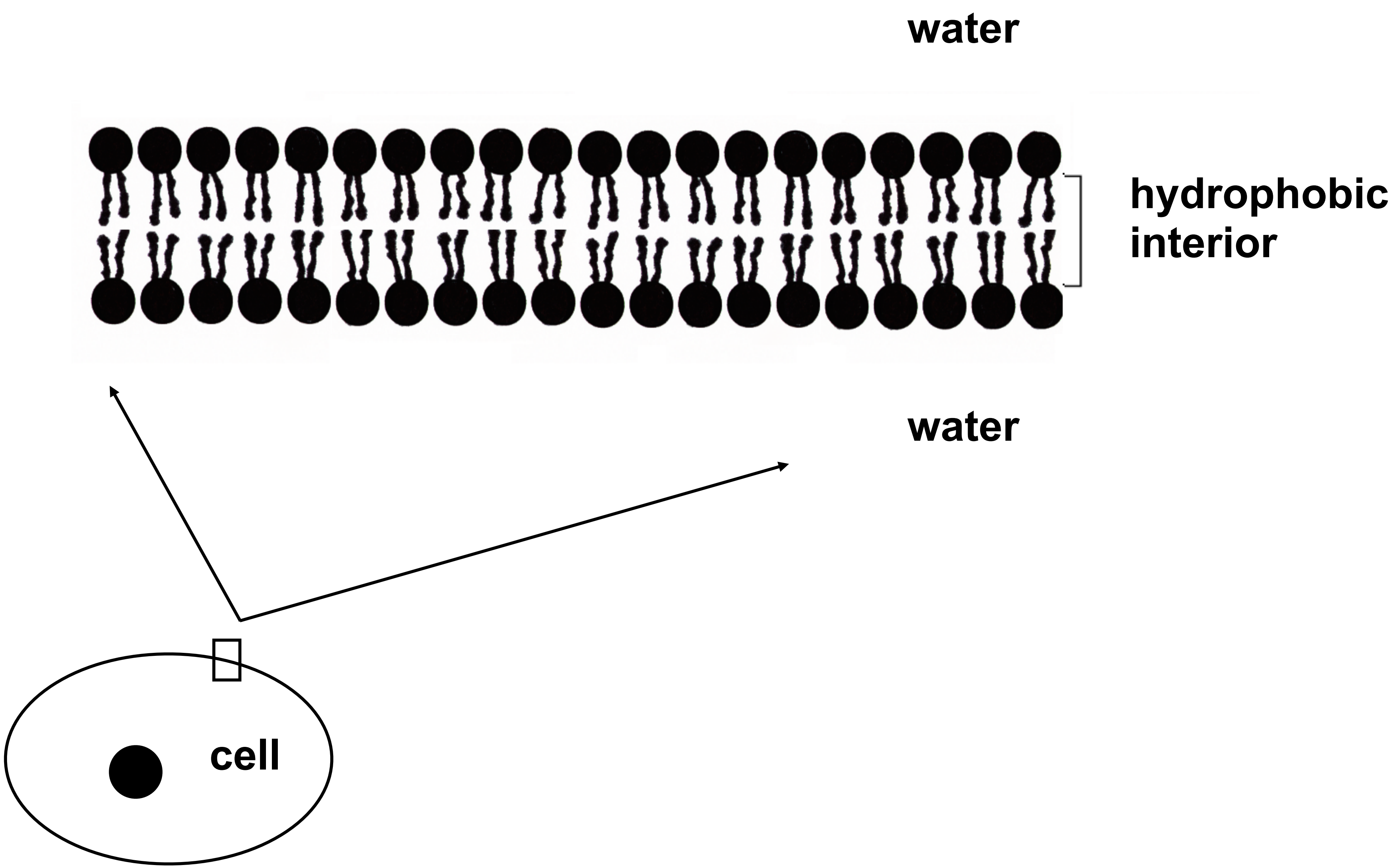

Figure 11-31 *Molecular Biology of the Cell* (© Garland Science 2008)


