

QUESTION	YOUR SCORE	FULL SCORE
I	13	20
II	16	20
III	7	20
IV	16	20
V	14	20
TOTAL	66 68	100

YOUR NAME: _____
 (Please PRINT your name in ink on this line as legibly as possible.)

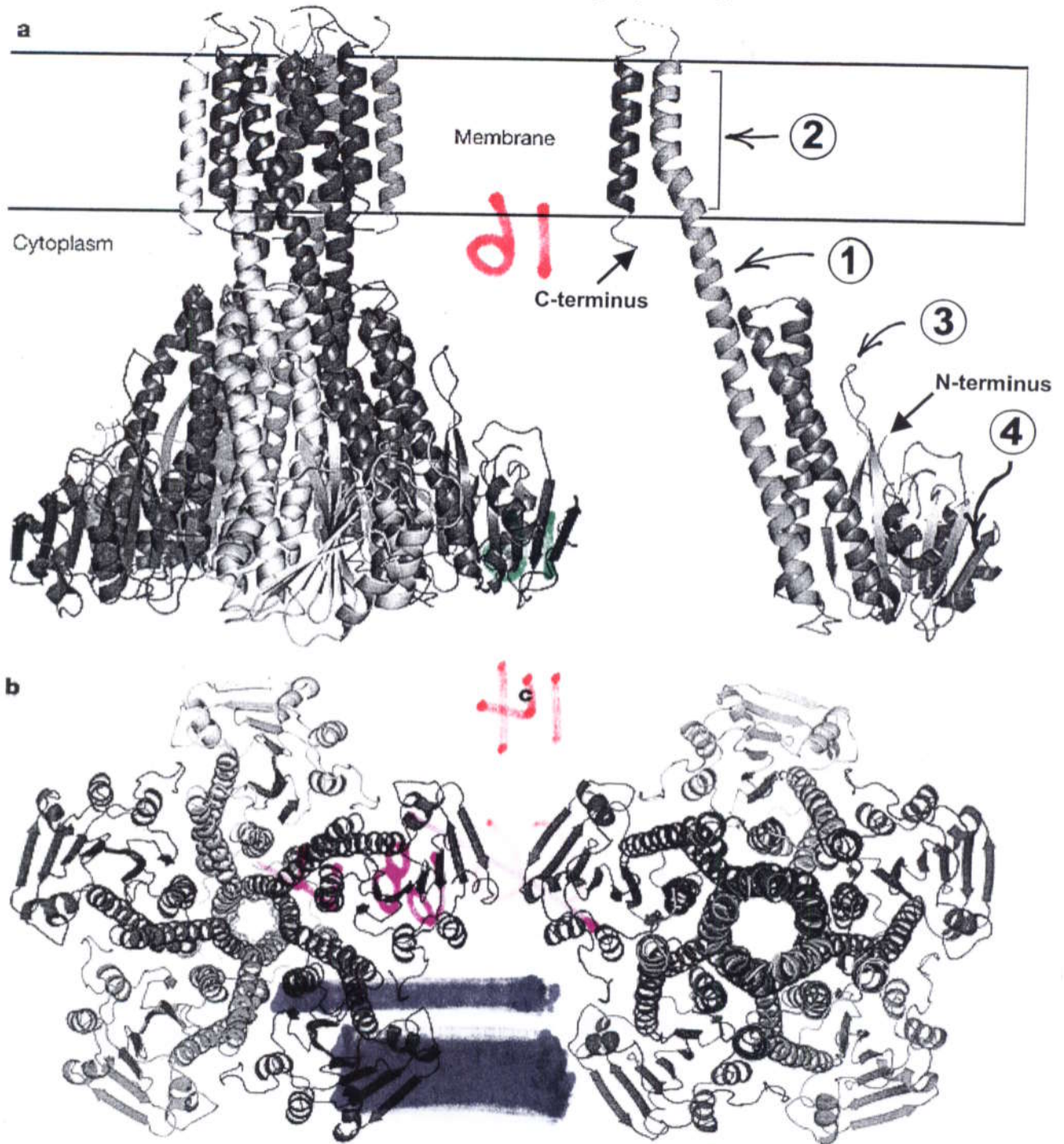
YOUR SIGNATURE: _____
 (Please SIGN your name in ink on this line.)

