Part A (2 pts each, 34 Pts) ; Multiple Choice. Please circle the best answer.

1. A buffer solution at pH 6.0 is made from an acid with a pK_a of 5.0. What is the ratio of $[A^-]$ to [HA] in this buffer.

a) 10:1
b) 5:1
c) 1:1
d) 1:10

2. The isoelectric point of any amino acid is defined as

a) the pH where the molecule carries no electric charge.
b) the pH where the carboxyl group is uncharged.
c) the pH where the amino group is uncharged.
d) the pH of maximum electrolytic mobility.

3. The peptide bond in proteins is

a) planar, and usually found in a cis conformation.
b) nonplanar, and rotates to three preferred dihedral angles.
c) nonpolar.
d) planar, and usually found in a trans conformation.

4. The denaturation of DNA or protein causes

a) little increase in the entropy of the DNA or protein.
b) large decrease in the entropy of the DNA or protein.
c) no change in the entropy of the DNA or protein.
d) large increase in the entropy of the DNA or protein.

5. If the Gibb's free energy, ΔG, is greater than zero then:

a) the reaction direction will form products.
b) the reaction direction will form reactants.
c) the concentration of the reactants and products must be equal.
d) you cannot predict anything about a reaction with a positive Gibbs energy.

6. A protein that shows infinite *negative* cooperative for binding of *n* ligands will

a) show a Hill coefficient, n_h, of 0
b) only be found in either the unliganded form or the fully liganded form.
c) show a linear Scatchard plot.
d) show a Hill coefficient, n_h, of n.

7. A competitive inhibitor of an enzyme is usually:

a) a highly reactive compound.
b) a metal ion such as Hg^{+2} or Pb^{+2}.
c) structurally similar to the substrate.
d) a drug.

8. An allosteric *activator* of an enzyme usually

a) binds to the active site.
b) participates in feedback regulation.
c) causes the enzyme to work faster.
d) precipitates the product.

9. The major problem in the use of drugs to treat HIV infections is:

a) Drugs that are good inhibitors cannot by synthesized.
b) The drugs interfere with normal digestion.
c) The drugs are rapidly degraded.
d) Virus particles with altered (mutant) proteases arise.

10. Fatty acids *and* phospholipids in water are organized such that the _____ face the solvent and the _____ are directed toward the ____ interior.

a) hydrophobic tails; hydrophilic heads, polar.
b) hydrophilic heads; hydrophobic tails, non-polar.
c) hydrocarbon chains; carboxylic acid groups, polar.
d) carboxylic acid groups; hydrocarbon chains, non-polar.

11. Which of the following membrane structures function in active transport?

a) peripheral proteins.
b) cholesterol.
c) integral membrane proteins.
d) trans-membrane proteins.

12. Which of the following would yield the most energy per gram when oxidized?

a) starch.
b) glycogen.
c) fat.
d) protein.

13. Amino acids whose side chains can interact with the DNA or RNA nucleotide bases via hydrogen bonding include the following:

a) Asn and Gln.

b) Val and Ala.

c) Lys and Leu.

d) Ile and Val.

14. DNA Gel Electrophoresis is similar to SDS-PAGE of proteins because

a) Both techniques rely on a constant charge to mass ratio.

b) Both techniques utilize the sieving properties of gels.

c) In both cases molecules migrate to the anode.

d) All of the above are correct.

15. During replication, overwinding or overtightening of DNA is caused by ____ and removed by ____.

a) DNA ligase, Gyrase

b) Dna B, DNA polymerase

c) DnaB, Gyrase

d) DnaA, Gyrase

16. The two features of the tRNA molecule involved in converting the triplet codon to an amino acid are

a) in the anticodon loop and the 3' CCA end.

b) in the anticodon loop and the D stem.

c) solely in the anticodon loop.

d) solely at the 3'CCA end.

17. A change in the *middle* base of the anticodon triplet would most likely

a) prevent the tRNA from becoming charged with an amino acid.

b) cause the tRNA to become charged with the incorrect amino acid.

c) cause the incorporation of the wrong amino acid into the protein sequence.

d) have no effect on the final protein sequence since this is the wobble position.

B (3 pts each/9 pts). Listed below are generalizations that apply to biochemical structures or functions. **Pick any three of the five**. In many, but not all, there are clear exceptions to the rule as it is stated. In those cases, provide a single good example of an exception in the space provided. If the rule actually applies without exception, state " No exceptions" in the space provided.

