

7.014 Problem Set 2

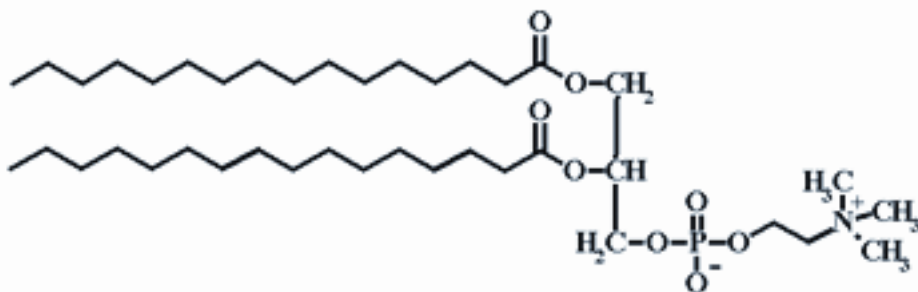
Please print out this problem set and record your answers on the printed copy. Answers to this problem set are to be turned in to the box outside 68-120 by 5:00pm on Friday March 2, 2007. Problem sets will not be accepted late. Solutions will be posted online.

1. Lipids

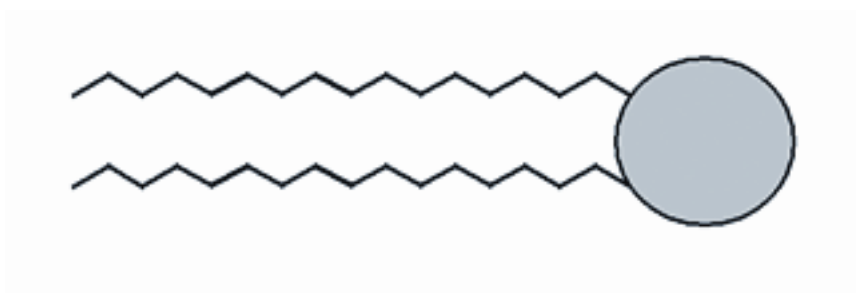
(a) Carbohydrates and lipids are both made up of long hydrocarbon chains, but they have very different properties. What is the major distinction between carbohydrates and lipids?

(b) Membranes are made up of many components and, in some membranes, phospholipids are the major component.

(i) Below is an example of a saturated phospholipid (Where the intersection of two lines represents a carbon atom with hydrogen atoms to fill available covalent bonds). Draw a square around the portion of the molecule that is hydrophobic and a circle around the portion that is hydrophilic.



Phospholipids are also sometimes represented like this:



Question 1 continued

(ii) Using the simplified structure above, draw what a lipid bi-layer looks like in an aqueous environment. Explain what causes the lipids to create this formation.

(iii) In your depiction of a lipid bi-layer drawn above, what type of force is acting...

- ...between the phosphoglycerol (hydrophilic) component and the fatty acid (hydrophobic) component of the phospholipids?

- ...between the phosphogroup and the surrounding aqueous environment?

- ...between the fatty acid groups of different phospholipids molecules?

(iv) In reality, membranes are composed of several different types of lipids, as well as proteins. One reason why there are multiple types of lipids is to ensure that the membrane remains fluid so that proteins, lipids and small molecules can move through and within the membrane. In particular, there is always a mixture of saturated and unsaturated phospholipids. Give a short explanation of why a membrane containing unsaturated phospholipids would be more fluid than a membrane made exclusively of saturated phospholipids.

2. There are thousands of different kinds of enzymes and each enzyme recognizes a specific substrate or substrates (substrates are the molecules upon which an enzyme acts). You are working in a lab that studies the activity of an enzyme called a nuclease that was purified from the gram-negative bacteria *Serratia marcescens*. A nuclease is an enzyme that cleaves or breaks apart DNA or RNA by hydrolyzing the phosphate-sugar backbone.

The work in your lab has shown that there are four residues important for binding or catalysis of the substrate.

Question 2 continued

(a) Below is the amino acid sequence of the *Serratia* nuclease. Those amino acids important for binding or catalysis are marked by being enlarged and bolded. These amino acids are Arg78, His110, Asn131 and Glu158.

