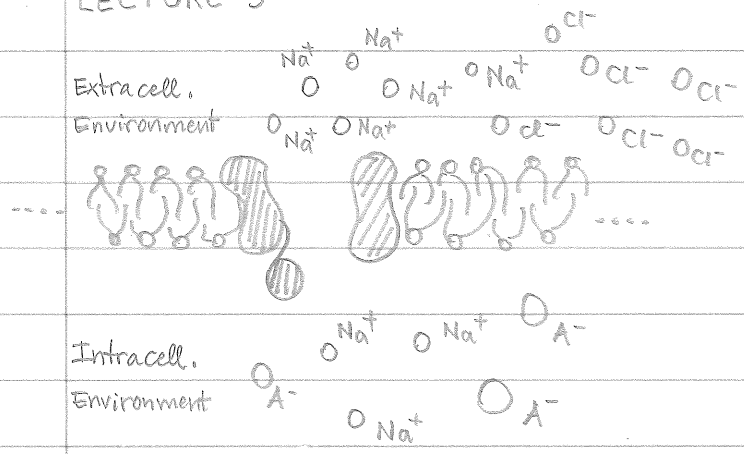


# LECTURE 5



In the previous lecture, we said that there are proteins across the cell membrane that selectively allow the passage of only one type of ions (e.g.,  $Na^+$ ,  $K^+$ ,  $Cl^-$ , etc.). Let us study what happens around these proteins, which are called "ion channels".

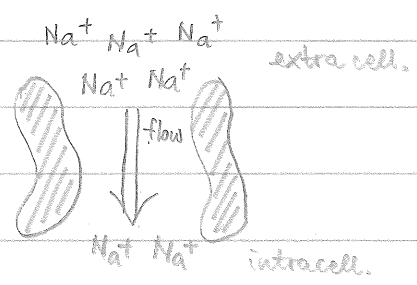
In a situation as in figure:

- Net charge inside and outside the cell is zero
- The concentration of  $Na^+$  is different inside and outside the cell
- The ion channel allows the passage of  $Na^+$  but nothing else

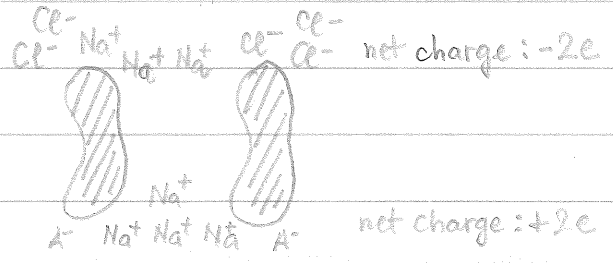


The following events occur:

a) A flow of  $Na^+$  through the ion channel according to the concentration gradient of  $Na^+$

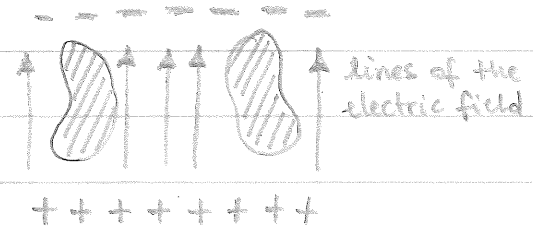


b) The ions of a type that cannot pass the channel (e.g.,  $Cl^-$ ) remain unbalanced and accumulate along the membrane



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c) The resultant electric field across the membrane opposes the transport of ions by gradient of concentration

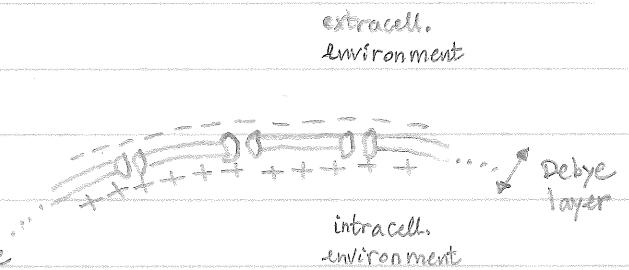


For any new  $\text{Na}^+$  ion moving from one side to the other, the intensity of the electric field increases

Equilibrium is eventually achieved when the electric field exactly balances the diffusion of  $\text{Na}^+$

