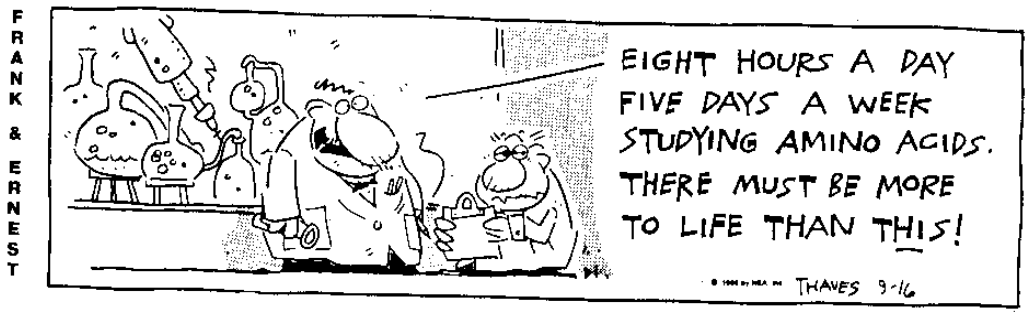
Introduction to Biochemistry **Your Name**_____
Final Examination - Individual (Part I)
Tuesday, 24 May 2011
7:00 – 8:45 PM
H. B. White – Instructor

130 Points

Important - Please read this before you turn the page.

- For the first hour, this is a closed-book examination. From 8 – 8:45 PM you may refer to your course notes and materials.
- This examination will assess your learning, problem-solving skills, and ability to communicate clearly. It is intended to be challenging even to the best students in the class. Writing reflects how you think. Among the "right answers" I will read, some will be better than others because they show greater depth of understanding, avoid extraneous or inaccurate information, provide a more logical structure, use appropriate examples, and choose words with precision. Better quality answers will receive higher marks. Therefore organize your thoughts before you write or draw. 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.
- This examination emphasizes work done in this course since Spring Break; however, knowledge is not so conveniently compartmentalized. Therefore, you should feel free to use any relevant example from your experience, if it is appropriate.
- There are 9 pages to this part of the examination (counting this cover page). There is also a worksheet distributed separately. Please write your name on each page. Feel free to use the backs of pages, if you need more space.
- Part I (130 points) This individual part of the examination, includes 11 problems and essay questions.
- Part II (35 points) The group part of the examination will require you to deal with new information collaboratively.
- If you complete Part I early, you may leave the room and move to 205 Brown Lab where the Group Part of the examination will begin about 8:50 PM.
- You may refer to your notes, course reader, handouts, or graded homework assignments after the first hour of the examination and for the group part of the examination.
- Attempt to draw a picture or diagram as part of your answer to every question.
- Have a productive and safe summer.



NAME _____

1. (5 points) You were asked to attend two seminars during the semester. Pick one and describe something about it even though it may have been pretty advanced.

2. (6 points) This is a variation of that recurrent end-of-the-semester nightmare students have—somehow you managed to go through the semester not realizing CHEM-342 had a lab and you discover yourself facing a lab final with a single practical problem. The TA hands you two tubes containing diluted oxygenated hemoglobin and says your grade depends on how soon you can convert one to deoxyhemoglobin and the other to methemoglobin. Any chemical you might want is in the laboratory. What would you do to pass this dream test and wakeup happy?

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3. (6 points) One of the objectives of the hemoglobinopathy assignment was to have you demonstrate your ability to research a topic and pursue it into areas of knowledge not covered in the course. Pick one significant new topic or concept you learned about as a result of doing your hemoglobinopathy assignment and write a short clear paragraph describing it.

4. (8 points) Is it possible to inhale a molecule of oxygen O_2 and have it have it reduced by the electron transport system in a mitochondrion in your big toe without ever being bound to hemoglobin? Explain your answer.