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      10           20           30           40           50           60
MRFNNKMLAL AALLFAAQAS ADTLESIDNC AVGCPTGGSS NVSIVRHAYT LNNNSTTKFA

      70           80           90           100          110          120
NWVAYHITKD TPASGKTRNW KTDPALNPAD TLAPADYTGA NAALKVDRGH QAPLASLAGV

      130          140          150          160          170          180
SDWESLNYLS NITPQKSDLN QGAWARLEDQ ERKLIDRADI SSVYTVTGPL YERDMGKLPG

      190          200          210          220          230          240
TQKAHTIPSA YWKVIFINNS PAVNHYAAFL FDQNTPKGAD FCQFRVTVDE IEKRTGLIIW

      250          260
AGLPDDVQAS LKSKPGVLPE LMGCKN
    
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If the role of this enzyme is to cleave DNA and RNA, why does it make sense that Arginine (R) and Histidine (H) are two of the amino acids important for binding the substrate?

(b) As stated above, it is known that these residues are important for binding or catalysis. You want to test for which of these functions (binding or catalysis) the amino acids Arg78 and His 110 is important. To perform this test you change Arg78 and His110 to different amino acids and then monitor if the nuclease can still cleave DNA. Below is the outline for the assay:

1. Incubate either or wild-type (wt) or mutant (mt) enzyme with DNA.
2. After several minutes, you isolate the DNA from the reaction.
3. Run the DNA pieces through an agarose gel matrix (see the Research Method box on pg. 319 in *Purves et. al.*) using an electric current. At this time it is not important to understand the technique, but it is important to understand that the electric current causes DNA fragments of different sizes to separate from each other and that this separation can be visualized.

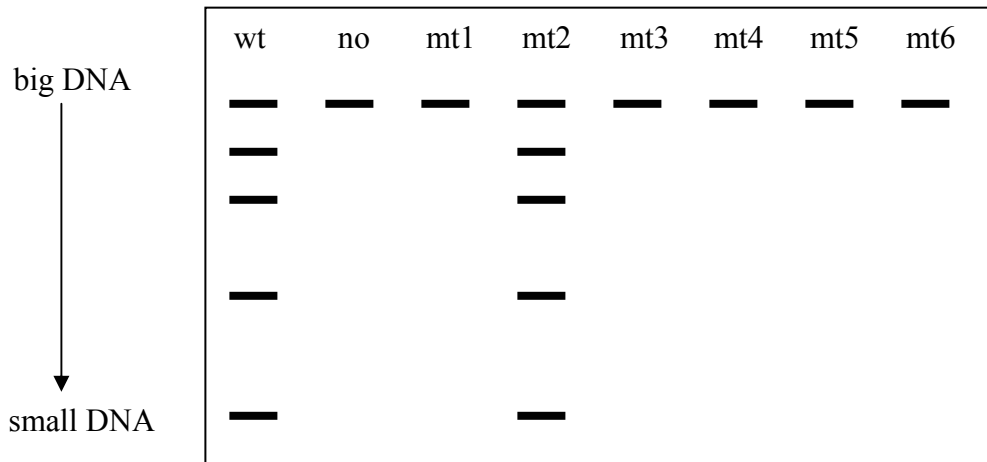
The chart below shows which enzymes you will use in the assay:

Name of Enzyme	Amino Acid	Position of Amino Acid
wt*	Arg	78
mt1	Ala	78
mt2	Lys	78
mt3	Trp	78
wt*	His	110
mt4	Ala	110
mt5	Lys	110
mt6	Trp	110

* note that there is only one “wt” enzyme. “wt” is listed one time for each of the different amino acids you are studying.

Question 2 continued

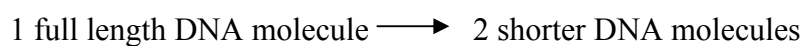
Below is a representation of your agarose gel after you have separated the DNA. There are multiple lanes on the gel. Each lane contains the DNA from one of your different incubations. The version of the enzyme (wt or mt) that was incubated with the DNA in a specific lane is noted above the lane (no = no enzyme added to that DNA).



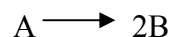
(i) Based on your results above do you think that Arg88 is more likely to be important for binding the DNA or for cleaving the DNA? Why?

(ii) Based on your results above do you think that His110 is more likely to be important for binding the DNA or for cleaving the DNA? Why?

