

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 γ D crystallin (H γ 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 γ D-Crys). H γ D-Crys is a soluble human eye lens protein.

I. The true 3-dimensional structure of H γ 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 γ D-Crys:

i. Secondary:

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

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

Ribbon:

Spacefill:

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

II. We will now look at some interactions important in maintaining the tertiary structure of H γ 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!)

Question 1, continued

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

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

iv. Select “**Central Interaction with All Atoms Shown.**” This view shows all the atoms of HyD-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 HyD-Crys is a soluble protein?

e) Choose “**Interaction I**”

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

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

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

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.

III. H γ D 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 H γ D 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.

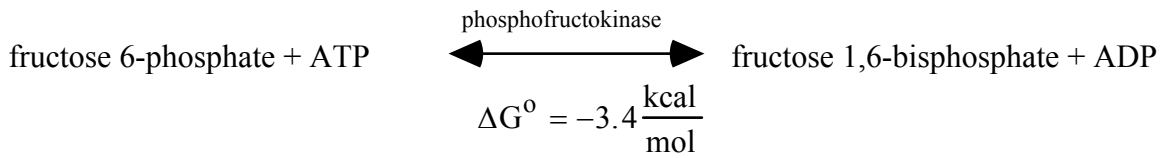
f) Are the amino acids at positions 14 and 110 found in the inside or on the outside of the H γ D-Crys structure?

g) Consider two molecules of mutant H γ D 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?

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

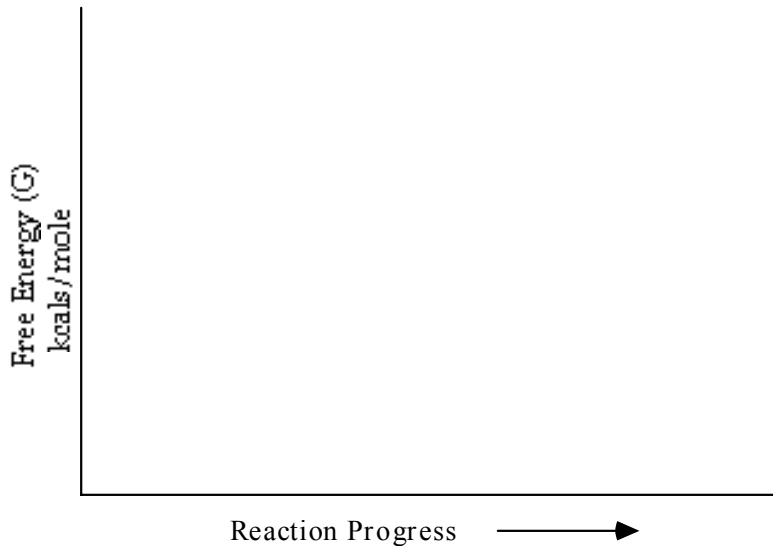
Question 2

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?

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 ΔG° 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?

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?

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

f) Why does this mechanism of regulation make sense?

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?

Question 3

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

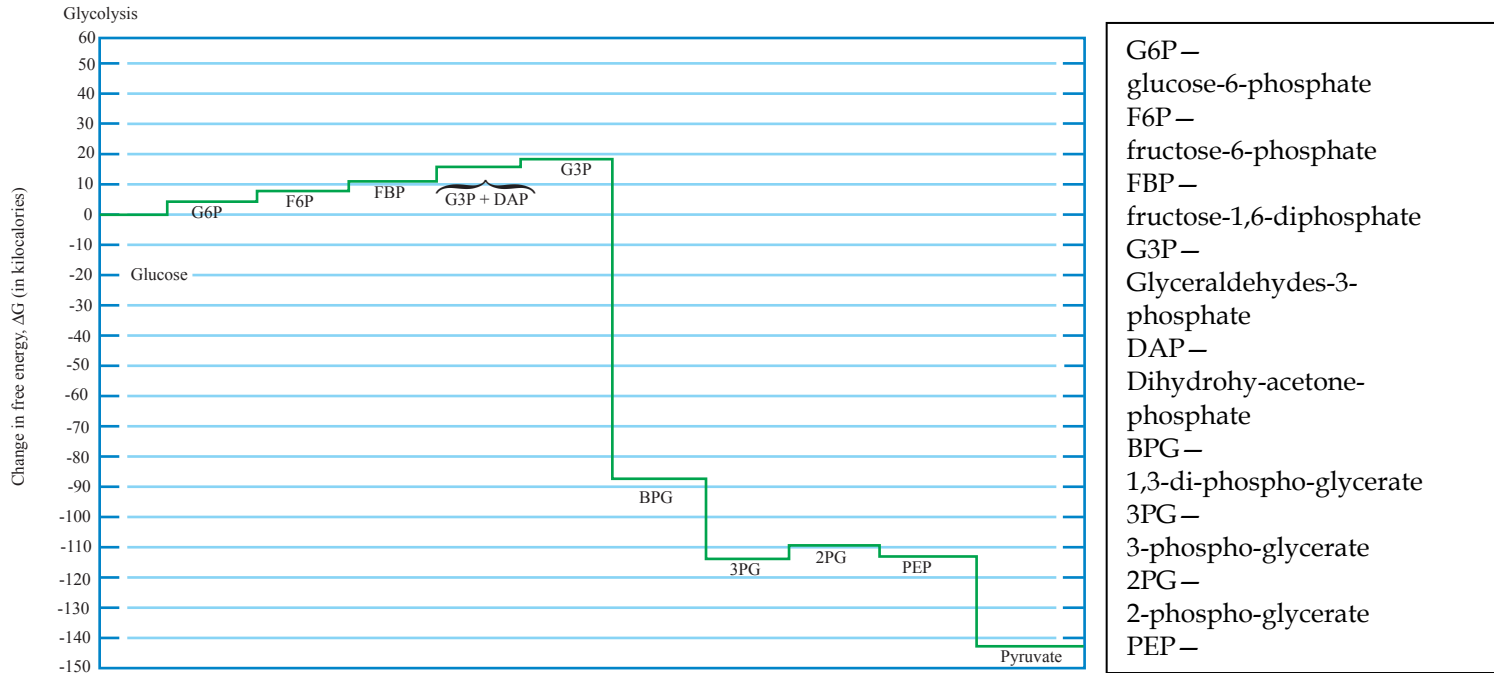


Figure by MIT OCW.