Note: If we zoom out and we approximate the membrane as a continuum of  $\text{Na}^+$  ion channels, then we have:

- both sides of the membrane are electrically not-neutral
- at steady-state, the excess of charges accumulates in a thin layer around the membrane (Debye layer)



Let us remember that we are dealing with dilute solutions  $\Rightarrow$  Ions on any side of the membrane have a chemical potential, which is defined as the energy that can be absorbed or released during a chemical reaction

Hence the  $\text{Na}^+$  ions at the interface with the cell membrane have a chemical potential that is determined by the free energy they would have in an electrically neutral condition AND the electric potential energy determined

by the electric field:

$$\text{INSIDE: } G_{\text{Na},i} = \underbrace{G_{\text{Na}}^{\circ} + RT \ln([\text{Na}^+]_i)}_{\text{Gibbs free energy}} + \underbrace{zF\psi_i}_{\text{Electric potential energy}}$$

$$\text{OUTSIDE: } G_{\text{Na},e} = G_{\text{Na}}^{\circ} + RT \ln([\text{Na}^+]_e) + zF\psi_e$$

$G_{\text{Na}}^{\circ} \triangleq$  chemical potential at 1M in an electrically neutral condition

$R \triangleq$  universal gas constant

$T \triangleq$  temperature (in  $^{\circ}\text{K}$ )

$[\text{Na}^+]_i; [\text{Na}^+]_e \triangleq$  concentration of  $\text{Na}^+$  inside and outside the cell, respectively

$zF \triangleq$  charge (it is the valence of  $\text{Na}^+$  ( $z$ )  $\times$  Faraday constant ( $F$ ))

$\psi_i, \psi_e \triangleq$  electric potential inside and outside the cell, respectively

The potential difference is:

$$\begin{aligned} \Delta G_{\text{Na}} &\triangleq G_{\text{Na},i} - G_{\text{Na},e} = \\ &= RT \ln \left( \frac{[\text{Na}^+]_i}{[\text{Na}^+]_e} \right) + \underbrace{zF(\psi_i - \psi_e)}_{\triangleq V\text{-membrane voltage}} \end{aligned}$$

$$\text{At equilibrium: } \Delta G_{\text{Na}} = 0 \Rightarrow zFV = -RT \ln \left( \frac{[\text{Na}^+]_i}{[\text{Na}^+]_e} \right)$$

$$\Rightarrow V = \frac{RT}{zF} \ln \left( \frac{[\text{Na}^+]_e}{[\text{Na}^+]_i} \right) - \text{Nernst potential}$$

Note that the definition of

Nernst potential only depends on  $\Rightarrow$

the ratio of  $\text{Na}^+$  ion concentrations

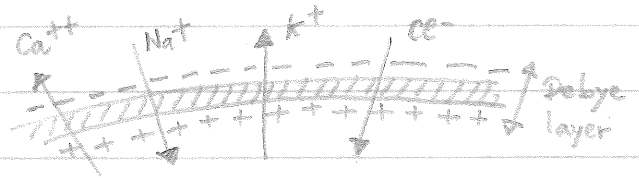
- There is one Nernst potential for each type of ions crossing the cell membrane
- The Nernst potential does not depend on how ions move and is time-varying

④

In a more realistic scenario, where several types of ions are allowed to cross the membrane (each one through its own set of ion channels) we have:

- Each flow of ions has its own Nernst potential, e.g.:

$$\left. \begin{aligned} V_{Na} &= 56 \text{ mV} \\ V_K &= -77 \text{ mV} \\ V_{Cl} &= -68 \text{ mV} \end{aligned} \right\} \text{value for the squid axon at } 27^\circ\text{C}$$



- But  $V = V_i - V_e$  is approximately constant at steady-state along the membrane  $\Rightarrow$  There will be ion flows through the ion channels

To study these flows let us consider that these ions are moved by two forces:

- concentration gradient  $\Rightarrow$  Fick's Law:  $\vec{J}_a = -D \nabla \vec{u}$

$\uparrow$  flux density                       $\uparrow$  concentration gradient of ions

