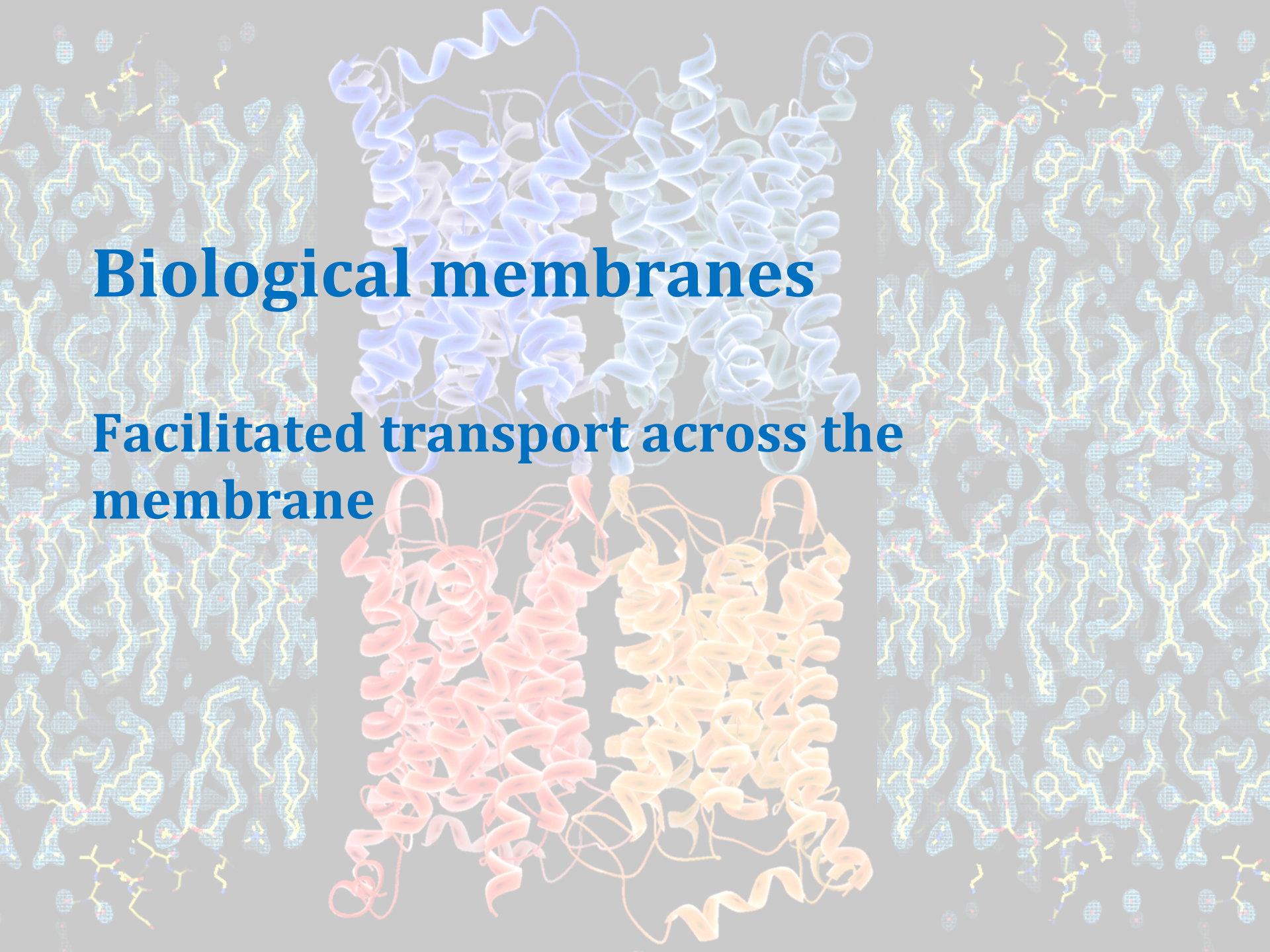


Biological membranes

Facilitated transport across the membrane

Molecule must shed their water of hydration before they can cross the membrane

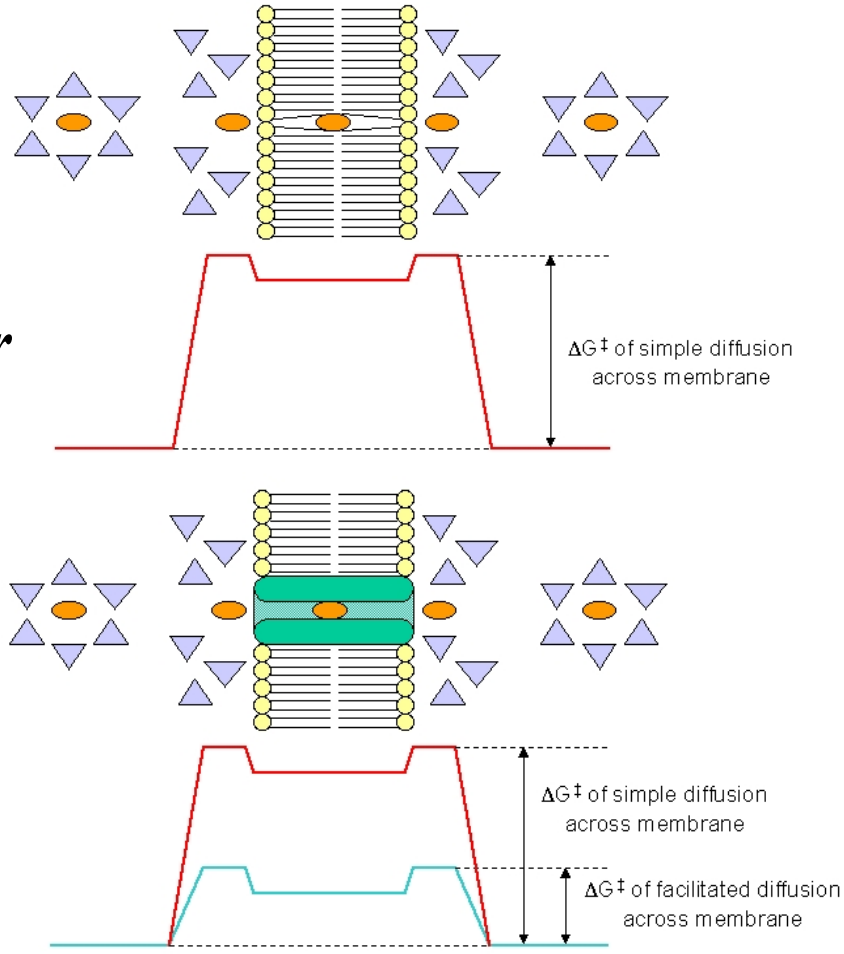
Amino acid residues of the transporter interact with "dehydrated" solute

