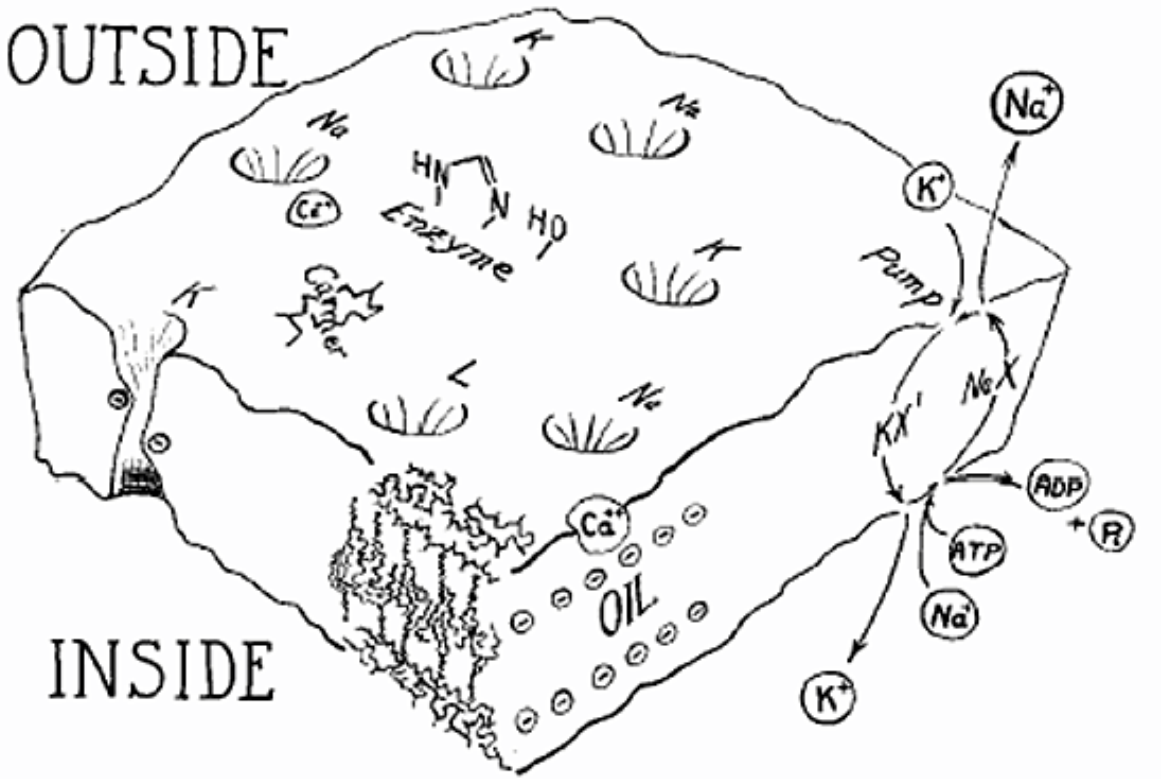
# Biology 5357: Chemistry and Physics of Biomolecules

# Sensing and Gating





## Baron Chanda

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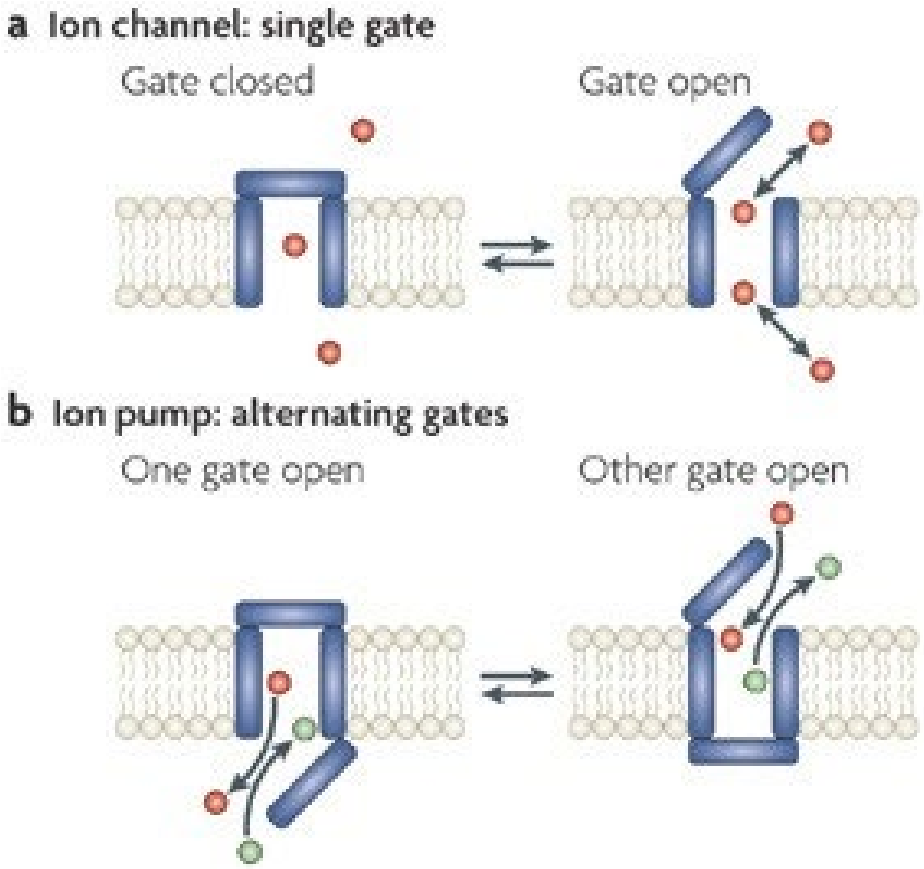
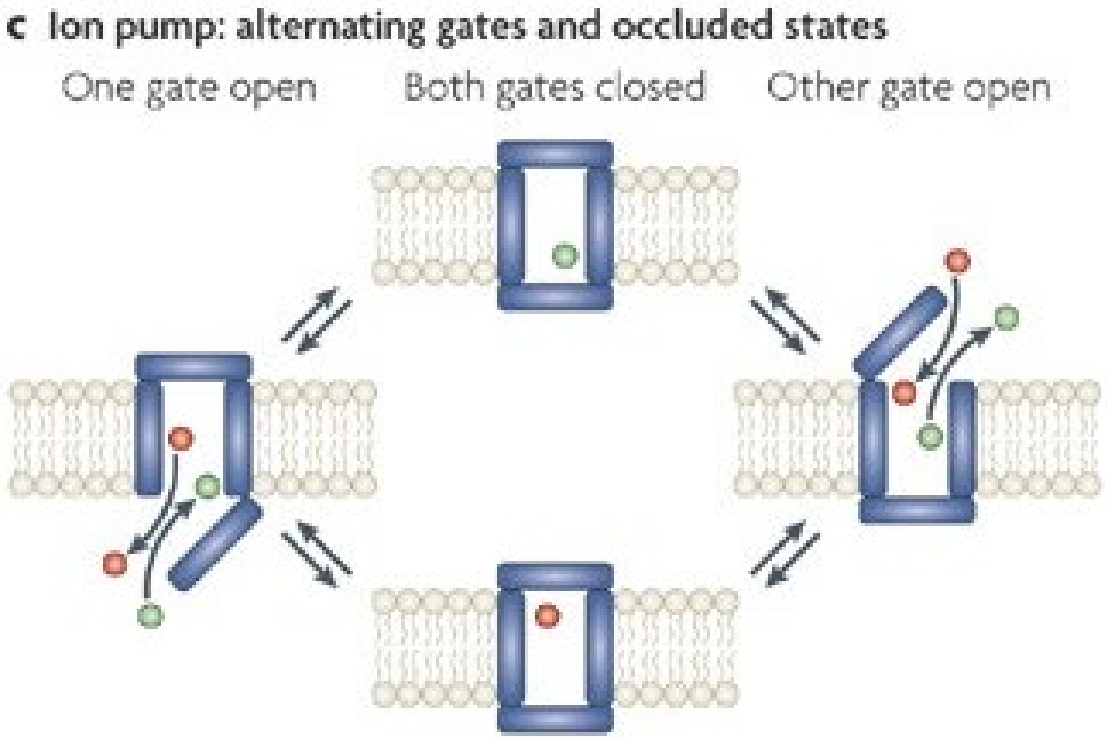
Washington University School of Medicine

# Ion Channels and Transporters




Ion channels typically transport ions at the rate of 10 million per second whereas transporters move 1000 ions per second.

Ion channels can have multiple gates but both gates are opened at the same time unlike in a transporter.

In most ion channels, ligand and substrate are distinct. Ligand activates the channel and substrate gets transported.

# Membrane signaling complexes are physiological sensors of physical and chemical stimuli

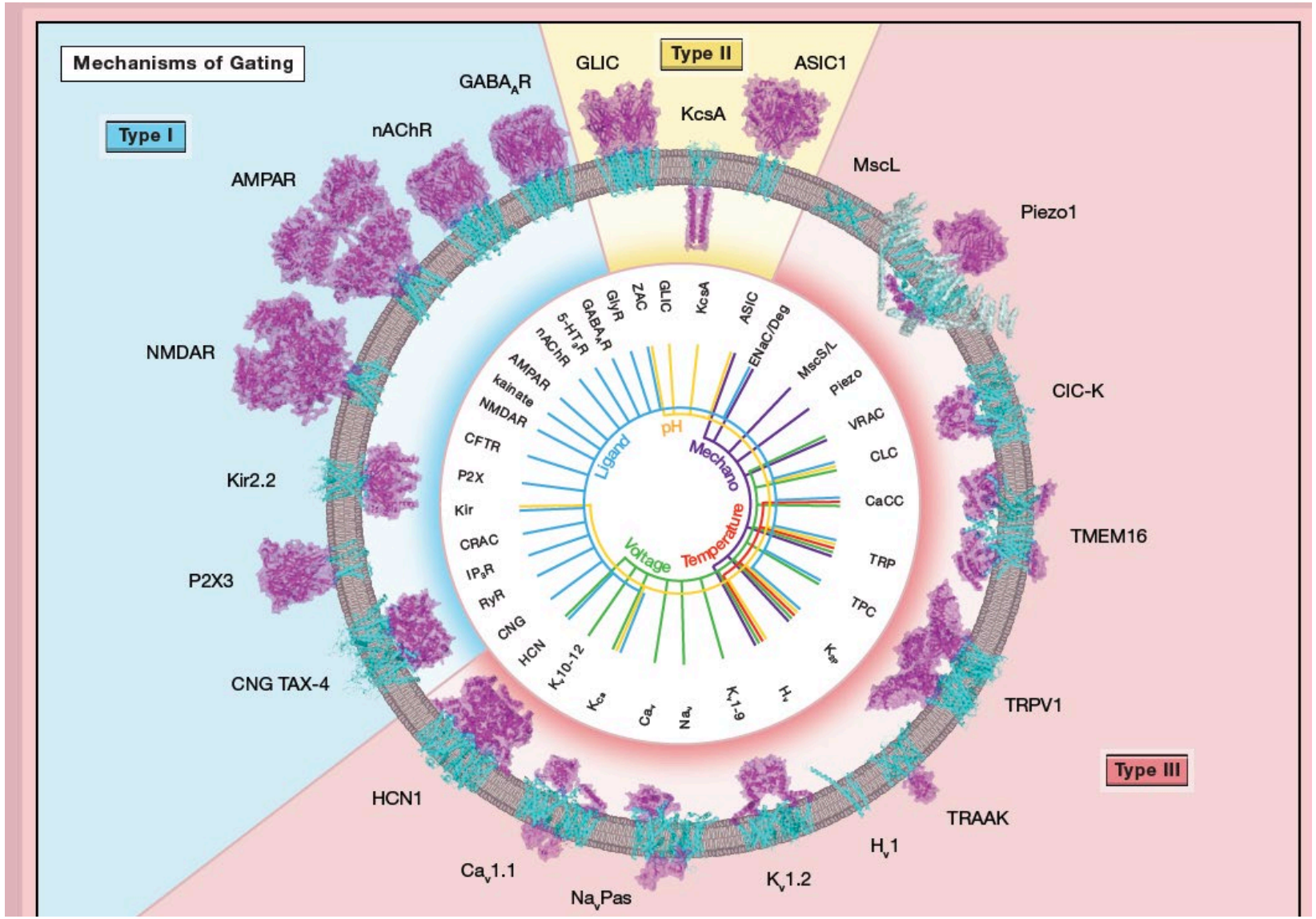
## Ion Channels

- Ligand activation channels – Neurotransmitter receptors.
- Voltage-gated ion channels – $Na^+$ and $K^+$ channels.
- Light activated ion channel – Holorhodopins in microbes.
- Mechanically activated - MET channel in hair cells.
- Membrane stretch activated- PIEZO channels.
- Temperature-activated ion channel – TRPV1 and TRPM8 ion channels.

