Biophysics I (BPHS 3090)

Instructors: Prof. Christopher Bergevin (cberge@yorku.ca)

Website: http://www.yorku.ca/cberge/3090W2015.html

York University
Winter 2015
Lecture 4

Reference/Acknowledgement:
- TF Weiss (Cellular Biophysics)
- D Freeman

Diffusion Through Cell Membranes: History 101

Diffusion through Cell Membranes

Charles Ernest Overton (late 1800s): first systematic studies

- qualitative:
  - put cell in bath with solute
  - wait, rinse, squeeze
  - analyze to see how much got in (+ = some; +++ = a lot)
- 100's of solutes, dozens of cell types
- surprising results: previously cell membranes had been thought to be impermeant to essentially everything but water

Overton's Rules:

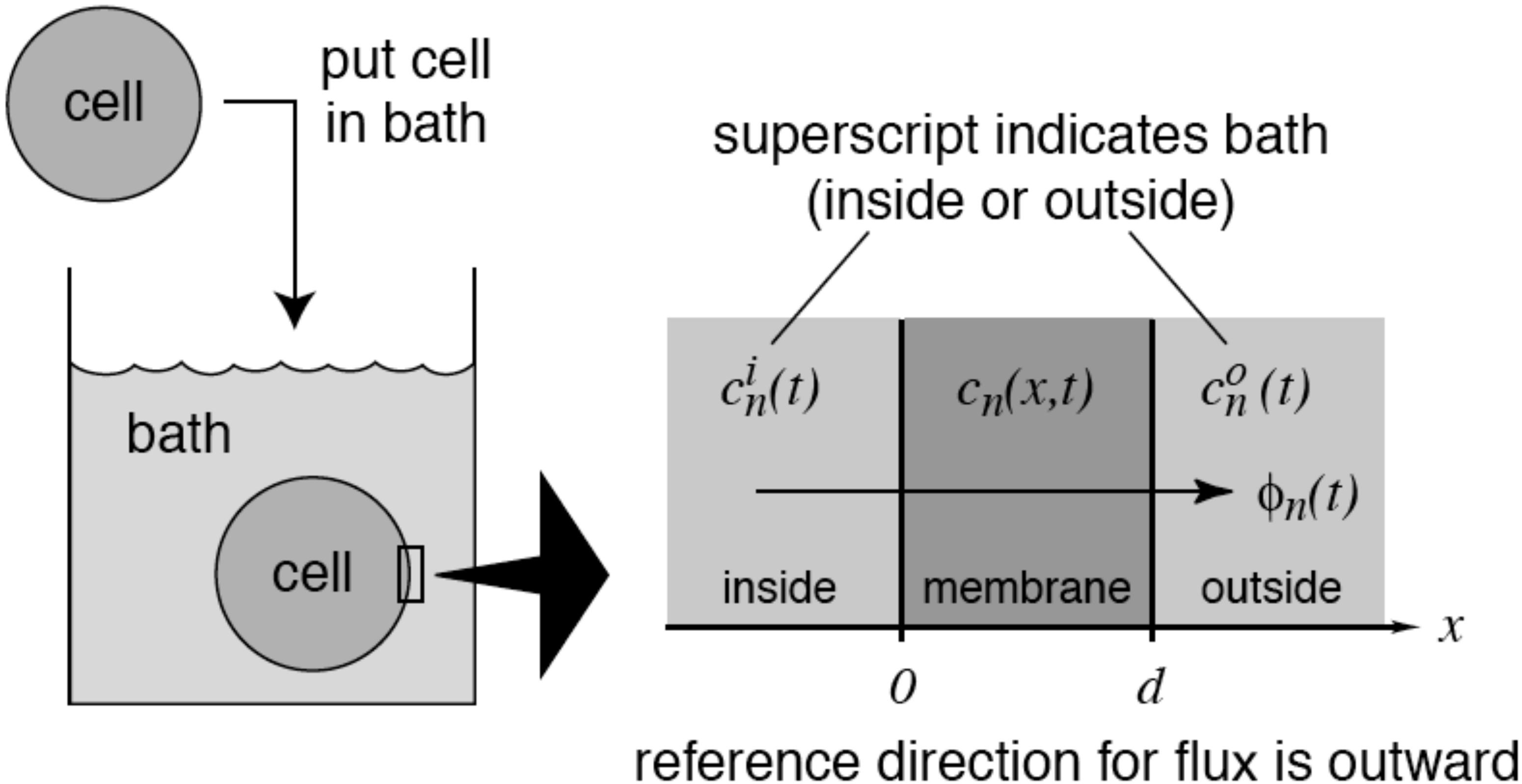
- cell membranes are semi-permeable
- relative permeabilities of plant and animals cells are similar
- permeabilities correlate with solubility of solute in organic solvents
  $\rightarrow$ membrane is lipid (specifically cholesterol and phospholipids)
- certain cells concentrate some solutes $\rightarrow$ active transport
- potency of anesthetics correlated with lipid solubility
  $\rightarrow$ Meyer-Overton theory of narcosis
- muscles don't contract in sodium-free media

