# 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.

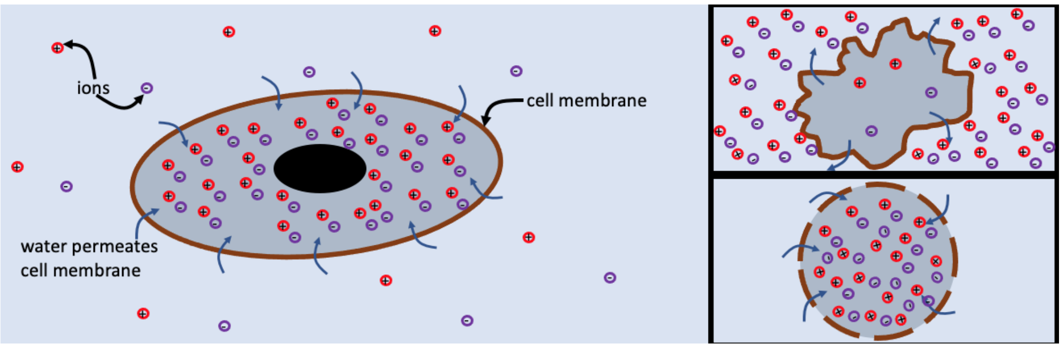

Figure 1: Cell in saline water. Ions can not permeate the cell membrane, but the water molecules can (blue arrows). When the solute (ion) concentration is high inside the cell this drives water permeation into the cell creating a pressure difference that can rupture the cell membrane (lower right image). 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,...,$\nu$.

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

**Why** is equation (1) the right definition of chemical potential to use in the case of osmotic flow out of a cell?

$$\mu_i = \left(\frac{\partial G}{\partial N_i}\right)_{p,T,N_{j\neq i}}, \tag{1}$$

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

**Use** the total differential of the Gibbs free energy

$$G(N, \mu) = \sum_{i=1}^{\nu} N_i \mu_i, \tag{2}$$

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

$$\sum_{i=1}^{\nu} N_i d\mu_i = -SdT + Vdp, \tag{3}$$

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

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

In ideal solutions the chemical potential can be expressed as

$$\mu_i = \mu_{i,0} + kT \ln n_i, \tag{4}$$

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 $\Delta n_i$ assuming that the solution is ideal:

$$\Delta p = kT \sum_{i=2}^{\nu} \Delta n_i. \tag{5}$$

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:

$$\Delta p = \frac{N}{V} kT. \tag{6}$$

**Use** this to argue how you should count NaCl in equation (5) and relate this to the Van't Hoff formula for osmosis

$$\Delta p = \gamma c R T, \tag{7}$$

where $\gamma$ 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 $\gamma$ should be for NaCl, glucose and $CaCl_2$.

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?

# 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 $\Delta T = T - T_0$:

$$\Delta\mu = -\frac{\Delta H_m}{N}\frac{\Delta T}{T}, \tag{8}$$

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

**Find** an expression for the final concentration difference of solutes across the membrane by using equation (3) and assuming that the solutions are ideal.

**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.

## 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)

$$\Delta T = -\frac{\gamma_2 N_2 kT}{\Delta S_m} = -\frac{\gamma_2 N_2 kT^2}{\Delta H_m}, \tag{9}$$

where $\Delta S_m$ and $\Delta H_m$ are the entropy and heat (enthalpy) of melting that you measured in Lab2 and $\gamma$ 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 $\Delta T_{\text{fpd}}$ as a function of $T_{\text{imposed}}$?