## GPCR

- Almost all GPCRs are ligand activated.
- Activity of muscarinic receptor is modulated by voltage.
- Light activated receptor –human rhodopsins

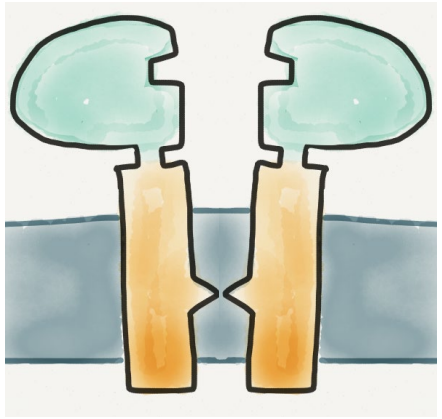
# Physical stimuli sensing channels lack conserved structural motifs



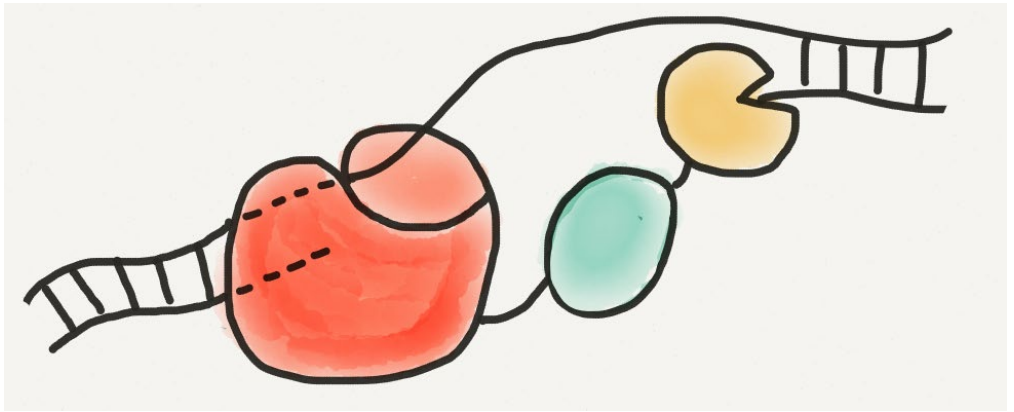

Julius and Patapoutian,
2021 Nobel Prize in Physiology

*Goldschen-Ohm and Chanda (2017) Cell*

# Proteins are modular in structure and function but..



Ligand activated ion channel



DNA polymerase Type I

Discrete domains encode specialized functions.

In almost all instances, they are involved in catalysis or binding which requires recognition of specific stereochemistry of a ligand or substrate.

Proteins responding to physical stimuli are not constrained by stereochemistry and a variety of structural arrangements may have evolved to sense these forces.

Understanding the mechanisms of sensing and gating in ion channels is a major driving question in Biophysics.

# Basic Principles of Sensing and Gating

# Consider a two state model for Ligand gating

$$C + L \underset{}{\overset{K_A}{\rightleftharpoons}} O$$

$$K_A = \frac{[O]}{[C][L]}$$

$$K_A[L] = \frac{[O]}{[C]}$$

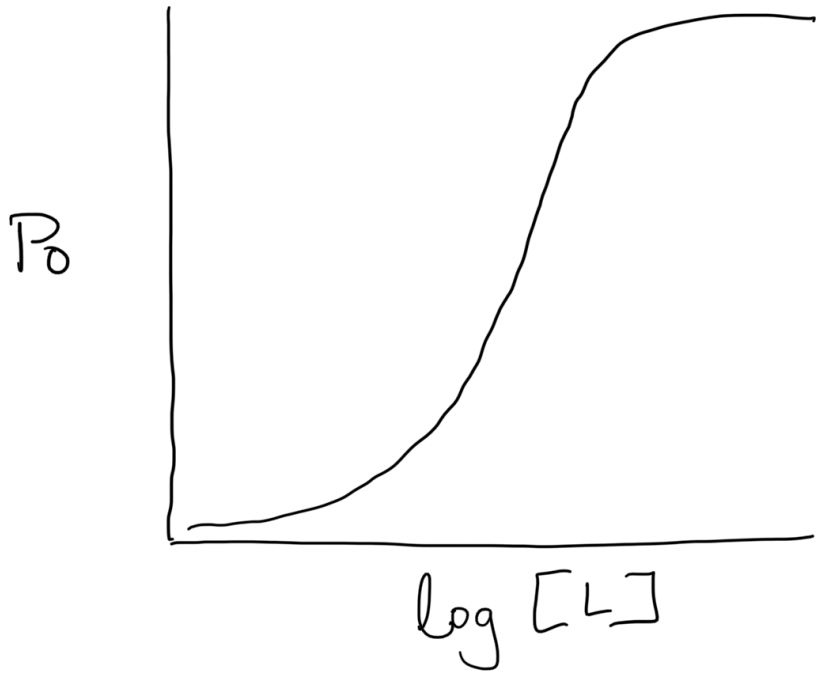
$$P_O = \frac{O}{O + C} = \frac{K_A[L]}{1 + K_A[L]}$$

$$= \frac{[L]}{[L] + \frac{1}{K_A}} = \frac{[L]}{[L] + K_D}$$

$$\Delta G = RT \ln K_A$$

$$-\Delta G = RT \ln K_D$$

$$P_O = \frac{[L]}{[L] + e^{\frac{-\Delta G}{RT}}}$$



# Consider a two-state model for Ligand gating

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$$-\Delta G = RT \ln K_D$$

$$P_O = \frac{[L]}{[L] + e^{\frac{-\Delta G}{RT}}}$$

# Two state model for voltage-gating

$$C \underset{}{\overset{K_V}{\rightleftharpoons}} O$$

$$P_O = \frac{K_V}{1 + K_V} = \frac{1}{1 + \frac{1}{K_V}}$$

$$-\Delta G_V = RT \ln K_V$$

$$P_O = \frac{1}{1 + e^{\frac{\Delta G}{RT}}}$$

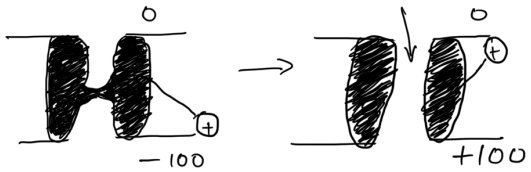
$$\Delta G_V = \Delta G_C - qFV$$

$\Delta G_C$ = Intrinsic chemical bias in absence of voltage

$qFV$ = electrical work done by applied membrane voltage.

$q$ = net charge displaced

$F$ = Faraday constant




$q = 1.0$

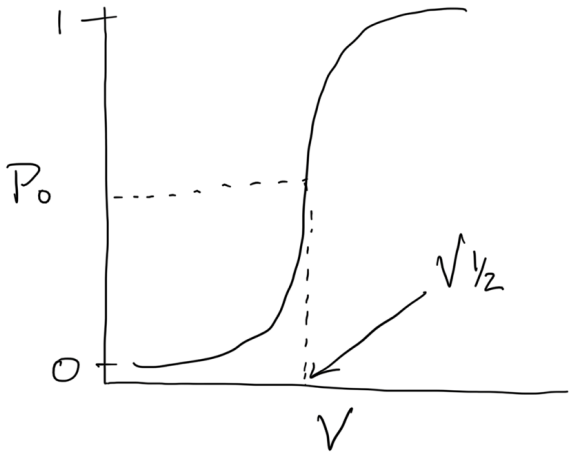
$$P_O = \frac{1}{1 + \exp\left(\frac{\Delta G_C - qFV}{RT}\right)}$$

when $P_O = 0.5$

$\Delta G_C = qFV \rightarrow V_{1/2}$

$$= \frac{1}{1 + \exp\frac{qF(V_{1/2} - V)}{RT}}$$

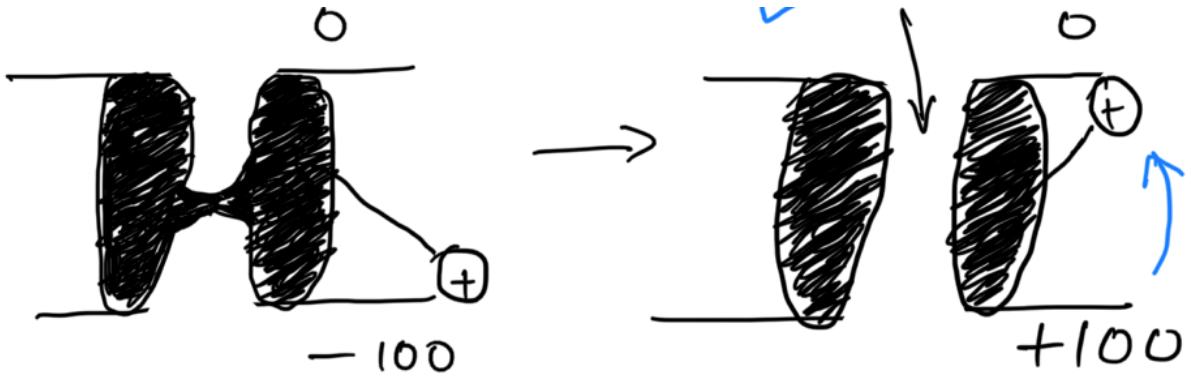
Two free parameter $q$ & $V_{1/2}$.