Diffusion through Cell Membranes

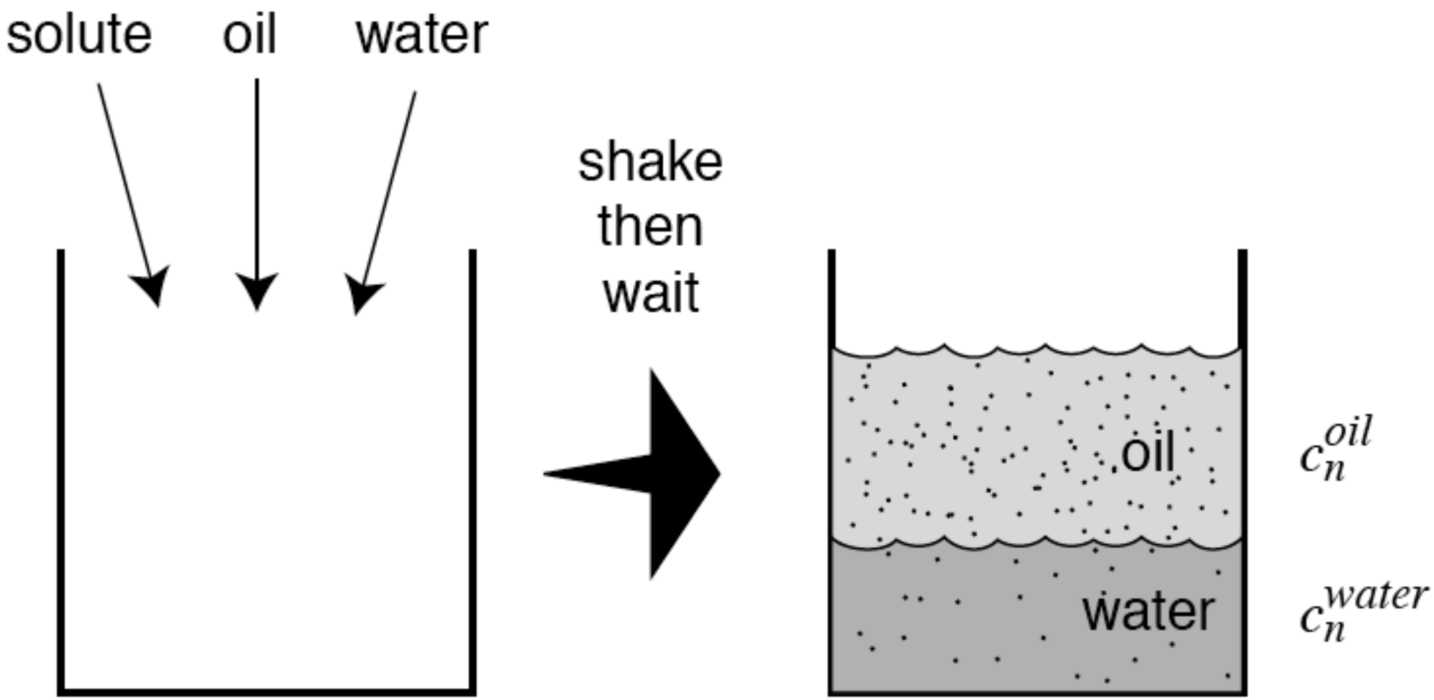
Paul Runar Collander (1920-1950): first quantitative studies

- large cells (cylindrical algae cells, 1 mm diameter, 1 cm long)
- bathe cell in solute for time t_1, squeeze out cytoplasm, analyze
- repeat with new cell and new time t_2
- plot intracellular quantity versus time
- fit with exponential function of time (two-compartment theory)
- infer permeability from time constant