YOUR STUDENT ID NUMBER: _____
 (If taking the course via UC Extension, then please so state.)

YOUR DISCUSSION SECTION NO.: 101

YOUR TA's NAME: Laura Lombardi

QUESTION I. Examine the figure below and read the corresponding legend beneath it carefully, and then answer the questions about this protein on the following pages. [20 pts total]



Mg^{2+} is a divalent metal cation that is an essential cofactor for the physiological function of many enzymes and other proteins. Such highly charged and H_2O -solvated ions cannot diffuse across the hydrophobic bilayer of biological membranes. Therefore, protein carriers (transporters, pumps, and channels) provide the conduit by which ions translocate across membranes. Transmembrane delivery of Mg^{2+} is carried out by members of the CorA family of proteins in both prokaryotic and eukaryotic organisms. Just this year (2006), two different groups reported the three-dimensional structure of the CorA channel isolated from *Thermotoga maritima*, a thermophilic (grows best near $80^\circ C$) Gram-negative eubacterium. Shown above is a ribbon diagram of the CorA channel viewed in the plane of the membrane (a, left) and a single subunit of the CorA protein (a, right). Also, shown is a "worm's eye" view of the CorA channel, looking from the cytoplasm outward (b) and a "bird's eye" view of the CorA channel, looking from periplasm (extracellular space) inward (c).

13
20

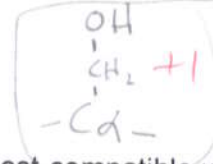
(1) What element of secondary structure spans all the way from the membrane-associated domain of each subunit to its cytosolic domain?

α-helix +1 [1 pt]

Name two different amino acid residues whose side-chains would be most compatible with this element of secondary structure and would be well tolerated projecting into the cytoplasm (aqueous phase) in the region marked with a number 1? Then, draw the structure (every atom and every bond) of the side-chain of one of these two.

(a) Serine, Ser +1 [1 pt] I have chosen to draw the structure of Ser, S below: [1 pt]

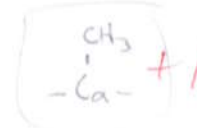
(b) Threonine, Thr [1 pt]



(2) Name two different amino acid residues whose side-chains would be most compatible with the element of secondary structure indicated with the bracket (and numbered 2) and would be well tolerated projecting into the hydrophobic core of the lipid bilayer of the plasma membrane? Then, draw the structure (every atom and every bond) of the side-chain of one of these two.

(a) Alanine, Ala +1 [1 pt] I have chosen to draw the structure of Ala, A below: [1 pt]

(b) Valine, Val [1 pt]



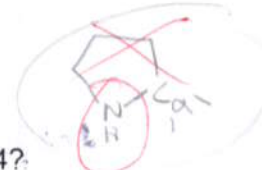
(3) What element of secondary structure is indicated with the number 3?

β-strand +1 [1 pt]

Name two different amino acid residues whose side-chains would be most compatible with this element of secondary structure and would be well tolerated when situated at the apex (top) of this structural element, as draw? Then, draw the structure (every atom and every bond) of the side-chain of one of these two.

(a) Proline, Pro +1 [1 pt] I have chosen to draw the structure of Pro, P below: [1 pt]

(b) Glycine, Gly +1 [1 pt]



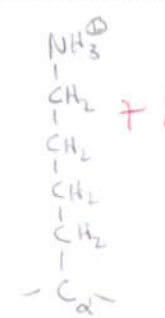
(4) What element of secondary structure is indicated with the number 4?

β-sheet +1 [1 pt]

Name two different amino acid residues whose side-chains would be most compatible with this element of secondary structure? Then, draw the structure (every atom and every bond) of the side-chain of one of these two.

