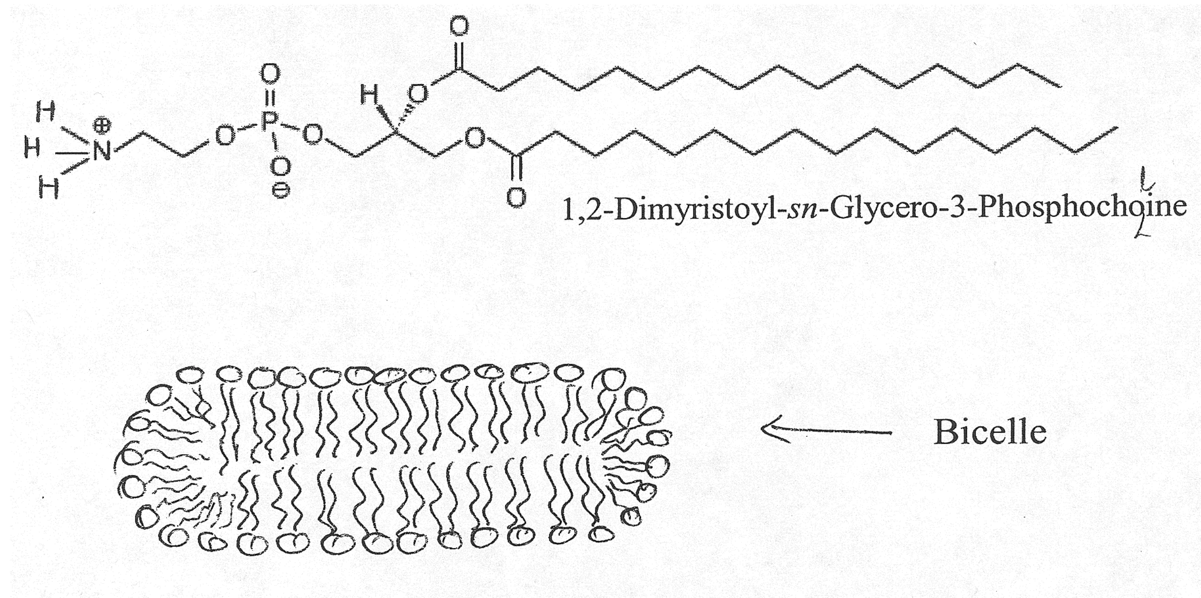
Problem 1. (25 points total)
Disc-shaped phospholipid particles, called *bicelles*, can be used to mimic membrane bilayers, in order to study protein-membrane interactions *in vitro*. Bicelles are flattened micelles that look like a pancake (~ 40A thick and hundreds A in diameter). They can be prepared by adding buffer to a mixture of glycerol-phosphocholine lipids (see Figure) with short and long acyl chains and shaking them overnight. Assume that the pK_a of the amino group is 9.0, and the pK_a of the phosphate group (a strong acid) is around 1.0.



a) (10 pts) Assuming that each bicelle contains 1000 lipid molecules, calculate the charge of such a particle at pH 9.0 and at neutral pH. Show your calculation and explain your reasoning.

In this pH range, the phosphate groups on all lipids in the bicelle are negatively charged. The charge will then depend on the ionization state of the amino group: the lipid molecule will be neutral when protonated, and will be negatively charged when it is deprotonated. In general, since at pK_a half of the molecules are protonated ($[A^-] = [HA]$), the total charge of the bicelle at pH 9.0 should be negative and equal half of the number of molecules in the bicelle, i.e. (-1)*1000 /2 = -500. The pH7.0 case requires a somewhat more detailed calculation, so let's first do it for a general case.
Let $N_A \sim [A^-]$ be the amount of lipids in the bicelle that are deprotonated (hence have charge e^- – this amount we want to know) and $N_{HA} \sim [HA]$ is the amount that is protonated (i.e. neutral). The following equations then apply:
$N_{HA} + N_A = 1000$, and $N_A/N_{HA} = [A^-]/[HA] = 10^{pH-pKa}$.
Expressing N_{HA} ($N_{HA} = 1000 – N_A$) from the first and substituting it into the second equation then gives: $N_A/(1000 – N_A) = 10^{pH-pKa}$. From here we have
$N_A = 1000 / [1 + 10^{pKa - pH}]$.
At pH = 9.0, N_A = 1000/2 = 500 → half molecules are protonated at pH = pKa and the charge is - 500.
At pH = 7.0, N_A = 1000/101 = 9.9 → the net charge is –10..