Membrane Diffusion: Two-Compartment Geometry



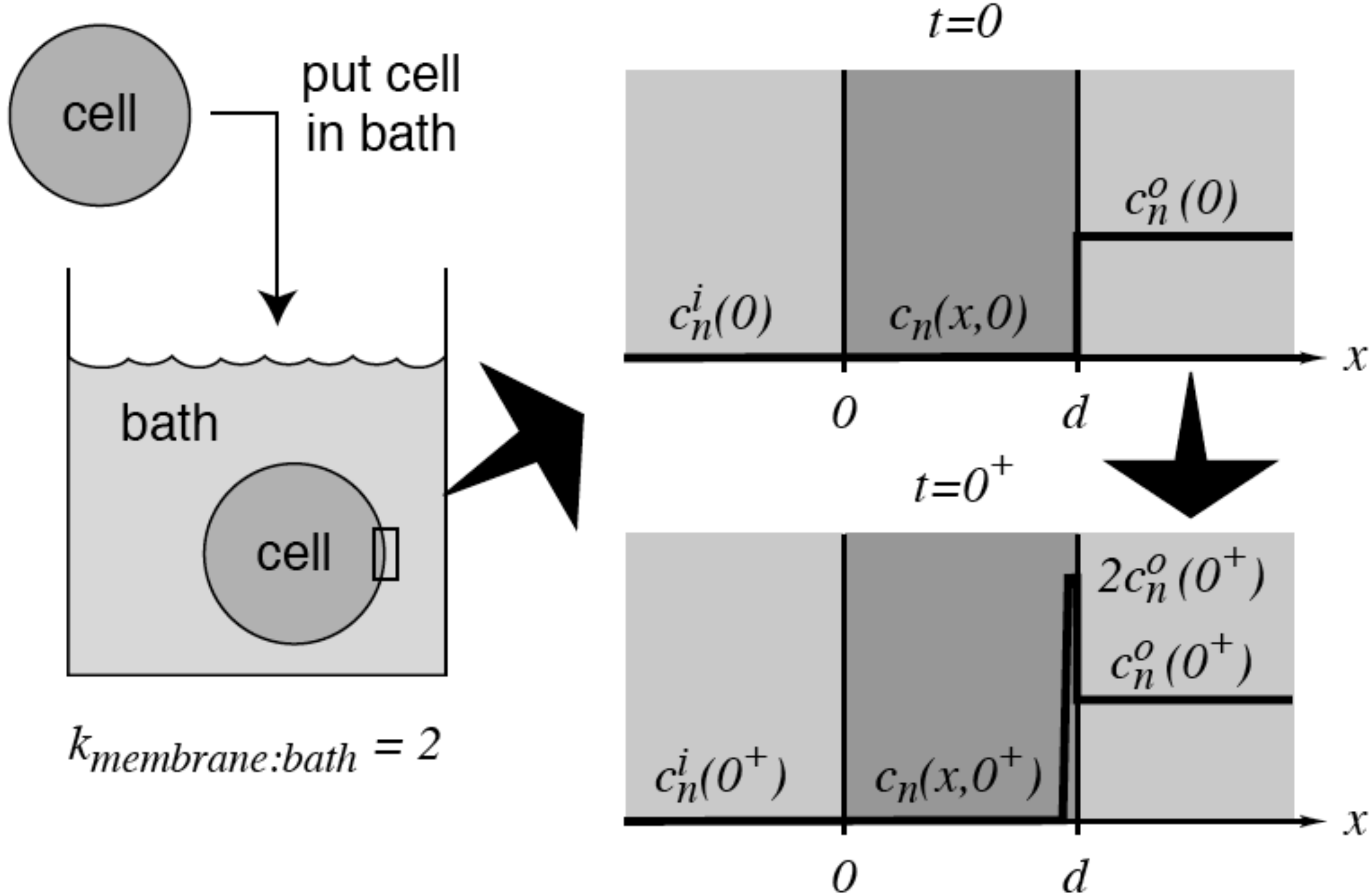
Step 1: Dissolve



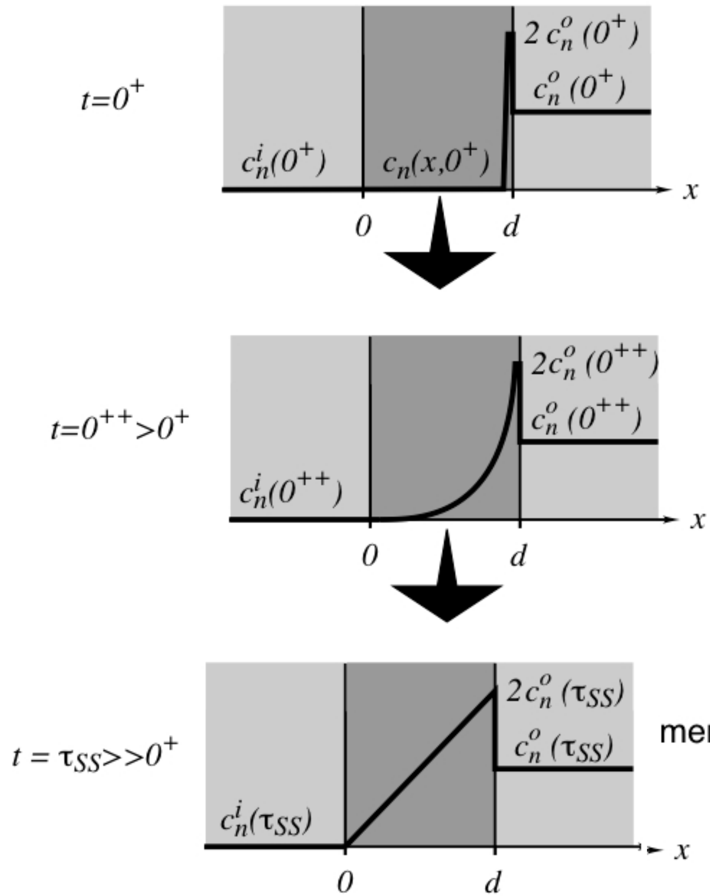
Equilibrium characterized by relative solubilities of solute n in oil and water

partition coefficient $k_{oil:water} = \dfrac{c_n^{oil}}{c_n^{water}}$

Assume Dissolving is fast relative to diffusing



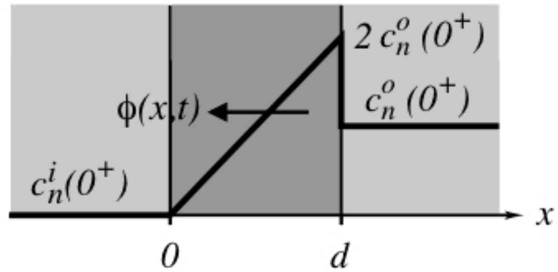
Step 2: Solute diffuses though membrane



steady state
membrane time constant

$$\tau_{SS}= \frac{d^2}{\pi^2 D}$$

Step 3: Solute enters the cell



$$c_n(x,t) = c_n(0,t) + \frac{x}{d}(c_n(d,t) - c_n(0,t))$$
$$= k_n c_n^i(t) + \frac{k_n x}{d}(c_n^o(t) - c_n^i(t))$$

$$k_n = k_{membrane:bath}$$
$$= k_{membrane:cytoplasm}$$

Fick's law: $\phi_n(t) = -D_n \dfrac{\partial c_n(x,t)}{\partial x}$

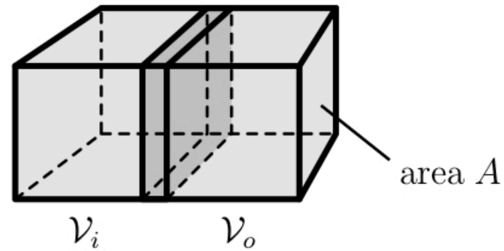
$$= -D_n \frac{c_n(d,t) - c_n(0,t)}{d}$$

$$= \frac{D_n k_n}{d}(c_n^i(t) - c_n^o(t))$$

$$\boxed{\phi_n(t) = P_n\,(c_n^i(t) - c_n^o(t)) \;\; ; \;\; P_n = \frac{D_n k_n}{d}}$$

Fick's law for membranes
P_n = permeability of membrane to solute n

Step 4: Concentration in cell changes: two-compartment diffusion



Assume

- $\mathcal{V}_i$ and $\mathcal{V}_o$ constant
- well-stirred baths: $c_n^i(t)$, $c_n^o(t)$
- solute is conserved and membrane is thin: $c_n^i(t)\mathcal{V}_i + c_n^o(t)\mathcal{V}_o = N_n$
- membrane always in steady state: $\phi_n(t) = P_n(c_n^i(t) - c_n^o(t))$

By continuity,

$$A\phi_n(t) = -\frac{d}{dt}(c_n^i(t)\mathcal{V}_i) = \frac{d}{dt}(c_n^o(t)\mathcal{V}_o)$$

$$\frac{d}{dt}c_n^i(t) = -\frac{AP_n}{\mathcal{V}_i}(c_n^i(t) - c_n^o(t)) = -\frac{AP_n}{\mathcal{V}_i}\left(c_n^i(t) - \frac{1}{\mathcal{V}_o}N_n + c_n^i(t)\frac{\mathcal{V}_i}{\mathcal{V}_o}\right)$$

$$\frac{d}{dt}c_n^i(t) + AP_n(\frac{1}{\mathcal{V}_i} + \frac{1}{\mathcal{V}_o})c_n^i(t) = \frac{AP_nN_n}{\mathcal{V}_i\mathcal{V}_o}$$

