W09

Name [redacted] Student number [redacted]

Section 1: Multiple Choice Questions
Answer questions 1-25 on the Scantron Card provided with pencil.

1. What observation is an example of the **Building Block Principle** of Life?

   A. Collagen is a fibrous protein found in tendons.
   B. Protein folding reactions terminate at the lowest energy.
   C. Van der Waals bonds are formed in secondary structure.
   D. Proteins are chains of amino acids.

2. Which statement best describes the chemical basis for **polarity** of molecular groups in amino acids?

   A. Their atoms donate valence electrons to form a new bond.
   B. Their atoms possess a negative charge.
   C. Their atoms tend to hold on to their valence electrons.
   D. Their atoms form hydrogen bonds with oxygen or nitrogen.

3. Which of the following is the largest **completely non-polar** amino acid?

   A. Trp
   B. Tyr
   C. Phe
   D. Pro

4. An atom does **NOT** employ a lone pair of valence electrons _____

   A. as a general acid.
   B. as a general base.
   C. as a nucleophile.
   D. as a hydrogen-bond acceptor.

5. A new amino acid, called Wall•ine, has an amino group on its sidechain with a **pKa of 7.8**. At what pH will **two-thirds** of Wall•ine sidechains be **protonated**?

   $pH = pKa + \log_{10} 2/3$

   A. 7.5
   B. 7.6
   C. 8.0
   D. 8.1

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6. A mixture of all naturally occurring amino acids is applied to a gel filtration column. Which amino acid will be eluted **first**?

A. Ala
B. Phe
C. Trp
D. Gly

7. Aminocellulose paper is made from modified cellulose fibres with bound ($-NH_3^+$) groups at neutral pH. In a thin layer chromatography experiment, a mixture of amino acids is applied to a sheet of aminocellulose paper and dipped in an aqueous buffer at pH 7.0. Which of the following amino acids would possess the **lowest** relative mobility (R_f)?

A. Ala
B. Glu (Glutamic)
C. Arg (Arginine)
D. Trp

↳ more polar.

8. Which technique does **NOT** separate proteins on the basis of **size**?

A. Gel filtration chromatography
B. Analytical ultracentrifugation
C. SDS-polyacrylamide gel electrophoresis
D. Metal affinity chromatography

9. Why did Sanger and Edman both perform the first step of their amino acid analysis methods at **pH 9.0**?

A. To make the N-terminus an effective nucleophile.
B. To achieve the V_{max} of chymotypsin.
C. To activate the attacking electrophile.
D. To weaken the peptide bond being broken.

10. How many **peptides** result from **cyanogenbromide** treatment of the following peptide?

DEPSMRQGSHIKLWMPILASNCMSIIAVG

A. 2
B. 3
C. [redacted]
D. 5

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11. What **key observation** did Linus Pauling make that allowed him to **predict** the structure of α-helices and β-strands?

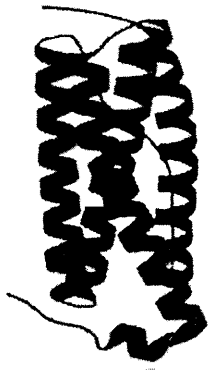
A. The peptide bond oxygen atom is most susceptible to hydrolysis.
B. The peptide bond has an atomic length between that of a single or double bond.
C. The peptide bond O atom is an excellent H bond donor.
D. The X-ray diffraction patterns of proteins are different from those of DNA.

12. What is the **rise per amino acid** in an α-helix? (Å = angstrom)

A. 1.5 Å
B. 3.5 Å
C. 5.4 Å
D. 7.0 Å

— MAKE SURE YOUR STUDENT ID IS ON THE SCANTRON CARD! —

13. The structure on the right belongs to which **family** of proteins?



A. β-barrel
B. β-sandwich
C. α–helix bundle
D. α/β barrel

14. Which of the following amino acids is most likely to be found in the **interior** of a protein?

A. Serine
B. Isoleucine
C. Lysine
D. Aspartic Acid

15. Which of the following atomic interactions contributes most to the formation of **secondary** structures in proteins?