b) (10 pts) In what pH range will the bicelle be completely neutral (i.e. zero net charge)? Explain.

The full answer is: $4.0 < pH < 6.0$.

The number of charged molecules per bicelle should be less than 1. For $N_A = 1$, $N_{HA} = 999$, and from Henderson-Hasselbalch equation: $pH = pKa + \log(N_A/N_{HA}) = 9.0 + \log(1/999) = 6.0$. Below pH6.0 all amino groups on the bicelle will be protonated. Lowering the pH further will eventually reach the point when the phosphate group on at least one molecule gets protonated: $N_A = 999$, $N_{HA} = 1$. This will happen (for phosphate group) at $pH = pKa + \log(999/1) = 4.0$.

c) (5 pts) List all interactions that you think are important for forming bicelles and for their stability in aqueous solution at neutral pH. Will raising the pH to 9.0 increase or decrease the stability of these particles, and how will this affect their shape? Explain your reasoning.

A less detailed answer, based on calculation of pI = 5.0 should get partial credit, say ???

c) (5 pts) List all interactions that you think are important for forming bicelles and for their stability in aqueous solution at neutral pH. Will raising the pH to 9.0 increase or decrease the stability of these particles, and how will this affect their shape? Explain your reasoning.

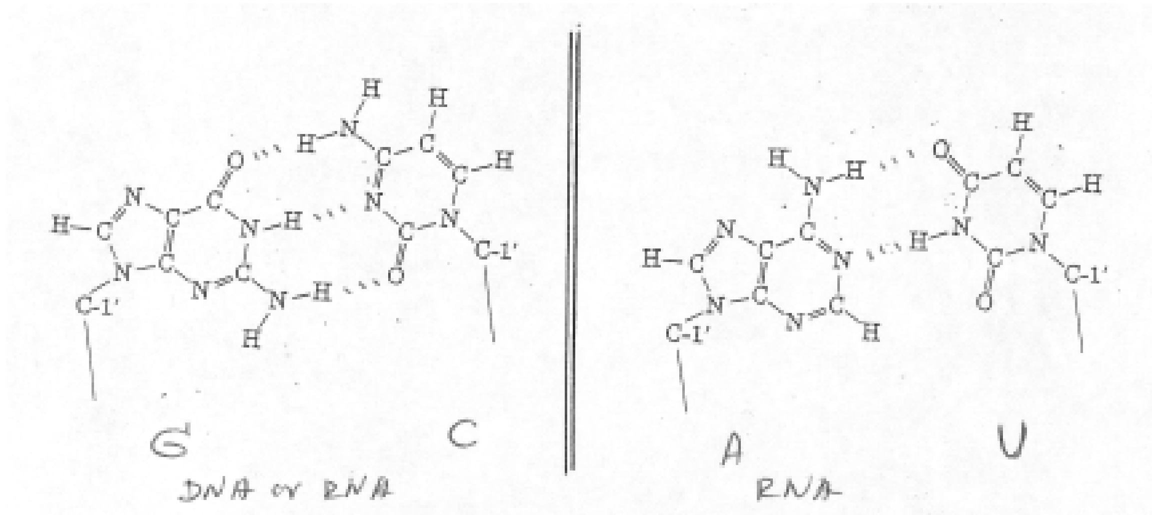
Stabilizing interactions: hydrophobic effect; Van der Waals interactions between the nonpolar tails; hydrogen and ion bonds between water and polar head groups.