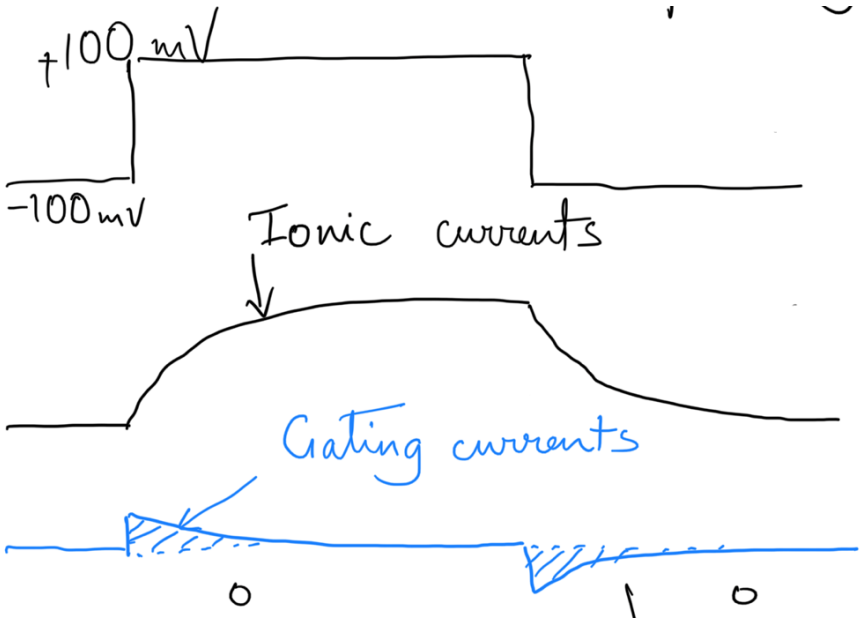



The chemical free-energy of gating of a voltage-dependent ion channel

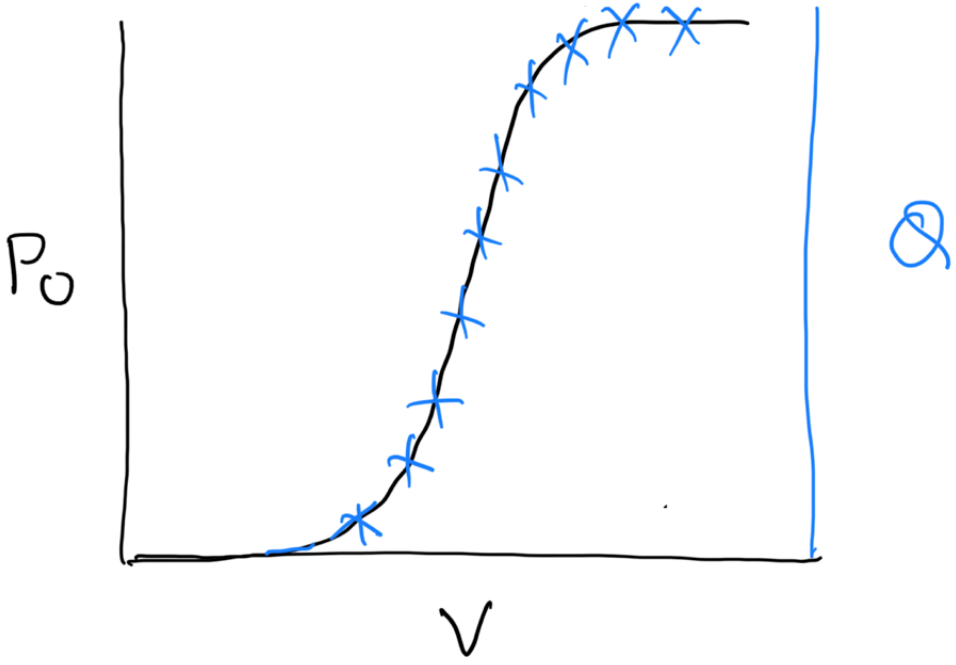
$$\Delta G_c^o = qFV_{1/2}$$

# Gating current



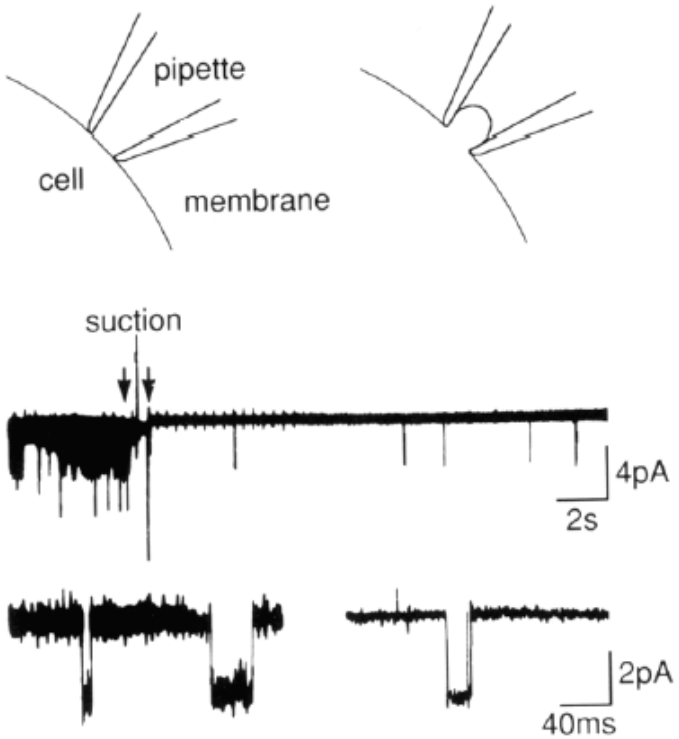



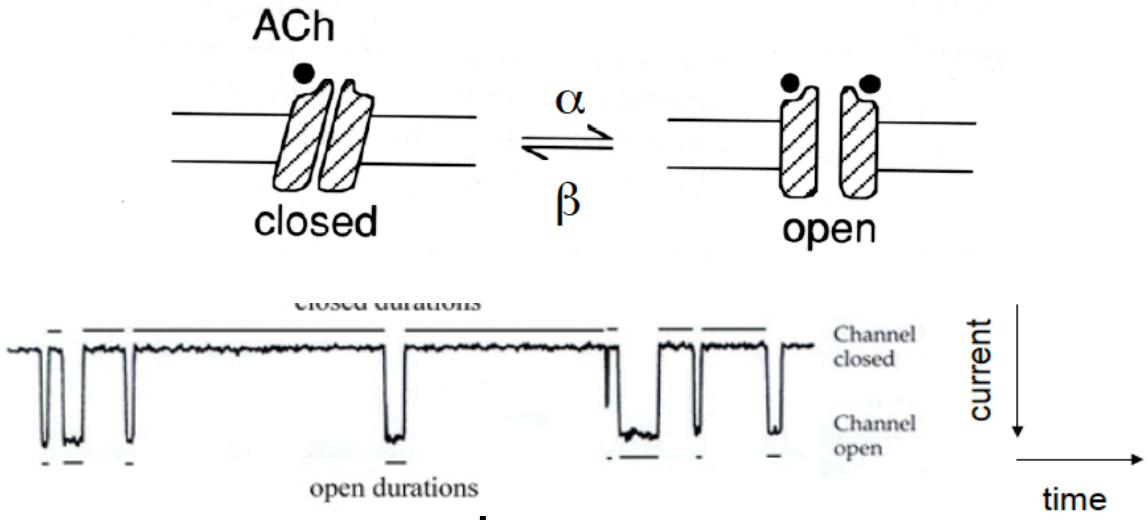

$$C \underset{}{\overset{K_r}{\rightleftharpoons}} O$$

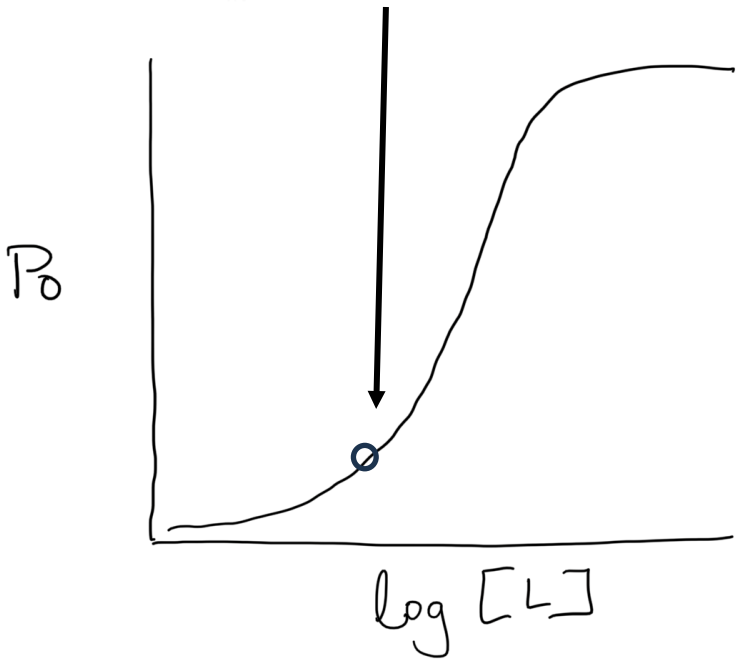


# Patch clamp method enable measurement of activity of single ion channels

Neher and Sakmann, 1983 Nobel Prize in Physiology



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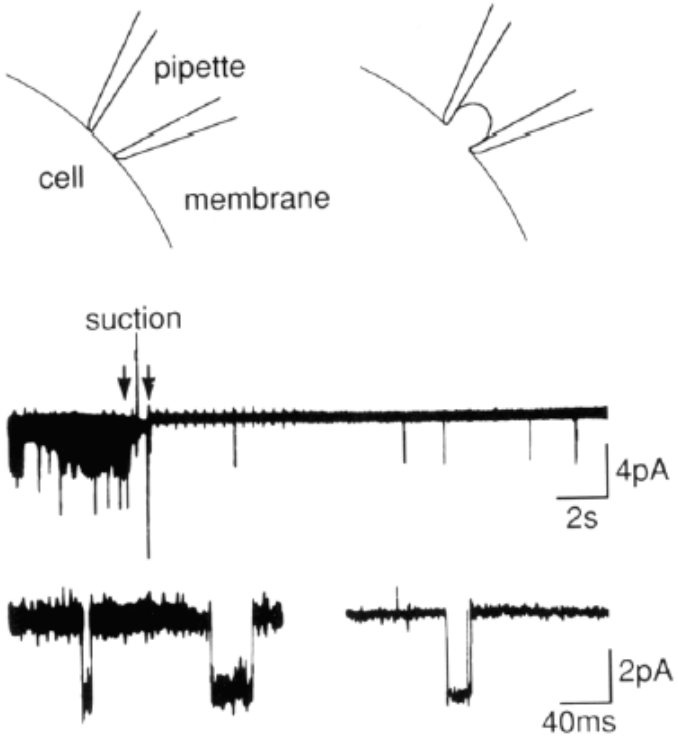
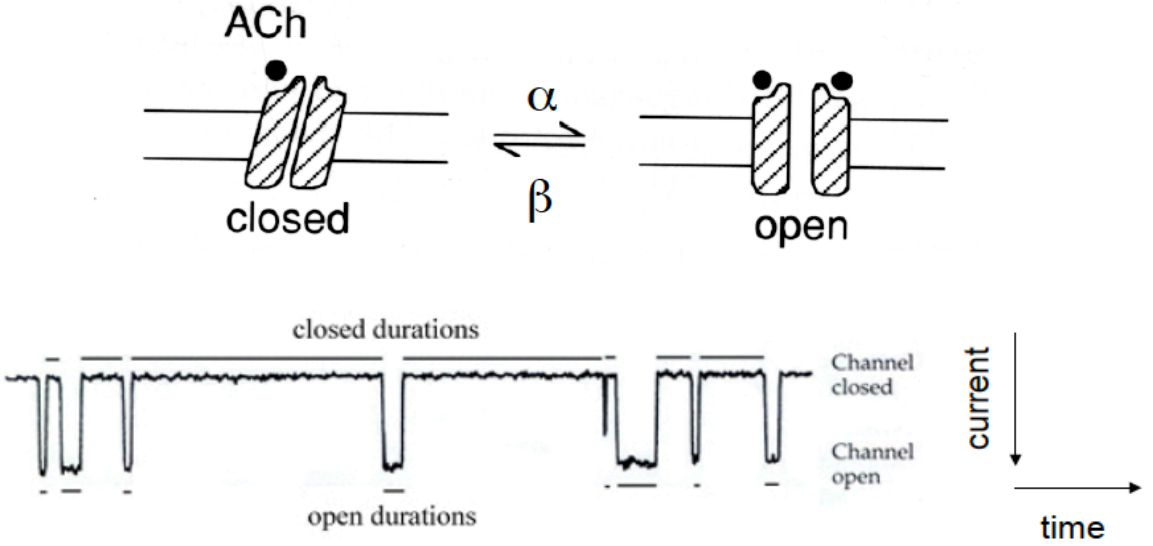
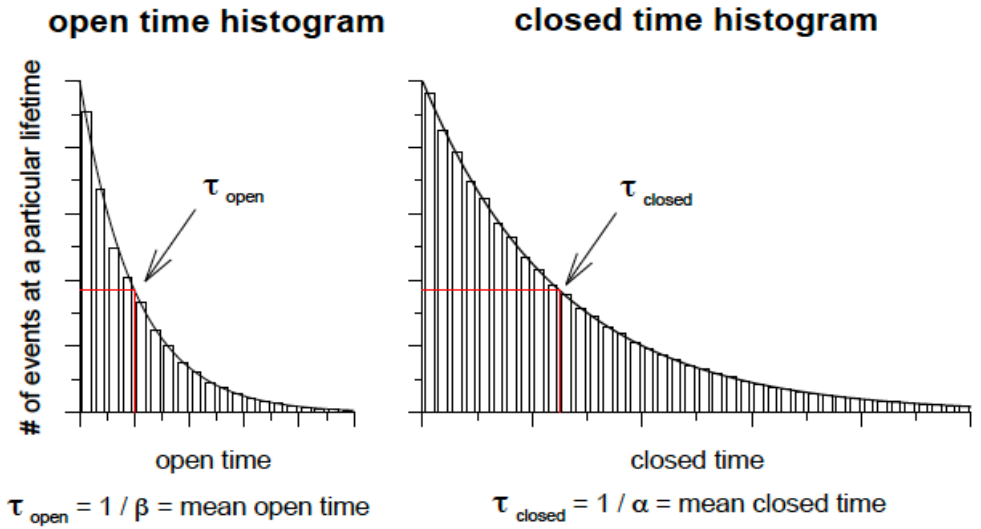
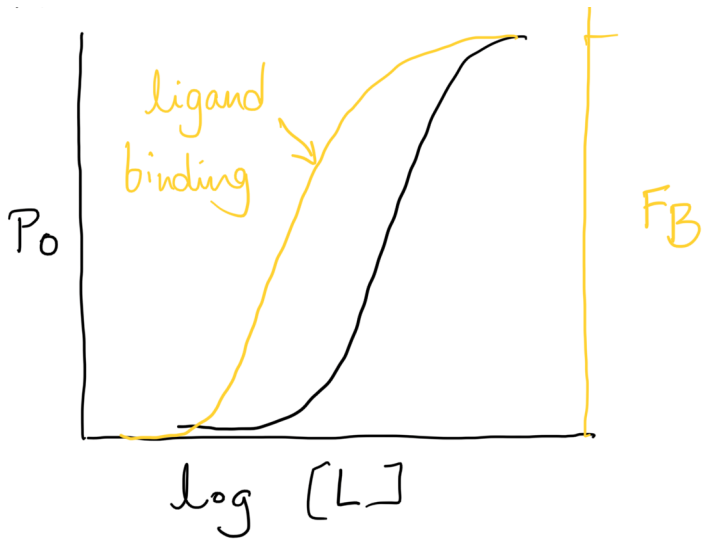
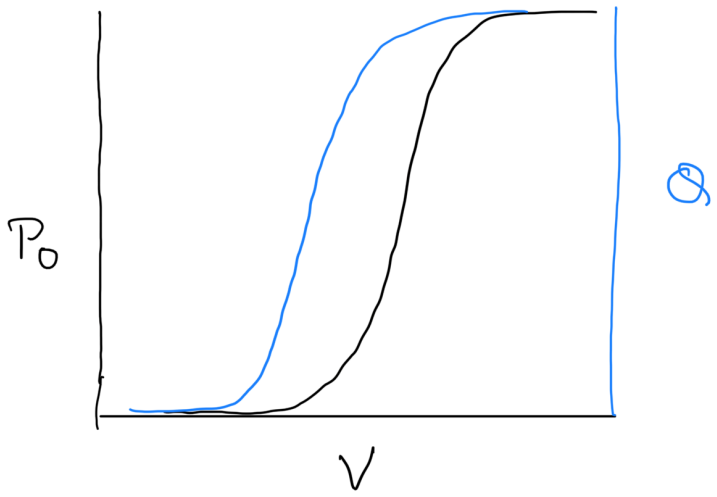