⇓

Forming hydrophilic passageway or package through membrane

⇓

Reduce energy barrier



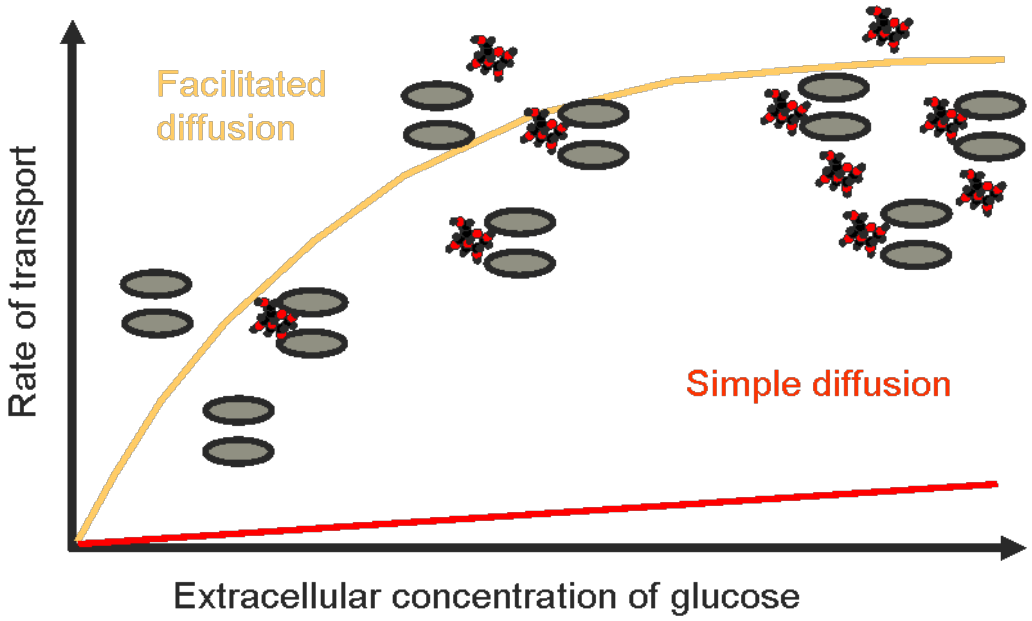
$$\Delta G = \sum_i n_i \Delta \mu_i = \sum_i n_i \left(\mu_i^{in} - \mu_i^{out} \right) = \sum_i n_i \left[RT \ln \left(\frac{C^{in}}{C^{out}} \right) + z_i F \psi_m \right]$$

Properties of facilitated transport

- ***Passive** – down concentration gradient - energy-independent.*

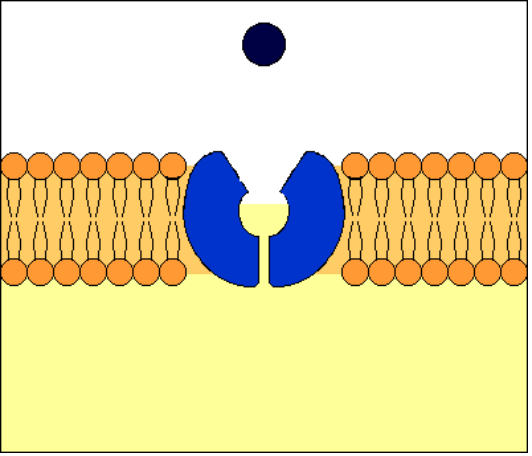
- ***Like enzymes** - bind and transport substrate molecules, ONE at a time.*

- *A rate of solute movement across the membrane is **saturable**.*



- ***Specific***
- ***Dependent on temperature***
- ***Can be inhibited***

- ***Fast – the flow may approach diffusion limit e.g. 10^7 ions/sec.***



Michaelis -Menten Kinetics Applies to Transport Activity

$$T_0 + S_0 \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} TS \xrightarrow{k_2} T_i + S_i$$

The Michaelis constant, K_M, is the concentration of substrate at which the velocity of transport is one-half the maxima.

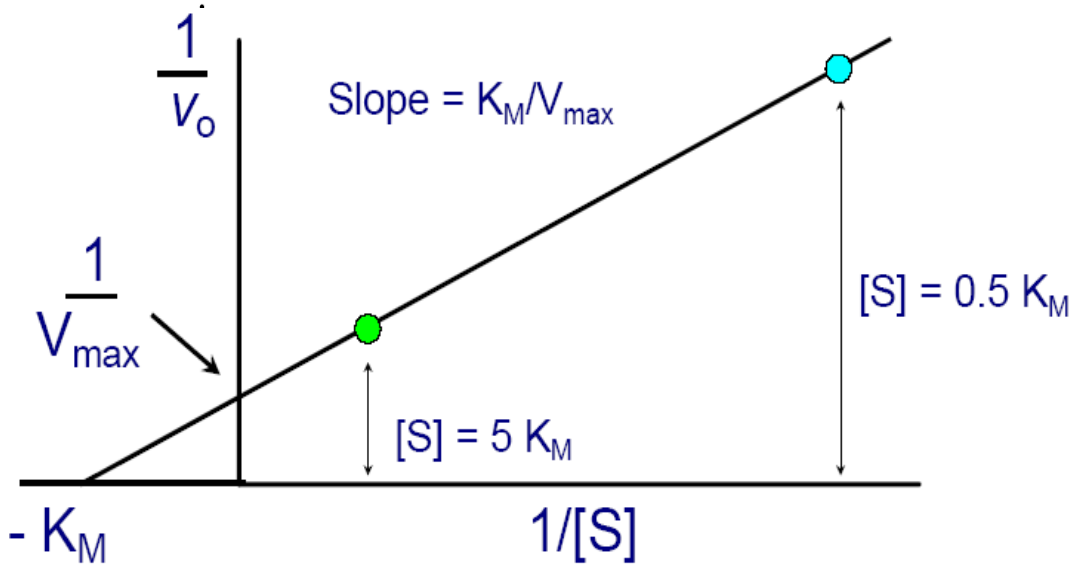
Michaelis-Menten equation

$$v_0 = \frac{v_{\max}[S]}{K_M + [S]}$$

Dissociation constant

$$K_D = \frac{k_{-1}}{k_1} = \frac{[T][S]}{[TS]}$$

Michaelis complex



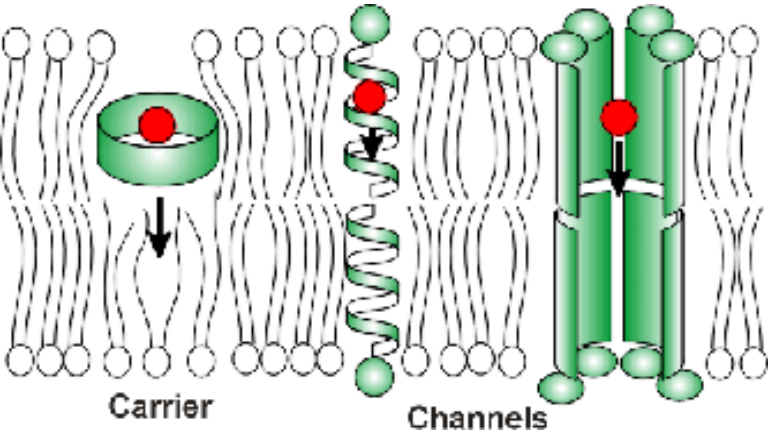
$$\frac{1}{v_0} = \frac{K_M}{V_{\max}} \times \frac{1}{[S]} + \frac{1}{V_{\max}}$$

Lineweaver - Burk plot

Ionophores

Small agents produced by microorganisms to kill other microorganisms

They are hydrophobic compounds which can complex an ion and carry it across a lipid bilayer.