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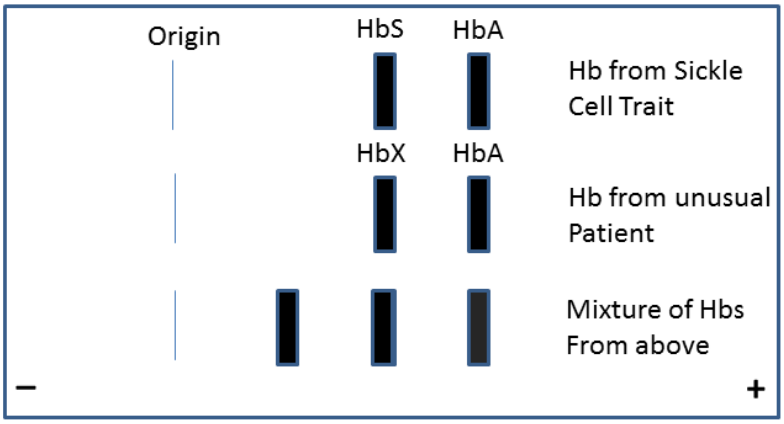
5. (10 points) The electrophoretic pattern of blood from a patient indicated that she had sickle cell trait. However, when a sample of her blood was subjected to low oxygen pressure, the red blood cells did not sickle. A researcher suggested that a sample of the patient's blood should be mixed with that from a person with sickle cell trait and the mixture subjected to electrophoresis. The results are shown below. Given that HbA has a subunit composition of $\alpha_2\beta_2$ and HbS has a subunit composition of $\alpha_2\beta^S{}_2$, explain each of the bands observed when the two were mixed and subjected to electrophoresis as shown below?



6. (10 points) Pauling and coworkers did not know the exact cause of the electrophoretic difference between HbA and HbS. Knowing the $\alpha_2\beta_2$ structure of hemoglobin and given that amino acid side chains can have a charge ranging from -1 to +1, ***what is the maximum range of charge difference*** that could be observed between HbA and a hemoglobin variant differing from HbA at ***one amino acid position in either the α or β chain***? Show with a diagram how you arrived at your answer.

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7. (10 points) A Kenyan hospital tested 1000 newborn infants for the sickle cell disease and found the following: 15 had sickle cell disease, 233 had sickle cell trait, and 752 did not have the sickle cell gene, Estimate the frequency of the sickle cell gene in this population. Given the frequency you calculate, what would be the expected number of newborns with each genotype?

8. (6 points) A group of CHEM-342 students were arguing in the absence of their omniscient tutor:

   Alphie "I think the sickle cell gene is recessive because sickle cell anemia occurs only in people with two copies of the gene."

   Betty "How can that be? The sickle cell gene has to be dominant because at low oxygen pressure cells will sickle whether they are homozygous or heterozygous for the sickle cell gene."

   Gammy "You are both wrong." The sickle cell gene and the allelic normal beta globin gene are expressed together each producing functional protein. The alleles must be codominant."

   Della "So which is it? Recessive, dominant, or codominant?"

   How would you answer Della's question?

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9. (12 points) The quality of experimental results usually depends on careful design and planning. Both Shemin and Rittenberg (1946) and Dintzis (1961) were interested in understanding protein synthesis. However, they used different labeled amino acid precursors to label proteins in their respective experiments. Shemin and Rittenberg used ^{15}N-glycine, while Dintzis used ^{3}H and ^{14}C-Leucine. Discuss the reasons why a good or fortuitous choice (A-C) for one experiment might be inappropriate or less than ideal (a-c) for the other experiment.

	^{15}N-Glycine	**^{14}C and ^{3}H-Leucine**
Shemin & Rittenberg (1946)	A. B. C.	a. b. c.
Dintzis (1961)	a. b. c.	A. B. C.

Bonus questions (1 point each)

What is human body temperature in ºC?

What is the pO_2 at sea level in mmHg?

