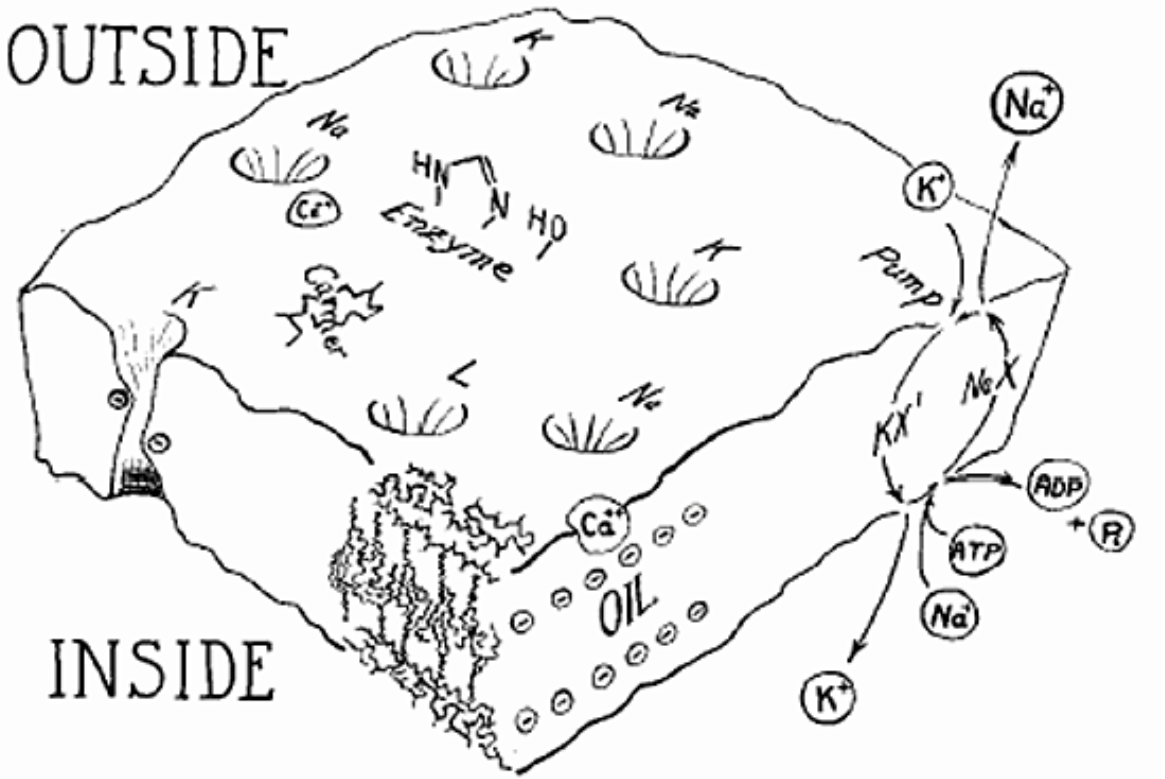
Biology 5357: Chemistry and Physics of Biomolecules

Electrical signaling and ion permeation



Baron Chanda

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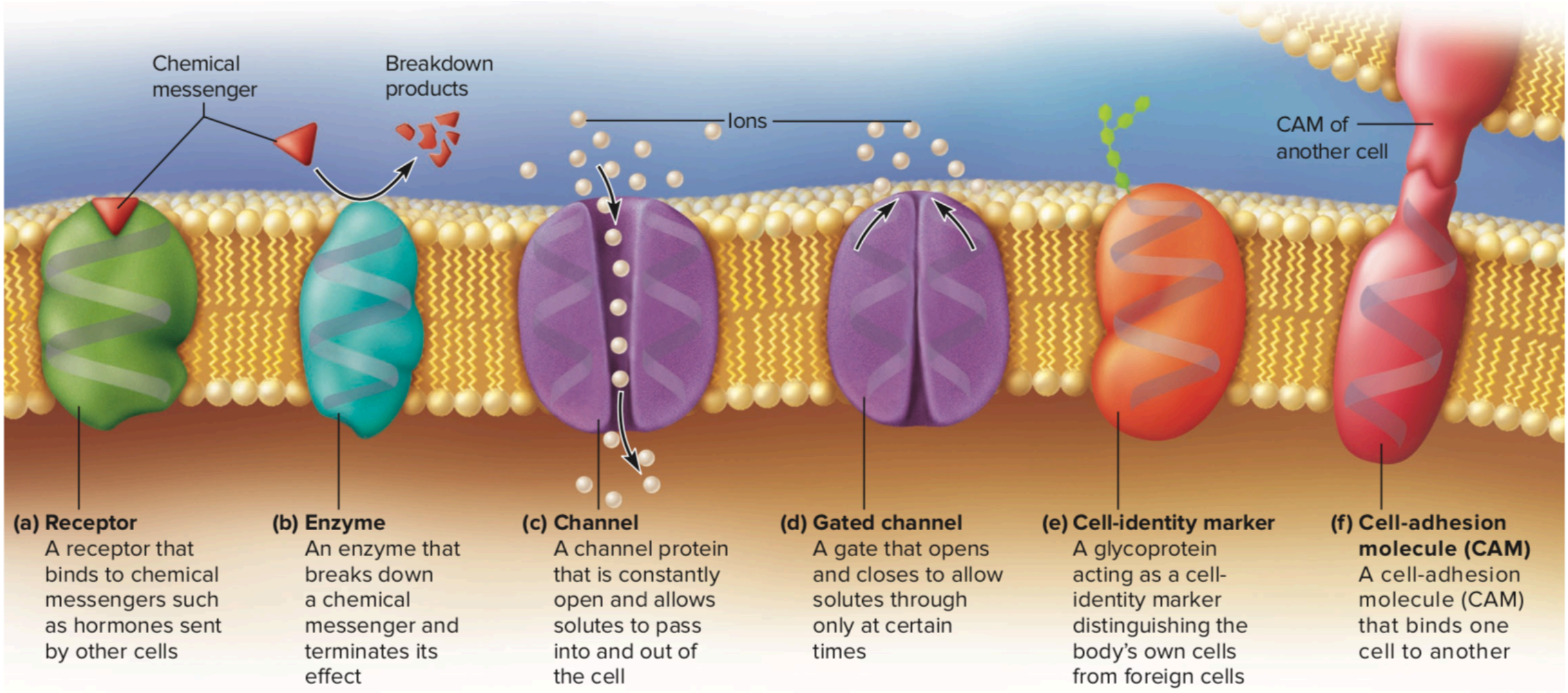
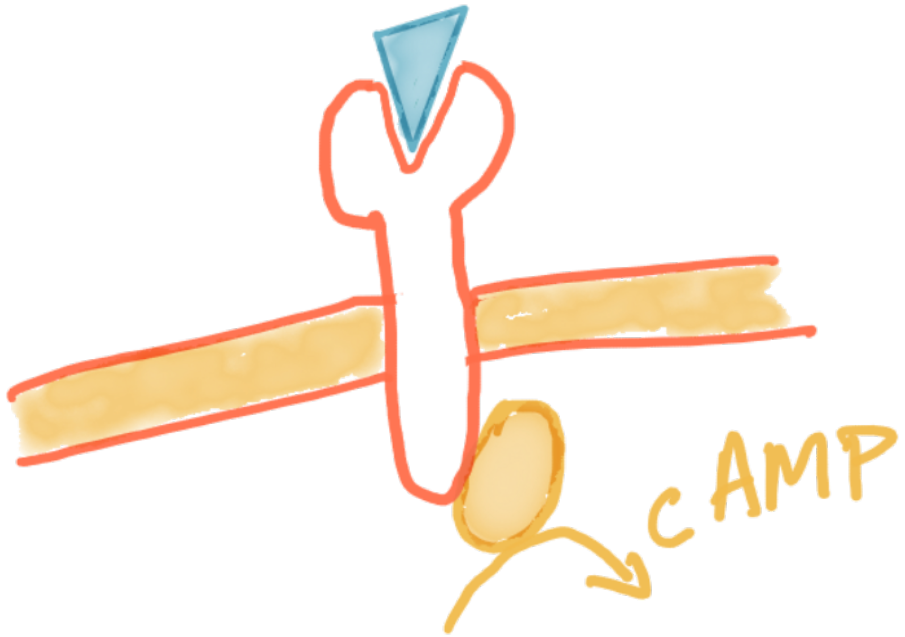
Different types of Membrane Proteins