G79
D80
1

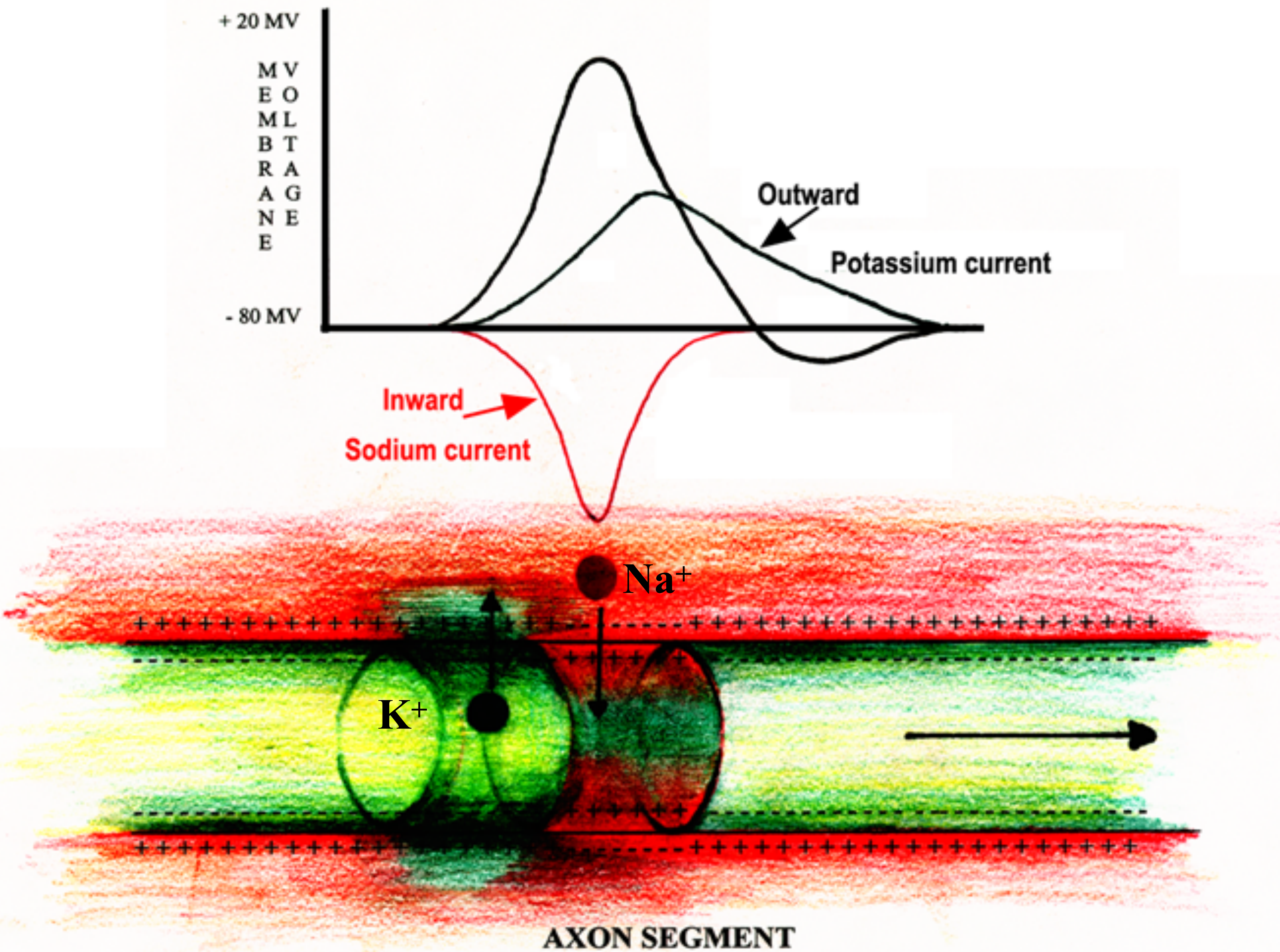


Number of ions
3
2.5
2
1.5
1
0.5
0
0
50
100
150
200
250
[Tl+] (mM)
Low-Tl+
conformation
High-Tl +
conformation