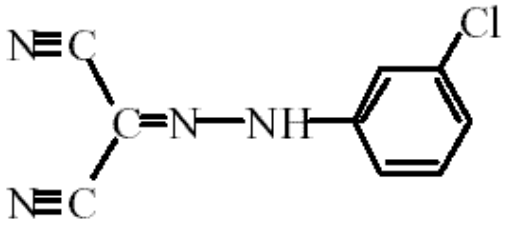
Two basic types: mobile carriers & pores

- ***Pores are not affected by temperature.***
- ***Carriers depend on the fluidity of the membrane, so transport rates are highly sensitive to temperature, especially near the phase transition of the membrane lipids***

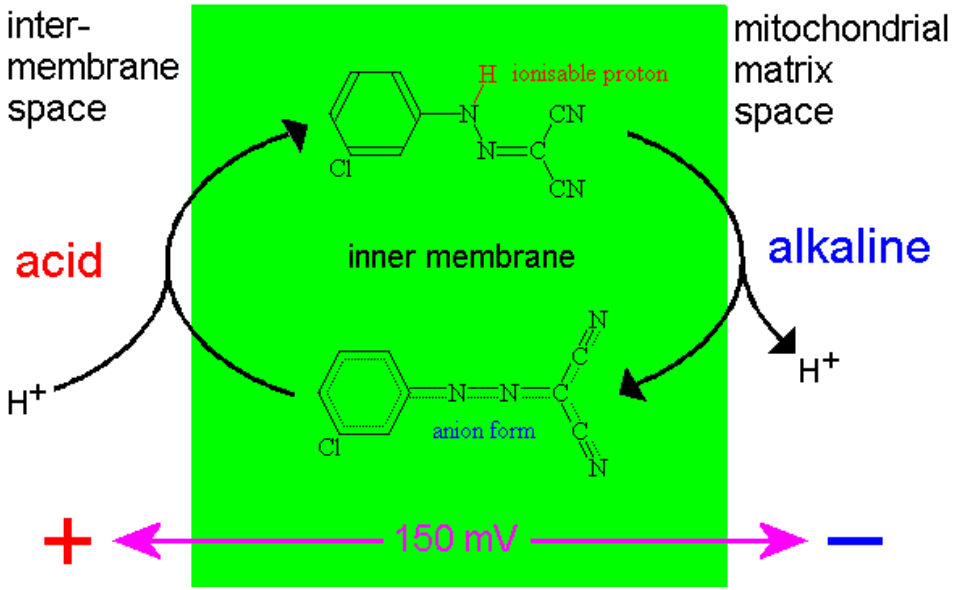
Classification of ionophores

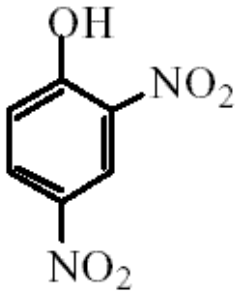
- ***neutral ionophores*** (e.g. Valinomycin)
- ***carboxylic ionophores*** (e.g. Nigericin)
- ***protonophores***

Protonophores

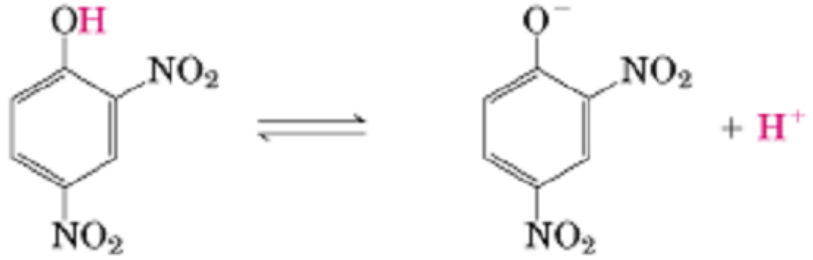


Carbonylcyanide m-chlorophenyl hydrazone (CCCP)





2,4-Dinitrophenol (DNP)

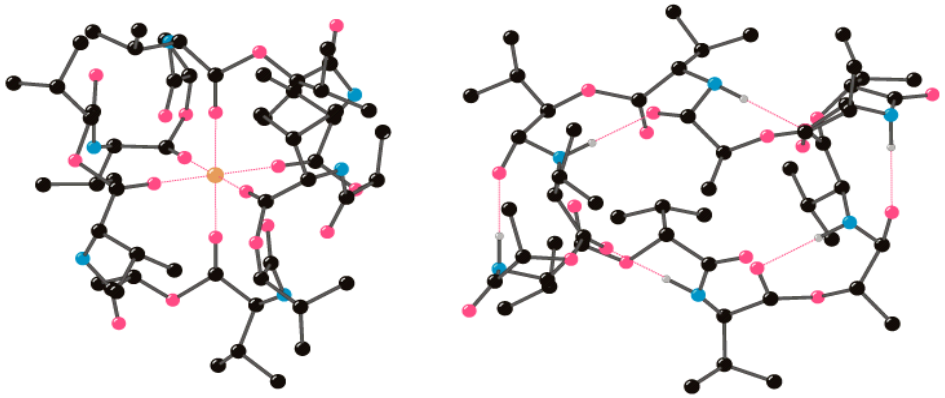


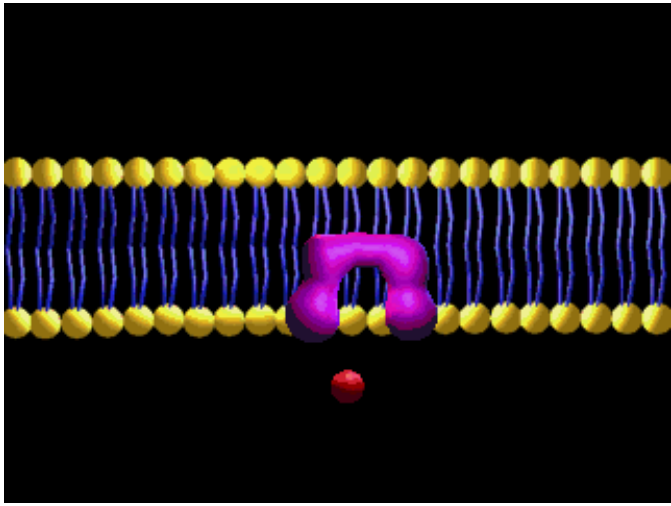
Both DNP and CCCP have a dissociable proton (weak acids) and are hydrophobic.

$$\psi_m = \frac{RT}{F} \ln\left(\frac{[H^+]_{out}}{[H^+]_{in}}\right)$$

At the equilibrium

Valinomycin – neutral ionophore



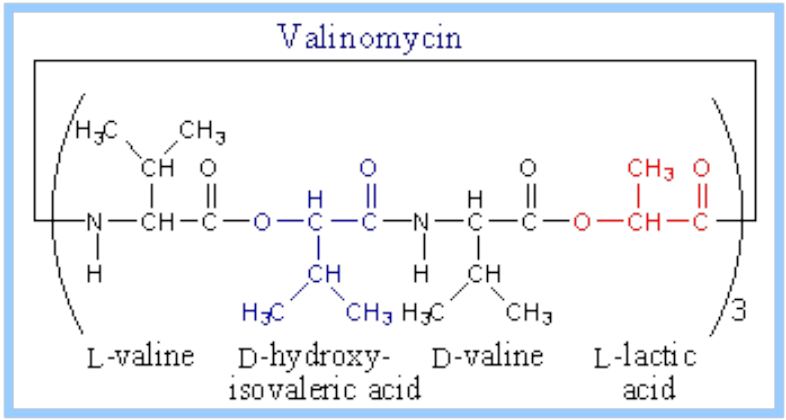


•A dodecadepsipeptide (has both peptide and ester bonds). It is a cyclic structure composed of 4-unit sequence repeated three times