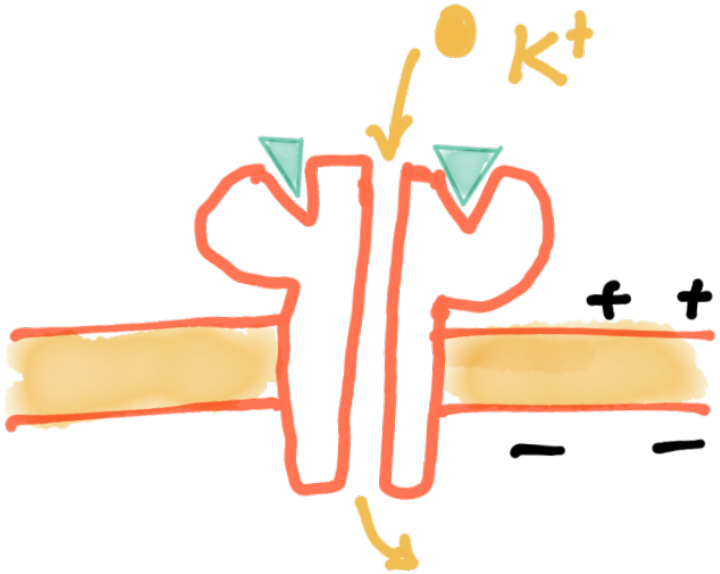


Pumps or transporters

Anatomy & Physiology—The Unity of Form and Function
Ken Saladin

Plasma membrane signaling involves both chemical and electrical signals



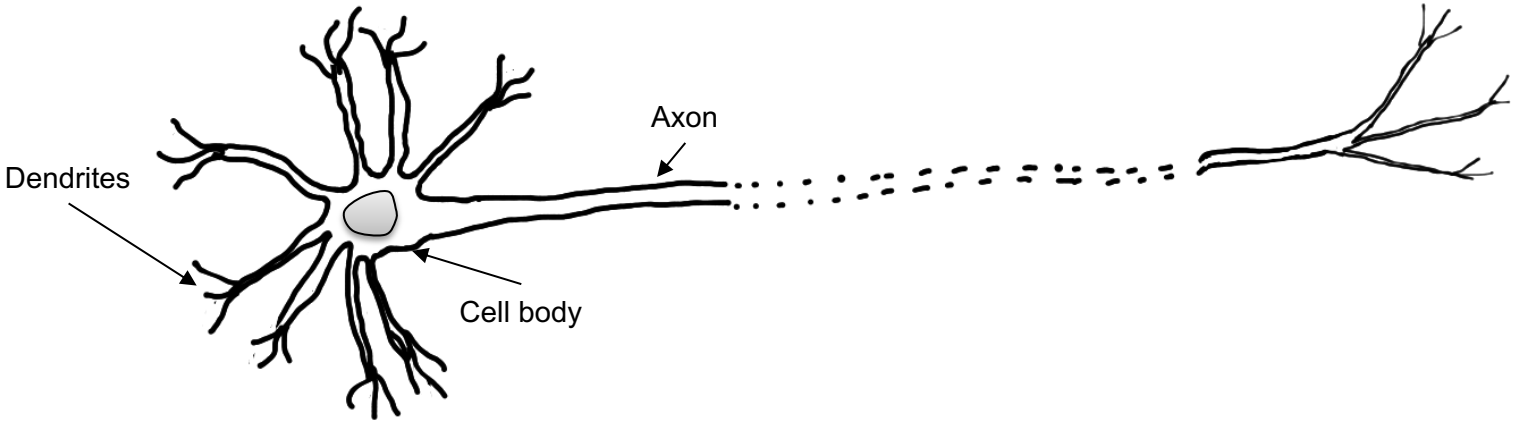


GPCRs, Receptor kinases and cell adhesion molecules

Ion Channels and Ion Transporters

Why is there electrical signaling in biology?

Electrical signaling is essential for rapid communication over large distances



Longest nerve in the human body is the Sciatic nerve. It's length is about 1.5 meter

If the cell body is the size of a football, then length of the axon 15 football fields!

In blue whale, the longest nerve is 30 meters in length.

Necessary for evolution of large multi-cellular organisms in the animal kingdom. However, they have been discovered in all kingdoms of life including archaea and plants.

Bioelectricity is observed in non-excitable cells. Mitochondria uses proton electrochemical gradient for energy generation

Diffusion in two dimension is slow and not suitable for signaling over long range

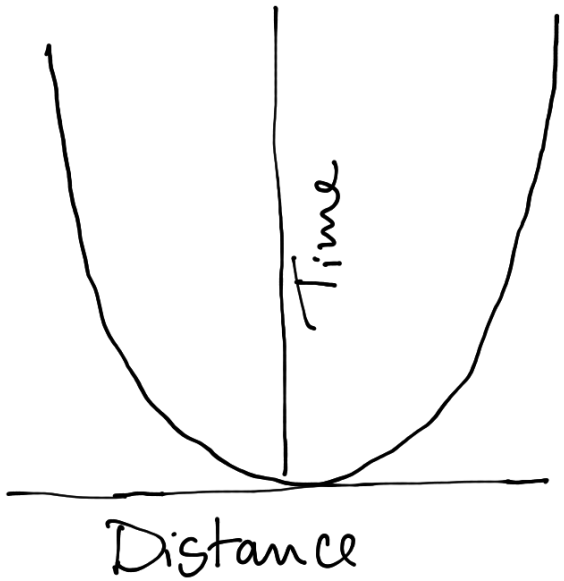
Diffusion in 2D

$$T = \frac{1}{2D}(\Delta x)^2$$

Δx is the distance to diffuse

D diffusion Coefficient

T time required.

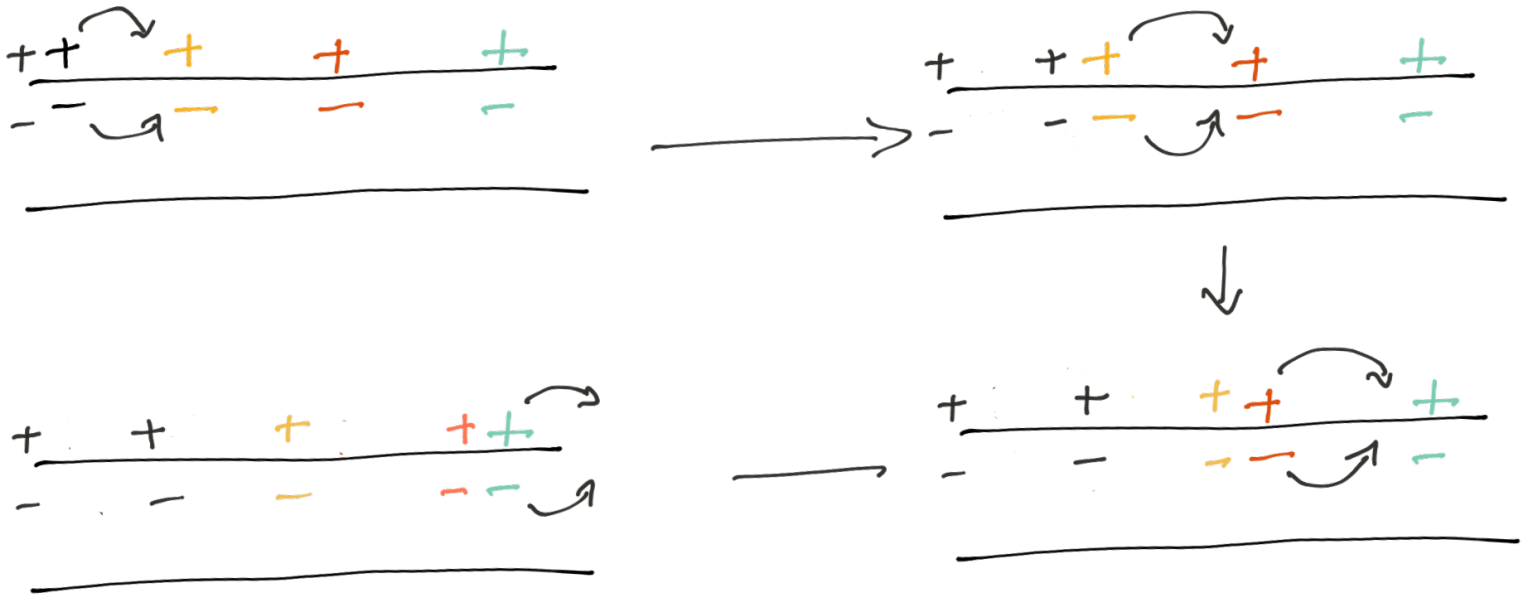


0.01 cm = 5 sec

0.1 cm = 500 sec

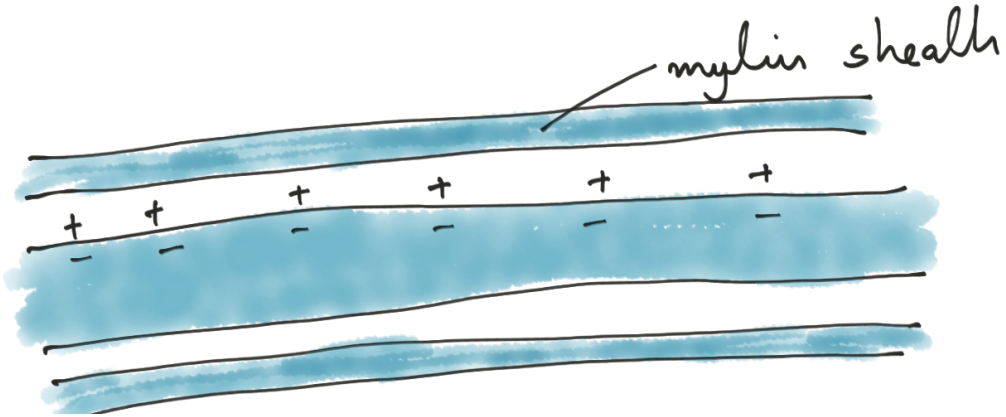
1 cm = 50,000 sec

Electrical signaling involves propagation of voltage signal rather than ions.



Ions physically move a short distance but the electric potentials propagates rapidly

Saltatory conduction- Mechanism similar to conduction of electrons through conductor. Ions instead of electrons

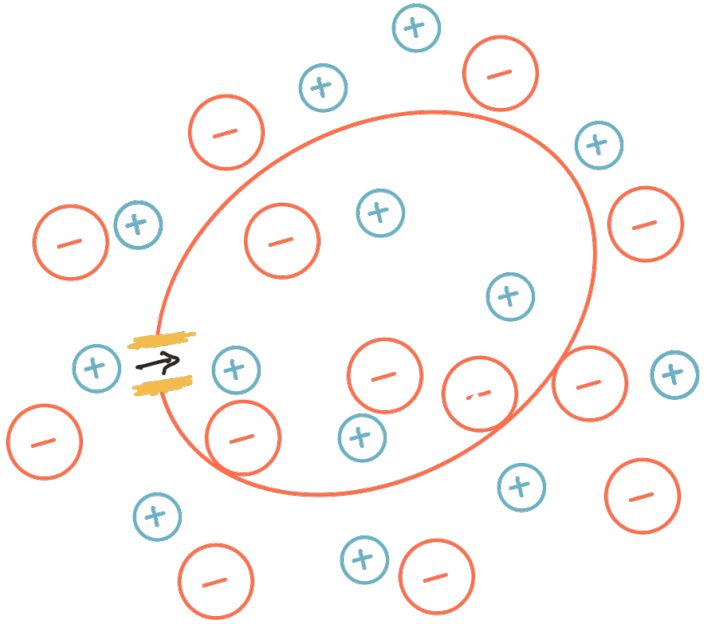


Myelin sheath – acts as an insulator surrounding a conductor. Reduces the extracellular space and restricts the diffusion of ions into free space. Increase the speed of transmission of action potential.

How is membrane potential generated in cells?

