

Solutions to 7.012 Problem Set 2

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.

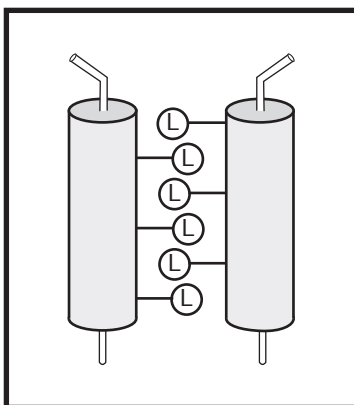


Figure by MIT OCW.

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

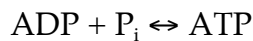
b) DNA-binding proteins often associate with the charged sugar and phosphate backbone of DNA. Which specific amino acids might you find in a DNA-binding protein on the surface that associates with DNA? What kind of interaction do you expect between DNA and these amino acids of the binding protein?

You would expect to find positively charged amino acids (arginine, lysine, histidine) interacting with the negatively charged phosphate backbone.

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? *Segment 1 has more A-T base pairs. A-T base pairs are held together by 2 H-bonds, whereas C-G base pairs share 3 H-bonds. The amount of thermal energy, i.e heat, required to dissociate each segment is directly proportional to the overall number of H-bonds holding the 2 stands of the DNA segment together. Therefore, since Segment 1 required less thermal energy to cause dissociation, it must have had fewer overall H-bonds than Segment 2. As both strands are comprised of an equal number of base pairs, Segment 1 must have had more A-T base pairings.*

Question 2

Consider the following two biologically relevant reactions:



$$\Delta G^\circ = +7.5 \text{ kcal/mol}$$



$$\Delta G^\circ = +9.5 \text{ kcal/mol}$$

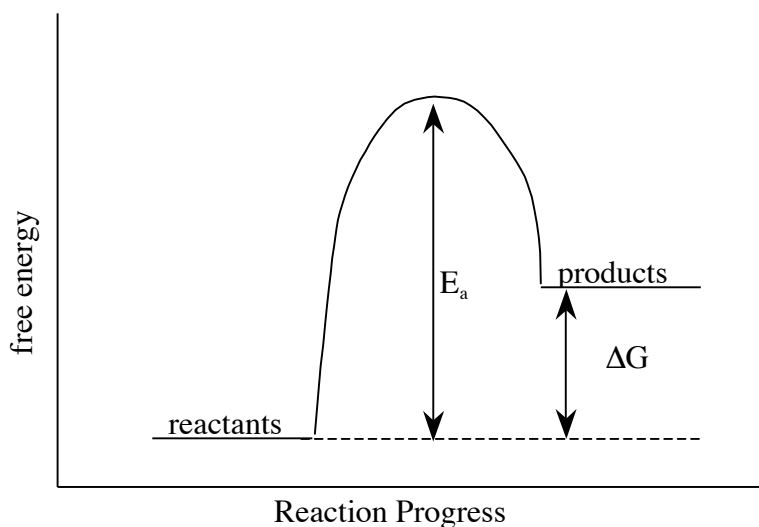
Creatine is used as a storage form of high-energy phosphate in both the muscles and the brain. The enzyme, creatine phosphokinase, transfers the phosphate of ATP to creatine generating creatine phosphate. The reaction is reversible such that when energy demand is high (e.g. during muscle exertion) creatine 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 ΔG° for the overall reaction. Would you expect the reaction to be spontaneous as written?



As written above, the ΔG° is positive and thus the reaction would be non-spontaneous.

b) Draw and label a free energy diagram for the overall reaction as written in (a). Be certain to include the relative energy levels of the substrates and the product, the activation energy and the ΔG° 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?

$$R = 1.987 \times 10^{-3} \text{ kcal mol}^{-1} \text{ K}^{-1}$$

$$T = 273 + 37 = 310 \text{ K}$$

$$\Delta G = \Delta G^\circ + RT \ln \left(\frac{[\text{products}]}{[\text{reactants}]} \right)$$

$$\Delta G = 2.0_{\text{kcal mol}^{-1}} + 1.987 \times 10^{-3}_{\text{kcal mol}^{-1} \text{ K}^{-1}} \times 310 \text{ K} \times \ln \left(\frac{[0.02][26.0]}{[3.0][8.0]} \right)$$

$$\Delta G = -0.36 \text{ kcal/mol}$$

Under the above cellular conditions, the synthesis of creatine phosphate is spontaneous.

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

ΔG° describes the free energy of the reaction under standard conditions when the concentrations of the reactants and products are 1M. ΔG is different from ΔG° , because the reaction is not being carried out 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?