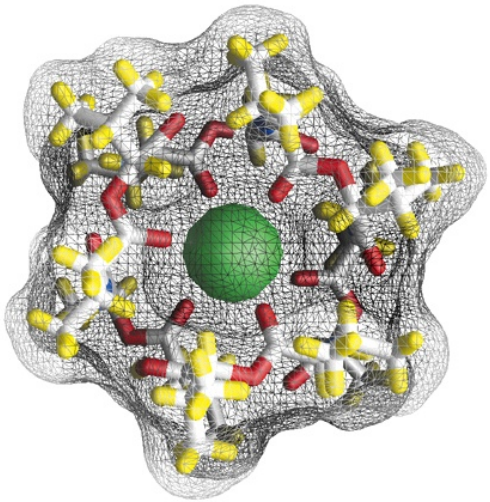
•A potassium ionophore – highly selective

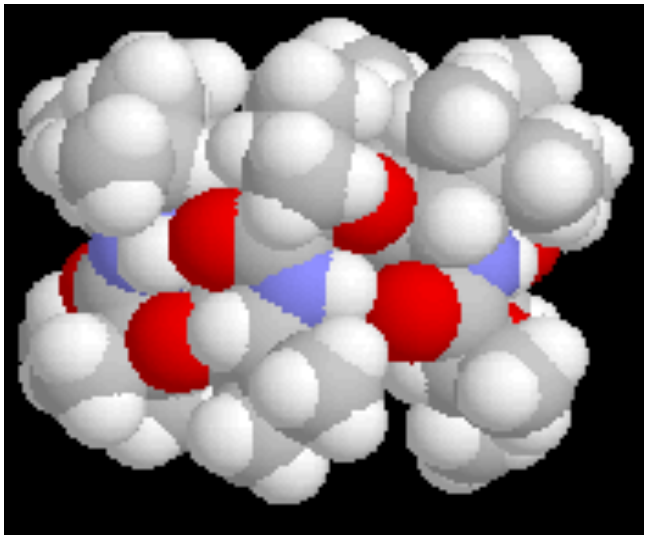
• Increases K-permeability up to 10,000 K-ions/sec

•Destroys K^+-gradient without affecting ΔpH



*The **valinomycin** surronds the potassium ion with a hydrophobic surface which allows the ion to cross the membrane.*

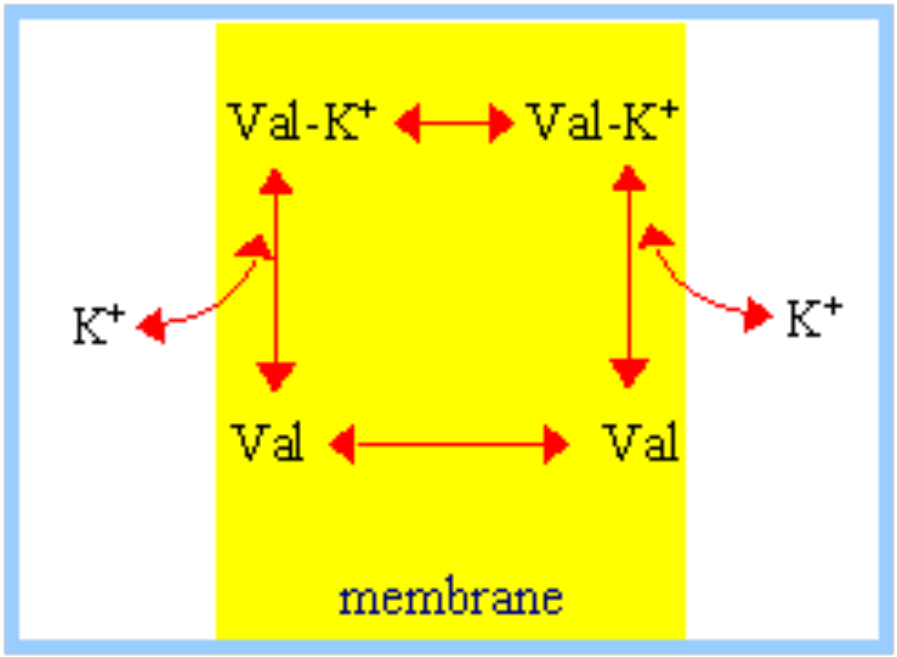




K^+ is 6-coordinated when in complex with Valinomycin.

- It crosses the membrane either with or without a bound ion.

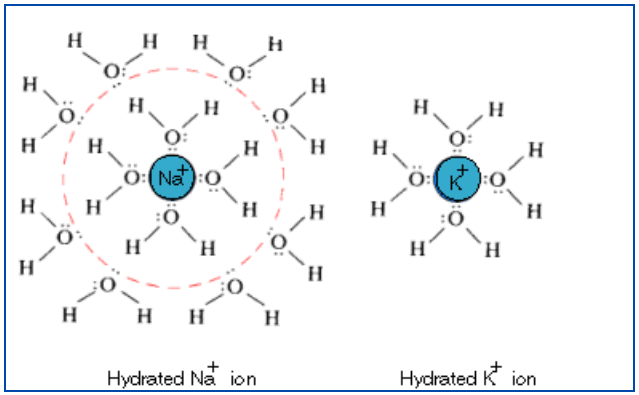
- It depends on the membrane potential.



The selectivity of valinomycin for K^+

Affinities for Na^+ and Li^+ are about a

10 000 - fold lower.



Factor 1: Ionic radius ($K^+ > Na^+ > Li^+$).

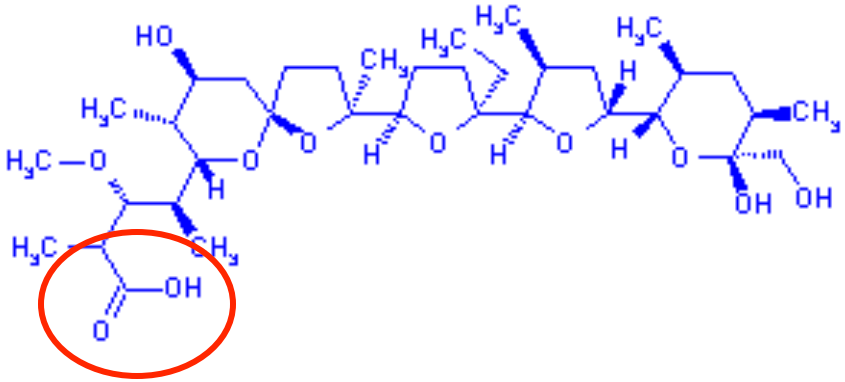
Factor 2: desolvation energy: water molecules surrounding the ion must be stripped off before it binds to the carrier:

Ion	Atomic Number	Ionic Radius (nm)	Hydration Free Energy, ΔG (kJ/mol)
Li^+	3	0.06	−410
Na^+	11	0.095	−300
K^+	19	0.133	−230
Rb^+	37	0.148	−210
Cs^+	55	0.169	−200

It "costs more" energetically to desolvate Na^+ and Li^+ than K^+

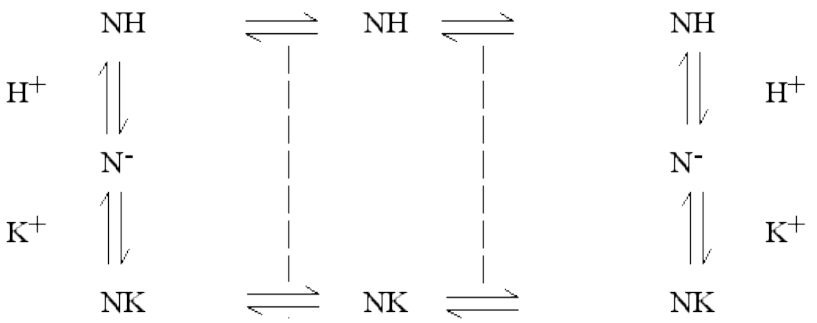
The carboxylic ionophores -Nigericin

- It has linear structure with a carboxyl group on one end and hydroxyls on the other.



- It is a K^+/H^+ exchanger.

- It cyclize by head-to-tail hydrogen bonding and will cross the membrane with the carboxyl group either protonated or complexed to an ion.



- Nigericin does not carry a net charge across the membrane.

$$\Delta G = RT\ln\left(\frac{[H^+]_{in}}{[H^+]_{out}}\right) + F\psi_m - RT\ln\left(\frac{[K^+]_{in}}{[K^+]_{out}}\right) + F\psi_m$$

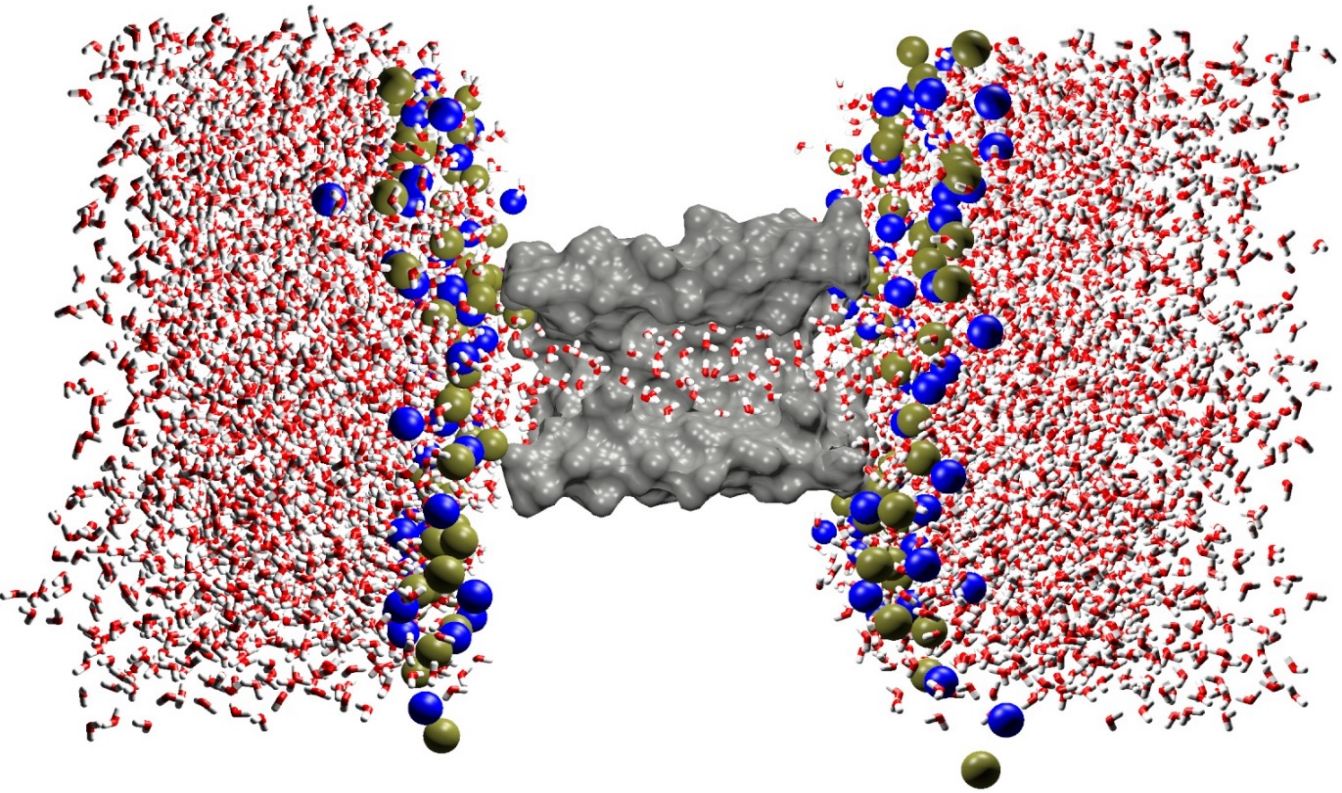
$\Delta G = 0$

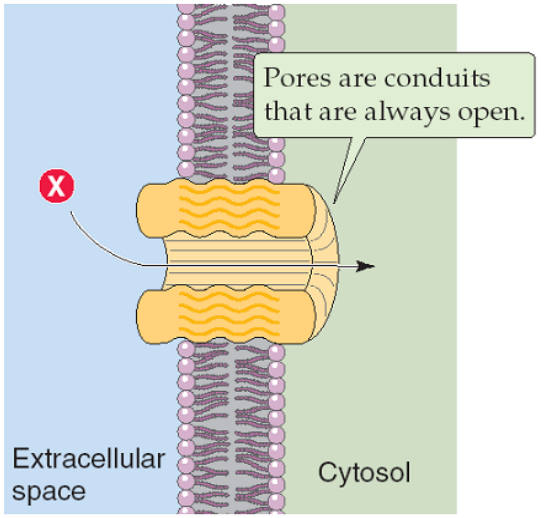
$$\frac{[H^+]_{in}}{[H^+]_{out}} = \frac{[K^+]_{in}}{[K^+]_{out}}$$

Nigericin will reach equilibrium when the $[H^+]$ and $[K^+]$ gradients are proportional.