- electric field  $\Rightarrow$  Planck's Equation:  $\vec{J}_e = -v \text{ sign}(z) u \nabla \psi$

$\uparrow$  flux density                       $\uparrow$  velocity of ions under a unit electric field                       $\uparrow$  gradient of the electrical potential

Note that  $\text{sign}(z)$  accounts for the fact that currents have a conventional orientation and that  $v$  is related to the Fick's constant  $D$  via the relationship:

$$D = \frac{vRT}{|z|F}$$

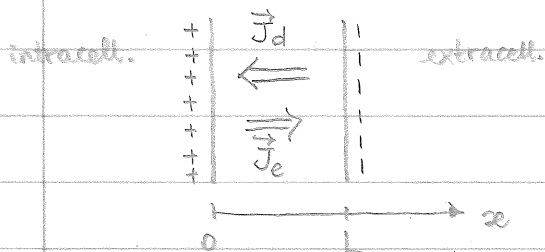
Hence, given that  $\text{sign}(z) \triangleq \frac{z}{|z|}$ , we can write:

$$\vec{J}_e = -v \frac{z}{|z|} u \nabla \vec{V} = -\frac{zF}{RT} D u \nabla \vec{v}$$

and the total flux density is:

$$\vec{J} = \vec{J}_d + \vec{J}_e = -D \left( \nabla \vec{u} + \frac{zF}{RT} u \nabla \vec{v} \right)$$

Let us consider a uni-dimensional case with one type of ions (e.g.,  $\text{Na}^+$ ):



$$J_d = -D \frac{du}{dx}$$

$$J_e = -\frac{zF}{RT} D u \frac{dv}{dx}$$

$\Rightarrow$

$$u \triangleq [\text{Na}^+]; u = u(x)$$

$v = v(x)$  - electric potential

$$V_i \triangleq v(0); V_e \triangleq v(L); V \triangleq v(0) - v(L)$$

$$\Rightarrow J_{\text{Na}} = -D \left( \frac{du}{dx} + \frac{zF}{RT} u \frac{dv}{dx} \right)$$

Let us make a quick check: is this expression for  $J_{\text{Na}}$  consistent with the definition of Nernst potential?

$$J_{\text{Na}} = 0 \Leftrightarrow \frac{du}{dx} + \frac{zF}{RT} u \frac{dv}{dx} = 0 \Leftrightarrow \frac{du}{u} = -\frac{zF}{RT} dv$$

↑  
integration  
by parts

$$\int_{u(0)}^{u(L)} \frac{du}{u} = -\frac{zF}{RT} \int_{v(0)}^{v(L)} dv \Leftrightarrow \ln(u) \Big|_{[\text{Na}^+]_i}^{[\text{Na}^+]_e} = -\frac{zF}{RT} \underbrace{(V_e - V_i)}_{-V}$$

$$\Leftrightarrow V = \frac{RT}{zF} \ln \left( \frac{[\text{Na}^+]_e}{[\text{Na}^+]_i} \right) = V_{\text{Na}} - \text{Nernst potential}$$

⑥

Let us consider a steady-state condition where the electric field is constant through the membrane, but  $V \neq V_{Na}$ :

$$\bullet \frac{d\psi}{dx} = \text{const} \Rightarrow V = - \int_0^L \frac{d\psi}{dx} dx = - \frac{d\psi}{dx} \int_0^L dx \Rightarrow \frac{d\psi}{dx} = -\frac{V}{L}$$

$$\bullet V \neq V_{Na} \Rightarrow J_{Na} = -D \left( \frac{du}{dx} - \frac{zF}{RT} u \frac{V}{L} \right) \neq 0$$

