

Name: \_\_\_\_\_

Section : \_\_\_\_\_

# 7.014 Problem Set 2

Answers to this problem set are to be turned in. Problem sets will not be accepted late.  
Solutions will be posted on the web.

## Question 1

Open the Human  $\gamma$ D crystallin (H $\gamma$ D-Crys) structure html file by clicking on the link on the Problem Sets page. Rotate the molecule and get an idea of the three dimensional structure of (H $\gamma$ D-Crys). H $\gamma$ D-Crys is a soluble human eye lens protein.

I. The true 3-dimensional structure of H $\gamma$ D-Crys can be observed using the “**Show Spacefill**” button, and a trace of the peptide backbone can be observed using the “**Show Ribbon**” button.

a) If possible/applicable, describe the following types of structural features of H $\gamma$ D-Crys:

i. Secondary:

*The secondary structure is described as  $\alpha$ -helical or  $\beta$ -sheet. Secondary structure is controlled by backbone hydrogen bonds between amino acids. H $\gamma$ D-Crys is primarily  $\beta$ -sheet, but has two small  $\alpha$ -helices; one at the top of each domain.*

ii. Quaternary (Hint: Use the “Color Ribbon by Protein Subunit” button):

*The quaternary structure of a protein created by a number of distinct interacting amino acid chains. If we color H $\gamma$ D-Crys by chain we find there is only one amino acid chain and, therefore, no quaternary structure. There are two  $\beta$ -sheet domains in the protein, but they are both from one amino acid chain.*

b) Does there appear to be a gap between the two protein domains in the ribbon and spacefill structure representations?

Ribbon: Yes

Spacefill: No

c) Which of the two representations (spacefill or ribbon) more accurately represents the actual structure of H $\gamma$ D-Crys in the cell? Why?

*The spacefill model is a more accurate representation of what H $\gamma$ D-Crys looks like in the cell. The spacefill representation shows the van der Waal radius or electron density clouds of all non-hydrogen atoms in the protein. The ribbon structure simply shows a trace of the polypeptide backbone without showing any amino acid side chain atoms.*

II. We will now look at some interactions important in maintaining the tertiary structure of H $\gamma$ D-Crys.

d) Choose “**Central Interaction**”

i. What are the names of the amino acids involved in this interaction? (Hint: It may help to change the representation of the residues to ball and stick by clicking in the box labeled “Ball and Stick on/off” below the 3D window. Keep in mind that in cpk coloring, gray is carbon, blue is nitrogen, and red is oxygen. Hydrogen is not shown!)

*The interaction involves a methionine, a phenylalanine, two valines, an isoleucine, and a leucine.*

## Question 1, continued

- ii. The central cluster is composed of what type of amino acids (acidic, basic, hydrophobic, or polar)?

*All of the residues are hydrophobic amino acids.*

- iii. What type(s) of interaction(s) are formed between residues in this cluster?

*All of the amino acids form a hydrophobic cluster and, therefore, are involved in hydrophobic interactions.*

- iv. Select “**Central Interaction with All Atoms Shown.**” This view shows all the atoms of H $\gamma$ D-Crys (not including hydrogens) as transparent dots. Is the central interaction located on the inside or the outside of the protein? Why is this reasonable given that H $\gamma$ D-Crys is a soluble protein?

*These amino acids are primarily found on the interior of H $\gamma$ D-Crys. There are a few areas that are solvent exposed, but most of the cluster is shielded from solvent. It is energetically favorable for this cluster to be buried from a hydrophilic solvent like the cytoplasm or water because the amino acid side chains are hydrophobic and like nonpolar environments.*

- e) Choose “**Interaction I**”

- i. What are the names of the amino acids involved in this inter-domain interaction?

*There is an arginine and a glutamine residue in this interaction.*

- ii. What types of amino acids are involved in this interaction (acidic, basic, hydrophobic, or polar)?

*Glutamine is a polar residue, while arginine is basic.*

- iii. What type(s) of bonding interaction(s) are found between these two residues?