1. Oxidative phosphorylation in *all* organisms requires the presence of oxygen as an electron acceptor.

   Exception, many organisms can use alternative compounds to accept electrons.

2. *All* heme containing proteins transport oxygen.

   Exception, numerous heme containing proteins, such as cytochromes, carry electrons.

3. *All* polymerases have a 5'-3' polymerase activity.

   No exceptions.

4. *All* membrane proteins are α-helical.

   Exception, a beta-barrel will also satisfy all hydrogen bonds, thus forming a stable structure in membranes.

5. *All* carbohydrates can be found in 5 or 6-membered ring forms.

   Exception, 3 and 4 carbon carbohydrates exist, but do not form rings.

C1 (5 pts): Do part a or part b, but not both.

Choice a: Sketch the overall structure of **either** an α-helix **or** a β-sheet. Please indicate your choice. You do not need to show a detailed drawing of the chemical structure, but do indicate the direction of hydrogen bonds relative to the mainchain direction in your drawing.

This required a simple cartoon drawing, in the case of an α-helix the hydrogen bonds should point in the direction of the helix axis, while in a β-sheet they are between the strands and are perpendicular to them as well.

Choice b: Compare and contrast tertiary and quaternary structures of proteins. Give an example of each type.

Tertiary structure is the folded form of a single chain, Quaternary structure is comprised of multiple chains. Examples of proteins that assume tertiary structures are myoglobin, lysozyme, trypsin. Examples of proteins that are found in quaternary structure are hemoglobin, HIV protease, immunoglobulins.

C2 (6 pts): Do **one** of the following **two** questions:

Choice a: Select **one** of the following two intermolecular forces and compare and contrast their importance to the stability of folded proteins and double stranded DNA..

- Van der Waals interactions: Play in important role in the stability of both proteins and DNA due to the well packed core in proteins and due to base-stacking in DNA.
- Electrostatic interactions: Play little role in protein stability because the surface charge density of proteins is generally small. Play a large role in destabilizing DNA due to the repulsion of like charges on the phosphodiester backbone.

OR

Choice b: Provide a brief description of the hydrophobic effect. Indicate whether this effect is favorable or unfavorable for protein folding and phospholipid bilayer formation.

Exposed non-polar groups, either non-polar amino acids or non-polar acyl chains, order water and thereby lowering its entropy. When these groups are buried when proteins fold or lipid bilayers assemble, this water is released. The released water raises the entropy of the system. The change in the entropy of the solvent is the hydrophobic effect.

C3 (8 pts): Provide a *general* conceptual framework that explains the underlying molecular mechanism of allosteric affects. Briefly describe how allosteric effects are used to regulate **either** phosphofructose kinase activity **or** hemoglobin **or** glycogen synthesis. Give an example of an allosteric regulator for your chosen example and briefly indicate its regulatory role.

Allosteric regulators bind elsewhere on the enzyme/protein and modify the enzyme kinetics or binding of the protein due to conformational change induced by the binding of the regulator. Allosteric regulators can stabilized the tense(T), or inactive, form of the enzyme/protein if they are allosteric inhibitors. Alternatively, allosteric activators stabilize the relaxed (R), or active, form of the enzyme/protein. Some examples:

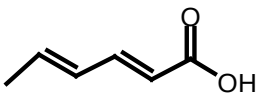
- PFK: Activated by F-2,6-P, AMP, inhibited by ATP. Turns on glycolysis if glucose levels are high (F2,6P) or energy (AMP) stores are low. ATP inhibits PFK , indicating high cellular energy reserves.
- Hemoglobin: Tense, or low oxygen binding form, stabilized by bis-phosophoglycerate. [BPG] is increased at high altitudes to enhance the delivery of oxygen to the tissues.
- Glycogen synthesis: Protein phosphorylation is an example of a more permanent allosteric effect due to the formation of a covalent bond between Ser and Tyr residues on a protein and the attached phosphate group. Phosphorylation activates glycogen breakdown and inhibits glycogen synthesis. Phosphorylation occurs under conditions of low glucose, causing glucose to be released from glycogen stores.

C4 (8 pts): Do only **one** of the following two questions:

Choice a: Most bacteria can produce lactate when fed glucose. Lactate is a valuable commercial product. What growth conditions would you employ to maximize the production of lactate from bacterial cultures? Support your answer by a brief description of the metabolic steps involved in lactate production.

