

Solution to Biomembranes Problem Set

Problem 1

$$\Delta G = RT \ln \left(\frac{A_{OUT}}{A_{IN}} \right) = 58.6 \text{ meV} = 5.65 \text{ kJ / mole}$$

a) If $A_{OUT} = 10 \text{ mM}$ and $A_{IN} = 1 \text{ mM}$,

b) If the concentrations on the inside and outside are reversed, the same answer is obtained with the opposite sign (the reaction is favorable in this case).

c) If the concentration on the inside and outside is the same, the log is zero and thus the free energy is zero.

d) An additional imbalance of B across the membrane results in no change unless the movement of B across the membrane was coupled to the movement of A across the membrane.

e) If there is no voltage across the membrane, then it does not make any difference if A is charged or not.

f) If A is not charged, then a membrane voltage has no effect on the free energy of transfer across the membrane.

g) If A is charged and there is a membrane voltage of 100 meV, then we must include the zFV term in the equation:

$$\Delta G = RT \ln \left(\frac{A_{OUT}}{A_{IN}} \right) + zFV = 58.6 \text{ meV} + 100 \text{ meV} = 158.6 \text{ meV} = 15.29 \text{ kJ / mole}$$

Problem 2

If we assume that $[ADP]=10\text{mM}$, $[ATP]=5\text{mM}$ and $[Pi]=1\text{mM}$ then

$$\Delta G = 30 \frac{\text{kJ}}{\text{mole}} + RT \ln \left(\frac{[ATP]}{[ADP][Pi]} \right) \approx 46 \frac{\text{kJ}}{\text{mole}}$$

The minimum proton gradient that would provide such a free energy is given by:

$$\Delta G = RT \ln \left(\frac{[H^+]_{out}}{[H^+]_{in}} \right)$$
$$\left(\frac{[H^+]_{out}}{[H^+]_{in}} \right) = \exp \left(\frac{\Delta G}{RT} \right) = 5.68 \times 10^7$$

$$\Delta G = RT \ln \left(\frac{[H^+]_{out}}{[H^+]_{in}} \right) = -46 \text{kJ} / \text{mole}$$

Note here we switched the sign of the free energy from above because we need to balance that free energy. We now solve using 37C:

$$\frac{[H^+]_{out}}{[H^+]_{in}} = 1.77 \times 10^{-8}$$

Nearly 8 pH units difference would be required between the inside and outside of the mitochondrion or chloroplast!!! This cannot be, yet ATP is made by these organelles through the use of a proton-motive force. How? The above calculation assumed that the transfer of only one proton powers the production of one ATP from ADP and phosphate. In reality, the number of protons used is more like four and thus the proton gradient required is only about 2 pH units. This shows what can be done by choosing the right molecular gear ratio. The study of how one invents a molecular gear box (rather like a turbine engine in this case -- it is called ATP synthase) won several people the Nobel prize.

Problem 3

a) Under these circumstances, the A just diffuses everywhere and so the membranes are essentially not important. There is no pH gradient, so the pH is the same everywhere and therefore we can just view it as 1 ml of weak acid at pH 8.0 with a pK_A of 6.0.

$$8.0 = 6.0 + \log \frac{[A]}{[AH^+]}$$

$$[A] + [AH^+] = 10^{-4}$$

solving

$$[A] = 9.9 \times 10^{-5}$$

$$[AH^+] = 1.1 \times 10^{-6}$$

b) Now with the light we start pumping protons in. We generate a free energy of 200 meV which we stated was going entirely, in this case, into the formation of a pH gradient (not membrane voltage – this is almost true because chloroplasts are leaky to Cl^- which ends up balancing out the voltage).

Not that 1eV is 96.5 kJ/mole so 0.200meV is 19.3 kJ/mole. So at 37 C we have

$$\Delta \bar{G} = (19.3 \text{ kJ / mole}) = (8.314 \text{ J / kmole})(310 \text{ K}) \ln \left(\frac{[H^+]_{in}}{[H^+]_{out}} \right)$$

$$\left(\frac{[H^+]_{in}}{[H^+]_{out}} \right) = 1790$$

Remember that the external pH is held constant at 8.0. Now we know the pH inside (comes to 4.75) so we can calculate the concentrations of all the A and AH^+ components. What do we know? We know that the acid base equilibrium holds. We know that the neutral form of the acid can go across the membrane. We know how much total weak acid we added.