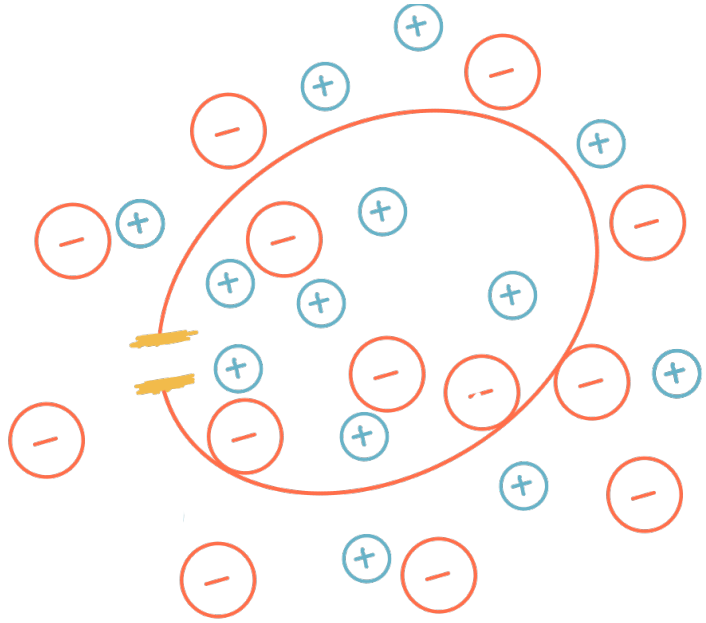
Selective Ion permeation and concentration gradient determines membrane potential

Initial Condition



Electroneutral but Concentration difference of salt between inside and outside

At Equilibrium

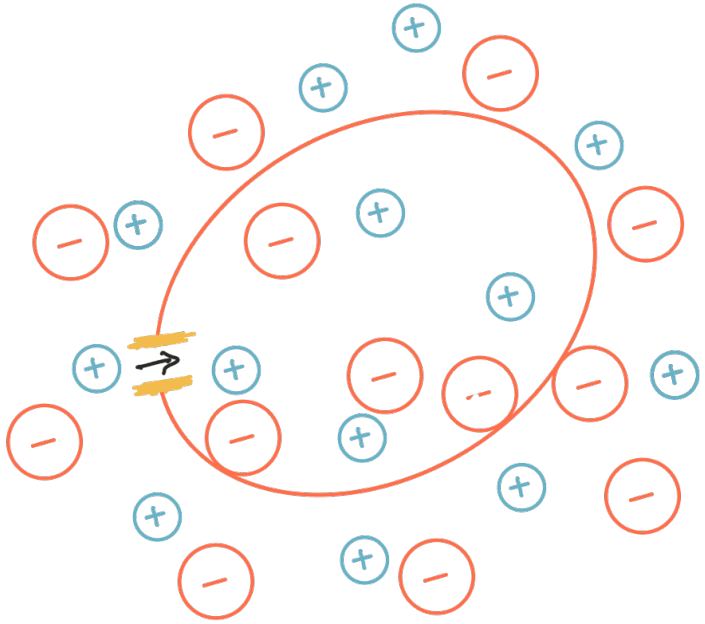


Electronegative inside with some dissipation of concentration gradient

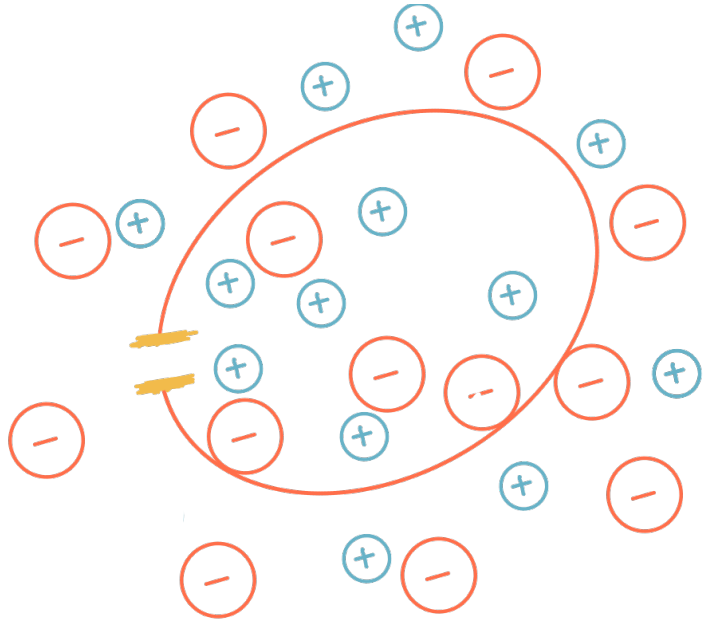
Extent of dissipation of the gradient depends on the ratio of membrane surface area to total cell volume because charge separation occurs only over the membrane. The change in concentration gradient due to membrane potential can be be 0.01% in large neurons to 10% in small membrane compartments.

Membrane potential at equilibrium can be calculated using Nernst equation

Initial Condition



At Equilibrium



$$\Delta G = -RT \ln \frac{C_{out}}{C_{in}}$$ Chemical free energy

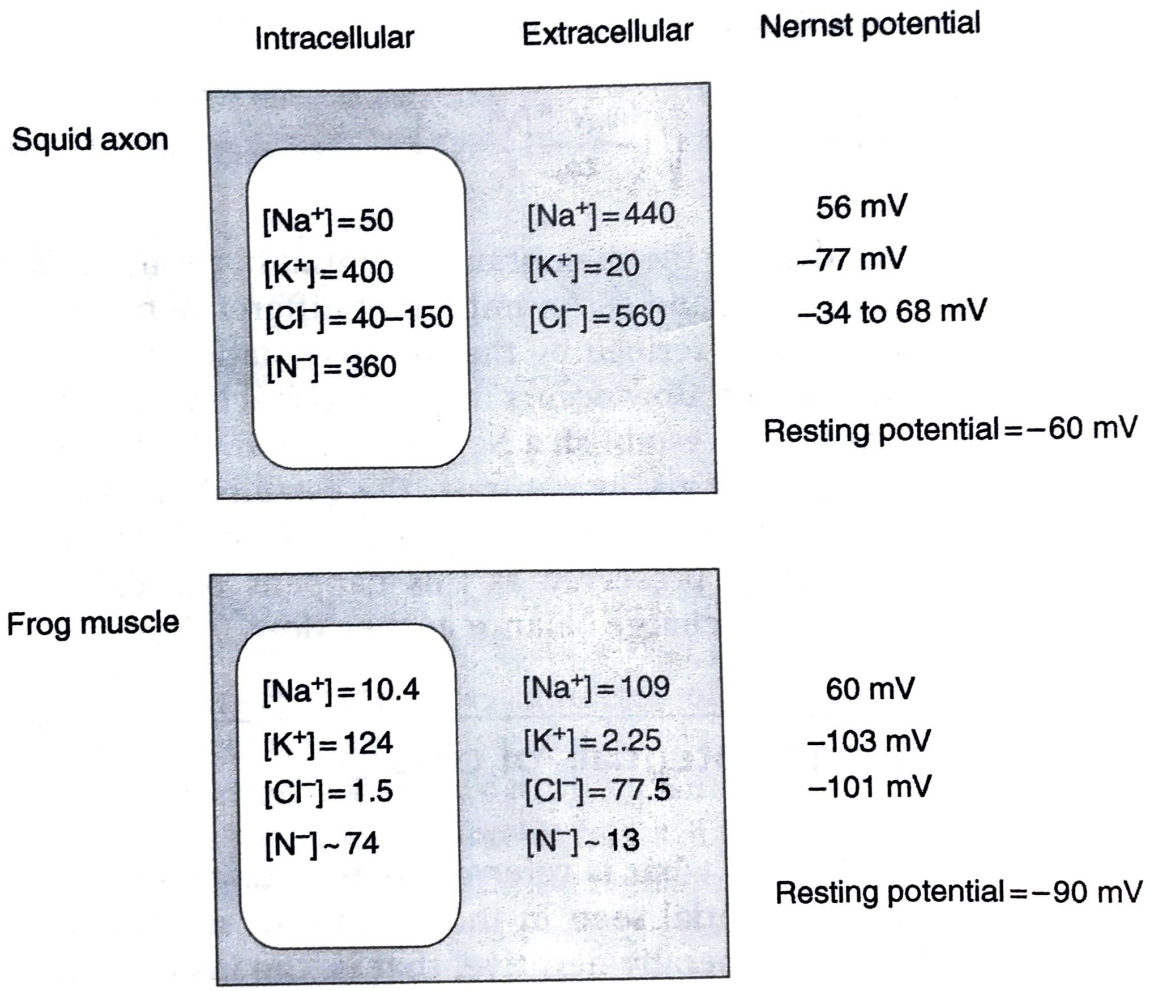
$$\Delta U = zF(V_{out} - V_{in})$$ Electrical free energy

C_{in} and C_{out} are concentrations of ions in A and B. V_{out} and V_{in} are the voltages, z is the charge, F is Faraday constant.

$$At\ equilibrium,\ \Delta U + \Delta G = 0$$

$$\Delta V = -\frac{RT}{zF} \ln \frac{C_{out}}{C_{in}}$$ Nernst Equation

Cellular membrane potential is determined by permeabilities of multiple ions



Goldman Hodgkin Katz Equation

$$\Delta V = -\frac{RT}{zF}\ln\left(\frac{P_{Na}[Na_b^+] + P_K[K_b^+] + P_{Cl}[Cl_b^-]}{P_{Na}[Na_a^+] + P_K[K_a^+] + P_{Cl}[Cl_a^-]}\right)$$

P_{Na} P_K P_{Cl} are the permeabilities of various ions.