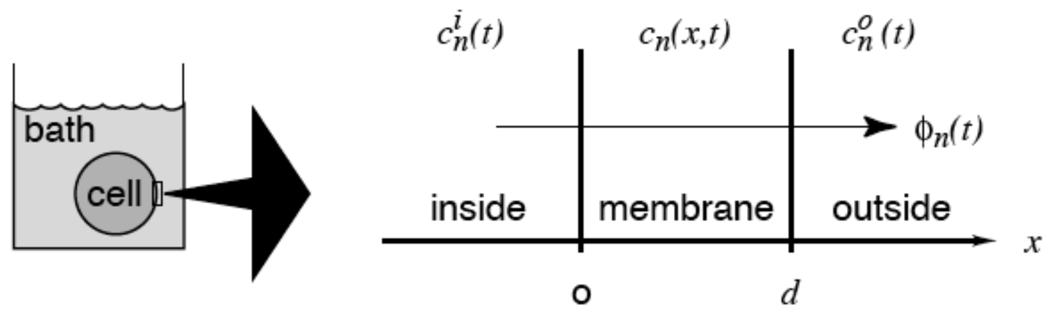
First-order linear differential equation with constant coefficients, therefore

$$c_n^i(t) = c_n^i(\infty) + [c_n^i(0) - c_n^i(\infty)]e^{-t/\tau_{EQ}}$$

$$c_n^i(\infty) = \frac{N_n}{\mathcal{V}_i + \mathcal{V}_o} \qquad \tau_{EQ} = \frac{1}{AP_n(\frac{1}{\mathcal{V}_i} + \frac{1}{\mathcal{V}_o})}$$

Membrane Diffusion: Summary

Membrane diffusion



Dissolve and diffuse model
- solute outside cell dissolves into cell membrane
- solute diffuses through membrane
- solute dissolves into cytoplasm

Membrane time constant $t_{SS} = \dfrac{d^2}{\pi^2 D_n}$

Fick's law for membranes: $\phi_n(t) = P_n\,(c_n^i(t) - c_n^o(t))\ ;\ \ P_n = \dfrac{D_n k_n}{d}$

Two-compartment diffusion

Cell time constant $t_{EQ} = \dfrac{1}{AP_n\left(\dfrac{1}{V_o} + \dfrac{1}{V_i}\right)}$

Freeman

Dynamics of Membrane Diffusion

- Numerical solution to eqns.

- Arbitrary initial condition (top)

- Fast dynamics (middle)

- Steady-state set up (middle)

- Eventually, the two compartments change (bottom)

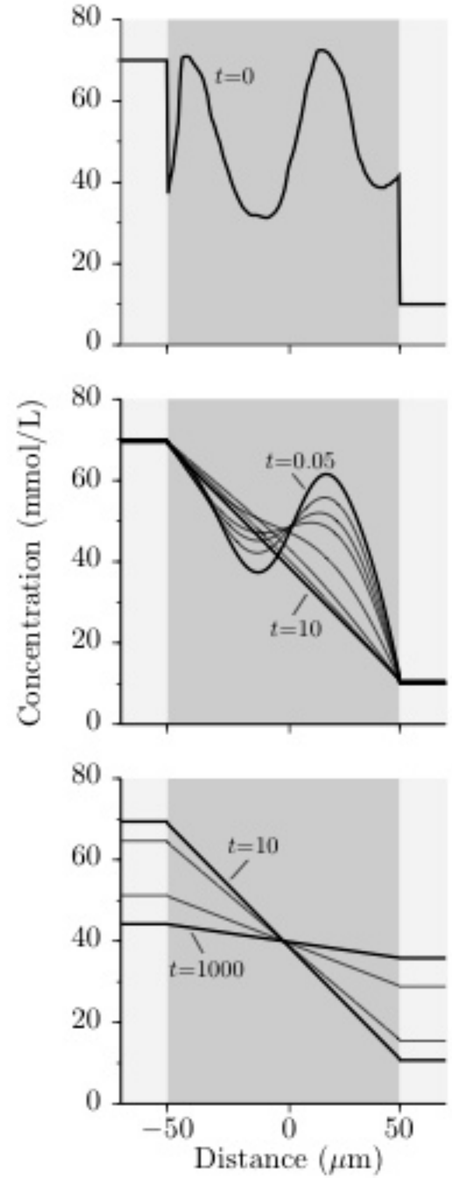
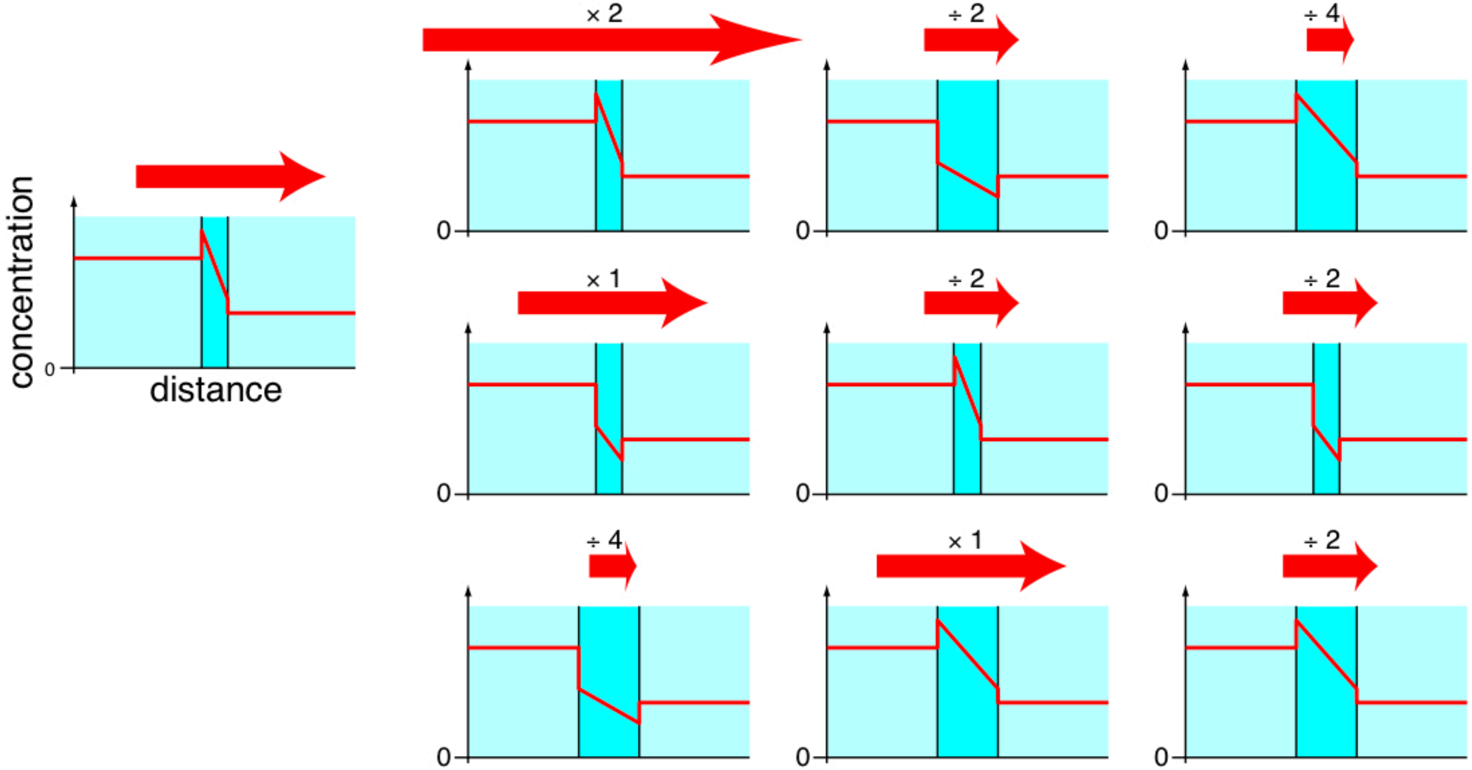


