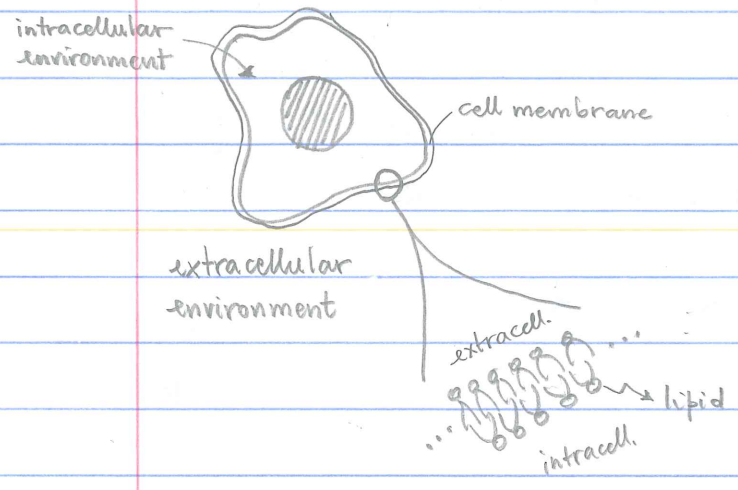
* How are substrates carried into a cell? How can we model the process?



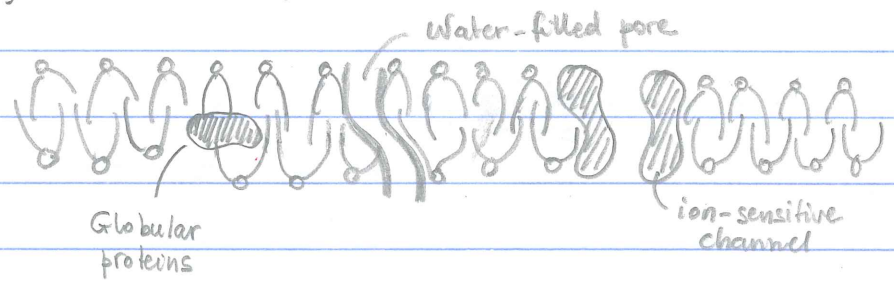
The "cell membrane" is the component that separates the internal workings of the cell (where the biochemical reactions take place) from the extracellular environment

The cell membrane is made of lipids (water-insoluble fat molecules)

⇓

It works as a "wall" preserving the internal environment from the outside

Irregularly dispersed throughout the lipidic layers (2 layers) we have:



- Globular proteins ⇒ They give the fluid-like appearance to the membrane
- Water-filled pores ⇒ They are responsible for the passage of water (H_2O) molecules, urea (a waste product), and Cl^- ions
- Ion channels ⇒ They are protein-lined pores that selectively allow the passage of specific types of ions (e.g., Ca^{++}, Na^+, K^+)

The presence of pores and channels suggests that the cell membrane implements a variety of mechanisms to prevent the free flow of ions and to maintain the concentration differences of these ions

Given the configuration of the cell membrane, the transport of molecules and ions through the cell membrane may occur in one of these ways:

- **Diffusion**: It is a passive (i.e., no consumption of energy) transport driven by the concentration gradient

  It applies to small molecules passing through pores

  It applies to water (H_2O molecules) and it is called "OSMOSIS"

- **Facilitated Diffusion**: It is a diffusion process that becomes amplified by a biochemical reaction that occurs in the diffusing medium

- **Carrier-mediated Transport**: It is a diffusion process wherein the transported molecule is insoluble in the cell membrane but it passes by bonding to special proteins (carriers) ⇒ Ex: transport of glucose

- **Active Transport (Pump)**: It is a process that consumes energy to transport molecules against the concentration gradient

- ## Models of Diffusion

Because diffusion is only driven by concentration gradient, it must obey a conservation law, i.e., for any chemical substance U inside the cell it must be possible writing:

$$\Delta U = \Delta U_p + \Delta U_a \qquad (*)$$

ΔU: change in amount of U

ΔU_p: amount of U produced or consumed in the cell

ΔU_a: amount of U transported in or out of the cell

Note that ΔU_p and ΔU_a can be either positive or negative, depending on whether there is a net production or consumption of U inside the cell (ΔU_p) and wheter there is a net accumulation or removal of U inside the cell (ΔU_a), respectively

Because it is a conservation law, (*) must be satisfied at any time t. Hence, it must hold in the differential form:

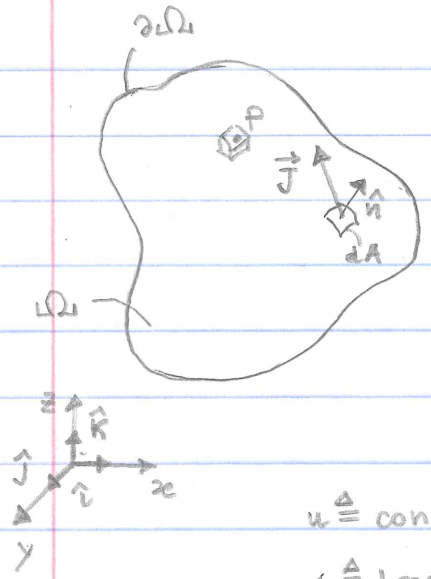
$$\frac{dU}{dt} = \frac{dU_p}{dt} + \frac{dU_a}{dt}$$

$\frac{dU}{dt}$: rate of change of U

$\frac{dU_p}{dt}$: rate of production/consumption of U

$\frac{dU_a}{dt}$: rate of accumulation/removal of U due to transport

Now let us assume that a cell has a generic volume Ω in the 3D space and let us assume that the cell membrane can be approximated as a boundary $\partial\Omega$ (i.e., no thickness for the lipidic bi-layer). Then we have:



$$U = \int_{\Omega} u \, dV$$

$$\frac{dU_P}{dt} = \int_{\Omega} f \, dV$$

$$\frac{dU_a}{dt} = -\int_{\partial\Omega} \vec{J} \cdot \hat{n} \, dA$$

$u \triangleq$ concentration $[U]$ of U within Ω

$f \triangleq$ production/consumption density of U per unit volume and unit of time

$\hat{n} \triangleq$ outward-pointing unit vector normal to surface dA

$\vec{J} \triangleq$ flux density of U (i.e., amount of U per surface unit that passes through dA per unit of time)

$$\frac{d}{dt}\int_{\Omega} u \, dV = \int_{\Omega} f \, dV - \int_{\partial\Omega} \vec{J} \cdot \hat{n} \, dA$$

Because $\vec{J}$ is typically smooth over the surface $\partial\Omega$ (i.e., the vector field is continuously differentiable), then we can write:

(Divergence Theorem) $$\int_{\partial\Omega} \vec{J} \cdot \hat{n} \, dA = \int_{\Omega} \nabla \cdot \vec{J} \, dV$$

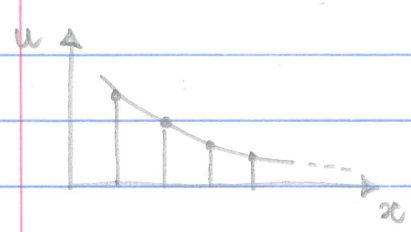
$\nabla \cdot \vec{J} \triangleq$ divergence of $\vec{J}$, i.e., it is defined as:

$$\nabla \cdot \vec{J} \triangleq \frac{\partial J_x}{\partial x} + \frac{\partial J_y}{\partial y} + \frac{\partial J_z}{\partial z} \quad \text{where } \vec{J} \triangleq J_x \hat{i} + J_y \hat{j} + J_z \hat{k}$$

The use of the divergence theorem allows us to drop the integral, i.e., regardless of what shape Ω has, we can always write:

$$\frac{du}{dt} = f - \nabla \cdot \vec{J} \qquad (1)$$

Let us focus on the flux density of U in one direction, e.g., J_x along the x-direction in the Cartesian coordinate system, at time t:



If the concentration u of U decreases with x but there is no different consumption or production of U along the x-axis, then the variation of u must entirely depend on J_x

In particular: $\frac{\partial u}{\partial x} < 0 \Rightarrow J_x > 0$ (flux is out going)

$\frac{\partial u}{\partial x} > 0 \Rightarrow J_x < 0$ (flux is incoming)

In many cases, the following assumption can be made:

$$J_x = -\underset{\text{constant}}{D} \frac{\partial u}{\partial x}$$

And more in general:

$$\underbrace{\vec{J} = -D \frac{\partial u}{\partial x}\hat{i} - D \frac{\partial u}{\partial y}\hat{j} - D \frac{\partial u}{\partial z}\hat{k} = -D \overset{\text{concentration gradient}}{\vec{\nabla} u}}_{\text{Fick's Law}}$$