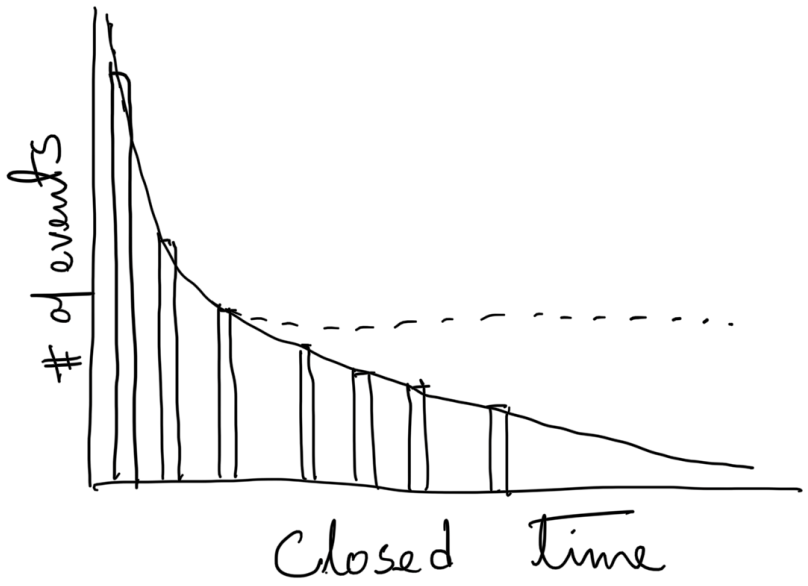



# But gating in most ion channels is more complicated…



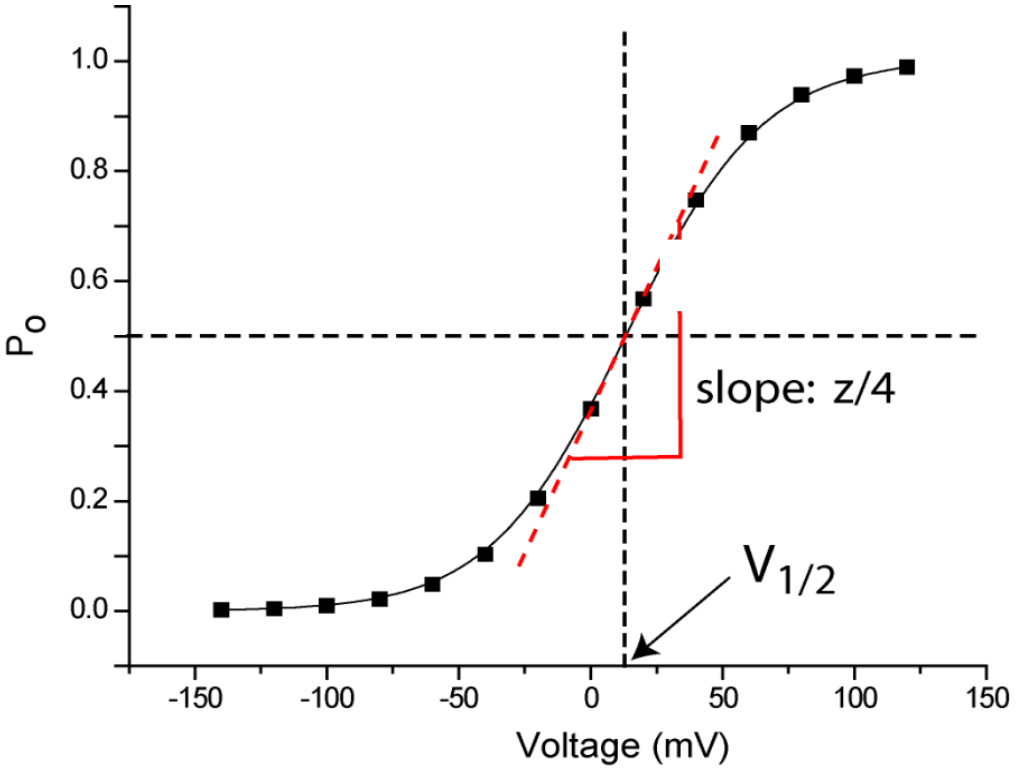

$$C + L \underset{}{\overset{K_A}{\rightleftharpoons}} CL \overset{K_P}{\rightleftharpoons} OL$$

$$C_1 \overset{K_V}{\rightleftharpoons} C_2 \overset{K_P}{\rightleftharpoons} O$$

# Free-energy of channel activation is model-dependent

## Method I

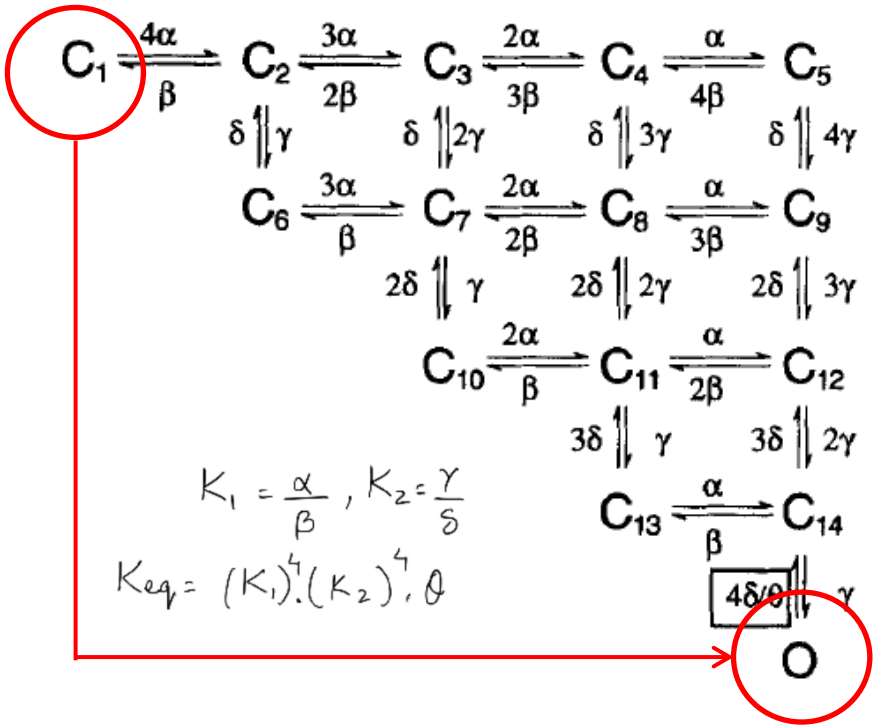
Treat this as a two-state model.
Fit to Po-V curve to Boltzmann function.




$\Delta G_{act}$ = -2.8 kcal/mol

## Method II

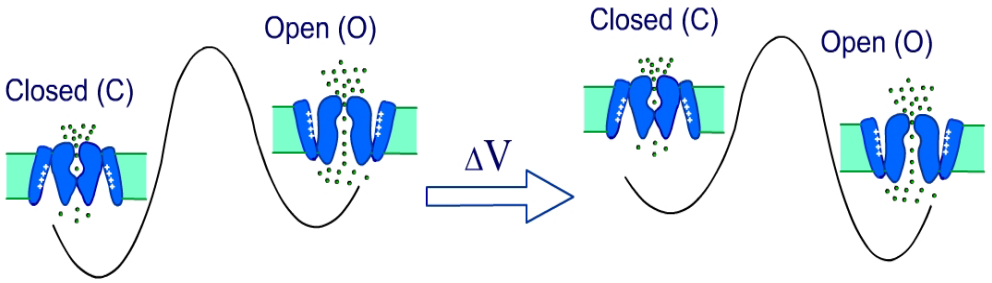
Kinetic description of Shaker $K^+$ channel based on single channel recordings and gating current measurements




$\Delta G_{act}$ = -15.4 kcal/mol

Two state assumption underestimates the free-energy of channel activation.

# Free-energy of channel activation




For any reversible process:

$\Delta G$ = Work done! = '**Force**' **X** 'Conjugate **displacement**'

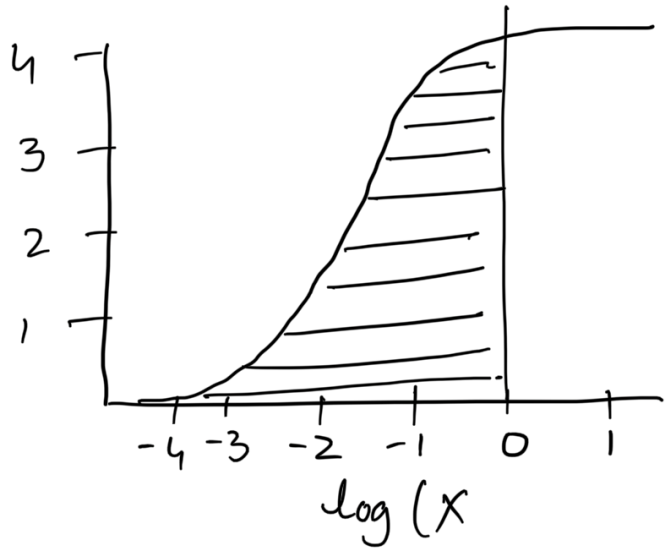
| Type of work | Force | Conjugate |
|---|---|---|
| Mechanical | Force ($\gamma$, P) | Displacement |
| Electrical | Voltage | Gating-charge |
| Chemical | Ligand conc. | Ligand occupancy |
| Thermal | Temperature | Entropy |

$$\delta G_x = (RT \ln x)(\delta n_x)$$

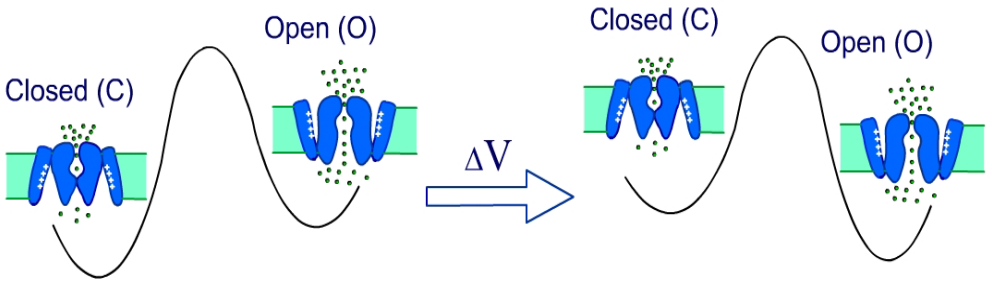
Force — displacement

In thermodynamic terms, these are called conjugate variables.

$$\Delta G_x = RT \int_0^{\bar{x}} \ln x \, \delta \bar{x}$$



# Free-energy of channel activation

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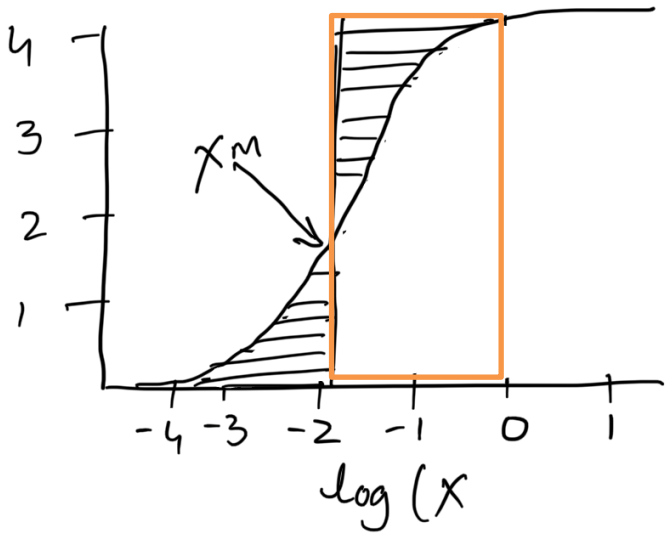


$$\Delta G_x = RT N_{max} \ln x_m$$

$$\Delta G_v = \theta_{max} F V_m$$

Where $X_m$ and $V_m$ is the median ligand activity and median voltage activity respectively. Nmax and Qmax correspond to maximum number of ligand bound and max charge.

