Freezing cells

October 17, 2023

Abstract

This exercise will lead you to the research front where you will construct and solve models that use and explain some recent advances. Large parts of biological research and regenerative medicine relies on freezing cells and thawing them again. SciGroup, a research company that does their research in the Physics building on Blindern is trying to develop new methods to freeze cells without damaging them.

Try to answer all tasks that are indicated by first word in bold face. Do not copy from this text, but formulate your answers in full sentences, equations and figures with full explanations. You should make assumptions and simplify the problems so that you can do at least order of magnitude calculations or qualitative explanations. Specify clearly your assumptions and simplifications.

1 Osmosis and cell membranes

A cell is a "bag" containing water, ions, large molecules like proteins and "organelles" (structures that are separated from the liquid in the cell). The "bag" is made of a soft membrane that is permeable to water, but where ions and larger molecules have to be transported through specialized channels. The cells are small and soft and have the same temperature, T, and (most often) the same pressure, p as it's environment.

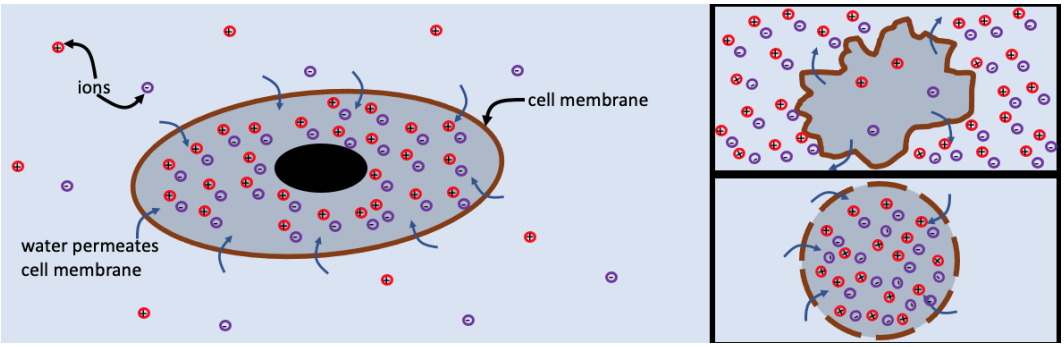


Figure 1: Cell in saline water. Ions can not permeate the cell membrane, but the water molecules can (blue arrows). When the solute (ion) concentration is high inside the cell this drives water permeation into the cell creating a pressure difference that can rupture the cell membrane (lower right image). If the ion concentration is higher outside the cell the water will permeate out of the cell and the cell will shrink and the soft cell membrane will buckle.

Osmosis is the motion of solvent molecules (in our case: water molecules) through a semi-permeable membrane from a region of low solute concentration to a region of high solute concentration. We will study what happens to the cell when it comes in contact with water with a different solute concentration. We will designate the permeable water molecules by 1 and other species are enumerated 2,...,ν.

When does water stop flowing through the cell membrane? Write down the corresponding equilibrium condition.

Solution:
The water can permeate the membrane wereas the other molecules can not. The driving force for water transport through the membrane is therefore the chemical potential of water, not the concentrations of water or ions. The equilibrium condition, that is, water stops flowing through the membrane when the difference in chemical potential of water over the membrane is zero:

$$\Delta\mu_1 = (\mu_{1,in} - \mu_{1,out}) = 0. \tag{1}$$

Why is equation (2) the right definition of chemical potential to use in the case of osmotic flow out of a cell?

$$\mu_i = \left(\frac{\partial G}{\partial N_i}\right)_{p,T,N_{j\neq i}}, \tag{2}$$

where G is the Gibbs free energy and N_i is the number of particles of species i.

Solution:
Since all processes inside the cell happen at constant T and p we have to use the derivative of the Gibbs free energy to evaluate the chemical potential:

Use the total differential of the Gibbs free energy

$$G(N,\mu) = \sum_{i=1}^{\nu} N_i\mu_i, \tag{3}$$

and the thermodynamic identity for $G(p,T,N)$ to show that

$$\sum_{i=1}^{\nu} N_i d\mu_i = -SdT + Vdp, \tag{4}$$

Where S is entropy, and V is the volume. Comment the result.