Under the Fick's Law one can write (1) as below:

$$\frac{du}{dt} = f - \nabla \cdot \vec{J} = f + \nabla \cdot (\vec{\nabla} u) =$$

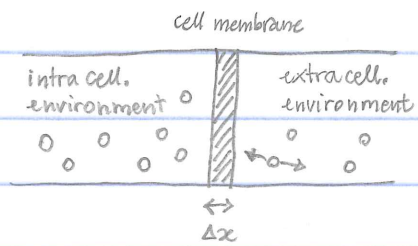
$$= f + \frac{\partial}{\partial x}\left(D \frac{\partial u}{\partial x}\right) + \frac{\partial}{\partial y}\left(D \frac{\partial u}{\partial y}\right) + \frac{\partial}{\partial z}\left(D \frac{\partial u}{\partial z}\right) =$$

$$= f + D\left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial z^2}\right) =$$

$$= f + D \underbrace{\nabla^2 u}_{\text{Laplacian}} \quad (2)$$

The Key-assumption of the Fick's Law is that the relationship between J_x and $\frac{\partial u}{\partial x}$ is linear and that the constant D applies to any other component of $\vec{J}$. An intuitive explanation is here:

The Fick's Law has a thermodynamic origin:



- A molecule would be free to move in a completely random way if in isolation with a velocity that depends on:
  - temperature ($T \uparrow \Rightarrow v \uparrow$)
  - radius of the molecule ($a \uparrow \Rightarrow v \downarrow$)

- In a more realistic situation, the moving molecule will hit other molecules while moving, thus transferring some kinetic energy and deflecting the direction of the movement (Brownian motion)

- If we focus on the overall behavior of all the available molecules, we will see that those in the regions with higher concentration will likely collide more often and be deflected more frequently $\Rightarrow$ The direction where the collision is least probable is opposite to the direction of the concentration gradient

- In a more realistic situation, the velocity of the molecules is also influenced by:
  - friction of the molecule ($\mu_f \uparrow \Rightarrow v \downarrow$)
  - density of the molecule ($\rho \uparrow \Rightarrow v \uparrow$)

As a result, studies have shown that D relates to the velocity of the molecules and it is therefore:

$$D \propto T, \rho, \frac{1}{\mu_f}, \frac{1}{a}$$

For instance: $D = \frac{kT}{\mu_f}$ with $k \triangleq \frac{R}{N_A}$

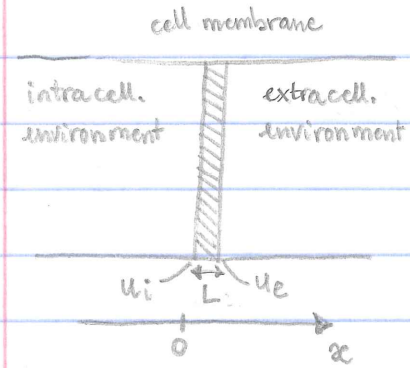
In case of a spherical molecule: $\mu_f = 6\pi a \underset{\substack{\uparrow \\ \text{viscosity} \\ \text{coefficient}}}{\mu}$ $\quad M = \frac{4}{3}\pi a^3 \underset{\substack{\uparrow \\ \text{mass of} \\ \text{molecule}}}{\rho} \Rightarrow$

$$\Rightarrow \frac{1}{\mu_f} = \frac{1}{6\pi\mu} \cdot \left(\frac{4}{3}\pi \rho / M\right)^{1/3} = \frac{1}{3\mu}\left(\frac{\rho}{6\pi^2 M}\right)^{1/3}$$

$\Rightarrow$ Hence: $D = \frac{kT}{3\mu}\left(\frac{\rho}{6\pi^2 M}\right)^{1/3}$

Let us now consider a simple unidimensional scenario:

8



Assumptions:

a) The concentration of U depends on x only, i.e., $u = u(x)$

b) The width L of the cell membrane is small (e.g., zoom in around a pore)

c) No production/consumption of U, i.e., $f = 0 \quad \forall x$

$u_i \triangleq u(x=0)$ - at the interface cell membrane - intracellular environment

$u_e \triangleq u(x=L)$ - at the interface cell membrane - extracellular environment

First, we write the conservation law:

$$\frac{\partial u}{\partial t} = +D\nabla^2 u \iff \frac{\partial u}{\partial t} = D\frac{\partial^2 u}{\partial x^2}$$

Second, we solve the equation under steady-state conditions (SSC):

$$\text{SSC}: \frac{\partial u}{\partial t} = 0 \Rightarrow D\frac{\partial^2 u}{\partial x^2} = 0 \Rightarrow J = -D\frac{\partial u}{\partial x} = \text{constant}$$

At SSC the flux density is the same everywhere along the x-axis.