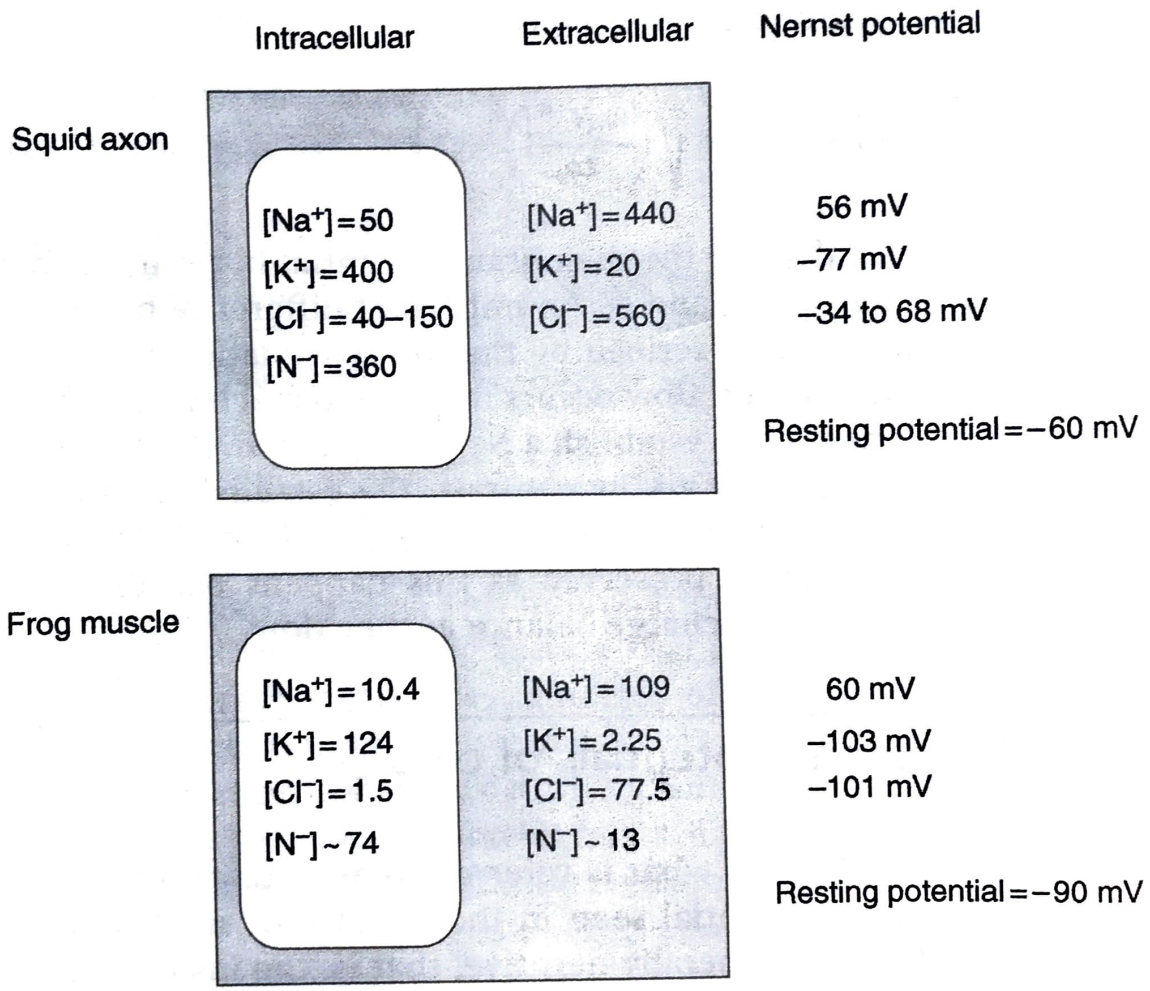
In a cell, membrane potential is a weighted mean of Nernst potentials of various permeant ions.

If $P_{Na} >> P_K$ or P_{Cl}, then ΔV (membrane potential) will be 56 mV.

If $P_K >> P_{Na}$ or P_{Cl}, then ΔV (membrane potential) will be -77 mV.

Changes in ionic permeability underlie action potential

Cellular membrane potential is determined by permeabilities of multiple ions



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P_{Na} P_K P_{Cl} are the permeabilities of various ions.

Pumps like Na-K-ATPases are responsible for maintaining the ionic gradient and its contribution to membrane potential is cell-type dependent.

Contribute to about 10-15 mV in a typical cell. In neutrophils and mitochondria, all of membrane potential is generated by pumps.

What is the mechanism for generation of action potentials?

Brief history of bioelectricity

In 1780, Luigi Galvani discovered that dead frog's leg twitched when connected to electricity generating circuit. Named it animal electricity. Inspired Mary Shelley's book Frankenstein.

Luigi Galvani

Between 1881 to 1887, Sydney Ringer showed that isolated frog heart must contain salts sodium, potassium and calcium mixed in definite proportion if the heart were to continue beating

In 1912, Julius Bernstein found that at rest, cell membrane is highly permeable to potassium but during excitation, membranes become transiently permeable to other ions such as sodium. "Membrane breakdown" hypothesis.

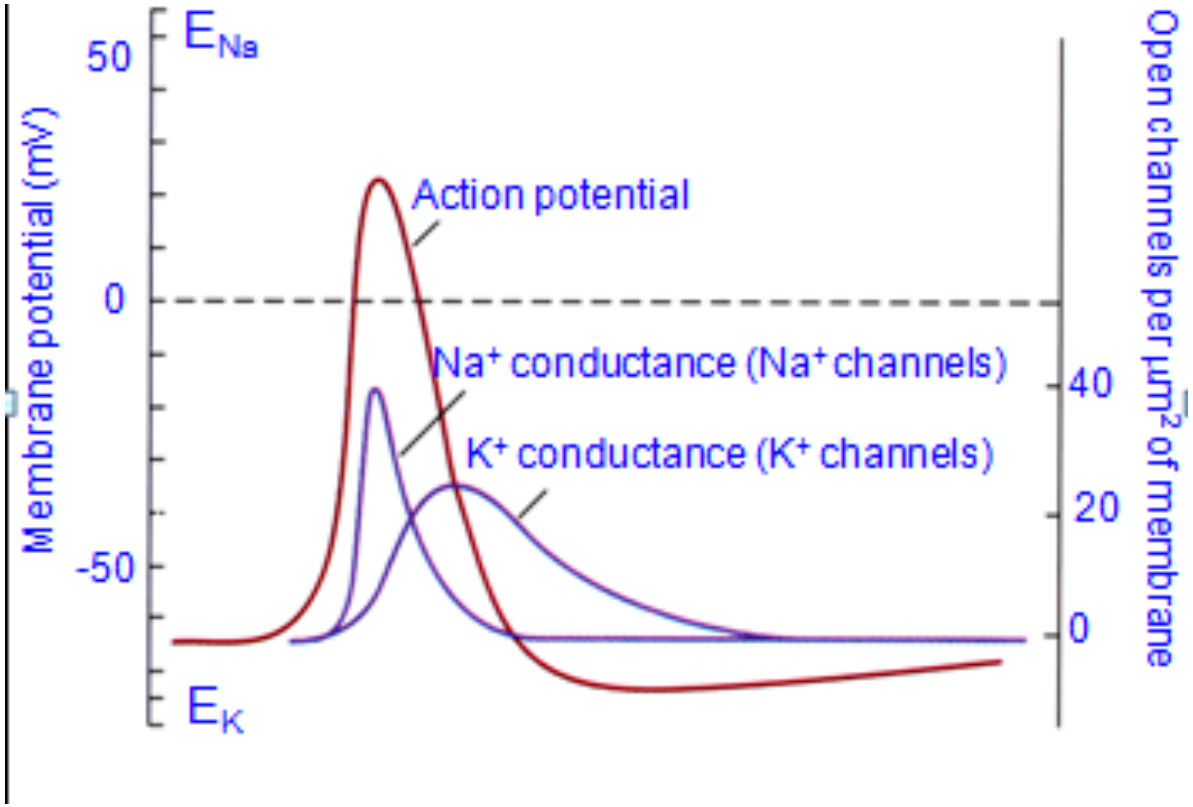
In 1935, Erlanger and Gasser built Cathode ray tube amplifier to accurately measure and record nerve action potentials. Described a relationship between thickness of nerve fiber and conduction velocity.