# The cell membrane

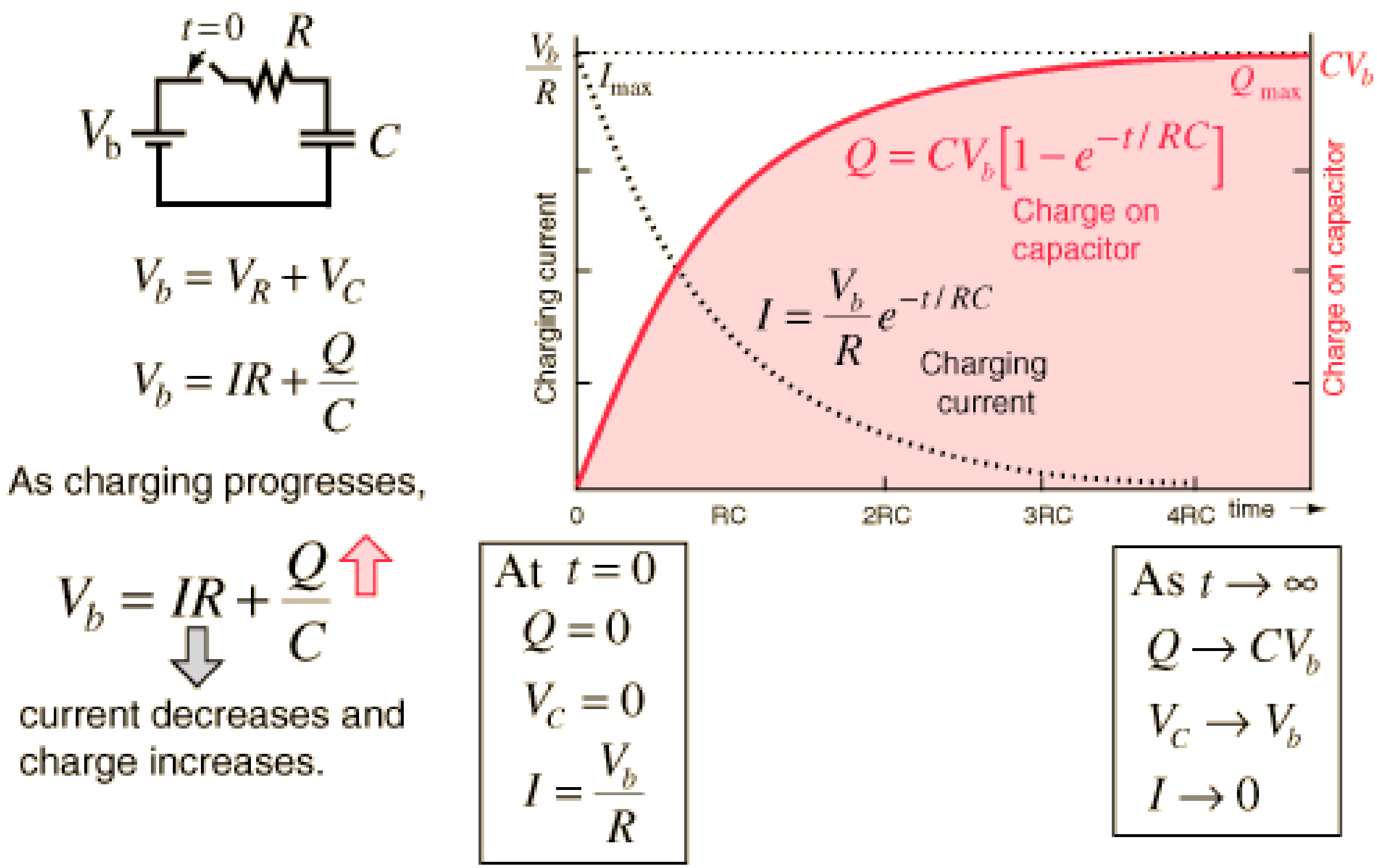


# THE ELECTRICAL IMPULSE DEPENDS ON THE FLOW OF IONS ACROSS THE CELL MEMBRANE

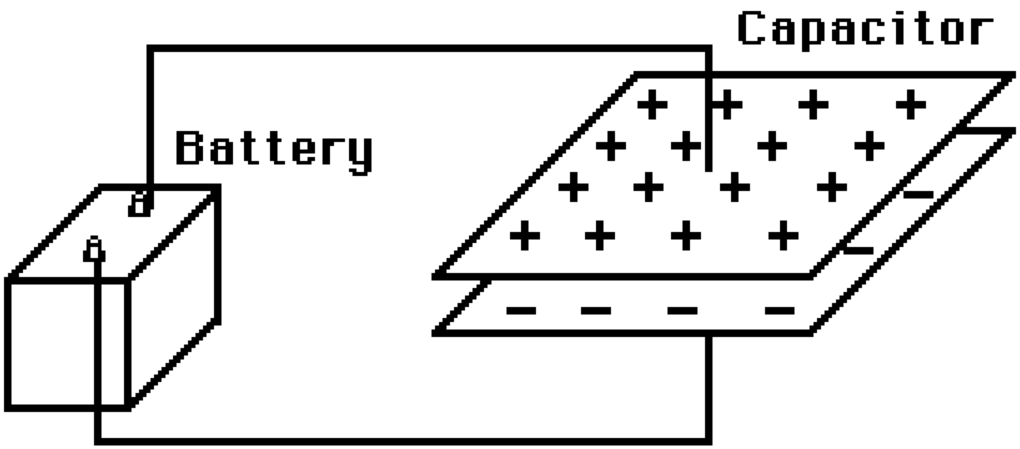


# Charging a Capacitor

When a battery is connected to a series resistor and capacitor, the initial current is high as the battery transports charge from one plate of the capacitor to the other. The charging current asymptotically approaches zero as the capacitor becomes charged up to the battery voltage. Charging