A. Hydrophobic effect
B. Hydrogen bonding interactions
C. Van der Waals interactions
D. Ionic interactions

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16. Completely pure barnase enzyme can be rendered **inactive** at 80° C. Upon cooling to room temperature, the protein becomes **completely active** again. What general principle of protein structure is illustrated by this example?

A. Thermal denaturation is an effective means of purification of proteins.
B. The formation of tertiary structure follows a positive cooperative pathway.
C. The entropy of tertiary structure formation is equal to or greater than the enthalpy.
D. The tertiary structure of barnase is dictated by its primary structure.

17. Enzymes do **NOT** increase reaction rates by _____

A. bringing reactants into close proximity.
B. orienting functional groups so that they can react.
C. providing a binding pocket that is complementary to the transition state.
D. binding to reactant mimics that contain a chromophore.

18. Given that the specific activity of pure prolylpeptidase is 8.2 $mmol.mg^{-1}.min^{-1}$, what percentage of a sample with a specific activity of 16.4 $umol.mg^{-1}.min^{-1}$ consists of prolylpeptidase?

16.4
$0.0164 mmol \cdot mg^{-1} \cdot min^{-1}$

A. 0.2%
B. 0.5%
C. 2%
D. 50%

$100\% \times \frac{0.0164}{8.2}$ 0.002

19. A sample of pure actin protein was placed in a thin cuvette with a pathlength of 0.75 cm and an absorbance of 0.45 at 290 nm was measured. Given that the extinction coefficient of actin is 42680 $M^{-1}cm^{-1}$, what is the concentration of actin in the sample?

A. 1.40 µM
B. 7.91 µM
C. 10.5 µM
D. **14.0 µM**

$A = \varepsilon c l$

$.45 = 42680 (c)(.75)$

$0.45 = 42680\ M^{-1} \cdot cm^{-1} \cdot 0.75 cm \cdot C$

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20. Why was it important for Michaelis and Menten to measure **initial rates** of enzyme-catalyzed reactions at time zero?

A. So they could ignore the reformation of the enzyme-substrate complex from product and enzyme.
B. So they could perform many measurements in a small amount of time to obtain a complete progress of reaction curve.
C. So they were not limited by the V_{max} of the enzyme, thereby obtaining more accurate velocity measurements.
D. So they could make the steady state assumption regarding the formation and breakdown of the enzyme-substrate complex.

21. At what percentage of the V_{max} is an enzyme functioning in the presence of a substrate concentration that is three-times that of the K_M?

A. 50%
B. 66%
C. 75%
D. 80%

75%

$\frac{V_0}{V_{max}} = \frac{(S)}{K_M + (S)}$ $\frac{V_0}{V_{max}} = \frac{S}{K_M + (S)}$ $\frac{3}{1+3}$

22. On a Lineweaver-Burk plot, what is the label of the **y-axis**?

A. 1/[S]
B. $1/v_o$
C. $[S]/v_o$
D. $1/V_{max}$

$1/V_{max}$

3 km
1 km + 3 km
3 km
4 km

23. What is a working definition of K_M?

A. The concentration of substrate that yields $V_{max}/2$.
B. The turnover number for the enzyme.
C. The concentration of enzyme that yields maximum reaction rate.
D. The rate of reaction given by enzyme with highest specific activity.