$$[A]_{OUT} = [A]_{IN}$$

$$K_A = \frac{[A]_{OUT} [H^+]_{OUT}}{[AH^+]_{OUT}}$$

$$K_A = \frac{[A]_{IN} [H^+]_{IN}}{[AH^+]_{IN}}$$

There are two ways to do this problem. The exact way is to write down the mass balance equation, and solve the four equations and four variables. Remember that we know the total amount of the weak acid that was added. That is we know the moles. We cannot simply write this in terms of concentrations, because there are two different parts of our

sample (inside and outside) that have different concentrations. Thus we must write it in terms of moles. To do this, we need to know the volumes of the two compartments (inside and outside). Lets call these V_{IN} and V_{OUT} , just to be imaginative. We can now see that:

$$A_{tot} = [AH^+]_{OUT}V_{OUT} + [AH^+]_{IN}V_{IN} + [A]_{OUT}V_{OUT} + [A]_{IN}V_{IN}$$

Remember that A_{tot} in this equation is the total number of moles of A, not the concentration. It is now easy to substitute the first three equations into the last one and come up with an expression in terms of $[AH]_{OUT}$ and $[H^+]$:

$$A_{tot} = \frac{[H^+]_{OUT}[A]_{OUT}}{K_A}V_{OUT} + \frac{[H^+]_{IN}[A]_{OUT}}{K_A}V_{IN} + [A]_{OUT}V_{OUT} + [A]_{OUT}V_{IN}$$

$$A_{tot} = [A]_{OUT} \left(V_{tot} + \frac{[H^+]_{OUT}}{K_A}V_{OUT} + \frac{[H^+]_{IN}}{K_A}V_{IN} \right)$$

$$[A]_{OUT} = \frac{A_{tot}}{\left(V_{tot} + \frac{[H^+]_{OUT}}{K_A}V_{OUT} + \frac{[H^+]_{IN}}{K_A}V_{IN} \right)}$$

We can measure the internal volume of the chloroplast (0.001 mls total) and external volume in the cuvette (0.999 mls). The external pH is constant at 8.0 and with the light on we have determined that the internal pH is 4.75. Thus, we can determine the external A concentration. From the equilibrium relations, we can then get the other concentrations:

$$[A]_{out} = [A]_{in} = 9.90 \times 10^{-5} \text{ M}$$

$$[AH^+]_{out} = 9.90 \times 10^{-7} \text{ M}$$

$$[AH^+]_{in} = 1.76 \times 10^{-3} \text{ M}$$

Notice that in fact the concentration of [A] did not change from its value before we turned on the light. We could have seen this was going to be true from the beginning in that 99.9% of the solution is at pH 8.0 where A is not going to bind a proton. Thus, we could have done a much simpler calculation (as I did in class) assuming that [A] stays at 0.099 mM and the concentration of $[AH^+]$ in the chloroplasts was just given directly from the equilibrium relationships. We would have gotten the same answer. Many times you can greatly simplify such problems by thinking about what terms are important.

You should note something else about this. Because the pH is more alkaline on the outside than on the inside, more AH^+ is formed on the inside. Since A is at a constant concentration between the inside and outside, but AH^+ must be much higher on the inside, this means we are effectively pumping the weak acid into the cells via the pH gradient. There is therefore a much, much higher total weak acid concentration inside the cell than outside under these conditions (assuming that there is a substantial pH gradient). This is an important and useful way to concentrate materials inside of cells without a specific molecular pump.

Problem 4

In this problem we will investigate the assumption that we usually make that the concentration gradient of an ionic species is not significantly disturbed if we allow it to come to equilibrium with an electric potential across the membrane. Consider the following system, we have a membrane vesicle with 1 mM KCl on the inside and 10 mM KCl on the outside. KCl is, of course neutral overall since it dissociates into a anion and a cation. Thus, we start without a charge difference on the two sides of the membrane. Now we add valinomycin. This specifically let's K^+ go through the membrane freely. After equilibration we know that:

$$V = - \frac{RT}{nF} \ln \left(\frac{[\text{K}^+]_{\text{OUT}}}{[\text{K}^+]_{\text{IN}}} \right)$$

a) So far, we have not made any assumptions about the effect of transferring charges across the membrane on the internal concentration of K^+ . If we did make the assumption that the number of charges required to set up the voltage was small compared to the concentration of K^+ , then we could easily calculate the voltage as -58.6 meV.

b) If we do not make any assumptions about the K^+ concentrations, the problem gets considerably more difficult, though still possible. In order to calculate what V would be if we did not make that assumption, we must go back to what we discussed in the notes about how much charge goes across a membrane to generate a particular voltage. Recall that $V=Q/C$ where Q is the number of charge difference between the two sides of the capacitor (in coulombs) and C is the capacitance. What we really want to know is Q because the number of charges is the number of K^+ ions that went across the membrane.