Destabilizing interactions: electrostatic repulsion between negatively charged head groups.

The total negative charge of the bicelle will increase with pH, hence strong electrostatic repulsion, which will cause an increase in the energy of the bicelle – it will become less stable. The repulsion will be stronger between the (close in space) flat parts of the bicelle surface – one could expect a tendency to change the shape to a more spherical one, like for a ‘normal’ micelle.

Problem 2. (35 pts total)

a) (6 pts) Two base pairs taken from two pieces of nucleic acids are shown below. Identify the bases shown in the figure (you can use one-letter abbreviations) and the type of nucleic acid.



b) (10 pts) What are the interactions that are responsible for the base pairing? When reproducing the figure, the bonds between the interacting atoms in each pair did not come out. Restore (draw) them.

Hydrogen bonds

c) (5 pts) A fragment of DNA strand that is used as a template for mRNA synthesis has the following sequence: 5' ACTGTCGCACAGT 3'. Write the sequence of the mRNA molecule (starting from the 5'-end) synthesized from this DNA. Can this mRNA adopt a 3-dimensional structure by itself?

5' ACUGUGCGACAGU 3'

Since the sequence is a palindrome, it will form a double-strand RNA hairpin structure.

d) (10 pts) You have analyzed an unknown fragment of mRNA and discovered that adenine makes 12% of the total bases and uracil 10%. Based on these data, calculate the percentage (relative proportion) of all bases in the corresponding double-stranded fragment of its DNA template. Explain your reasoning.

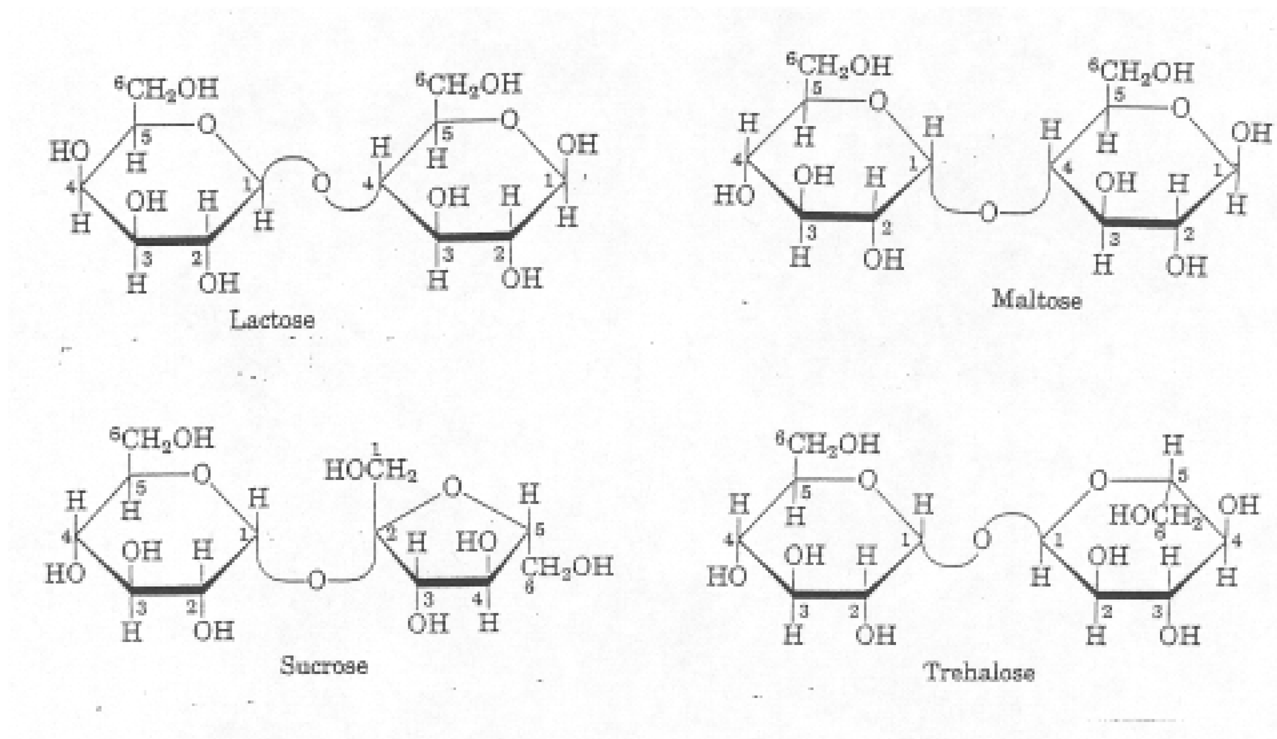
Assume that the transcription was perfect. From these data, the content of A in the transcribed strand from the DNA template was 10% and of T was 12%. The content of A for the other, complementary strand of DNA was 12%. The total content of A in both DNA strands was (10%+12%) /2 = 11%. Same content for T. The remaining 78% is C and G, and because of complementarity, the content of each of them is 78/2=39%.