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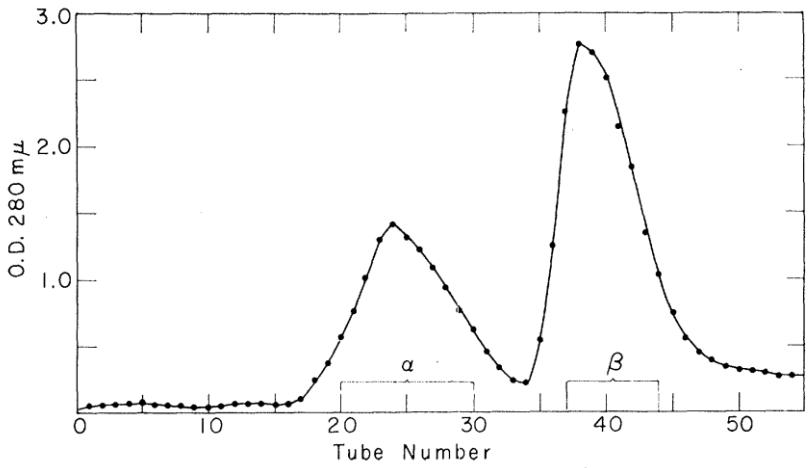
10. (12 points total) In Dintzis' experiment, he separated the α and β globin chains of rabbit hemoglobin using carboxymethyl-cellulose column chromatography. The elution profile Dintzis observed is shown below.



A. (4 points) Dintzis detected the globin chains by measuring "O.D. 280 mμ". What is this?

What chemical aspect of the globin chains is being detected by "O.D. 280 mμ"?

B. (4 points) The α and β globin chains form an $\alpha_2\beta_2$ stoichiometric complex in normal HbA. Thus one would expect equal amounts of the two subunits when they are separated as in Dintzis' experiment; however, the areas under the peaks in the diagram shown are not equal. Provide a reasonable explanation for this observation.

C. (4 points) What property of the α-chain would likely cause it to elute from carboxymethyl-cellulose before the β-chain?

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11. (43 points total) This problem has a number of parts in which you will apply what you have learned about protein structure and synthesis from the Pauling et al., Ingram, and Dintzis articles as well as your hemoglobinopathy assignment.

When Dintzis published his first paper on the direction of protein synthesis, the amino acid sequence of hemoglobin was not known from any organism. He made the presumption that the labeled peptides he observed could be ordered in sequence by their increasing $^{3}H/^{14}C$ ratios. Soon after the sequence of human (not rabbit) hemoglobin was determined. Dintzis, with Naughton, published a second paper because the previous paper did not "constitute a rigorous proof of the sequential assembly of amino acids into hemoglobin." Now the sequence of rabbit hemoglobin is known. It is aligned and displayed with human hemoglobin below. (You will be marking up this figure for your answer. ***You may use the worksheet handout to develop your answers.***)

Alpha Chain Globins

```
                        10                  20                  30                  40
RABBIT  VLSPADKTNIKTAWEKIGSHGGEYGAEAVERMFLGFPTTKTY→
HUMAN   VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTY→

          50                  60                  70                  80                  90
FPHFDFTHGSEQIKAHGKKVSEALTKAVGHLDDLPGALSTLSDLHAHKLR→
FPHFDLSHGSAQVKGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLR→

         100                 110                 120                 130                 141
VDPVNFKLLSHCLLVTLANHHPSEFTPAVHASLDKFLANVSTVLTSKYR
VDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSKYR
```

Beta Chain Globin

```
                    10                  20                  30                  40
RABBIT  VHLSSEEKSAVTALWGKVNVEEVGGEALGRLLVVYPWTQRFFES→
HUMAN   VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFES→

        50                  60                  70                  80                  90
FGDLSSANAVMNNPKVKAHGKKVLAAFSEGLSHLDNLKGTFAKLSELHCDK→
FGDLSTPDAVMGNPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDK→

       100                 110                 120                 130                 140       146
LHVDPENFRLLGNVLVIVLSHHFGKEFTPQVQAAYQKVVAGVANALAHKYH
LHVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH
```

