

1. Some proteins, like the jellyfish green fluorescent protein that we used in the lab as a marker in transgenic tobacco cells, contain alpha helices that are entirely buried within the interior of the protein.
- a. (4 pts) What general type of amino acid (in terms of the chemical properties of the side chain) would you expect to find oriented toward the outside of such a buried alpha helix?

The interior of proteins is usually occupied predominantly by hydrophobic (non-polar) amino acid side chains.

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- b. (3 pts) What type of amino acid would have its side chains oriented toward the inside of the buried alpha helix?

None (with the arguable exception of glycine). Side chains of amino acids found in alpha helical structures are oriented toward the outside of the helix. The interior of an alpha helix is crowded by the atoms of the peptide backbone in Van der Waals contact with one another.

2. Consider the DNA sequence:  
GAACTAGCCATCGCGAT

- a. (4 pts) What is the 5' base in this strand?

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- b. (4 pts) Give the sequence of the mRNA that would be produced by utilizing this strand as the template for transcription.

AUCGCGAUGGCUAGUUC

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- c. (4 pts) How many amino acids could be encoded by this region of the mRNA?

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- d. (4 pts) For this region of mRNA, which part would encode the amino acid closest to the amino terminal end of the protein?

AUC

3. (10 pts) Succinctly describe the evidence that DNA replication is semi-conservative.

Replication of DNA in the presence of physically dense analogues of the deoxynucleoside triphosphate precursors (the example we discussed involved using bromo-deoxyuridine as a dense analogue of thymidine) yields a product that is intermediate in density between the unlabeled DNA and DNA that has been labeled for many generations (fully labeled). Physical density of DNA can be determined by equilibrium sedimentation in a density gradient of a salt such as CsCl. This intermediate density is expected in the case of semi-conservative replication, but a mixture of unlabeled and fully dense DNA would be expected if replication were conservative. The semi-conservative replication pattern can be confirmed by observation of the DNA generated in a further round of replication in the presence of the dense label. The semi-conservative mechanism predicts that the DNA of intermediate density (one unlabeled strand and one labeled strand) would replicate to yield one intermediate density duplex (one labeled and one unlabeled strand) and one fully dense duplex (both strands labeled).

- 4a. (4 pts) Identification of the amino-terminal amino acid in a wide variety of cellular enzymes reveals a range of different amino acids. I presume that you find this experimental finding surprising. If so, explain why. If not, explain why (no points for an answer like "I don't find anything surprising.").

All protein synthesis inserts the amino acid methionine as the first (hence amino-terminal) amino acid. This mechanism would suggest that methionine should always be the amino-terminal amino acid of any protein.

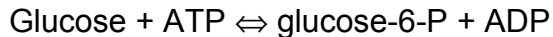
- 4b. (4 pts) What does this finding imply about the relationship between the process of protein synthesis and the process of producing functioning enzymes in cells (Hint: there is a general analogy with the relationship between primary RNA transcripts and functional RNA molecules)?

If the amino-terminal amino acid is not methionine, then there must be some post-translational processing of the protein that removes at least the methionine (often many more amino acids are removed). RNA molecules are also often modified after transcription before they become functional.

5. (10 pts) Synthesis of macromolecular polymers (DNA, RNA, protein) involves the generation of highly ordered molecules from comparatively disordered monomers. For one of these polymers, give a succinct and specific rationale for why we think its synthesis does not violate the second law of thermodynamics.

In each case, the production of ordered structures (the polymer) is coupled to the release of substantial enthalpy from high energy covalent bonds. The enthalpy release exceeds the entropy gain, giving a net release of free energy in the reaction, in full compliance with the second law of thermodynamics. In nucleic acid synthesis, two phosphoanhydride bonds are broken for each phosphodiester bond formed. For protein synthesis, two phosphoanhydride bonds from ATP ( $\text{ATP} + \text{H}_2\text{O} \rightarrow \text{AMP} + 2\text{Pi}$ ) and two from GTP ( $2\text{GTP} + 2\text{H}_2\text{O} \rightarrow 2\text{GDP} + 2\text{Pi}$ ) are broken for each peptide bond formed.

