EXAM I
This exam will be taken apart for grading. Please PRINT your name on each page.
If you do not have sufficient room for your answer in the space provided, please continue on the back of the page on which the question appears.

<table>
<thead>
<tr>
<th>Question</th>
<th>Maximum Points</th>
<th>Earned Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>6</td>
<td>--------------</td>
</tr>
<tr>
<td>II.</td>
<td>5</td>
<td>--------------</td>
</tr>
<tr>
<td>III.</td>
<td>4</td>
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</tr>
<tr>
<td>IV.</td>
<td>5</td>
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<tr>
<td>V.</td>
<td>12</td>
<td>--------------</td>
</tr>
<tr>
<td>VI.</td>
<td>11</td>
<td>--------------</td>
</tr>
<tr>
<td>VII.</td>
<td>5</td>
<td>--------------</td>
</tr>
<tr>
<td>VIII.</td>
<td>10</td>
<td>--------------</td>
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<tr>
<td>IX.</td>
<td>11</td>
<td>--------------</td>
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<td>X.</td>
<td>9</td>
<td>--------------</td>
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<tr>
<td>XI.</td>
<td>14</td>
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<tr>
<td>XII.</td>
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I. [6 points]
Match the amino acids in the column on the left with the appropriate side chain type(s) in the column to the right. [The point count corresponds to the number of responses expected.]

a) K __3__ 1) Nonpolar aliphatic
b) E __4__ 2) Nonpolar aromatic
c) L __1__ 3) Basic
d) C __5__ 4) Acidic
e) W __2__ 5) Sulfur-containing
f) S __6__ 6) Hydroxyl-containing

7) Thioether-containing

II. [5 points]
Would you use an anion exchanger [anions bind reversibly] or a cation exchanger [cations bind reversibly] to purify bovine histone [pI = 10.0] at pH 11.5? Your rationale must be clear.

Above the pI the basic (+) charged groups will be dissociated; e.g., K will have no charge on the side chain. D and E side chains however will be negatively charged, therefore histone should bind to an anion exchanger.
III. [4 points]
A. Primary sequences predicted from DNA analyses sometimes differ significantly from sequences determined by more traditional procedures of protein biochemistry (even in the absence of experimental error). Explain. (2 points)

*Sequences predicted from DNA analyses often fail to predict post-translational modifications.*

B. Primary sequences of proteins that contain both D and N residues as well as E and Q residues that are predicted from DNA analyses can place each of these four residues unambiguously. If the same protein is analyzed the more traditional methods of protein biochemistry, the locations of N and Q residues are often ambiguous. Explain. (2 points)

*The more traditional procedures often include hydrolytic procedures that cleave the amide side chains of Q and N. These residues then show up as D and E.*
IV. [5 points]
Answer A OR B but not both; only your first answer will be graded.
A. You have isolated two samples of collagen, one from fibroblasts of a normal individual, and one from fibroblasts of an individual who is thought to produce a mutant form of collagen in which several G residues have been replaced by A. Unfortunately the labels have come off during rearrangement of the -70°C freezer, before you have had time to perform the relevant analyses. A bright Bio500 student suggests that you can identify the two samples by performing a thermal transition analysis (‘melting point’) of aliquots of the two collagen samples. Is this a good suggestion? Explain.

Yes. The stability of collagen depends on the close association of the polypeptides in the triple helix. Glycine is the only amino acid that has a sufficiently small R group to allow maximum stability. An unstable collagen will "melt" at a lower temperature.

B. [5 Points]
Explain the following observations:
Mature collagen fibers contain a very significant number of hydroxyproline residues, but if radiolabeled hydroxyproline is
added to a culture system that is actively engaged in collagen synthesis, none of the added hydroxyproline is incorporated into the newly-synthesized collagen fibers.

*The Hyp are produced by post-translational hydroxylation of Pro residues in the polypeptide chains.*

V. (12 points)
The composition of a peptide secreted by a culture of bacterial cells is:

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<thead>
<tr>
<th>Amino Acid</th>
<th># Residues/peptide</th>
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<tr>
<td>Cys</td>
<td>2</td>
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<td>Arg</td>
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<td>Glu</td>
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<td>Leu</td>
<td>1</td>
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Considering ALL of the information listed below, deduce the most likely structure of the peptide.