Lactate is produced during anaerobic metabolism, therefore you would want to grow the bacteria in the absence of oxygen. Pyruvate is reduced to lactate in order to reoxidize NADH to NAD^+ for consumption in glycolysis.

Choice b: How many $FADH_2$, NADH, and acetyl-CoA molecules would be produced by β-oxidation of the fatty acid shown to the right. Note the presence of the double bonds. Please show your work.



This is a six-carbon fatty acid, therefore it will undergo two cycles of oxidation, producing three acetyl-CoA molecules. Due to the presence of the double bonds, the 1st oxidation step that produces $FADH_2$ would not be required, hence only two NADHs would be produced.

C5 (5 pts): After eating several candy bars the glucose level in the blood can rinse to 1 mM. In a normal individual, these levels drop to about 0.5 mM within 30-45 min and then a constant glucose level is maintained. *Briefly* discuss how the regulation of blood glucose is accomplished by **hormonal control**. (You need **not** give extensive details about phosphorylation pathways, etc.)

When blood sugar levels are high, the hormone insulin is released from the pancreas. This hormone binds to the insulin receptor on the surface of liver cells. This binding event leads to general dephosphorylation of

enzymes. This dephosphorylation leads to the incorporation of glucose into glycogen since glycogen synthase is active with dephosphosrylated. When blood glucose levels fall, the pancreas releases the hormone glucagon. When glucagon binds to its receptor on liver cells it activates a G-protein, which activates adenyl-cyclase. Adenyl-cylase produces the second messenger cAMP. cAMP activates a protein kinase, leading to phosphorylation of many enzymes. This phosphorylation results in the release of glucose from glycogen since glycogen phosphorylase is activated by phosphorylation.

C6 (3 pts): Do only **one** of the following two questions:

Choice a: Distinguish between ΔG^o and $\Delta G^{o'}$ (the latter is ΔG^o *prime).*

ΔG^o is the free energy change when one mole of A is converted to one mole of B, for the reaction A→B. If A or B were protons, their concentration would be 1M. In the case of $\Delta G^{o'}$, the proton concentration is 10^{-7}.

Choice b: Distinguish between ΔG and ΔG^o.

$\Delta G=\Delta G^o+RT ln K_{eq}$, is the amount of energy that can be obtained from a system when it proceeds from a non-equilibrium position to an equilibrium position.

C7 (10 pts): Do **either** part a **or** part b, but not both!

Choice a: In question C5, the concentration of glucose will initially be different across the cell membrane, with a concentration of 1 mM on the outside and 0.5 mM on the inside. The membrane potential across the cell membrane is -0.01 volts, with the inside more negative.

i) Calculate the Gibbs free energy, ΔG, associated with this concentration gradient (5 pts).

The voltage gradient across the membrane does not affect the free energy because glucose is not charged. Defining the inside as the 'product' of the reaction: $\Delta G=+RT ln$[0.5 mM]/[1 mM] = 2.5 kJ/mol *ln (0.5) = -1.73 kJ/mol*

ii) Based on the sign of ΔG, is the movement of glucose into the cell spontaneous or not (2 pts)?

Spontaneous, since $\Delta G<0$ and we defined 'inside' as the product of the reaction.

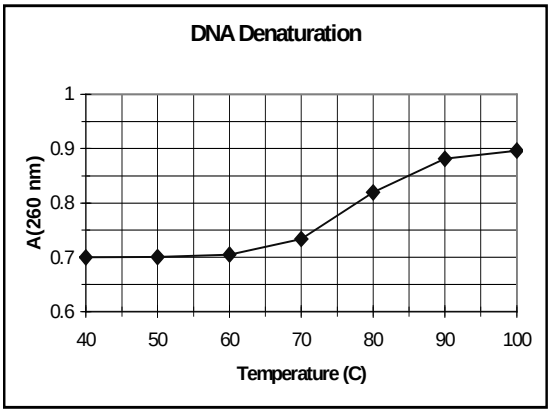
iii) How many glucose molecules would have to be transported to provide sufficient energy to synthesize an ATP (2 pts)?

It takes 30 kJ/mol to synthesize one ATP, therefore about 17 glucose molecules would have to be transported to produce one ATP.

iv) If the transport of glucose does indeed lead to ATP synthesis, what *must* exist in the membrane for this to occur (1 pts)?

