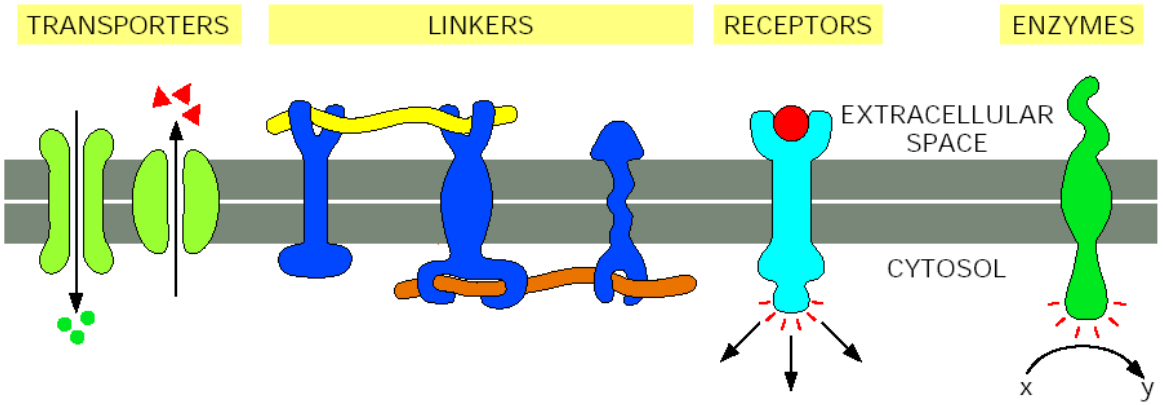
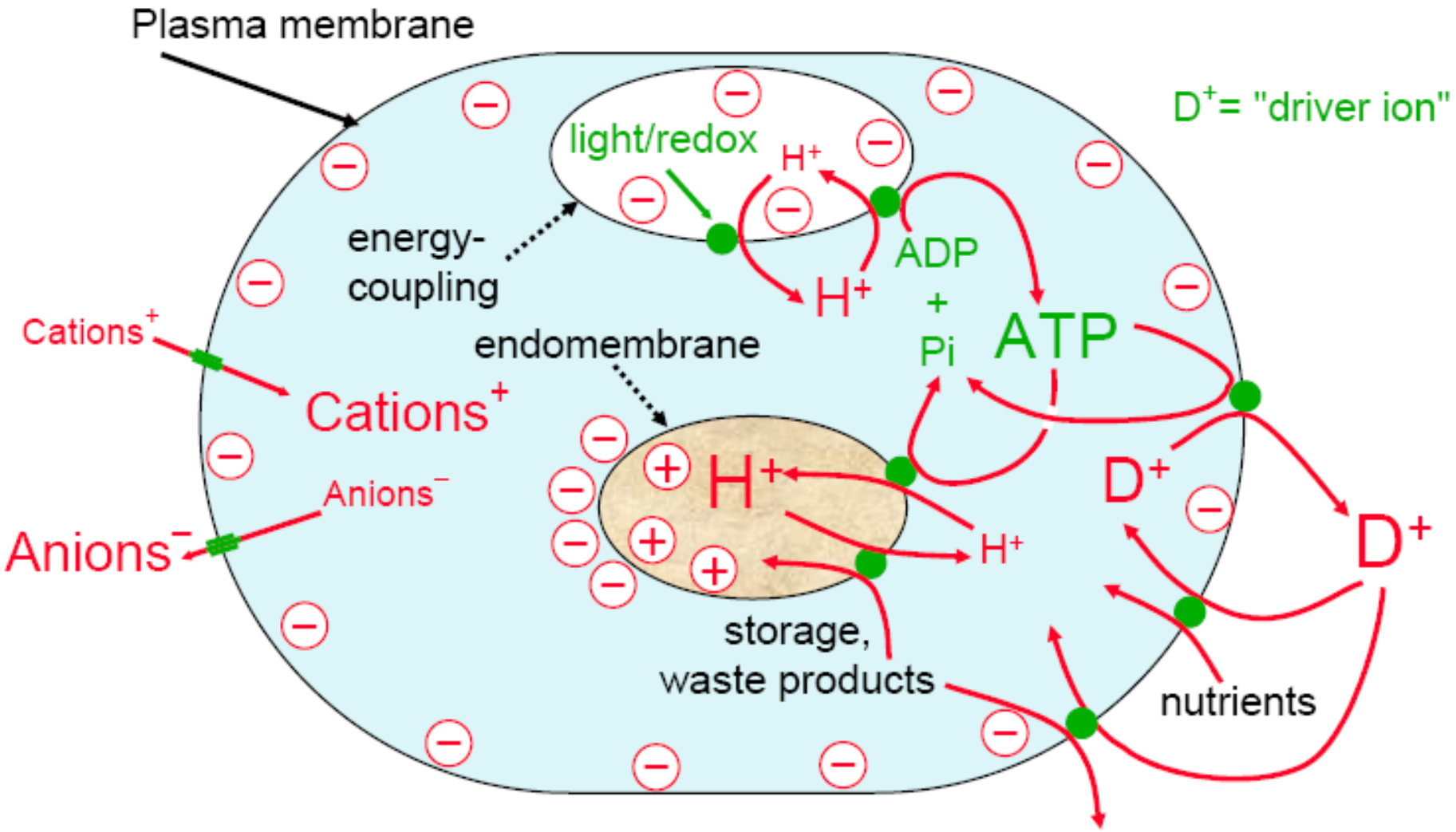
Membrane Proteins