HINTS: Please use the single letter codes for the amino acids to write your final answer. ALSO: It helps (partial credit) to jot down what EACH observation tells you about the peptide.

a. The titration curve for the peptide, over the range pH 2 thru 13, shows two, well-defined inflection points.

b. The peptide:
   1) does not react with iodoacetate

   2) does not react with phenylisothiocyanate or dansyl chloride

   3) does not change in size when treated with trypsin
c. The product of tryptic digestion reacts with fluorodinitrobenzene.

d. After treatment with trypsin, three rounds of Edman degradation yielded the following partial sequence: Cys-Leu-Glu (Equipment malfunction prevented further analysis).

e. Following incubation with excess 2-mercaptoethanol, the peptide binds two carboxymethyl groups when treated with excess iodoacetate.

For full credit you must demonstrate that the structure you deduce is consistent with EACH of the stated experimental observations.

A. The titration data indicate that there are only two ionizable groups. [One would expect up to four for a linear peptide with the composition given.]

B.  
1. Failure to react with iodoacetate indicates no free –SH’s. Probably a disulfide present.  
2. Either the N-terminus is blocked or the peptide is cyclic.

C. The exposure of an FDNB-reactive group indicates that trypsin does cleave, in this case at R.

D. We now know that the tryptic product (C-terminal R) has the sequence CLE. This means that there was an R–C bond. Now can deduce RCLE  

We still have 1C and an 1I to place. We do not have sufficient data to say whether it is ICRCLE or CIRCLE.

What is left is to close the loop between E and I or between E and C.

CIRCLE works with a line connecting the two endpoints PLUS a SS between the two C’s.
In this type of question it is essential to test your answer to be sure it is consistent with ALL of the data.

E. confirms the disulfide

VI. (11 points)
The points assigned for this question reflect the number of correct answers which are possible. One-half point off for each incorrect guess. Choose the answer(s) which is (are) correct.

A. With respect to enzymatic catalysis, the term steady state is best described as an experimental condition in which:
--- $-d[S]/dt = 0$
--- the rate of change in the concentration of ES is approximately zero.
--- initial velocity is zero order with respect to [S].
--- $[E_{total}]$ remains unchanged.
--- $d[P]/dt = 0$

B. For an enzyme which follows Michaelis-Menten kinetics, the substrate concentration required to reach a velocity which is only 1% lower than $V_{max}$ is:
--- 10K.
--- 30K.
--- 80K.
--- 99K.
--- 300K.
C. Those noncovalent interactions which affect $k_\text{cat}$
- $X$— are most important in the transition state.
- $X$— have little to do with recognition or affinity of substrate
  --- enhance $K_M$ by several orders of magnitude
- $X$— can include hydrophobic and ionic interactions as well as hydrogen bonds
  --- cannot be explored by site-directed mutagenesis

D. Transition state analogs
- $X$— Mimic the size, configuration and charge distribution of the postulated transition state.
  --- Increase the rate of product formation
- $X$— Can be used to produce catabolic antibodies
- $X$— Are typically very unstable molecules
- $X$— Are potent inhibitors of enzymes

E. Characteristics of the Bohr effect include
- $X$— Lowering the pH shifts the oxygen dissociation curve of hemoglobin to the right
  --- The acidic environment of an exercising muscle allows hemoglobin to bind $O_2$ more strongly
- $X$— The affinity of hemoglobin for $O_2$ is diminished by high concentrations of $CO_2$
  --- In the lung, the presence of higher concentrations of $H^+$ and $CO_2$ allows hemoglobin to become oxygenated
- $X$— In the lung, the presence of higher concentrations of $O_2$ promotes the release of $CO_2$ and $H^+$

VII. [5 points]
Answer A OR B, but not both; only your first answer will be graded.
A. What are the structural and functional bases of the observation that although the amino acid residues that constitute the catalytic triad of chymotrypsin [ChT] are also present in chymotrypsinogen [ChTg], ChTg does not bind either DIFP or TPCK? Full credit will be granted for a concise but specific answer.