# Free-energy of channel activation

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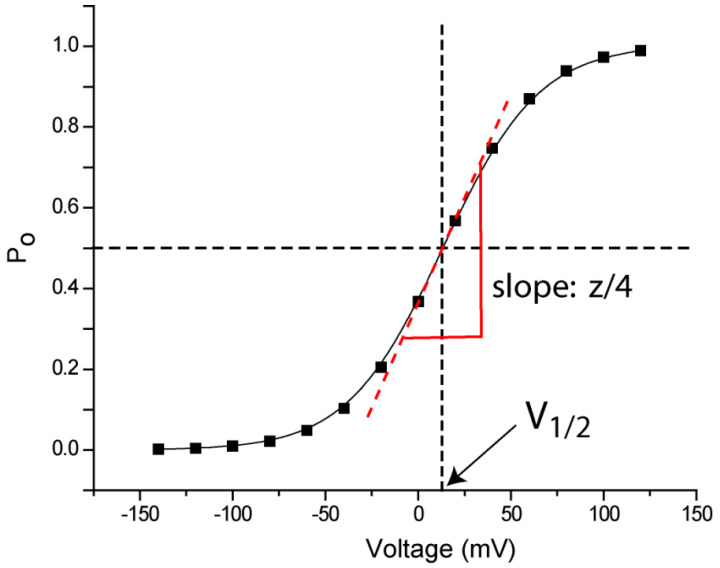
Treat this as a two-state model.
Fit to Po-V curve to Boltzmann function.

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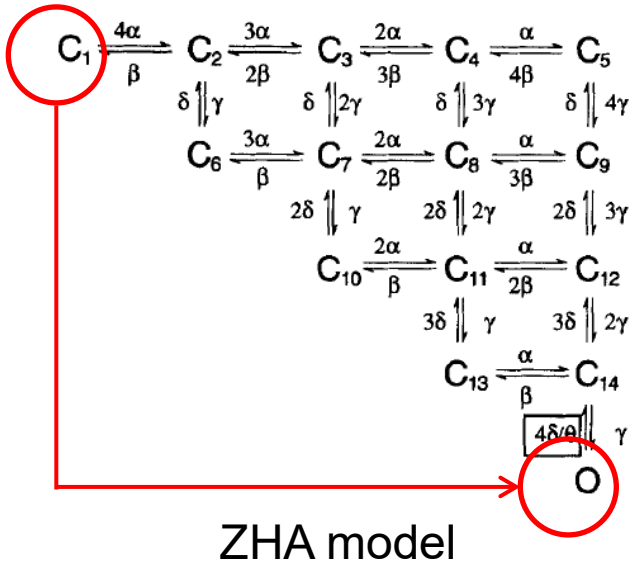


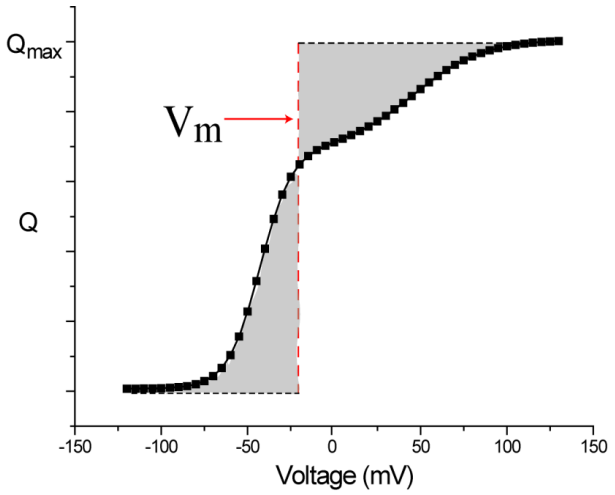

ZHA model

$\Delta G_{act}$ = -15.4 kcal/mol

*Zagotta et. al. (1994) J. Gen Physiol.*

## Method III

Estimate free-energy of activation from Force-displacement curves. For voltage-dependent system, Q-V curves

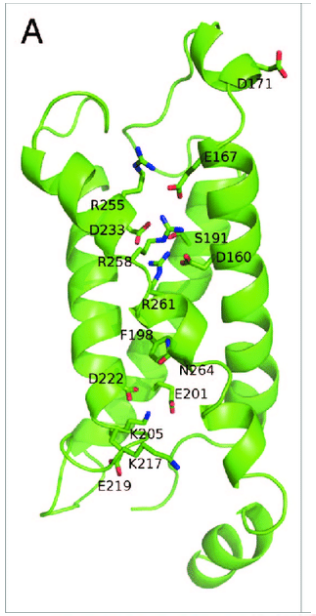



$\Delta G_{act}$ = -14.7 kcal/mol

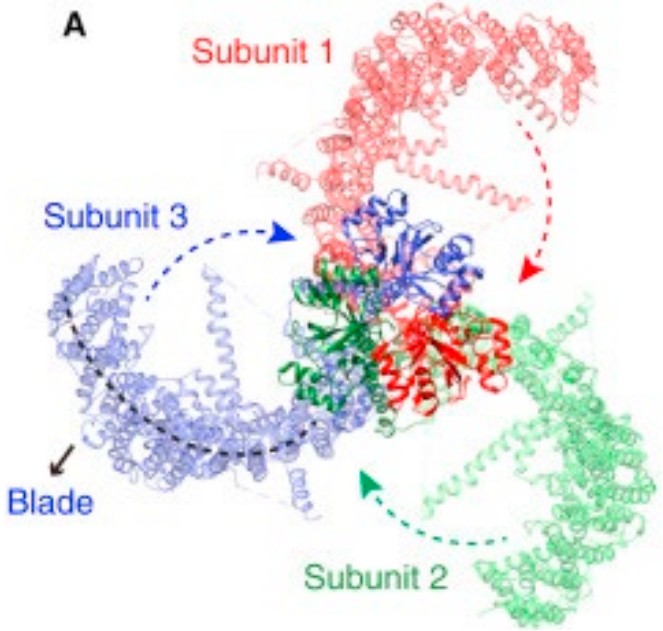
*Chowdhury and Chanda (2012) J. Gen Physiol.*

# Probing the mechanisms of ion channel gating

# Structural analysis reveal tremendous diversity of ion channels



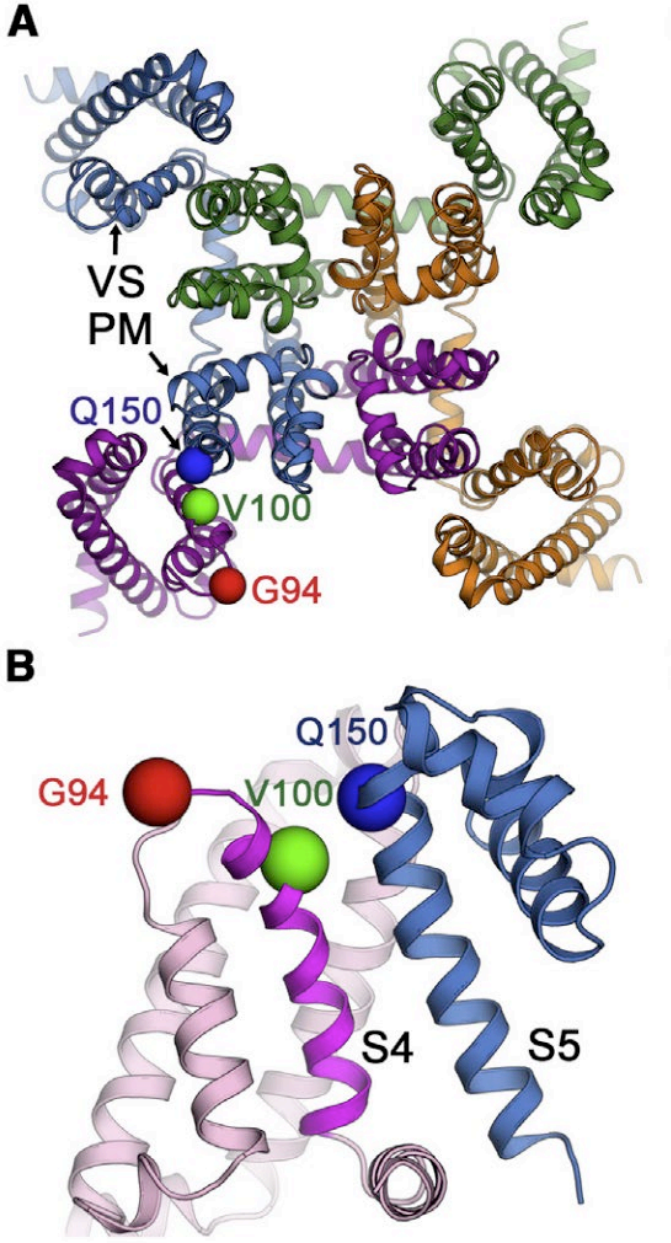

Voltage-gated proton channel, HV1




PIEZO ion channel

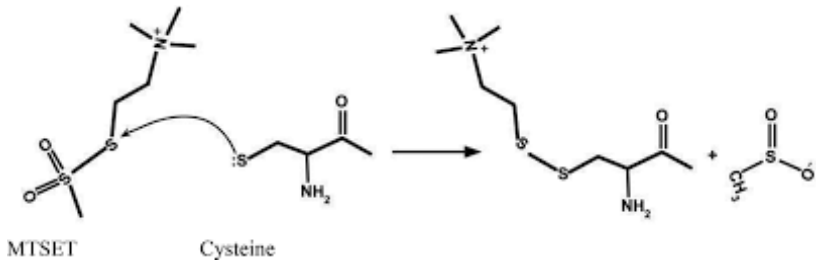
| Oligomerization states | Ion Channels |
|---|---|
| Monomer | Hv1, Holorhodopsins |
| Dimer | Chloride channels |
| Trimer | P2X receptor, Piezo channel |
| Tetramer | Voltage-gated ion channels, TRP channels, NMDA receptor |
| Pentamer | Cys loop receptor family, GABA, AChR, Serotonin receptor |
| Hexamer | LRRC1 (SWELL) ion channels |
| Heptamer | Pannexin ion channels |
| Octamer, Decamer, Undecamer | CALHM1, CALHM6, CALHM4 |

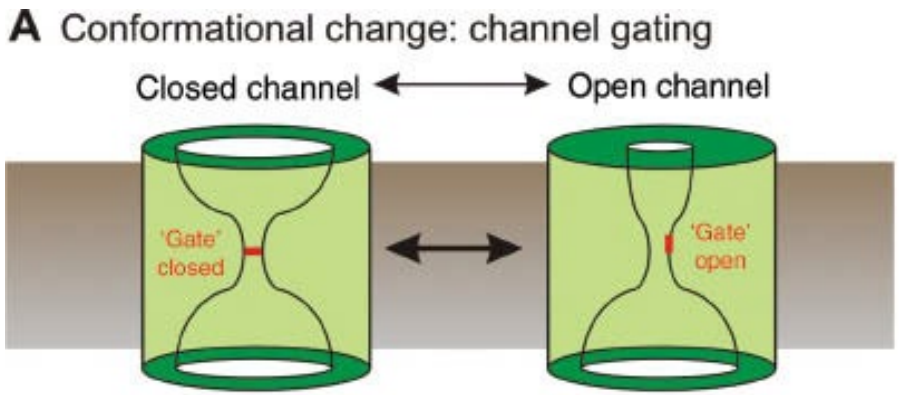
# Structures in various conformational states