(a) Lysine, Lys +1 [1 pt] I have chosen to draw the structure of Lys below: [1 pt]

(b) Arginine, Arg [1 pt]



+2

+2

+2

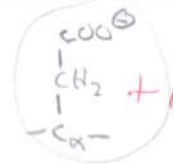
+2

5

QUESTION I (continued)

(5) Given that Mg^{2+} needs to shed its water of solvation to enter this channel and needs to be attracted to the funnel-shaped opening (see **c** on previous page) that then tapers into a size-selective pore (see **b** on previous page), name two different amino acid residues whose side-chains would be most compatible projecting into the center of the pore (i.e. serving as its lining)? Then, draw the structure (every atom and every bond) of the side-chain of the one you have chosen.

- (a) Aspartic Acid +1 [1 pt] I have chosen to draw the structure of Asp below: [1 pt]
- (b) Glutamic acid +1 [1 pt]



(6) What is the oligomeric state of this protein? That is, of how many subunits is the native protein composed? Explain your reasoning briefly. [2 pts]

5 subunits. The worms-eye view of (b) shows five distinct groups of α -helices and β -sheets + strands arranged radially about the channel.

QUESTION II. Multiple Choice. Please CIRCLE clearly the letter that corresponds to the most correct conclusion to each of the following statements or queries. [20 pts total]

- (1) Compared to the radius of the covalent single bond formed by an atom, the length of the van der Waals radius for the same atom is: [2 pts]
- (a) ~~The same~~
 (b) Shorter
 (c) Longer
 (d) ~~Depends on the atom~~
- (2) When the dielectric constant of the surrounding medium is lower, the strength of the attractive force (electrostatic interaction) between two charges of opposite sign is: [2 pts]
- (a) Weaker
 (b) Stronger
 (c) Unaffected
 (d) ~~Becomes independent of the distance between the charges~~
- (3) Enantiomers differ from each other in all of the following respects, except: [2 pts]
- (a) ~~Chirality~~
 (b) Configuration
 (c) Conformation
 (d) ~~Optical activity~~
- (4) High concentrations of all of the following compounds act as protein denaturants, except: [2 pts]
- (a) ~~Urea~~
 (b) ~~H⁺~~
 (c) NaCl
 (d) Ethanol

- (5) During the purification of an enzyme, its specific activity should: [2 pts]
- (a) Stay the same
 - (b) Increase
 - (c) Decrease
 - (d) Cannot be determined from the information provided
- (6) The least appropriate method to determine the approximate molecular weight of a purified protein is: [2 pts]
- (a) ~~Isopycnic~~ banding
 - (b) ~~SDS-polyacrylamide~~ gel electrophoresis
 - (c) Size exclusion chromatography
 - (d) Electrospray ionization mass spectrometry
- (7) The standard free energy changes at pH 7 for the following enzyme-catalyzed hydrolysis reactions are given:
- Reaction #1: $\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{PO}_4^{3-} + \text{H}^+$ $\Delta G'' = -30.5 \text{ kJ/mole}$
- Reaction #2: $\text{Glucose-6-phosphate} + \text{H}_2\text{O} \rightarrow \text{Glucose} + \text{PO}_4^{3-}$ $\Delta G'' = -13.8 \text{ kJ/mole}$
- Which of the following statements about these two reactions is correct: [2 pts]
- (a) ~~Rate~~ of Reaction #1 is greater than that of Reaction #2
 - (b) ~~Rate~~ of Reaction #2 is greater than that of Reaction #1
 - (c) Neither reaction ~~can~~ occur spontaneously
 - (d) Rate of neither reaction can be determined from the information provided
- (8) The approximate diameter (in Å) of the roughly globular hemoglobin molecule is about: [2 pts]
- (a) ~~0.55-0.65~~
 - (b) ~~5.50-6.50~~
 - (c) 55.00-65.00
 - (d) ~~550.00-650.00~~
- (9) Mutations in the coding sequence of a gene that would cause all of the following amino acid changes within the open-reading-frame are considered "conservative" substitutions, except: [2 pts]
- (a) ~~Ser for Thr~~
 - (b) Met for Arg
 - (c) ~~Tyr for Phe~~
 - (d) ~~Gln for Asn~~
- (10) All of the following unusual amino acids occur as the result of naturally-occurring crosslinks found in certain (especially fibrous) proteins, except: [2 pts]
- (a) ~~Cystine~~
 - (b) ~~Lysinonorleucine~~
 - (c) ~~Desmosine~~
 - (d) Hydroxyproline