Also, because we are in a steady-state and there is no production of  $Na^+$  ions, the flux density  $J_{Na}$  must be constant (it comes from the Fick's Law)  $\Rightarrow$  Hence:

$$\frac{J_{Na}}{D} + \frac{du}{dx} - \frac{zFV}{RTL} u = 0 \quad \text{- To solve this ODE let us observe:}$$

constant  $\neq 0$

$$\alpha \triangleq \frac{zFV}{RTL}; \quad \frac{du}{dx} - \alpha u = -\frac{J_{Na}}{D} \quad \text{- By multiplying both sides by } e^{-\alpha x} \text{ we have:}$$

$$e^{-\alpha x} \frac{du}{dx} - \alpha e^{-\alpha x} u(x) = -\frac{J_{Na}}{D} e^{-\alpha x} \quad \text{- Integration between } x=0 \text{ and } x=L:$$

$$\underbrace{\frac{d}{dx} (e^{-\alpha x} u)}_{\text{left}} \quad \underbrace{\frac{J_{Na}}{\alpha D} \frac{d}{dx} (e^{-\alpha x})}_{\text{right}}$$

$$e^{-\alpha x} u \Big|_{x=0}^{x=L} = \frac{J_{Na}}{\alpha D} e^{-\alpha x} \Big|_{x=0}^{x=L}$$

$$[Na^+]_e e^{-\alpha L} - [Na^+]_i = \frac{J_{Na}}{\alpha D} (e^{-\alpha L} - 1)$$

$$\text{Note: } \alpha L = \frac{zFV}{RTL} \cdot L = \frac{zF}{RT} V$$



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$$I_{Na} + I_K + I_{Cl} = 0 \Leftrightarrow P_{Na} \frac{[Na^+]_i - [Na^+]_e e^{-\left(\frac{zF}{RT} V^*\right)}}{1 - e^{-\left(\frac{zF}{RT} V^*\right)}} + P_K \frac{[K^+]_i - [K^+]_e e^{-\left(\frac{zF}{RT} V^*\right)}}{1 - e^{-\left(\frac{zF}{RT} V^*\right)}} + P_{Cl} \frac{[Cl^-]_i - [Cl^-]_e e^{+\left(\frac{zF}{RT} V^*\right)}}{1 - e^{+\left(\frac{zF}{RT} V^*\right)}} = 0$$

Let us call:  $n \triangleq e^{-\left(\frac{zF}{RT} V^*\right)}$   $p \triangleq e^{+\left(\frac{zF}{RT} V^*\right)}$

Let us note:  $p \cdot n = 1$

$$(1-p) \cdot n = -(1-n)$$

Hence we can write:

$$P_{Cl} \frac{[Cl^-]_i - [Cl^-]_e p}{1-p} \cdot \frac{n}{n} = -P_{Cl} \frac{[Cl^-]_e - [Cl^-]_i n}{(1-n)}$$

$$I_{Na} + I_K + I_{Cl} = 0 \Leftrightarrow P_{Na} ([Na^+]_i - [Na^+]_e n) + P_K ([K^+]_i - [K^+]_e n) + P_{Cl} ([Cl^-]_e - [Cl^-]_i n) = 0$$

$$\Leftrightarrow (P_{Na} [Na^+]_i + P_K [K^+]_i + P_{Cl} [Cl^-]_e) - n (P_{Na} [Na^+]_e + P_K [K^+]_e + P_{Cl} [Cl^-]_i) = 0$$

$$\Leftrightarrow n = \frac{P_{Na} [Na^+]_i + P_K [K^+]_i + P_{Cl} [Cl^-]_e}{P_{Na} [Na^+]_e + P_K [K^+]_e + P_{Cl} [Cl^-]_i}$$

$$P_{Na} [Na^+]_e + P_K [K^+]_e + P_{Cl} [Cl^-]_i$$

$$\Leftrightarrow \begin{matrix} \uparrow \\ n = e^{-\left(\frac{zF}{RT} V^*\right)} \end{matrix} - \frac{zF}{RT} V^* = \ln \left( \frac{P_{Na} [Na^+]_i + P_K [K^+]_i + P_{Cl} [Cl^-]_e}{P_{Na} [Na^+]_e + P_K [K^+]_e + P_{Cl} [Cl^-]_i} \right)$$

$$\Leftrightarrow V^* = - \frac{RT}{zF} \ln \left( \frac{P_{Na} [Na^+]_i + P_K [K^+]_i + P_{Cl} [Cl^-]_e}{P_{Na} [Na^+]_e + P_K [K^+]_e + P_{Cl} [Cl^-]_i} \right) \quad \begin{matrix} \text{GHK} \\ \text{Potential} \end{matrix}$$