- On the diagram, circle the two steps that require ATP. How did you determine that ATP was required for these steps?
- 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?
- On the diagram, box the energy harvesting steps.
- The harvested energy is stored in what molecule(s)?
- What is/are the molecule(s) listed in (d) used for?

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 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 | carbohydrates |
| Mostly in the chloroplasts | 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?

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 | carbohydrates |
| Mostly in the chloroplasts | nucleic acids |
| Mostly in the nucleus | proteins |
| Mostly in the mitochondria | lipids |

Justify your answer(s)

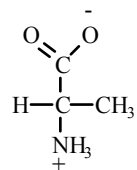
Question 4, continued

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

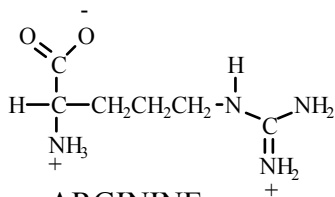
Rubisco is a very inefficient enzyme – it only processes about three CO_2 molecules per second. Inside a plant chloroplast it forms large complexes with eight active sites in each. The complex does not appear to be allosterically regulated – each subunit appears to be very rigid, and each active site 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.

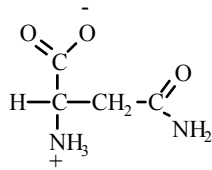
STRUCTURES OF AMINO ACIDS at pH 7.0



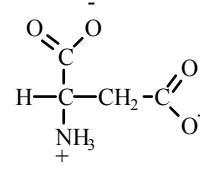
ALANINE
(ala)



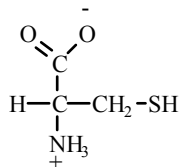
ARGININE
(arg)



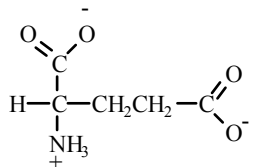
ASPARAGINE
(asN)



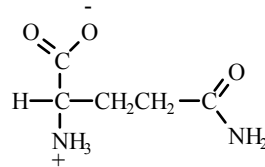
ASPARTIC ACID
(asp)



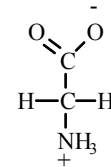
CYSTEINE
(cys)



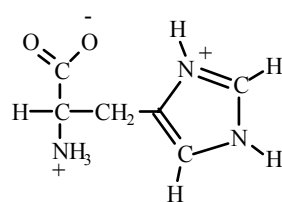
GLUTAMIC ACID
(glu)



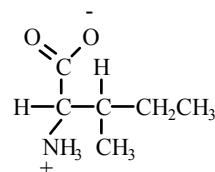
GLUTAMINE
(glN)



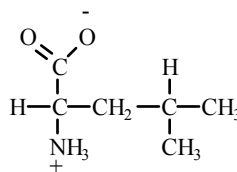
GLYCINE
(gly)



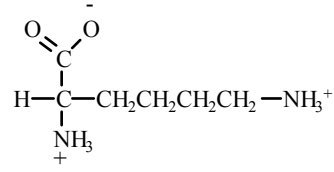
HISTIDINE
(his)



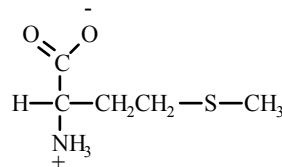
ISOLEUCINE
(ile)



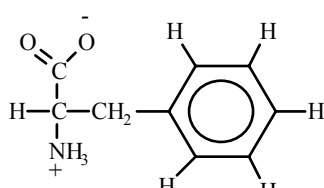
LEUCINE
(leu)



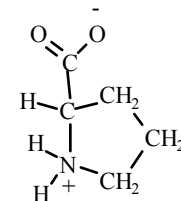
LYSINE
(lys)



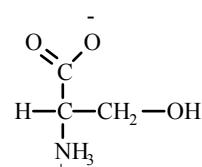
METHIONINE
(met)



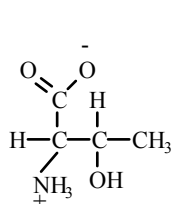
PHENYLALANINE
(phe)



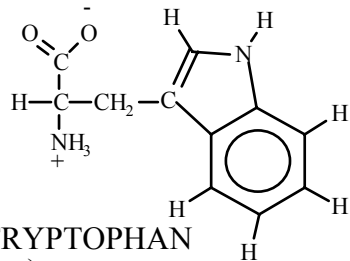
PROLINE
(pro)



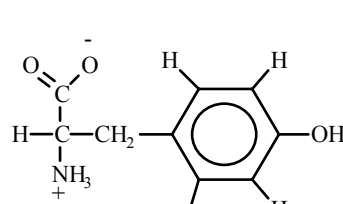
SERINE
(ser)



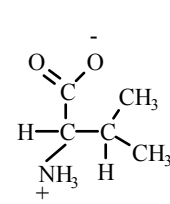
THREONINE
(thr)



TRYPTOPHAN
(trp)



TYROSINE
(tyr)



VALINE
(val)