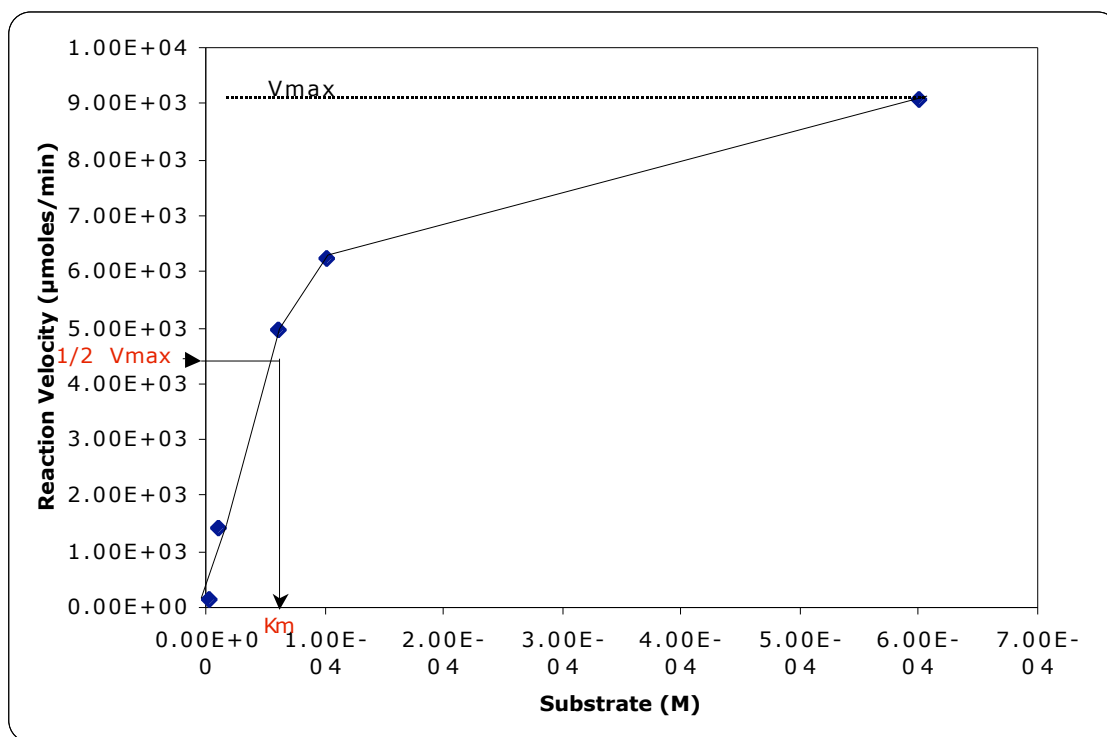
If the equilibrium concentration of ADP were to increase in the cell by an order of magnitude, the synthesis of creatine phosphate would become non-spontaneous as the ΔG would be positive. This illustrates the importance of maintaining the proper balance of various metabolites within the cell, as small changes in concentration of just a few metabolites can have a significant impact upon the behavior and function of the cell.

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 solid line through the 5 reaction coordinates provided that best approximates the activity of a general single-subunit enzyme.
- 2) Draw a dashed 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?

K_m describes the substrate concentration at which the overall rate of the reaction is $1/2 V_{max}$. The value of K_m is proportional to the affinity of the substrate for the enzyme and is thus of significant physiological relevance.

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?

V_{max} would increase.

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?

At high substrate concentrations, e.g. near V_{max} , the enzyme is saturated with substrate. Under these conditions, the binding of substrate is no longer rate limiting as the enzyme can immediately bind new substrate after the release of product. Thus, at high substrate concentrations (the top portion of the graph)

the rate of the enzymatic reaction is limited only by the actual rate of catalysis, i.e. how long it takes to physically perform the chemical reaction.

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	Sinuuous	+ / +	+ / +
2	Dumpy	Sinuuous	<i>dpy</i> / <i>dpy</i>	+ / +
3	Dumpy	Uncoordinated	<i>dpy</i> / <i>dpy</i>	<i>unc</i> / <i>unc</i>
4	Round	Uncoordinated	+ / +	<i>unc</i> / <i>unc</i>

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

	<i>dpy, unc</i>	<i>dpy, unc</i>
+ , +	+/ <i>dpy</i> , +/ <i>unc</i>	+/ <i>dpy</i> , +/ <i>unc</i>
+ , +	+/ <i>dpy</i> , +/ <i>unc</i>	+/ <i>dpy</i> , +/ <i>unc</i>

All F1 progeny are +/*dpy*, +/*unc* in genotype and have a round (wild-type), uncoordinated phenotype.

- Individual from plate 2 × Individual from plate 3

	<i>dpy, unc</i>	<i>dpy, unc</i>
<i>dpy</i> , +	<i>dpy</i> / <i>dpy</i> , +/ <i>unc</i>	<i>dpy</i> / <i>dpy</i> , +/ <i>unc</i>
<i>dpy</i> , +	<i>dpy</i> / <i>dpy</i> , +/ <i>unc</i>	<i>dpy</i> / <i>dpy</i> , +/ <i>unc</i>

All F1 progeny are *dpy*/*dpy*, +/*unc* in genotype and have a dumpy, uncoordinated phenotype.

