Multiple Choice Questions

1. The site in the cell at which cellular respiration occurs is the ___________.
   a. endoplasmic reticulum
   b. Golgi complex
   c. ribosome
   d. nucleus
   e. mitochondria

2. One molecule of water is capable of forming ___________ hydrogen bonds.
   a. two
   b. three
   c. four
   d. five
   e. six

3. The pKa of glutamic acid is around 4 in an aqueous environment. When that same glutamic acid is located in the nonpolar interior of a protein, its apparent pKa _________ and it becomes a __________ acid.
   a. remains the same; stronger
   b. decreases; stronger
   c. decreases; weaker
   d. increases; stronger
   e. increases; weaker

4. What buffer system is predominantly used in the blood?
   a. phosphate
   b. bicarbonate
   c. citrate
   d. borate
   e. Tris

5. Which set of the following amino acids are all polar?
   a. W  K  Q
   b. H  M  N
   c. T  N  Y
   d. M  T  C
   e. P  C  S

6. When you measure the amount of a protein in a solution by UV absorbance, you are actually detecting the UV absorbance of predominantly which amino acid?
   a. W
   b. P
   c. R
   d. M
   e. H
7. In what order will the amino acids E, H, I, K be eluted from a column of DEAE-cellulose ion exchange resin (an anion exchanger) by a buffer at pH 6?
   a. E I H K
   b. I E K H
   c. H K E I
   d. K H I E
   e. I K H E

8. When separating proteins using gel filtration chromatography, the first proteins to be eluted will be ________________.
   a. smallest
   b. largest
   c. negatively charged
   d. positively charged
   e. more spherical in shape

9. The pH of a solution is 8.3. What is the [OH⁻]?
   a. 5 x 10⁻⁹
   b. 1 x 10⁻⁷
   c. 2 x 10⁻⁶
   d. 5 x 10⁻⁶
   e. 9 x 10⁻¹

10. What is the approximate pI of the following polypeptide: ANYDGHERM?
    a. 4
    b. 5
    c. 7.5
    d. 9.5
    e. 11

11. Which of the following can best be related to tertiary protein structure?
    a. AIVRKYP
    b. α helix
    c. Greek key
    d. myoglobin
    e. hemoglobin
12. If the protein below were digested with trypsin, how many fragments would you expect to find? (Trypsin cleaves on the C-terminal side of Lysine and Arginine, unless the next amino acid is proline)

AVMFRLSGCKPV

TCLKWCQRECMI

a. two
b. three
c. four
d. five
e. six

13. Edman reagent cleaves polypeptides ____________.

a. after M residues
b. after positively charged residues
c. after bulky nonpolar residues
d. at the C-terminus
e. at the N-terminus

14. What best summarizes the MALDI method by which gas phase ions are produced for mass spectrometry?

a. Sample is hit by a low energy xenon beam.
b. Sample is forced through a narrow capillary tube and solvent rapidly evaporates.
c. Sample is embedded in a crystalline matrix and bombarded by laser beams.
d. Sample is heated and then bombarded by electrons
e. Sample is forced through a narrow capillary tube and then bombarded by electrons.

15. In an α helix,

a. side chain residues point up and down the axis of the helix.
b. the helix is right-handed.
c. there are five residues per helical turn.
d. the oxygen of the carbonyl carbon in a peptide bond points out toward the exterior of the helix.
e. there are usually many proline residues present.

16. Collagen

a. has a very regularly repeating primary protein structure.
b. is a dimer of two intertwined helices.
c. is composed almost entirely of β-pleated sheets.
d. has a very compact, “springy” structure.
e. is a protein found in hair, among other things.
17. Molecular chaperones, such as the GroEL/ES system, have been proposed to have which function(s)?
   a. They shuttle the protein to its appropriate location within the cell.
   b. They provide the folding protein with a protected environment from the aqueous interior of the cell.
   c. They unfold and release misfolded proteins.
   d. Two of these statements are true.
   e. All of these statements are true.