(c) Your lab also is interested in understanding how much energy is required to cleave the phosphodiester bond. To do this, you perform an assay similar to the one described above, but this time you allow the reaction to reach equilibrium. You then measure the amount of full-length DNA you started with and the amount of cleaved DNA after the reaction reaches equilibrium. The reaction can be described using the following equation:



or



Question 2 continued

The final concentrations of your reactants and products are:

$$\begin{aligned}A &= 3 \text{ nM} \\B &= 4.5 \text{ mM}\end{aligned}$$

(i) Based on these measurements, determine the amount of standard free energy (ΔG°) for $A \longrightarrow 2B$. Show your work.

(ii) What does your answer tell you about how the reaction proceeds under standard biological conditions?

(iii) Why is the concentration of enzyme not important in determining ΔG° ?

(iv) Draw an energy diagram for the hydrolysis of the phosphodiester bond with and without the presence of the nuclease enzyme. Make sure to label the reactants, the products, the value of ΔG° and the activation energy. The only labeled item that needs an exact number is ΔG° .

3. To answer the problem below, you need to use the StarBiochem, a java viewer for macromolecules. To get access to and directions for this viewer, go to the expanded problem statement online at <http://web.mit.edu/viz/>. The questions are reproduced below. To obtain credit for the problems, you must write your answers down in the spaces provided on the problem set that you will turn in to be graded.

We examine the primary, secondary, and tertiary structure of a specific protein, nitrogenase Fe (1XD8).

(a) Chain A consists of 289 amino acids.

(i) List in order the 12 amino acids numbered 16 through 28 in chain A.

(ii) What level of protein structure does this represent?

(b) These 12 amino acids also make up an α -helix in nitrogenase.

(i) What level of protein structure do α -helices and β -sheets represent?

(ii) Do the side chains of the amino acids in a helix point into or out of the helix?

(iii) What type of bond is primarily responsible for maintaining secondary structure?

(iv) What part of the amino acid participates in this bond (side chain or backbone)?

(c) The tertiary structure of a protein is formed by bending and folding of the amino acid chain, with the interactions between the amino acid side chains determining this structure. Below, we list four kinds of tertiary interactions between side chains that are possible and four sets of residues.

(i) Match up which set of residues belongs to which type of bond

- | | |
|-------------------------|--------------------------------|
| A. Hydrogen Bond | I. Residues 129, 10 |
| B. Ionic bond | II. Residues 8, 10 |
| C. Disulfide bridge | III. Residues Chain B; 132, 97 |
| D. Hydrophobic clusters | IV. Residues 8, 126, 135 |

(ii) Which represents the strongest interaction: A, B, C or D?

(iv) Which represents the weakest interaction: A, B, C or D?

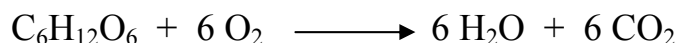
(d) The residue found in chain B at position 129 in this protein is known to be involved in catalytic activity of this nitrogenase. Residue 129 helps with the binding of ADP.

(i) What type of residue is at position 129 (polar, nonpolar, acidic or basic)?

(ii) What is likely the strongest bond found between residue 129 and ADP?

(iii) A mutant version of this nitrogenase that is unable to bind ADP has a glutamic acid in this position instead of the wild-type residue. How would this substitution affect ADP binding?

4. The cell harvests energy by metabolizing the carbohydrate glucose. The overall reaction for the breakdown of glucose (under aerobic conditions) is:



(a) When, during the process of glucose metabolism is O_2 used?

(b) When, during the process of glucose metabolism is CO_2 released?

(c) When, during the process of glucose metabolism is H_2O released?

(d) What is/are the oxidizing agents during glycolysis?

(e) Which process of the metabolism of glucose is blocked under anaerobic conditions?

(f) Do you think that the cell could use galactose ($\text{C}_6\text{H}_{12}\text{O}_6$) as a substrate for glycolysis? Why or why not?

5. Back at your summer job at the Venter Institute, you are still working on characterizing Species M, Species I and Species T. Currently, you are working on determining the composition of the nucleotides in the DNA for two of the species, M and I.

(a) Before you can even begin your experiment for the day, your annoying bench-mate suggests that you should determine if DNA or RNA is the stable, heritable genetic material for these organisms. Give two reasons (based on the structure and/or chemistry of DNA or RNA) why DNA is a better choice to be the stable, heritable genetic material than RNA.