Solution:
The chemical potential is the partial Gibbs free energy with respect to the number of particles. Therefore the Gibbs free energy of the system can be calculated by adding together molecules at the specified T and p and constant ratio of the components so that the chemical potential does not change (see explanation of eq (5.37) in Schroeder):

$$G = \sum_{i=1}^{\nu} N_i\mu_i. \tag{5}$$

The total differential of G is then:

$$dG = \sum_{i=1}^{\nu} \mu_i dN_i + \sum_{i=1}^{\nu} N_i d\mu_i. \tag{6}$$

We may equate this by the thermodynamic identity of the Gibbs free energy

$$dG = -SdT + Vdp + \sum_{i=1}^{\nu} \mu_i dN_i \tag{7}$$

which yields the Gibbs-Duhem relation:

$$\sum_{i=1}^{\nu} \mu_i dN_i + \sum_{i=1}^{\nu} N_i d\mu_i = -SdT + Vdp + \sum_{i=1}^{\nu} \mu_i dN_i \tag{8}$$

$$\sum_{i=1}^{\nu} N_i d\mu_i = -SdT + Vdp. \tag{9}$$

This relation says that when there are ν components in a system only $\nu - 1$ components have independent values for the chemical potential.

Use this to derive an expression for the osmotic pressure Δp at a constant temperature in terms of the differences in chemical potential of the solutes, $\Delta\mu_i, i \in \{2, \ldots, \nu\}$.

Solution:
(Alternatively:
Use this to derive this expression for the osmotic pressure:

$$\Delta p = \sum_{i=2}^{\nu} n_i \Delta\mu_i. \tag{10}$$

.)
In an isothermal system $dT = 0$ and we can rewrite this as

$$dp = \sum_{i=1}^{\nu} n_i d\mu_i, \tag{11}$$

where $n_i = N_i/V$ are the number densities (or concentrations) of the species i. We may now write the chemical potential for water (species 1):

$$d\mu_1 = \frac{1}{n_i}(dp - \sum_{i=2}^{\nu} n_i d\mu_i) \tag{12}$$

We may now return to the osmosis over the cell membrane and express this as differences Δ between inside and outside the cell:

$$\Delta\mu_1 = \frac{1}{n_i}(\Delta p - \sum_{i=2}^{\nu} n_i \Delta\mu_i) \tag{13}$$

As stated initially, equilibrium is reached when $\Delta\mu_1 = 0$ which yields an expression for the osmotic pressure:

$$\Delta p = \sum_{i=2}^{\nu} n_i \Delta\mu_i. \tag{14}$$

In ideal solutions the chemical potential can be expressed as

$$\mu_i = \mu_{i,0} + kT \ln n_i, \tag{15}$$

where $n_i = N_i/V$ is the concentration of species i.

Derive the expression for the osmotic pressure in terms of the concentration differences Δn_i assuming that the solution is ideal:

$$\Delta p = kT \sum_{i=2}^{\nu} \Delta n_i. \tag{16}$$

Solution:
Inserted in the previous expression for the osmotic pressure we get:

$$\Delta p = \sum_{i=2}^{\nu} n_i kT \Delta \ln n_i \tag{17}$$

$$= kT \sum_{i=2}^{\nu} \Delta n_i. \tag{18}$$

In the above sum we have not defined which particles are counted as solutes. Dissolving NaCl in water should we count $N_{Na^+} + N_{Cl^-}$ or only N_{NaCl}?

If we consider N independent, freely moving solute particles in a dilute solution as contributing to the pressure as in an ideal gas, the ideal gas equation of state gives:

$$\Delta p = \frac{N}{V} kT. \tag{19}$$

Use this to argue how you should count NaCl in equation (16) and relate this to the Van't Hoff formula for osmosis

$$\Delta p = \gamma c R T, \tag{20}$$

where γ is a counting factor, c the molar concentration and R the ideal gas constant. Give an argument why we may consider dilute solutes as ideal gas particles and what the counting factor γ should be for NaCl, glucose and $CaCl_2$.