*These residues interact via a hydrogen bond.*

- iv. Based on the 3-D structure of amino acid pairs, what parts of the amino acid are involved in forming this interaction (circle the correct answer)?

*Only the amino acid side chains*

*Only the amino acid backbones*

*Both the amino acid side chains and backbones*

## Question 1, continued

- v. What one amino acid substitution could make this interaction stronger? What one amino acid substitution could make this interaction weaker? Justify your answer in each case.

*Substituting glutamic acid for glutamine would make the interaction stronger. Glutamine and glutamic acid have very similar size and shape, and glutamic acid and arginine would then make an ionic bond, that is stronger than the hydrogen bond that the glutamine and arginine make.*

*Substituting phenylalanine for arginine would make the interaction weaker. Phenylalanine is a large nonpolar amino acid that would only be able to interact with glutamine by van der Waals bonds, a bond that is weaker than the hydrogen bond that the glutamine and arginine make. Although phenylalanine and arginine are both large amino acids, their shapes are not too similar. However, since the interaction in question is happening on the outside of the protein, the shape differences will likely not play a significant role.*

III. HyD crystallin protein is known to be involved in cataract formation in the eye. Cataracts form when several proteins in the eye bond to one another to create a very large insoluble multi-protein complex.

There is a point mutation that changes the arginine normally found at position 14 to a cysteine. Children with this mutation develop cataracts. The overall 3-D structure of the protein remains the same despite this substitution.

Both in the wild type and this mutant version of the HyD crystallin protein there is also a cysteine at position 110. Click on the button labeled "**Residues 14 and 110**" to see the location of these two amino acids.

- f) Are the amino acids at positions 14 and 110 found in the inside or on the outside of the HyD-Crys structure?

*They are found on the outside, solvent-exposed surface of the protein.*

- g) Consider two molecules of mutant HyD crystallin protein. Recall that these molecules would have cysteine residues in both positions 14 and 110. What type of interaction could form between these two molecules?

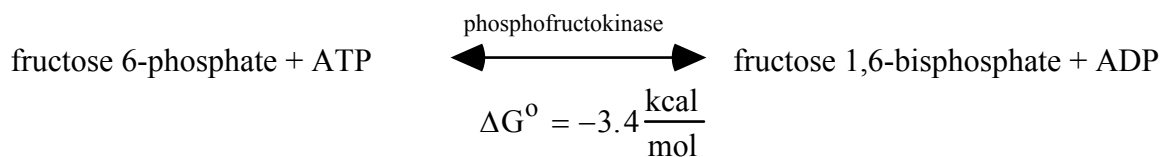
*A disulfide bond could form between two nearby mutated crystallin molecules.*

- h) How could interaction in part g above lead to the development of a cataract?

*The cysteine at position 14 in crystallin molecule A could bond to the cysteine at position 110 of crystallin molecule B. The cysteine at position 14 in crystallin molecule B then could bond to the cysteine at position 110 of crystallin molecule C. This could continue on and on until a very large multi-protein complex formed. Disulfide bonds are covalent bonds and are, therefore, very strong. Covalent bonding between many adjacent crystallin proteins would eventually produce a huge protein complex composed of many different crystallin chains that would become insoluble and fall out of solution simply because of its enormous size.*

## Question 2

The enzyme Phosphofructokinase (PFK) catalyzes the conversion of fructose 6-phosphate (F6P) to fructose 1,6-bisphosphate (FBP), in step 3 of glycolysis.