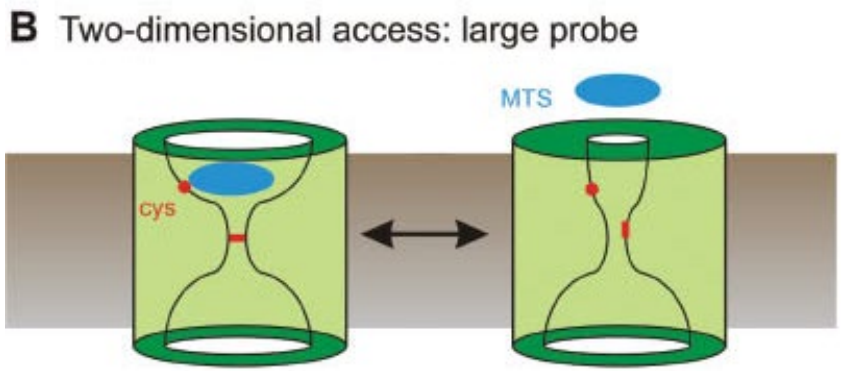


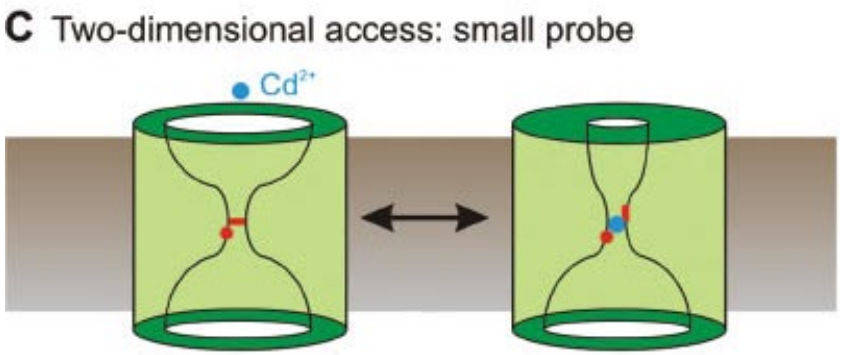

Structures are extremely useful for testing detailed mechanistic models. (Needed validation from experiments in physiological conditions)

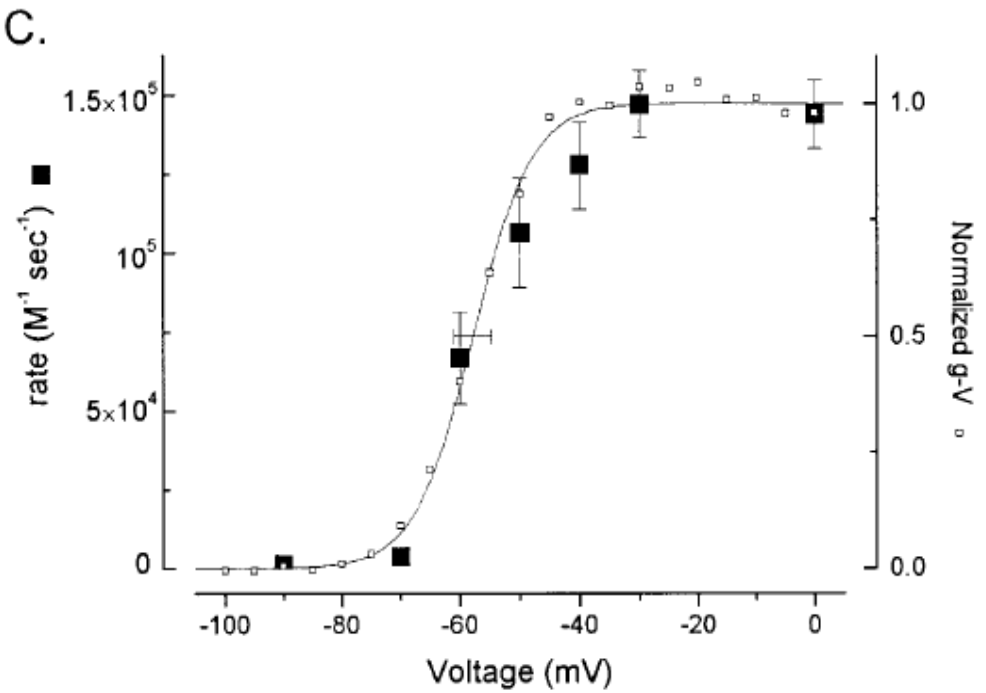
# Biochemical methods to probe structural changes



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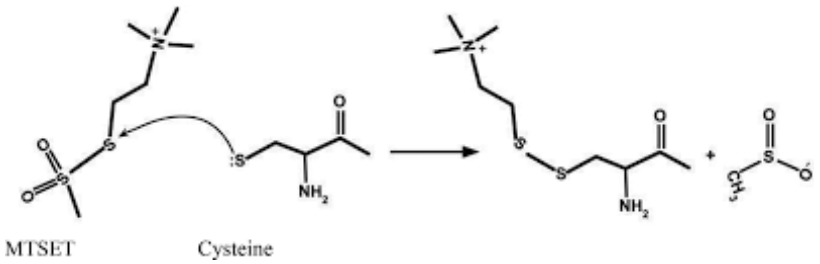
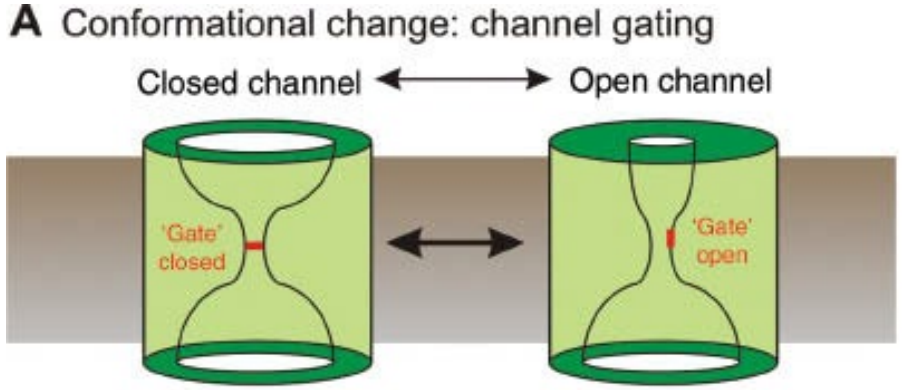
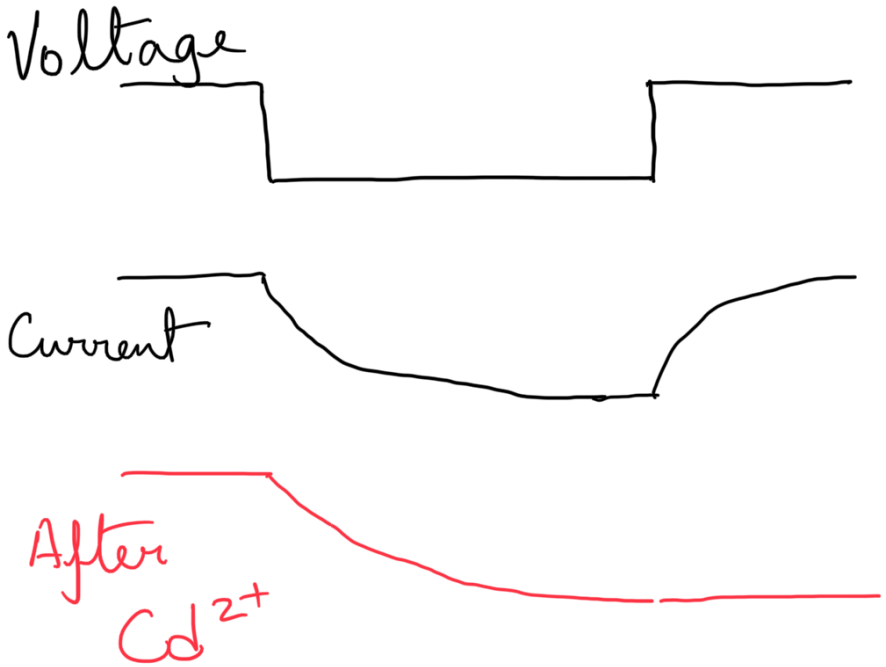
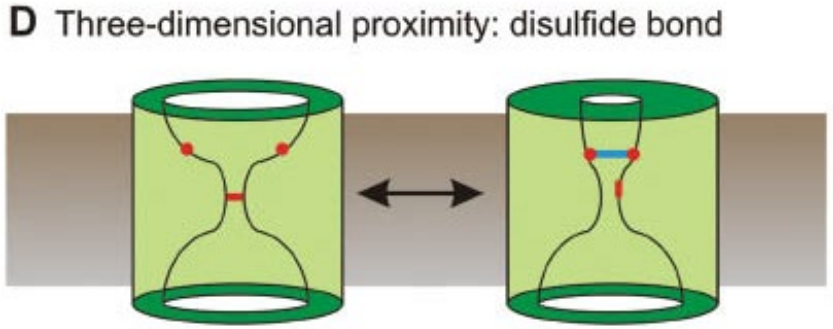
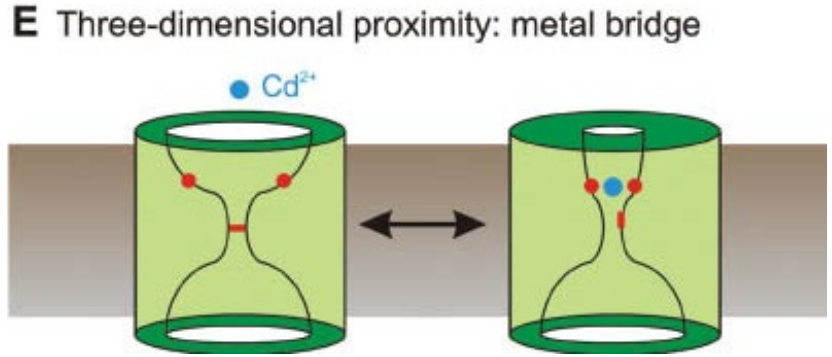