Solution:
Each Na^+ ion is moving independently of each other and of the Cl^- ions although they on average have to respect electroneutrality. When they are dilute they will only move around in the sea of water and virtually never interact with other ions. The ideal gas is just that, particles moving around without interacting with each other. So the contribution of these solute particles to the pressure are additive like in the ideal gas. This argument tells us how to count in equation (16): count all independently moving particles. That means that the counting factor γ is 2 for NaCl, 1 for a glucose molecule, 3 for $CaCl_2$, etc.

A physiological saline solution with concentration $c_{2,p}$ contains 9 g (gram) NaCl per liter water has been defined to give no osmotic pressure in our cells if it is injected in our blood or for rinsing our eyes.

How large osmotic pressure would our cells reach (if they can withstand it) if they were subject to a) pure water b) seawater that has approximately 0.6 mol/l NaCl?

Solution:



- *Physiological saline solution: 9g Nacl/1000 g H2O*
- *How do we calculate molar concentration?*
- 

22 

35
- *Units? g/mol, R=8.3 J/(K mol)*
- $c_2 = \frac{9g}{(23+35)g/mol1l} = 0.15\ mol/l = 0.15\ M = 150\ mol/m^3$

$$\Delta p = 2cRT = 2 * 150 * 8.3 * 300 = 7.2 \cdot 10^5 Pa = 7.2bar$$

More than in the tyres of a racing bike!

The last is a trick question. $\Delta p = 0$, but water would be removed from the cell until the salinity inside the cell reached 0.6 M.

2 Cells at low temperature

In practically every laboratory for life science research, biological cells are stored in liquid nitrogen at -196°C. Ice formation inside the cells (intracellular ice formation, IIF) has a high probability of damaging the cells so that they will not live when they are thawed. How to avoid IIF during freezing? Basically there are two ways: 1) extremely rapid cooling that transforms water to a glass instead of crystalline ice or 2) controlled freezing of water outside the cells to remove water from inside the cells. We will study the second path. The two thermodynamic effects we will study are A) water permeation through the cell membrane due to the lower chemical potential of ice and B) freezing point depression of the solution inside the cell.

2.1 Ice driving water permeation

The cell is suspended in a physiological solution. The temperature of the cell and the surrounding solution is lowered slowly so that they have the same temperature, T, below the freezing temperature, T_0. Assume that the solution outside the cell freezes and the solution inside the cell is still liquid. Since only the water permeates we will simplify to consider pure water right now.

Use the thermodynamic identity for the Gibbs free energy to derive the chemical potential difference between pure water inside the cell and pure ice outside the cell as function of the temperature change $\Delta T = T - T_0$:

$$\Delta\mu = -\frac{\Delta H_m}{N}\frac{\Delta T}{T}, \tag{21}$$

where $\frac{\Delta H_m}{N}$ is the latent heat of melting per molecule water.

Solution:

Consider two systems with pure water at the same temperature and pressure with no exchange of particles. At the freezing temperature, T_0 the water and ice have the same chemical potential: $\Delta\mu(T_0) = 0$. Since temperature and pressure is the same in both systems the Gibbs TDI is

$$dG = Vdp - SdT + \mu dN \quad \Rightarrow \quad dG = -SdT \tag{22}$$

Now we are interested in the difference in chemical potential between the two systems as function of temperature.

$$T = T_0 \qquad \Delta_{ls}\mu(T_0) = \mu_l(T_0) - \mu_s(T_0) = 0 \tag{23}$$
$$T < T_0 \qquad \Delta_{ls}\mu(T) = \mu_l(T) - \mu_s(T) = ? \tag{24}$$

