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.

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I. [18 points]
Identify and state the significance of the following items to protein structure and/or function. 

CHOOSE SIX. Only your first SIX answers will be graded.

A. $k_{\text{CAT}}/K_M$

B. Peptidylprolyl isomerase

C. Vitamin C

D. Western blot

E. 6M Guanidine hydrochloride

F. Cyanogen bromide

[continued]
G. Tropocollagen

H. Homoserine lactone

I. Diisopropylphosphofluoridate

J. Ser 195

K. Proximal Histidine

L. $T \leftrightarrow R$

M. Bohr effect
II. [16 points]
A peptide hormone was isolated from the posterior pituitary. In order to determine its primary
structure three forms of the peptide were examined: 1) the untreated peptide, 2) the peptide after
performic acid oxidation, and 3) the alkylated peptide, produced by reduction and alkylation with
iodoacetate. Analysis of the performate oxidized peptide yielded the following composition:
(Cysteic acid)$_2$, Gln, Pro, Glycaminde, Asn, Ile, Leu, Tyr

Explain EACH of the following observations and deduce as much of the structure of the peptide
as is consistent with the information provided. Be as specific as the observations allow.

A. The untreated peptide does not react with iodoacetate.

B. Following treatment of with mercaptethanol, the peptide bound two moles of iodoacetate per
   mole peptide [yielding the alkylated peptide].

C. Treatment of the alkylated peptide with dansyl chloride followed by hydrolysis yielded the
dansyl derivative of carboxymethyl-Cys [CMCys].

D. Analysis of the alkylated peptide yielded the following composition:
   (CMCys)$_2$, Leu, Pro, Tyr, Ile, Gln, Asn, Glycinamide.

[continued]
E. Titration of the untreated peptide revealed only two pK’s, at pH 8, and 10.

F. Digestion of the untreated peptide with chymotrypsin did not change the size of the peptide significantly, but it now reacted with dansyl chloride and yielded Dansyl-Ile on acid hydrolysis.

G. Edman degradation of a chymotryptic fragment of the alkylated peptide yielded the following sequence:
   Ile-Gln-Asn-CMCys-Pro-Leu-Gly(NH$_2$)

The structure of the untreated peptide is:
III. [6 points]
Lactate dehydrogenase from beef heart is comprised of two electrophoretic forms, each of which is a tetramer. One form $H_4$ is comprised of four identical subunits; the other form $H_3M$ contains one $M$ subunit and three $H$ subunits. Relevant features of the amino acid compositions of these individual subunits are listed below.

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<th>H</th>
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<tbody>
<tr>
<td>Lys</td>
<td>102</td>
<td>103</td>
</tr>
<tr>
<td>Arg</td>
<td>34</td>
<td>42</td>
</tr>
<tr>
<td>Glu</td>
<td>131</td>
<td>121</td>
</tr>
<tr>
<td>Asp</td>
<td>130</td>
<td>127</td>
</tr>
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A. Which of the native enzymes $H_4$ or $H_3M$ has the lower isoelectric point? Your rationale must be clear. [[NOTE: YOU ARE NOT ASKED TO CALCULATE THE pI’s]]

B. Which of these forms would bind tighter to an anion exchanger [positively charged] at pH 7.0? Your rationale must be clear.
IV.  [9 points]
Hemoglobin Helsinki [Hb\textsubscript{Helsinki}] has a single amino acid substitution in each of the $\beta$ chains; the $\beta$82 residue, which is Lys in HbA, is replaced by Met. Illustrate your familiarity with structure/function relationships for Hb by addressing the following questions.

A. The mutation responsible for Hb\textsubscript{Helsinki} alters the binding of BPG, relative to that of HbA? Explain.

B. Sketch the oxygen saturation curves for HbA and Hb\textsubscript{Helsinki}, labeling each carefully. Explain.

C. How does Hb\textsubscript{Helsinki} compare with HbA as an oxygen carrier? [Hint: Think about the physiological role of Hb.]
V. [8 points]
Answer **A OR B**, but not both. Only your first answer will be graded.