Pores

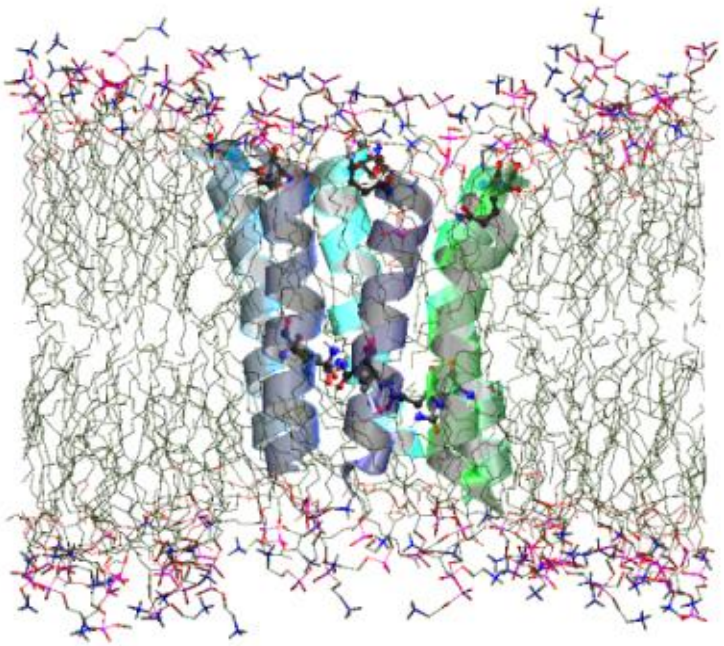
Solutes with appropriate size and charge can pass rapidly in either direction by diffusion.





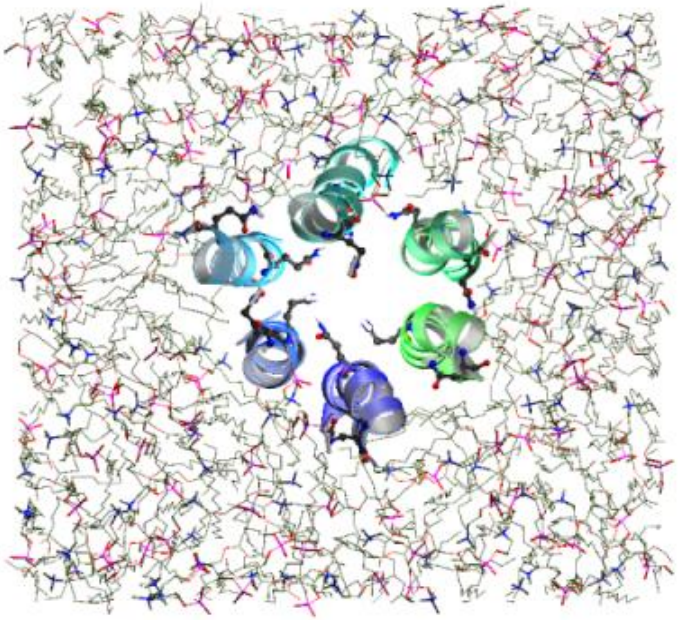
Pore

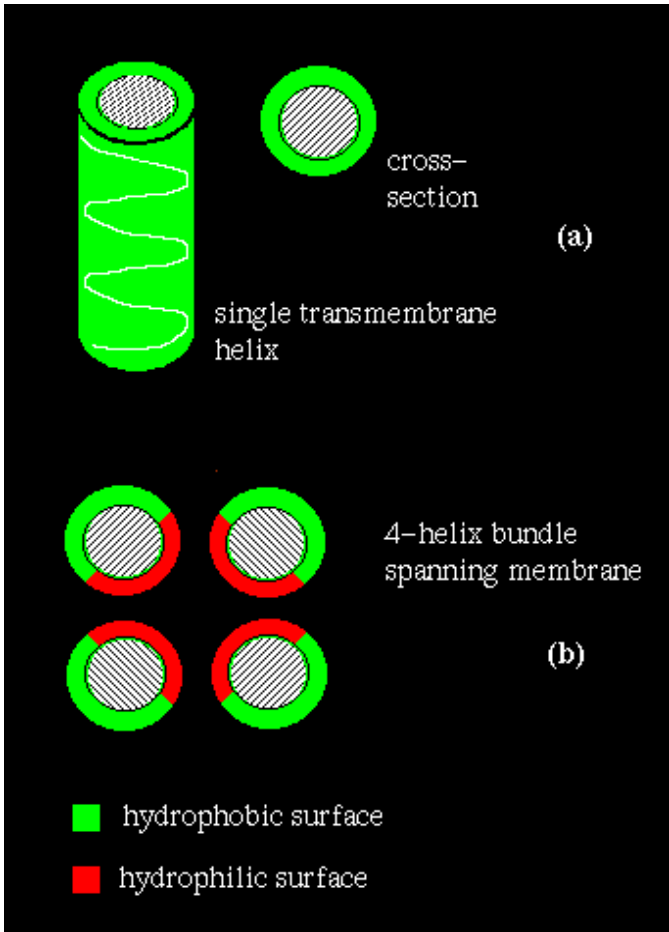
(non-gated channel)



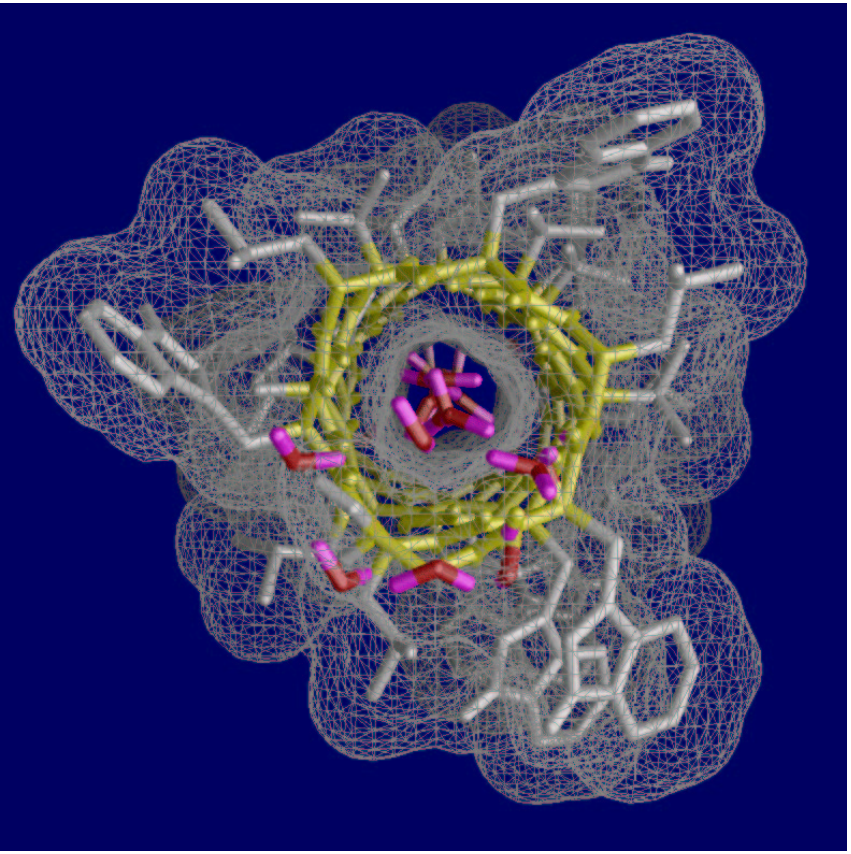
Alamethicin – a weakly selective channel

