UNIVERSITY OF OSLO

Faculty of Mathematics and Natural Sciences

Exam in:	**Fys2160,**
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Exam time:	**09:00 - 17:00**
Number of pages:	**4**

The exam you deliver should be your individual product. That is, text, code and calculations must be done by yourself, even if you have collaborated with others to understand the tasks. We will compare the exams and we reserve the right to pick out candidates for an oral discussion of their exams.

Introduction - transport through cell membranes

This exam 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. The 2021 Nobel Prize in physiology and medicine was given for identifying the genes of 2 important mechanosensitive channels: Piezo1 and Piezo2. Mechanosensitive channels are important for our sensation of touch and proprioception and they regulate pressure in certain cell types to avoid that they explode from osmotic pressure.

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 slightly 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.

1 Two state model of mechanosensitive channels

Many bacteria have evolved to survive different environments, both saline water and fresh water. To avoid exploding from osmotic pressure they have mechanosensitive channels in their membrane as relief valves. You will model a mechanosensitive channel.

The channel is a two-state system with microstates open, $\sigma = 1$, and closed, $\sigma = 0$. The energy change ΔE of the membrane loading system upon opening the channel is illustrated in Figure 1.

Show and discuss the expression for the energy change assuming that the tension, τ, in the membrane does not change upon opening the channel:

$$\Delta E = -\tau \Delta A, \tag{1}$$

where ΔA is the area change of the channel in the membrane.

In addition to the work done on the system to open a channel, $W = \tau \Delta A$, the channel itself is a two state system with energies of open, ϵ_o, and closed, ϵ_c, channels and $\Delta\epsilon = \epsilon_o - \epsilon_c$.

Write the partition function of the channel and the probability of the channel being open, P_o.

The so-called patch-clamp technique allows the measurement over time of the current passing through a single channel. A large negative value means that the channel is open, a small negative value (close to zero) means that the channel is closed. Since the channel is a very small system it fluctuates and the measurements over N times yield the same probability of open and closed states as the measurement of the probability of N channels. The file `channel_current.txt` contains 9 columns of currrents through a mechanosensitive channel at 9 different tensions $\tau = \{0.20, 0.45, 0.70, 0.95, 1.20, 1.45, 1.70, 1.95, 2.20\}$ pN/nm. The area