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YOUR NAME: _____

QUESTION III. [20 pts total]

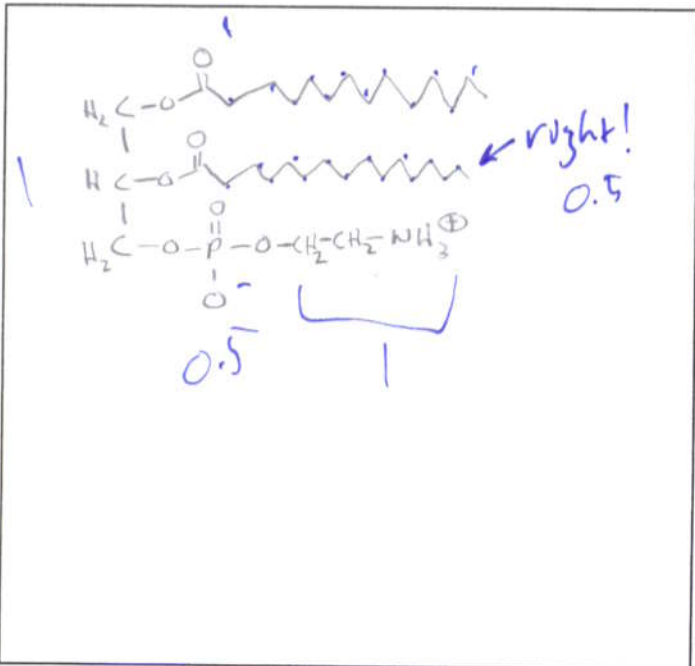
2 (a) On the right is a list of over a dozen different physical and chemical properties that might be possessed by a biomolecule or conditions to which a biomolecule might be exposed. On the left is a list of separation methods that are commonly used for the fractionation of the complex mixture of proteins in a crude cell extract and are therefore used for the purposes of preparing (purifying) a protein of interest. In the blank space provided, write in the NUMBER (or NUMBERS) of the property (or properties) and/or condition (or conditions) that best represent the physico-chemical properties or conditions that constitute the basis for each separation method indicated. [10 pts total] [PLEASE note: One pt will be subtracted for each incorrect response.]

- (a) Affinity Chromatography 7+1
ligand or inhibitor
- (b) Ammonium Sulfate Fractionation 11+1
- (c) Cation Exchange Chromatography 2+1
- (d) Differential Centrifugation 1+1-1
6, 13, 4
- (e) Gel Permeation Chromatography 8+1
(size exclusion)
- (f) Hydrophobic Interaction Chromatography 5-1
- (g) Isoelectric Focusing 3-1-1
10

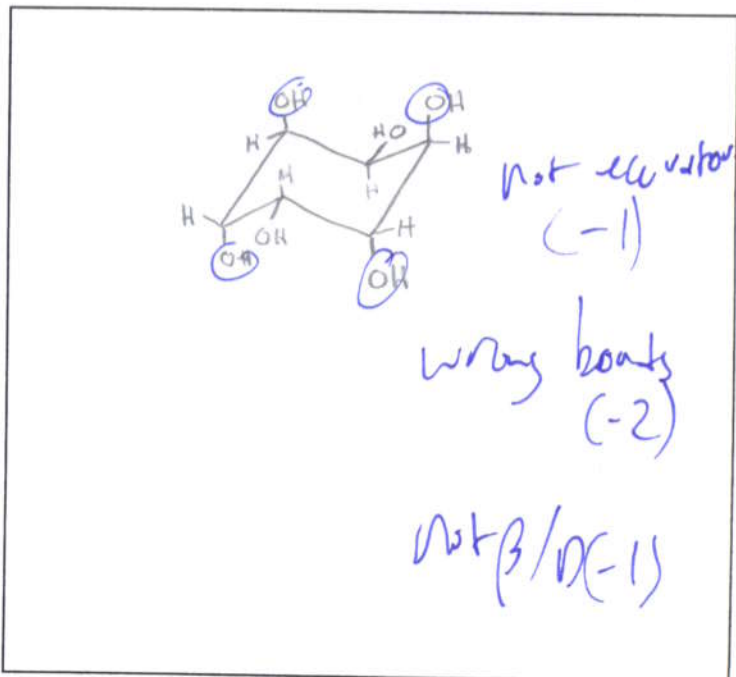
1. Absorbance
2. Charge
3. Conductivity
4. Density
5. Dipole Moment
6. Frictional Coefficient
7. Ligand Binding
8. Mass
9. Osmolarity
10. Refractive Index
11. Solubility
12. Tensile Strength
13. Viscosity