18. What type of glycosidic bond is present in lactose?
   a. $\alpha(1\rightarrow2)$
   b. $\beta(1\rightarrow2)$
   c. $\alpha(1\rightarrow4)$
   d. $\beta(1\rightarrow4)$
   e. $\alpha(1\rightarrow6)$

19. How would you classify D-erythrose by its chemical nature?
   a. aldotriose
   b. ketotriose
   c. aldotetrose
   d. ketotetrose
   e. aldopentose

20. The heme group found in hemoglobin
   a. coordinates the iron atom in the plane of the heme only when oxygen is bound.
   b. contains a centrally bound Fe(III) atom.
   c. is covalently bound to the molecule.
   d. is held within the central cavity formed between the four hemoglobin subunits.
   e. is not found in myoglobin.
Written Answer Questions

1. (10 points total)
1 a) Translate the following amino acid sequence into one letter code:
   LeuGluAlaArgAsnIleAsnGlySerCysIleGlu AsnCysGluIleSerGlyArgGluAlaThr. Note the message.

   LEARNING SCIENCE IS GREAT   (+3 points)

1 b) A protein was purified to homogeneity. Determination of the molecular weight by molecular exclusion chromatography yields 60 kd. Chromatography in the presence of 6 M urea yields a 30 kd species. When the chromatography is repeated in the presence of 6 M urea and 10 mM mercaptoethanol, a single molecular species of 15 kd results. Determination of the N-termini with Dansyl chloride yielded Dansyl-ala and Dansyl-leu. Describe the structure of the molecule.

   The 60 kd protein is a dimer of dimers. It consists of four 15 kd subunit chains, each chain has either an alanine or a leucine residue at the N-terminus. Each 30 kd dimer consists of two subunit chains held together by one or more disulfide bridges. (+3 points).
   (Urea is a denaturant that causes the 60 kd protein to separate into two 30 kd dimers. Mercaptoethanol is a reducing agent that breaks the disulfide bridges between the two 15 kd subunits of each dimer. Dansyl chloride reacts with primary amines, thus will react to the amino acid at the N-terminus.)

1 c) Determine the sequence of a hexapeptide based on the following data. Note: when the sequence is not known, a comma separates the amino acids. (see the table below for enzyme specificities)
   Amino acid composition: (2R,A,S,V,Y)
   N-terminal analysis of the hexapeptide: A
   Trypsin digestion: (R,A,V) and (R,S,Y)
   Carboxypeptidase A digestion: No digestion
   Chymotrypsin digestion: (A,R,V,Y) and (R,S)

   Trypsin cleaves on the C-terminal side of lysine and arginine residues(except before proline)
   Carboxypeptidase A cleaves on the amino side of C-terminal residues (except arg, lys or pro)
   Chymotrypsin cleaves on the C-terminal side of tyr, trp, phe, leu and met

   The sequence is: AVRYSR  (+4 points)
2. (9 points total)
   2. a). Sketch the pH titration curve for glutamic acid. Label both axes and the equivalence points
   ![Titration Curve Diagram]
   (+3 points)

2 b). Draw chemical structures of the different Glu ionization states. Indicate on your sketch of part a) where each Glu structure would be the principal species present.

\[
\begin{align*}
\text{H} & \quad \text{H}_3\text{N}-\text{C}-\text{COOH} & \leftrightarrow & \quad \text{H} & \quad \text{H}_3\text{N}-\text{C}-\text{COO}^- & \leftrightarrow & \quad \text{H} & \quad \text{H}_2\text{N}-\text{C}-\text{COO}^- \\
\text{CH}_2 & \quad \text{CH} & \quad \text{CH}_2 & \quad \text{CH} & \quad \text{CH}_2 & \quad \text{CH}_2 & \quad \text{CH}_2 & \quad \text{CH}_2 \\
\text{CH}_2 & \quad \text{COOH} & \quad \text{COO}^- & \quad \text{COOH} & \quad \text{COO}^- \\
A & \quad B & \quad C & \quad D \\
\end{align*}
\]
(+3 points, including designation of principal species in above graph)
2 c. Draw the structure of the following peptide with the R-groups as they occur at pH 7.

\[ ^{1}H_{3}N\text{-Met-Trp-Arg-Asp-Leu-COO}^{-} \]

3. (6 points total)
3. a) The partial pressure of CO\(_2\) in the lungs can be varied rapidly by the rate and depth of breathing. For example, a common remedy to alleviate hiccups is to increase the concentration of CO\(_2\) in the lungs. This can be achieved by holding one's breath, by very slow and shallow breathing (hypoventilation), or by breathing in and out of a paper bag. Under such conditions, the partial pressure of CO\(_2\) in the air space of the lungs rises above normal. Qualitatively explain the effect of these procedures on the blood pH.