the capacitor stores energy in the electric field between the capacitor plates. The rate of charging is typically described in terms of a time constant RC.



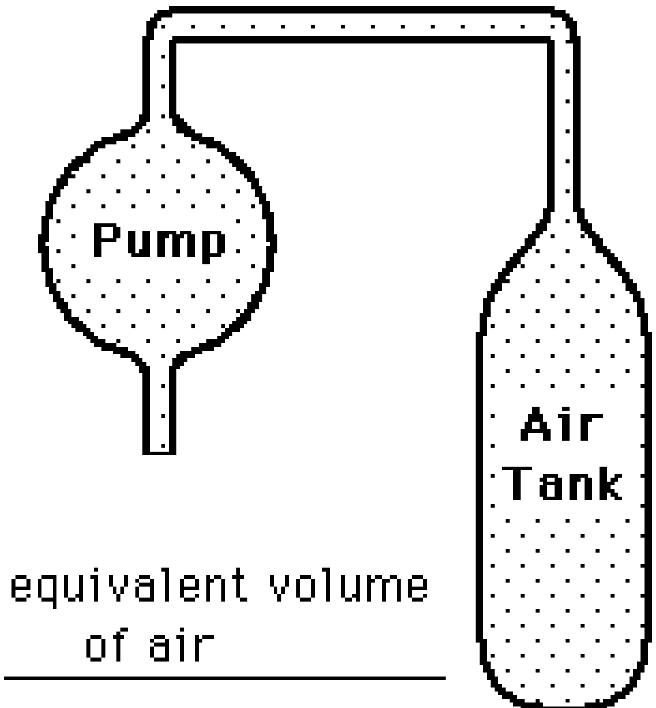
# Air Tank Analogy for a Capacitor




$$\text{Capacitance} = \frac{\text{charge stored}}{\text{voltage applied}}$$

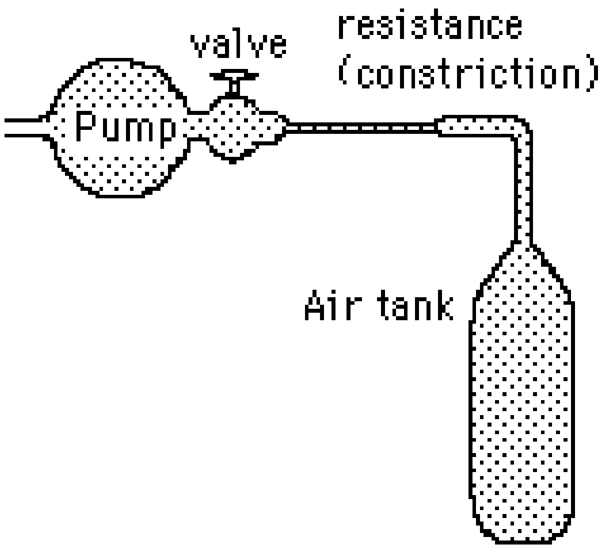
Since more charge can be stored by forcing it with a higher voltage, it makes sense to define capacitance as charge stored per unit voltage.