Figure 3.30

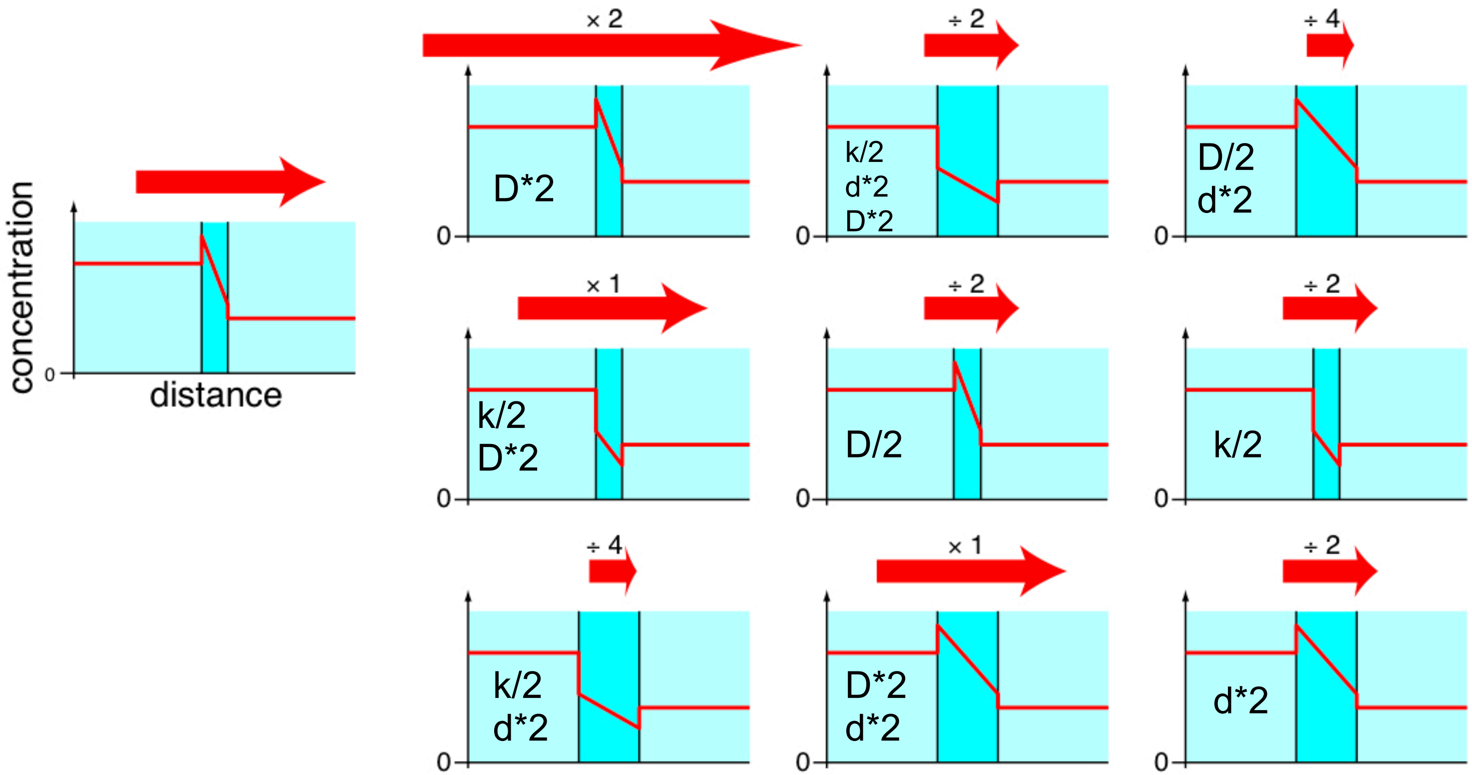
Effect of changing parameters on flux: What is being changed?



$$\phi_n(t) = P_n\,(c_n^{\,i}(t) - c_n^{o}(t)) \;\; ; \;\; P_n = \frac{D_n k_n}{d}$$

Fick's law for membranes

P_n = permeability of membrane to solute n



$$\phi_n(t) = P_n\,(c_n^{\,i}(t) - c_n^{o}(t)) \;\; ; \;\; P_n = \frac{D_n k_n}{d}$$

