1. To further investigate HIV infection you decide to study the process of the viral recognition of host cells. The strain of HIV you have available, HIV-1 NL4-3, normally infects T cells that have the CD4 receptor protein and the T-cell chemokine co-receptor on their surfaces. To dissect the requirements for the entry process, you decide to test whether HIV can infect cheek cells.

a) First you add HIV-1 NL4-3 to the cheek cells growing in culture. You find that the HIV viral particles don’t bind to the cells. Is this what you would expect? Briefly explain why.

b) Next, you engineer your cheek cells to express the CD4 receptor and the T-cell chemokine co-receptor. Do you think that HIV-1 NL4-3 will bind to these engineered cheek cells?

c) (*extra*) Your friend gives you a second strain of HIV-1, HIV-1 YU-2, which normally infects macrophages instead of T-cells. When you try to infect your engineered cheek cells that express CD4 and the T-cell co-receptor with this strain of HIV, you find that HIV-1 YU-2 does not bind to the cells. Give one possible explanation for this result.
d) Since you know that the membrane is a heterogeneous and dynamic structure, you are interested in determining if membrane components are important in the binding of HIV to the cell. You deplete your engineered cheek cells of glycosphingolipids. Why have you chosen to target this type of lipid for depletion?

e) Interestingly, you find that HIV-1 NL4-3 can no longer bind to and infect the glycosphingolipid-depleted cheek cells, although these cells still express CD4 and the T-cell chemokine receptor on their cell surface. Give one possible explanation for these results.
2. In your studies of cells, you discover two new transmembrane proteins, A and B, which are present on the cell surface. To further investigate their distribution in the membrane, you use fluorescent tags to label and observe these proteins. You first examine Protein A.

a) You label Protein A with a fluorescent tag and examine its localization in the cell’s membrane using a microscope. You find it is distributed diffusely across the cell surface. You then use FRAP (fluorescence recovery after photobleaching) to determine the mobility of protein A in the membrane. You notice that after bleaching an area of the cell membrane 90% of the fluorescence is recovered in this area within 5 minutes. Draw the recovery graph you would expect and briefly explain these results.

b) Next you label protein B with a fluorescent tag and find it is also distributed diffusely across the cell surface. However the FRAP results are very different. After bleaching an area of the cell membrane, only 25% of the fluorescence is recovered in this area within 5 minutes. Draw the recovery graph you would expect and briefly explain these results.
c) You further examine the mobility of protein A in cells that have a defect in cholesterol production. The membranes of these cells have very little cholesterol compared to the cells used above. If you repeat your FRAP experiments from part (a) using these cells, how do you predict the mobility of protein A would compare with your previous observation? Briefly explain and include any assumptions.
3. Organisms can regulate their lipid composition in response to temperature. Fish are particularly rich in the healthful “Omega-3” and “Omega-6” fatty acids, which are unsaturated fatty acids containing cis-double bonds (the “omega-#” names refer to the location of the cis-double bond). On the other hand, poultry and mammalian meat sources (chicken, beef, etc) tend to be richer in saturated fats, which do not contain any double bonds.

a) In light of what you know about plasma membranes, why is it not surprising that fish membranes are rich in unsaturated fats while poultry and mammalian membranes are rich in saturated fats? (Hint: Fish are cold-blooded, whereas birds and mammals are warm-blooded).

Regulating membrane fluidity is especially important for organisms such as bacteria that cannot regulate their own temperature.

b) Consider a colony of bacteria that suddenly undergoes a drastic drop in temperature. What consequence would this have on the fluidity of the membranes of the bacteria?

c) If this temperature shift were gradual, what could the bacteria do to combat the change in membrane fluidity? (Hint: some bacteria have enzymes that can adjust the length and saturation of the fatty acid chains)

d) If the bacteria were suddenly shifted to a very high temperature, how would the membrane permeability be affected?
4. Transport of cations, such as Na\(^+\), K\(^+\) and Ca\(^{2+}\), across membranes is necessary for many processes that occur inside a cell. However, the phospholipid bilayer that comprises the cell membrane is impermeable to these cations.

a) Why are cations unable to cross the phospholipid bilayer in the absence of channels?

The Na\(^+\)-K\(^+\) ATPase is an ion pump that is present in the plasma membrane of most animal cells. It pumps 3 Na\(^+\) out of the cell and 2 K\(^+\) into the cell. The force that drives an ion across a membrane is made up of two components: one due to the electrical membrane potential and one due to the concentration gradient.

b) Are Na\(^+\) and K\(^+\) ions being pumped against their concentration gradients?

c) Are Na\(^+\) and K\(^+\) ions being transported with or against the membrane potential? (Hint: a typical membrane potential for an animal cell is -60mV \{inside negative\})

The Na\(^+\)-K\(^+\) ATPase uses the energy from the hydrolysis of ATP to drive the thermodynamically unfavorable process of ion transport. The energy (\(\Delta G\)) from the hydrolysis of ATP to ADP and Pi is roughly 12 kcal/mole.

d) What is the sign of \(\Delta G\) for ATP hydrolysis?

e) What is the sign and value of \(\Delta G\) for the unfavorable ion transport that one mole of ATP can drive?
For transport into the cell the free energy change ($\Delta G_{\text{in}}$) per mole of K\(^+\) moved across a plasma membrane with a membrane potential of -60mV at 37°C is:

$$\Delta G_{\text{in}} = -1.41 \text{ (kcal/mole) log } \frac{C_o}{C_i} + 1.38 \text{ kcal/mole}$$

f) What is the maximum concentration gradient that can be achieved by ATP driven active transport of K\(^+\) into the cell, assuming that one ATP is hydrolyzed for each solute molecule that is transported?

g) How does this maximum compare with the actual concentration gradient observed in animal cells? (Hint: see your Lecture notes!!)
5. Nerve impulses are transmitted across neurons through the release of neurotransmitter molecules. The neurotransmitter molecules diffuse across the space between the neurons (synapse) and bind to neurotransmitter receptors that in turn propagate the nerve impulse into the neurotransmitter receiving neuron.

Neurons are often pre-labeled with lipophilic dyes to visualize all of their processes. The structure for one such dye, DiI is shown below. These dyes are specifically introduced to very small regions of a cell's membrane, however the dye will eventually diffuse to label the entire plasma membrane.

\[ \text{DiI} \]

a) Given DiI’s chemical properties and the features of the plasma membrane, explain this observation.

b) Given an infinite amount of time, DiI fluorescence is never observed in the cytosol of a DiI labeled cell. Explain.