As the partial pressure of CO\(_2\) rises in lungs, levels of H\(_2\)CO\(_3\) rise and this thereby causes proton concentration to rise (leChatlier’s principle). Therefore, blood pH lowers and becomes more acidic.

3 b). During a short-distance run the muscles produce a large amount of lactic acid (CH\(_3\)CH(OH)COOH, \(K_a = 1.38 \times 10^{-4}\)) from their glucose stores. In view of this fact, why might hyperventilation before a dash be useful?

Hyperventilation would have the opposite effect to above.
More O\(_2\), less CO\(_2\) → blood becomes less acidic and so it can more easily absorb build-up of lactic acid without bringing on acidosis.
4. (6 points total)
4. As the newest and least experienced student in a biochemistry research lab, your first few weeks are spent washing glassware and labeling test tubes. You then graduate to making buffers and stock solutions for use in various laboratory procedures. Finally, you are given responsibility for purifying a protein. It is a citric acid cycle enzyme, citrate synthase, located in the mitochondrial matrix. Following a protocol for the purification, you proceed through the steps below. As you work, a more experienced student questions you about the rationale for each procedure. Supply the answers.

4 a) You pick up 20 kg of beef hearts from a nearby slaughterhouse. You transport the hearts on ice, and perform each step of the purification on ice or in a walk-in cold room. You homogenize the beef heart tissue in a high-speed blender in a medium containing 0.2 M sucrose, buffered to a pH of 7.2. Why do you use beef heart tissue, and in such large quantity? What is the purpose of keeping the tissue cold and suspending it in 0.2 M sucrose, at pH 7.2? What happens to the tissue when it is homogenized?

Beef is easily obtained and inexpensive. It also works all of the time and so is rich in mitochondria compared to white muscle tissue. However, we still need a large quantity because there are many purification steps required with a loss of yield at each step. We keep the tissue cold to slow down tissue degradation by proteases, and the sugar prevents mitochondrial lysis. Homogenization breaks cell membranes but allows mitochondria to stay intact.

(+2 points)

4 b) You subject the resulting heart homogenate, which is dense and opaque, to a series of differential centrifugation steps. What does this accomplish?

Differential centrifugation allows for the precipitation and discarding of entities larger (more dense) than mitochondria. After sequential runs or through the use of a density gradient, i.e. zonal centrifugation (see pp. 153-154), relatively pure mitochondria can be obtained.

(+2 points)

4 c) You proceed with the purification using the supernatant fraction that contains mostly intact mitochondria. Next you osmotically lyse the mitochondria. To this lysate you add ammonium sulfate, a highly soluble salt, to a specific concentration. You centrifuge the solution, decant the supernatant, and discard the pellet. To the supernatant, which is clearer than the lysate, you add more ammonium sulfate. Once again, you centrifuge the sample, but this time you save the pellet because it contains the protein of interest. What is the rationale for the two-step addition of the salt?

The first step is to raise ammonium sulfate concentration to just below that which would precipitate out the desired protein, citrate synthase in this case. These precipitated, unwanted proteins can then be removed by centrifugation. The second addition of ammonium sulfate is to bring the salt concentration just above that required to precipitate out the citrate synthase, which is then recovered in the pellet of the centrifuged sample.

(+2 points)
5. (5 points total)
In X-ray studies of crystalline peptides, Linus Pauling and Robert Corey found that the C-N bond in the peptide link is intermediate in length (1.32 Å) between a typical C-N single bond (1.49 Å) and a C=N double bond (1.27 Å). They also found that the peptide bond is planar (all four atoms attached to the C-N group are located in the same plane) and that the two α-carbon atoms attached to the C-N are always trans to each other (on opposite sides of the peptide bond):

5 a). What does the length of the C-N bond in the peptide linkage indicate about its strength and its bond order (i.e., whether it is single, double, or triple)?