(b) After explaining to your bench-mate why RNA is a poor macromolecule to encode the precious genetic information of a cell, you begin your experiment. You first isolate DNA from each of the two species and aliquot some of the DNA into four tubes per strain. You label these tubes:

M-A M-G M-C M-T I-A I-G I-C I-T

The DNA in each tube will be used to identify the percentage of one of the four nucleotides (A, G, C or T). For example, the DNA in tube M-A will be used to identify the percentage of adenine in the DNA of species M.

You go to another room to gather some reagents you will need to perform the different reactions. When you come back, you find that your bench-mate is not only annoying but clumsy too! While carrying some ethanol, he knocked over your tubes and spilled the ethanol on top. This has caused all the writing on your tubes to come off, and you no longer know which tube contains the DNA from the two different species. You pick up the tubes and re-label them #1- #8. You decide to perform the experiment anyway by choosing two tubes at random on which to perform each nucleotide-determining reaction. Below are your results:

Tube #	1	2	3	4	5	6	7	8
nucleotide determined	A	A	G	G	C	C	T	T
% A/G/C/T	30	30	20	17	17	17	33	30

What is the nucleotide composition for each of the two different species?

Species #1	Species #2
A _____	A _____
G _____	G _____
C _____	C _____
T _____	T _____

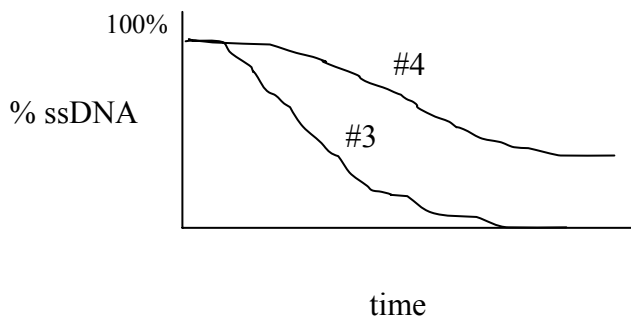
(c) Now, you need to identify which nucleotide composition belongs to which species. To do this, you use a small amount of DNA that is still labeled as being from Species M or Species I. To determine which species has which nucleotide composition, you do a DNA re-naturing experiment. Below is the outline of the experiment:

1. Combine DNA from Species M or Species I with DNA from at least two of your tubes labeled #1- #8.
2. Break the DNA into smaller pieces using a technique called sonication. The smaller pieces will have, on average, 500 base pairs.
3. Heat the DNA in the tube so that the hydrogen bonds between the two strands of the helix break and the strands come apart.
4. Incubate the tubes at room temperature, which will allow hydrogen bonds to reform.
5. Monitor the percentage of single-stranded DNA (ssDNA) that is present in the samples at different times during the room temperature incubation.
6. Graph the percentage of ssDNA over time.

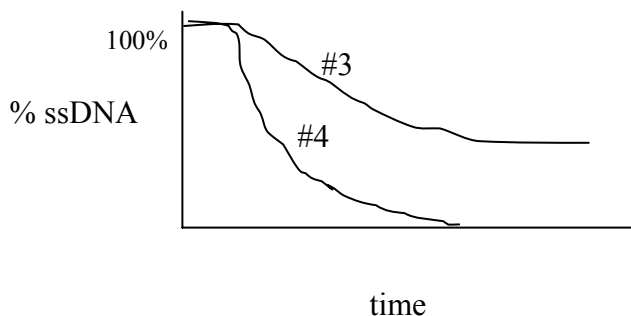
(i) Before you begin, your bench-mate suggests that you use tubes #1 and #2. Why is this idea bad?

(ii) You choose to use tubes #3 and #4 instead. Below are the results from your experiment:

For tubes containing Species M DNA and DNA from tube #3 or #4:



For tubes containing Species I DNA and DNA from tube #3 or #4:



Which tube, #3 or #4 contains which species' DNA (M or I)? Explain your reasoning based on your data.