For pure water $\Delta\mu = \frac{\Delta G}{N}$ *and we can write*

$$N\Delta_T\mu_l = \Delta_T G_l = G_l(T) - G_l(T_0) = -S_l\Delta T \tag{25}$$
$$N\Delta_T\mu_s = \Delta_T G_s = G_s(T) - G_s(T_0) = -S_s\Delta T \tag{26}$$
$$N(\Delta_T\mu_l - \Delta_T\mu_s) = \Delta_T G_l - \Delta_T G_s = (S_s - S_l)\Delta T = -\frac{H_m\Delta T}{T} \tag{27}$$
$$= N(\Delta_{ls}\mu(T) - \Delta_{ls}\mu(T_0)) = N\Delta_{ls}\mu(T) \tag{28}$$
$$\Delta_{ls}\mu(T) = -\frac{H_m}{N}\frac{\Delta T}{T} \tag{29}$$

Here we have used the latent heat of melting $dQ = H_m = TdS$ *that is used in the Clausius-Clapeyron relation and lab2. This is the chemical potential difference between pure ice outside the cell and pure water inside the cell.*

In reality, what will happen is that the water will permeate through the membrane until the solute concentration is large enough to balance this chemical potential difference (29).

Find an expression for the final concentration difference of solutes across the membrane by using equation (4) and assuming that the solutions are ideal.

Solution:
The Gibbs-Duhem relation

$$N_1 d\mu_1 + N_2 d\mu_2 = -SdT + Vdp. \tag{30}$$

simplifies since $dT = 0$ *and* $dp = 0$ *over the membrane:*

$$-N_1\Delta\mu_1 = N_2\Delta\mu_2. \tag{31}$$

Here we insert $\Delta\mu_1 = \Delta_{ls}\mu$ *from (29) and use the ideal solution expression for the chemical potential* $\mu_2 = \mu_{2,0} + kT\ln N_2$*:*

$$N_1\frac{\Delta H_m}{N}\frac{\Delta T}{T} = N_2 kT\Delta\ln N_2 = kT\frac{N_2\Delta N_2}{N_2} = kT\Delta N_2 \tag{32}$$
$$\Delta N_2 = \frac{\Delta H_m\Delta T}{kT^2} \tag{33}$$
$$\Delta c_2 = \frac{\Delta N_2}{N_A V} = \frac{\Delta H_m}{V}\frac{\Delta T}{RT^2} = \frac{\Delta H_{m,mol}}{V_{mol}}\frac{\Delta T}{RT^2} \tag{34}$$

Plot the final solute concentration inside the cell for temperatures down to -20°C? and comment. Use data from the reference data in Schroeder and Lab2 or find values on the internet.

Solution:
An alternative way of reaching the same solution is the following. Use the same starting point as above

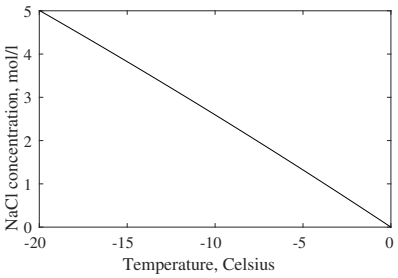


Figure 2: Solute concentration inside the cell as function of temperature when the water in the cell is in equilibrium with the ice outside the cell. The concentration is translated to NaCl concentration. The total concentration of free solute particles inside the cell is twice as high.

(equation (4)) and simplify equation (4) to use only one solute. For each phase, liquid and solid the changes when temperature is lowered are still governed by equation (4) and we can write

$$\Delta\mu_1 = \frac{1}{N_1}(-S\Delta T + V\Delta p - N_2\Delta\mu_2) \tag{35}$$