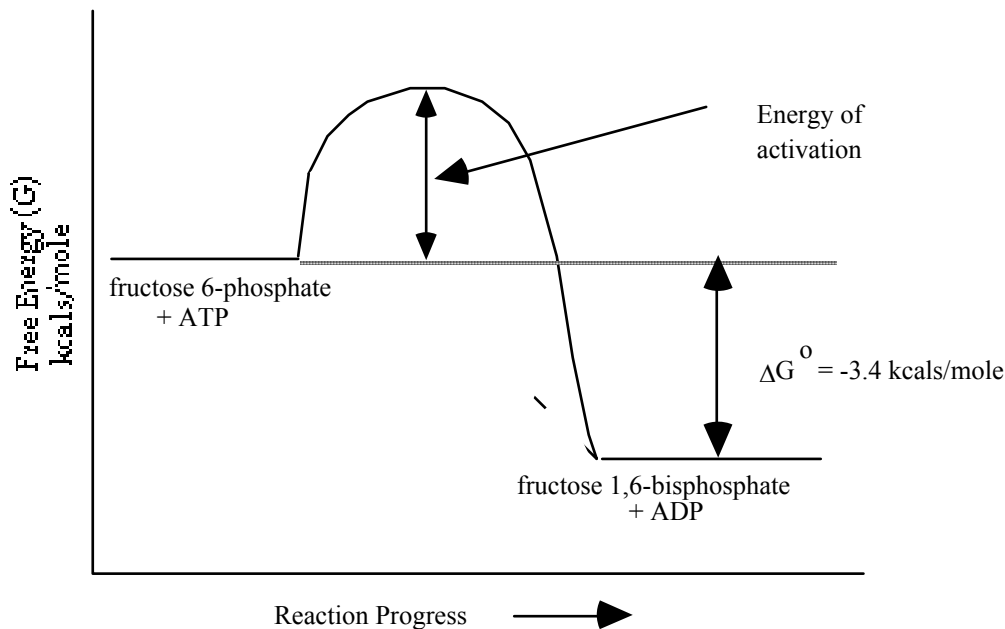


a) What two functions does ATP serve in this coupled reaction?

*The hydrolysis of ATP provides the energy to drive this coupled reaction.*

*ATP also serves to donate a phosphate to fructose 1,6-bisphosphate from fructose 6-phosphate.*

b) Draw and label an energy diagram for this reaction. Include the relative energy levels of the substrates and the products, the activation energy and the  $\Delta G^{\circ}$  for the reaction.



In erythrocytes, the following intracellular concentrations of metabolites are found:

Metabolite	Concentration
Fructose 6-phosphate	.014 mM
Fructose 1,6-bisphosphate	.028 mM
AMP	1 mM
ADP	0.2 mM
ATP	2 mM
Pi	1 mM

## Question 2, continued

c) What is the free energy change of the phosphofructokinase reaction under these cellular conditions (37°C)? Show your work. Is the reaction spontaneous under these conditions?

$$\Delta G = \Delta G^0 + RT \ln \frac{[\text{products}]}{[\text{reactants}]}$$

$$\Delta G = -3.4 \text{ kcal/mole} + 0.61 \text{ kcal/mole} \ln \frac{[\text{FBP}] [\text{ADP}]}{[\text{F6P}] [\text{ATP}]}$$

$$\Delta G = -3.4 \text{ kcal/mole} + 0.61 \text{ kcal/mole} \ln \frac{[.028 \text{ mM}] [0.2 \text{ mM}]}{[.014 \text{ mM}] [2 \text{ mM}]}$$

$$\Delta G = -3.4 + 0.61 \ln (0.2)$$

$$\Delta G = -4.38 \quad \text{Spontaneous}$$

Phosphofructokinase (PFK) is the primary site for regulation of the glycolytic pathway. The activity of PFK is dependent on the energy status of the cell.

d) Why would a cell regulate glycolysis?

*The end result of glycolysis is the generation of ATP. If ATP levels in the cell are high, then the cell would not need to spend resources to generate more ATP. However, if the levels of ATP are low, then the cell would utilize the glycolytic pathway to generate the needed energy.*

e) What is the specific signal or molecule(s) to which PFK responds?

*PFK responds to the decreased ATP to ADP ratio (increased levels of ADP).*

f) Why does this mechanism of regulation make sense?