The single-letter abbreviations for the 20 amino acids are:

A = Alanine	I = Isoleucine	R = Arginine
C = Cysteine	K = Lysine	S = Serine
D = Aspartic Acid	L = Leucine	T = Threonine
E = Glutamic Acid	M = Methionine	V = Valine
F = Phenylalanine	N = Asparagine	W = Tryptophan
G = Glycine	P = Proline	Y = Tyrosine
H = Histidine	Q = Glutamine	

Questions on next page

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(continued) Pay attention to detail.

a. (6 points) Using a vertical line between letters to indicate peptide bond hydrolysis, identify all of places in the rabbit and human alpha and beta globin chains that are susceptible to trypsin hydrolysis.

b. (8 points) Identify one trypsin-susceptible peptide bond by chain/number and draw the chemical structure of the dipeptide that includes it.

c. (6 points) Draw a box around every rabbit tryptic peptide that would contain radioactivity in Dintzis' experiment.

d. (4 points) Draw a line under every amino acid that is different in the human alpha and beta globins compared to the homologous rabbit sequences.

e. (8 points) Using the standard genetic code on the handout sheet, identify with a star under those differences in part 1d that would require more than a one-base change in a codon.

f. (6 points) How many sulfur atoms per iron atom are there in:

Human hemoglobin?	Rabbit hemoglobin?
S/Fe =	S/Fe =

Referring to your answers to 1a & 1c the previous section:

g. (5 points) Do you find any discrepancies between your analysis and what Dintzis reported in his first paper? If so, describe.

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Work sheet

Alpha Chain Globins

```
                      10                  20                  30                  40
RABBIT  VLSPADKTNIKTAWEKIGSHGGEYGAEAVERMFLGFPTTKTY→
HUMAN   VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTY→

          50                  60                  70                  80                  90
FPHFDFTHGSEQIKAHGKKVSEALTKAVGHLDDLPGALSTLSDLHAHKLR→
FPHFDLSHGSAQVKGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLR→

         100                 110                 120                 130                 141
VDPVNFKLLSHCLLVTLANHHPSEFTPAVHASLDKFLANVSTVLTSKYR
VDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSKYR
```

Beta Chain Globin

```
                     10                  20                  30                  40
RABBIT  VHLSSEEKSAVTALWGKVNVEEVGGEALGRLLVVYPWTQRFFES→
HUMAN   VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFES→

        50                  60                  70                  80                  90
FGDLSSANAVMNNPKVKAHGKKVLAAFSEGLSHLDNLKGTFAKLSELHCDK→
FGDLSTPDAVMGNPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDK→

       100                 110                 120                 130                 140       146
LHVDPENFRLLGNVLVIVLSHHFGKEFTPQVQAAYQKVVAGVANALAHKYH
LHVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH
```

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G = Glycine	P = Proline	Y = Tyrosine
H = Histidine	Q = Glutamine	

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Genetic Code Chart

UUU	PHE	UCU	SER	UAU	TYR	UGU	CYS
UUC	F	UCC	S	UAC	Y	UGC	C
UUA	LEU	UCA		UAA	End	UGA	End
UUG		UCG		UAG		UGG	TRP W
CUU	L	CCU	PRO	CAU	HIS	CGU	ARG
CUC		CCC	P	CAC	H	CGC	R
CUA		CCA		CAA	GLN	CGA	
CUG		CCG		CAG	Q	CGG	
AUU	ILE	ACU	THR	AAU	ASN	AGU	SER
AUC	I	ACC	T	AAC	N	AGC	S
AUA		ACA		AAA	LYS	AGA	ARG
AUG	MET M	ACG		AAG	K	AGG	R
GUU	VAL	GCU	ALA	GAU	ASP	GGU	GLY
GUC	V	GCC	A	GAC	D	GGC	G
GUA		GCA		GAA	GLU	GGA	
GUG		GCG		GAG	E	GGG	