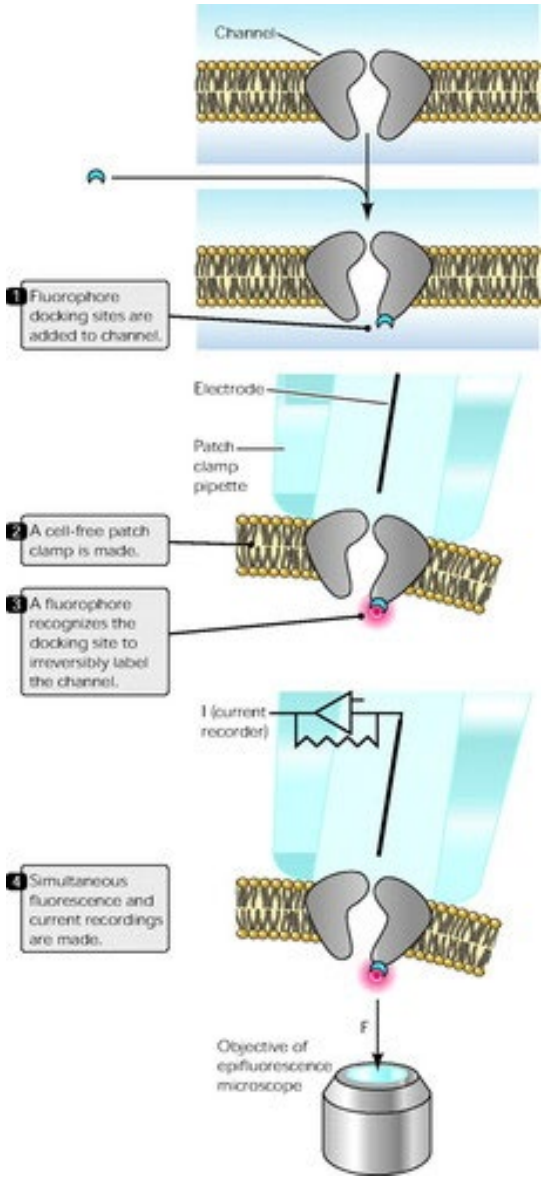



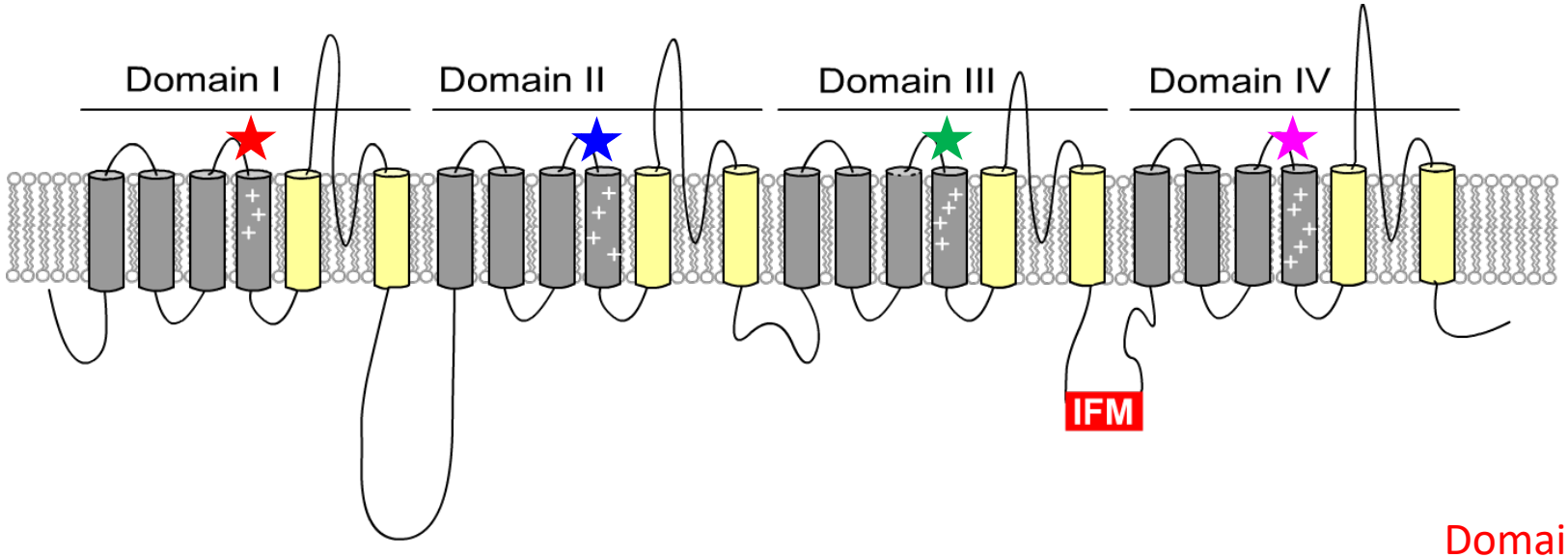



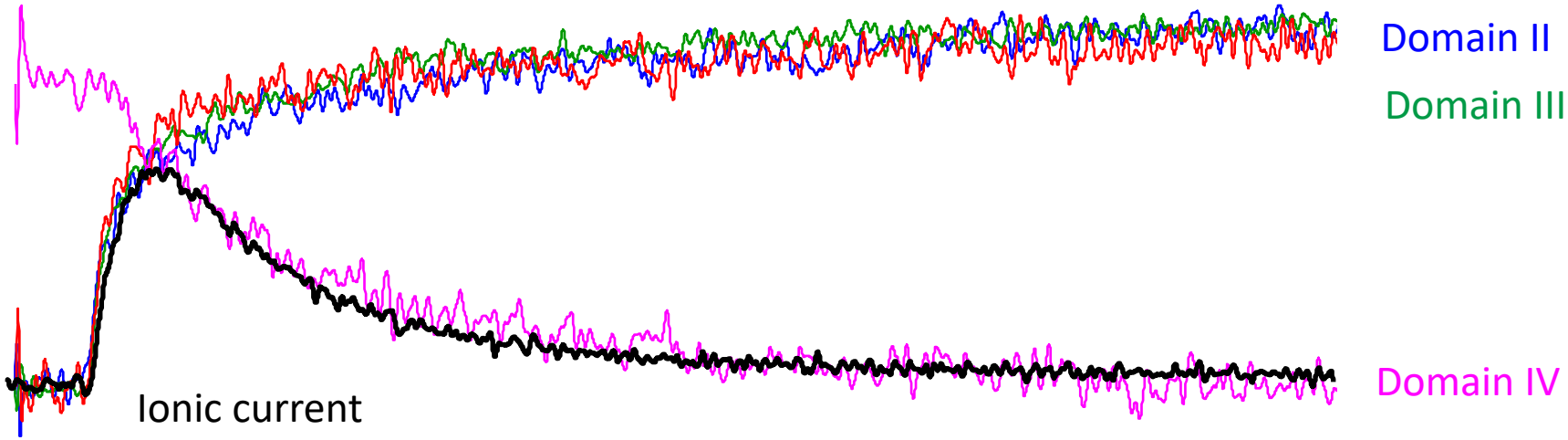



# Patch Clamp Fluorometry



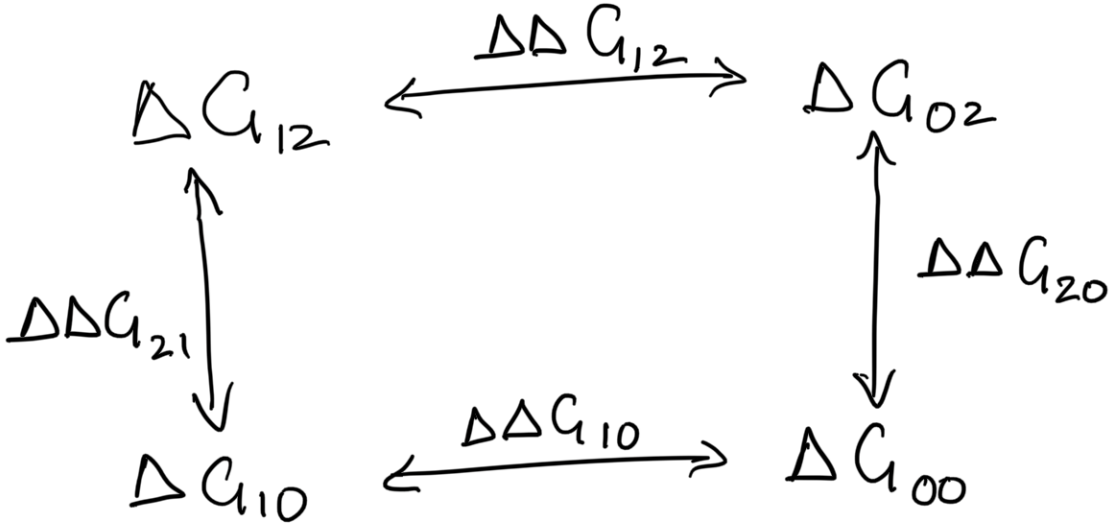




Chanda and Bezanilla (2002) J Gen. Physiol.

Goldschen et. al. (2013) Nature Comms

# Mapping interaction pairs using mutant cycle analysis




If sites 1 & 2 are independent

$$\Delta\Delta G_{12} = \Delta\Delta G_{10} \;\&$$

$$\Delta\Delta G_{21} = \Delta\Delta G_{20}$$

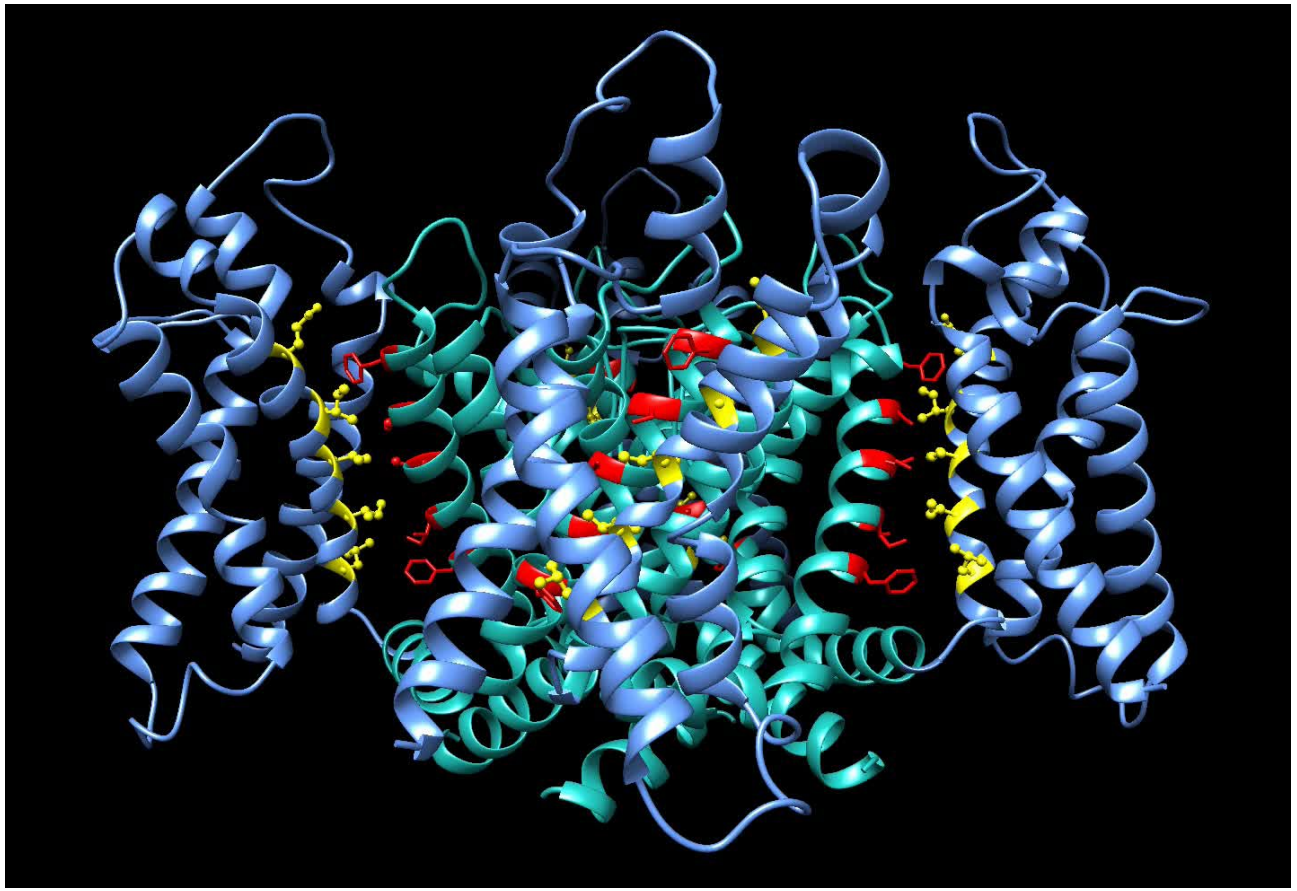
If sites 1 & 2 interact, then

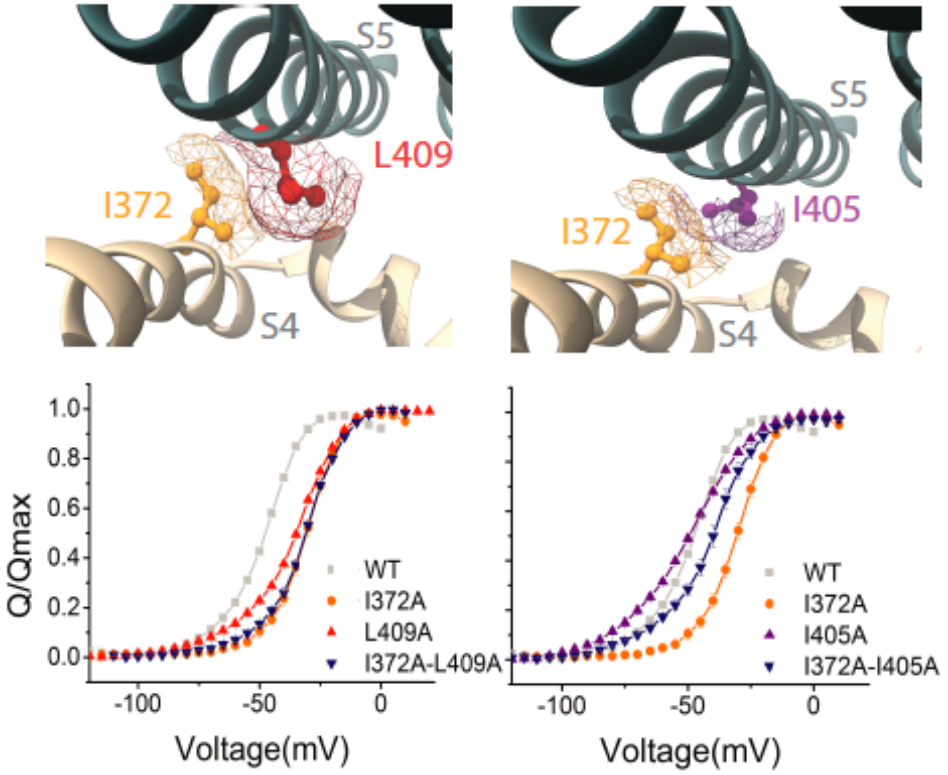
$$\Delta\Delta G_{12} - \Delta\Delta G_{10} = \Delta G_{12}^{INT}$$

$$\Delta\Delta G_{21} - \Delta\Delta G_{20} = \Delta G_{12}^{INT}$$

Free-energy calculations should either use median method or take into account detailed kinetic model. Two state approximation is not meaningful.