A few remarks:

- The GHK equation and the GHK potential are determined under the assumptions of: (i) constant electric field and (ii) steady-state conditions and (iii) channel always open.
- "Constant electric field" implies that the electric field is not affected by the transport of charges across the cell membrane.
- "Constant electric field" implies that  $dV/dt = 0$  (it is an electrostatic field)

Let us now study the ionic currents under more realistic conditions:

- Non-constant electric field  $\Rightarrow \frac{dV}{dt} \neq 0 \Rightarrow$  There is a capacitive current density through the membrane



$$\Rightarrow I_c = C_m \frac{dV}{dt} \Rightarrow \text{At steady-state we must replace the condition:}$$

↑  
equivalent capacity of the membrane per unit of area

$$\sum_i I_{ion_i} = 0$$

with the condition:

$$C_m \frac{dV}{dt} + \sum_i I_{ion_i} = 0$$

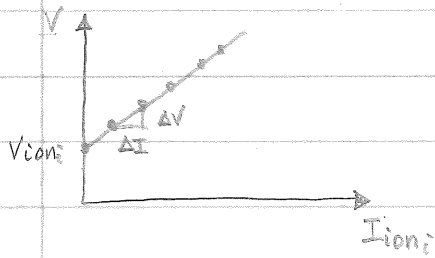
where " $I_{ion_i}$ " denotes the ionic current density for ion type  $i$  (e.g.,  $Na^+$ ,  $K^+$ , etc.)

$C_m \cong 1.0 \mu F/cm^2$  (a calculation is in section 2.6.4 of the textbook  $\Rightarrow$  check it!)

\* What model for  $I_{ion_i}$  under non-constant electric field conditions?

- Option 1 (linear): let us assume that  $I_{ion_i}$  (e.g.,  $I_{Na}$ ) varies linearly with the membrane voltage

10

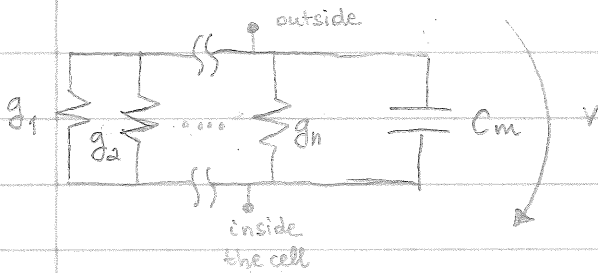


$$\frac{\Delta V}{\Delta I} = r_i - \text{constant}$$

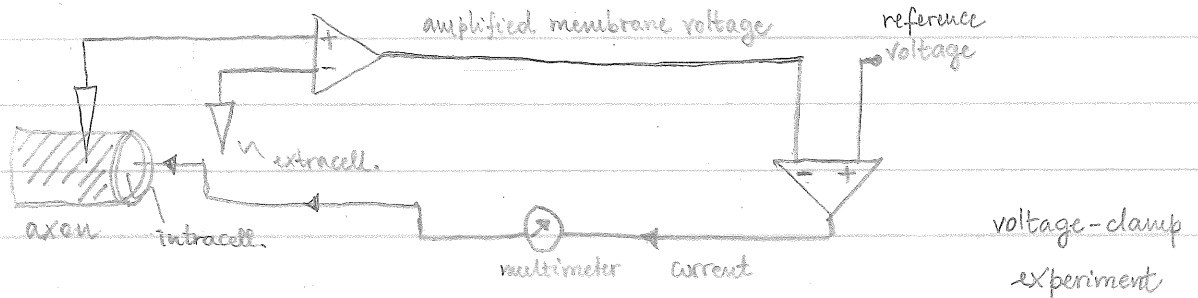
$$V = V_{ion_i} \text{ (Nernst potential)} \Rightarrow I_{ion_i} = 0$$

Hence:  $I_{ion_i} = \frac{1}{r_i} (V - V_{ion_i}) \Rightarrow g_i \triangleq \frac{1}{r_i}$  is a conductance per unit of area

As a result, the cell membrane can be modeled as an ohmic circuit:

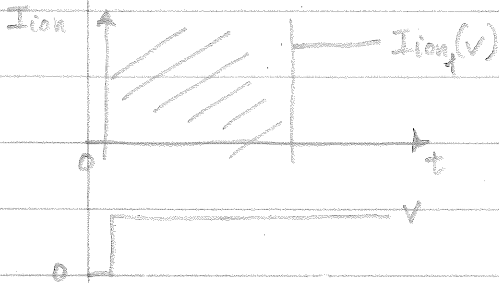


The linear model is motivated by the fact that the I-V curve estimated under steady-state conditions for open channels is almost linear in cells from invertebrates (e.g., giant squid axon).