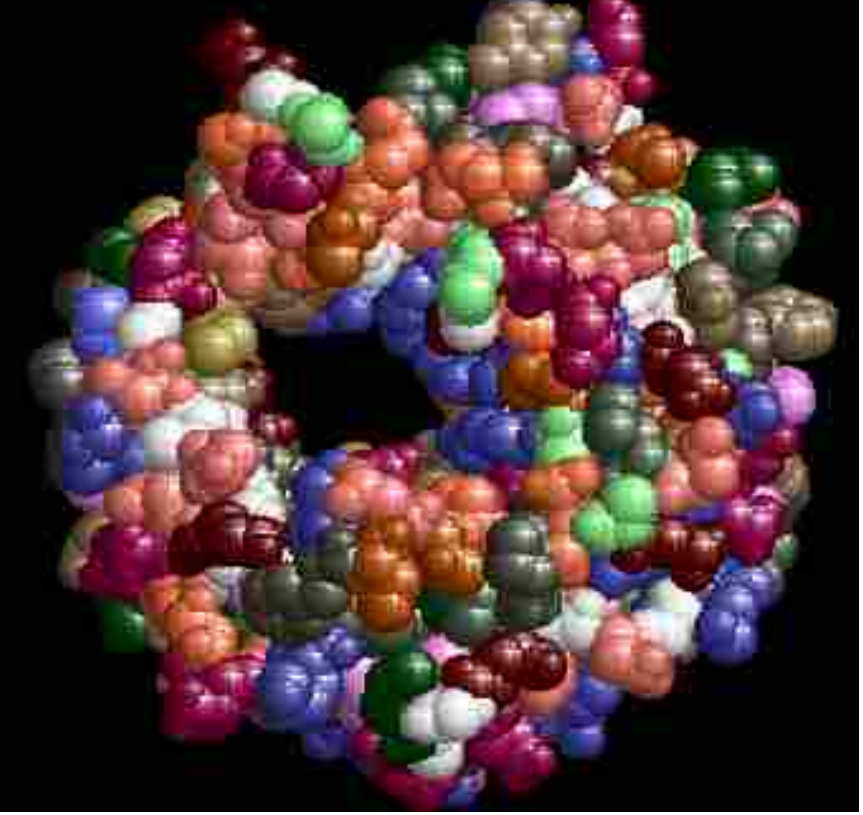
MEMBRANE PROTEINS

can be categorized according to

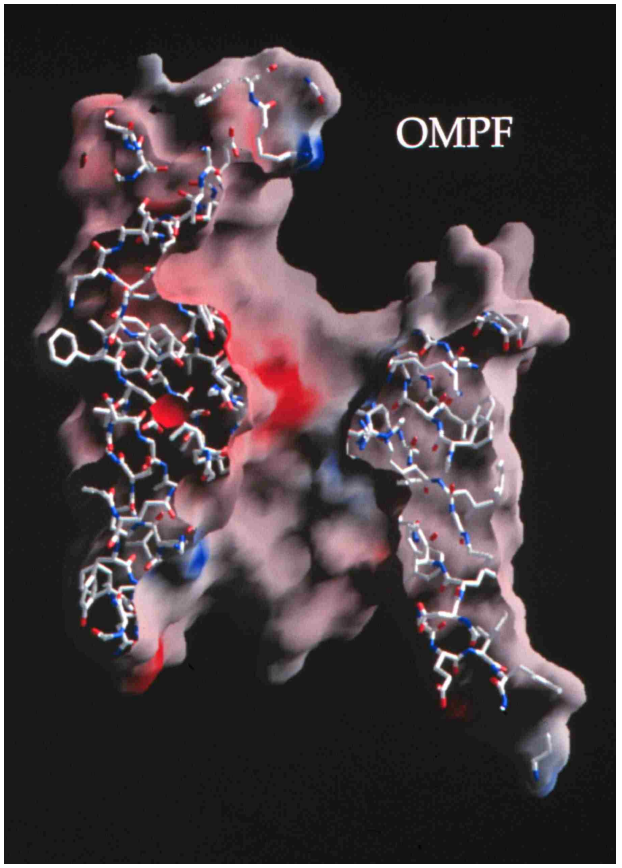
- **Structure**
  - Integral proteins
  - Associated proteins
- **Function**
  - Membrane transporters
    - Carrier proteins
      - change conformation
    - Channel proteins
      - form
        - Open channels
        - Gated channels
          - Mechanically gated channel
          - Voltage-gated channel
          - Chemically gated channel
  - Structural proteins
    - are found in
      - Cell junctions
      - Cytoskeleton
  - Membrane enzymes
    - are active in
      - Metabolism
      - Signal transfer
  - activate
  - Membrane receptors
    - are active in
      - Receptor-mediated endocytosis
      - Signal transfer
      - open and close