# Identifying a non-canonical coupling pathway using interaction analysis



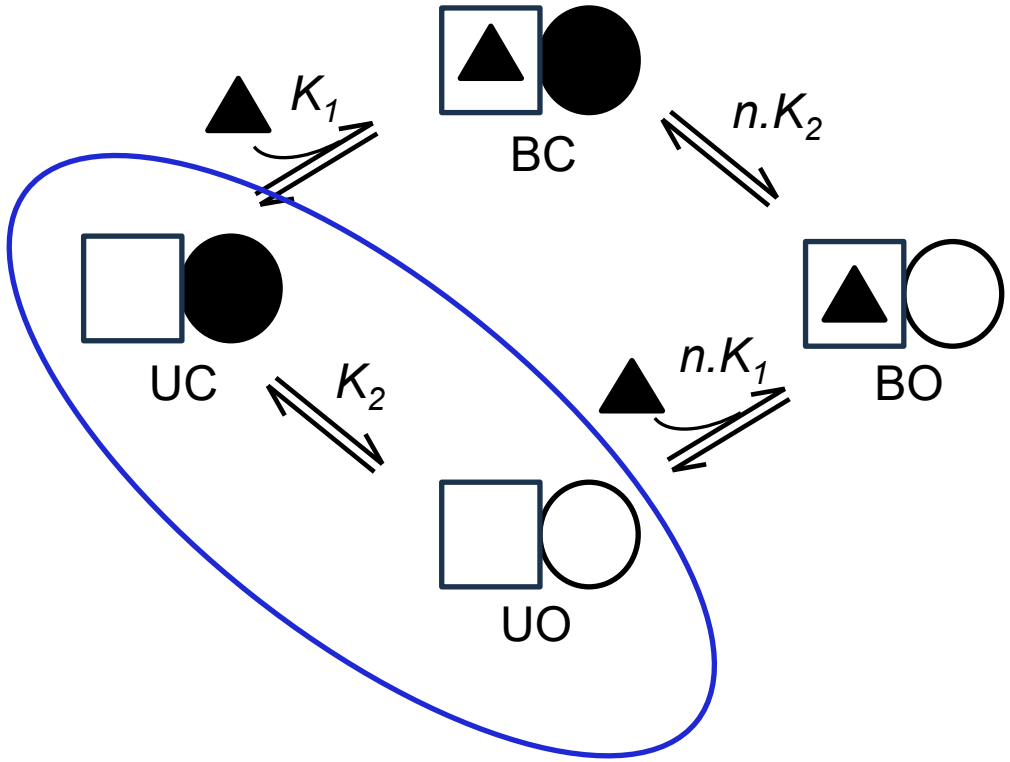



$\Delta\Delta G_{I372\text{-}F409}$= -4.15 kcal/mol  $\Delta\Delta G_{I372\text{-}I405}$= -2.45 kcal/mol

Marino- Fernandez et. al. NSMB (2018)

How do we identify key residues involved in coupling sensor movement to pore gate conformation?

# Analysis of simple allosteric model




$$\frac{P_o}{P_c} = \frac{[BO] + [UO]}{[UC] + [BC]}$$

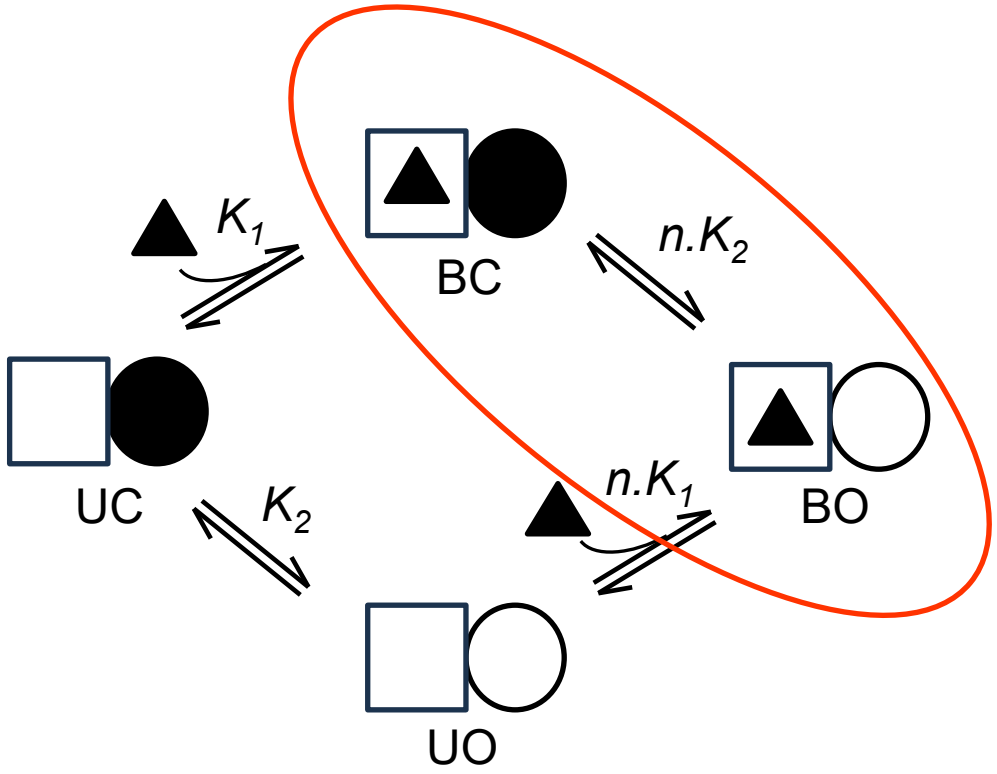
$$\frac{P_o}{P_c} = \frac{nK_2K_1[L] + K_2}{K_1[L] + 1} = \frac{K_2(nK_1[L] + 1)}{(K_1[L] + 1)}$$

At zero ligand concentration

$$\frac{P_o}{P_c} = \frac{K_2(0 + 1)}{(0 + 1)}$$

$$\frac{P_o}{P_c} = K_2 = K_{eq}^0$$

# Analysis of simple allosteric model




$$\frac{P_o}{P_c} = \frac{[BO] + [UO]}{[UC] + [BC]}$$

$$\frac{P_o}{P_c} = \frac{nK_2K_1[L] + K_2}{K_1[L] + 1} = \frac{K_2(nK_1[L] + 1)}{(K_1[L] + 1)}$$

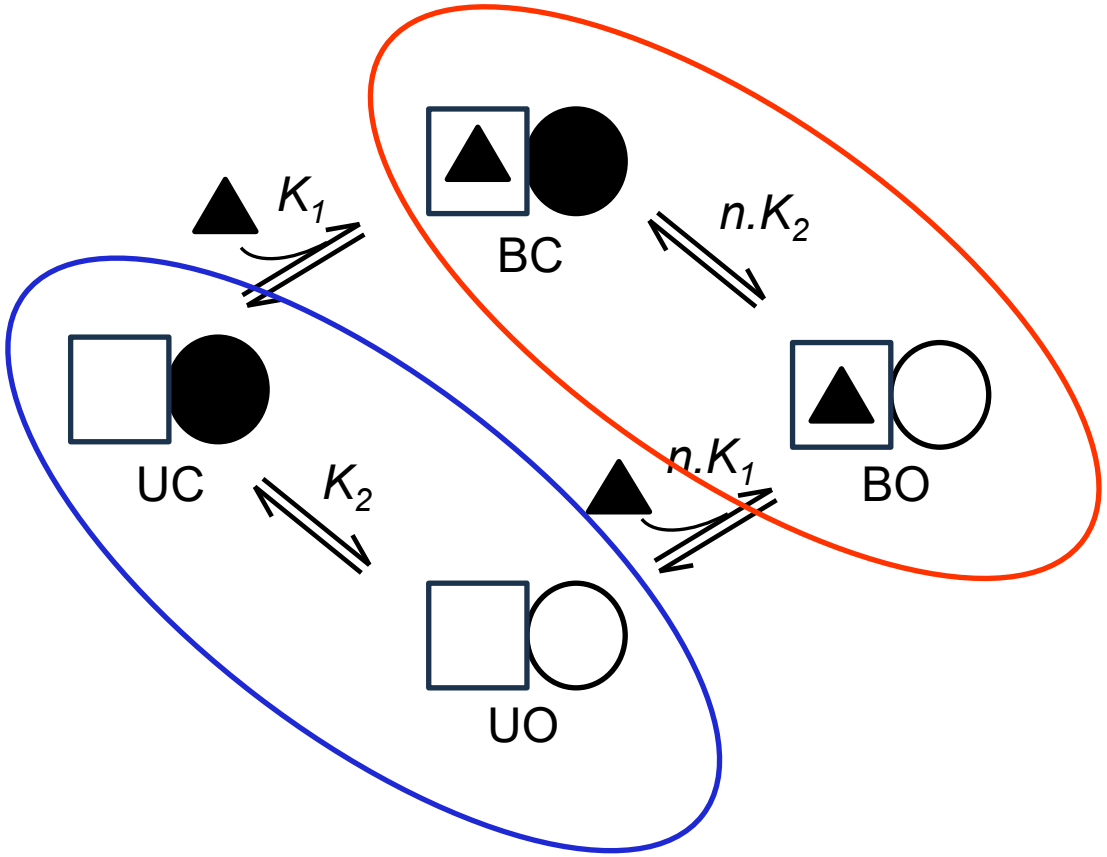
At saturating ligand concentration

$$nK_1[L] + 1 \approx nK_1[L]$$

and $K_1[L] + 1 \approx K_1[L]$

$$\frac{P_o}{P_c} = nK_2 = K_{eq}^{\infty}$$

# Analysis of simple allosteric model




$$\frac{P_o}{P_c} = \frac{[BO] + [UO]}{[UC] + [BC]}$$

$$\frac{P_o}{P_c} = \frac{nK_2K_1[L] + K_2}{K_1[L] + 1} = \frac{K_2(nK_1[L] + 1)}{(K_1[L] + 1)}$$

$$\frac{K_{eq}^{\infty}}{K_{eq}^{0}} = \frac{nK_2}{K_2} = n$$

$$Allosteric\ coupling, n = \frac{Channel\ opening\ equil.\ with\ saturating\ stimulus}{Channel\ opening\ equil.\ without\ stimulus}$$

This type of allosteric analysis is limited to certain models and there are other manifestations of allosteric interactions…

# Future Outlook

Ion channels are outstanding model systems for studying allosteric regulation in membrane proteins for many reasons:

1. Many of them are physiologically important signaling molecules. Regarded as one of most important family of signaling complexes for development of new drugs.
2. Tremendous diversity of structures and conformations. Cryo-EM resolution revolution started with the structure of an ion channel. High resolution structures in a variety of conformational states.
3. Ability to measure both activity (patch clamp recordings) and conformational changes (smFRET) at single molecule resolution. Especially important for identifying allosteric networks involved in transduction of conformational changes.
4. Understanding how physical and chemical properties of lipids influence structure, function and allostery.
5. Do same rules govern physical and chemical sensing? What are the key determinants of sensing and gating by physical stimuli?

# Thank You!