+6
-4
-2

4 (b) In the box provided beneath draw the structure of dipalmitoyl phosphatidylethanolamine [5 pts]



1 (c) In the box provided beneath draw the structure of β-D-glucose in its chair conformation [5 pts]



16/20

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YOUR NAME: _____

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QUESTION IV. Problems. [20 pts total]

(1) Tyrosine is one of the twenty naturally-occurring amino acids.

(a) Based on your knowledge of the approximate pK_a values of the ionizable groups in tyrosine, what is the approximate pI value for this amino acid? SHOW your calculations and/or EXPLAIN your reasoning. [3 pts]

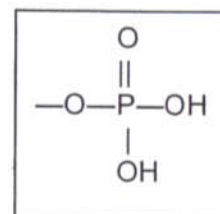
pH ranges	$-NH_3^+$ $pK_a=9$	-R side chain $pK_a=10$	$-COO^-$ $pK_a=2$	net charge
2-9	\oplus	no charge	\ominus	zero!
<2	\oplus	no charge	no charge	+1
9-10	no charge	no charge	\ominus	-1

we therefore know that the pI must be located halfway between the pK_a 's of the carboxyl and amino groups:
 $pI \approx \frac{2+9}{2} = \frac{11}{2} = 5.5$

(b) The side chain of tyrosine can be phosphorylated. Based on the fact that the approximate pK_a 's of the two ionizable groups present on an esterified phosphate (see box) are $pK_{a1} = 1$ and $pK_{a2} = 7$, what is the approximate pI value for phospho-tyrosine? SHOW your calculations and/or EXPLAIN your reasoning. [3 pts]