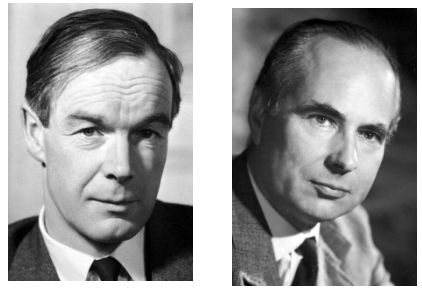
Joseph Erlanger

Herbert Gasser

Nobel prize in physiology 1944

Voltage-dependent and selective ion permeabilities underlie action potential



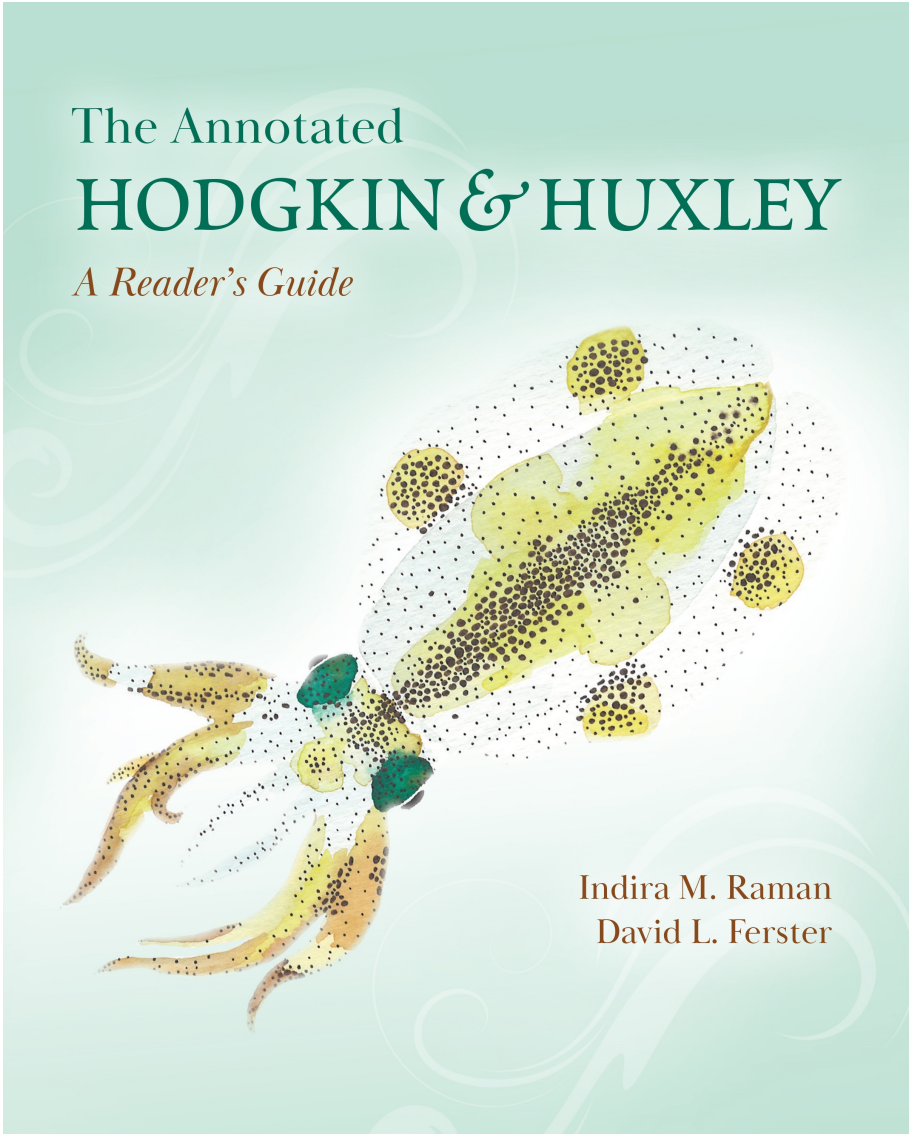


Hodgkin and Huxley, 1963 Nobel Prize in Physiology

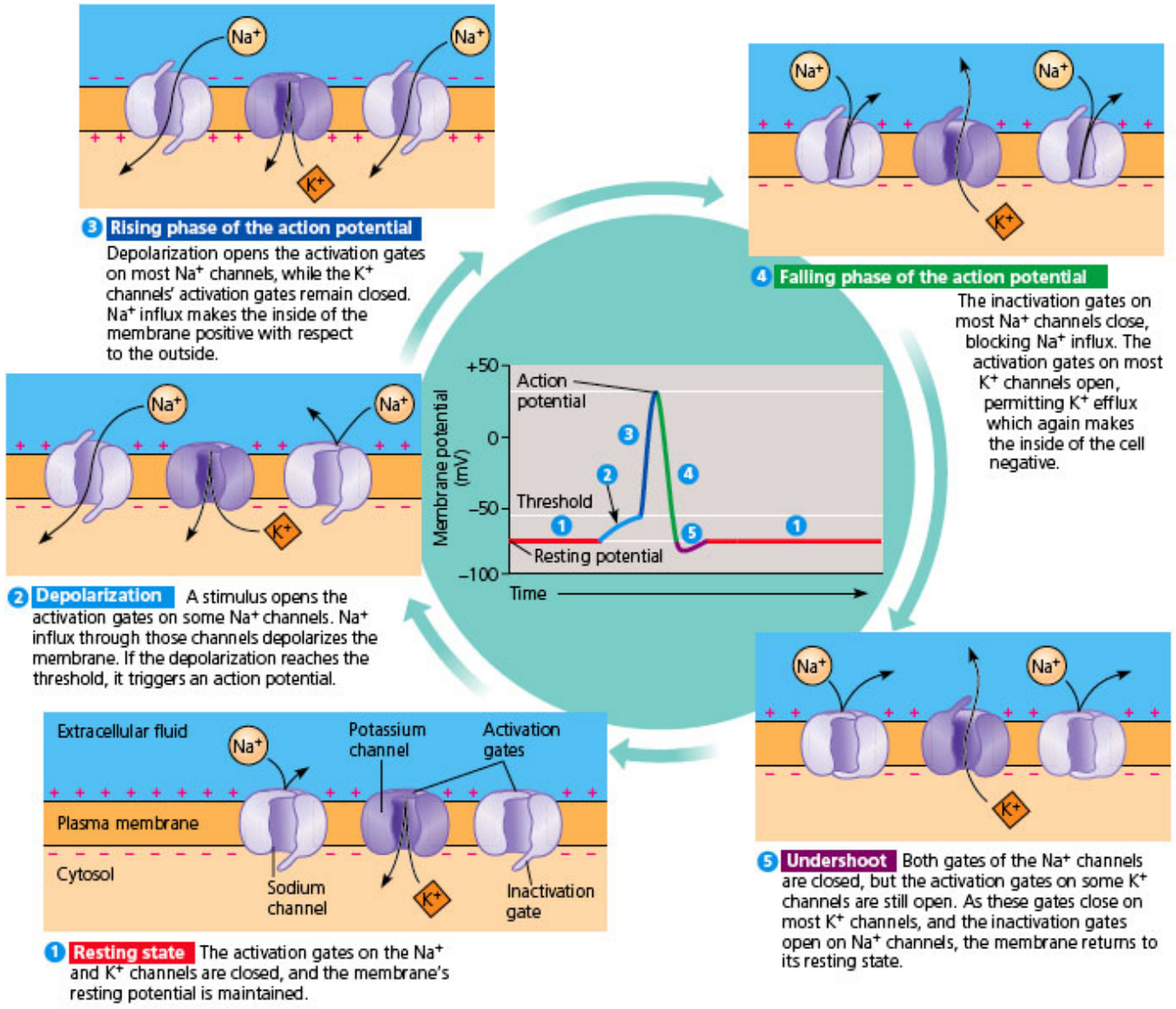
Provide quantitative description of action potential in terms of voltage and time-dependent changes in sodium and potassium conductances

The Annotated Hodgkin and Huxley: *A Reader's Guide*
Indira M. Raman and David L. Ferster

The first annotated edition of the scientific papers that created the foundation of modern neuroscience and physiology



Voltage-dependent and selective ion permeabilities underlie action potential



Key features of biological ion channels

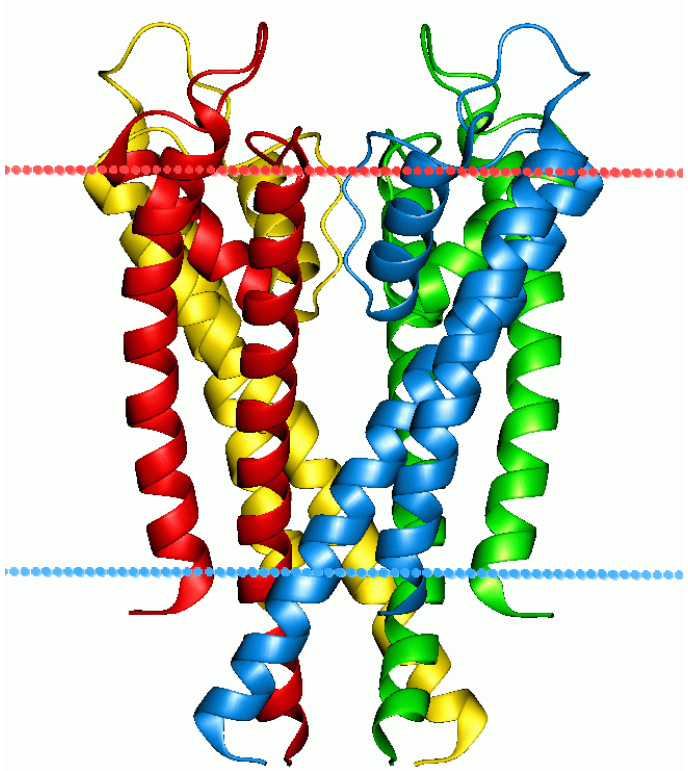
High ion selectivity. Potassium ion channels are most selective. P_K/P_{Na} is more than 100 even though Na^+ ion is 0.4 Å smaller than K^+.

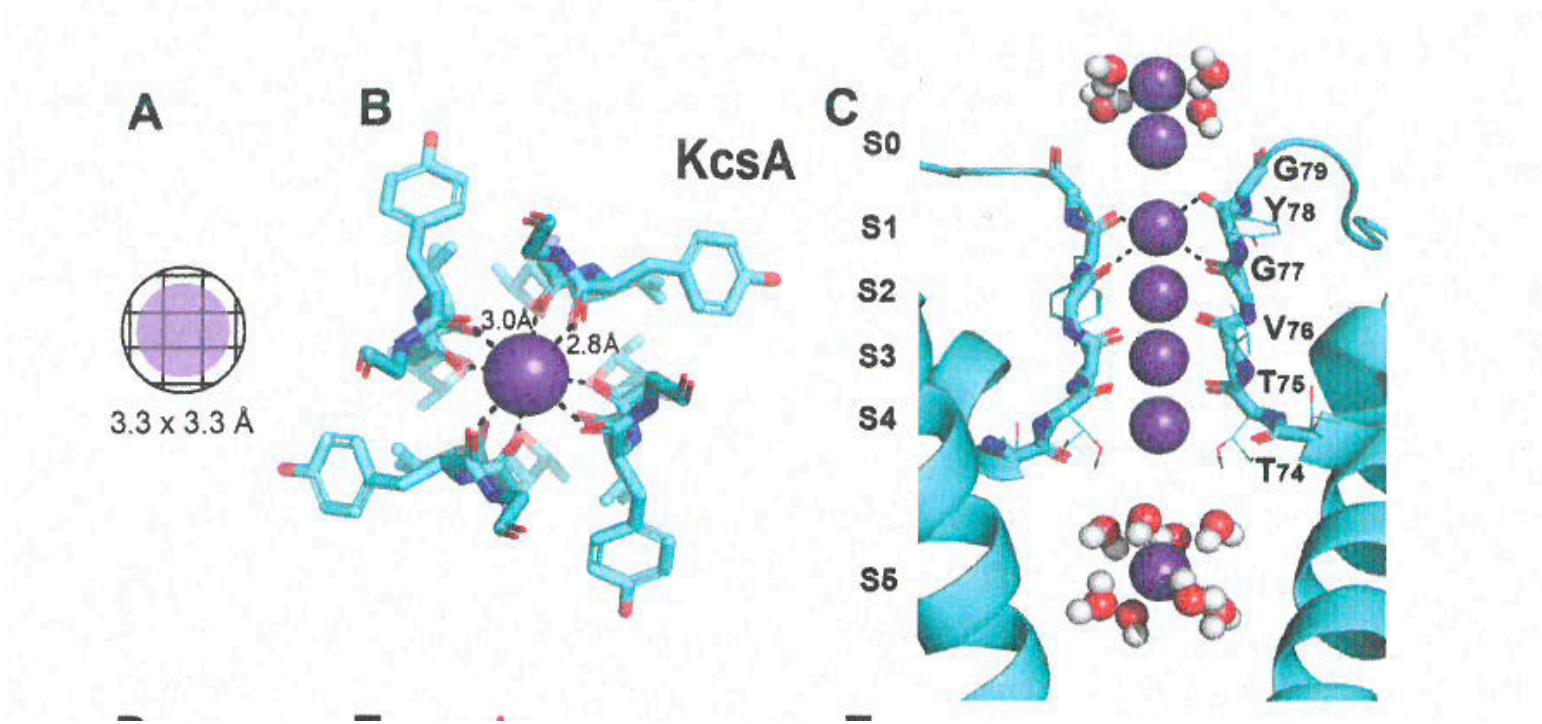
High conductance essentially diffusion limited. 10 million ions per second.