Similar logic: the total content of A+T for the transcribed (template) strand of DNA is 10% + 12% = 22%. Same T+A content should be for the complementary strand, and therefore same content for both DNA strands. Because of complementarity, the content of each of them is 22% / 2 = 11%. The calculation for C,G is same as above.

e) (4 pts) Tyrosine is, in general, specified by two codons: UAU and UAC. Which of them is likely to be found more frequently in thermophilic bacteria (those that live e.g. in volcanic hot springs)?

The corresponding DNA sequences are ATA and ATG. The second has higher content of G hence will have higher melting temperature.

Problem 3. (25 pts total)
Four different disaccharides are shown in the Figure below.



a) (8 pts) Name the monosaccharides that these disaccharides are composed of (don't forget to indicate their stereoisomer form)

Lactose β-D-galactose_and β-D glucose________________________________

Sucrose β-D glucose and β-D-fructose____________________________________

Maltose α–D glucose and β-D glucose ____________________________________

Trehalose two molecules of α–D glucose ___________________________________

b) (4 pts) Which of these disaccharides are composed of two identical monosaccharides?

trehalose

c) (3 pts) Which of these disaccharides are composed of two stereoisomers?

lactose, maltose

d) (10 pts) which of these disaccharides (if any) contain

a pentose none

an aldose all

a pyranose all

a furanose sucrose

a ribose none

a ketose sucrose

a L-stereoisomer none

Problem 4. (30 pts total)

a) (12 pts) What is the general principle that describes how enzymes work as catalysts? How does this affect the free energy difference between the initial and the final states of the reaction? What is the major source of free energy required for the catalytic power of enzymes?

Enzymes lower the activation barrier of the reaction.
The free-energy difference between the initial and the final states is not affected.
The energy source is the binding energy, i.e. the energy released from enzyme-substrate interactions.

c) (8 pts) Trypsin and chymotrypsin are members of the family of serine proteases. They cleave peptide bonds at the C-terminal end of specific residues. Chymotrypsin recognizes aromatic residues, while trypsin recognizes lysine and arginine. The recognition of a particular side chain (side chain specificity) is fully determined by the structure and properties of the binding pocket. In the case of chymotyipsin the binding pocket is hydrophobic and is wide enough to accommodate an aromatic ring. Given what you know about the properties of Lys/Arg side chains, what can you say about the size/shape and possible interactions that would provide the substrate specificity in the case of trypsin? What amino acid residues from the trypsin molecule would you expect to find in the binding site?

