CHEM-342 Introduction to Biochemistry Name _____
Midterm Examination - Individual Part (75%)
Wednesday, 21 March 2012
H. B. White – Instructor

Range 34-121/132 Average ± SD = 77.7 ± 30.9 N=27

Important - Please read this before you turn the page.

Write your name on every page.

There are 8 pages to this examination including this page.

This individual part of the midterm examination is worth 132 points.

The examination is closed book until 8:15 AM. Thereafter you may refer to your notes, course reader, handouts, or graded homework assignments. Textbooks and reference books cannot be used. The examination ends promptly at 9AM.

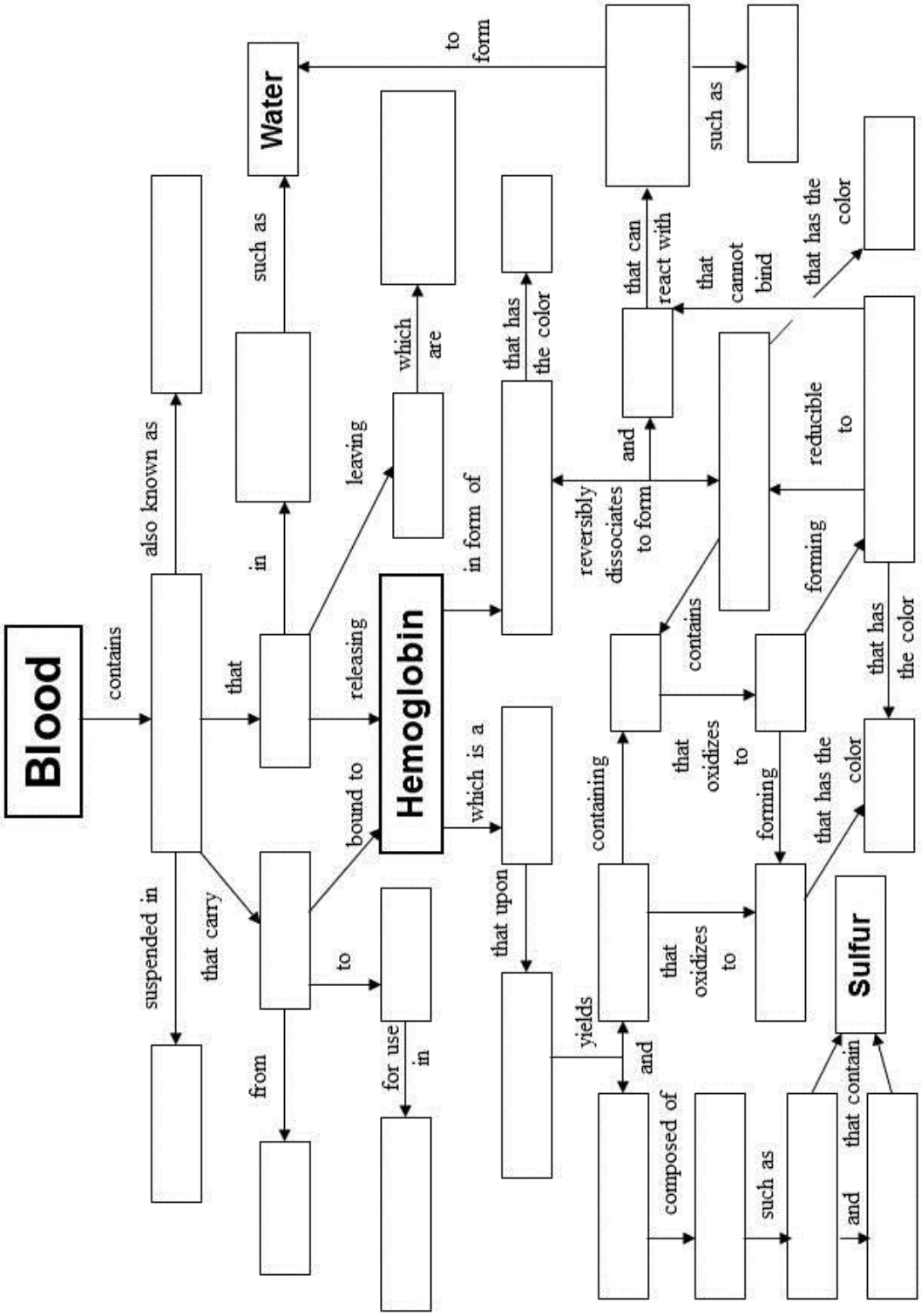
This examination will assess your learning, problem-solving skills, and ability to communicate clearly. Parts are intended to be challenging even to the best students in the class.

Writing reflects how you think. Better quality answers will receive higher marks. Therefore organize your thoughts before you write and draw. Among the "right answers" I will read, some will be better than others because they:

- show greater depth of understanding,
- provide a more logical structure,
- use appropriate examples,
- include appropriate illustrations,
- avoid extraneous or inaccurate information, and
- choose words with precision.

Strive to write not that you may be understood, but rather that you cannot possibly be misunderstood. Stream of consciousness answers are rarely well organized or clearly presented. Also, **USE YOUR OWN WORDS**, transcription of words from your notes does not show me that you understand.

Complete the Concept Map (30 Points) Most of the terms have been removed from the concept map below. Based on the linking words associated with the arrows, fill in the missing terms in the boxes provided



Part II (37 Points) Short Answer about Equilibrium, Equality, and Equations

2. (25 Points Total) It could be argued that the most important chemical concept needed for understanding biochemistry is the concept of equilibrium. In a living cell, innumerable reversible association-dissociation reactions are coupled and shift in response to a changing chemical environment. The association-dissociation equilibrium between hemoglobin and molecular oxygen is a good example of this foundational concept. Based on your readings so far, provide **five specific examples** where the condition in the body (*in vivo*) or imposed by an experimenter (*in vitro*) shifted the equilibrium position of the oxygen-binding reaction. **How** was the equilibrium perturbed in each case?