In a more realistic scenario, though, channels are not "pipes" always open to the passage of ions, as we have hypothesized thus far  $\Rightarrow$  Ion channels are sensitive to the membrane voltage too, i.e., they can let ions pass or can block the ionic flux completely depending on the value of  $V \Rightarrow$  Ion channels must be studied on two different time scales:

1) Slow time scale (e.g., like during the voltage-clamp experiment in figure)

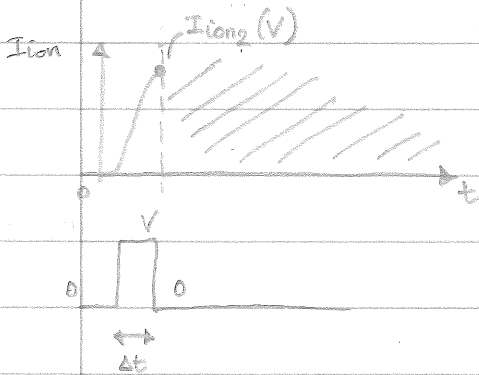


If we apply a constant membrane voltage  $V$  and wait long enough that the ion channels adjust to  $V$ , then the measured current  $I_{ion}(V)$  is determined by the number of channels open at voltage  $V$ , each channel being at its max capacity

Note: The I-V curve is determined by the number of fully-open ion channels

A "steady-state" I-V curve is estimated

2) Fast time scale (i.e., the membrane voltage is varied for a time  $\Delta t$



much shorter than the time the ion channels need to open or close)

The measured current  $I_{ion}(V)$  is due to a change in the flux per channel

Note: We assume that the flux adjusts instantaneously within the channel

An "instantaneous" I-V curve is estimated

Hence a more complete model must have a "fast" component and a "slow" component

(12)

- Option 2 (nonlinear): In order to combine instantaneous and steady-state I-V curves in one model, one can write:

$$I_{\text{ion}} = N g(v, t) \varphi(v)$$

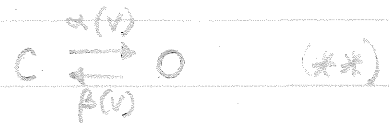
Number of ion channels  $\nearrow$

Instantaneous I-V relationship for one fully-open channel  $\nwarrow$

Proportion of open channels in the population of N channels  $\uparrow$

Example on how to model  $g(v, t)$ :

- Let us assume that each ion channel can be in one of two states (i.e., "open" [O] or "closed" [C])
- The rate of conversion from O to C is a function of V only



Note that (\*\*), formally looks like a biochemical reaction  $\Rightarrow$  We can think of  $g$  as the concentration of open channels (i.e.,  $g = [O]$ ) under the constraint  $[O] + [C] = 1$  at all time  $\Rightarrow$  We can translate (\*\*), into the ODE:

$$\frac{dg}{dt} = \alpha(v)(1-g) - \beta(v)g$$

where:

$g \triangleq$  proportion of open channels

$1-g \triangleq$  proportion of closed channels

$$\frac{dg}{dt} = -(\alpha(V) + \beta(V))g + \alpha(V)$$

By defining:  $\tau_g(V) \triangleq \frac{1}{\alpha(V) + \beta(V)}$

$$g_{\infty}(V) \triangleq \frac{\alpha(V)}{\alpha(V) + \beta(V)}$$

We can rewrite:  $\rightarrow$  steady-state value of  $g$

$$\frac{dg}{dt} = \frac{g_{\infty}(V) - g}{\tau_g(V)} \quad \text{- First order ODE}$$