The difference between the binding pockets in trypsin and chymotrypsin should reflect the difference in the shape and properties of the side chains of the corresponding amino acids. Lys and Arg have long side chains that are positively charged (at neutral pH) and not as bulky as the aromatic rings. Therefore, the binding pocket in trypsin should be deep and narrow, and have a negative charge at the bottom, to form an ion bond with the positive charge on the amino (Lys) or guanidino (Arg) groups. You could expect Asp (which is indeed there, in trypsin!) or Glu to be located close to the bottom of the binding pocket in trypsin.

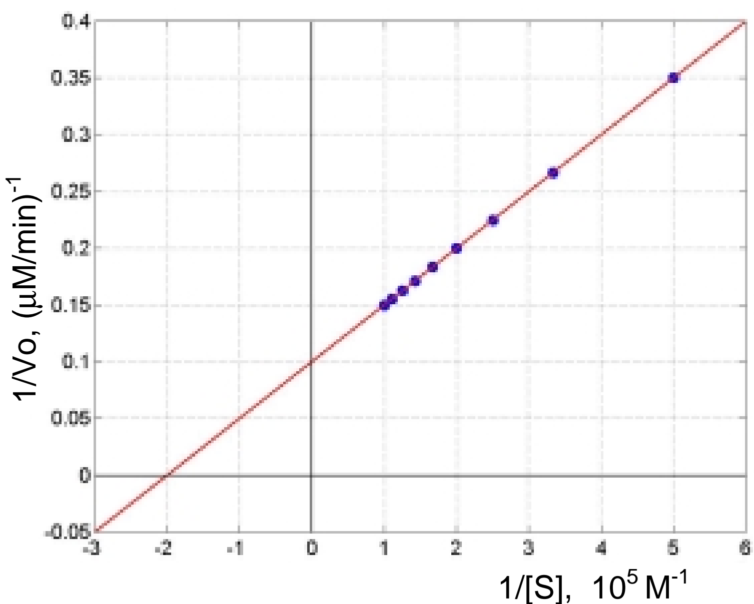
d) (10 pts) A particular chemical reaction, A ↔ B, is characterized by the difference in the free energy $\Delta G_{A \rightarrow B}$ = -20 kJ/mol. The uncatalyzed reaction is, however, slow, because of the high activation barrier, $\Delta G^{\ddagger}$ = 50 kJ/mol, therefore, you need an enzyme to speed up the reaction. What should be the activation barrier for the catalyzed reaction, $\Delta G^{\ddagger}_{cat}$, in order to achieve a 1000 fold increase in the reaction rate at standard conditions? How will this affect the rate of the backward reaction, B → A?
Is the required change in the ΔG a big or a small number? Compare it with the typical energies associated with a hydrogen bond or a covalent bond.

Since the rate of the reaction (all other conditions being equal) is proportional to $e^{-\Delta G^{\ddagger} / RT}$, the difference in the ΔG's can be written as $\Delta\Delta G^{\ddagger} = \Delta G^{\ddagger}_{cat} - \Delta G^{\ddagger} = - RT * \ln 1000 = -17.1$ kJ/mol. Therefore, $\Delta G^{\ddagger}_{cat}$ = 32.9 kJ/mol.
The rate of the backward reaction will also increase 1000 fold.
The $\Delta\Delta G^{\ddagger}$ is about or slightly higher than the energy of one hydrogen bond (-12 to -30 kJ/mol), and is a small fraction of the energy of a typical covalent bond.

Problem 5. (35 pts total)

Step 1. (10 pts) You measure the kinetics of an enzyme E as a function of substrate concentration first without any inhibitor (see Table) and plot the data using the double-reciprocal (Lineweaver-Burk) plot (Figure below). The enzyme concentration is maintained constant at a level of 1 μM (=10^{-6} M)