Now the water is transported out of the cell, the cell is shrinking and there is no pressure difference over the cell membrane, $\Delta p = 0$. Initially the salt concentration is the same on both sides of the membrane, that is $\Delta\mu_2 = 0$, but the water will continue to flow until $\Delta\mu_1 = 0$. Thus for each phase

$$\Delta\mu_{1,ini} = \frac{-S\Delta T}{N_1} \tag{36}$$

$$\Delta\mu_{2,fin} = \frac{-S\Delta T}{N_2} \tag{37}$$

Thus the final difference in chemical potential between the liquid in the cell and the ice outside is

$$N_2\Delta\mu_2 = \Delta H_m\frac{\Delta T}{T} \tag{38}$$

$$RT\frac{N_2}{VN_A}\Delta\ln c_2 = \frac{\Delta H_m}{N_A}\frac{\Delta T}{T}\frac{N_A}{V} \tag{39}$$

$$RT\Delta c_2 = \frac{\Delta H_{m,mol}}{V_{mol}}\frac{\Delta T}{T} \tag{40}$$

$$c_{NaCl} = c_2/2 = 0.15\ M + \frac{\Delta H_{m,mol}}{2RV_{mol}}\frac{\Delta T}{T^2} = 5.7\ M \tag{41}$$

This is approximately the solubility of NaCl in water at room temperature and basically means removing all free water inside the cell. But this is outside the validity of the dilute solution approximation that we have used so a real treatment will need to be done with a complete solution model.

2.2 Freezing point depression

It is difficult to determine the exact mechanisms of damage to freezing cells. In order to get closer to an answer we must explore all mechanisms we understand. Freezing point depression has been mentioned and is something we can calculate.

The change in freezing point, $\Delta T_{fpd} = T_0 - 273.15$ K due to adding N_2 solute particles is (see problem 81 in Schroeder)

$$\Delta T = -\frac{\gamma_2 N_2 k T}{\Delta S_m} = -\frac{\gamma_2 N_2 k T^2}{\Delta H_m}, \tag{42}$$

where ΔS_m and ΔH_m are the entropy and heat (enthalpy) of melting that you measured in Lab2 and γ is the same counting factor as before.

What is the freezing temperature of a human cell at normal physiological salinity, $c_{2,p}$?

Assume that you are starting with a physiological solution concentration, $c_{2,p}$ both outside and inside your cells. As the temperature is lowered $\Delta T_{\text{imposed}} = T - T_0$ below the freezing point, T_0, of the physiological solution, water is driven out of the cell and thereby changing the concentration $\Delta c_2 = c_2 - c_{2,p}$ inside the cell.

How large is the resulting freezing point depression ΔT_{fpd} as a function of T_{imposed}?

What can this tell us about freezing cells while avoiding intercellular ice formation?

Solution:

In Lab2 we measured $\Delta H_{m,mol} = 6$ kJ/mol. We transform equation (42) to molar concentration and simplify with $N = \sum_j \gamma_j N_j$:

$$\Delta T = -\frac{\frac{N}{VN_A} N_A k T^2}{\frac{N_A}{V}\Delta\frac{H_m}{N_A}} = -\frac{V_{mol} c R T^2}{\Delta H_{m,mol}} \tag{43}$$

$$= -\frac{18\cdot 10^{-3}\ l/mol\ 0.3\ M\ 8.3\ kJ/mol\ K\ 273^2\ K}{6\ kJ/mol} = -0.56\ K, \tag{44}$$

where N_1 is the number of water molecules, $c = N/(VN_A)$ is the molar concentration (M), Physiological saline solution has $c = 0.3$ M ($\gamma = 2$ is included). The molar volume of water $V_{mol} = 18\cdot 10^{-3}$ can be found in the reference data of Schroeder. Thus the freezing temperature of a cell is -0.56° C. Experimentally (Johlin, J Biol Chem **91**,*551,(1931)) it is found that for 0.2 M NaCl solution the freezing point is in the interval [-0.74 -0.69]° C. Using our formula we get -0.75° C.*

If we insert Δc_2 from (40) into (43) it yields

$$\Delta T_{fpd} = \Delta T_{imposed}. \tag{45}$$

In some sense this only says: If what's inside the cell is liquid, it is liquid. But from another point of view it says: If nucleation of ice does not happen while we are waiting for water to leave the cell and equilibrium to be established, we can always reach an equilibrium where the liquid inside the cell will not freeze. This means that the main problem of freezing is the ice nucleation probability during the time it takes to dessicate the cell. And when thawing the cell it is probably important to get the solute concentration back to normal physiological concentration before the cell is warm enough for processes to start and go wrong because of the high solute concentration.