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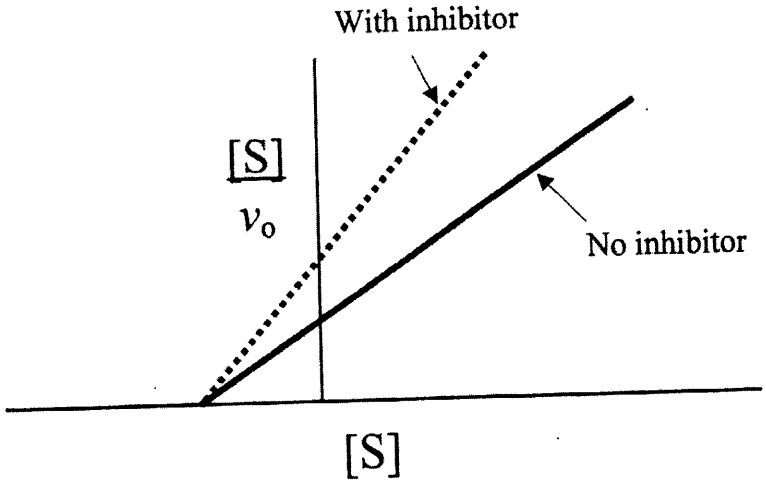
24. In the presence of 7.8 μM competitive inhibitor, the apparent K_M of an enzyme is seven (7) times that seen in the absence of inhibitor. What is the K_i of the inhibitor?

A. 0.98 μM
B. 1.3 μM
C. 7.8 μM
D. 9.8 μM

25. Shown below is a linear transformation of Michaelis-Menten plot data for an enzyme in the presence and absence of an inhibitor. What kind of **linear transformation** plot **AND** kind of **inhibitor** is shown?

A. Lineweaver-Burk plot and competitive inhibitor
B. Lineweaver-Burk plot and uncompetitive inhibitor
C. Woolf-Hanes plot and competitive inhibitor
D. Woolf-Hanes plot and uncompetitive inhibitor



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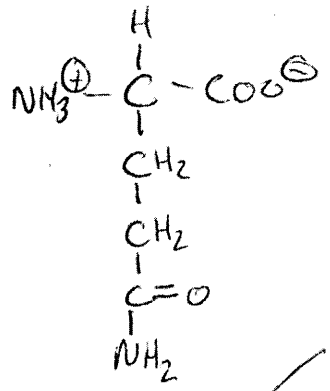
Section 2: Short Answer Questions

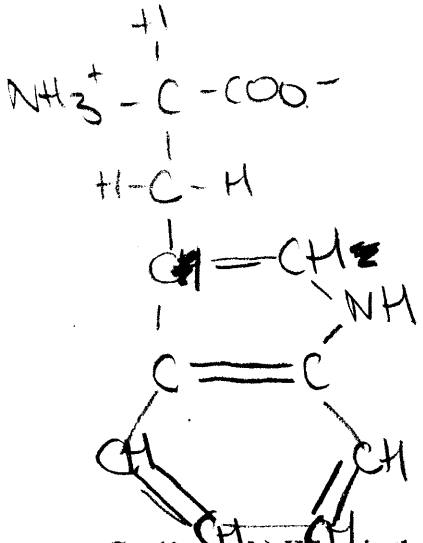
26. Amino acid structures 3

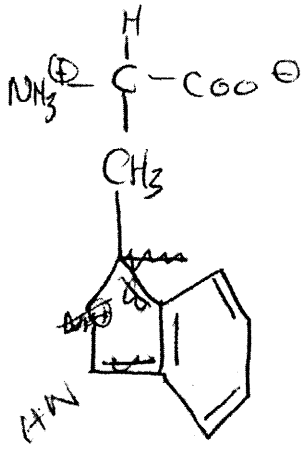
A. **(1 mark)** Draw the complete structure of the amino acid with the single letter code "Q" at pH 7.0:

Glutamine



B. **(2 marks)** Draw the complete structure of tryptophan at pH 7.0:





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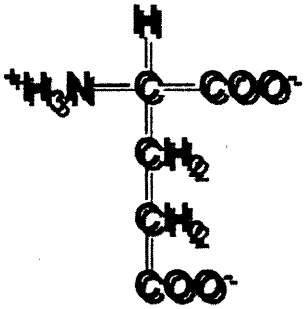
C. **(1 mark)** What is the full formal name of this amino acid?