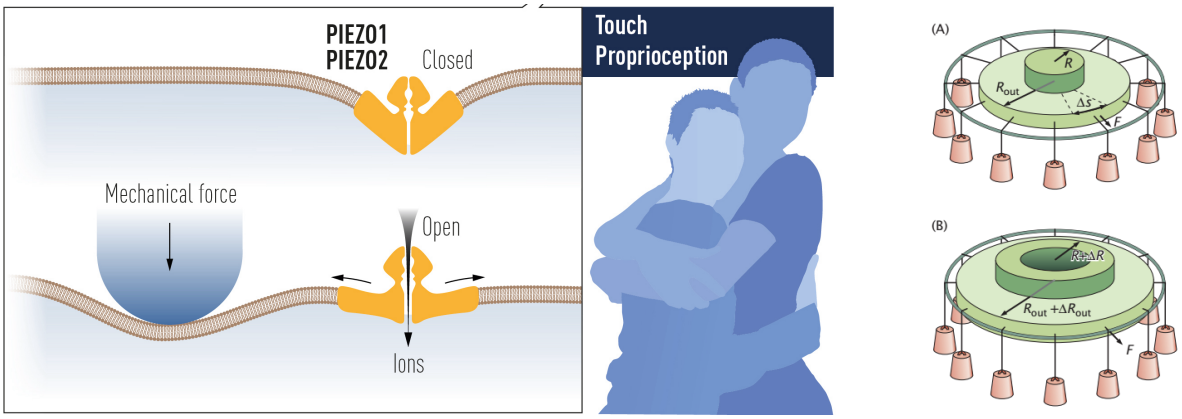


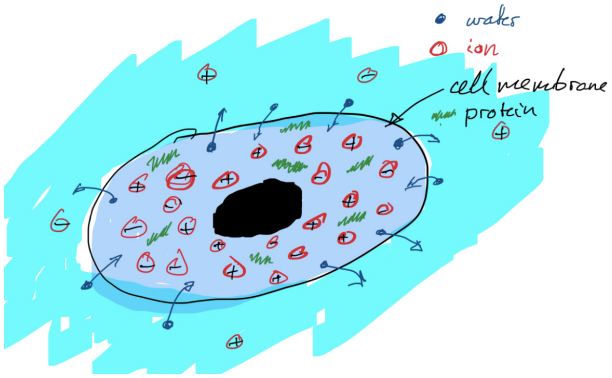
Figure 1: Mechanosensitive channels are found in most cells and have many functions in living organisms. **Left:** The 2021 Nobel Prize in physiology and medicine was given for identifying the genes of 2 important mechanosensitive channels: Piezo1 and Piezo2 that, among other functions are responsible for our sensation of touch and proprioception. **Right:** Sketch of how channel opening is related to relaxation of the loading device. Here the loading device is represented by weights attached to the membrane far from the channel. (A) closed channel (B) Open channel resulting in a reduction in the potential energy of the loading device.

change ΔA, upon opening the channel is of the order $\mathcal{O}(10\ \text{nm}^2)$ and the energy difference between open and closed channels is $\Delta\epsilon = -5.0 \pm 1.1\ k_BT$.

Determine the experimental open probability, P_o, by choosing a current threshold between open and closed. Plot the experimental and model P_o and explain and discuss your results.

2 Osmosis and cell membranes

2.1 Thermodynamics of osmosis



Osmosis is the motion of solvent 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.

Why is equation (2) the right definition of chemical potential to use in the case of osmotic flow into/out

of a cell:

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

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

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

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.

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, $\Delta\mu_i, i \in \{2, \ldots, \nu\}$.

Assume that the solution is dilute and derive the expression for the osmotic pressure in terms of the concentration differences n_i:

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

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

$$\Delta p = icRT, \tag{7}$$

where i 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 i should be for NaCl, glucose and $CaCl_2$.

A physiological saline solution of 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 M NaCl?

2.2 Mechanosensitive channels relieving osmotic pressure

How large concentration difference between the inside and outside do you need to completely open the mechanosensitive channels you studied previously? Assume they are in the membrane of a spherical cell with radius $R = 3$ micrometer? Use the Laplace formula for the difference in pressure between inside and outside of a spherical membrane under tension τ with radius, R:

$$\Delta p = \frac{2\tau}{R}. \tag{8}$$

Will the mechanosensitive channels open when water freezes outside the cell and the solution inside the cell remains liquid?

3 Freezing of cells

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.

3.1 Ice driving water permeation

Estimate the final concentration difference of solutes across a membrane with the same temperature on both sides. Use the same starting point as above (equation (4)) and simplify equation (4) to use only one solute.

What will the final solute concentration become for a temperature of -20°C?

3.2 Freezing point depression

Even after reading many scientific papers on freezing of cells it is not entirely clear to us (or the researchers in SciGroup) if anyone knows the exact mechanism of avoiding damage to the cells at liquid nitrogen temperature. 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, ΔT due to adding N_2 solute particles is

$$\Delta T = -\frac{\sum_j i_j N_j k T}{\Delta S_m} = -\frac{\sum_j i_j N_j k T^2}{\Delta H_m}, \tag{9}$$

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

What is the freezing temperature of a human cell at normal physiological salinity? Assume that you are starting with a physiological solution both outside and inside your cells. As the temperature is lowered $\Delta T_{\text{imposed}}$ below the freezing point of the physiological solution water is driven out of the cell changing the concentration Δc_2 inside the cell.

How large is the resulting freezing point depression ΔT_{fpd}? What can we judge from this?