There must be an enzyme in the membrane that could somehow couple the movement of 17 glucose molecules to the phosphorylation of ADP to from ATP. In a manner similar to how ATP synthase makes ATP from transporting 3 protons.

Choice b: The thermal denaturation curve for the melting of a double stranded DNA molecule is shown in the figure to the right. The absorbance of the double stranded DNA is 0.7 and the absorbance of the denatured DNA is 0.9.



i) Draw a *simple* cartoon that illustrates the change in the structure of the DNA as a result of this experiment (1 pt).

This would just be a double stranded piece of DNA going to two separated single strands.

ii) What is the T_M for this DNA? Justify your answer.

When the absorbance is 1/2 way between 0.7 and 0.9 one-half of the DNA will be melted, this occurs at about 78C.

iii) What is the GC content of this DNA? Show your work. (2 pts).

Using the expression: $T_M= 70 + 0.41$ (%GC), %GC=19. [This is a slightly different formula than in 2003 because the salt concentration is different for PCR experiments]

iv) What is the ΔG^o for denaturation at 90°C (363K)? (5 pts)

At 90C, the absorbance of the solution is 0.875. Therefore the fraction unfolded is (0.875-0.70)/0.2 = 0.87.

$f_u = K_{EQ}/(1+K_{EQ})$, $f_u+f_u K_{EQ} = K_{EQ}$, $K_{EQ}(1-f_u)=f_u$, $K_{EQ} = f_u/(1-f_u) = 0.87/0.13 = 6.73$.

$\Delta G = -(8.3)(90+273)\times\ln 6.73 = -5.8$ kJ/mol

C8 (10 Pts): Do **either** part a **or** part b, but not both.

Choice a: Discuss the role of transition state stabilization in enzyme catalysis. Provide one example of transition state stabilization from any of the enzymes that were discussed in this course.

The transition state is an high energy unstable intermediate in the reaction pathway. It is stabilized by two factors. First, the enzyme may form bonds with characteristic features of the transition state, for example trypsin forms hydrogen bonds with the tetrahydral intermediate formed when Ser attacks the C=O group. Since bonds are formed this is an enthalpy effect.

In addition, the transition state is stabilized due to the fact that the chemically reactive groups are held in the correct position for catalysis by the enzyme, thus the decrease in entropy that would occur if these groups were free in solution does not occur. All enzymes 'utilize' this method of reducing the transition state energy.

Choice b: Discuss general methods by which enzymes bind specific substrates. Illustrate your answer by providing one example of a specific interaction between an enzyme and its substrate.

Enzymes form specific interactions with functional groups on the substrate. These interactions can involve h-bonds, the hydrophobic effect, electrostatics, and van der Waals. As an example:

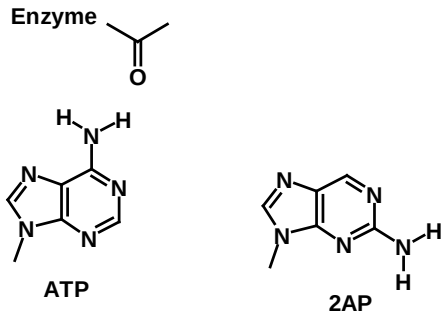
- Trypsin: Negative charge of the Asp on enzyme interacts with the positively charged Arg or Lys on substrate
- Chymotrypsin: the large non-polar pocket in the enzyme binds large non-polar sidechains of the substrate due to hydrophobic as well as van der Waals interactions.
- Lysozyme: Formation of h-bonds with N-acetyl group on NAG or NAM
- Eco R1: Formation of h-bonds with bases in the major groove.

C9 (10 pts): Do **either** part a **or** part b, but not both!

Choice a: Describe, *in general*, how you would obtain the inhibition constant, K_I, for any competitive inhibitor. Your answer should include a description of the actual experiments that would be necessary to acquire the raw data. You should also show how a double reciprocal plot is used to obtain K_I by graphical analysis of the data.