Transport processes in an idealized eukaryotic cell



Ion Channels
Proteins with a Hole



~30 Å

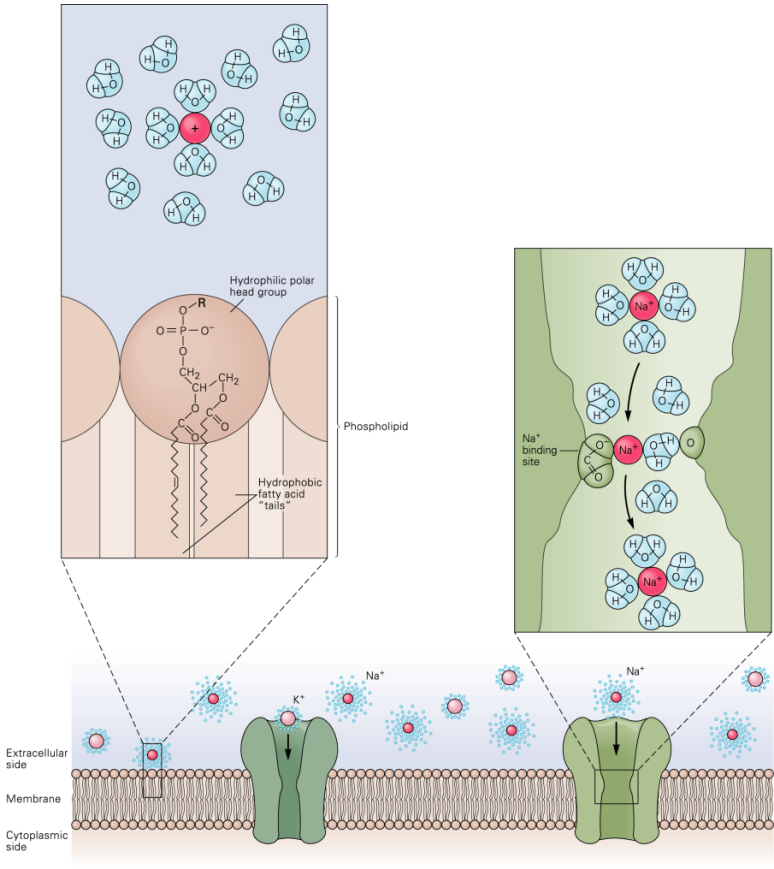
Ion Channels
are the
Main Molecular Controllers
"Valves"
of Biological Function

Channels

The downhill diffusion of ions through biological membranes occurs through ion channels.

- an ion channel has a resistance of ~ 10^{10} Ω,
- a patch of lipid bilayer of similar cross section has a resistance of ~ 10^{23} Ω.

For every ion that crosses the membrane through the lipid bilayer, 10^{13} do so by traversing an ion channel.



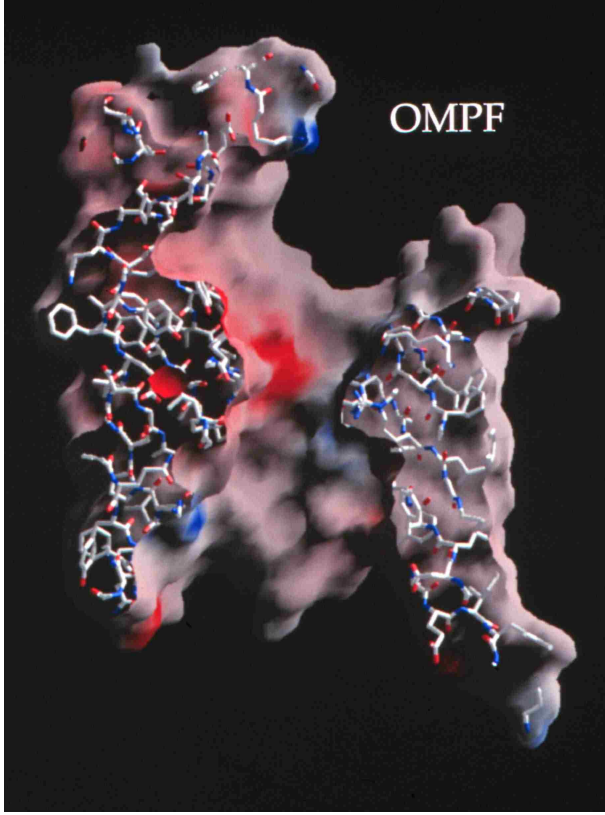
Ion channel

ion-permeation pathway through the membrane

Selectivity filter
(narrowest constriction in the 'open' conformation)

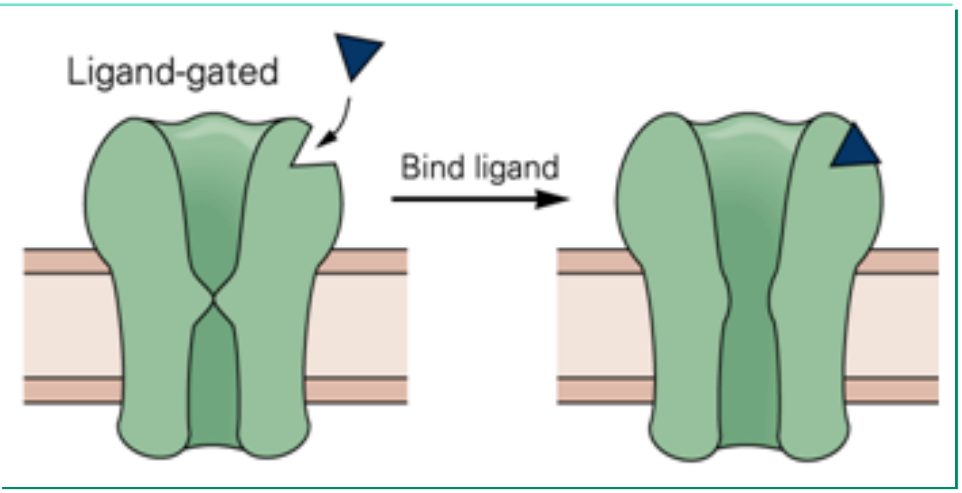
Gate
(narrowest constriction in the 'closed' conformation)