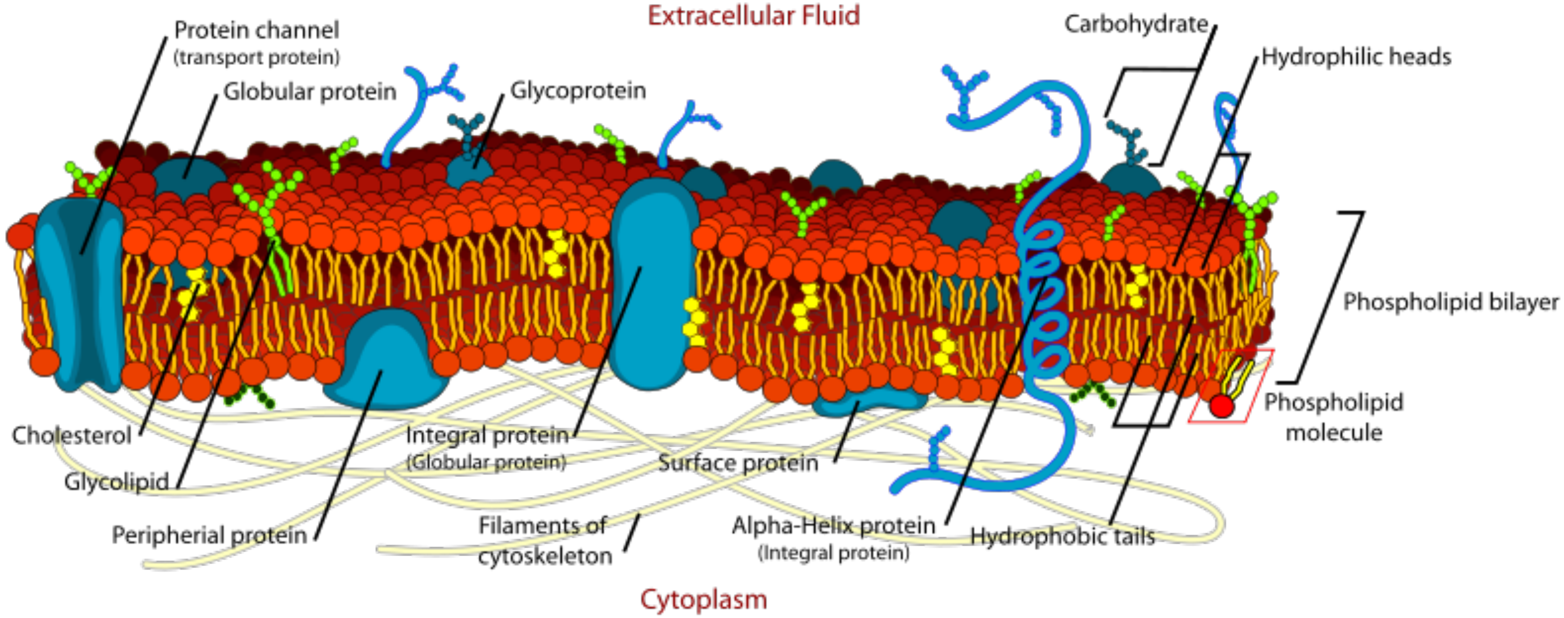
Fick's law for membranes

P_n = permeability of membrane to solute n

Question(s)

→ What are cell membranes made of?

→ How does one go about determining such?



→ It is only relatively recently we had a picture such as this!!

Wikipedia

Empirical means to estimate cell diffusion?

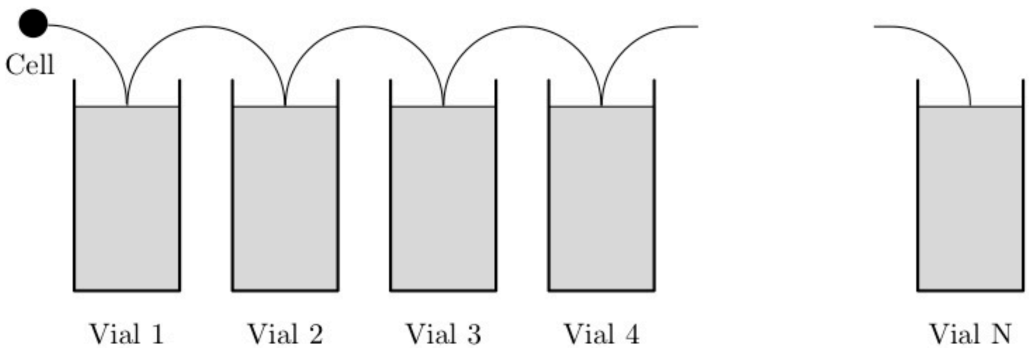


Figure 3.34

Flow cytometer

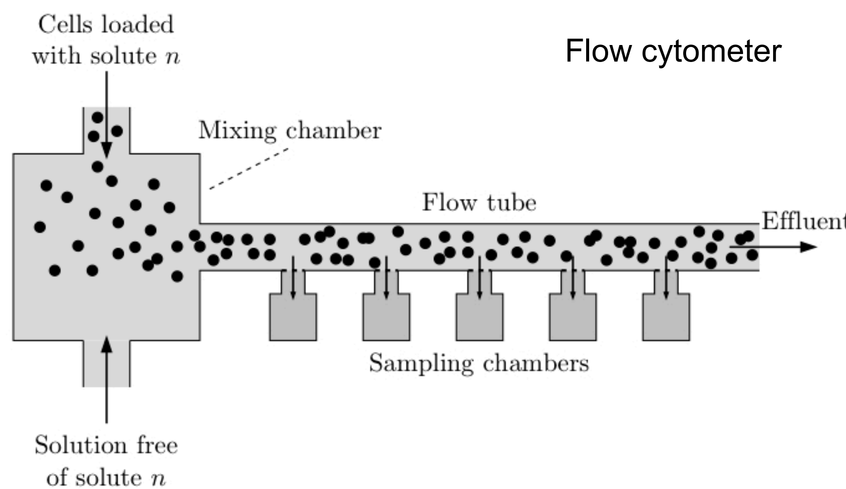


Figure 3.35

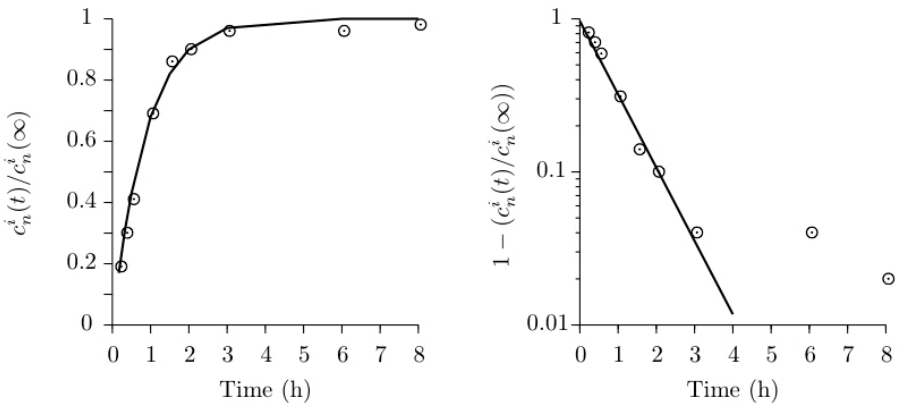


Figure 3.33

Diffusion of ethylene glycol through *Chara* membrane (Collander)
(see Weiss eqns. 3.56, 3.58, 3.60)