A. Chymotrypsinogen [ChTg] can be readily renatured following reduction and treatment with 8M urea, whereas the yields of native chymotrypsin [ChT] following reduction and denaturation of ChT under similar conditions are vanishingly small. Explain. Your answer must reflect your familiarity with the differences in structure between ChT and ChTg, as well as the principles which govern protein folding.

B. The primary structures of aspartate aminotransferases isolated from mitochondria and cytosol are only 40% identical, but the three dimensional structures of the NATIVE enzymes are very similar. The two enzymes were denatured in 6M guanidine hydrochloride and reactivated by dilution. The effect of the molecular chaperones GroEL/GroES on the yield of reactivated [renatured] aminotransferase activity was explored. GroEL/GroES increased considerably the yield of reactivated mitochondrial aspartate aminotransferase but the effect of these chaperones on renaturation of the cytosolic enzyme was significantly less.

Offer a reasonable hypothesis for the differential extents of chaperone-mediated renaturation of the mitochondrial and cytosolic aminotransferases. Your answer must be consistent with the postulated mode of action of chaperones and the information provided above.
VI. [6 points]
One approach to the detection of intermediates during protein folding involves enhanced exchange of backbone amide hydrogens at alkaline pH. A student in a biochemistry course argued however that the rate of alkali-enhanced exchange of amide hydrogens should be diminished in a region of a polypeptide in which a run of lysine residues is found.

Is this a reasonable hypothesis? Explain. [More than one answer is possible.]
A recent Medline search for adenosine deaminase yielded 2,742 publications since 1985. Interest in this enzyme arises largely from the fact that a defect in this enzyme is associated with severe immunodeficiency.

Mouse adenosine deaminase (ADA) contains an active site glutamate residue at position-217 that is highly conserved in other adenosine and AMP deaminases. Previous research has suggested that proton donation to position N-1 of the substrate occurs prior to catalysis and supports the mechanism as proceeding via formation of a tetrahedral intermediate at position C-6. The proposed catalytic mechanism of ADA based on the recent elucidations of the crystal structure of this enzyme with transition and ground-state analogs hypothesized that Glu-217 was involved in this proton donation step.

To further study the importance of this residue, site-directed mutagenesis was used to generate four mouse ADA mutants. Glu-217 was mutated to Asp, Gly, Gln, or Ser, and all four mutants were successfully expressed and purified.

A. Circular dichroism analysis showed no significant differences between the mutant and normal enzymes. What is the significance of this observation?

B. The mutants showed only a slight variation in $K_M$. What is the significance of this observation?

C. The mutant enzymes, however, exhibited dramatic reductions in $k_{cat}$, [to less than 0.2% of the normal enzyme]. What do these data say about the role of Glu-217 in the mechanism of action of ADA?
A. Which line represents the result obtained without inhibitor? Explain your rationale.

B. What is the $K_M$ for the enzyme? Explain, using an appropriate equation.

C. What is the mode of inhibition of the inhibitor? Explain.

EXTRA CREDIT [5 POINTS]
D. Would the plot be qualitatively different for a subsaturating concentration of an irreversible
IX. [6 points]
Explain the following observations.
A. A competitive inhibitor of chymotrypsin, β-phenylpropionate, protects His57 against alkylation by TPCK.

B. Noncompetitive inhibitors do not provide such protection.
Your research advisor has asked you to design a catalyst that will accelerate the following lactonization reaction:

The proposed transition state is indicated. You decide to try to prepare a catalytic antibody.

A. Given the postulated structure of the transition state, and the structural features common to many known transition state analogs, design a transition state analog for the above reaction. [Note: You are to indicate an appropriate structure, NOT outline its synthesis.]

B. Describe the rationale behind your design.

C. Outline how you would use this analog and immunochemical techniques to generate a potential catalyst.