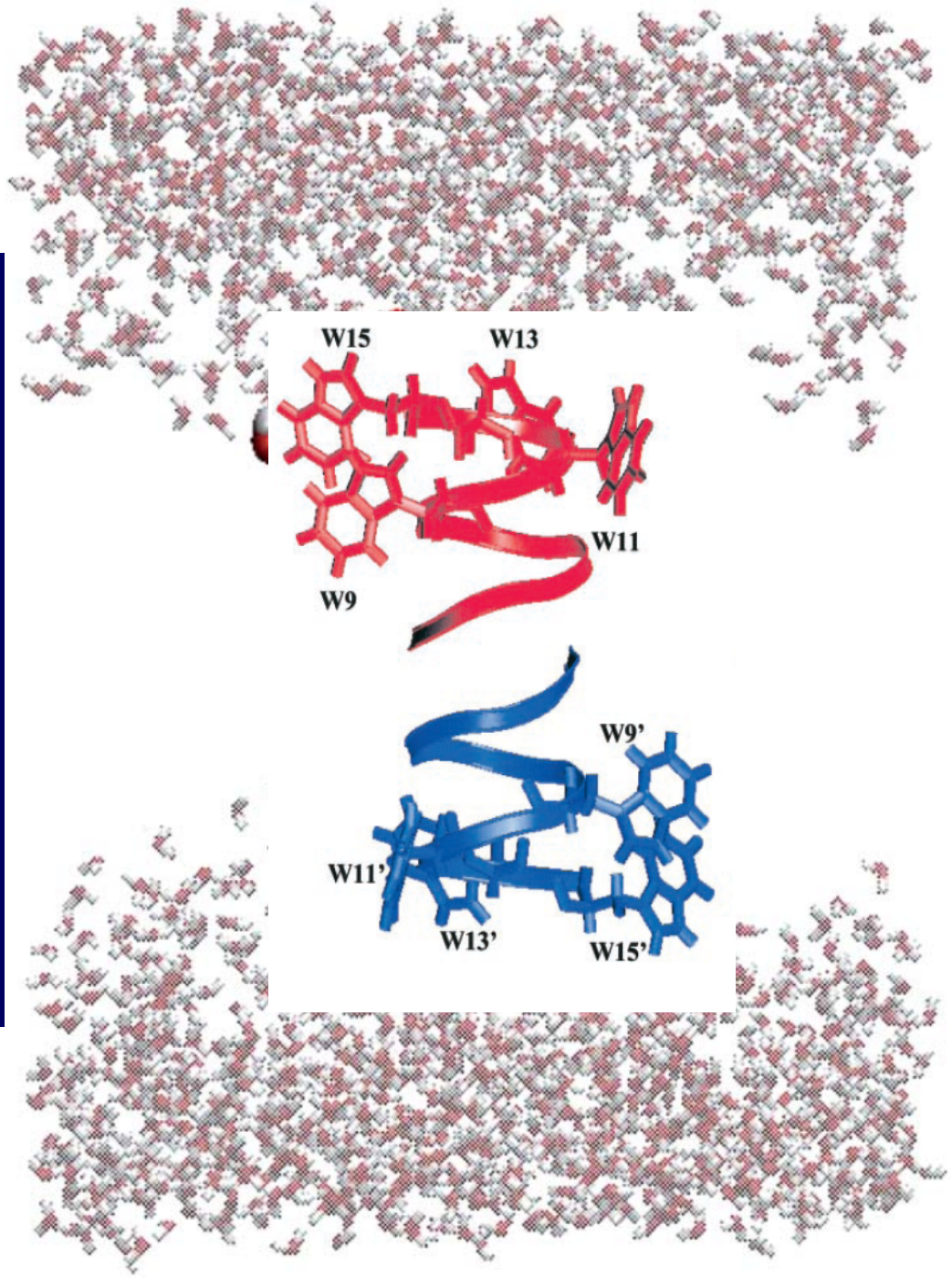
- *Multi-conductance level channels,*
- *Rapid switching between conductance levels,*
- *Weakly cation selective (ca. 4:1 cations:anions)*