'Collander Plot'
(see Weiss sec.3.8.4)

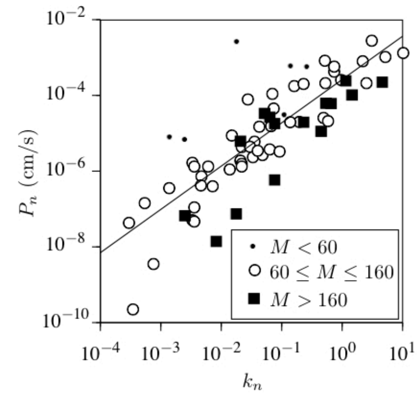


Figure 3.38

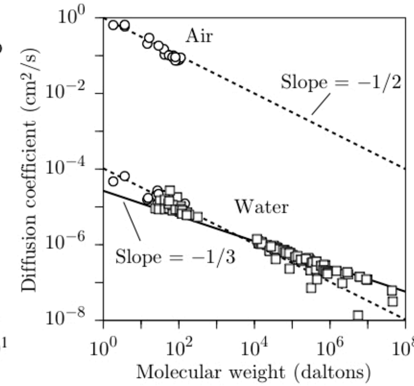


Figure 3.11

- strong correlation between solute permeability and solute ether:water partition coefficient

- supports Overton's rules & dissolve-diffuse mechanism

- relates in molecular weight (i.e., there is a strong 'physical' aspect to line of thought)

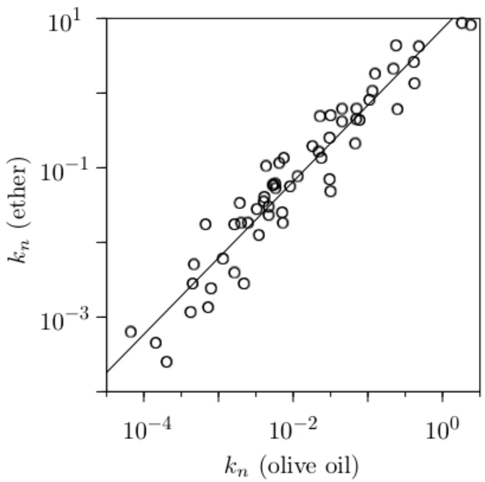


Figure 3.39

→ Raises question as to what solvent best resembles partitioning in actual membranes