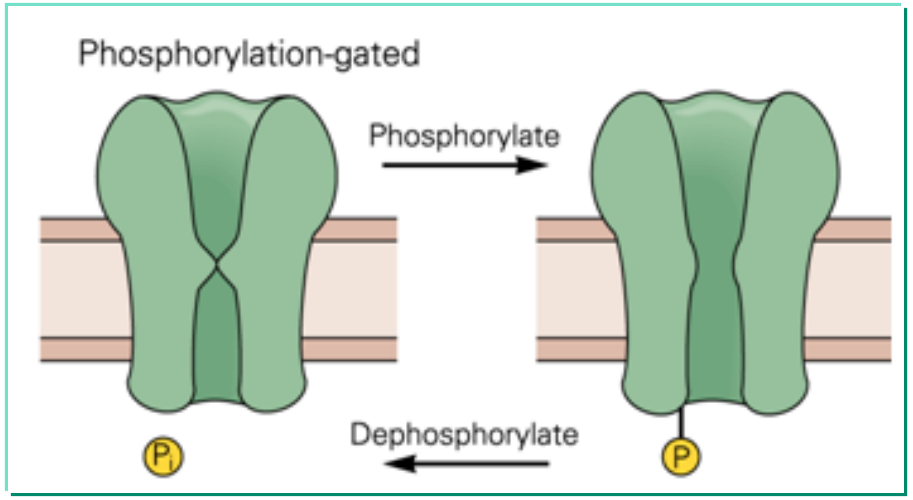
Elements that control the gate
(ligand-binding sites, voltage-sensor, pH-sensor, temperature-sensor, mechanical-deformation sensor)

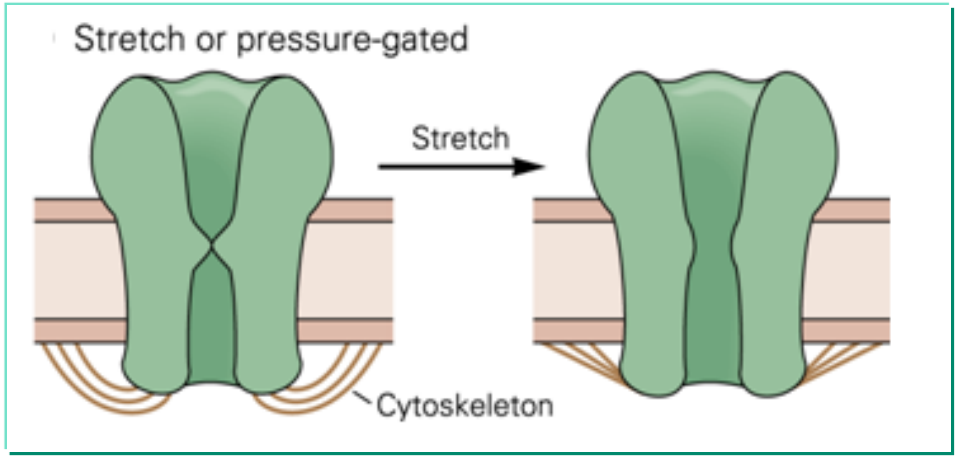


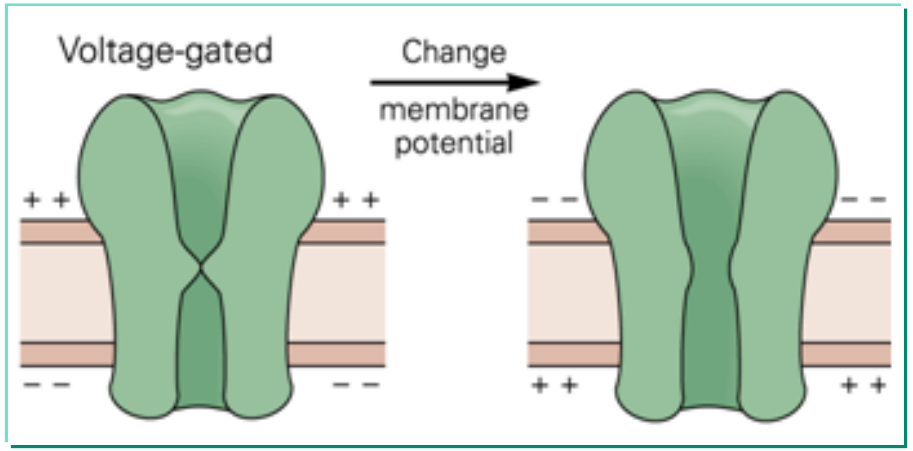
~30Å

Depending on the type of the channel, this gating process may be driven by:



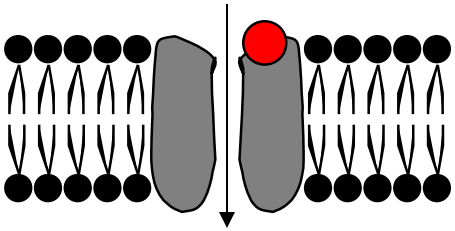




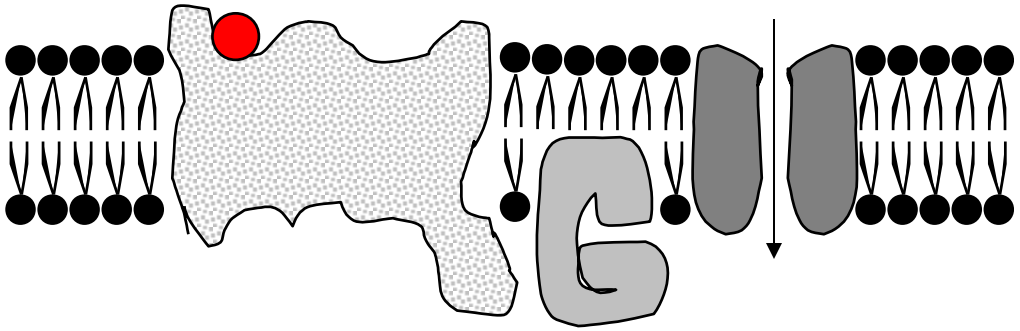


Ligand-gated ion channels – response time

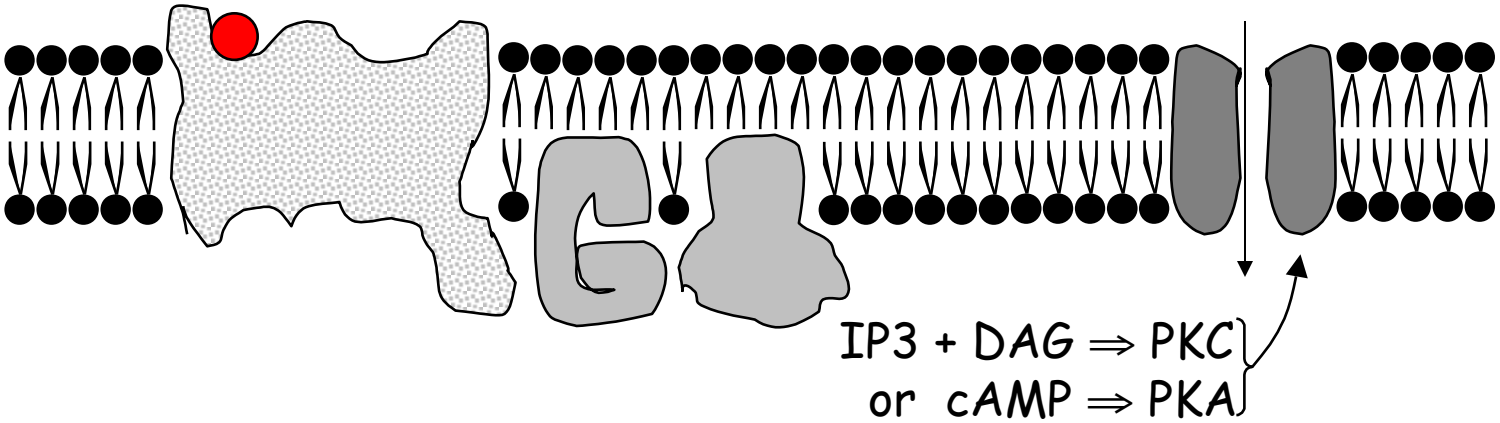
Receptor with integral ion channel



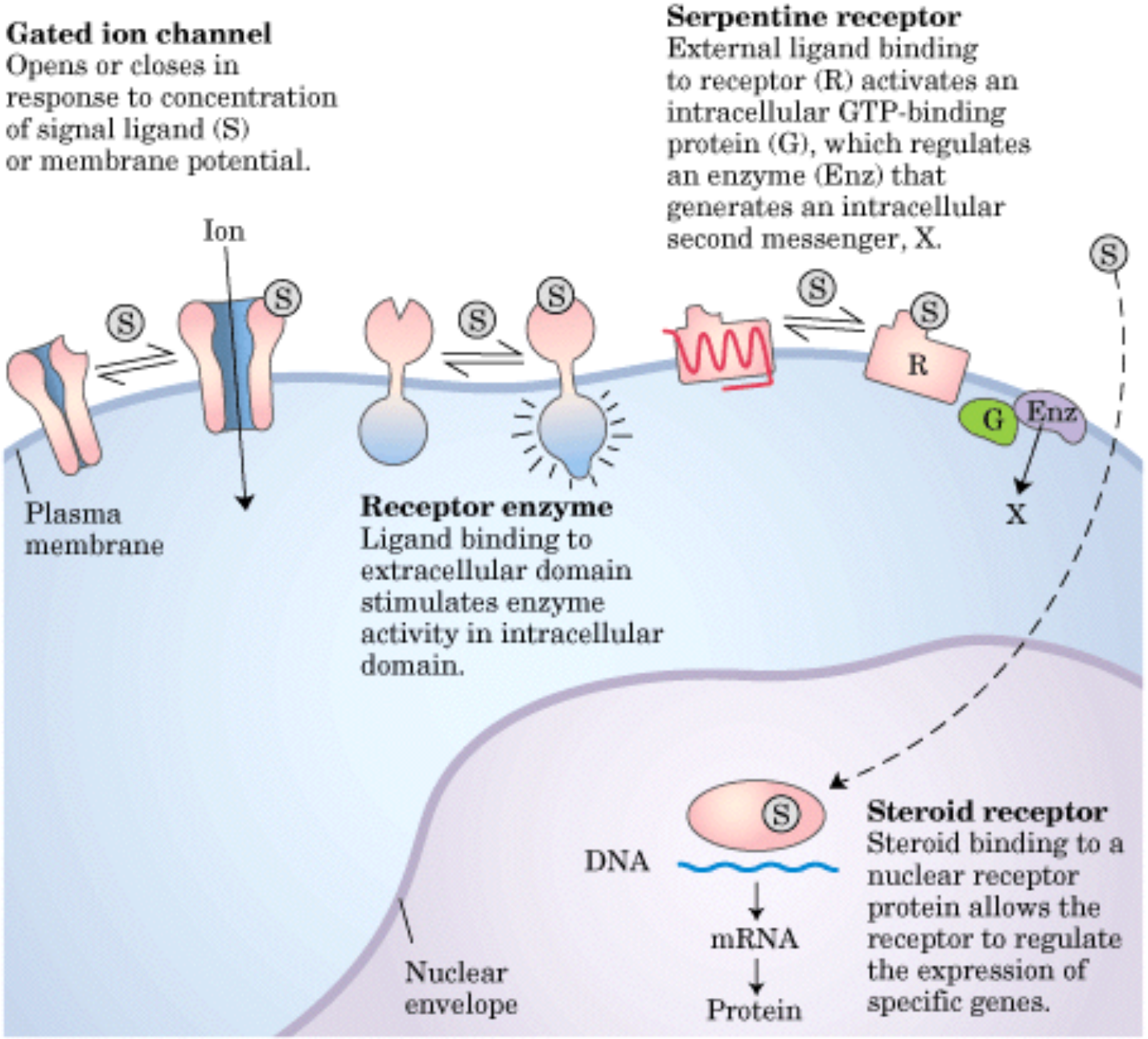
Receptor linked to ion channel via g-protein