$\uparrow$   
time constant

For a fixed value of  $V$ ,  $\tau_g(V)$  can give insights on how long it takes to approach steady-state conditions. In fact, the solution would be:

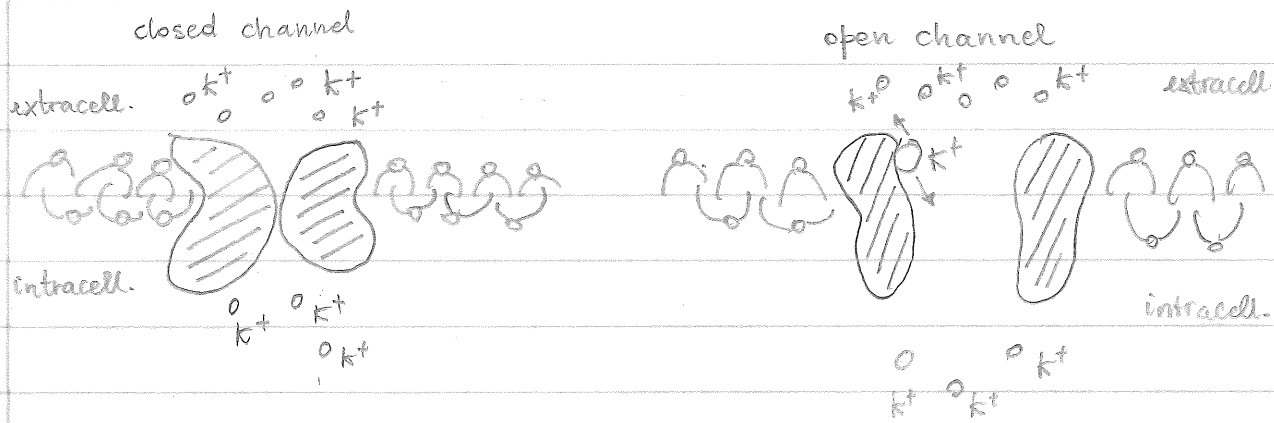
$$\frac{dg}{g_{\infty}(V) - g} = \frac{dt}{\tau_g(V)} \Leftrightarrow \int_{g(V,0)}^{g(V,t)} \frac{dg}{g_{\infty}(V) - g} = \frac{t}{\tau_g(V)} \Leftrightarrow -\ln \left( \frac{g_{\infty}(V) - g}{g_{\infty}(V) - g(V,0)} \right) \Bigg|_{g(V,0)}^{g(V,t)} = \frac{t}{\tau_g(V)}$$

$$\Leftrightarrow \ln \left( \frac{g_{\infty}(V) - g(V,0)}{g_{\infty}(V) - g(V,t)} \right) = \frac{t}{\tau_g(V)} \quad \text{- By assuming that } g(0) = 0, \text{ we have.}$$

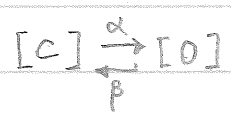
that  $g(V,t)$  will reach 99% of the value of  $g_{\infty}(V)$  at time:

$$t = \ln \left( \frac{g_{\infty}(V)}{g_{\infty}(V) - 0.99g_{\infty}(V)} \right) \cdot \tau_g(V) = \ln \left( \frac{1}{0.01} \right) \tau_g(V) \approx 4.6 \tau_g(V)$$

The function  $g_{\infty}(V)$  can be estimated from data. Alternatively one can obtain an analytical form by pursuing the analogy between the opening of ion channels and a biochemical reaction:



Opening a channel and letting ions (e.g.,  $K^+$ ) pass, in fact, mean that the ions can bind to the proteins of the channel and roll in or out the cell  
 $\Rightarrow$  The chemical potential of an open channel  $\neq$  the chemical potential of a closed-channel



generic chemical reaction



It can be showed that:

$$\frac{k_-}{k_+} = e^{\frac{\Delta G^0}{RT}}$$

By analogy we expect that:

$$\frac{\beta}{\alpha} = e^{\frac{\Delta \hat{G}^0}{RT}}$$

where  $\Delta \hat{G}^0$  is change in chemical potential at the concentration of 1M from substrate S, B to product SB