~~Glutamic Acid~~

Glutamate

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27. Ionization of peptides

(3 marks) Calculate the total charge on the peptide below at **pH 7.1** (refer to last page for pKa values of the ionizable groups). Show all your calculations to earn full marks.

Leu-Ala-Met-Phe-Val-Arg-Tyr-His-Trp-Asn-Ser-Lys-Cys-His-Lys-Asp-Tyr-Ile-Cys-Glu-Gly-Val

+1 +1 +1 +1 -1 -1

1

Total Charge = Arg + His + Lys + Lys - Asp - Glu

= +1 +1 +1 +1 -1 -1

= +2

His → only one within 1 pH unit of 7.1 ∴ use His pKa of 6.5

Total Charge = 3.98 + 2

= 5.98

$pH = pKa + \log_{10}$

$7.1 = 6.5 + \log_{10}$

$10^{7.1-6.5} = \frac{[His]}{[HisH^+]}$

$3.98 = \frac{[His]}{[HisH^+]}$

His, Lys, Arg

Glu, Asp.

∴ the total net charge is 5.98.

28. Protein sequencing.

(2 marks) One sample of a polypeptide was treated with **Trypsin**, and a second sample of the same polypeptide was treated with **Elastase**. The sequences of the resulting fragments are listed below (in single-letter code):

Trypsin: Arg, Lysine

~~TVK~~

~~ASGWI~~ R K

~~TCQGWR~~

~~NFAPHR~~

Elastase: Ala, Gly

~~WI~~

~~SG~~

~~WRA~~ A, G

~~TVKNFA~~

~~PHRTCQG~~

Use this information to write out the original complete sequence of the polypeptide **using single-letter code**: (use end page for rough work, if needed)

2

~~TVKNFAPHRTCQGWRASGWI~~

TVKNFAPHRTCQGWRASGWI

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29. Protein Structure

Use **three-letter codes** to answer the following questions dealing with beta-strand structure:

A. **(1 mark)** In a sequence of six (6) amino acids, what is the **minimum number** of beta-strand-favouring amino acids required to predict that the sequence will be a beta-strand?

0

~~3~~ 4

B. **(1 mark)** What is the pattern of amino acid polarity observed for beta-strands that are part of a protein with beta-barrel tertiary structure?

1

nonpolar, polar, non-polar, polar, etc

C. **(2 marks total for i. and ii.)**

i. Which amino acid(s) are **completely non-polar** AND prefer to adopt **beta-strand structure**? Ile, Val

1 1/2

F, M

ii. Which amino acid(s) are **polar AND uncharged** AND prefer to adopt **beta-structure**?

Thr + Cys.

D. **(3 marks)** Using the information you have described in sections A-C of this question, design a primary sequence for a **beta-strand** consisting of **six (6) DIFFERENT amino acids** that is found in protein with a **beta-barrel** tertiary structure.

