Biochemistry Exam II Fall 2012 Dr. Stone
Name_____

There are 11 short answer/ multiple choice questions worth 2 points each. There are six long answer questions worth a total of 68 points.

Short, 2 point questions. Be brief, but not vague. Specfic details are needed.

1. Why is SDS (sodium dodecyl sulfate) added to the acrylamide gel for the electrophoresis of proteins?

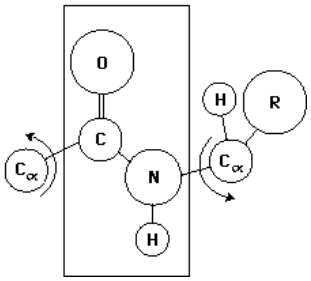
2. Why is a peptide treated with dithiothreitol and iodoacetate prior to SDS PAGE?

3. What is isoelectric focusing used for?

4. What chemical is used to only determine the N-terminus amino acid?

5. What chemical is used to sequence all of the amino acids in a peptide?

6. In the diagram below, what does the plane drawn behind the peptide bond indicate?



7. Where are the hydrogen bonds in an alpha helix?

8. Where are the amino acid R (side chains) groups in an alpha helix?

9. In an ELISA, what binds to the protein of interest? (The protein that is being detected?)

10. What two amino acids are commonly found in the middle of β turn?

11. Which of the following binding constants is for a protein that has the lowest affinity for its ligand?

A.) $K_a = 1.0 \times 10^8 M^{-1}$

B) $K_d = 1.0 \times 10^{-10} M$
C) $K_d = 1.5 \times 10^{-9} M$
D) $K_a = 2.0 \times 10^{8} M^{-1}$
E) None of these, they are all the same

This section requires longer answers, be specific and give details.

12. (10 points) Describe the functions and structures of the proteins listed below:

    a. Collagen

    b. Keratin

13. (10 points) In hemoglobin, the pKa value for histidine (His HC-3) changes depending on the conformation of hemoglobin. When hemoglobin is in the T form, the pKa for this histidine is 8.1, in the R form, the pKa is 6.0. In the T form, this histidine forms a salt bridge. In the R form, it does not.

    a. Draw the structure of histidine showing the equilibrium between the protonated and deprotonated forms.

    b. Explain why this histidine has two different pKa values.

14. (8 points) The following peptide was determined to have a threonine as the N-terminus aminot acid. It was then cleaved with an enzyme and the resulting peptides were separated by anion exchange chromatography. For all of the peptides, the N terminus amino group pKa is 8 and the C terminus carboxyl pKa is 3.4.

The fragments are:
EES SWQMPYANK TIGER

a. What is the order of elution from the anion exchange column:
1.

2.

3.

b. What enzyme was used to cleave the peptide?

c. Can you determine the sequence of the original peptide? What is it? (Or, what are the possibilities?)

15. (10 points) The following mutations in hemoglobin have been detected. Predict how each change will affect the structure and function of hemoglobin. Then briefly describe your rationale.

    a. Normal hemoglobin has L at position 88 on the beta chain. The mutant has R.

    b. Normal hemoglobin has A at position 110 on the beta chain. The mutant has P.

    c. Normal hemoglobin has N at position 57 on the beta chain. The mutant has K.

d. Normal hemoglobin has E at position 6 on the beta chain. The mutant has V.