Ionic permeability is regulated by stimulus. Voltage, ligand, mechanical stretch, membrane tension, heat, cold, light.

What are the structural differences in the selectivity filter between sodium and potassium ion channels?

High-resolution structures of potassium channels

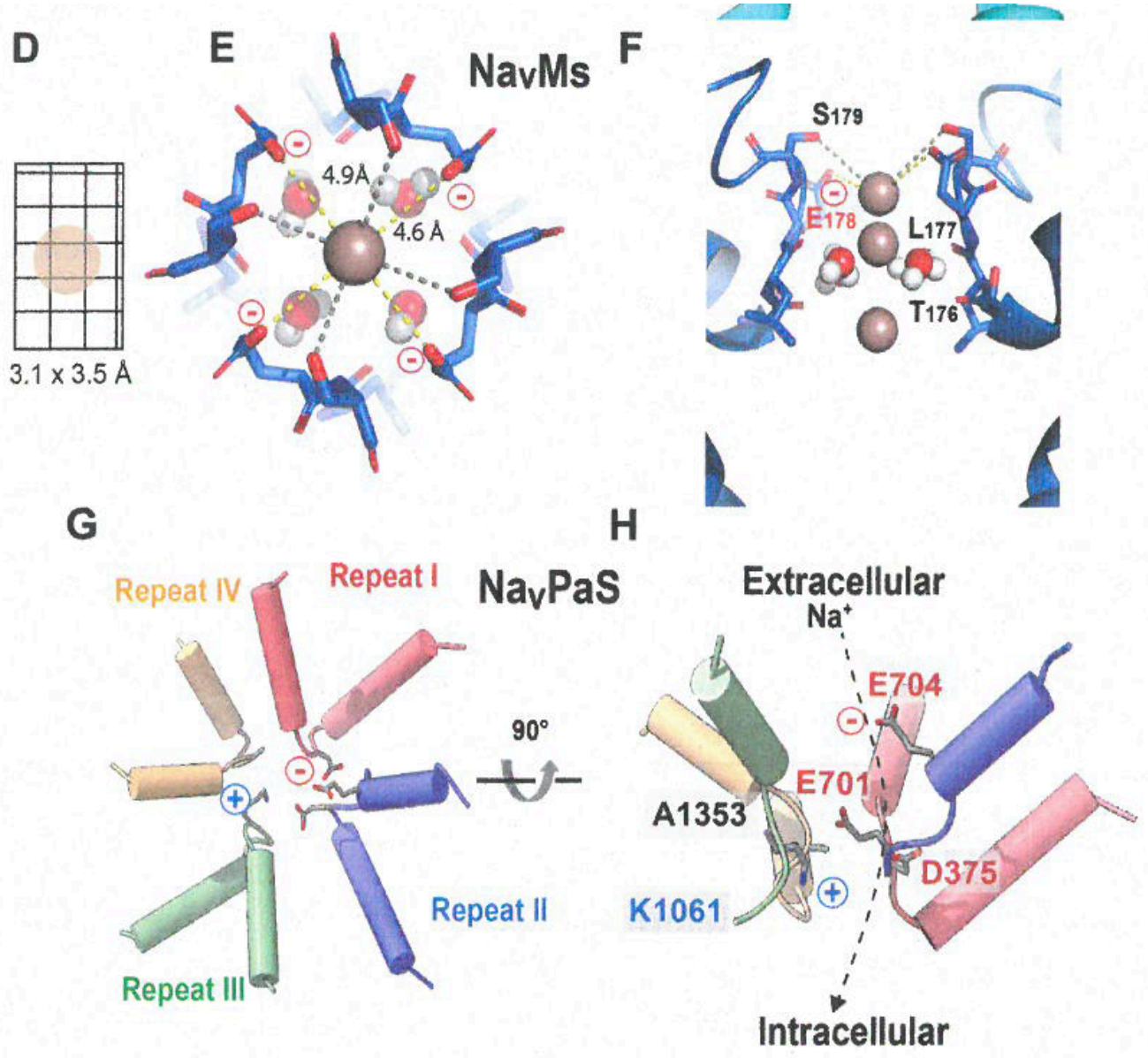




- **K+ ion channels are tetrameric in nature.**
- **Selectivity filter distinct from the pore gate.**
- **Four K^+ ion binding site.**
- **Backbone amide carbonyl coordinate with potassium ions**

Mackinnon, 2003 Nobel Prize in Chemistry

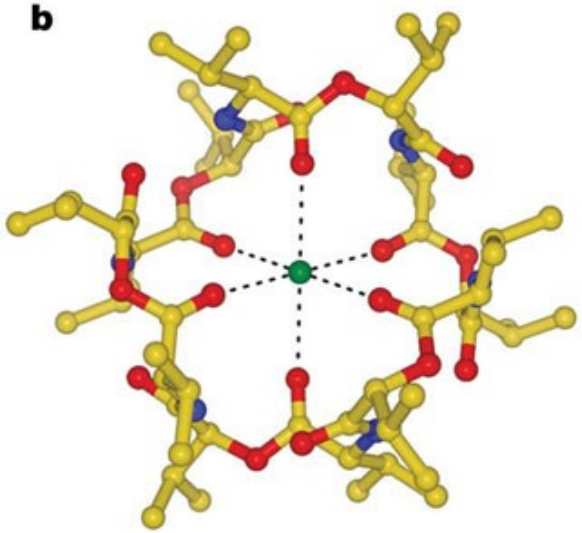
High-resolution structures of Sodium ion channels

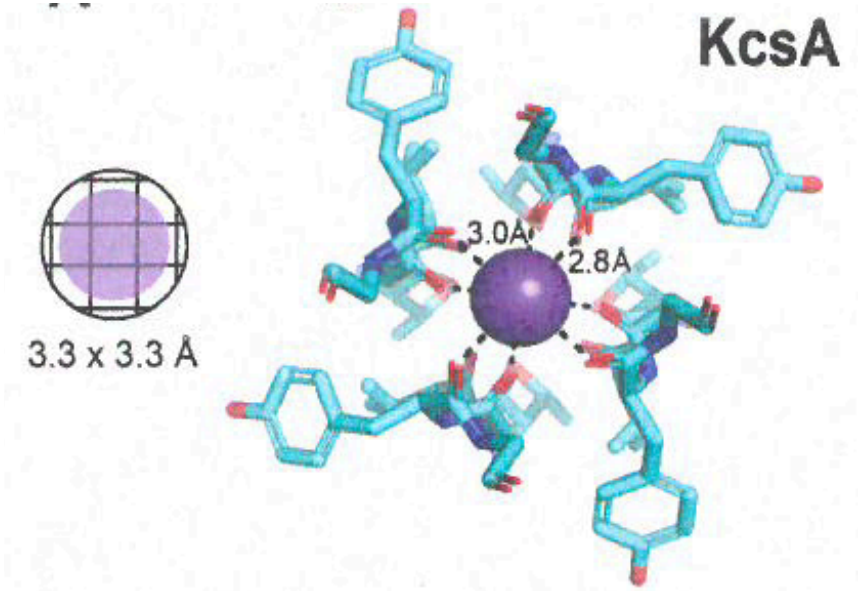


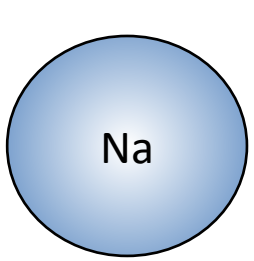
Mechanisms of selectivity in cation channels

Close-fit model

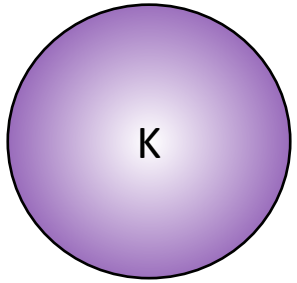
Valinomycin







0.95 Å



1.33 Å

Structure is optimized so that coordination distance between backbone oxygen atoms and K^+ ion is 2.8 to 3.0 Å. Nearly identical to that observed in highly selective ionophore such as valinomycin.

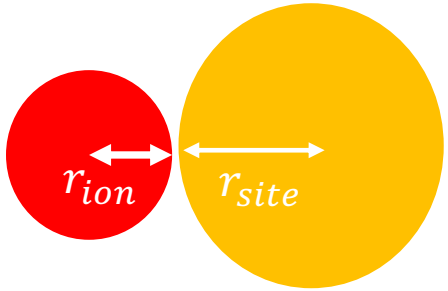
Cabral et al. (2001) Nature

Field strength model

B-factor in the X-ray structures is around 0.5 A which means that the extent of fluctuations around the mean position. Hence, tight fit model is not sufficient to account for differences in ion selectivity.

Eisenman's Theory of Ion selectivity

Ion	Radius (Ang)	ΔH_{hyd} (kcal/mol)
Li^+	0.6	-131
Na^+	0.95	-105
K^+	1.33	-85
Rb^+	1.48	-79
Cs^+	1.69	-71
Ca^{++}	0.99	-397



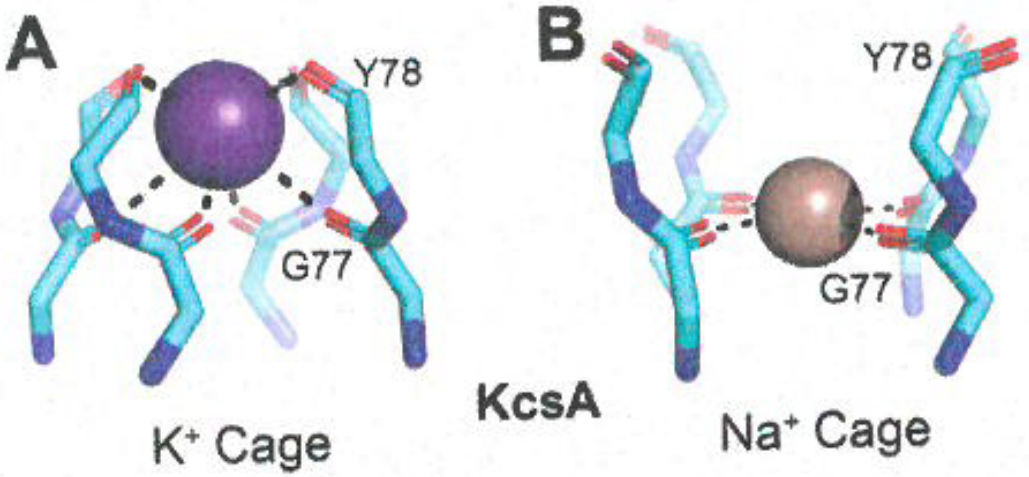
$$E_{site} = \frac{q_{site} q_{ion}}{\varepsilon(r_{site} + r_{ion})}$$

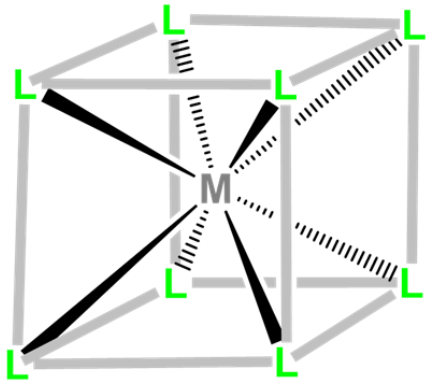
Cost of dehydration of ion is balanced by favorable interaction with the binding site. Rsite is the parameter that is optimized in the selectivity filter. If Rsite is too small, strong interaction with the site and it will inhibit ion flux.