~~Ile-Phe~~

~~Ile-Trp-Cys-Val-Tyr-Thr~~

Ile-Cys-Val-Thr-Phe-Trp

N P N P N P

2 1/2

5

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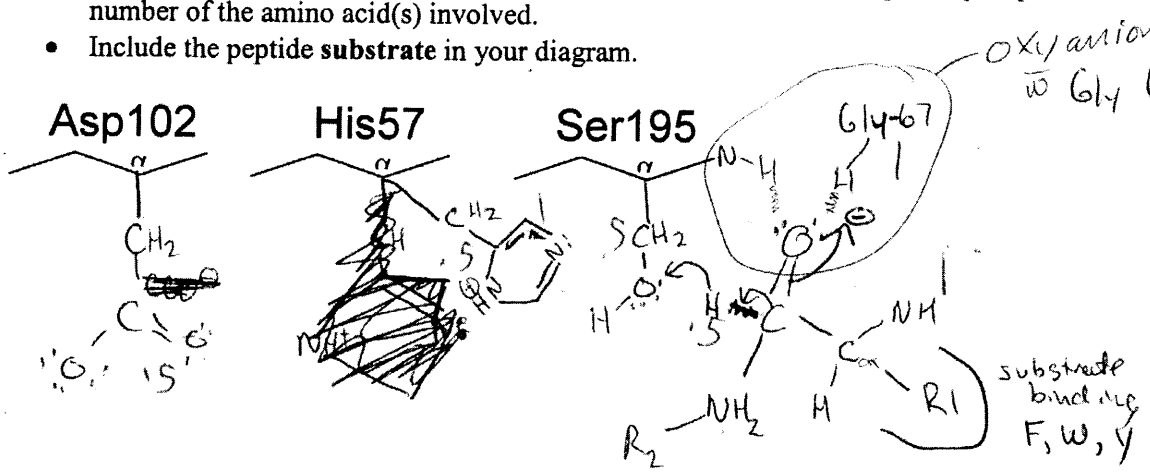
30. Enzyme Mechanisms: Chymotrypsin

A. **(5 marks)** Draw the structure of the **first transition state** of a peptide hydrolysis reaction catalyzed by chymotrypsin, following these instructions:

- The **alpha-carbons** of the main chain are indicated (α) on the schematic; only draw the side chains of the amino acids.
- Include **lone pairs of electrons** and **formal charges** on the appropriate atoms in these structures.
- Indicate where the **oxyanion hole** exists, including the identity and primary sequence number of the amino acid(s) involved.
- Include the peptide **substrate** in your diagram.



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stabilizes transition state

B. **(1 mark)** What is the role of the **oxyanion hole** in the transition state of the chymotrypsin catalysis reaction?

To [redacted] use hydrogen bonding to take off double bond on oxygen of substrate to allow for 1st group to break away.

C. **(1 mark)** What property of **histidine** is the central feature of the chymotrypsin catalytic cycle?

general acid base

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31. Enzyme Kinetics: Michaelis-Menten.

Given the chemical reaction:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

(2 marks) Derive the Michaelis constant (K_M), starting from the steady state assumption. Show all your work for full credit.

rate of break = rate of formate

$k_2[ES] + k_{-1}[ES] = k_1[E][S]$

$(k_2 + k_{-1})[ES] = k_1[E][S]$

$\frac{(k_2 + k_{-1})[ES]}{k_1} = [E][S]$

$K_M = [E][S]$

$K_M = \frac{k_2 + k_{-1}}{k_1}[ES]$

$K_M = \frac{k_2}{k_{-1}}[E][ES]$

$[E][S]\,k_1 = [ES]\,k_1$ 0.5

$[ES]\,k_1 = [E][P]\,k_2$

$k_2 = \frac{k_1}{k_{-1}}$

$K_M = \frac{k_1}{k_{-1}}(k_2)$

$V_0 = k_2[E]^{k-1}$

$K_M[ES] = (E)(S)$

$K_M(ES) = ((E)_{tot} - (ES))(S)$

$K_M(ES) + (ES)(S) = E_{tot}$

$(ES)(K_M + [S]) = E_{tot}(S)$

$(ES) = \frac{E_{tot}(S)}{K_M + [S]}$

$V_0 = \frac{k_2(E)_{tot}(S)}{K_M + (S)}$

$V_0 = \frac{V_{max}[S]}{K_M + (S)}$

pKa Values Chart:

Side chain pKa values of amino acids:

Asp 4.0	Glu 5.0	His 6.5
Cys 8.5	Tyr 10.0	Lys 10.2
Arg 12.5		

Average N-terminal amino group has pKa = 9.5

Average C-terminal carboxylic acid group has pKa = 2.5