In fact there is one process that we have skipped here: Solidification rejects solutes and increases the solute concentration in the adjacent liquid. It is thought that it is this increased solute concentration that drives osmotic flow through the cell membrane to dessicate the cell. The solute concentration in the liquid phase outside the cell depends on diffusion, how quickly the solidification front advances and the volume ratio between solution outside the cells and inside the cells. This is a highly non-equilibrium process that is much more complex to model.

2.3 Rate of water flow through the cell membrane

The permeability P of a membrane is defined

$$J = P\Delta n, \tag{46}$$

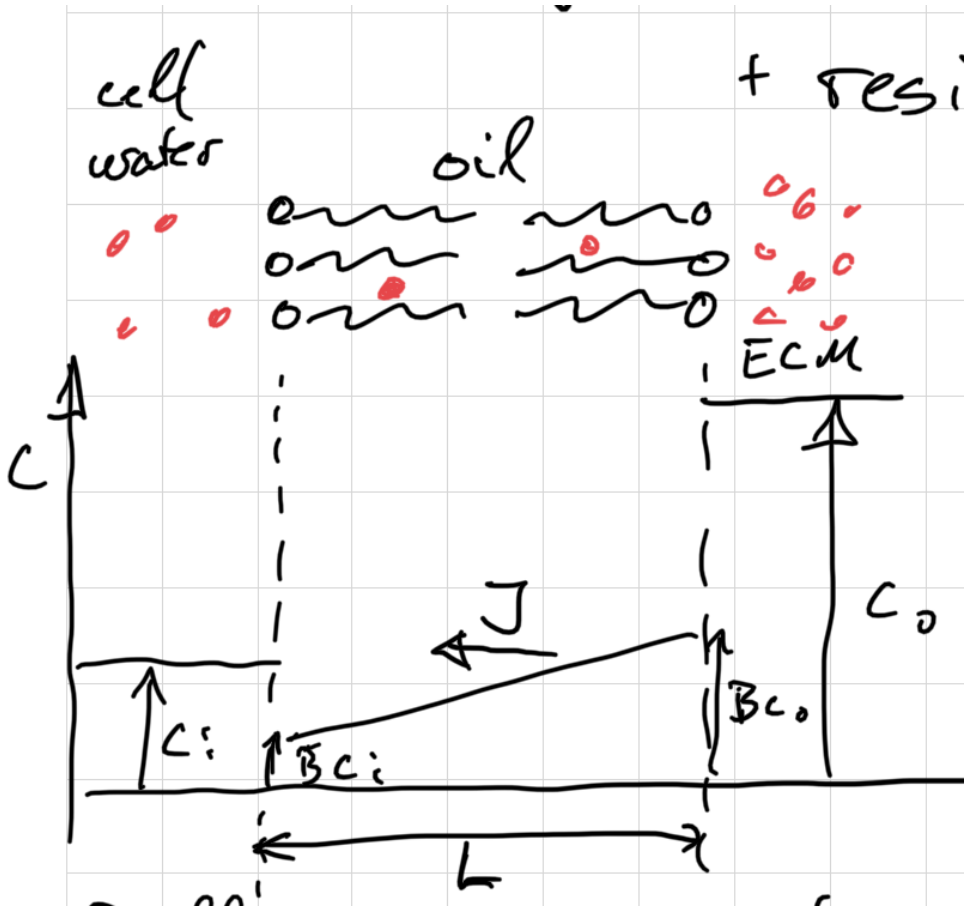


Figure 3: Diffusion through membrane with low partition coefficient.

where $n = N/V$ is the number density water. In fact, the permeation of water molecules through a membrane is diffusion through the membrane and resistance to entry/exit (we will neglect the latter). We have to take into account that the solubility of water is very low inside the cell membrane. The so-called partition coefficient B is

$$B = \frac{\text{solubility in oil}}{\text{solubility in water}} \tag{47}$$

Diffusion in membrane is

$$J = -D\frac{\Delta n_{\text{inside}}}{dx} = -\frac{DB\Delta n}{L} = P\Delta n \quad \Rightarrow \quad P = \frac{DB}{L} \tag{48}$$

Describe the flux through the membrane in terms of the chemical potential difference $\Delta\mu$, assuming that the solutions are dilute.