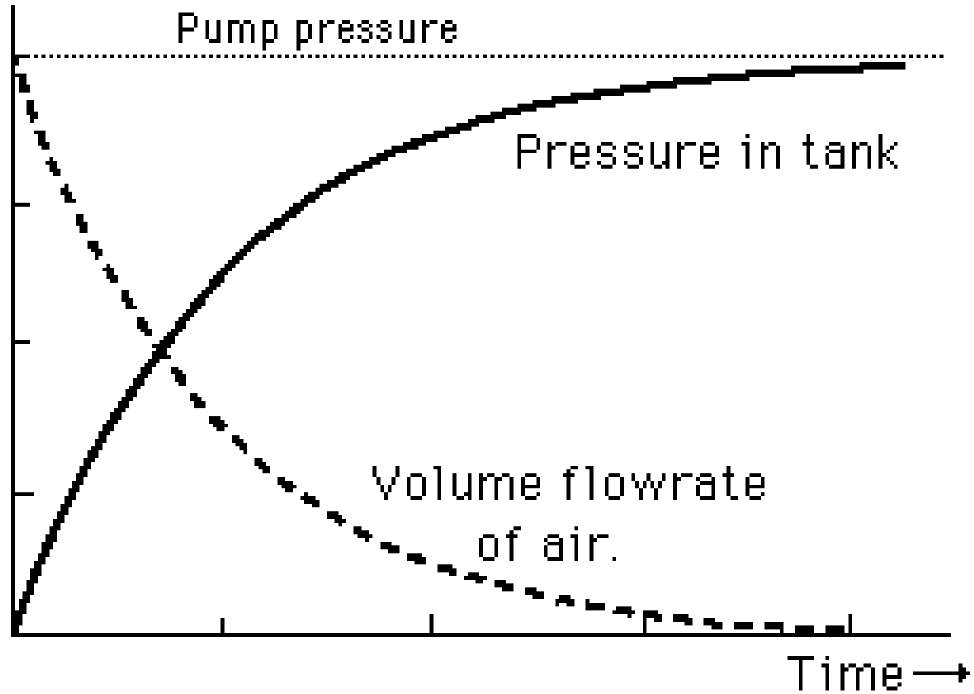
Since you can force more air in the tank with a higher pressure, you might define the capacity of the tank as standard volume of air per unit pressure.




$$\text{capacity} = \frac{\text{equivalent volume of air}}{\text{pressure applied to force air into tank}}$$

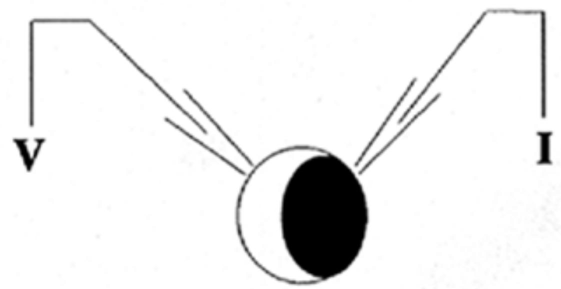
# Airtank Analogy to Charging a Capacitor



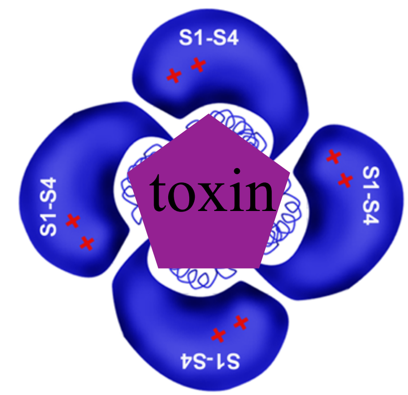

As pumping continues, volume flowrate decreases as pressure in tank builds up. When tank pressure equals pressure supplied by the pump, the flow ceases.

If you have more constriction in the tubing leading to the tank, it will take longer to fill the tank.

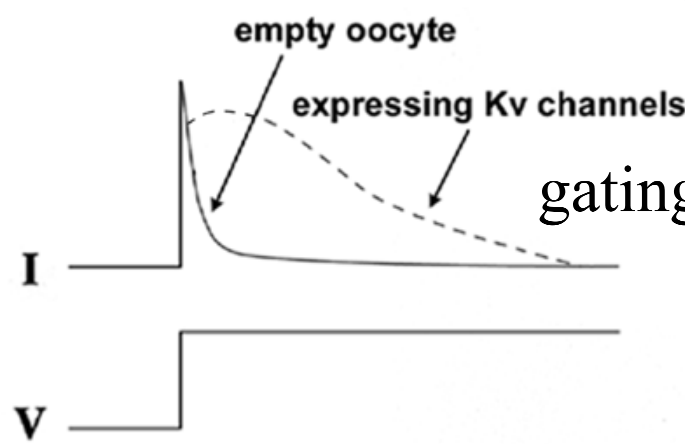



<u>Two-electrode voltage clamp</u>

Measure voltage inside the cell (V).
Use an amplifier to inject (or subtract) current (I) into the cell so as to keep the voltage at the voltage setpoint that you desire.
Voltages inside the cell are measured with respect to outside (ground).




ionic current




gating current