So, $Q = VC = V \frac{\epsilon A}{d}$. For a membrane vesicle, $\epsilon = \epsilon_0 = 8.85 \times 10^{-12}$ coul/(volt m), A is $4\pi r^2$ where r is the radius of the vesicle (the surface area of a sphere with radius 1×10^{-6} m is

$1.26 \times 10^{-11} \text{ m}^2$), and d is the width of the membrane (roughly 50 angstroms or $50 \times 10^{-10} \text{ m}$). Thus C is $2.23 \times 10^{-14} \text{ coul/volt}$. Therefore $Q = (2.23 \times 10^{-14})(V)$. This is the number (in coulombs) of charges that must go across the membrane to generate the voltage V . To translate that into a change in concentration, we must convert coulombs to moles and then divide by the volume. A coulomb is 6.24×10^{18} charges. A mole is 6.02×10^{23} . So to find moles of charges we need to multiply our expression for Q by $6.24 \times 10^{18} / 6.02 \times 10^{23} = 1.04 \times 10^{-5} \text{ moles/coulomb}$. So, the moles of charge should be given by $(1.04 \times 10^{-5} \text{ moles/coulomb})(2.23 \times 10^{-14} \text{ coulomb/volt})(V) = (2.31 \times 10^{-19} \text{ moles/volt})(V)$. To convert this to a concentration change, we divide by the volume. The volume outside the vesicles is always going to be very large and therefore the change in concentration must be negligible. However, the volume inside the vesicle is small and given by

$$\frac{4}{3} \pi r^3 = 4.19 \times 10^{-18} \text{ m}^3 = 4.19 \times 10^{-15} \text{ l}$$

so the concentration change due to the influx of potassium ions is $(2.31 \times 10^{-19} \text{ moles/volt})(V) / (4.19 \times 10^{-15} \text{ l}) = (5.52 \times 10^{-5} \text{ M/volt})(V)$. Plugging that into the equation for V above we find:

$$V = -\frac{RT}{nF} \ln \left(\frac{[K^+]_{OUT}}{[K^+]_{IN} + (5.52 \times 10^{-5} \frac{M}{V})V} \right)$$

Here $[K^+]_{IN}^0$ is the initial concentration of K^+ on the inside of the vesicle before addition of valinomycin. Right off the bat we can see that the additional term is going to be pretty small. The concentration initially of K^+ on the inside was 1 mM. Our voltages are well under a Volt. Therefore, the changes in the K^+ concentration will be on the order of 10 micromolar -- 1% of the initial concentration. There are two ways to proceed from here. We could give up and say that there is no effect and our original assumption was good (that's what a real biochemist would do). We could solve for V in this equation (that's what a real theoretic physical chemist would do) or we could perform an iterative solution, calculating the change in the concentration for a particular V and then recalculating V . We will perform an approximate solution (the middle path and all that). Since the correction to the internal concentration is fairly small, we can write an approximation for the log:

$$V = -\frac{RT}{nF} \ln([K^+]_{OUT}) + \frac{RT}{nF} \ln([K^+]_{IN} + (5.52 \times 10^{-5} \frac{M}{V})V)$$

$$V = -\frac{RT}{nF} \ln([K^+]_{OUT}) + \frac{RT}{nF} \ln([K^+]_{IN}) + \frac{RT}{nF} \ln \left(1 + \frac{(5.52 \times 10^{-5} \frac{M}{V})V}{[K^+]_{IN}} \right)$$

I have expanded the equations such that the V term is inside of a log term that looks like $\ln(1+x)$ where x is less than 1. The expansion of $\ln(1+x)$ is $x - x^2/2 + x^3/3 - \dots$. We will just use the first term which is a reasonable approximation for a small x. Thus:

$$V = -\frac{RT}{nF} \ln([K^+]_{OUT}) + \frac{RT}{nF} \ln([K^+]_{IN}) + \frac{RT}{nF} \frac{(5.52 \times 10^{-5} \frac{M}{\text{vol}})V}{[K^+]_{IN}}$$

$$V - V \frac{RT}{nF} \frac{(5.52 \times 10^{-5} \frac{M}{\text{vol}})}{[K^+]_{IN}} = V \left(1 - \frac{RT}{nF} \frac{(5.52 \times 10^{-5} \frac{M}{\text{vol}})}{[K^+]_{IN}} \right) = -\frac{RT}{nF} \ln \left(\frac{[K^+]_{OUT}}{[K^+]_{IN}} \right)$$

$$V = -\frac{\frac{RT}{nF} \ln \left(\frac{[K^+]_{OUT}}{[K^+]_{IN}} \right)}{\left(1 - \frac{RT}{nF} \frac{(5.52 \times 10^{-5} \frac{M}{\text{vol}})}{[K^+]_{IN}} \right)}$$

Thus, for 10 mM K^+ outside and 1 mM K^+ inside the true voltage is not -58.6 volts but rather -58.7 mV (note that the RT/nF term in the above equation in units of volts, which it must be to cancel the rest of the units in the equation, is 25.4 mV)