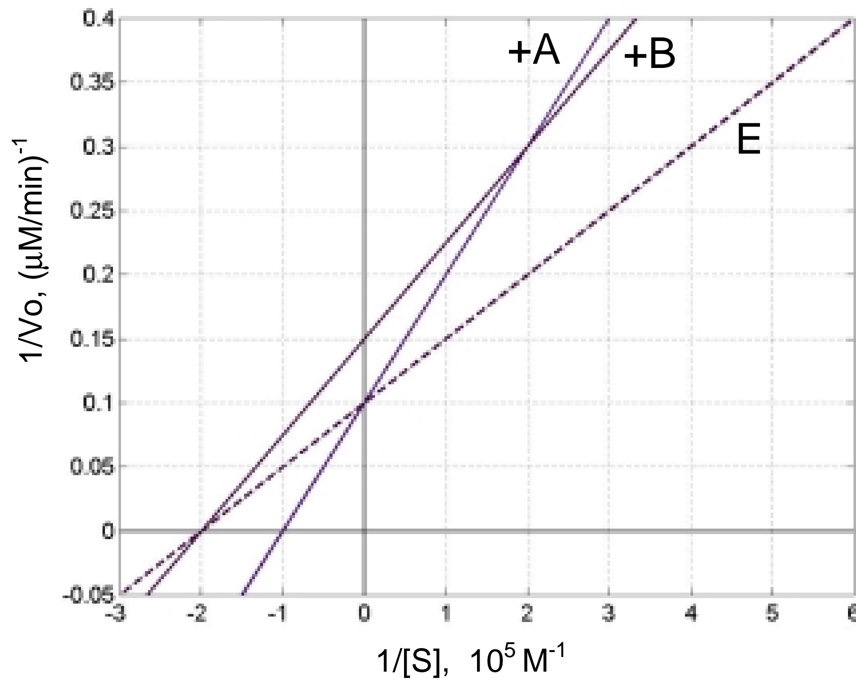
[S], μM	Vo, μM/min
2	2.9
3	3.8
4	4.4
5	5.0
6	5.4
7	5.8
8	6.2
9	6.4
10	6.7



From these data, determine V_{max}, K_M, k_{cat}, and the turnover number for the enzyme.

From the intercepts in the plot:
V_{max} = 10 μM/min;
Km = 5 10^{-6} M = 5 μM;
The turnover number, $k_{cat} = V_{max}/[E]_t = 10\ min^{-1}$.

Step 2. (25 pts) Now you study enzyme inhibition by measuring enzyme kinetics in the presence of 10 mM of inhibitor A or inhibitor B (separately). The Lineweaver-Burk plots in the presence of these inhibitors are indicated by "+A" or "+B" in the Figure below.



a) (5 pts) From these data determine the type of inhibition for:

A competitive_________

B noncompetitive______

b) (5 pts) Which of the two inhibitors is more efficient at high substrate concentrations? At low substrate concentrations? Show you reasoning.

At high substrate concentrations (1/[S] values close to zero), inhibitor B is more efficient: the reaction velocity is lower than for A (the '+B' line is higher than the '+A' line). At lower substrate concentrations, (higher 1/[S] values), inhibitor A becomes more efficient: the corresponding line is lower than that for B.

c) (10 pts) Using the data you have, determine the binding constant (K_I) for each of the inhibitors. Show the calculations.

Inhibitor A. Use equations for *competitive* inhibition: $Vo = V_{max}[S]/(\alpha K_m+[S])$ where $\alpha = 1 + [I] /K_I$..
α can be determined from the increase in the apparent K_m: $\alpha = K_m$('E+A')/ K_m('E') = 2 (see plot). Therefore, $K_I = [I] = 10$ mM

Inhibitor B. Use equations for *noncompetitive* inhibition: $Vo = (V_{max}/\alpha) [S]/(K_m+[S])$. We can determine α from the decrease in the apparent V_{max}: $\alpha = V_{max}(E) / V_{max}(E+A) = 0.15 / 0.1 = 1.5$. Therefore, $[I] / K_I = 0.5$, and we get $K_I = 2 [I] = 20$ mM.

d) (5 pts) Draw schematically the enzyme kinetic plots (in coordinates Vo vs. [S] on the graph below) in the presence of inhibitors A and B.