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

## 2.3 Rate of water flow through the cell membrane

The permeability $P$ of a membrane is defined

$$J = P\Delta n, \tag{10}$$

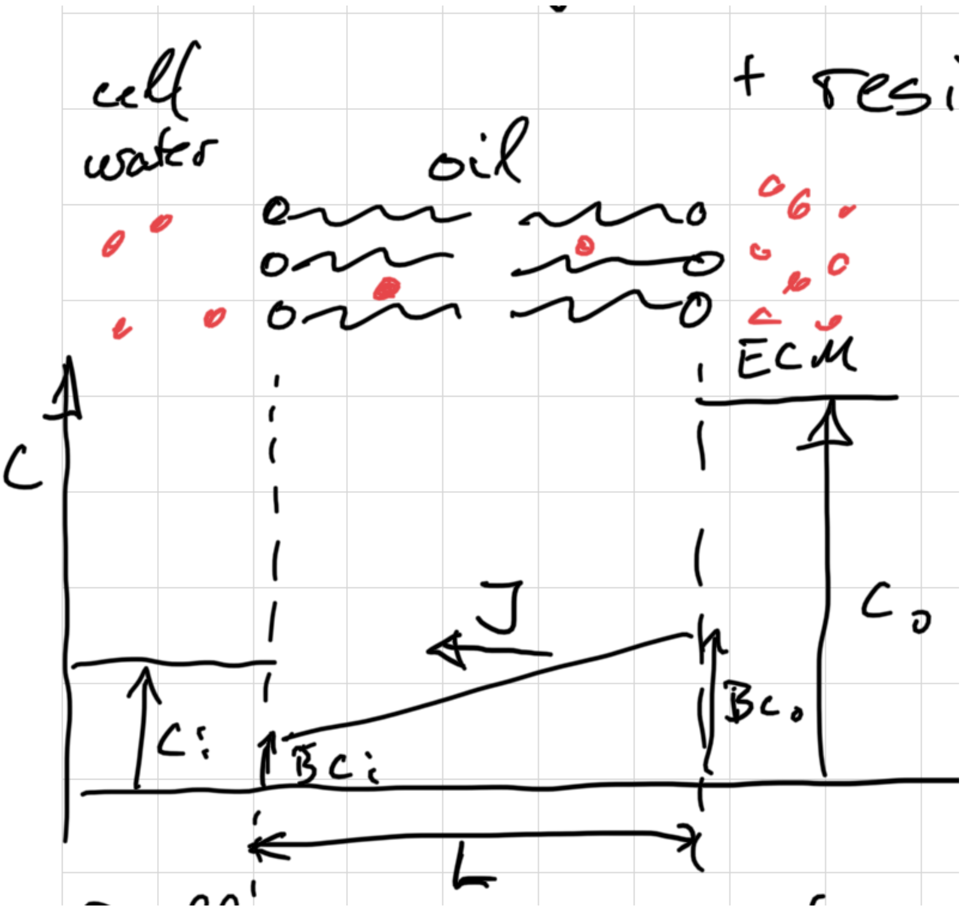

Figure 2: Diffusion through membrane with low partition coefficient.

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

$$B = \frac{\text{solubility in oil}}{\text{solubility in water}} \tag{11}$$

Diffusion in membrane is

$$J = -D\frac{\Delta n_{\text{inside}}}{dx} = -\frac{DB\Delta n}{L} = P\Delta n \quad \Rightarrow \quad P = \frac{DB}{L} \tag{12}$$

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

How quickly does water flow out of a cell? Figure 3 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:

$$J = \frac{1}{A}\frac{\Delta N_i}{\Delta t}, \tag{13}$$

where $N_i$ is the number of water molecules inside

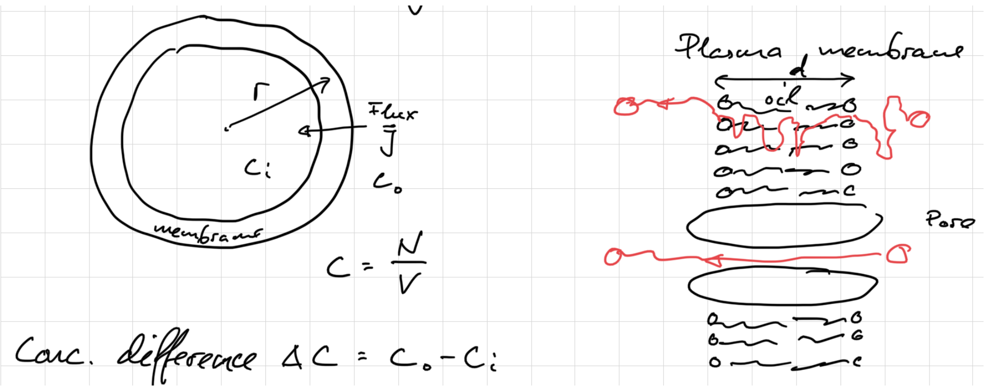

Figure 3: Permeation through a membrane.

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

$$\frac{\partial n_i}{\partial t} = -\frac{\partial \Delta n}{\partial t} = \frac{1}{V}\frac{\partial N_i}{\partial t} = \frac{3}{r}J_i \tag{14}$$

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:

$$-\frac{\partial \Delta n}{t} = \frac{3}{r}P\Delta n \tag{15}$$

$$\int_{\Delta n_0}^{\Delta n} \frac{\Delta n}{\Delta n} = -\frac{3P}{r}\int_0^t \partial t \tag{16}$$

$$\ln \Delta n - \ln \Delta n_0 = -\frac{3P}{t} \tag{17}$$

$$\frac{\Delta n}{\Delta n_0} = e^{-t/\tau}, \qquad \tau = \frac{r}{3P}. \tag{18}$$

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$\approx 4$ $\mu$m/s.
- And a time constant of permeation of water into a cell with radius 10 $\mu$m: $\tau = r/(3P) \approx 1$ s. That's pretty fast!
- Partition coefficient of monovalent ions: $B \approx 10^{-60}$. That's why cells need ion channels.