The unique reactivity of Ser195 (with DIFP) and His57 (with TPCK) depends on close proximity of the groups in the catalytic triad.
These groups are much further apart in the zymogen. Activation of the zymogen is associated with a large conformational change; residues that are far apart undergo a six-fold shift in position (from Molecular Graphics I).
B. A Bio 500 student who had been working with a crystallographer on a protein structure problem for a couple of summers was given the task of testing certain aspects of a structural model deduced from X-ray analysis. Having displayed the values of $R$ and $N$ based on this model as a Ramachandran plot using the Swiss-Pdb viewer, he was devastated to discover that a very significant number of residues in the sequence fell well outside the regions corresponding to maximum stability. The postdoc who had been working along side the student during most of the many hours that were required to obtain the original data was perplexed until he clicked on a few of the suspect residues. Both of them were delighted to find that while each of the residues was in a different position in the sequence, each spot corresponded to the same amino acid.

What was the residue? 

**Glycine**

Explain why it is OK for this amino acid residue to fall in an ‘unstable’ region of a Ramachandran plot.

*The small side chain \([H]\) allows for conformations that would be sterically hindered for larger ones.*

VIII. [10 Points]
Several terms related to protein isolation/characterization, structure or function are listed below. Select the single statement that most clearly defines the term or describes its significance. One point for each correct response; one-half point off for each incorrect guess.

A. Salting out

------- a method of protein purification that is based primarily on differences among proteins in surface charge.
---X---- a separation method in which one or more proteins in a mixture are separated on the basis of differential requirements for water of hydration.

B. Iodoacetamide

---X-- An alkylating agent that reacts with C residues.

------ Used to inactivate serine proteases

C. Molecular chaperone

---X--- typically interacts with hyrophobic patches; release sometimes requires an input of energy.

------ catalyzes the formation of the proper orientation of X-Pro bonds during protein folding.

D. $\$_$ loop

---X--- a supersecondary structure comprised of a parallel $\_-$ strand, connected to an "$_-$ helix.

------ a supersecondary structure comprised of an anti-parallel $\_-$ strand, connected to an "$_-$ helix

E. Vitamin C

------ one of the many water soluble vitamins; it functions as a structural component of NAD.

---X--- an essential cofactor for the iron-containing hydroxylases that are involved in the biosynthesis of collagen.

F. Proximal histidine

---X--- occupies the fifth coordination position for Fe(II) in the heme of Mb and Hb.

------ its location precludes (presumably by steric hindrance) the formation of linear complexes between CO or O$_2$ and the iron atom in the heme of Mb or Hb.
G. 2-Mercaptoethanol

-------- A reagent used to produce oxidative cleavage of disulfide bonds
--X-- A reagent that carries out reductive cleavage of disulfide bonds.

H. The catalytic triad for chymotrypsin

--X--- Asp\textsubscript{102}, His\textsubscript{57} and Ser\textsubscript{195}

-------- Asp\textsubscript{57}, His\textsubscript{102} and Ser\textsubscript{195}.

I. Cyanogen bromide

--X-- A reagent that cleaves polypeptides at internal M residues
----- A reagent that cleaves polypeptides at C-terminal M residues

J. Chaotropic agents

--X--- urea and guanidine hydrochloride

----- used to disrupt noncovalent interactions as well as disulfides but not peptide bonds

IX. [11 points]
A. Identify the three most important physiological regulators of the oxygenation of hemoglobin. (3 points)

$H^+, BPG, CO_2$

B. Choose one of these and describe both its effect on oxygen binding and the molecular basis of its regulatory effect in as much detail as you are able. (8 points)

*E.g.*, Having a carboxyl group as well as two phosphoryl groups, BPG has a high density of negative charge at physiological pH. The N-terminal regions of both of the $\delta$-chains of Hb have three positively charged and are in close proximity to one another. One BPG binds very tightly to this site; this crosslinking of the $\delta$-chains stabilizes the deoxyconformation and converts the oxygen saturation curve from a more nearly hyperbolic one to the sigmoid curve characteristic of Hb as an efficient oxygen carrier.

$$Hb: nH^+ + 4O_2 = Hb(O_2)_4 + nH^+$$

Physiologically this means that in the tissues, protons promote the release of molecular oxygen — favoring oxygen unloading, driving the reaction to the left. The conformation of Hb changes, the carboxyl of an Asp residue moves close to the His146, the pK of His146 is raised, a proton binds to both chains.