• What is  $\Delta \hat{G}^0$  in case of ion channels?

$$\Delta \hat{G}^0 = \underbrace{\Delta G^0}_{\text{change in Gibbs free energy if no electric field is applied}} + \underbrace{zFV}_{\text{change due to the application of an electric field}}$$

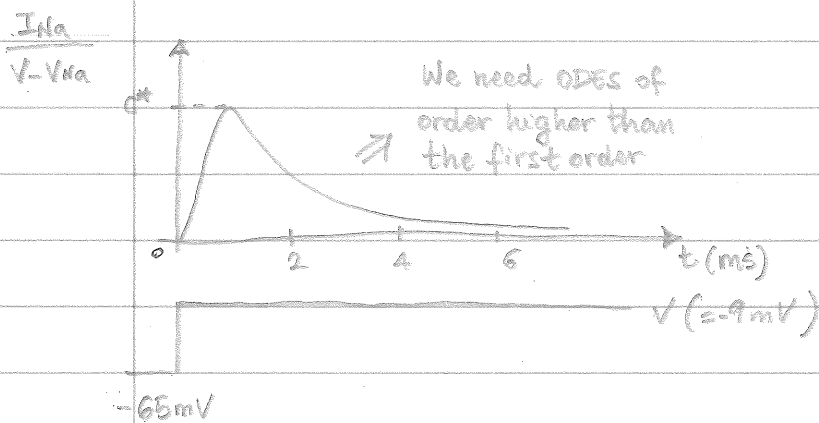
$$\frac{\beta(V)}{\alpha(V)} = e^{-\left(\frac{\Delta G^0}{RT} + \frac{zFV}{RT}\right)} = e^{-\frac{\Delta G^0}{RT}} \cdot e^{-\frac{zFV}{RT}}$$

Because  $\Delta G^0$  does not change with  $V$ , it can be estimated at  $V=0$  and it results in:  $k_0 = e^{-\frac{\Delta G^0}{RT}}$  - constant

$$\frac{\beta}{\alpha} = k_0 e^{-\frac{zFV}{RT}} \Rightarrow g_{\infty}(V) = \frac{\alpha(V)}{\beta(V) + \alpha(V)} = \frac{1}{\frac{\beta}{\alpha}(V) + 1} = \frac{1}{1 + k_0 e^{-\frac{zFV}{RT}}}$$

Problem: beside a few (simple) cases like  $K^+$  ion channels, the transition between open and closed state cannot be modeled by using an elementary model like:  $[C] \xrightleftharpoons[\beta]{\alpha} [O]$

For instance:



In this case, one possible approach consists of assuming that the ion channel is made of parts (subunits) and that each subunit must be open in order to have the whole channel open

This approach may lead to very complicated models, though, as the number of subunits grows. For instance, if there are 3 subunits involved, the model should include transitions among these conditions:

Subunit # 1 2 3

open/close :

o ≡ open  
c ≡ closed

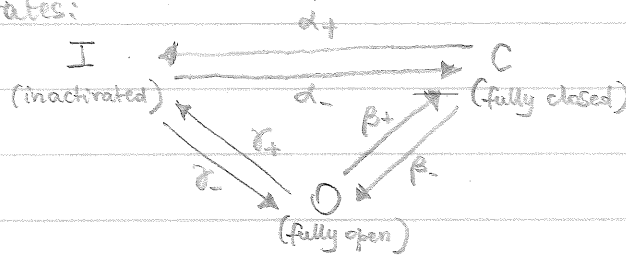
o	c	c	} Inactivated states ⇒ ions cannot pass
c	o	c	
c	c	o	

c c c → fully closed state (ions cannot pass)

o	o	c	} Inactivated states ⇒ ions cannot pass
o	c	o	
c	o	o	

o o o → fully open state (ions can pass)

Alternatively, one can consider 3 macro-states and estimate the transition rates:



REFERENCE:

Textbook (volume 1): chapter 1, sec. 1.2

chapter 2, sec. 2.6; 2.6.1; 2.6.3; 2.6.4

chapter 3, sec. 3.1; 3.1.1; 3.5; 3.5.1

chapter 3, sec. 3.5.2 (reading only)