6. The enzyme hexokinase catalyzes the addition of phosphate to the 6-position of glucose in the following overall reaction:



a. (5 pts) In the absence of hexokinase, in which direction do you expect the equilibrium of this reaction to lie? (give your reasons)

The equilibrium is toward the right since the products on that side contain one less phosphoanhydride bond and one more phosphate ester bond. The enthalpy released from the phosphoanhydride bond is more than that stored in the phosphate ester bond that is formed.

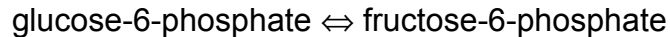
b. (4 pts) Is it possible, in the presence of the enzyme, to arrange this reaction so that it yields net ATP synthesis? If you think not, explain why (succinctly) and if so, explain how.

If the concentrations of the "products" on the right side of the equation relative to those on the left are greater than those predicted by the standard free energy change in the reaction, then the net direction of the reaction will be toward ATP synthesis. [One way to arrange this would be to continually remove glucose by some other reaction (such as oxidation).]

c. (4 pts) How is your answer to parts a and b affected by the presence or absence of the enzyme?

Unaffected. Enzymes, as catalysts, do not affect the equilibrium of reactions, only their rates.

7. The enzyme phosphoglucose isomerase catalyzes the following reaction that is important in glycolysis:



- a. (4 pts) Do you expect this enzyme to be found in eukaryotes only, prokaryotes only or both? (give your reason)

Glycolysis is a universal pathway found in both prokaryotes and eukaryotes.

- b. (4 pts) Using the isolated enzyme and starting with no fructose-6-phosphate present in the solution, under what conditions would you expect the enzyme reaction rate to be unchanged when we double the concentration of glucose-6-phosphate (concentrations  $x$  and  $2x$ )?

When the enzyme is saturated with substrate at concentrations well above the  $K_m$ , the net rate of the reaction will be unaffected by changes in the substrate concentration.

- c. (3 pts) Under what conditions (again no fructose-6-phosphate present) would you expect the enzyme reaction rate to be twice as fast when the concentration of glucose-6-phosphate is doubled (concentrations  $y$  and  $2y$ ). Compare concentration  $y$  with concentration  $x$  from part b.

When the substrate is limiting for the reaction (at concentrations well below the  $K_m$ ), the net reaction rate can double when the concentration of substrate is doubled. Concentration  $y$  is substantially lower than concentration  $x$ .

- d. (3 pts) Why does the enzyme reaction rate decrease when the temperature is raised above a certain threshold?

Above a certain temperature, thermal fluctuations in the solution will produce forces that can overcome the non-covalent forces that hold the protein's 3D structure together and it will "denature." The denatured protein will no longer catalyze the reaction and the rate will decline.

8. (10 pts) In mitochondria, the enzymes that make up the metabolic pathway known as the tricarboxylic acid cycle catalyze the complete oxidation of carbon from pyruvic acid to CO<sub>2</sub>. Although this set of reactions leads to the loss of carbon from the cell, it also supports more rapid growth (carbon compound accumulation) than in its absence. Please give a brief but specific explanation for this apparent paradox.

Carbon oxidation releases energy that is conserved in the form of reduced metabolic cofactors (mainly NADH+) in the TCA cycle. This energy can then be coupled (through various steps) to the biosynthetic reactions that are necessary for forming the diverse compounds essential for growth that are not obtained directly from the outside. Thus, a portion of the carbon compounds obtained from the outside contribute energy when they are oxidized to yield CO<sub>2</sub> while the remainder contribute the carbon skeletons that will be modified in reactions that are coupled to this energy to yield the necessary compounds (and growth).

9a. (4 pts) Place the following cellular components in order of decreasing size: Ribosome, amino acid, hexokinase, ATP, mitochondrion, tRNA, nucleus, golgi vesicle, actin filament, water.

Actin filament length > Nucleus > mitochondrion > golgi vesicle > ribosome > hexokinase, tRNA, actin filament width > ATP > amino acid > water molecule.

9b. (2 pts) Given two of each component (e.g. two water molecules) separated from each other by a space equal to their smallest dimension (e.g. 0.15nm for water), which could be resolved in the light microscope? (Circle the resolvable components in your list in part a.)

9c. (2 pts) Aside from adequate separation, what other general property would the components have to have in order to be distinguished from one another in the microscope?

Contrast

9d. (4 pts) Which of these components are not found in prokaryotic cells?

Nucleus, mitochondrion, golgi apparatus, actin filaments