(1 pt) Circle direction of equilibrium shift. (2 pt) Show the chemistry of the perturbation.	(2 pt) Describe in words the circumstances where this perturbation happens or happened.
a. $HbO_2 \leftarrow\rightarrow Hb + O_2$	
b. $HbO_2 \leftarrow\rightarrow Hb + O_2$	
c. $HbO_2 \leftarrow\rightarrow Hb + O_2$	
d. $HbO_2 \leftarrow\rightarrow Hb + O_2$	
e. $HbO_2 \leftarrow\rightarrow Hb + O_2$	

3. (Two points each, 12 total) When a system is at equilibrium, there is no change in some measurable parameter over time. That applies in chemistry, biology, and physics. Yet what is measured and what is equal can be quite different and thus confusing. Equilibrium conditions lead to powerful mathematical equations that enable predictions and hypothesis testing. You have encountered the equilibrium concept in chemistry, biology, and physics. Please fill in the following table as it applies to each discipline.

Discipline	What doesn't change over time?	What is "equal" at equilibrium?	Bonus: Provide an appropriate mathematical equation for each.
Chemistry (à la Stokes, Zinoffsky, Bohr et al. and others			
Biology (à la Diggs et al. and population genetics)			
Physics (à la Svedberg and Fåhraeus and ultracentrafugation)			

Part III Short Essay and Problems (59 points)

4. (5 points) Peters used ferricyanide to oxidize hemoglobin to methemoglobin and release the bound oxygen, which he measured as a gas. But, oxygen is soluble in water and was released from hemoglobin in solution. How is it possible to quantitatively measure oxygen volumetrically as a gas when some of it is dissolved in solution?

5. Questions about Zinoffsky's article.

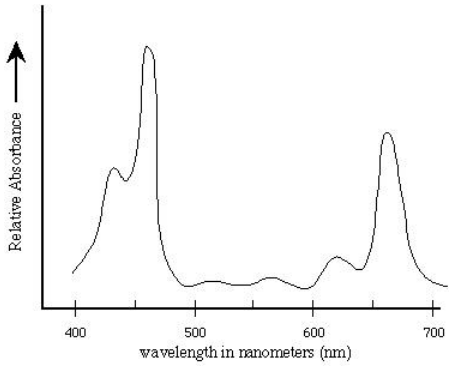
a. (5 points) Explain why the stoichiometry between iron an sulfur was important for Zinoffsky's estimation of the size of horse Hemoglobin.

b. (3 points) Zinoffsky was concerned about the formation of methemoglobin. Why would methemoglobin interfere with his purification?

c. (3 points) If Zinoffsky had done his elemental analysis on methemoglobin, would he have gotten different results?

d. (3 points) What would be a good reason why Zinoffsky worked with oxyhemoglobin, rather than deoxyhemoglobin?

6. (10 points) The following is an absorption spectrum of a common biological molecule. If, instead of hemoglobin, Stokes had studied this molecule using a spectroscope, what would a figure in his publication look like?



a. (7 points) Draw a figure that would correspond to his woodcut for this molecule.

b. (3 points) Based on what you know, what color would this molecule be?

7. (10 points total, 5 points each) Describe the experimental observations and the logic that led to **two of the three** following conclusions:

a. Oxygen forms a specific chemical combination with hemoglobin.

b. Oxygenation of hemoglobin is different than oxidation.

c. Hemoglobin binds four molecules of oxygen, not one.

8. (10 points total) The Hill Equation, $y/100 = Kx^n/(1 + Kx^n)$ is a mathematical model derived from the following chemical model of oxygen binding to hemoglobin ("y" is the percent saturation of hemoglobin; "x" = pO_2; "K" is the equilibrium constant; "n" is the number of oxygen-binding units in the aggregate):

$$Hb_n + nO_2 \overset{K_{eq}}{\leftrightarrow} Hb_nO_{2n}$$

Barcroft (1913) showed that the Hill Equation fit the data for oxygen binding to hemoglobin, e.g. the sigmoid data from Bohr et al., better than other mathematical models of the day. He argued for the generality of the model because it also accommodated CO binding, CO binding in the presence of O_2, and the effect of CO_2 on the binding of CO and O_2.
Answer **one** of the following two questions.

a. Derive the Hill Equation from the chemical model.

b. Hill's chemical model does not recognize intermediates such as $Hb_4(O_2)_2$, yet solutions of hemoglobin that are 50% saturated are clearly possible. Assume, as is now known, that hemoglobin occurs as aggregates of four oxygen-binding subunits (n = 4). Draw a molecular representation (that would satisfy Hill and Barcroft) of a solution in which hemoglobin is 50% O_2-saturated.

9. (10 points) Answer **one** of the following two choices.

a. The title of the article by Svedberg and Fåhraeus does not mention hemoglobin. Rather, it focuses on a method and on proteins in general. Given that there are thousands of proteins, make a convincing case that the authors used good judgment in selecting hemoglobin as the specific example to illustrate their method.

b. Adair (1925) published his result that hemoglobin had a molecular weight four times that estimated by Zinoffsky before Svedberg and Fåhraeus (1926) published the same conclusion. Yet, it was Svedberg, not Adair, who received the Nobel Prize for the work. Explain.