Ligand dipole of Carbonyl group is between 2.5 to 4.5 debye. Changing this alters K^+ selectivity.

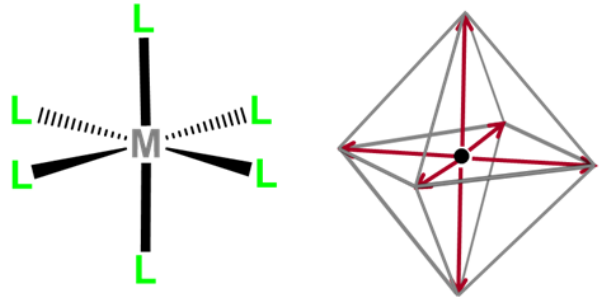
Noskov et al. (2004) Nature

Coordination model





Cubic



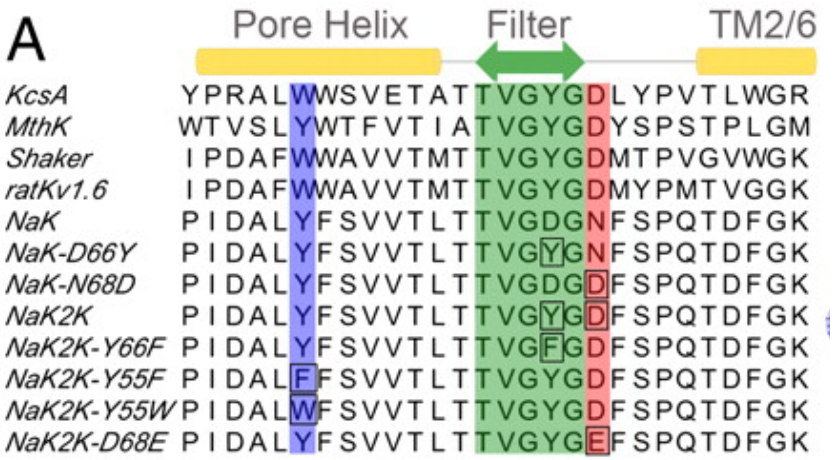
Octahedron

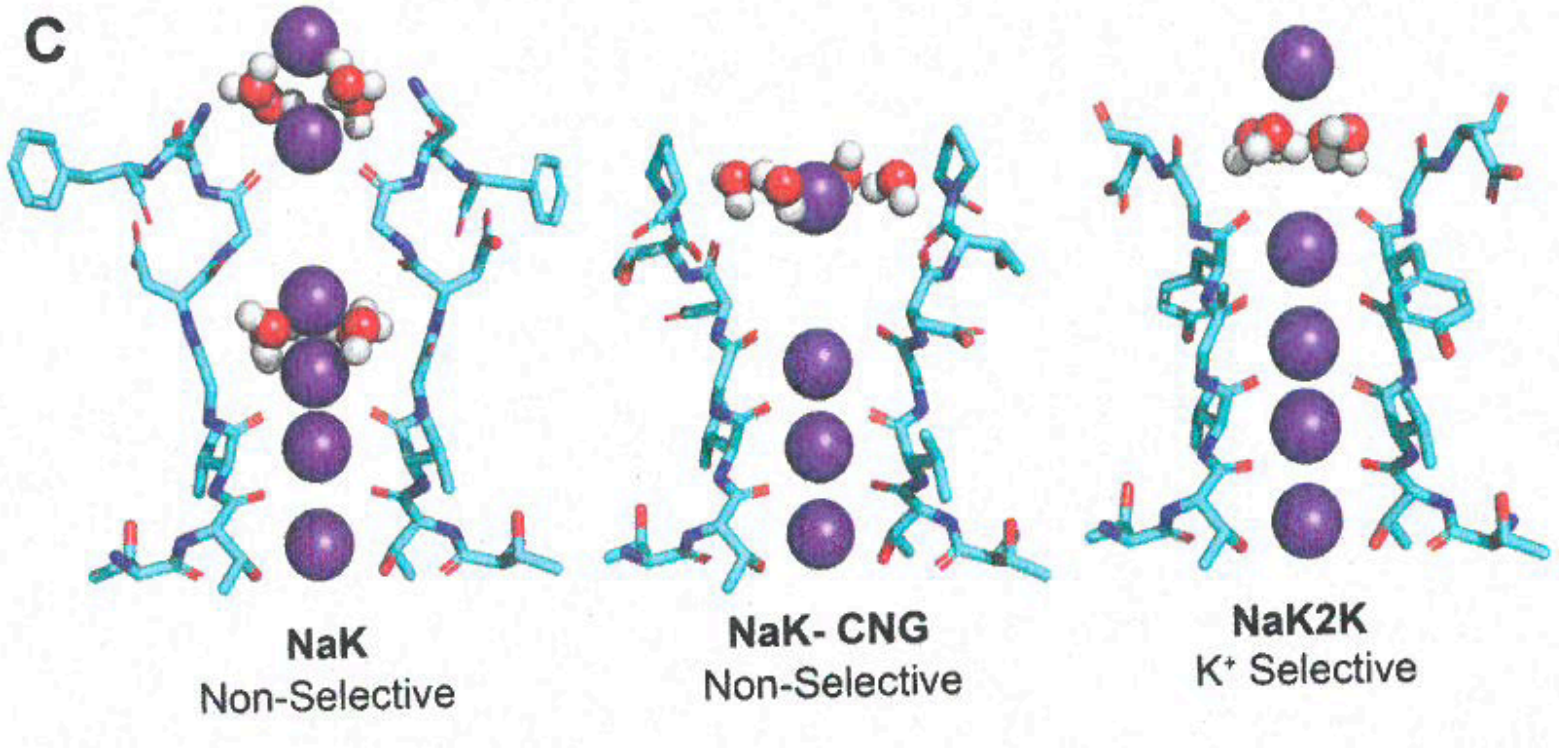
Eight liganding moieties forming a cubic coordination around the K^+ ion which is rare and seen in transition metals and rare-earth metals. In water, K^+ ion is in octahedral coordination. Cubic coordination selects K^+ over Na^+.

Bostick and Brooks (2007) PNAS

Verma, Sabo and Rempe (2008) JMB

Site number model





Number of K+ ion binding site is important. Four contiguous ion binding site is necessary for ion selectivity.

Sauer et al (2011) PNAS

Outstanding/ unresolved questions

Challenging to model computationally. Probably polarizable force fields will be needed to accurately model these interactions.

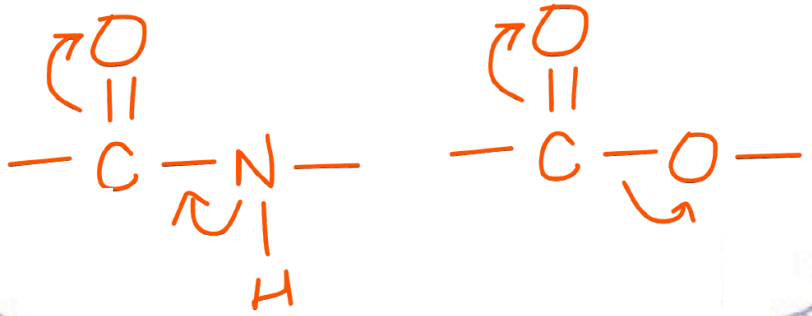
Mutations outside the selectivity filter seem to change ion selectivity.

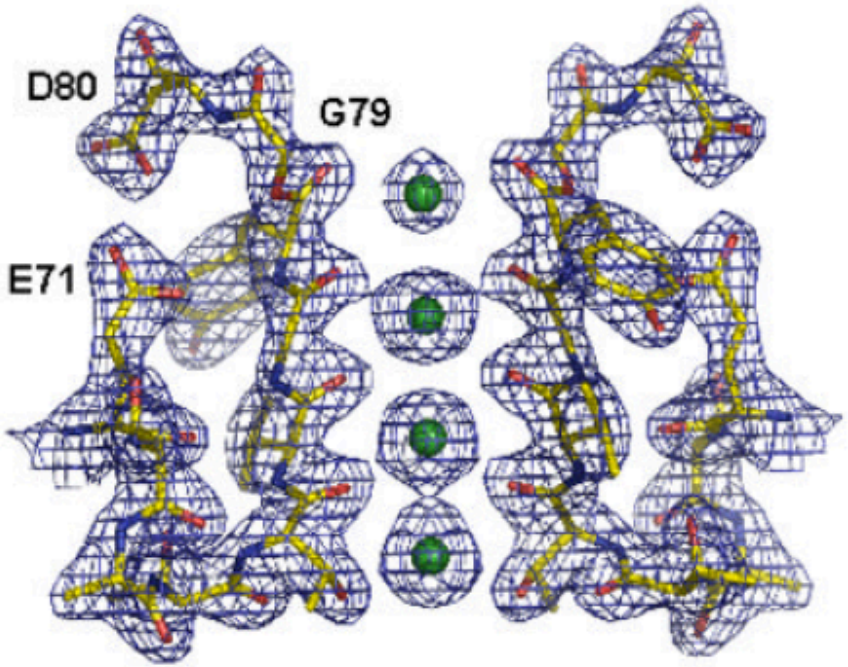
Recent smFRET studies suggest that mutations that result in non-selective pores is accompanied by dynamic or dilated pore conformations.