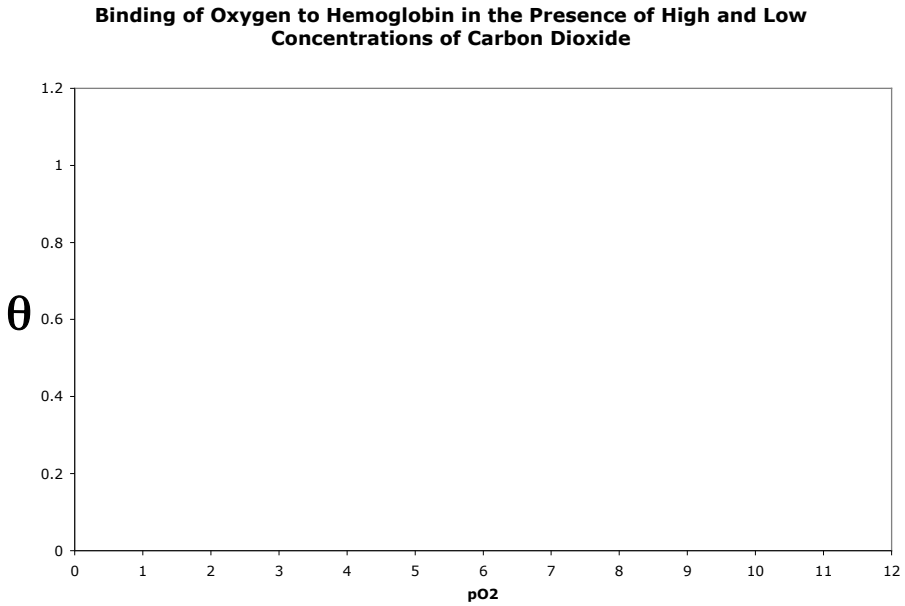
e. Normal hemoglobin has V at position 20 on the beta chain. The mutant has I.

16. (20 points) Carbon dioxide is produced in the tissues during metabolism.

a. Fully describe how carbon dioxide is transported from the tissues to the lungs.

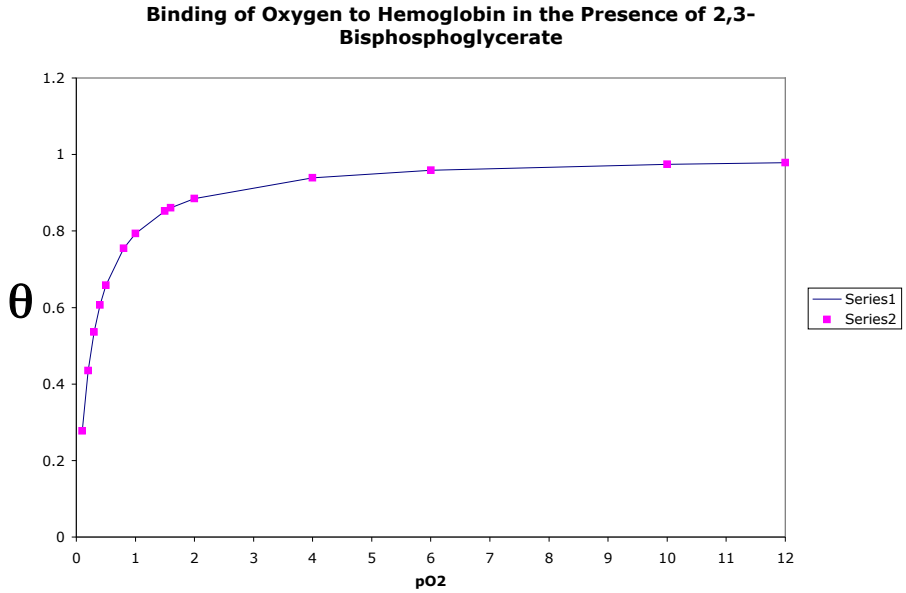
b. "Houston, we have a problem": Lack of fresh air (like being stuck on Apollo 13, or not breathing due to an overdose of narcotics) will cause carbon dioxide levels to rise. Why is this dangerous?

d. Draw the graph of θ vs pO_2, showing the binding of oxygen in high (1.3mM) and low (1.0mM) carbon dioxide. (one line for each condition)



e. How does Carbon dioxide change the shape of hemoglobin and thus alter its affinity for oxygen?

17. (10 points) You did an experiment to determine the effect of 2,3-bisphosphoglycerate on oxygen binding to hemoglobin. Unfortunately, your cat spilled diet pepsi on your graph and it washed away your hand draw labels. Now, you have to remember which line went with each experiment.
    i. Label each line on the graph below.
        A. 0 mM 2, 3-bisphosphoglycerate
        B. 5 mM 2,3-bisphosphoglycerate
        C. 8 mM 2,3-bisphosphoglycerate



ii. Describe how 2,3 bisphosphoglycerate binds to hemoglobin to alter its affinty for oxygen.