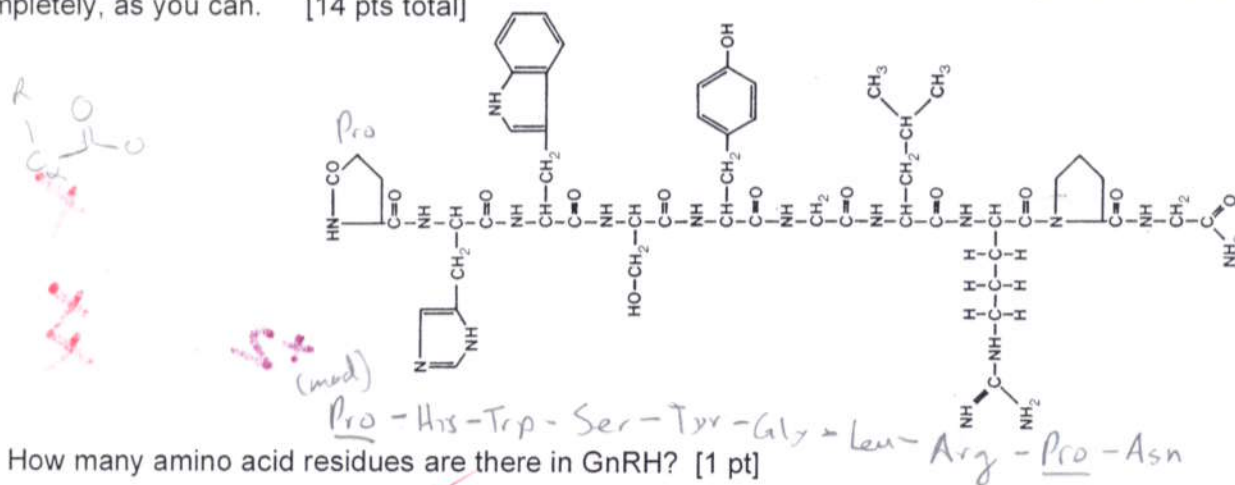
2 additional pK_a 's to consider

pH ranges	$-NH_3^+$ $pK_a=9$	side chain $pK_a=10$	$pK_{a1}=1$	$pK_{a2}=7$	$-COO^-$ $pK_a=2$	net charge
2-7	\oplus	no chg	\ominus	no charge	\ominus	-1
7-9	\oplus	no charge	\ominus	\ominus	\ominus	-2
1-2	\oplus	no chg	\ominus	no charge	no charge	zero!



$\therefore pI \approx \frac{1+7}{2} = 1.5$

(2) Immediately below is shown the primary structure of an important mammalian peptide hormone, called gonadotropin-releasing hormone (or GnRH), which has interesting chemical modifications at both its N- and C-terminal ends. Below are specific questions about this peptide. Answer these queries as succinctly, but as completely, as you can. [14 pts total]



(a) How many amino acid residues are there in GnRH? [1 pt]

10

(b) What is different about the amino-terminal end of GnRH, compared to the α -amino group found in a typical / conventional peptide? [1 pt]

There is no free, \oplus charged amino group. Instead, there is a cyclic proline side chain with an additional oxygen atom modification.

(c) What is different about the carboxyl-terminal end of GnRH, compared to the ω -carboxyl found in a more typical / conventional peptide? [1 pt]

Instead of having a charged carboxyl end ($-COO^-$), there is an uncharged amide ($-CONH_2$) characteristic of an AA such as asparagine.

acid

QUESTION IV (continued)

YOUR NAME: _____

(d) If GnRH were subjected to standard conditions for acid-catalyzed hydrolysis and then the resulting hydrolyzate was examined using an automated amino acid analyzer, what would be the apparent amino acid composition of GnRH? Explain your reasoning. [2 pt]

- 2 proline (one of which has a modification, not an additional oxygen atom)
- 1 each of histidine, serine, tyrosine, glycine, leucine, arginine, and asparagine
- Trp will not be visible from the analyzer b/c it's destroyed by the acid.

(e) Will the GnRH peptide (PUT AN "X" MARK IN THE APPROPRIATE SPACE): [9 pts]

+7

PITC

FDNB

	YES	NO
Be cleaved by trypsin? (Lys, Arg) C-side		X
React with phenylisothiocyanate? N-terminus		X
Be degraded by carboxypeptidase A? C-terminus NO Arg, Lys, Pro		X
Be cleaved by chymotrypsin? Phe, Tyr, Trp, indol C	X	
React with fluoro-dinitro-benzene?	X	X
Be cleaved by cyanogen bromide? Met		X
Be degraded by aminopeptidase M? Trp	X	X
Be cleaved by thermolysin? Phe, Ile, Leu, Trp, Trp, Val	X	
Be cleaved by <i>S. aureus</i> V8 protease? Glu, Asp		X

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QUESTION V. True or False. [20 pts total]

	TRUE	FALSE
Heme is only found in the oxygen-binding proteins of air-breathing organisms.		X
Hemoglobin can only bind oxygen when the iron in its heme groups is in the ferric state. ^{Fe³⁺}		X
Like all proteins with aromatic side chains, hemoglobin only absorbs light in the UV range.		X
Under <u>all</u> conditions, O ₂ binding by hemoglobin displays sigmoidicity due to cooperativity.	X	X
The toxicity of CO ₂ to humans is due to its ability to block O ₂ binding to hemoglobin.		X
2,3-bis-phosphoglycerate is only made in red blood cells, where it can act on hemoglobin.	X	X
The Hill coefficient (n _H) cannot be a negative number. ⁺²	X	X
Hemoglobin helps remove the H ⁺ generated in metabolically-active tissues.	X	
Sickle cell anemia is a genetic disease.	X	
Fetal hemoglobin has a higher affinity for O ₂ than maternal hemoglobin under all conditions.		X