1. Obtain enzyme velocities at different substrate concentrations, without inhibitor present.
2. Obtain enzyme velocities at differenct substrate concentrations in the presence of a *fixed* concentration of inhibitor.
3. Plot 1/V versus 1/S for both data sets.
4. Obtain the ratio of the slopes, which gives α, the effect of the inhibitor on the kinetics.
5. $K_I = (\alpha-1)/[I]$

Choice b: The enzyme adenyl cyclase converts ATP to cAMP. The interaction of the adenine ring with the enzyme is shown to the right. This diagram also shows the structure of 2AP, an inhibitor of the reaction. The K_M for ATP is 1×10^{-6} M.



i) Is 2AP likely to be a competitive or non-competitive inhibitor? Why? (2 pts)

Competitive, since it has a structure similar to the substrate.

ii) Using the K_M for ATP, estimate the ΔG^o for the binding of ATP to adenyl cylase. State any assumptions that you made (4 pts)

You have to assume that k_2 is small, such that $K_M \sim K_D$. If so, then $K_A = 1/K_M$, $K_A = 10^{+6}$ M^{-1}, giving $\Delta G^o = -RT\ln K_A = -2.5$ kJ/mol 13.81 = -34.5 kJ/mol.

iii) Based on the difference in the molecular interaction between the enzyme and ATP versus 2AP and your answer to part *ii*, estimate the K_I for 2AP. State any assumptions you have made in solving this problem.(4 pts).

The inhibitor is missing one H-bonding interaction with the enzyme, this is probably worth about 4 kJ/mol, therefore $\Delta G^o = -30$ kJ/mol, giving a K_A of $1.6\text{x}10^5$ M^{-1}.

C10: (7 pts) You should do **one** of the following three questions:

Enzyme Name	Recognition Sequence
*Natrat*I	$^{5'}$AA$^{\downarrow}$CGTT
*Tartan*II	$^{5'}$AAA$^{\downarrow}$TTT
*Cut*I	$^{5'}$GGC$^{\downarrow}$GCC
*Cohen*II	$^{5'}$CG$^{\downarrow}$CGCG
*BstE*II	$^{5'}$G$^{\downarrow}$GCGCG

Choice a: The list on the right shows a number of restriction endonucleases and their associated recognition sequences and cleavage sites. Only one strand is shown and the cleavage position is indicated by a downward arrow: $^{\downarrow}$.

i) For *Natrat*I, write the complete sequence (i.e. both strands) and draw the individual products (both strands) after treatment of its restriction sequence with the enzyme (3 pts).

```
AACGTT      Product->        AA      CGTT
TTGCAA                       TTGC     AA
```

ii) Which of the other four restriction sites would produce fragments that could be readily joined to the fragments produced by cleavage with *Natrat*I? Briefly justify your answer with a diagram of the resultant joined fragment.(4 pts.)

Cohen II could be used since its sticky ends (CG) are the same as NatratI.

Choice b: Describe the steps involved in converting an mRNA to a cDNA molecule. You should clearly indicate the enzymes involved and any DNA primers that would be required.

Prime with oligodT, synthesize 1st DNA strand with reverse transcriptase, digest RNA with RNAase, add G's to 3' end with terminal transferase, prime with oligodC, synthesize 2nd strand with DNA polymerase.
[In 2003 we did HIV RNA to double stranded (ds) DNA]

Choice c: You have just made a cDNA library from mRNA isolated from human cells. The size of the entire human DNA is approximately 10^9 basepairs. You are interested in isolated the gene for hemoglobin to produce human hemoglobin from bacteria. You probe your cDNA library with a radioactive probe that is 12 nucleotides in length and identical in sequence to a portion of the DNA sequence of the hemoglobin gene. You identify approximately 50 different plasmids that contain this 12 base sequence. What is the likelihood that almost all 50 of these plasmids contain the gene for human hemoglobin? Justify your answer.

The probability that a 12 nucleotide sequence will occur at random in 10^9 basepairs is:

$(1/4)^{12}x10^9 = 59$. Therefore most of the plasmids will *not* contain the hemoglobin gene, but some other random piece of the human genome.