Solution:

$$\mu = \mu_0 + kT\ln n, \qquad \frac{d\mu}{dn} = kT\frac{d\ln n}{n} = \frac{kT}{n} \tag{49}$$

Then we get

$$J = -\frac{DB}{L}\frac{n}{kT}\Delta\mu \tag{50}$$

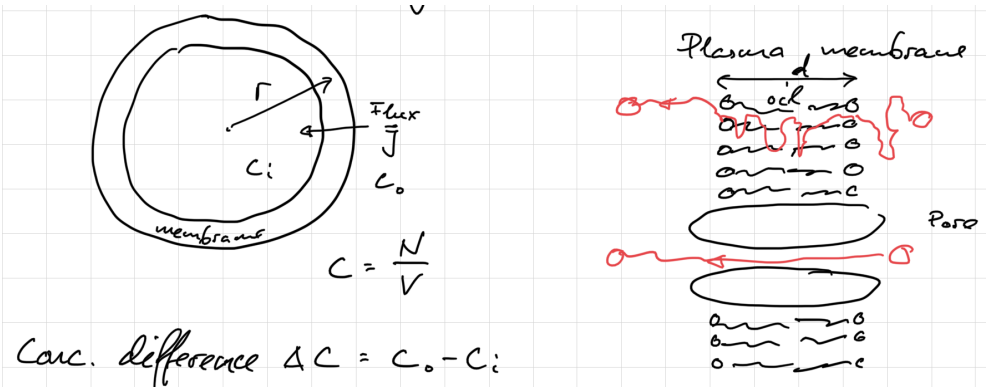


Figure 4: Permeation through a membrane.

How quickly does water flow out of a cell? Figure 4 depicts a round cell of radius r with a changing concentration n_i inside and constant concentration n_o outside. The flux (flow rate per unit area) of water through the cell membrane is:

$$J = \frac{1}{A}\frac{\Delta N_i}{\Delta t}, \tag{51}$$

where N_i is the number of water molecules inside

How does $n_i = N_i/V$ change with time? (n_0 is const.)

$$\frac{\partial n_i}{\partial t} = -\frac{\partial \Delta n}{\partial t} = \frac{1}{V}\frac{\partial N_i}{\partial t} = \frac{3}{r}J_i \tag{52}$$

because $A = 4\pi r^2$, $V = \frac{4}{3}\pi r^3$, $V/A = r/3$.

We can enter the definition of permeability into the above equation and solve:

$$-\frac{\partial \Delta n}{t} = \frac{3}{r}P\Delta n \tag{53}$$

$$\int_{\Delta n_0}^{\Delta n}\frac{\Delta n}{\Delta n} = -\frac{3P}{r}\int_0^t \partial t \tag{54}$$

$$\ln \Delta n - \ln \Delta n_0 = -\frac{3P}{t} \tag{55}$$

$$\frac{\Delta n}{\Delta n_0} = e^{-t/\tau}, \qquad \tau = \frac{r}{3P}. \tag{56}$$

Some numbers:

- Partition coefficient of water in lipid bilayer membrane: $B = 4.2 \cdot 10^{-5}$ (A Finkelstein, J. Gen. Phys. **68**, 127 (1976))
- Thickness of cell membrane: $L = 7.5 - 10$ nm. (B10NUMB3R5)
- This gives a permeability of $P = 10^{-9}4 \cdot 10^{-5}/10^{-8}$ m/s≈ 4 μm/s.
- And a time constant of permeation of water into a cell with radius 10 μm: $\tau = r/(3P) \approx 1$ s. That's pretty fast!
- Partition coefficient of monovalent ions: $B \approx 10^{-60}$. That's why cells need ion channels.