Receptor linked via g-protein & 2nd messenger



Types of signal transducers



Two approaches to study ion channels

Reconstitution: channels from organelles, channels present in membranes that are difficult to 'seal' (e.g., highly-invaginated epithelia), channels from cells that are too small (e.g., bacteria) or that move too much (sperm cells). Experiments that require a defined chemical composition of the membrane. Experiments that require changes in the solutions that bathe *both* sides of the membrane.

Patch-clamp: channels from native cells, channels heterologously-expressed in cell-lines or in *Xenopus* oocytes. Experiments that require a fast perfusion of the membrane. Experiments that require a high time resolution (tens to a few hundred microseconds).

Methods for Studying Ion Channels

Biochemistry

- **agonist, antagonist or drug binding**
- **isolation and purification**
- **reconstitution**
- **radioactive ion flux**

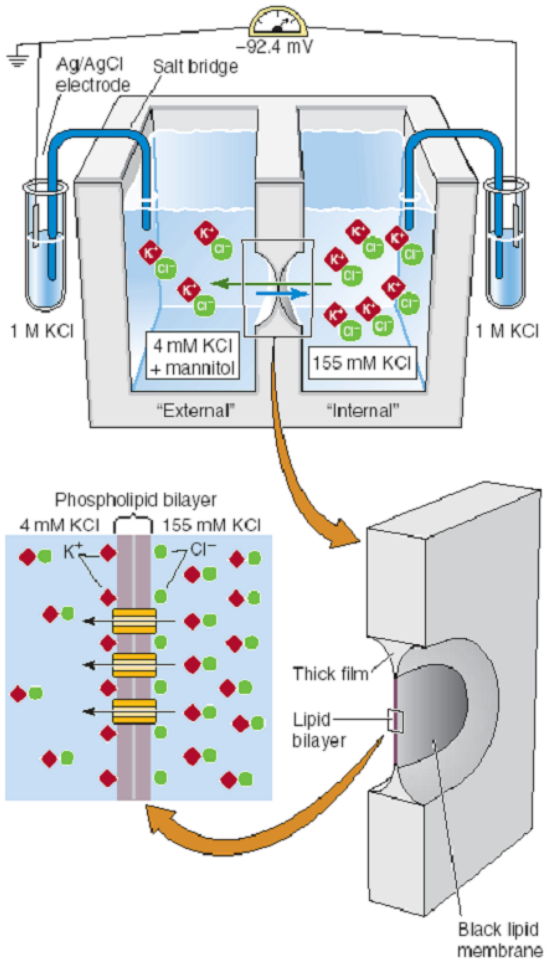
Molecular biology

- **sequencing, cloning, mutagenesis**

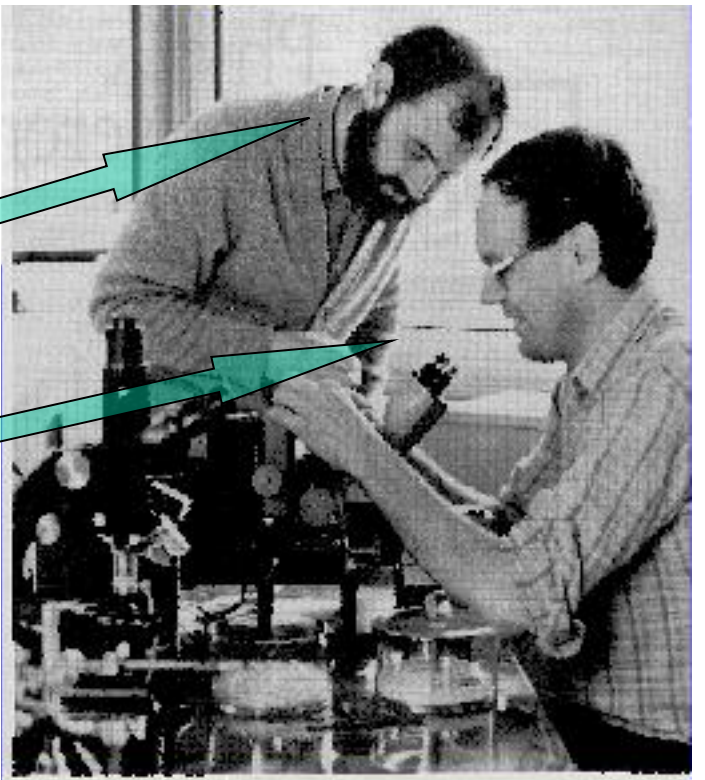
Structural biology

- **microscopy, crystallography, NMR, ...**

Chris Miller
Brandeis University



Patch clamp technique

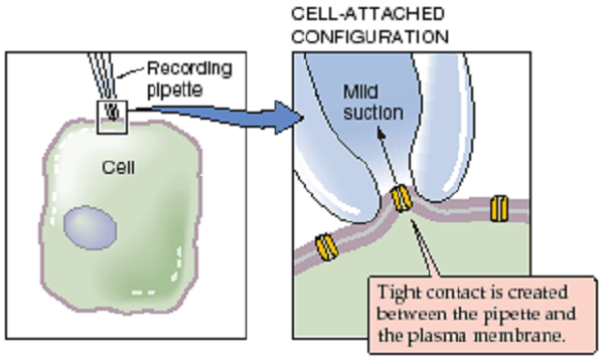


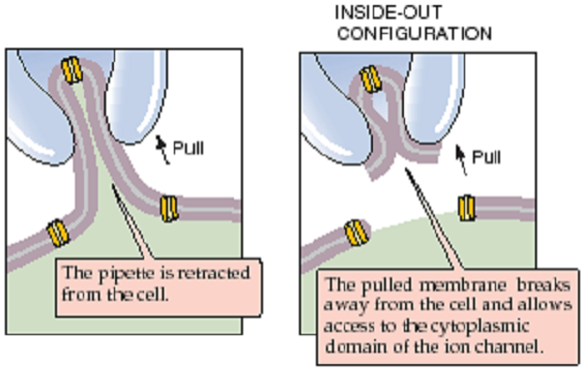
Erwin Neher

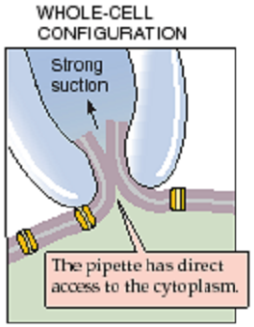
Bert Sakmann

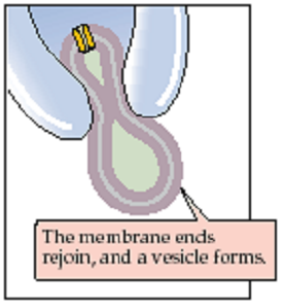
Germany
(1991 Nobel Laureates)