Hence: $$\frac{\partial u}{\partial x} = \underbrace{-\frac{J}{D}}_{\text{constant}} \Rightarrow \int_0^L \frac{\partial u}{\partial x}\,dx = -\frac{J}{D}\int_0^L dx$$

$$\Rightarrow u(x=L) - u(x=0) = -\frac{J}{D}(L-0) \Rightarrow u_e - u_i = -\frac{JL}{D}$$

Assuming that u_e, u_i, D are measured and L is known, we can determine

the flux density: $J = \frac{D}{L}(u_i - u_e)$

Note: L/D is a measure of the resistance of the membrane to the passage of the molecules:

$L \uparrow \Rightarrow J \downarrow$, i.e., the resistance increases

$D \uparrow \Rightarrow J \uparrow$, i.e., the resistance decreases

If we define: $R_d \triangleq \frac{L}{D}$ - membrane resistance

Then: $\frac{D}{L} = 1/R_d$ - conductance per unit area $\Rightarrow$ $J = \underbrace{\frac{1}{R_d}(u_i - u_e)}$

It is formally like the Ohm's law with J instead of I (current) and u instead of V (voltage)

- Models of Facilitated Diffusion

The utility of a model based on Conservation Law and Fick's Law is that we can drop the integral and focus on instantaneous relationships $\Rightarrow$ The implicit assumption (which is carried by the smoothness of $\vec{J}$) is that the membrane has a uniform behavior $\Rightarrow$ This mainly applies to molecules that can pass through the lipidic layers (e.g., O_2, CO_2)

Some of these molecules can also bind to proteins before diffusing and this phenomenon can enhance the amount of molecules diffused

Ex: Muscle Respiration

O_2

Diffusion into muscle fibers

Reaction with myoglobin (Mb) and diffusion as oxymyoglobin (MbO_2)

Two processes to be modelled:

- Diffusion of O_2
- Reaction: $O_2 + Mb \underset{k_-}{\overset{k_+}{\rightleftharpoons}} MbO_2$ and diffusion of MbO_2

Let us show the modeling paradigm for the facilitated diffusion of O_2 in muscle fibers. The paradigm can then be applied to other examples of facilitated diffusion

Step 1: Let us model the biochemical reaction

$$Mb + O_2 \underset{k_-}{\overset{k_+}{\rightleftharpoons}} MbO_2$$

$s \triangleq [O_2] \quad e \triangleq [Mb] \quad c \triangleq [MbO_2]$

$$\begin{cases} \dfrac{ds}{dt} = -k_+ se + k_- c \\[2ex] \dfrac{de}{dt} = -k_+ se + k_- c \qquad (**) \\[2ex] \dfrac{dc}{dt} = k_+ se - k_- c \end{cases}$$

Step 2: Model (**) does not take into account that the rate of change of s, e, c also depend on the diffusion process $\Longrightarrow$ Modify model

(**) by using the Fick's Law and Conservation Law:

$$\begin{cases} \dfrac{ds}{dt} = D_s \dfrac{\partial^2 s}{\partial x^2} - (k_+ se - k_- c) \\[2ex] \dfrac{de}{dt} = D_e \dfrac{\partial^2 e}{\partial x^2} - (k_+ se - k_- c) \\[2ex] \dfrac{dc}{dt} = D_c \dfrac{\partial^2 c}{\partial x^2} + (k_+ se - k_- c) \end{cases} \qquad (***)$$

Note that we are still assuming that the diffusion occurs along the x-axis and that myoglobin may diffuse both bound to O_2 and unbound