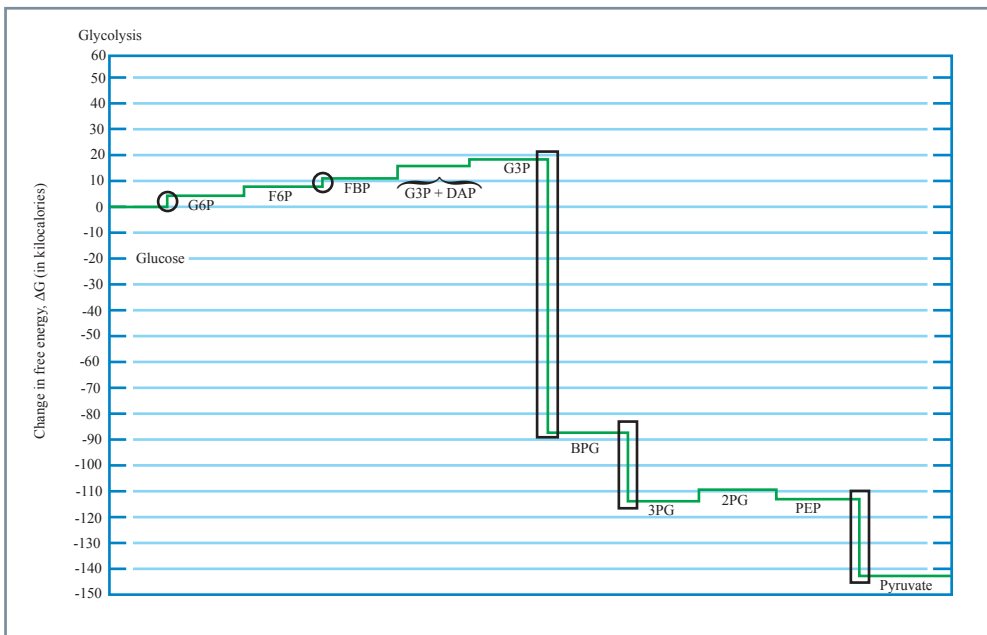
*Normally, a low ATP to ADP ratio indicates that cells need more energy currency available ready to use. By speeding up glycolysis, more ATP can be formed from ADP to raise the ATP to ADP ratio.*

g) In certain tumor cells, an enzyme called ATPase becomes abnormally active, resulting in increased hydrolysis of ATP to ADP. What would be the effect on the overall rate of glycolysis in these cells?

*The activity of the glycolytic enzymes would increase, increasing the overall rate of glycolysis.*

### Question 3

The diagram below shows the change in free energy during the course of glycolysis.



G6P –  
 glucose-6-phosphate  
 F6P –  
 fructose-6-phosphate  
 FBP –  
 fructose-1,6-diphosphate  
 G3P –  
 Glyceraldehydes-3-  
 phosphate  
 DAP –  
 Dihydroxy-acetone-  
 phosphate  
 BPG –  
 1,3-di-phospho-glycerate  
 3PG –  
 3-phospho-glycerate  
 2PG –  
 2-phospho-glycerate  
 PEP –

Figure by MIT OCW.

a) On the diagram, circle the two steps that require ATP. How did you determine that ATP was required for these steps?

*The circled steps lead to the products that have an extra phosphate with respect to the reactant (glucose or the product of the previous step of the pathway)*

b) There are steps where the product has a higher free energy than the reactants and yet these steps do not require ATP. What drives these reactions?

*The steps that do not require ATP are driven by the relative concentrations of reactants (higher) with respect to that of the product (low). The way the cell keeps the concentration of products low is by immediately using the products as reactants in the next step of the pathway.*

c) On the diagram, box the energy harvesting steps.

d) The harvested energy is stored in what molecule(s)?

*NADH and ATP*

e) What is/are the molecule(s) listed in (d) used for?