Tube #3 contains DNA from Species _____

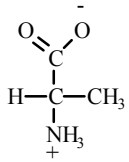
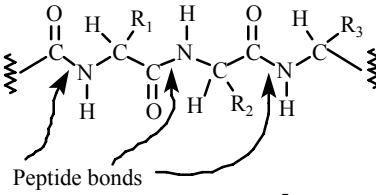
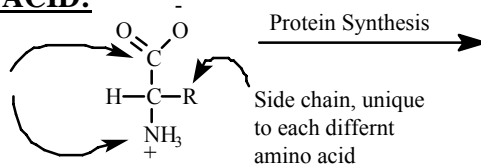
Tube #4 contains DNA from Speices _____

Bonus thought question (ie – not worth extra points!): Can you provide a reason for why the ssDNA went away *faster* for the incubation of DNA from Species I with DNA from tube #4 than the ssDNA for the incubation of the DNA from Species M with DNA from tube #3?

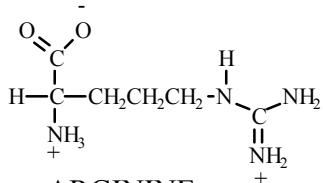
STRUCTURES OF AMINO ACIDS

GENERIC AMINO ACID:

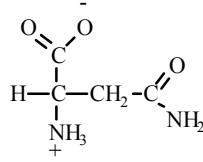
Individual amino acids are linked through these groups to form the backbone of the protein.



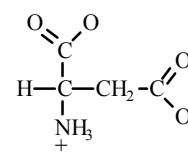
ALANINE
(ala)



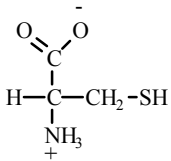
ARGININE
(arg)



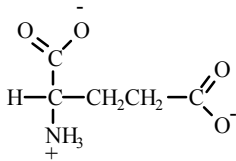
ASPARAGINE
(asN)



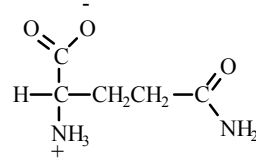
ASPARTIC ACID
(asp)



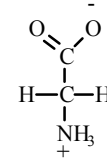
CYSTEINE
(cys)



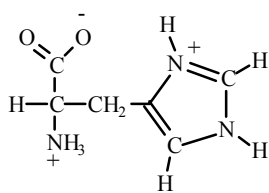
GLUTAMIC ACID
(glu)



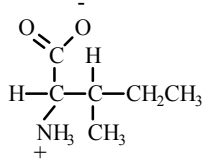
GLUTAMINE
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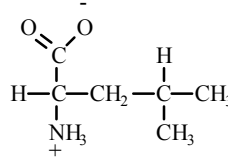
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(gly)



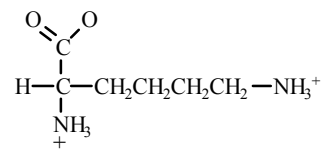
HISTIDINE
(his)



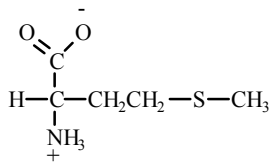
ISOLEUCINE
(ile)



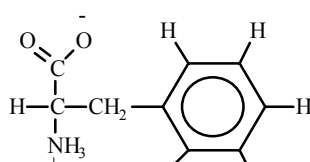
LEUCINE
(leu)



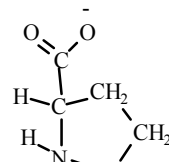
LYSINE
(lys)



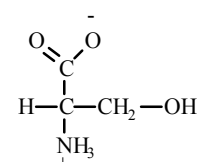
METHIONINE
(met)



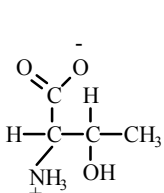
PHENYLALANINE
(phe)



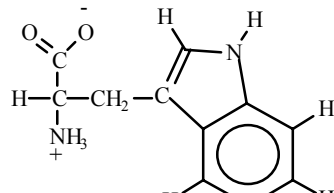
PROLINE
(pro)



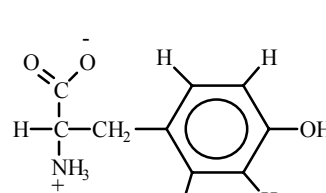
SERINE
(ser)



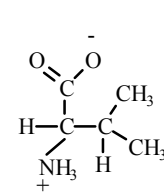
THREONINE
(thr)



TRYPTOPHAN
(trp)



TYROSINE
(tyr)



VALINE
(val)