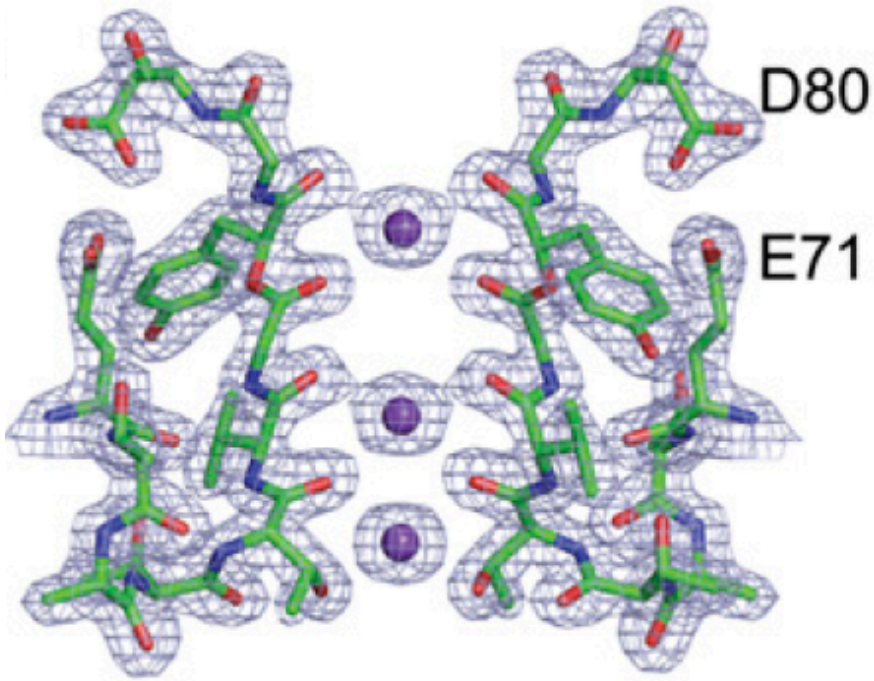
Wang et al (2019) Nat. Chem. Biol.

Experimental approaches to test the various models. Ester substitution of the amide linkage.

Experimental test of the field strength model







Ester substitution on S1 site

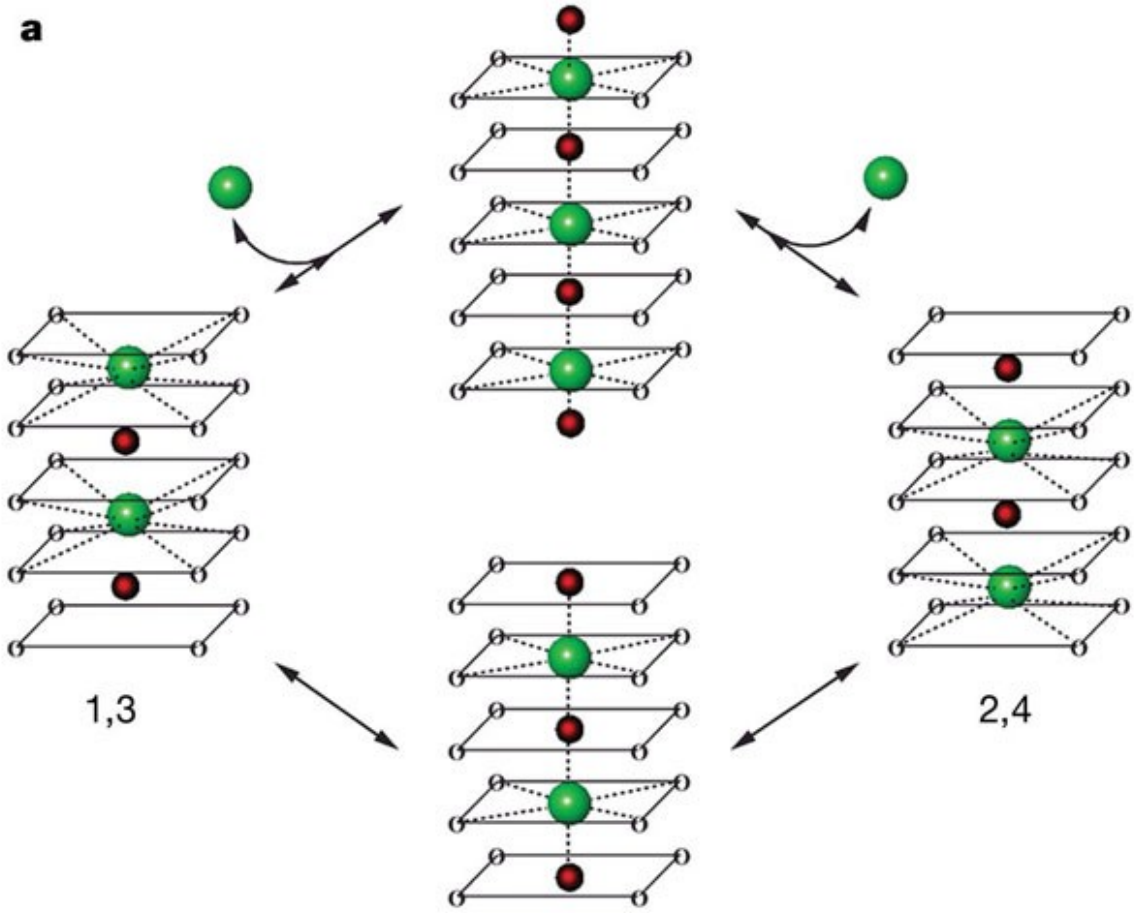
Ester substitution on S2 site

Vilyaveetil et al. (2006) JACS

Matulef et al. (2013) PNAS

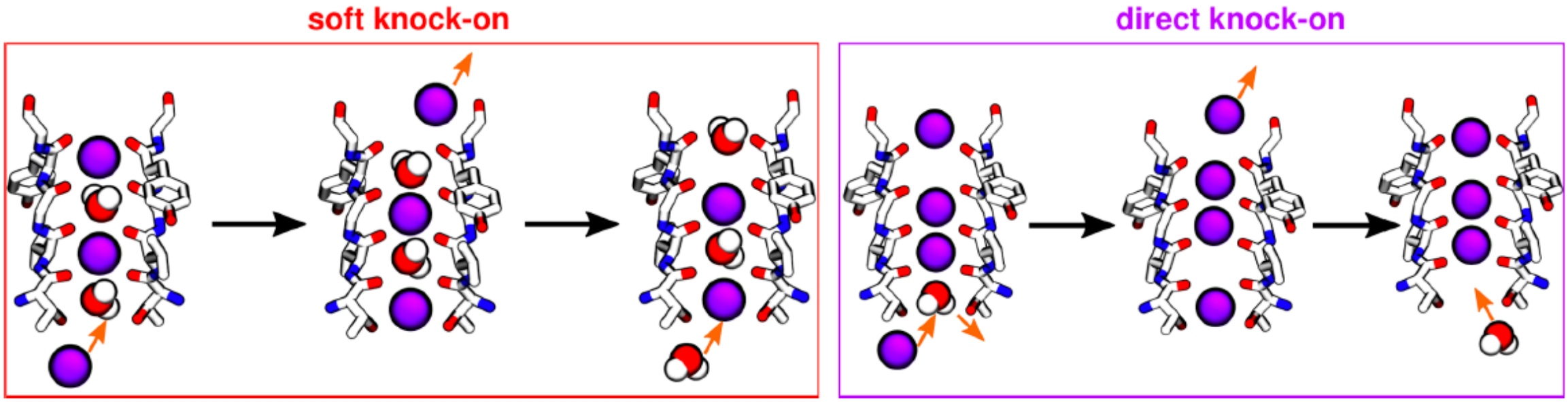
Mechanisms of high conductance in cation channels

Multi-ion pore is necessary for obtaining high flux with high selectivity



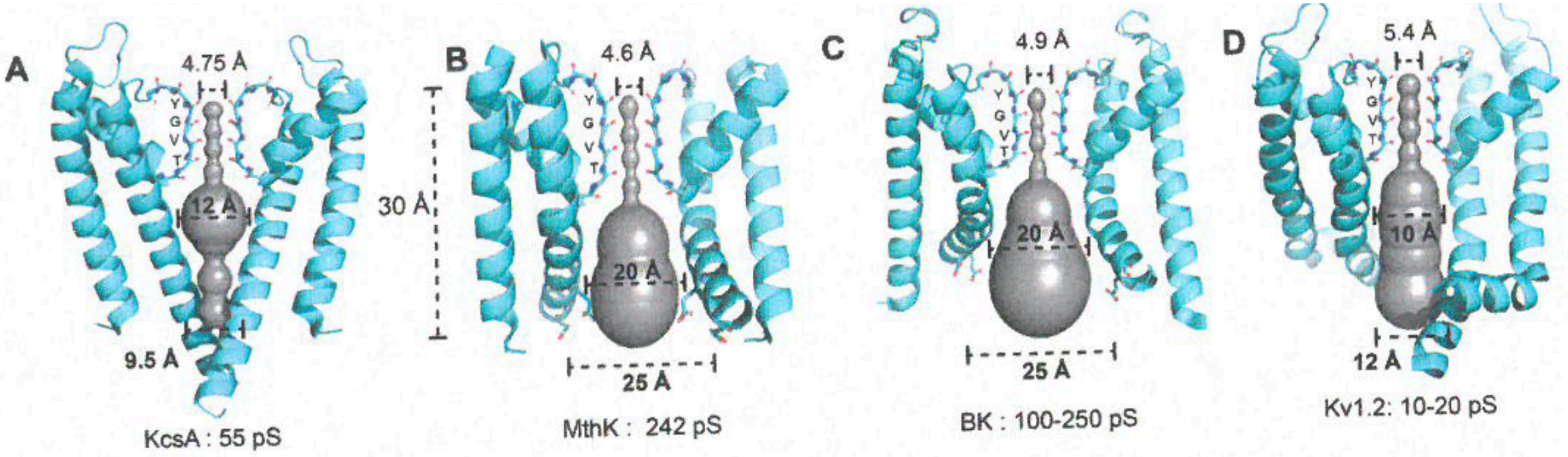
Cabral et al. (2001) Nature

Hard knock-on vs. Soft knock-on



Kopec et al. (2014) Science

Other factors that determine the conductance



Negative charges at the entrance of the selectivity filter increases single channel conductance by increasing the local concentration of cations

Thank You!