C11 (5 pts):

a) Indicate the following on the nucleic acid molecule shown on the right:

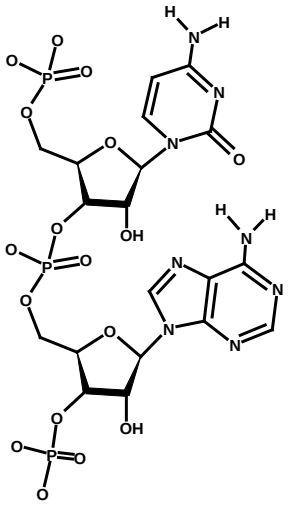
i) The location of the 5' *and* 3' ends. *The 5' end is at the top.*

ii) Any charges that are omitted. *Each phosphate would have a neg. charge.*

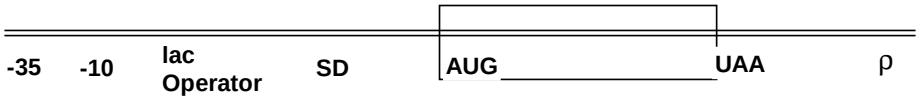
iii) Draw an 'X' through a ribose or deoxyribose. *The 5-membered rings.*

iv) Draw a circle around the purine base. *A&G are purines, A is the lower base*.

b) Is this a DNA or RNA molecule? Why? *RNA, since there is a 2' hydroxyl.*



C12 (10 pts): The following diagram shows a segment of DNA with the necessary control elements for the production of a recombinant protein (e.g. human insulin) in



bacteria.

i) Briefly discuss the role of **three** of the following five features in the production of recombinant protein:

-35 & -10 region: Promoter region, RNA polymerase binds here to initiate mRNA synthesis.

lac operator: The lac repressor protein binds here and prevents RNA polymerase binding and thus production of the recombinant protein. Addition of IPTG causes the lac repressor to come off of the DNA, initiating production of the recombinant protein.

SD: Site on mRNA that binds to 16s RNA on the 30s (small) ribosomal subunit). Positions mRNA so that the correct reading frame is selected.

AUG & UAA: Start (AUG) and stop (UAA) codons for protein synthesis.

ρ: Site were rho protein binds to mRNA, terminating DNA transcription.

ii) List in the space to the right the feature(s) that act as signals in protein synthesis?(2 pts)

The SD, AUG and UAA.

iii) Indicate, by drawing a *neat* box on the above diagram, the region of the DNA that encodes the information required for the amino acid sequence of the protein (2 pts)

The box has been drawn on the figure.

C13 (8 pts): Do part a **or** part b. You can answer this question using simple diagrams or a sequential list of events.

Choice a: Describe the steps involved in the replication of the lagging strand in DNA replication.

1. Opening up of double stranded DNA by DNA helicase (DNA B) .
2. Synthesis of RNA primer by primase
3. Replication in 5'-3' direction by pol III until previous RNA primer is reached.
4. Removal of previous RNA primer by pol I 5'-3' exonuclease activity.
5. Synthesis of DNA to fill gap by pol 1 5'-3' polymerase activity
6. Ligation of DNA by DNA ligase.

Choice b: Describe the steps involved in the addition of one amino acid to a polypeptide chain by the ribosome.

1. The existing peptide would be attached to the tRNA in the P-site.
2. A charged tRNA binds to the A-site by virtue of the codon-anticodon interaction.
3. Peptide bond formation occurs, moving the peptide from the P-site to the A-site.
4. Translocation of the ribosome occurs, the peptide is now back in the P-site, still attached to tRNA.
5. The uncharged tRNA exits from the E-site.

C14 (25 pts): The binding of Single Stranded Binding protein (SSB) to dA_{10} (10 adenosine residues in a single chain) was measured at five different pH values. The binding data for one pH value (8.0) is shown below (left table), as are the K_{EQ} (=K_A) values for four of the five pH values (right table).

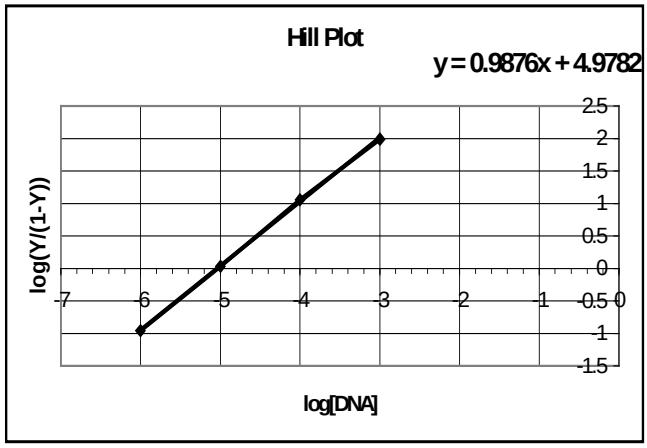
Experiment #	**[DNA]**	**Y (pH = 8.0)**
1	1×10^{-6}	0.10
2	1×10^{-5}	0.52
3	1×10^{-4}	0.92
4	1×10^{-3}	0.99