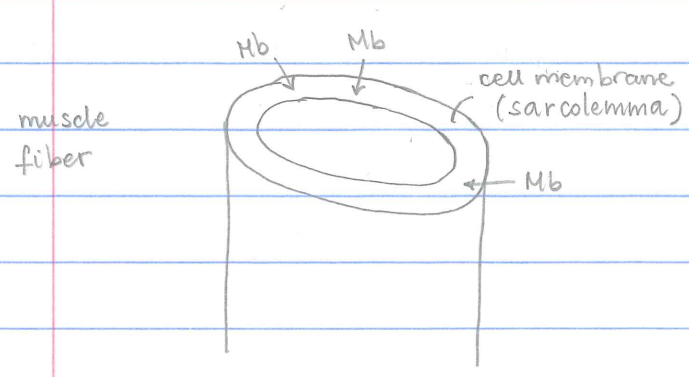
Note that $D_s \gg D_e$ and $D_e \cong D_c$ because Mb molecules are much larger than O_2 molecules. Typical values are:

$D_s = 1.2 \times 10^{-5}\ cm^2/s$

$D_e = 4.4 \times 10^{-7}\ cm^2/s$

<u>Step 3</u>: Let us use model (***) to understand whether this facilitated diffusion process brings more O_2 into the muscle fibers than just diffusion

$$\frac{de}{dt} + \frac{dc}{dt} = \frac{\partial}{\partial x}\left(D_e \frac{\partial e}{\partial x} + D_c \frac{\partial c}{\partial x}\right)$$



In the muscle fiber, Mb molecules are mainly in the sarcolemma

$\Downarrow$

$$\left.\frac{\partial e}{\partial x}\right|_{x=0,L} = \left.\frac{\partial c}{\partial x}\right|_{x=0,L} = 0$$

We therefore have:

$$\left.\frac{de}{dt} + \frac{dc}{dt}\right|_{x=0,L} = 0 \Rightarrow$$ The total amount of Mb is conserved. Hence:

$e + c = e_0 - \text{constant} \Rightarrow$ We can drop one equation in (***)

By combining 1st and 3rd equation in (***) we have:

$$\frac{ds}{dt} + \frac{dc}{dt} = D_s \frac{\partial^2 s}{\partial x^2} + D_c \frac{\partial^2 c}{dx^2}$$

At steady-state conditions we have:

$$\frac{ds}{dt} + \frac{dc}{dt} = 0 \Rightarrow \frac{\partial}{\partial x}\left(D_s \frac{\partial s}{\partial x} + D_c \frac{\partial c}{\partial x}\right) = 0 \Rightarrow J = -D_s \frac{\partial s}{\partial x} - D_c \frac{\partial c}{\partial x} = \text{const.}$$

By integrating by parts we have:

$$\int_0^L J\,dx = -D_s \int_{s(0)}^{s(L)} ds - D_c \int_{c(0)}^{c(L)} dc$$

$s_0 \triangleq s(0)$ $\quad c_0 \triangleq c(0)$

$s_L \triangleq s(L)$ $\quad c_L \triangleq c(L)$

$$JL = -D_s(s_L - s_0) - D_c(c_L - c_0) \Rightarrow J = \frac{D_s}{L}(s_0 - s_L) + \frac{D_c}{L}(c_0 - c_L)$$

This relationship is not enough yet because c_0 and c_L are unknown. So let us go back to the equations:

$$\frac{ds}{dt} = 0 \Leftrightarrow D_s \frac{\partial^2 s}{\partial x^2} - (k_+ s e - k_- c) = 0 \underset{e = e_0 - c}{\Leftrightarrow} D_s \frac{\partial^2 s}{\partial x^2} = k_+ s e_0 - (k_+ s + k_-) c$$

$$\frac{D_s}{k_+ e_0}\frac{\partial^2 s}{\partial x^2} = s\left(1-\frac{c}{e_0}\right) - \frac{k_-}{k_+}\frac{c}{e_0}$$

Analogously, we have

$$\frac{dc}{dt}=0 \iff D_c\frac{\partial^2 c}{\partial x^2} + \left(k_+ s e - k_- c\right) = 0 \iff D_c\frac{\partial^2 c}{\partial x^2} = -k_+ s e_0 + \left(k_+ s + k_-\right)c$$

$$\iff \frac{D_c}{k_+ e_0}\frac{\partial^2 c}{\partial x^2} = -s\left(1-\frac{c}{e_0}\right) + \frac{k_-}{k_+}\frac{c}{e_0}$$

$$\left.\begin{array}{l} D_s = 1.2\times 10^{-5}\ cm^2/s \\ k_+ = 1.4\times 10^{10}\ cm^3/Ms \\ k_- = 11\ s^{-1} \\ e_0 = 1.2\times 10^{-5}\ M/cm^3 \\ D_c \cong D_e = 4.4\times 10^{-7}\ cm^2/s \end{array}\right\} \Rightarrow \begin{array}{l} \dfrac{D_s}{k_+ e_0} \cong 7.1\times 10^{-10}\ cm^2 \Rightarrow \dfrac{D_s}{k_+ e_0}\dfrac{\partial^2 s}{\partial x^2} \cong 0 \\[2ex] \dfrac{D_c}{k_+ e_0} \cong 2.6\times 10^{-12}\ cm^2 \Rightarrow \dfrac{D_c}{k_+ e_0}\dfrac{\partial^2 c}{\partial x^2} \cong 0 \end{array}$$