*ATP is used to drive reactions in the cell, and NADH is used to regenerate NAD<sup>+</sup> for the next rounds of glycolysis.*

## Question 4

Rubisco is a key enzyme in the dark phase of photosynthesis, and is the most abundant protein on Earth. It makes up roughly 50% of the protein content of chloroplasts. Rubisco catalyzes the addition of one molecule of  $\text{CO}_2$  to a five carbon sugar ribulose 1,5-biphosphate. The resulting six-carbon sugar is unstable, and splits into two molecules of 3-phosphoglyceric acid.

a) You happen to have some  $^{14}\text{CO}_2$  around your lab, so you decide to place your favorite ficus into a large hermetically sealed container, and pump some  $^{14}\text{CO}_2$  into the container to mix with regular atmosphere already in there. After some time, you move the ficus to a non-radioactive environment. A quick read with a Geiger counter reveals that the plant is now slightly radioactive.

i. If you moved the plant after 1 minute, and analyzed the cells immediately, where would you expect to find radioactivity? Circle all that apply:

- |  |                      |
|--|----------------------|
| Evenly distributed throughout the cell | <i>carbohydrates</i> |
| <i>Mostly in the chloroplasts</i>      | nucleic acids        |
| Mostly in the nucleus                  | proteins             |
| Mostly in the mitochondria             | lipids               |

ii. If you moved the plant after 1 minute and analyzed the cells the next day, would your answer change? Why or why not?

*After 1 minute, we would expect most of  $^{14}\text{C}$  to be either in the form of 3-phosphoglyceric acid, ribulose 1,5-biphosphate (that is regenerated from 3-phosphoglyceric acid), or in another carbohydrate in the glucose-generating pathway. If the plant is removed and the leaves are taken for analysis after 1 minute, but are not actually analyzed until the next day, we would expect some metabolic processes to happen in the leaves in the intervening time, such that some  $^{14}\text{C}$  would end up in other molecules in the cells.*

iii. If you moved the plant the next day, where would you expect to find radioactivity in a ficus leaf cell? Circle all that apply:

- |  |                      |
|--|----------------------|
| Evenly distributed throughout the cell | <i>carbohydrates</i> |
| Mostly in the chloroplasts             | <i>nucleic acids</i> |
| Mostly in the nucleus                  | <i>proteins</i>      |
| Mostly in the mitochondria             | <i>lipids</i>        |

Justify your answer(s)

*$\text{CO}_2$  that is fixed from the atmosphere is used to make glucose that is then used in many intersecting pathways in the cell. Glycolysis is used to make ATP, while other pathways lead to making of nucleotides, amino acids, and fats. In short, all mass was (at some point) gas.*

## Question 4, continued

- iv. You dream that your pet goat eats your experimental plant. In such a scenario, where would you now find  $^{14}\text{CO}_2$ ?

*The goat would use  $^{14}\text{C}$  acquired from the plant in all aspects of its metabolism. Some of it would exit in excrement, some would be used to build new molecules in goat's cells, and some would be respired out in the form of  $^{14}\text{CO}_2$ .*

Rubisco is a very inefficient enzyme – it only processes about three  $\text{CO}_2$  molecules per second. Inside a plant chloroplast it forms large complexes with eight active sites in each. The complex does not appear to be allosterically regulated – each subunit appears to be very rigid, and each active site operates independently from all other active sites in the complex. Recall that chloroplasts are small organelles.

- b) Propose a hypothesis for why Rubisco forms large complexes, and an experiment to test this hypothesis. Assume that you can create cells with any kind of mutant Rubisco protein you would like.

*One hypothesis would be that because the room inside the chloroplasts is limited, Rubisco forms tight complexes such that the most active sites can be exposed in the least amount of space.*

*One way to test this hypothesis is by creating cells where the only Rubisco protein is the mutant that can not form these large complexes. The model behind our hypothesis would then predict that in such cells, a lot less  $\text{CO}_2$  would get fixed, resulting in a lot less energy and raw materials available for the cell. It is reasonable to expect that an organism consisting of this type of cells would have stunted growth.*

*Therefore, if the mutant plant exhibits stunted growth, the result would be consistent with our hypothesis. If not, our hypothesis would be proven wrong, and we would have to start again.*