pH	**K_{EQ}**	**ΔG^o** (Optional calculation for part iv.)
11.0	$3.0 \times 10^3\ M^{-1}$	
10.0	$4.4 \times 10^3\ M^{-1}$	
9.0	$2.2 \times 10^4\ M^{-1}$	
8.0	$1.0 \times 10^5\ M^{-1}$	
7.0	$1.6 \times 10^5\ M^{-1}$	

i) Estimate the binding affinity (K_{EQ}) at pH 8.0 by direct inspection of the data, enter your value into the right-hand table above. Briefly justify your approach (4 pts).

When Y=0.5 the ligand concentration equals K_D, this occurs at [DNA]=$10^{-5}\ M^{-1}$, giving a K_A of $10^5 M^{-1}$.

ii) Is the binding of dA_{10} to SSB at pH = 8.0 cooperative or not? Justify your answer by a *quantitative* analysis of the data. If you feel the need to construct a Hill plot, please use the graph to the right, although such a plot may not be necessary (4 pts).



It would appear that the binding is non cooperative, since a 10 fold increase in [DNA] from 10^{-5} M to 10^{-4} M only raises the fractional saturation to 0.92, an increase expected for non-cooperative binding. This is confirmed by the Hill plot, which shows a slope of 1, indicative of non-cooperative binding.

iii) Does the binding of SSB to dA_{10} become stronger or weaker as the pH is increased? Justify your answer using the above data. (3 pts)

As the pH is increased the binding becomes weaker, K_{eq} (= K_A) decreases at higher pH.

iv) Based *entirely* on the effect of pH on the binding affinity, speculate on the nature of the interaction between SSB and the DNA. Make specific reference to the types of amino acid involved and the region of the DNA with which it interacts. (Sample answer: The hydrophobic residues, such as Ala and Val, in SSB interact with the methyl group on the T base). Make specific reference as to why the pH would affect the interaction. (5 pts)

The reduction in binding affinity at higher pH indicates the loss of a favorable interaction that involves a titratible group. Since the change occurs at high pH, a Lys or Arg residue is involved. This indicates that SSB binds to the DNA by virtue of electrostatic interactions between lysine residues on the protein and the phosphate charges on the DNA.

v) Based on your answer to part iv, is the interaction between SSB and DNA likely to be specific for certain base sequences? Speculate why the nature of the interaction between SSB and DNA is important for the function of SSB during DNA replication.(2 pts)

SSB could bind to any base because all have phosphate groups. This is important for its function since SSB coats single stranded DNA during DNA replication and must be able to bind to any sequence.

vi) Assuming that the binding was measured by equilibrium dialysis and that the concentration of the SSB was $1x10^{-6}$ M, calculate the UV absorbance at 270 nm of the solution **inside** the dialysis bag for experiment #1 in the above table (Y=0.1, [DNA]=$1x10^{-6}$ M). Please show your work. SSB contains 2 Trp residues, recall that this particular DNA (dA_{10}) contains 10 dA bases.(3 pts)

The following extinction coefficients may be useful: $\varepsilon_{270}^{Trp} = 5{,}000$ $\varepsilon_{270}^{dA} = 10{,}000$

Both DNA and protein contribute to the UV absorption. The protein contribution is:

$A_{PROTEIN} = 1x10^{-6}\ M \times (2 \times 5{,}000) = 0.01$

the contribution from the DNA is from both the bound and free DNA. The amount bound is $0.1 \times 1x10^{-6} = 1x10^{-7}$M, giving a total DNA concentration of $1.1x10^{-6}$. The extinction coeffecient of the DNA is 10,000 x 10 = 10^5/M-cm. Therefore the absorption from the DNA is $1.1x10^{-6}$ x 10^5 = 0.11, therefore the total absorption from both protein and DNA is 0.12.

vii) On the basis of the above binding data, design an affinity chromatography step to purify SSB. Briefly describe the nature of the column material and how you would elute the bound SSB off of the column(4 pts).

The column could have either single stranded DNA or even poly-phosphate on the beads. The SSB would bind to the column at low pH and could be eluted off of the column at high pH.