Biology 5357 Problem Set – Membrane & Membrane Proteins

1. Calculate the thickness of the oil layer in Benjamin Franklin's experiment on Clapham pond. Estimate how many molecules thick was in the layer. Use the quantities that are provided in his report and state your assumptions in your calculations.

2. Plot the following relationships for the phase transition temperature (T_m) dependency on lipid structure/chemistry. Find the values in your notes or in published literature.

a. Tm vs. headgroup – PE, PC, PS, PG, cardiolipin
b. Tm vs. chain length
c. Tm vs. chain saturation
d. Tm vs. chain modification

3. Adding cholesterol to the lipid bilayer has a "buffering" effect on the phase transition – it makes gel membranes more fluid, and fluid membranes more ordered (see Fig). Considering the molecular interactions in the lipid bilayer, provide a rationale for the buffering effect of cholesterol.

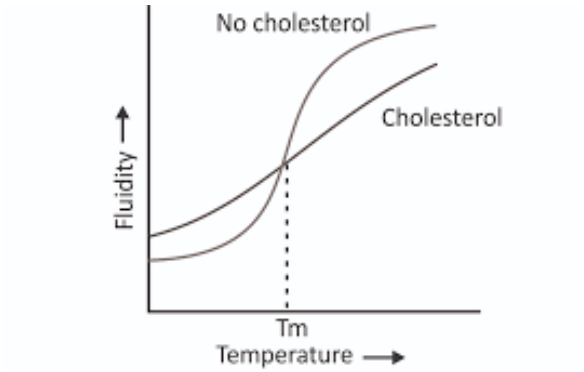

Fig. : Effect of Temperature and Cholesterol on Fluidity

Draw the structures of the following membrane phases with accurate chemical representations:

a. L_β
b. L_α
c. L_β + cholesterol
d. L_α + cholesterol

4. One method of reconstituting membrane proteins into liposomes is to combine the detergent purified protein with detergent solubilized lipids, and then dialyze the detergent away. For example:

A 1 mL lipid sample is prepared containing 20 mg/mL of POPC, and 35 mM CHAPS. Protein (MW=25 kDa) is added to the lipids from a 10 μM stock of purified protein in buffer containing 5 mM of n-Decyl-β-D-Maltopyranoside (DM), for a final ratio of 1 μg of protein per mg lipids. The sample is mixed and placed into a dialysis cassette (MWCO = 10 kDa), in 2 L of dialysis buffer. Calculate the following (you may need to look things up online):

a. Draw the structures of POPC, CHAPS and DM.
b. Look up the CMCs for each amphiphile.
c. Look up the aggregation numbers for two detergents.
d. Calculate the number of protein, lipid and detergent molecules in the sample
e. Consider CHAPS only. Calculate N_{free}, $N_{interface}$, and $N_{micelle}$ for the detergent molecules in the initial sample. The dialysis container is cylindrical with a diameter of 30 mm (i.e. like a 50 mL falcon tube).
f. At the end of many dialysis changes, assume that the detergent concentration has become negligible and that there are only lipids left in the system. Also assume that you have the

same total concentration of lipids as when you started. Assume that the system is at equilibrium, and calculate N_{free}, $N_{interface}$, and $N_{liposome}$ for the POPC in the final sample. A 2 L beaker has a diameter of ~130 mm.

5. In the "Cl^- dump" assay, a CLC Cl^-/H^+ antiporter is reconstituted into liposomes and the movement of chloride out of the liposome is measured by a silver chloride electrode.

Reference cell: 3 mL of 1 M KCl
Measurement cell: 1.8 mL of 299 mM K^+-isethionate, 1 mM KCl, 20 mM citrate, pH 4.5

Note, the two cells are electrically connected by a salt bridge containing agar prepared in the measurement cell solution.

a. Calculate the chloride concentration in the measurement cell when 15 μL of 10 mM KCl is added.
b. Calculate the expected change in voltage relative to the reference cell.
c. The proteoliposomes are prepared in 300 mM KCl, 20 mM citrate, pH 4.5. Right before adding the proteoliposomes to the measurement cell, the extraliposomal solution is changed to 299 mM K^+-isethionate, 1 mM KCl, 20 mM citrate, pH 4.5. Why doesn't the chloride leak out of the liposomes?
d. Why does adding valinomycin initiate the net transport of Cl^- out of the liposome?
e. Set the baseline voltage, i.e. prior to addition of valinomycin, to zero. Once all of the chloride in protein containing liposomes has been transported, the remaining chloride in unoccupied liposomes is released by adding detergent. The voltage difference relative to the reference cell is now 6 mV. What is the final concentration of chloride in the measurement cell?
f. Assuming that each liposome has a radius of 50 nm and is spherical, how many liposomes were added to the measurement cell?
g. Prior to the addition of detergent, the transport signal saturated to 3 mV. How many liposomes contained protein?