In the lungs, the oxygenated (R) state is favored, the Asp moves away from the His 146, the pK drops, the protons are released; the released protons help drive the carbonic anhydrase reaction toward release of carbon dioxide.

His146 is among the proton binding sites on Hb that have a higher affinity (greater pK) in the deoxy (T) state than in the R state.

For carbon dioxide — see class notes.
X. [9 points]
Consider the following experiment: An enzyme exhibits a $V_\text{m}$ of 500 mol/min/mg and a $K_\text{m}$ of 6 mM. Each molecule of enzyme can bind one molecule of an irreversible inhibitor. 10 mol of enzyme was treated with 6 mol of the irreversible inhibitor. Those molecules of enzyme that bind inhibitor are totally inactive.

A. When the kinetics of the untreated and untreated enzymes were studied as a function of substrate concentration, the data were consistent with classical noncompetitive inhibition. Explain.

*NCI is characterized by a lower maximum velocity but the same $K_\text{m}$. The fraction of the enzyme that is not bound by the inhibitor will show the same $K_\text{m}$; the fraction of the enzyme to which inhibitor is bound will exhibit zero activity.*

B. What is the $V_\text{m}$ of the treated enzyme? Explain.

$10 - 6 = 4$ mol of uninhibited enzyme or 40% of the untreated preparation $= 500 \times 0.4 = 200$ mol/min/mg

C. What is the $K_\text{m}$ of the treated enzyme? Explain

*See part A — it will be the same as the untreated enzyme.*
XI. [14 points]
Lactate dehydrogenase [LDH] catalyzes the following reaction:

\[
\text{NADH} + \text{Pyruvate} \rightleftharpoons \text{NAD}^+ + \text{L-lactate}
\]

Oxamic acid is a structural analog of pyruvate, and an inhibitor of LDH activity.

A. What would you expect to observe in plots of \(v_v\) vs \([\text{Pyruvate}]\) ± oxamic acid,? Why? [3 points]

**Being structurally similar to pyruvate, oxamate would be expected to be competitive with respect to pyruvate.**

B. When oxamate is covalently coupled to an insoluble matrix (agarose), and the suspension is poured into a column, the derivative can be used to purify LDH from crude extracts.

AFTER READING ALL of the following observations, provide a rational explanation — based on the principles of protein structure/function that have been presented in class and/or your text for EACH observation and answer any questions that are posed.

1. If an extract containing LDH in an appropriate buffer is poured over the column, little if any protein binds to the column, including LDH. [HINT: Answer after completing the questions below.] [2 points]

   **None of the proteins recognize oxamate in the absence of NADH.**

2. If NADH is added to the extract before pouring it into the column, virtually all of the LDH is bound to the column, but most of the non-LDH protein remains unbound [does not stay on the column]. [2 points]
The enzyme does not bind oxamate unless oxamate is bound, presumably due to a conformational change on forming the binary [LDH:NADH] COMPLEX.

Continued

3. If the column is now washed with the same buffer, but in the absence of NADH, LDH is found in the eluate. [2 points]

Removing the NADH diminishes the affinity for oxamate; see part 2.

4. The specific activity of the eluted LDH is much higher than it is in the crude extract. Why? [2 points]

Only LDH binds to the column in the above protocol [with NADH]. SA is expressed as a ratio of activity/[protein]. Since most (if not all) of the non-LDH protein washes off this leaves only LDH. If the subsequent recovery of LDH is good than the ratio of activity/[protein] will increase.

5. Which of the mechanisms of inhibition that were discussed in class would you expect to observe if you explored LDH activity as a function of [NADH] ± Oxamic acid at constant [PYRUVATE]? Explain? [3 points]

The mechanism that is consistent is uncompetitive; i.e. the oxamate binds preferentially to the LDH:NAD complex.
XII. [8 points]

Answer A OR B but not both; only your first answer will be graded.

A. Dihydrofolate reductase [DHFR; an enzyme that is essential for nucleotide biosynthesis] is a small protein that has been used to explore details of the mechanism of action of GroEL. The following observations are taken from a paper by Dr. Carl Frieden, Chairman of the department of Biological Chemistry of our School of Medicine.