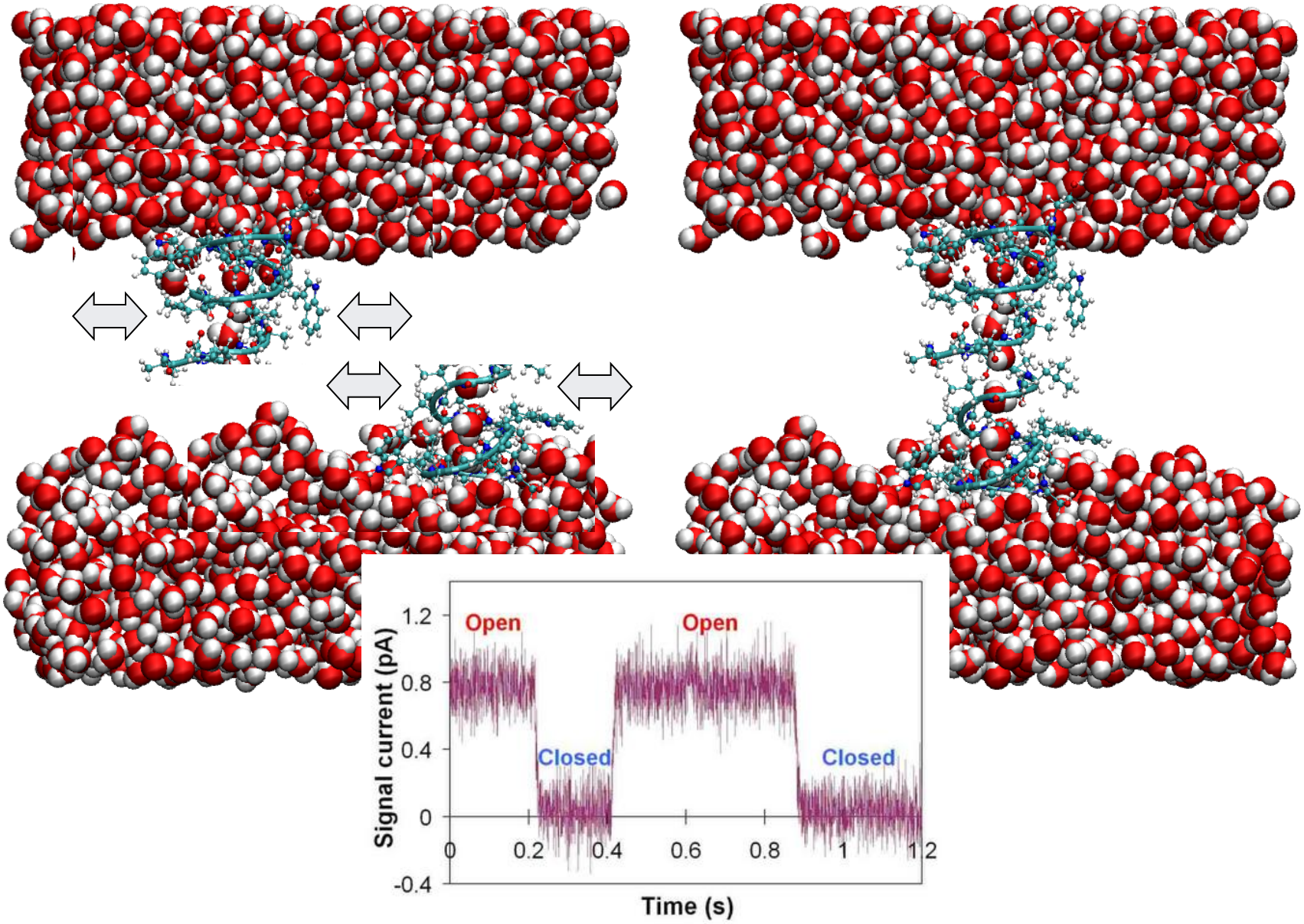


Gramicidins



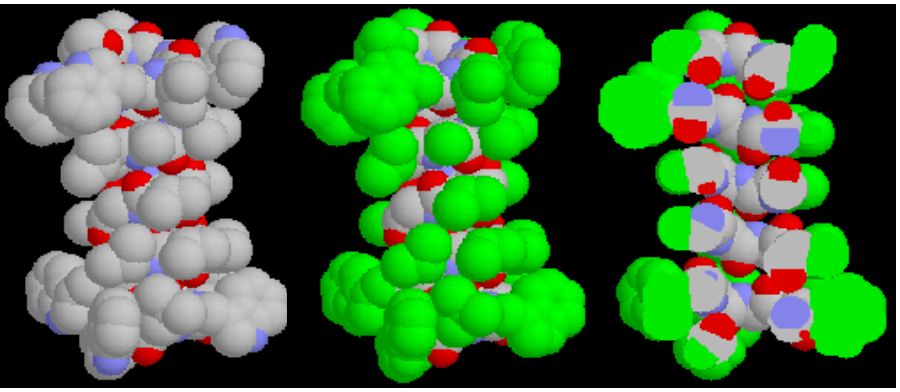


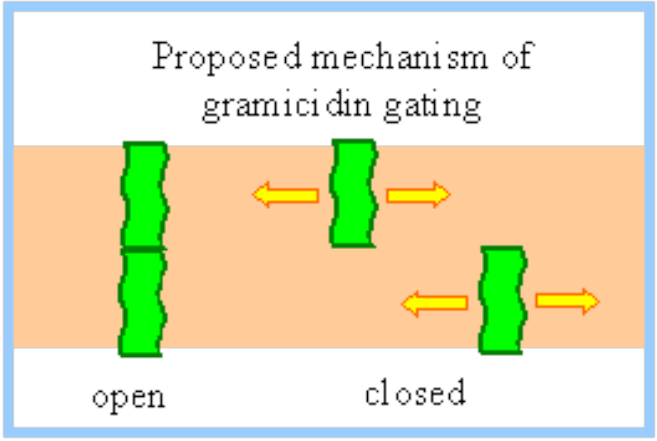
Gramicidin A: a diffusive carrier that forms pores



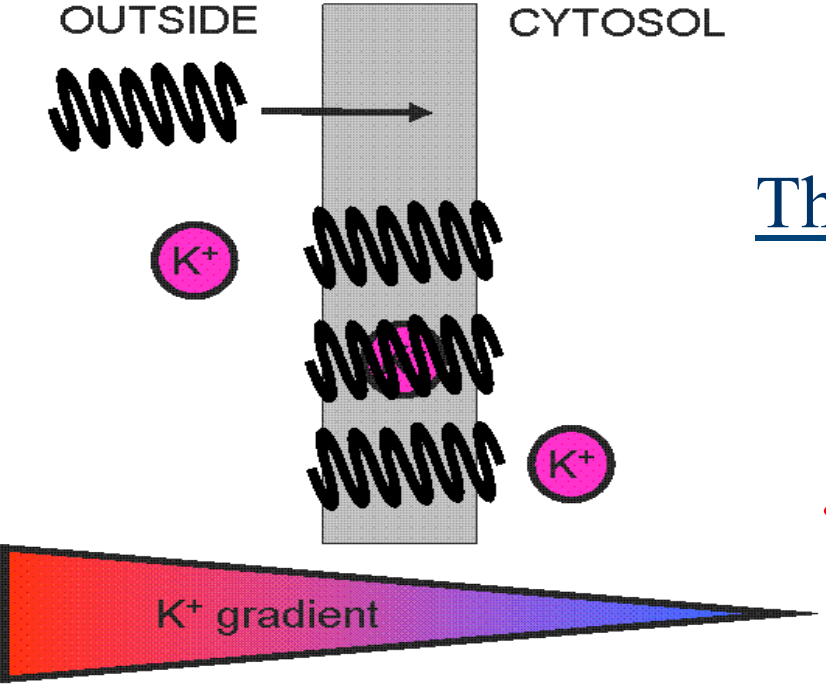
Gramicidin pore

- Channels constantly assemble and dissociate (lifetime ~1 sec)





- At high [gramicidin] overall transport rate depends on **[gramicidin]^2**.



The rate of transport:

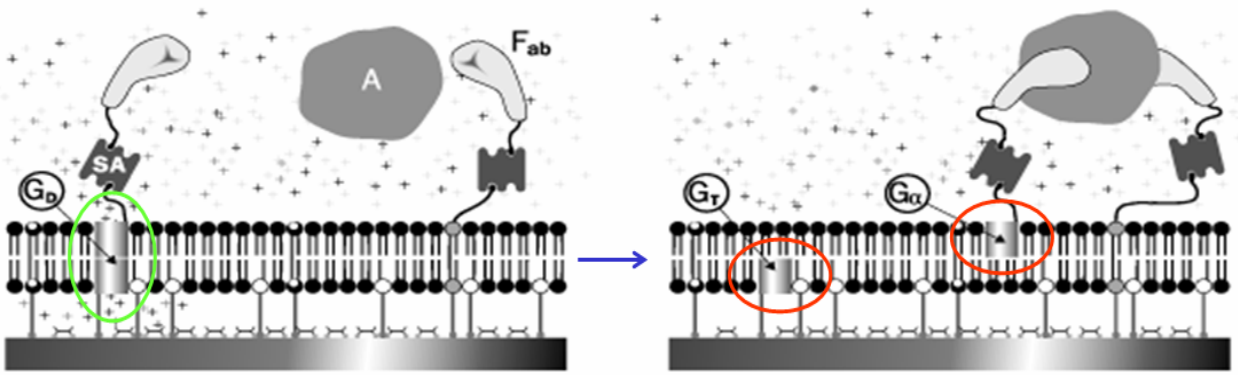
valinomycin **(carrier) transports up to 10^4 K^+/sec**

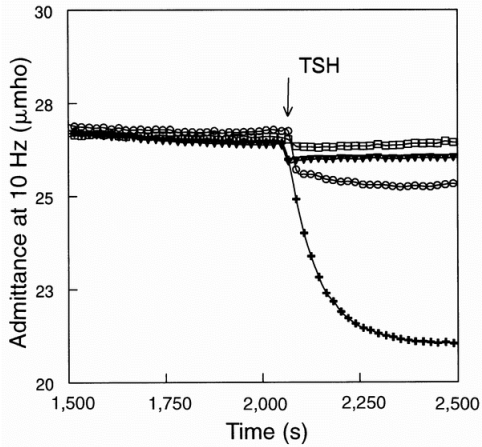
gramicidin **(channel) permeability is up to 10^7 K^+/sec**

Gramicidin Based Biosensor - Design

Mode A

- *Analyte binding disrupts channels*
- *Analyte reduces conductance*





Mode B

- *Analyte competes for Fab binding, allowing channels to reform*
- *Analyte increases conductance*