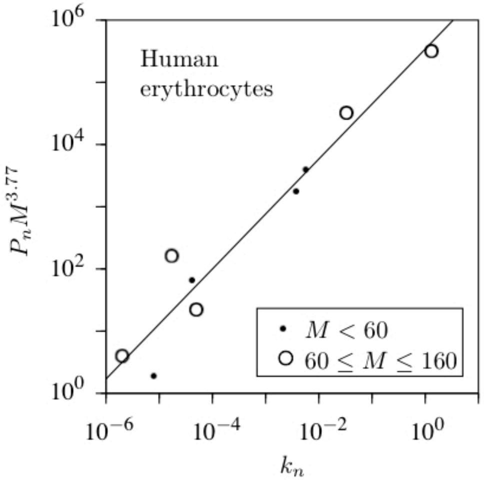


Figure 3.42

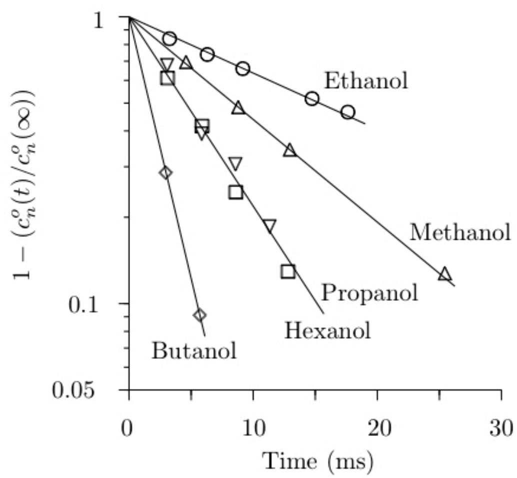


Figure 3.36

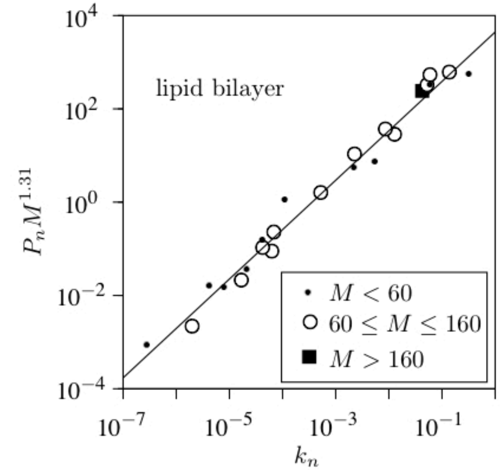


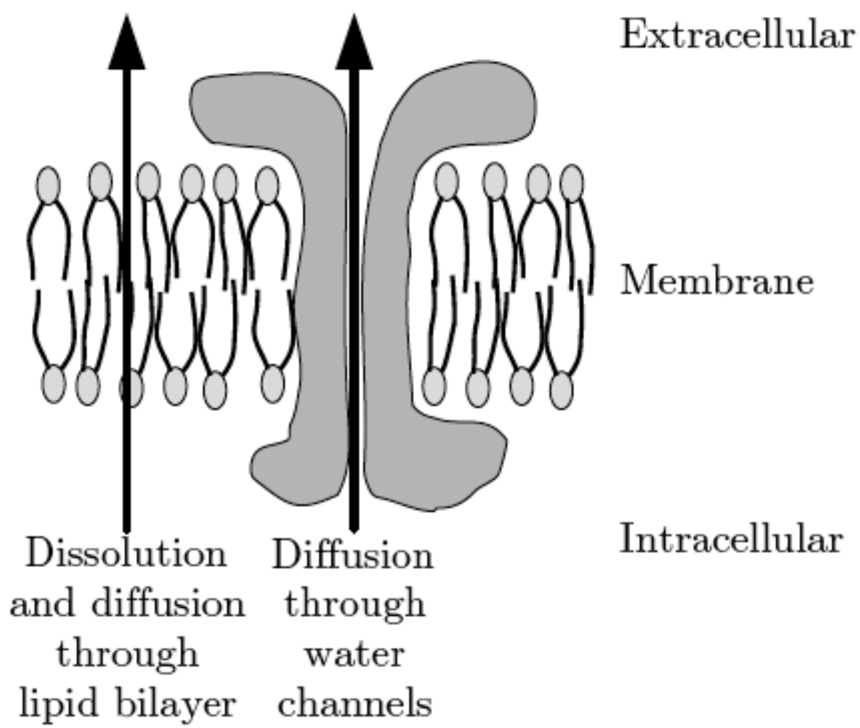
Figure 3.43

→ These figures represent the key empirical observations leading up to the deduction of what constitutes the cell membrane

- Diffusion is slow over long distances (e.g., neuron carrying information to and from the toe to the base of the spinal cord)

- So how else might things get across a cell membrane? Could such a mechanism speed up ‘transport’?

⇒ Specialized ion channels (permeability unique to different ions)



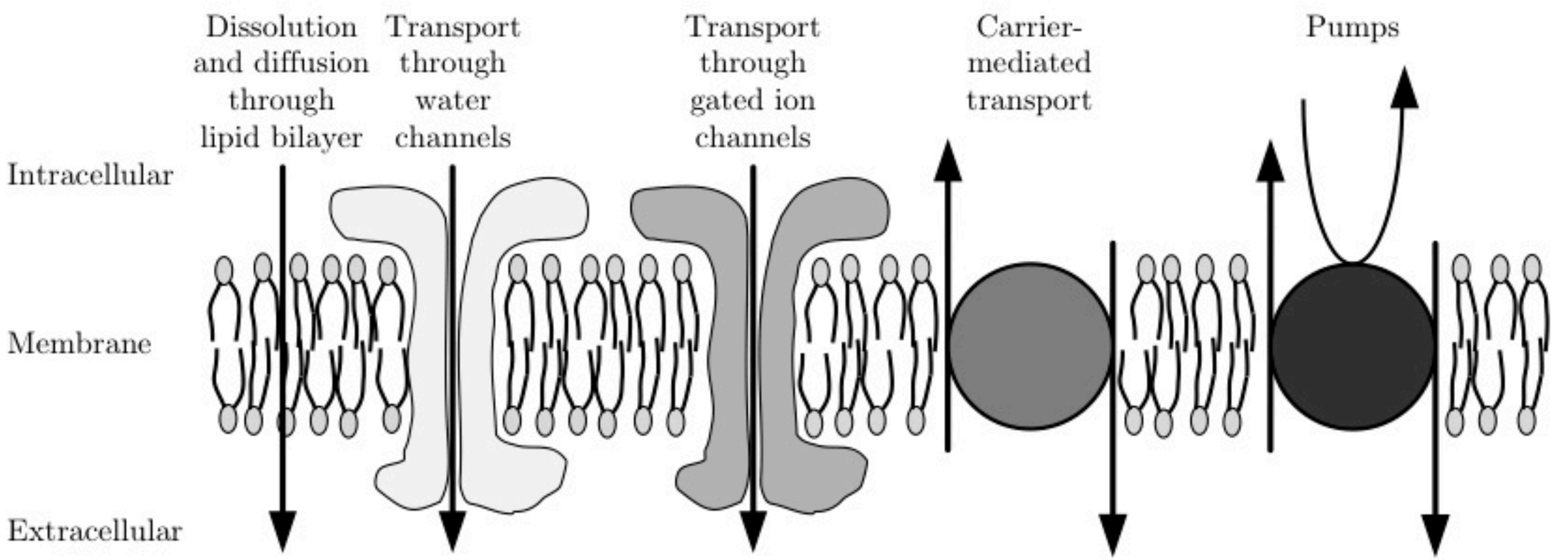
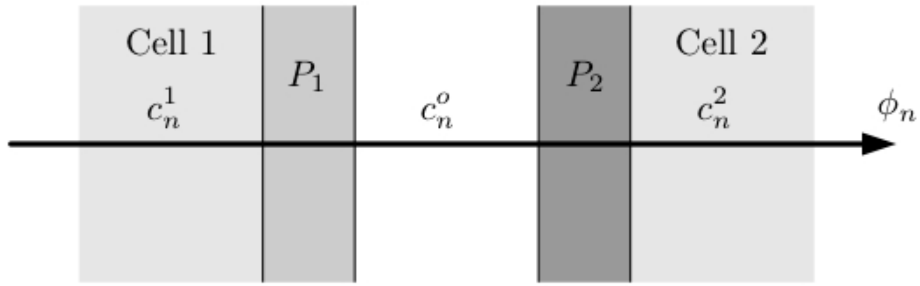

Dissolution and diffusion through lipid bilayer
Transport through water channels
Transport through gated ion channels
Carrier-mediated transport
Pumps
Intracellular
Membrane
Extracellular

Figure 2.19

Exercise



Two adjoining cells have closely apposed membranes. The concentrations of uncharged solutes n are c^1_n and c^2_n inside cells 1 and 2, respectively, and c^o_n in the intercellular space. The membrane permeabilities for this solute are P_1 and P_2 for the membranes of cell 1 and 2, respectively. Find the net permeability, P , between the inside of cell 1 and the inside of cell 2 in terms of P_1 and P_2, where

$$\phi_n = P\left(c_n^1 - c_n^2\right)$$

and φ_n is the steady-state flux of n in mol/(cm^2·s) across both membranes.