Dihydrofolate reductase (DHFR) from wild type (wt) *Escherichia coli* (bacteria) does not interact with the molecular chaperonin GroEL [also from *Escherichia coli*] regardless of whether the interaction is initiated from the native or the unfolded state. In contrast, the native murine (mouse; eucaryotic) DHFR shows a strong interaction with GroEL. Structural analysis suggests that there are three distinct loops in the murine DHFR that are not present in the *E. coli* protein. Several mutant derivatives of the bacterial DHFR were produced by inserting segments of murine DHFR into the corresponding regions of *E. coli* DHFR. In mutant (EcDHFR-i7136), residues 136-139 (V-F-S-E) of *E. coli* DHFR were replaced with the seven amino acid sequence L-P-E-Y-P-G-V. The *E. coli* DHFR mutant formed a complex with GroEL starting from either the native or the unfolded states of DHFR. The binding was specific since the presence of MgATP caused the release of the proteins from GroEL. As with murine DHFR, the nonnative conformation of EcDHFR-i7136 are bound to GroEL. Both murine DHFR and EcDHFR-i7136 bind to GroEL very tightly, with equilibrium dissociation constants of less than 85 nanomolar.
In answering the question(s) below you need to recall the major features of the mode of action of GroEL. [YOU ARE NOT BEING ASKED TO REPRODUCE THE FIGURE FROM YOUR HANDOUT.]

1. Illustrate your familiarity with the major features of the mode of recognition GroEL for polypeptides by providing a rational explanation for binding of the mutant DHFR, but not the enzyme from wt E.Coli. For full credit you must be as specific as possible. [4 points]

*Initial binding (recognition) of polypeptides by GroEL requires hydrophobic interactions at the apical surface. The mouse enzyme must contain such residues on the surface of the native protein (as well as the unfolded polypeptide). The wt bacterial DHFR does not. The inserted peptide has several hydrophobic residues.*

2. What do the observations suggest about the location of the inserted loop in the mutant DHFR, as well as in the normal murine enzyme. [3 points]

*It must be on the surface of the native proteins.*

3. Can you infer anything from these observations about the role of GroEL in the folding of DHFR in wild type E. coli cells? Explain. [1 points]

*It seems that DHFR in E. coli does not need GroEL in order to fold DHFR.*
B. The paragraph below is from an abstract of a letter titled “Mice without myoglobin” that appeared in Nature in 1998 [Vol. 395 Pages 905 - 908].

Here we show that mice without myoglobin, generated by gene-knockout technology, are fertile and exhibit normal exercise capacity and a normal ventilatory response to low oxygen levels (hypoxia). Heart and soleus muscles from these animals are depigmented, but function normally in standard assays of muscle performance in vitro across a range of work conditions and oxygen availability. These data show that myoglobin is not required to meet the metabolic requirements of pregnancy or exercise in a terrestrial mammal, and raise new questions about oxygen transport and metabolic regulation in working muscles.

1. Comment on these observations in the light of the material covered in class and in your text (same as other texts and a very large body of literature) regarding the physiological role of myoglobin and hemoglobin. [4 points]

An appropriate comment could be as follows. There is a large literature that supports the following thoughts about the role...
of Mb in mammalian physiology. Myoglobin, an intracellular haemoprotein expressed in the heart and oxidative skeletal myofibres of vertebrates, binds molecular oxygen and facilitates oxygen transport from erythrocytes to mitochondria, thereby maintaining cellular respiration during periods of high physiological demand.

2. Assuming that these observations are accurate and reproducible, would you expect to observe essentially the same results in an elephant, a giraffe, a cheetah, or a whale? If not, why not? [4 points]

It seems unlikely that larger animals, could perform all of their normal aerobic activities without this auxiliary carrier. This seems particularly relevant in the whale.

<table>
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<tr>
<th>Amino Acid</th>
<th>Three-letter abbreviation</th>
<th>One-letter symbol</th>
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<tbody>
<tr>
<td>Alanine</td>
<td>Ala</td>
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<td>Arginine</td>
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<td>Aspartic Acid</td>
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