Therefore, O_2 and Mb are in quasi-steady state throughout the medium. We can write:

$$s\left(1-\frac{c}{e_0}\right) - \underbrace{\frac{k_-}{k_+}}_{k_{eq}}\frac{c}{e_0} = 0 \iff e_0 s - sc - k_{eq}c = 0 \iff c = \frac{e_0 s}{s + k_{eq}}$$

Let us replace this formula for $c(x)$ into the formula for J:

$$J = \frac{D_s}{L}\left(s_0 - s_L\right) + \frac{D_c}{L}\left(c_0 - c_L\right) =$$

$$= \frac{D_s}{L}\left(s_0 - s_L\right) + \frac{D_c e_0}{L}\left(\frac{s_0}{s_0 + k_{eq}} - \frac{s_L}{s_L + k_{eq}}\right)$$

$$J = \frac{D_s}{L}(s_o - s_L)\left(1 + \frac{D_c e_o}{D_s}\,\frac{1}{s_o - s_L}\,\frac{\cancel{s_o s_L} + s_o K_{eq} - \cancel{s_o s_L} - s_L K_{eq}}{(s_o + K_{eq})(s_L + K_{eq})}\right)$$

$$J = \frac{D_s}{L}(s_o - s_L)\left(1 + \frac{D_c}{D_s}\,\frac{e_o K_{eq}}{(s_o + K_{eq})(s_L + K_{eq})}\right)$$

Note this:

$$1 + \frac{D_c}{D_s}\,\frac{e_o K_{eq}}{(s_o + K_{eq})(s_L + K_{eq})} = 1 + \overbrace{\underbrace{\frac{D_c e_o}{D_s K_{eq}}}_{>0}}^{\rho} \cdot \overbrace{\underbrace{\frac{K_{eq}^2}{s_o s_L + (s_o + s_L)K_{eq} + K_{eq}^2}}_{>0}}^{\mu}$$

Hence the flux density J is augmented by the fraction $\rho \cdot \mu$ because of the reaction between O_2 and Mb. In fact, if no myoglobin were present, then $D_c = 0 \Rightarrow \rho = 0 \Rightarrow O_2$ would diffuse only passively and the flux density would be:

$$J_1 = \frac{D_s}{L}(s_o - s_L) < J$$

Note that for the values given above, we have:

$$K_{eq} \triangleq \frac{k_-}{k_+} = 7.9 \times 10^{-10}\ M/cm^3$$

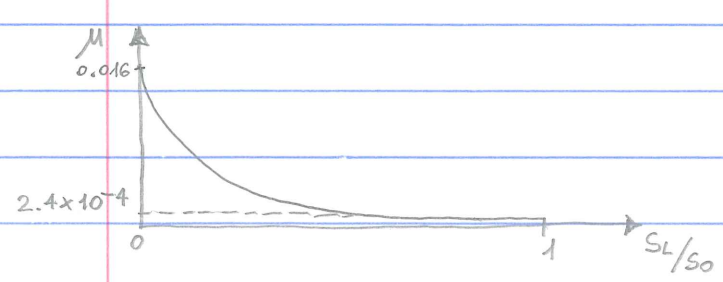
$$\frac{D_c e_o}{D_s K_{eq}} \cong 560$$

μ - non-trivially depends on the ratio s_L/s_o

For instance, a plot of possible values is obtained in matlab by using:

$k_{eq} = 7.9 \times 10^{-10}$ M/cm^3

$S_0 = 5.0 \times 10^{-8}$ M/cm^3.



Hence, provided that $S_L/S_0 \ll 1$, there is still enhancement, i.e., $\rho\mu > 1$ ∎

REFERENCE:

Textbook (volume 1): chapter 1, sec. 2.1 - 2.2. - 2.2.1 - 2.2.2 - 2.2.3 - 2.3 (till page 61)