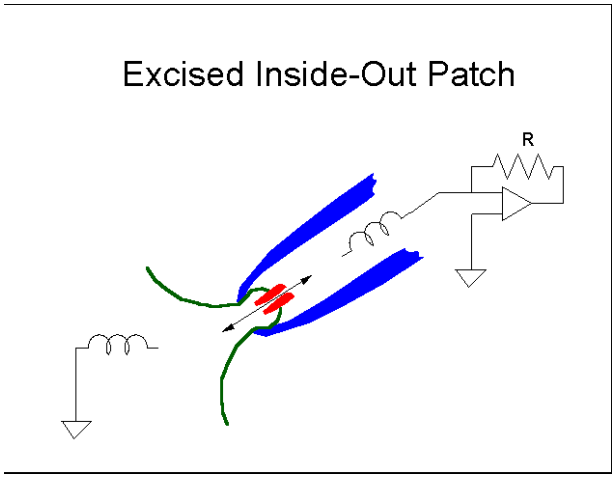
Patch clamp methods

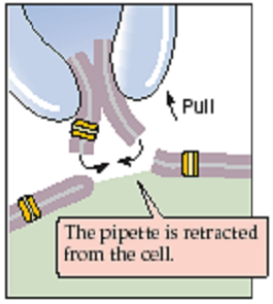


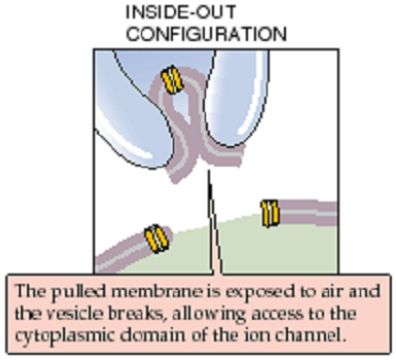


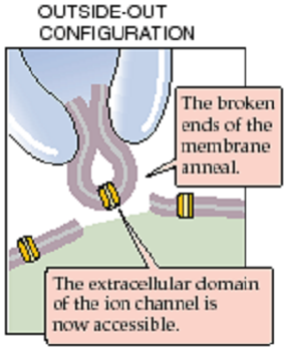


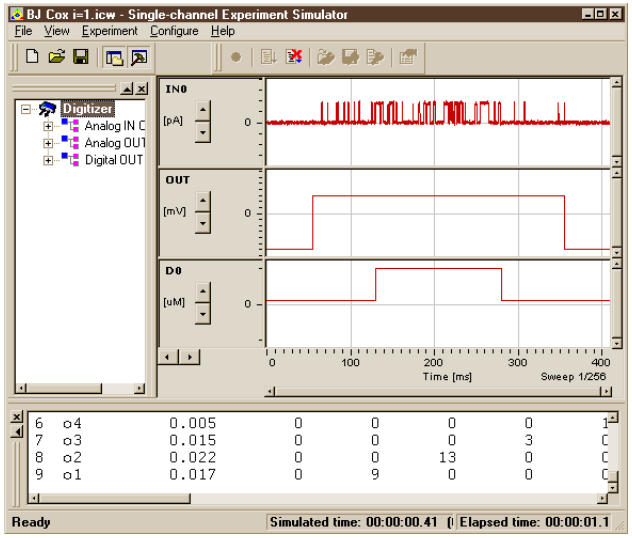




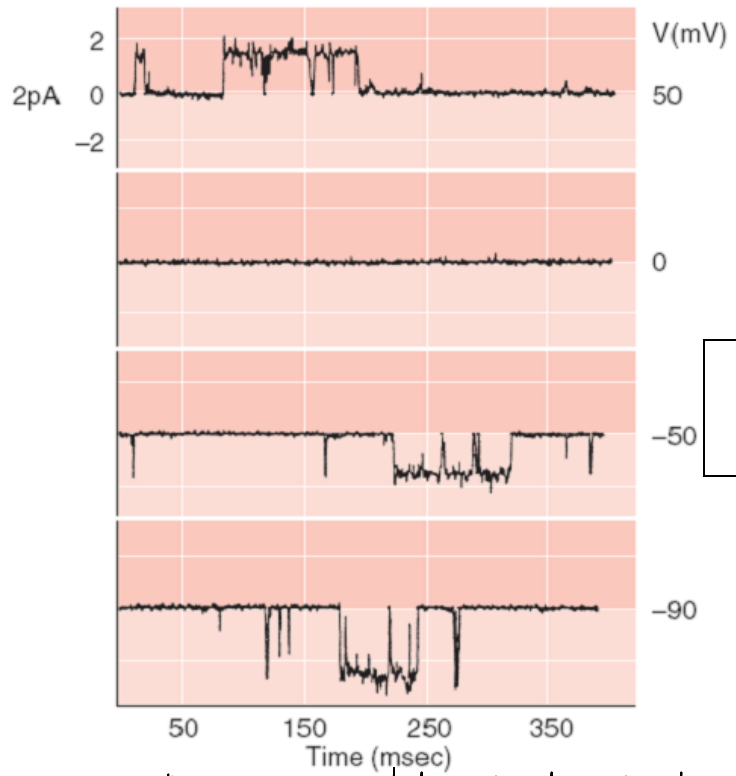


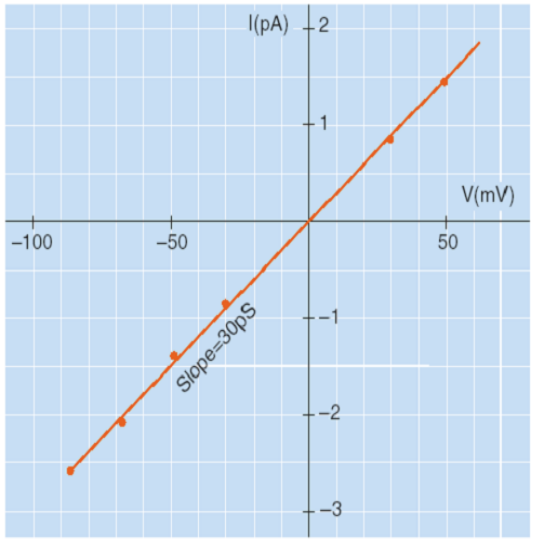






Gating and Permeation





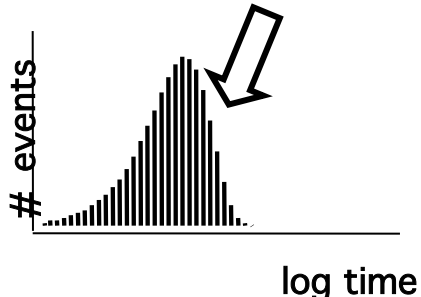
