Name:_____   Section:_____

7.012 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 at the box outside by 4:10 Wednesday, September 17. Problem sets will not be accepted late. Solutions will be posted on the web September 18, 2003.

Question 1

Many intracellular proteins are required to interact or bind to other macromolecules within a cell in order to function properly. One class of such proteins is comprised of proteins that can bind DNA. Such proteins often have conserved structural features, called motifs, which interact with DNA. One such motif consists of two closely aligned α-helices (shown as cylinders below) that each have leucine-rich regions. This motif is referred to as a leucine zipper.
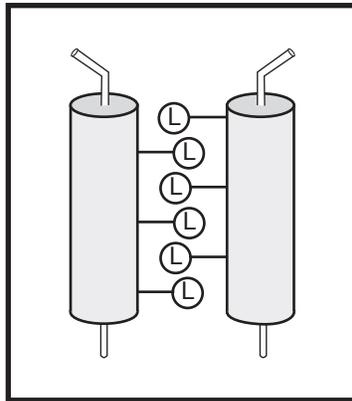

Figure by MIT OCW.

a) Based on what you know about leucine, what type of interaction is likely to be holding the two halves of the "zipper" together? Circle one of the following:

   Ionic   Covalent   Hydrophobic   Hydrogen bonds

b) DNA-binding proteins often associate with the charged sugar and phosphate backbone of DNA. Which specific amino acids might you find in a DNA-binding protein on the surface that associates with DNA? What kind of interaction do you expect between DNA and these amino acids of the binding protein?

c) In the lab you purify two short pieces of double-stranded DNA (segment 1 and segment 2). Each piece is exactly the same length (100bp), however the nucleotide sequence comprising each strand is different. You don't want to spend the money to sequence the strands, but you would like to know which 100bp strand has more A-T base pairs. You heat up each piece and find that

the two strands of segment 1 disassociate before those of segment 2. Based on what you know about the bonds between nucleotides, which DNA segment had more A-T base pairs? Why?

Question 2

Consider the following two biologically relevant reactions:

$$ADP + P_i \leftrightarrow ATP \qquad\qquad \Delta G° = +7.5 \text{ kcal/mol}$$
$$Creatine + P_i \leftrightarrow Creatine\ Phosphate \qquad\qquad \Delta G° = +9.5 \text{ kcal/mol}$$

Creatine is used as a storage form of high-energy phosphate in both the muscles and the brain. The enzyme, creatine phosphokinase, transfers the phosphate of ATP to creatine generating creatine phosphate. The reaction is reversible such that when energy demand is high (e.g. during muscle exertion) creatine phosphate donates its phosphate to ADP to yield ATP.

a) Write the equation for the reaction in which creatine phosphate is synthesized. Be sure to include the $\Delta G°$ for the overall reaction. Would you expect the reaction to be spontaneous as written?

b) Draw and label a free energy diagram for the overall reaction as written in (a). Be certain to include the <u>relative</u> energy levels of the substrates and the product, the activation energy and the $\Delta G°$ for the overall reaction.

Question 2, continued

In a typical muscle cell at rest the following concentrations are observed:

Metabolite	Equilibrium Concentration (mM)
ATP	8.0
ADP	0.02
Pi	3.0
Creatine	3.0
Creatine Phosphate	26.0

c) Calculate the ΔG for creatine phosphate formation in these cells at 37°C. Show all work. Under the above cellular conditions, is the synthesis of creatine phosphate spontaneous?

d) Briefly explain why the ΔG you calculated is different from the ΔG° given for the reaction under standard conditions.

e) What would happen to the spontaneity of the reaction if the equilibrium [ADP] was raised by an order of magnitude, i.e. from 0.02mM to 0.2mM? What does this suggest about the importance of maintaining a high ratio of ATP to ADP in the cell?

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Question 3

Most chemical reactions in biology take place on the surface of enzymes. Generally, such enzymes convert a substrate, or group of substrates, into a product, or set of products, that is crucial to the function of a cell. Below is a graph describing a general enzymatic reaction, in which the velocity of the chemical reaction is dependent upon the substrate concentration.
a) On the graph below complete the following steps:

      1) Draw a <u>solid</u> line through the 5 reaction coordinates provided that best approximates the activity of a general single-subunit enzyme.
      2) Draw a <u>dashed</u> line parallel to the x-axis at the abscissa corresponding to V_{max}.
      3) Find and label $1/2\ V_{max}$.
      4) Find and label K_m.
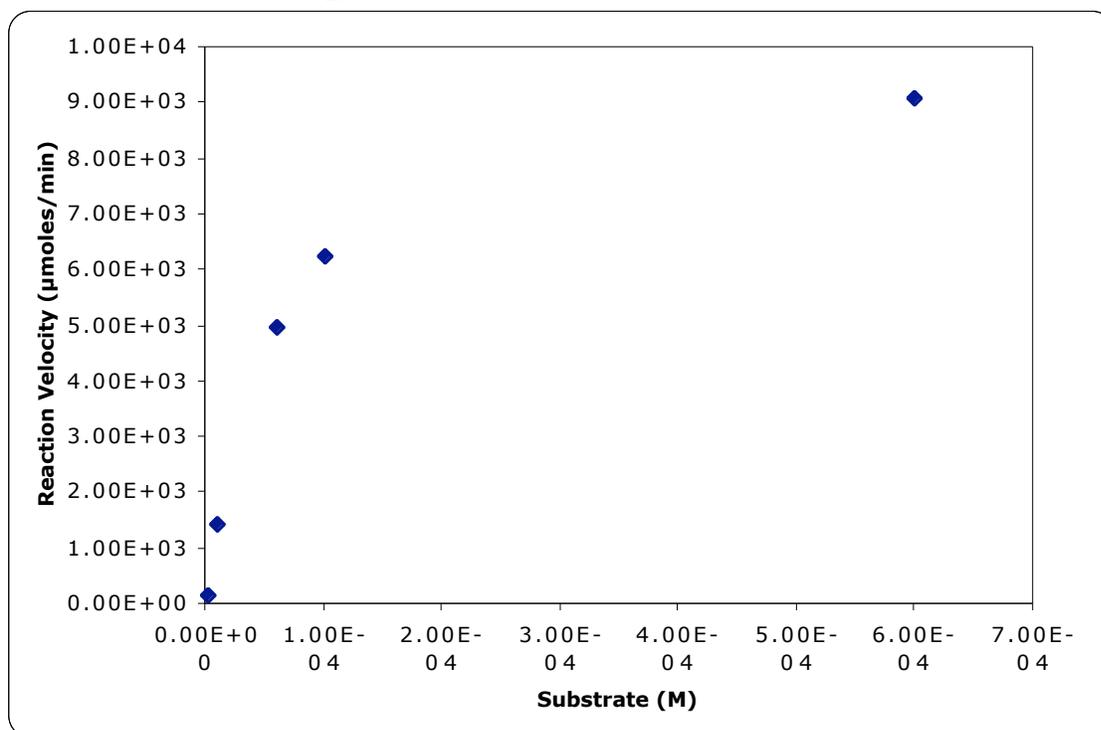
b) What does the value of K_m describe?

c) The above graph only describes a specific enzymatic reaction at a single given concentration of the enzyme. Qualitatively, what would one expect to happen to V_{max} if the amount of enzyme involved in the reaction were doubled?

d) Many enzymatic reactions can be broken down into 3 steps, each with its own specific rate: substrate binding, catalysis, and product release. In most cases product release is not rate limiting and can therefore be disregarded with respect to its effect on the rate of the overall reaction. This leaves substrate binding and catalysis, each of which is modeled by a different portion of the above graph. Describe which section of the above graph best models the rate of catalysis and why?

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Question 4

You have just joined a lab and are handed several plates containing strains of the worm, *C. elegans.* Your research advisor has asked you to perform several crosses and score the phenotypes. The plates are labeled as follows:

Plate	Phenotype		Genotype	
	Body	Movement	Body	Movement
1	Round	Sinuous	+/+	+/+
2	Dumpy	Sinuous	*dpy/dpy*	+/+
3	Dumpy	Uncoordinated	*dpy/dpy*	*unc/unc*
4	Round	Uncoordinated	+/+	*unc/unc*

Your advisor also tells you that the dumpy body type is a recessive phenotype, while uncoordinated movement is a dominant phenotype.

a) Predict the genotypes and phenotypes for F1s from the following crosses. (Be certain to include a Punnet Square):

- Individual from plate 1 × Individual from plate 3

- Individual from plate 2 × Individual from plate 3

- Individual from plate 1 × Individual from plate 4

b) If F1s from the last cross you performed (Plate 1 × Plate 4) were mated, what percentage of the progeny would have an uncoordinated phenotype?

STRUCTURES OF AMINO ACIDS

ALANINE
(ala)

ARGININE
(arg)

ASPARAGINE
(asN)

ASPARTIC ACID
(asp)

CYSTEINE
(cys)

GLUTAMIC ACID
(glu)

GLUTAMINE
(glN)

GLYCINE
(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)