- Individual from plate 1 × Individual from plate 4

	+ , <i>unc</i>	+ , <i>unc</i>
+ , +	+ / + , + / <i>unc</i>	+ / + , + / <i>unc</i>
+ , +	+ / + , + / <i>unc</i>	+ / + , + / <i>unc</i>

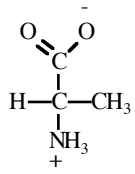
All F1 progeny are + / + , + / *unc* in genotype and have a round (wild-type), uncoordinated phenotype.

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?

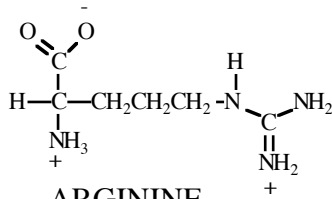
	+ , +	+ , <i>unc</i>
+ , +	+ / + , + / +	+ / + , + / <i>unc</i>
+ , <i>unc</i>	+ / + , + / <i>unc</i>	+ / + , <i>unc</i> / <i>unc</i>

In the F2 generation, 75% of the progeny would be expected to have the uncoordinated (*unc*) phenotype as 3/4 of the progeny carry the dominant *unc* mutation.

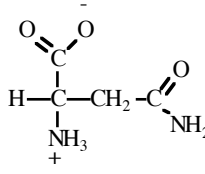
STRUCTURES OF AMINO ACIDS



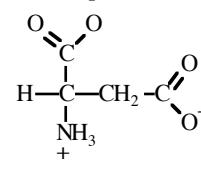
ALANINE
(ala)



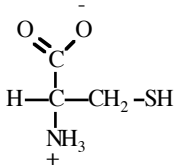
ARGININE
(arg)



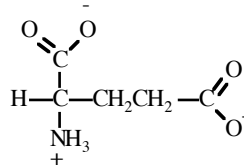
ASPARAGINE
(asN)



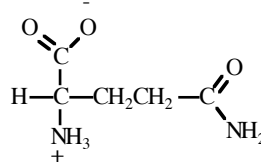
ASPARTIC ACID
(asp)



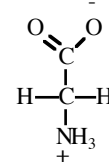
CYSTEINE
(cys)



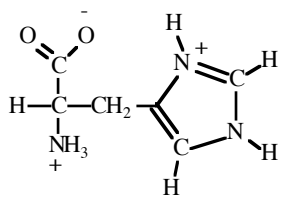
GLUTAMIC ACID
(glu)



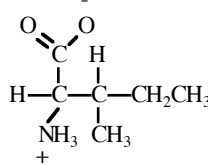
GLUTAMINE
(glN)



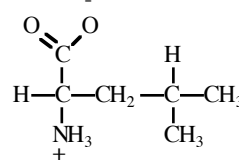
GLYCINE
(gly)



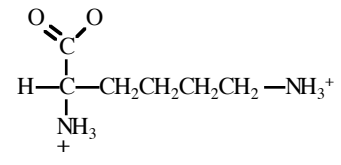
HISTIDINE
(his)



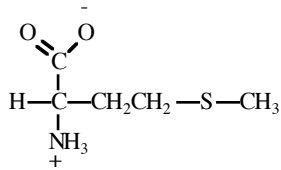
ISOLEUCINE
(ile)



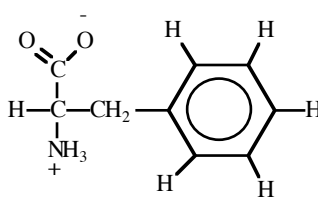
LEUCINE
(leu)



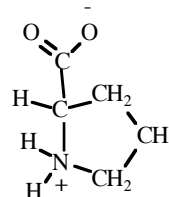
LYSINE
(lys)



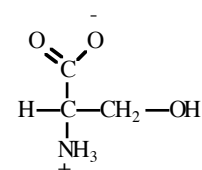
METHIONINE
(met)



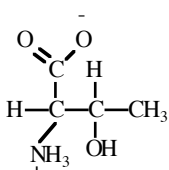
PHENYLALANINE
(phe)



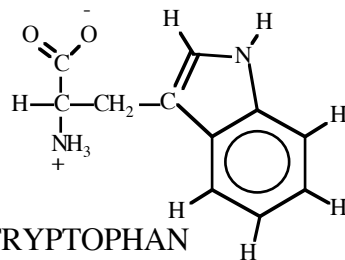
PROLINE
(pro)



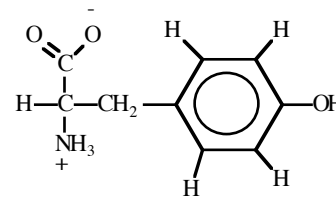
SERINE
(ser)



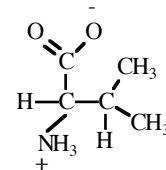
THREONINE
(thr)



TRYPTOPHAN
(trp)



TYROSINE
(tyr)



VALINE
(val)