(Bormann J, Hamill OP, Sakmann B; J Physiol (Lond) 385:243-286, 1987.)

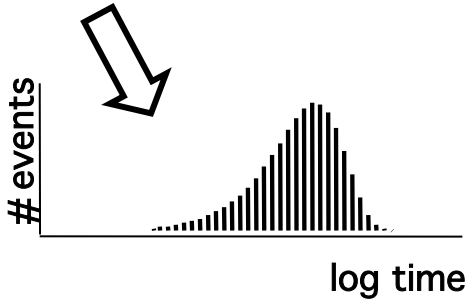
I-V plot of single Cl^- channel

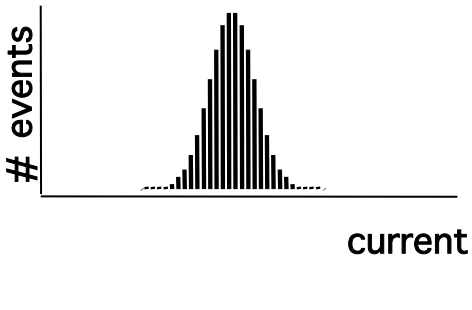
Current Amplitude in Picoamps (pA)

Open duration in Milliseconds (ms)

Closed duration in Milliseconds (ms)







OmpF and G119D Porin Trimer Current Voltage Curves

KCl Solutions