The C-N peptide bond is intermediate in strength between the single C-N bond and the double C=N bond; therefore, it has a partial double bond character.

(+2.5 points)

5 b). What do the observations of Pauling and Corey tell us about the ease of rotation about the C-N peptide bond?

The partial double bond character of the peptide bond makes rotation difficult.

(+2.5 points)

6. (7 points total)
6 a) The fructose in honey is mainly in the β-D-pyranose form. This is one of the sweetest carbohydrates known, about twice as sweet as glucose. The β-D-furanose form of fructose is much less sweet. The sweetness of honey gradually decreases at a high temperature. Also, high-fructose corn syrup (a commercial product in which much of the glucose in corn syrup is converted to fructose) is used for sweetening cold but not hot drinks.

What chemical property of fructose could account for both of these observations?

The equilibrium of the pyranose/furanose forms is affected by temperature with the β-D-furanose form being more predominant at high temperatures.

(+2 points)

6 b) Draw the structure of fructose in the β-D-furanose form.

(+2 points)
6 c). The almost pure cellulose obtained from the seed threads of *Gossypium* (cotton) is tough, fibrous, and completely insoluble in water. In contrast, glycogen obtained from muscle or liver disperses readily in hot water to make a turbid solution. Although they have markedly different physical properties, both substances are composed of (1→4)-linked D-glucose polymers of comparable molecular weight. What structural features of these two polysaccharides underlie their different physical properties? Explain the biological advantages of their respective properties.

The different properties result from whether the linkages are β(1→4) as in cellulose, which allows for extended, relatively flat sheets of highly hydrogen bonded chains or whether they are α(1→4) linkages with α(1→6) branches as in glucose. The α(1→4) configuration puts each glucose at an angle with respect to the preceding one inducing a loose coil structure, while the branches results in a shrub-like structure.

(+3 points)

7. (7 points total)
7 a) Many common parameters are input into the refinement program to produce a protein structure, whether by NMR or X-ray methods. The unique, experimentally derived information in the NMR method are the ___________ while the unique, experimentally derived information in X-ray crystallography are the ___________. In the equation below for calculating the electron density function, place a circle around the experimentally derived parameter and draw a square around the “missing” information that can often be recovered by one of the three major methods commonly used for this such as ___________.

\[
\rho(X,Y,Z) = \frac{1}{V} \sum \sum \sum F(hkl) \exp(i \alpha(hkl)) \exp(-2\pi i(hX + kY + lZ))
\]

(+1 point each)

7 b). Name two significant advantages of using synchrotron radiation in structural studies by crystallographic methods.

i) Very high beam intensity allows for small samples (+1 point)

ii) Tunable wavelengths for MAD phasing (+1 point)
8. (10 points total)
Consider structure determination by NMR methods. Examine the data given in the figure below and then answer the following questions. Note that the “Assignment” problem has been solved for you.

8a) What kinds of information can be obtained from this data?
NOESY provides distance information measured through space. Peaks represent protons that are “close” together in space (<6 Å). From the assignment of cross-peaks, a list of distance restraints can be generated to deduce the 3D structure.

(+3 points)

8b) Label all the peaks in the figure by using their association with the letters “a” through “g”.
(+3 points)

8c) Using your understanding of NMR methods of structure determination and the assignment information provided at the bottom of the figure, sketch a “probable” structure for this “molecule” based on the data provided in the figure. Use the labels given to indicate the direction of the molecule in your model.

NOESY spectra indicate that:
 b is close to g
e is close to f
d is close to e
(+4 points)
Bonus Question (2 points). A peptide with the primary structure Lys-Arg-Pro-Leu-Ile-Asp-Gly-Ala is sequenced by the Edman procedure. If each Edman cycle is 96% efficient, what percentage of amino acids liberated in the fourth cycle will be leucine?

\[(0.96)^4 = 84.9\%\]  
(+1 point)

Do the calculation a second time, but assume 99% efficiency for the cycle.

\[(0.99)^4 = 96\%\]  
(+1 point)